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THE EFFECT OF SLEEP FRAGMENTATION ON THE PERCEPTION OF EXPERIMENTALLY-INDUCED DEEP MUSCLE PAIN IN WOMEN WITH PRIMARY DYSMENORRHOEA AND HEALTHY CONTROLS

By

Chloe Eliza Flinn

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, 2018

Declaration

I, Chloe Eliza Flinn, declare that this Dissertation is my own work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

…….....
(Chloe Eliza Flinn)

……. Day of ……..... 20....

The procedures used in this dissertation were approved by the University of the Witwatersrand's Committee for Research on Human Subjects (Clearance no. M150211), which adheres to the principles of the Declaration of Helsinki.

Presentations arising from this research project

Oral presentations (local):

Flinn, C.E, Baker F.C., Iacovides, S. The effect of sleep fragmentation on the perception of experimentally-induced deep muscle pain in women with primary dysmenorrhoea and healthy controls. *Physiological Society of Southern Africa Congress, August 2017*, Groenkloof Campus, University of Pretoria, Pretoria, Gauteng.

Abstract

The relationship between sleep and pain is bidirectional: sleep disturbances lead to increased pain sensitivity and increased pain disrupts sleep. Women with primary dysmenorrhoea experience monthly recurrent menstrual pain, associated with sleep disturbances, which could contribute to increased pain sensitivity. The present study aimed to determine the effect of sleep fragmentation on the perception of experimentally-induced deep-muscle pain during a pain-free phase of the menstrual cycle in women with severe primary dysmenorrhoea compared to pain-free controls.

Following an interview process and one-week screening phase, ten women with primary dysmenorrhoea (22 ± 3y) and nine healthy controls matched for age (21 ± 3y), visited the Wits Sleep Laboratory on four occasions, in a randomised order, during the pain-free follicular phase; an adaptation, a baseline (uninterrupted sleep), and two consecutive sleep fragmentation nights (disrupted sleep). Each morning, perception of experimentally-induced deep-muscle pain was assessed using: a) the submaximal effort tourniquet test (forearm ischaemia), and b) intramuscular injection of hypertonic saline in an area within (lower back) and outside (forearm) of referred menstrual pain.

Sensitivity to deep-muscle pain did not differ between groups after the baseline night. However, after one night of sleep fragmentation, women with dysmenorrhoea experienced an increase in hypertonic saline-induced forearm muscle pain sensitivity compared with the controls ($p = 0.01$), and both groups of women experienced an increase in ischaemic pain sensitivity compared to baseline ($p \leq 0.05$). Sensitivity to hypertonic saline-induced pain within the lower back was not altered by sleep fragmentation. Pain sensitivity returned to baseline levels after the second night of sleep fragmentation.

These findings provide support for a relationship between disrupted sleep and pain sensitivity, however, effects of sleep fragmentation are mixed; depending on duration of sleep fragmentation, pain modality, and whether women have primary dysmenorrhoea.

Acknowledgements

I never dreamed of conducting and completing a Masters, but with the help of some great individuals and institutions the idea of expanding my knowledge in research became possible.

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Again, thank you so much to my parents. You have supported me both financially and emotionally during this extremely difficult endeavour, I truly cannot thank you enough for all the love and support. You are my favourite people.

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List of Abbreviations

Aδ	Alpha-delta
AUC	Area Under the Curve
BMI	Body Mass Index
CNS	Central Nervous System
EEG	Electroencephalogram/Electroencephalography
EMG	Electromyogram/Electromyography
EOG	Electro-oculogram/Electro-oculography
GHQ	General Health Questionnaire
IASP	International Association for the Study of Pain
IL	Interleukin
LEP	Laser-evoked Potential
LH	Luteinising Hormone
LOC	Left Outer Canthus
MPQ	McGill Pain Questionnaire
MRI	Magnetic Resonance Imaging
NREM	Non-rapid Eye Movement
PILL	Pennebaker Inventory of Limbic Languidness
PMS	Premenstrual Disorder
POMS	Profile of Mood States
PSG	Polysomnography
PSQI	Pittsburgh SQ Index
REM	Rapid Eye Movement
ROC	Right Outer Canthus
SD	Standard Deviation
SE	Sleep Efficiency
SNK	Student-Newman-Keuls
SOL	Sleep Onset Latency
SQ	Sleep Quality
SWS	Slow Wave Sleep
TIB	Time in Bed
TNFα	Tumour Necrosis Factor alpha
TSD	Total Sleep Deprivation
TST	Total Sleep Time
VAS	Visual Analogue Scale
WASO	Wake After Sleep Onset

CHAPTER 1 – INTRODUCTION

To understand the scope of the following study, including the selection of methods and clinical subgroup to be researched, an extensive review of the literature was conducted. Firstly, the physiology of sleep and pain is described, followed by an introduction into how sleep and pain influence each other within different populations. Secondly, the clinical subgroup chosen for the study is described, followed by an introduction into how pain and sleep are affected within the clinical subgroup.

1.1 The Relationship between Sleep and Pain

1.1.1 Understanding Sleep

Sleep is a reversible behavioural state characterised by temporary disengagement from the external environment, with the eyes normally closed and muscles relaxed[1]. Polysomnography (PSG) is an objective measure of sleep which makes use of electroencephalography (EEG) to record electrical brain activity, electro-oculography (EOG) to record eye movements and electromyography (EMG) to record muscle activity, which allows us to recognise, and distinguish between, the various stages of sleep. Using this method, two states of normal human sleep have been observed; namely non-rapid eye movement (NREM) and rapid eye movement (REM) sleep[1]. NREM sleep is further divided into stages 1, 2, 3 and 4 (stages 3 and 4 are commonly referred to as slow wave sleep (SWS), or deep sleep, in humans), with waveforms increasing in amplitude and decreasing in frequency from stage 1 to 4 (Figure 1.1a)[1]. Stage 1 sleep is characterised by relatively low-voltage, mixed-frequency activity of 4 – 7 Hz in the EEG channels, with slow eye movements observed in the EOG channels during the initial transition from wake to sleep. Stage 2 sleep is characterised by K-complexes (a sharp negative deflection with high amplitude, followed by a positive wave) and sleep spindles (short bursts of high frequency waves). When transitioning to SWS, EEG activity begins to slow, producing high-voltage, slow-wave patterns in both the EEG and EOG channels. SWS begins as waves of 0.5 – 2 Hz reach an amplitude of at least 75µV for more than 20% of an epoch. REM sleep EEG activity is similar to that of stage 1 with low-voltage, mixed-frequency activity, with the exception of 2 – 6 Hz waves known as sawtooth waves. REM sleep is mainly characterised by minimal activity in

the EMG channels, due to low muscle tone, and the presence of bursts of high amplitude waves in the EOG channels, due to rapid eye movements (Figure 1.1b)[1-3].

The pattern of sleep, in an 8-hour period, generally begins with NREM sleep; gradually moving from stage 1 to 4. REM sleep is entered thereafter, approximately 80 minutes after the initial NREM stage 1. Thereafter, NREM and REM alternate in a 90-minute cycle through the rest of the night. Although these 90 minute cycles, often displayed in a hypnogram (Figure 1.2), are the general expected sleep pattern in healthy individuals, cycles may vary[1].

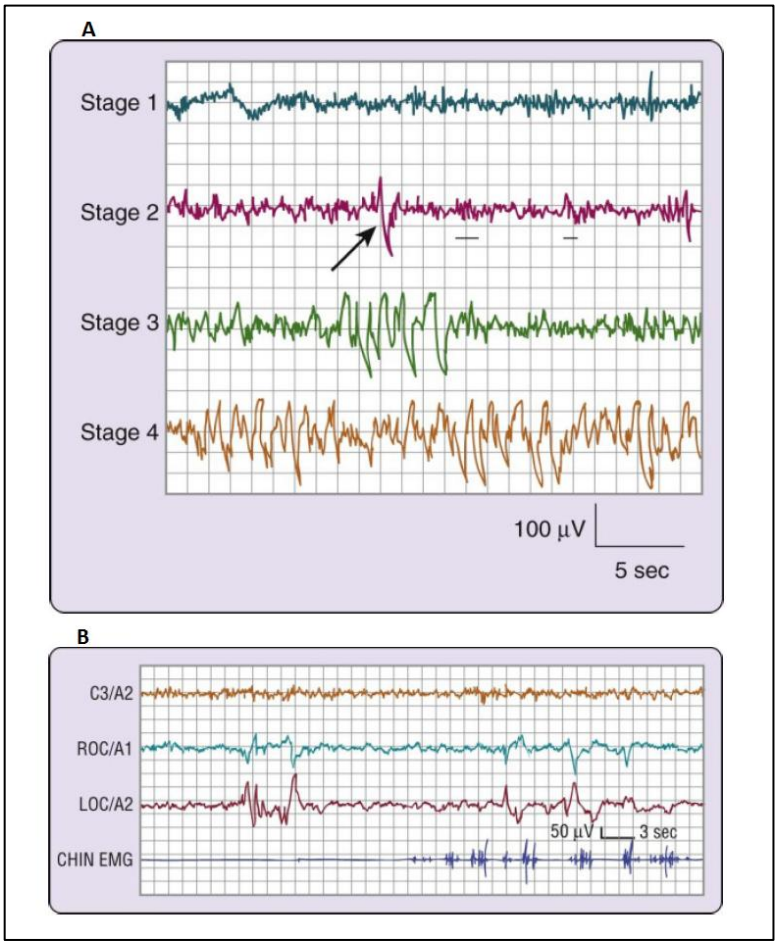


Figure 1.1: a) Non-rapid eye movement (NREM) sleep waveforms as recorded in an electroencephalogram (EEG); amplitude increases and frequency decreases from stage 1 to 4. The arrow indicates a K-complex and the underline shows two sleep spindles; characteristic of stage 2 sleep. b) Rapid eye movement (REM) sleep characterised by low-voltage, mixed-frequency activity in the EEG channel (C3/A2) and bursts of high amplitude waves in the electro-oculographic (EOG) channels [Right outer canthus of the eye (ROC/A1) and left outer canthus of the eye (LOC/A2)] due to rapid eye movements. Muscle tone is also low with phasic twitches as demonstrated in the electromyographic (EMG) channel (CHIN EMG).[1]

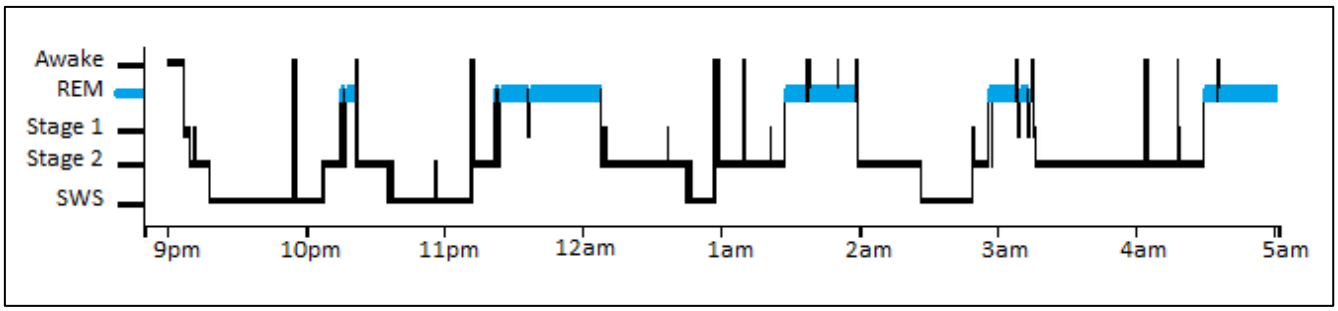


Figure 1.2: A hypnogram illustrating the sleep pattern of a healthy 22 year-old female. Sleep stages were assessed in 30 second epochs, for a night of normal sleep. Data collected during a night of 8-hours uninterrupted sleep in the present study.

While sleep takes up a large component of our lifetime, the reason as to why we sleep is poorly understood, however, researchers have proposed multiple theories regarding the reason as to why we sleep and the functions of sleep[4, 5]. With what we know today, sleep is believed to serve the following purposes: energy conservation whereby biological functions are reduced during sleep, leading to less energy expenditure than during quiet wakefulness[6, 7]; restorative function whereby cerebral glycogen stores depleted during wakefulness are replenished[8, 9] and neurotoxic products that accumulate during wakefulness are cleared[10, 11], allowing for the restoration of brain function; connectivity function whereby synapses are reorganised and reinforced to increase efficiency[12, 13], obsolete memories are erased[14], new memories are consolidated to facilitate learning[15], neuromuscular circuitries are reinforced[16] and plasticity is maintained[13, 17]; and immune system function whereby sleep has been found to facilitate the immune response, specifically to vaccinations[18]. One way to better understand the functions of sleep is to conduct studies to determine the effect of sleep deprivation on healthy individuals. Such studies have found that sleep deprivation leads to, among other things: an increase in appetite[19], salivary cortisol[20], sleepiness[21] and sympathetic tone (relating to an increased stress response), an increased heart rate, decreased insulin secretions[22], reduced inflammatory cytokines including interleukin-6 (IL-6) and tumour necrosis factor alpha (TNFα)[21], decreased psychomotor performance[21] and reduced carbohydrate tolerance[22]. In the long-term, the effects of sleep deprivation may ultimately lead to an increased risk of obesity[20, 22], depression[20], insulin resistance[21, 22], cardiovascular disease[21], osteoporosis[21], hypertension[22] and pain[23].

1.1.2 Understanding Pain

According to the International Association for the Study of Pain (IASP)[24], pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. This definition emphasises the fact that pain is subjective partly because it includes the emotional experience of pain, meaning, a stimulus that may cause pain in one individual may not necessarily cause pain in another, due to the emotional and therefore psychological factor of pain; which may be influenced by one's experience of pain throughout life[24, 25].

The physiology and anatomy of pain is well defined, with the transduction, conduction, transmission, modulation and perception of pain often being referred to as the pain pathway[25]. The pain pathway begins with nociceptive receptors (nociceptors) that respond to different types of noxious stimuli, such as thermal, chemical and mechanical stimuli[25]. Somatic nociceptors have been divided into two different fibre types due to anatomical and physiological differences; C-fibres, which are unmyelinated, polymodal nociceptors with slow conduction of signals (0.5 – 2.0 $m.s^{-1}$) leading to the typical throbbing or burning sensation associated with pain, and Alpha-delta (Aδ) fibres, which are myelinated, mechano-heat nociceptors with fast conduction of signals (5 – 20 $m.s^{-1}$) leading to the sharp stinging sensation associated with pain[25]. The pathway of visceral nociception is similar to that of somatic nociception; the major differences being that a) the organs are innervated more by C-fibres than Aδ-fibres, leading to diffuse and poorly localised sensations of pain, and b) the C-fibres are activated mostly by mechanical and chemical stimuli such as distension and inflammatory markers[26]. Most nociceptors express proteins when 'activated' by a noxious stimulus, leading to the transduction of a signal to the spine[25]. Within the spine, visceral afferent pathways may converge with somatic pathways leading to pain referral, such that visceral pain may be felt in somatic structures[26]. These processes are not 'hard-wired' and can therefore undergo functional changes and modulation, depending on the conditions of the body, such as inflammation[25], and may result in hyperalgesia – defined as an increased response to a noxious stimulus[24]. The signals within the spine then cross over the spinal cord and move up to the sensory cortex, via the midbrain and thalamus. Pain is also processed in other areas of the brain such as the hippocampus, limbic system and reticular formation. Interactions between these areas of the brain provide us with evidence that the perception

of pain is influenced by memory, cognition and emotion[25]; which also means that pain perception has high inter-individual differences.

1.1.3 The Reciprocal Relationship between Sleep and Pain

The relationship between sleep and pain is bidirectional, whereby sleep disturbances lead to an increase in pain sensitivity[23, 27-34], and increased pain leads to reduced quality and quantity of sleep[27, 35, 36]. The interactions between sleep and pain are complex and involve various physiological mechanisms, for example, the neurochemical and neuroimmune systems[37].

With regards to the neurochemical systems, serotonin has been found to be involved in the regulation of sleep[38] and pain modulation[39, 40]. While the exact mechanisms are unknown, Foo and colleagues (2003)[41] suggest that during the course of chronic pain, serotonergic raphe cells become dysregulated and contribute to sleep problems experienced by patients with chronic pain. In turn, REM sleep deprivation has been reported to decrease serotonin concentrations in the brains of rats[42], and reduced concentrations of serotonin metabolites have been found in patients with fibromyalgia[43], further contributing to the idea that sleep deprivation and chronic pain dysregulate the serotonergic pathways. Serotonin is also involved in pain modulation pathways, such as the descending pain inhibitory pathways[39, 40] which have been found to be disrupted in patients with chronic pain[44-46], and by sleep disruption in patients diagnosed with chronic temporomandibular joint pain[47]. In addition to serotonin; dopamine and endogenous opioids have been implicated in the sleep-pain relationship (For a full review on the role of dopamine and opioids in the sleep-pain relationship, see Finan and colleagues (2013)[48]). In summary, dopamine receptors are found in abundance in the reticular activating system, a critical region involved in sleep regulation, and due to the intricate interactions between serotonin and dopamine[49], it is possible that reduced dopamine metabolites and phasic response to pain observed in patients with chronic pain may affect sleep regulation via the reticular activating system[48]. Opioid receptors are also found in certain brain regions involved in sleep regulation, such as the suprachiasmatic nuclei, with studies suggesting that endogenous opioid systems are affected by sleep deprivation, leading to diminished opioid analgesia[48]. However, more research is required in order to determine the involvement, and interactions between, serotonin, dopamine and opioids in the sleep-pain relationship[37].

With regards to the neuroimmune system, one theory proposes an interesting link between sleep and chronic pain through the role of two specific central nervous system (CNS) cytokines; IL-1β and TNFα[37, 38, 50-52]. IL-1β and TNFα are two of very few substances to meet all the criteria of being defined as sleep regulatory substances involved in sleep homeostasis[38, 51, 52]. IL-1β and TNFα influence neurons located in brain regions involved in sleep-wake behaviour, such as the hypothalamus, hippocampus and brainstem[38], whereby somnogenic concentrations of IL-1β and TNFα increase SWS, however, high concentrations of either cytokines interrupt sleep by increasing fragmentation and decreasing NREM sleep[38, 52]. In patients with chronic pain, it has been observed that both cytokine (IL-1β and TNFα) concentrations increase as pain intensity increases[50], possibly leading to concentrations that interrupt, rather than facilitate, sleep. IL-1β and TNFα levels further increase during sleep deprivation[21, 52], possibly increasing pain through pro-inflammatory mechanisms[53] and contributing to the ongoing cycle between sleep and pain in these patients with chronic pain. Interestingly, both serotonin and IL-1 regulate sleep by engaging in reciprocal interactions[38], suggesting an interaction between the neurochemical and neuroimmune systems involved in the sleep-pain relationship.

Evidence of a bidirectional relationship between sleep and pain has been demonstrated in both insomniacs and patients with chronic pain, whereby a trend has emerged suggesting that sleep disturbance may predict future pain more so than existing pain may predict future sleep disturbances[48]. However, in order to fully understand the sleep-pain relationship, studies have focused on assessing the effect of sleep disturbances on both experimental and chronic pain, as well as the effect of both clinical and experimental pain on sleep in a unidirectional manner, as discussed in the following sections.

1.1.3.1 The Effect of Pain on Sleep

Clinical Pain

Multiple studies have been conducted to determine the effect of clinical pain on sleep. Many of these studies have shown that patients with pain do indeed experience regular sleep disturbances[35, 54-56]. The percentage of 'poor' sleepers within a chronic pain population in one such study was found to be as high as 70%, with only 10% claiming to be "good" sleepers[57]. It has also been documented that patients diagnosed with rheumatoid arthritis, chronic low back pain, musculoskeletal chronic widespread pain, fibromyalgia, ankylosing

spondylitis and migraines experience sleep disturbances compared with healthy controls[27, 35, 54, 55, 58, 59]. More specifically, subjective studies conducted on patients with chronic pain, such as chronic lower back pain, demonstrate that these patients experience worse sleep quality (SQ) compared to controls[35, 36, 60], with objective studies demonstrating lower sleep efficiency (SE) brought about by a longer sleep onset latency (SOL), more time spent in bed, longer wake after sleep onset (WASO) and increased sleep fragmentation, compared with controls[61-65].

Although it has been repeatedly shown that patients with chronic pain commonly experience sleep disturbances, the reason as to why this relationship exists is still unclear. The majority of studies show that as subjective pain intensity increases, subjective SQ decreases; with fragmentation and longer SOL most commonly reported[36, 55, 60, 66-68]. It is thought that increased fragmentation in these patients occurs due to the experience of pain which causes arousals throughout the night, which has been demonstrated in burn victims whereby patients reporting night time pain suffered more so with sleep disturbances than those reporting daytime pain[67]. Only a handful of studies have shown that no relationship exists between pain intensity and subjective SQ[69]. However, this may be explained by the fact that night-time pain severity, rather than overall pain severity, is more likely to be associated with sleep complaints. Also, night-time pain severity may affect specific sleep measures (ie. time spent awake), rather than overall sleep complaints[69]. Therefore, the different methodologies used to assess sleep and pain may lead to conflicting findings between studies. Disturbed sleep in patients with chronic pain could also be explained partly by other factors such as depression[55, 70] and attention to pain[27]. Nicassio and colleagues (2012)[55] demonstrated that rheumatoid arthritis patients who reported more pain experienced more depressive symptoms, and that depression and pain intensity were independently associated with an increase in sleep disturbances. These findings contribute to the growing body of literature suggesting that sleep, pain and negative mood, such as depression, share a three-way relationship whereby mood plays an integral role in the sleep-pain relationship[48, 71]. Affleck and colleagues (1996)[27] found that attention to pain is associated with a decrease in SQ and that this relationship is not explained by pain intensity in women with fibromyalgia. It has also been suggested that patients with chronic pain may spend more time being inactive by spending more time in bed (TIB) or napping during the day, which in turn leads to poorer sleep at night[69]. Chronic pain has also been shown to have

long-term effects on sleep, where patients diagnosed with chronic widespread pain have an increased risk of insomnia onset 3-years after their initial check-up[56]. Similarly, chronic arthritic pain has been found to predict sleep disturbances two years later[66].

Experimental Pain

For experimental studies, a wide range of pain modalities have been developed for use in human participants, including electrical, thermal (heat or cold), mechanical (pressure), and ischaemic pain, just to name a few. These different pain modalities target different nociceptors and may be applied to the skin surface or deep muscle tissue of different anatomical regions[72]. Thus, findings may differ across experiments based on the type and location of the pain induction method used. Typically, a noxious stimulus is applied acutely, until the participant reaches their pain threshold and/or tolerance. Pain threshold is described as the measurement of a noxious stimulus at which pain is first felt, while pain tolerance is described as the measurement of a noxious stimulus at which pain becomes unbearable[24]. Other indicators of perceived pain used in the literature include pain intensity, pain quality and pain distribution. Many different techniques are used to assess pain intensity, however, the most common techniques include the visual analogue scale (VAS; typically a 100-mm line with 100 being the worst pain possible)[72, 73] and numerical scale (typically a scale of 0 to 10, with 10 being the worst pain possible), which assess the level of present or past pain[72]. Pain quality is used to describe pain, such as a burning, itching, tight sensation, and is usually measured using the McGill Pain Questionnaire (MPQ)[72, 74]; while pain distribution is used to assess the anatomical location of pain using sketches or body diagrams[72].

Taking into account that chronic pain populations commonly suffer from insomnia and behavioural changes such as depression and anxiety[71], experimental studies have been designed in order to determine the effect of experimental pain on sleep in otherwise healthy individuals to eliminate any effects the comorbidities associated with chronic pain may have on sleep. The literature on the topic of the effect of experimentally-induced pain on sleep is limited, however, findings suggest that pain disrupts sleep, with effects dependent on stage of sleep. Noxious stimuli are more likely to induce arousals when applied during lighter stages of sleep, i.e. stage 2 sleep, compared to deeper stages of sleep, i.e. SWS[75-77]. Bentley and colleagues (2003)[76] also observed that arousals during SWS and REM sleep occurred

when heat pain reached tolerance levels, equivalent to those measured during the awake state, while lower levels of heat pain were sufficient to induce arousals during stage 2 sleep. There is some evidence to suggest that different experimental pain modalities exert different effects: one study found that deep-muscle pain or pressure pain induced arousals during SWS, whereas cutaneous pain did not[78]. Also, different intensities of pain elicit different responses across individuals, i.e. a noxious stimulus applied during SWS at the threshold level may be sufficient to cause an arousal in one participant, while pain tolerance is reached before an arousal in another[78]. Overall, it has been demonstrated that pain is modulated differently during different states of arousal[79]. Only one study has reported the effects of experimentally-induced pain on subjective SQ where it was found that SQ was significantly reduced during the nights where noxious stimuli, using hypertonic saline infusions, were applied compared to a night of habitual sleep (73 vs 90 mm, as measured on a 100-mm SQ VAS)[77]. In summary, clinical studies conducted on patients with chronic pain show that pain is associated with disrupted sleep, both subjectively and objectively measured, and experimental pain studies indicate directly that pain causes disrupted sleep, more specifically, continuity variables are affected such as overall SE and WASO.

1.1.3.2 The Effect of Sleep Disturbance on Pain

Trouble falling asleep, waking up at night, tiredness and other sleep problems have been found to strongly predict the onset of pain conditions later in life[80-85]. One study demonstrated that girls who experienced general sleeping problems at 16 years of age were likely to develop neck pain 2 years later, and those who had insufficient quality or quantity of sleep were at risk of developing neck and lower back pain[81]. Sleep deprivation has also been found to increase the odds of developing chronic somatoform pain[82], and disrupted sleep has been found to be associated with the development of chronic pain in previously pain-free patients, as well as the development of chronic widespread pain in patients previously only reporting chronic regional pain[84]. Severe sleep problems are associated with the development of headaches one year later[80], and patients with sleep disorders such as primary insomnia and restless leg syndrome tend to experience more headaches than those without any sleep complaints[58]. Difficulties falling asleep and/or self-reported sleep disorders have also been found to increase the risk of developing fibromyalgia[83], and patients diagnosed with fibromyalgia who experience sleep problems tend to have an

increase in pain symptoms[86]. Pain associated with rheumatoid arthritis, chronic somatoform pain, temporomandibular joint disorder and juvenile polyarticular arthritis, as well as many other chronic pain conditions, has also been found to be exacerbated by sleep restriction, sleep deprivation, sleep disruption, an increase in insomnia severity, poor SQ and, in general, poor sleep[55, 82, 86-89].

Furthermore, experimental sleep deprivation has been found to increase sensitivity to pain; whereby, as early as 1934 it was reported that 60 hours of sleep deprivation lowered the cutaneous pain threshold of otherwise healthy individuals[90]. Since then, many experimental sleep deprivation and pain protocols have been carried out to determine whether sleep deprivation affects pain perception, with majority of studies finding that sleep deprivation increases pain sensitivity[28-31, 33, 34, 91, 92], while others report no effect of sleep deprivation on pain[32, 93]. Studies assessing the effect of sleep deprivation on experimentally-induced pain employ different experimental techniques for the induction of pain and different sleep deprivation protocols. The different pain assessment techniques employed in studies include, but are not limited to; thermal pain which may include cold[29, 34] and/or heat[28-30, 32-34, 93] pain, pressure pain[28, 31, 34, 93], cutaneous pain[34, 90, 92], forearm ischaemic pain[92], and spontaneous pains[31, 34, 91, 92] such as muscle soreness, headache, stomach pains and any other bodily pains not induced by a physical noxious stimulus[31, 92]. In addition, pain may also be assessed using pain threshold, pain tolerance and/or pain intensity. The different sleep deprivation protocols employed include, but are not limited to: total sleep deprivation (TSD), where a participant is kept awake for a period of at least one night (24 hours)[28-31, 33, 34, 90, 91, 93]; sleep restriction, where a participant is allowed a limited period of sleep usually under 6 hours[30-32]; selective sleep stage interruption, where a participant is woken up each time they enter a specific stage of sleep such as SWS or REM[28, 30, 33]; and more recently, sleep fragmentation where a participant is woken up randomly, multiple times throughout the night[31, 92]. Table 1.1 gives a summary of the findings of the effect of each type of sleep deprivation protocol on experimentally-induced pain in healthy human participants.

Table 1.1: A summary of studies, in chronological order, assessing the effect of experimentally-induced sleep deprivation on experimentally-induced pain sensitivity in healthy participants.

Authors (Year)	Number of Participants	Sleep Deprivation/Disruption Protocol	Pain Protocol	Pain Outcome
Cooperman *et al.* (1934)[90]	6 males	TSD (60 hours)	Cutaneous pain threshold	Pain threshold reduced*
Moldofsky *et al*. (1976)[94]	6 males	Three consecutive nights of SWS interruption	Pressure pain thresholds and spontaneous bodily complaints	Pressure pain thresholds decreased after SWS interruption but not REM interruption. SWS interruption group reported more spontaneous bodily complaints (musculoskeletal pain and stiffness)
	6 males, 1 female	Three consecutive nights of REM sleep interruption		
Drewes *et al.* (1997)[93]	10 students[#]	TSD (24 hours)	Heat and joint pressure pain thresholds	No change*
Older *et al.* (1998)[95]	10 males, 3 females	Three consecutive nights of SWS interruption	Pressure pain thresholds and spontaneous bodily complaints	No change to pressure pain thresholds. Increased bodily complaints after the 3rd night
Lentz *et al.* (1999)[96]	12 females	Three consecutive nights of SWS interruption	Pressure pain thresholds and spontaneous bodily complaints	Decreased pressure pain thresholds after 2nd and 3rd night. Increased bodily complaints after 3rd night (musculoskeletal discomfort)
Arima *et al.* (2001)[97]	10 males	Three consecutive nights of SWS interruption	Pressure pain thresholds and spontaneous bodily complaints	No change
Onen *et al.* (2001)[28]	9 males	TSD (40 hours) followed by REM sleep interruption	Pressure and heat pain tolerance	Pressure pain tolerance decreased after TSD, but no change after SWS or REM interruption. No change in heat pain tolerance was observed after TSD and SWS or REM interruption
		TSD (40 hours) followed by SWS interruption		
Kundermann *et al.* (2004)[29]	15 males, 9 females	Two nights TSD separated by a night of recovery sleep.	Heat and cold pain thresholds, and spontaneous pain	Heat pain threshold decreased after both TSD nights. No significant change observed for cold pain threshold or spontaneous pain

*No statistics employed

[#] Gender not specified

LEP = Laser-evoked Potential; NREM = Non-rapid Eye Movement sleep; REM = Rapid Eye Movement sleep; SWS = Slow Wave Sleep; TIB = Time in Bed; TSD = Total Sleep Deprivation; TST = Total Sleep Time; VAS = Visual Analogue Scale

Authors (Year)	Number of Participants	Sleep Deprivation/Disruption Protocol	Pain Protocol	Pain Outcome
Roehrs *et al.* (2006)[30]	1 male, 6 females	One night of sleep restriction (4 hours TIB, early morning) and TSD separated by 3 – 7 days recovery	Heat pain threshold	Pain threshold significantly reduced by sleep restriction, TSD and REM interruption, with no change after one night of NREM interruption, however, a significant reduction was observed after a second consecutive night of NREM interruption
	2 males, 4 females	Two nights of sleep restriction (2 hours TIB, early morning), REM interruption and NREM interruption separated by 3 – 7 days recovery		
Smith *et al.* (2007)[31]	10 females	Three nights of sleep fragmentation (multiple awakenings = 4 hours 40 minutes TST with 8 hours TIB) followed by one night TSD	Pressure pain threshold, spontaneous pain and pain inhibition	No significant change in pressure pain threshold across any of the nights for either group. Ability to inhibit pain was significantly reduced by all three sleep fragmentation nights, but not the sleep restriction nights and TSD. Spontaneous pain increased after two and three nights of sleep fragmentation, as well as after TSD
	10 females	Three nights of sleep restriction (4 hours 40 minutes TIB, early morning) followed by one night TSD		
Tiede *et al.* (2010)[32]	8 males, 2 females	One night of sleep restriction (4 hours TIB, early morning)	Heat pain threshold	No significant change
Azevedo *et al.* (2011)[33]	9 males	TSD (48 hours)	LEP threshold and subjective VAS rating of heat pain	Subjective VAS ratings and LEP threshold increased significantly after TSD, but not REM interruption
	9 males	Four nights of REM interruption		
Schuh-Hofer *et al.* (2013)[34]	8 males, 6 females	One night of TSD	Cutaneous pain sensitivity, spontaneous pain, cold, heat and pressure pain thresholds	No change in spontaneous pain. Cutaneous pain sensitivity increased while cold, heat and pressure pain thresholds decreased
Iacovides *et al.* (2017).[92]	11 females	Two nights of sleep fragmentation (multiple awakenings = 4 hours 40 minutes TST with 8 hours TIB)	Cutaneous pain threshold, spontaneous pain, and ischaemic pain over a 10-minute interval	Decreased cutaneous threshold and increased ischaemic pain sensitivity after one and two nights of sleep fragmentation. No change in spontaneous pain

LEP = Laser-evoked Potential; NREM = Non-rapid Eye Movement sleep; REM = Rapid Eye Movement sleep; SWS = Slow Wave Sleep; TIB = Time in Bed; TSD = Total Sleep Deprivation; TST = Total Sleep Time; VAS = Visual Analogue Scale

As illustrated in Table 1.1, the most common studies evaluating the effect of sleep deprivation on pain sensitivity have been conducted on healthy subjects using heat[28-30, 32, 34, 78] and pressure[28, 31, 34, 94-97] pain protocols. Studies conducted on heat[29, 30, 34] and pressure[28, 34] pain generally report that TSD leads to decreased pain thresholds and/or tolerance, and therefore demonstrates that noxious stimuli become more painful after one or more nights of sleep deprivation. Additionally, sleep restriction and REM sleep interruption have been shown to increase sensitivity to heat pain[30], while only SWS interruption has been shown to increase sensitivity to pressure pain[94, 96]. Other types of pain assessment techniques that have been used to assess the effect of sleep deprivation on experimental pain sensitivity include cutaneous, cold and ischaemic pain; whereby cutaneous pain sensitivity has been shown to increase after TSD[34, 90] and sleep fragmentation[92]; similarly, ischaemic pain sensitivity has been shown to increase after sleep fragmentation[92]; and, in contrast, cold pain sensitivity has been shown to be unaffected by sleep deprivation[29]. It could be argued that increased pain sensitivity, due to sleep deprivation, is the result of increased sensitivity of the general somatosensory system and, hence, an increase in overall touch perception, however, evidence suggests that TSD, and sleep fragmentation, leads to hyperalgesia without actually altering the detection thresholds[29, 34, 90, 92]. Therefore it can be concluded that sleep deprivation causes a hyperalgesic effect that cannot be explained by changes in the general somatosensory system[29].

To my knowledge, only one study has assessed the effect of sleep deprivation on pain modulation, specifically pain inhibition – the ability to inhibit a noxious stimulus. Smith and colleagues (2007)[31] studied the effect of sleep restriction and sleep fragmentation on diffuse noxious inhibitory controls (a phenomenon whereby one noxious stimulus inhibits the perception of pain induced by a second noxious stimulus applied to a distant anatomic site) in young, healthy women. The sleep fragmentation protocol, which consisted of interrupting sleep across an 8-hour period with forced awakenings of different durations (e.g., 60 min for one awakening, and 20 min for others), was employed due to its similarity to the sleep pattern of patients with chronic pain, whose sleep is generally disrupted randomly during a part of the night, or in multiple bouts throughout the night[60]. Women who underwent sleep fragmentation had significantly lower diffuse noxious inhibitory control scores than the groups obtaining habitual (uninterrupted) or restricted sleep, indicating that sleep fragmentation specifically disrupts the endogenous pain-inhibitory system[31].

One of the most recent studies assessing the effect of sleep deprivation on pain was conducted on healthy women, and demonstrated that sleep disruption exacerbates experimentally-induced pain in healthy pain-free women. Specifically, the study assessed the effect of two nights of sleep fragmentation on cutaneous, deep-muscle and spontaneous pain, using Smith and colleague's (2007)[31] sleep fragmentation protocol, where it was found that both one and two nights of sleep fragmentation increased both deep-muscle and cutaneous pain sensitivity, with a greater effect being observed after the second night[92] (see Iacovides *et al.*[92] in Table 1.1).

In summary, studies have produced mixed findings regarding the effects of experimentally-induced sleep deprivation on pain. Some of the variability in the literature is likely due to the methodological differences across studies, with a mix of pain modalities applied, different types and durations of sleep manipulations, and small sample sizes. There is, therefore, a need for further studies to investigate the effect of sleep disruption on pain. Taking this into account, and given the clinical relevance of sleep fragmentation in patients with chronic pain[31, 55], I have decided to extend the investigation of the effect of sleep fragmentation on experimentally-induced pain to not only healthy pain-free women, but also to otherwise healthy women who experience recurrent monthly pain and are believed to have increased central sensitivity to pain[98] – primary dysmenorrhoea.

1.2 The Menstrual Cycle and Dysmenorrhoea

1.2.1 The Menstrual Cycle

Women undergo cyclic hormonal changes known as the menstrual cycle. The menstrual cycle includes both the ovarian and uterine cycles, whereby physical and hormonal changes occur monthly (± 28 days; normal range 24 – 35 days); with the first day of menstrual bleeding taken as day 1[99, 100].

Changes to the ovaries occur during the ovarian cycle due to three distinct hormonal changes[99, 100]; 1) the follicular phase occurs from day 1 until ovulation (day 14) - during the early follicular phase reproductive hormone concentrations are low, including oestrogen and progesterone, as this phase progresses oestrogen levels steadily increase until day 14 where a luteinising hormone (LH) surge occurs; 2) ovulation usually occurs on day 14, 16-24 hours

after the LH surge, whereby oestrogen synthesis is diminished and progesterone is secreted; and 3) the luteal phase occurs from day 14, after ovulation, until day 28, before the onset of menses - during the early luteal phase there is a steady increase of both oestrogen and progesterone, however, progesterone is the dominant hormone at this stage. During the late luteal phase both oestrogen and progesterone concentrations decrease, leading to menstrual bleeding.

The uterine cycle consists of three distinct physical changes; 1) menstrual bleeding (day 1), which occurs during the early follicular phase when oestrogen levels are still rising; 2) the proliferative phase, which occurs during the mid-to-late follicular phase in order to prepare the endometrial lining for pregnancy; and 3) the secretory phase, which occurs after ovulation turning the endometrial lining into a secretory structure[99, 101]. If pregnancy does not occur, an ischaemic period during the last two days of the secretory phase occurs, whereby the arteries supplying the uterus constrict intermittently, leading to the next phase (menses)[101].

Due to the intricate workings of the hormonal and physical changes during the menstrual cycle, there are many disorders associated with the menstrual cycle such as pre-menstrual disorder (PMS), menorrhagia (abnormally heavy menstrual bleeding) and dysmenorrhoea, to name a few[102].

1.2.2 Dysmenorrhoea

Dysmenorrhoea, described as painful menstruation, has prevalence ranges of 50 – 90 % of the female population, depending on assessment techniques[103-109]. Dysmenorrhoea affects the daily lives of women during menstruation and has been found to contribute to pain-related work and school absenteeism in approximately 20 – 50 % of women[104, 107, 110, 111]. The pain associated with menstruation not only contributes to absenteeism, which leads to loss of productivity, and therefore income, it also leads to a decrease in quality of life by affecting mental and social-wellbeing[106, 109, 112, 113].

Dysmenorrhoea can be divided into two etiologically different subgroups: Primary Dysmenorrhoea and Secondary Dysmenorrhoea[103, 106, 108, 114]. Primary dysmenorrhoea refers to painful menstruation that is not associated with any identifiable pelvic pathology and is thought to be caused by elevated uterine prostaglandin production during menstruation.

This increases the frequency and strength of uterine contractions causing a lack of oxygen to the uterus, resulting in ischaemic pain[103, 107-109, 112-115]. The onset of primary dysmenorrhoea is usually soon (within 6 – 12 months) after menarche and pain begins just before or after the commencement of menstrual bleeding, usually lasting 24 – 72 hrs with greater severity on the first day[103, 105, 106, 108]. Pain originates in the lower abdomen, and may radiate around to the lower back and down into the inner thighs[103]. Symptoms other than pain may include nausea[103, 105-107, 110, 112, 115], vomiting[103, 105-107, 110, 115], headache[103, 105, 106, 110], diarrhoea[103, 105-107, 115], fatigue[103, 105, 106], irritability[105, 109], and less frequently, dizziness and collapse[103, 105]. Secondary dysmenorrhoea is defined as the presence of abdominal pain related to pelvic pathologies such as endometriosis, ovarian cysts and inflammatory pelvic disease[103, 108, 112, 114]. Pain onset occurs more than 2 years after menarche, and pain may also occur during other phases of the menstrual cycle, not just during menstruation[103].

The present study focuses on primary dysmenorrhoea due to the fact that primary dysmenorrhoea is a unique pain condition; such that pain is recurrent and occurs monthly in association with menstruation[103], allowing researchers to accurately predict times at which women are experiencing pain or are pain-free. The nature of primary dysmenorrhoea gives researchers the opportunity to assess both pain and sleep in the same women during painful conditions (menstruation) versus pain-free conditions (mid-to-late follicular phase), allowing them to better understand the sleep-pain relationship. Further, researching sleep and pain in women with primary dysmenorrhoea during pain-free phases of the menstrual cycle allows for the unique opportunity to ensure results are not complicated by the comorbidities often associated with chronic pain, due to the fact that mood[116, 117], quality of life[113] and sleep[116, 118] are only poorer in association with painful menses. However, as discussed next, several studies show that women with primary dysmenorrhoea appear to have altered pain responses throughout the menstrual cycle, even when not in pain, compared to women without dysmenorrhoea, possibly due to pain sensitisation.

1.2.3 The Perception of Pain in Women with Dysmenorrhoea

In 1944, Haman first suggested that women with dysmenorrhoea have lower pain thresholds to a pressure stimulus applied to the thumb, than pain-free women[119]. Since then, multiple studies have been conducted to determine whether women with dysmenorrhoea are hyperalgesic to experimental pain. However, studies assessing pain between

dysmenorrhoeic women and women without any menstrual-related complaints have used a host of different pain assessment techniques, which have produced mixed findings. Sensitivity to electrical stimulation, pressure, heat and ischaemic pain has been found to either be greater in women with dysmenorrhoea[120-124] or no difference has been observed between women with and without dysmenorrhoea[121, 125-127]. Laser stimuli[127] and experimentally-induced deep-muscle pain with the use of hypertonic saline injections[117] have demonstrated an increased perception to pain in women with dysmenorrhoea across the menstrual cycle. In contrast, cold pain[128] has demonstrated the opposite such that women with dysmenorrhoea are less sensitive to pain than pain-free women, however, this finding was observed during the follicular phase only.

Furthermore, differences in the results of various experimental studies have been attributed to a) the arbitrary division of menstrual cycle phase[98, 100, 129-131] and b) the location and depth of pain stimuli, whereby pain induced by electrical stimulation has been found to produce reduced pain thresholds in women with dysmenorrhoea, compared to controls, in the subcutaneous tissue and muscle, with greater differences being observed in the muscles[121]. Reduced pain thresholds in women with dysmenorrhoea have also been found to be more predominant in areas within menstrual pain referral (i.e. abdomen) as compared to areas outside menstrual pain referral (i.e. limbs)[121]. However, another study, using heat pain, reported that women with dysmenorrhoea are more sensitive to pain outside areas of referred menstrual pain[122].

Another factor to consider when assessing pain in women is menstrual cycle phase, as pain sensitivity may differ according to menstrual cycle phase in women with and without primary dysmenorrhoea as a result of the different hormonal milieu, although findings are mixed (See Iacovides *et al*. (2015)[100], for a review). Some studies have found changes in pain sensitivity in women with dysmenorrhoea according to menstrual cycle phase[121, 122, 128] although other studies reported no change in the perception of pressure[126], heat[123, 127], ischaemic[126] and deep-muscle[117] pain across the menstrual cycle. The presence of clinical pain may also impact pain thresholds to experimental pain in women with primary dysmenorrhoea, as it has been reported that during menstruation, when dysmenorrhoeic pain is present, pressure and heat pain thresholds have been found to be reduced when compared to the rest of the menstrual cycle[122], and a study conducted on women with and without dysmenorrhoea using electrical pain stimulation found that the lowest pain

thresholds, regardless of dysmenorrhoeic status, occurred around menstruation for the subcutaneous tissue and muscle (but not for skin)[121].

With advances in technology and study techniques, strong evidence from recent brain imaging studies that have investigated brain structure and function rather than only behaviour, supports the notion that women with primary dysmenorrhoea are more sensitive to pain than women without any menstrual-related complaints[123, 132-134]. The CNS differences observed in women with primary dysmenorrhoea include, but are not limited to abnormal cerebral metabolism[132], abnormal grey matter volume changes in areas associated with pain transmission and modulation[133], little or no deactivation of brain responses to noxious stimuli[123] and maladaptive neuroplasticity of descending pain modulatory systems[134]. Furthermore, a large study containing 330 women (163 with primary dysmenorrhoea) observed a substantially higher incidence of normal variants - rare but not abnormal findings - measured using structural brain magnetic resonance imaging (MRI), compared with control women, however, the meaning behind these findings warrant further research and only demonstrate that CNS differences are observed between women with primary dysmenorrhoea and healthy controls[135]. All of these changes suggest that the sensory and cognitive components of pain processing and/or modulation are physiologically and morphologically different in women with dysmenorrhoea compared to healthy controls, and not only during painful menstruation[132-135], which support the findings that women with primary dysmenorrhoea are hypersensitive to pain throughout the menstrual cycle. These findings, such that pain is processed differently in women with primary dysmenorrhoea, and that these women are hyperalgesic throughout the menstrual cycle and not only during menstruation, have led to the suggestion that women with primary dysmenorrhoea may have central sensitisation – defined as hypersensitivity to peripheral inputs due to abnormal pain processing in the CNS[98].

1.2.4 Sleep Architecture of Women with Dysmenorrhoea

Few studies, with small sample sizes, have investigated sleep architecture in women with primary dysmenorrhoea. Two studies found that sleep is negatively affected by dysmenorrhoeic pain during menstruation[116, 118]. In the one study, objective and subjective measures of sleep were compared between women with primary dysmenorrhoea and healthy controls during their menstruation, mid-luteal and mid-follicular menstrual cycle

phases[118]. Differences in sleep were observed in women with severe primary dysmenorrhoea during the painful menstruation phase, compared with pain-free healthy controls, and compared with their own pain-free mid-luteal and mid-follicular phase. More specifically, women with primary dysmenorrhoea reported significantly reduced subjective SQ, and objective measurements, using PSG, showed a significant reduction in SE attributed to more time spent moving, awake and in stage 1 sleep. Further, compared to controls, REM sleep was found to be reduced across the menstrual cycle in women with primary dysmenorrhoea, and even more so during menstruation compared with their own mid-luteal and mid-follicular phases[118]. Similar findings were observed by Iacovides and colleagues (2009)[116] where, using PSG recordings, women with severe primary dysmenorrhoea were found to have a reduced SE and amount of time spent in REM sleep, with an increase in stage 1 sleep, during menstruation as compared to the pain-free follicular phase. However, another study found that women with dysmenorrhoea had similar sleep architecture during menstruation, compared with controls[136]. However, no distinction as to the type of dysmenorrhoea (primary or secondary) was made and the study was cross-sectional, with women being assessed only once during their menstrual cycle, with comparisons made to other women at different phases (36 women were assessed during menstruation of which 16 women experienced menstrual pain, 90 women were assessed during the mid-to-late follicular phase, 69 during the luteal phase, 19 during an ovulatory phase and 114 were in an anovulatory menstrual cycle)[136]. Furthermore, dysmenorrhoea has been found to resolve with age[104] and the women in the study that reported no differences in sleep architecture between women with and without dysmenorrhoea[136] were older (35 – 37 yr[137] vs. 20 – 23 yr[116,118]) and reported less severe menstrual pain than those in the studies reporting differences in sleep relative to controls[116, 118]. The severity of menstrual pain may also play an important role in sleep disturbance during menstruation in women with dysmenorrhoea as it has been reported that women with severe dysmenorrhoea experience reduced subjective SQ and SE, with increased SOL, as compared to women with mild dysmenorrhoea[137]. As described earlier, in line with the theory that sleep and pain share a reciprocal relationship[23, 27-36, 69], it is possible that disturbed sleep contributes to heightened pain and vice versa, in women with dysmenorrhoea.

Given the evidence that sleep disruption, including fragmented sleep, increases experimentally-induced pain sensitivity in pain-free women[92], and that women with

dysmenorrhoea appear to be more sensitive to pain than pain-free healthy control women, and more so in the muscles than subcutaneous tissue and skin[121], I aim to determine whether women with severe primary dysmenorrhoea are also more sensitive to sleep disruptions by determining the effect of sleep fragmentation on the perception of deep-muscle pain in women with primary dysmenorrhoea compared to healthy control women during a pain-free phase of the menstrual cycle. This will allow for a better understanding of primary dysmenorrhoea by determining whether the reciprocal sleep-pain relationship exists in this population. Studies have shown that women with primary dysmenorrhoea suffer with sleep complaints during menstruation[98, 116, 118], are hyperalgesic throughout the menstrual cycle[117, 119-124, 127] and are prone to developing central sensitisation[98]. Therefore the current study aims to determine whether experimentally-induced sleep disturbances during the pain-free phases of the menstrual cycle contribute to pain sensitivity (Figure 1.3), driving the vicious cycle of the sleep-pain relationship in this group of women.

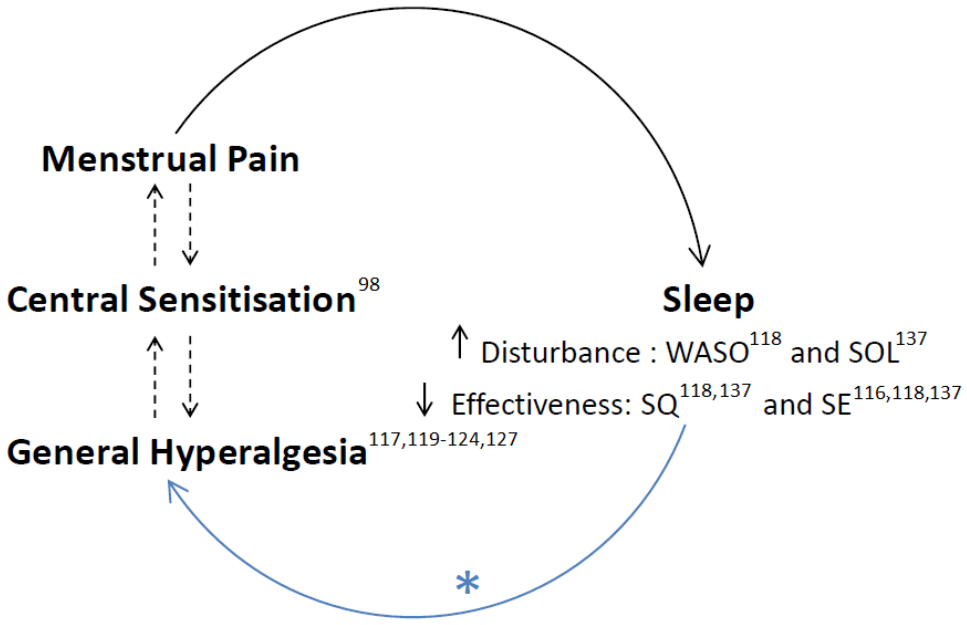


Figure 1.3: The proposed sleep-pain relationship in women with primary dysmenorrhoea, whereby menstrual pain leads to sleep disturbances and sleep disturbances lead to an increased sensitivity to pain. *The effect of sleep disturbance on pain sensitivity in women with dysmenorrhoea is unknown and is to be determined in the present study. --- Direction of cause and effect unknown.

The superscripted numbers denote the references of the studies supporting that menstrual pain affects sleep[116,118,137] and those showing that women with primary dysmenorrhoea are hyperalgesic[117,119-124,127] and prone to central sensitisation[98].

WASO, wake after sleep onset; SOL, sleep onset latency; SQ, sleep quality; SE, sleep efficiency.

1.3 Aim and Objectives

1.3.1 Aim

To determine the effect of sleep fragmentation on the perception of experimentally-induced deep-muscle pain in women with severe primary dysmenorrhoea during a pain-free phase of the menstrual cycle compared with pain-free controls.

1.3.2 Objectives

- To determine the effect of sleep fragmentation on the perception of deep-muscle pain, using an exogenous pain-producing technique (hypertonic saline injections), within and outside an area of referred menstrual pain in women with and without dysmenorrhoea.
- To determine the effect of sleep fragmentation on the perception of an experimentally-induced endogenous deep-muscle pain-producing technique (ischaemic pain) in women with and without severe dysmenorrhoea.
- To compare the effect of one and two nights of sleep fragmentation, relative to a baseline night, on the perception of deep-muscle pain, using both an endogenous and exogenous pain-producing technique in women with and without severe dysmenorrhoea.

1.3.3 Hypotheses

- Sleep fragmentation will exacerbate the perceived level of deep-muscle pain in both groups of women, both within and outside areas of menstrual pain referral. The exacerbation of pain will be greater in women with dysmenorrhoea, across all interventions and muscle-pain-producing techniques.
- An accumulative effect of sleep fragmentation on pain will occur, such that sensitivity to pain will be greater after a second, consecutive night of sleep fragmentation compared to the first sleep fragmentation night, for both groups of women.

CHAPTER 2 – METHODS

2.1 Subject Recruitment

All experimental procedures received ethical clearance from the University of the Witwatersrand's Committee for Research on Human Subjects (Clearance no. M150211, Appendix A), which adheres to the principles of the Declaration of Helsinki. Healthy women, between the ages of 18 and 30 were recruited to partake in this study. All volunteers were invited to an interview, during which, informed written consent was obtained from all the women prior to any experimental procedures. In addition, during the interview, volunteers completed a screening questionnaire (Appendix B) to ensure they were generally healthy and that they met all the inclusion criteria. To be included in the study the women were required to be non-smokers, nulliparous and to have regular menstrual cycle lengths (22 – 35 days) and sleep-wake cycles. Normal psychological status was determined using the 30-item version of the General Health Questionnaire (GHQ - Appendix C)[138], with volunteers having to score less than 6. As determined by the interview and screening questionnaire, volunteers were excluded from the study if they showed any signs of secondary dysmenorrhoea, suffered any chronic illness, and/or had been taking any chronic medication (including pain medication) more than once a week. Volunteers were also excluded if they had an irregular sleep-wake cycle or poor SQ; determined using the Pittsburgh Sleep Quality Index (PSQI - Appendix D)[139]. The PSQI is a self-rated measure assessing SQ over a one-month period by determining subjective SQ, SOL, sleep duration, SE, sleep disturbances, use of sleep medication, and daytime dysfunction[139]. To be included in the study women were required to have a global PSQI score of less than or equal to 6[140]. The women were also required to be free of any musculoskeletal disorders or any sensitive areas located in or around their lower back and/or arm[116, 117].

Thirty four (34) women met all the inclusion criteria and were then divided into two groups; women were included in the dysmenorrhoeic group if they had a history of severe menstrual pain, starting shortly (within 2 years) after menarche and had not been using any type of hormonal contraception for at least 6 months prior to participating in the study. The severity of menstrual pain was determined using a 100-mm VAS (Appendix B)[73] consisting of a 100 mm line anchored from "no pain at all" to "the worst pain I have ever felt", where each

woman was required to assess the intensity of her menstrual pain over the past 6 months. The distance, in millimetres, was measured from the beginning anchor point ''no pain at all'' to the mark filled in by the women, in order to obtain the VAS scores[73, 117]. According to Collins and colleagues (1997)[73], VAS scores less than 30 mm are considered as mild pain, and scores larger than 54 mm are considered as severe pain. Therefore, women who rated their menstrual pain as being higher than 54 mm on the VAS were considered to have "severe" dysmenorrhoeic pain and were placed in the primary dysmenorrhoeic group, whilst women who rated their menstrual pain as less than 30 mm were placed in the control group[73, 117]. Any women who met the inclusion criteria but scored between 30 and 54mm on the VAS were excluded from participation.

2.2 Screening Phase

The estimated date of the start of each woman's next menstrual cycle was calculated, with the commencement of menstruation being taken as day 1. Using these dates as a reference, the women then underwent a one-week screening phase before the start of menstruation. The screening phase was used to assess subjective measures of pain, as well as both objective and subjective measures of sleep.

2.2.1 Subjective Measures of Sleep and Pain – Questionnaires

During the week-long screening phase, the women were required to complete morning and evening questionnaires (Appendix E). The evening questionnaire, was to be completed every evening before bedtime and consisted of a McGill Pain Questionnaire (MPQ)[74] body chart and a 100-mm VAS anchored from "no pain" to "the worst pain ever" to assess the presence, location and intensity of any bodily pain, but also to ensure the absence of abdominal pain not associated with menstruation (possible secondary dysmenorrhoea), and to ensure the absence of pain in the lower back and arm (site of experimental pain assessments). The morning questionnaire, to be completed shortly after waking, every morning, contained a sleep diary that assessed various subjective sleep parameters such as bedtime, sleep time, TIB and wake-up time. The morning questionnaire also contained an MPQ body chart and pain VAS, again to assess body pain location and intensity.

2.2.2 Objective Measure of Sleep – Actiwatch

To obtain an objective measure of 24h daily activity, including sleep (particularly sleep-wake cycles) the women were required to wear an Actiwatch[141] (Spectrum device, Philips Respironics) on their non-dominant hand during the week-long screening phase. The Actiwatch provides data about body movement overtime; the data is then interpreted using Actiware v6.0.9 (Respironics Inc., Pennsylvania, USA, 2017). The actiwatch was used mainly to determine each woman's total sleep time (TST) by recording extended times of little or no activity, to confirm that each woman maintained a regular sleep-wake cycle (i.e. similar bedtimes and wake-up times each night), and to confirm the absence of sleep disturbances[141]. The Actiwatch was therefore used to confirm compliance with the sleep diary and together this data was used to ensure the women had normal sleep-wake cycles (i.e. a regular sleep-wake cycle with a sufficient amount of sleep; more than 6 but less than 9 hours sleep with few disturbances), as well as to calculate the lights-out and lights-on time for the experimental phase. Figure 2.1 is an example of the activity data received from the Actiwatch of one participant over one week.

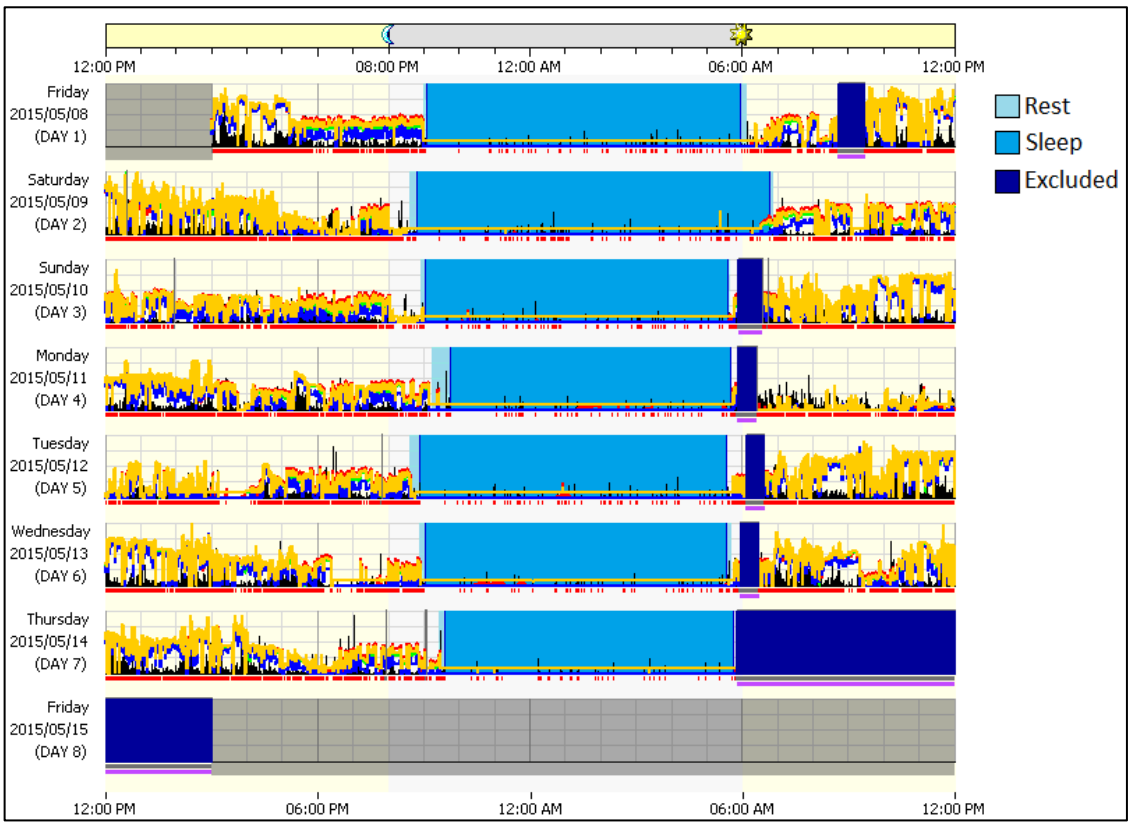


Figure 2.1: Spectrum Actiwatch data collected during the 1-week screening phase of the present study for one of the women who participated. The Actiwatch data shows that the woman has regular sleep-wake cycles, with an average bedtime of 20h58 and wake-up time of 05h54 and a total sleep time (TST) of 8 hours and 24 minutes.

Of the 34 women who met the study criteria, 15 women (5 women with dysmenorrhoea and 10 controls) were excluded from the study following the screening phase, for the following reasons: three women developed irregular menstrual cycles; one withdrew herself from the study due to time constraints; one woman was lost to contact; and ten women were unable to maintain regular sleep-wake cycles during the screening phase of the study. Therefore, ten women with severe primary dysmenorrhoea and nine healthy controls completed their participation in the study.

2.3 Experimental Phase

The experimental phase of this study always occurred during the pain-free follicular phase of each woman's menstrual cycle in order to eliminate any menstrual cycle-related and, in the dysmenorrhoeic women, any menstrual pain effects on experimental pain[98]. The women were required to visit the School of Physiology's Sleep Laboratory, University of the Witwatersrand on four occasions (Fig. 2.2). The first night was always the adaptation night, which always occurred on day 3 of each woman's menstrual cycle, and served to familiarise the women with the experimental procedures and with sleeping with PSG electrodes. The adaptation night was followed by two recovery nights at home, during which time the women were required to fill out the sleep diary and wear an Actiwatch. Thereafter, always between days 6 – 11 of the follicular phase, the women completed a baseline night and two consecutive sleep fragmentation nights, in a randomised order. In addition, the baseline and sleep fragmentation nights were always separated by two recovery nights, where the women were instructed to maintain their usual bedtimes, as determined from the screening phase. To ensure compliance the women filled out the sleep diaries and wore an Actiwatch between all laboratory visits.

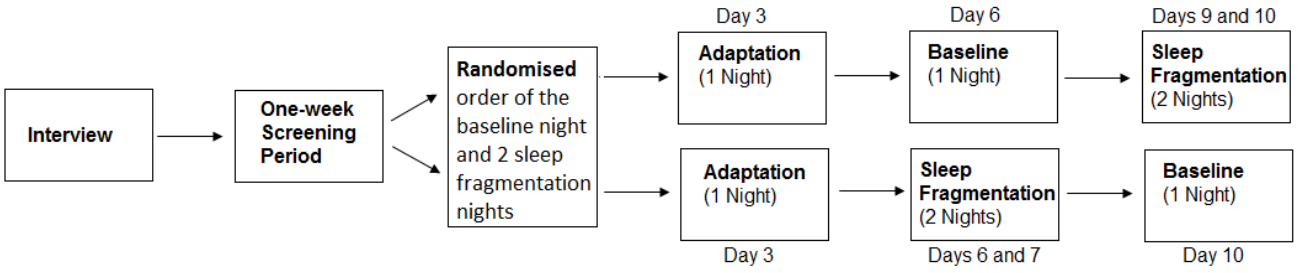


Figure 2.2: A flow diagram showing the randomisation of the baseline and two sleep fragmentation nights, in order to eliminate any effects of adaptation to pain. Participants either came into the laboratory on days 3,6,9 and 10 or on days 3,6,7 and 10 of their menstrual cycle.

The women were instructed to refrain from consuming caffeinated food/beverages and from taking any naps during the day prior to the experimental nights.

2.3.1 Adaptation Night

The women were asked to arrive at the sleep laboratory at least two hours before their bedtime on day 3 of their menstrual cycle. They were fitted with electrodes for PSG[116] in order to familiarise them with all the experimental procedures, as well as with the environment. Lights-out depended on the participant's usual bedtime, as determined during the week-long screening phase, and usually occurred between 21:00 and 00:00. Lights-on was then calculated 8 hours later, which allowed for an 8-hour period in bed. After waking, the women were given a standardised breakfast and reminded to arrive back at the sleep laboratory after two nights of recovery sleep, which were monitored using the sleep diary and Actiwatch.

The following sections detail the events of the baseline and two sleep fragmentation nights (points 2.3.2 – 2.3.3), with all the details of the questionnaires and experimental procedures described in the section thereafter (section 2.4).

2.3.2 Baseline Night

For the baseline night (occurring either on day 6 or on day 10 of each woman's menstrual cycle), the women were required to arrive at the sleep laboratory at least two hours before their bedtime. The women filled out the Profile of Mood States[142] (POMS) questionnaire and the Pennebaker Inventory of Limbic Languidness[143] (PILL). A von Frey hair[144, 145] assessment was conducted to assess touch, and the women were then fitted with electrodes for PSG recordings, as with the adaptation night. Lights-off and lights-on occurred at the same time as the adaptation night (according to each woman's habitual sleep-wake cycle) and the women were allowed 8 hours in bed. After waking, the women re-evaluated their mood (POMS) and spontaneous pain (PILL) and also completed two separate 100-mm VASs to assess SQ and morning vigilance[116]. A randomly selected arm, and the opposite side of the back, was then selected and an anaesthetic cream was applied to the areas (forearm and lower back)[117]. The PSG electrodes were then removed, and the von Frey hair assessment was repeated. Ischaemic pain was then assessed on the opposite arm to that randomly selected for the application of the anaesthetic cream. Thereafter, approximately 45 - 60

minutes after the application of the anaesthetic cream, hypertonic saline injections were carried out in the arm and back, in a randomised order. Once all the procedures were completed the women were given a standardised breakfast. If the women were required to visit the sleep laboratory for the sleep fragmentation nights, they were reminded to arrive back after two nights of recovery sleep at home. Sleep and activity at home were monitored using the sleep diary and Actiwatch.

2.3.3 Sleep Fragmentation Nights

Sleep fragmentation occurred on two consecutive nights (either on days 6 and 7 or on days 9 and 10 of each woman's menstrual cycle). Women were asked to arrive at the sleep laboratory at least two hours before their bedtime on both nights. As with the baseline night, the women completed the POMS and PILL questionnaires, underwent the von Frey hair assessment, and were fitted with the electrodes for PSG recordings. The women were again allowed 8 hours in bed, with the same bedtimes as the adaptation night (according to their habitual bedtimes). However, on these nights, they were woken-up randomly during the night using a sleep fragmentation protocol derived by Smith and colleagues (2007)[31]. The 8-hour sleeping period for each woman was divided into 8 one-hour blocks; one of which was randomly assigned as an hour-long awakening. The remaining 7 one-hour blocks were further divided into 3 x 20-minute blocks. During the randomly assigned one hour and during one of the randomly chosen 20-minute periods for each of the remaining 7 hour blocks, the women were woken up by the principal investigator and were required to remain awake and responsive for the entire one-hour/20-minute "forced awakening" period. The sleep fragmentation protocol therefore allowed for a maximum TST of 280 minutes[31]. The women were woken up by turning on a dim light (5 – 11 lux) and calling their name. They were then requested to sit upright, and responsiveness was ensured by the principal investigator by engaging the women in conversation. When the women struggled to remain awake, they had the option of playing card and board games with the principal investigator to help them stay awake. After the final waking time in the morning (lights-on), the women re-evaluated their mood (POMS) and spontaneous pain (PILL). As with the baseline night, the women assessed SQ and morning vigilance using two separate VASs[116]. A randomly selected arm, and the opposite side of the back, was then selected and an anaesthetic cream was applied to the areas (forearm and lower back)[117]. The PSG electrodes were then removed, and the

von Frey hair assessment was repeated. Ischaemic pain was then assessed on the opposite arm to that selected for the application of the anaesthetic cream. Thereafter, approximately 45 - 60 minutes after the application of the anaesthetic cream, hypertonic saline injections were carried out in the arm and back, in a randomised order.

Once all the procedures were completed the women were given a standardised breakfast. After the first sleep fragmentation night, the women were allowed to leave the laboratory and continue their day as normal, however they were asked to refrain from taking any naps and consuming any caffeine, and they were required to return to the laboratory that evening to repeat all the procedures described above for the second sleep fragmentation night. Due to safety concerns, women were driven to their day's destinations after having their sleep fragmented. Following all experimental procedures after the second night of sleep fragmentation, if the women were required to visit the sleep laboratory for the baseline night, they were reminded to arrive back after two days of recovery sleep at home. Sleep and activity at home were monitored using the sleep diary and Actiwatch.

Four women had to return to the laboratory during a different menstrual cycle due to incomplete data collection; one due to equipment failure and the rest due to unforeseen schedule changes.

2.4 Experimental Procedures and Questionnaires

2.4.1 Polysomnography (PSG)

Electrode placements for EEG, EOG and EMG recordings were used to record sleep during the experimental nights (Appendix F). According to the international 10-20 system, electrodes C3 and C4, referenced to A2 and A1, respectively, were used for the EEG electrode placements[2]. EEG signals were sampled at a rate of 200 Hz, and a high-pass filter at 0.53 Hz and low-pass filter at 30 Hz was used[116]. PSG recordings were stored on a computerised EEG system (Cadwell Easy EEG, version 2.0.2, Cadwell Laboratories Inc, Kennewick WA).

The PSG data were manually scored in 30 s epochs according to the Rechtschaffen and Kales (1968) criteria[3], with combining stage 3 and 4 sleep to represent SWS. These data were used to determine the TST of each woman (to confirm that the sleep fragmentation procedure

was successful); SOL, determined from lights-off to the first epoch of stage 1 sleep; SE, calculated as TST/TIBx100; as well as the percentage and time spent in each sleep stage.

2.4.2 Sleep Quality (SQ) and Morning Vigilance

SQ and morning vigilance were measured every morning after the experimental nights using two separate 100-mm VASs. The VAS for SQ was anchored from "no sleep" to "best sleep I have ever had" and the morning vigilance VAS was anchored from "not at all alert and fresh" to "most alert and fresh I have ever felt"[116]. The women were required to rate their SQ and morning vigilance by drawing a mark on the 100-mm line. A measurement, in mm, was then taken from the first anchor point to the mark filled in by the women.

2.4.3 Profile of Mood States (POMS)

The POMS[142] (Appendix G) is a validated questionnaire which consists of 65 adjectives that describe how a person is feeling according to 6 identifiable moods: Depression-Dejection; Tension-Anxiety; Confusion-Bewilderment; Fatigue-Inertia; Anger-Hostility and Vigor-Activity. Mood was assessed every evening and morning. In order to calculate a total mood score from the POMS, I added the negative mood scores, subtracted the vigor score and added a constant of 100 to avoid negative scores[116, 117].

2.4.4 Von Frey Hairs

Carrier-mounted optic glass von Frey hairs[145] (Optihair$_2$-Set, Marstock Nervtest, Germany) that exert forces between 0.25 and 512mN were used to assess touch threshold (Appendix H). Touch was assessed every evening and every morning before and after the ischaemic pain test. Women were blindfolded and von Frey hairs, starting with the smallest force (0.25 mN), were applied perpendicularly to the anterior skin surface of the distal phalanx of the index finger until the von Frey hair buckled in the middle. The first filament (with the smallest force) to be felt by each woman was recorded[124].

2.4.5 Pain Assessments

2.4.5.1 Ischaemic Pain

Experimental ischaemia was induced in the women's forearm (the forearm opposite to the arm to receive the hypertonic saline injection), using the submaximal effort tourniquet test[146]; a standard procedure involving the occlusion of blood flow to the forearm with a cuff placed above the elbow. Prior to inducing ischaemia, the women's maximal hand-grip strength was determined by having the women squeeze a hand-grip dynamometer with all their strength for a period of one second, thereafter, 30% of their maximal hand-grip strength was calculated and recorded. After inflating the tourniquet cuff to 200 mmHg, the women performed 20 hand-grip exercises, at 30% of their maximal hand-grip strength, using a hand-grip dynamometer. Thereafter, the cuff remained inflated, at 200 mmHg, for 10 minutes[92, 124, 146]. Every minute for a total of 10 minutes of ischaemia, the women were required to rate the intensity of the ischaemic pain on a 100-mm VAS anchored from "no pain at all" to "the worst pain ever". At the end of the 10 minutes, before deflating the cuff, to confirm that ischaemia was achieved, the women completed another non-painful somatosensory test using the von Frey hairs, as described above. Thereafter, the cuff was deflated.

2.4.5.2 Hypertonic Saline Injections

Experimentally-induced muscle pain was carried out on two distinct muscle groups: the extensor muscles of the forearm, and the erector spinae muscle of the lower back. The extensor muscle group of the forearm is located outside an area of menstrual pain referral and was located whilst each woman was seated and resting her arm, in a pronated position on an examination table next to her. Two thirds of the distance from the lateral condyle of the radius to the styloid process of the ulna was then measured, and the muscles were located after asking the woman to extend her wrist. The erector spinae muscle, at lumbar level (L4/L5), is a muscle located within an area of menstrual pain referral. It was located by having each woman lie prone on an examination table; the right and left iliac crests were then located by palpation, as the spinous process of L4 is located at the level of the iliac crests. The belly of the erector spinae muscle was then located on either side of the spinal column, by asking the woman to hyperextend her back. For each experimental session, the

order of the site of injection (forearm or back) was randomly selected, such that if the extensor muscles of the forearm were injected first, the injection into the spinae erector muscle of the lower back was carried out once all pain in the forearm had subsided. The side of the body (left or right) was also randomly selected, such that if the right forearm was injected then the left back was injected. Any superficial pain, caused by the injection itself, was removed using a topical anaesthetic cream (EMLA cream; prilocaine 2.5%, lignocaine 2.5%). As described above, the cream was applied soon after the participant had awoken (at least 45 minutes prior to the testing procedure) to the overlying skin of the site at which the intramuscular injection was to be carried out[117, 147].

Deep-muscle pain was induced using a 0.5 mL intramuscular injection of 5% sterile hypertonic saline. All injections were carried out by a certified phlebotomist or registered nurse. A sterile 25-gauge, 16-mm needle was used to inject the hypertonic saline at a depth of 0.5 cm into the extensor muscles of the forearm; and a sterile 23-gauge, 25-mm needle was used to inject the hypertonic saline at a depth of 1.5 cm into the erector spinae muscle. All injections were administered as a bolus over a maximum of 10 seconds and the needle was removed at the completion of the injection. The women were asked to rate the intensity of their muscle pain, using a 100-mm VAS anchored from “no pain at all” to “the worse pain ever”, immediately before each injection and then every 30 seconds thereafter, until the pain had completely subsided[117]. A measurement, in mm, was then taken from the first anchor point to the mark filled in by the women for each VAS.

2.4.5.3 Pennebaker Inventory of Limbic Languidness (PILL)

The PILL[143] is a 54-item questionnaire used to determine a person’s current experience of painful and non-painful somatic symptoms. The 54 items are separated into 44 non-painful somatic symptoms and 10 painful somatic symptoms (headache, back pain, cramps, heartburn, chest pain, severe pains or cramps in the stomach, toothache, sore throat, sore muscles, and joint pain). In this study, the PILL was adapted to only include the 10 items of painful somatic symptoms (Appendix I). Women rated the experience of a symptom using a 5-point likert scale ranging from 0 to 4, with 0 being “not at all” to 4 being “very much”[31]. A total spontaneous pain score was then calculated by adding the scores of each painful somatic symptom.

2.5 Statistical Analyses

All data were tested for normal distribution, and parametric or nonparametric tests were conducted where appropriate.

Unpaired t-tests were performed for the demographic and menstrual cycle data for the control women and women with dysmenorrhoea using GraphPad Instat (version 3.0 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com).

PSG data were analysed using STATISTICA [Dell Inc. (2016). Dell Statistica (data analysis software system), version 13. software.dell.com]. All graphs generated for the sleep data were drawn up using GraphPad Prism (version 5.02 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). TST, WASO, SOL, SE and the time and percentage of time (sleep stage/TSTx100) spent in each sleep stage for the baseline night was compared between the two groups of women using unpaired t-tests. TST, SE and the percentage of time spent in each sleep stage for all the experimental nights were compared across nights and between groups using a 2-way ANOVA and Student-Newman-Keuls (SNK) posthoc test where necessary.

Statistical analyses for all other data, including; morning vigilance, SQ, von Frey hairs, POMS, PILL and hypertonic saline-induced and ischaemic pain, were performed using R v3.4.0 (R Core Team. R: A language and environment for statistical computing. Vienna, Austria, 2014) in RStudio v1.0.143 (http://www.rstudio.com).

Two separate general additive mixed model regressions with an inflated β distribution were used to compare baseline 1) morning vigilance and 2) SQ scores to the scores on the morning of both sleep fragmentation nights, and between the two groups of women. An inflated β distribution describes (0, 1) bounded continuous probability distributions (VAS scores expressed as a proportion), and thus negates the need to transform the data to stabilize residual variance.

As the data were not normally distributed, linear mixed-effects models were used to compare log-transformed data for the evening and morning von Frey hairs, as well as transformed POMS data ($score^{-2}$), between the groups, across the nights (i.e. Fragmentation relative to baseline) and over time (i.e. Morning vs. evening).

A general additive mixed model regression with an inflated β distribution (VAS scores expressed as a proportion) was used to compare baseline ischaemic pain VAS scores to the scores on the morning of both sleep fragmentation nights, and between the two groups of women. Model fixed effects included: pain intensity overtime (the 10-minute assessment period), and intervention (baseline night, and fragmentation nights 1 and 2). Random effects included participants (random intercept) and time (random slope).

To determine whether forearm ischaemia was achieved or not, during the ischaemic pain test, a Wilcoxon signed rank test with continuity correction was used to assess a change in touch threshold before and after the cuff was inflated to 200 mmHg. In all cases the familywise error rate was controlled for using the Holm-Bonferroni method.

Hypertonic saline pain assessments in the arm and back were analysed by calculating area under the VAS-time curve (AUC, $mm.s^{-1}$), which incorporates both pain intensity and pain duration. An arcsine transformation was then carried out to normalise residuals, before data were compared between groups and across nights using linear mixed-effects models.

For the PILL data, majority of the questions had been scored as 0, therefore the data were organised into binary outcome variables by describing total scores of 0 as “no pain” and total scores larger than 0 as “the presence of spontaneous pain”. A generalised linear mixed model, using a Laplace approximation, was used to compare the presence of spontaneous pain between the two groups of women and across the experimental nights.

A two-tailed probability test of hypothesis was assumed, where a p-value ≤ 0.05 was considered to be statistically significant. All data are expressed as mean ± standard deviation (SD), unless stated otherwise.

CHAPTER 3 – RESULTS

3.1 Participant Demographics and Menstrual Cycle Characteristics

As displayed in Table 3.1, with the exception of menstruation length, and menstrual pain intensity, the women did not differ by age, body mass index (BMI), or menstrual cycle characteristics. Compared with controls, women with dysmenorrhoea had significantly longer menstruation phases ($t_{(17)}$ = 2.38, p = 0.03) and, as expected, they reported significantly higher ratings of menstrual pain in the past 6 months ($t_{(17)}$ = 13.63, p < 0.0001). Only one control was on oral contraceptives at the time of the experiment, while all other participants had either never taken oral contraceptives or ceased taking them more than 6 months prior to participating. The two groups of women did not differ according to their GHQ scores. The PSQI score was significantly higher in the women with dysmenorrhoea compared to the controls ($t_{(17)}$ = 2.34, p = 0.03), however, these values are not clinically significant, as all the women in the study were required to be "good sleepers" in order to partake in the study; i.e. they all scored ≤ 6 on the PSQI[140].

Table 3.1: Demographics and characteristics of the women who participated (n = 19).

	Women with Dysmenorrhoea (n = 10)	**Control Women** (n = 9)	**p-value** (Unpaired t-test)
Age (years)	22 ± 3	21 ± 3	0.91
BMI (kg/m^2)	22.9 ± 3.9	22.5 ± 2.4	0.82
Age at menarche (years)	13 ± 1	13 ± 1	0.12
Age at onset of Dysmenorrhoea (years)	13 ± 1	NA	NA
Years of menstruation	9 ± 2	8 ± 4	0.56
Length of menstrual cycle (days)	28 ± 2	26 ± 2	0.06
Length of menstruation (days)	5 ± 1	4 ± 1	0.03*
Menstrual pain (mm)	77 ± 8	14 ± 12	< 0.0001*
GHQ	1.3 ± 1.8	0.6 ± 0.7	0.27
PSQI	4.9 ± 1.0	3.8 ± 1.1	0.03*

Data expressed as mean ± standard deviation

**Statistical significance between groups (dysmenorrhoeic vs control women)*

BMI = body mass index; GHQ = general health questionnaire; PSQI = Pittsburgh Sleep Quality Index

3.2 Sleep Architecture - PSG

3.2.1 Comparison of Groups over the Baseline Night

As displayed in Table 3.2, the women did not differ according to SE, the amount and percentage of time spent in each sleep stage and the amount of time spent sleeping (TST) and awake (WASO), as measured during the baseline night with an 8-hour sleeping opportunity. The women did, however, differ significantly with regards to the amount of time taken to fall asleep (SOL), where control women took a longer time to fall asleep than the women with dysmenorrhoea ($t_{(17)} = 2.41$, $p = 0.03$).

Table 3.2: Comparison of sleep architecture between women with dysmenorrhoea and control women during the baseline night (uninterrupted sleep)

	Women with Dysmenorrhoea (n = 10)		**Control Women** (n = 9)		**p - value** (Unpaired t-test)
	Minutes	**% TST**	**Minutes**	**%TST**	
Stage 1	18.85 ± 12.39	4.20 ± 2.86	19.33 ± 8.06	4.33 ± 2.00	0.92[b]/0.91[c]
Stage 2	232.20 ± 28.99	50.80 ± 6.39	218.67 ± 19.27	48.11 ± 4.08	0.25[b]/0.30[c]
SWS	91.75 ± 32.06	21.8 ± 8.07	93.72 ± 21.18	20.56 ±4.45	0.88[b]/0.69[c]
REM	114.25 ± 16.85	24.80 ± 3.65	124.44 ± 21.54	27.22 ± 4.41	0.26[b]/0.21[c]
SOL (min)	4.80 ± 2.55		10.56 ± 7.07		0.03*
WASO (min)	17.25 ± 10.54		13.17 ± 11.78		0.44
TST (min)	457.05 ± 12.19		456.17 ± 14.55		0.89
SE[a](%)	95.30 ± 2.45		95.00 ± 3.04		0.81

Data expressed as mean ± standard deviation

[a]*Sleep efficiency (SE) = total sleep time (TST) /time in bed (TIB) x 100*
[b]*p-value comparing the amount of time (min) spent in each stage of sleep*
[c]*p-value comparing the percentage of time (%) spent in each stage of sleep*

**Statistical significance between groups (dysmenorrhoeic vs. control)*

REM = rapid eye movement; SE = sleep efficiency; SOL = sleep onset latency; SWS = slow wave sleep; TST = total sleep time; WASO = wake after sleep onset

3.2.2 Comparison of Groups and Nights over the Sleep Fragmentation Nights

3.2.2.1 Sleep Efficiency (SE) and Total Sleep Time (TST)

As expected, during both sleep fragmentation nights in both groups of women, TST and SE were significantly reduced compared with baseline sleep ($F_{(2,34)}$= 991.54, p <0.001 and $F_{(2,34)}$ = 994.15, p <0.001, respectively)(Fig. 3.1). This is not surprising, because according to the protocol, during the sleep fragmentation nights the women were given the opportunity to sleep for maximum of 4 hours 40 minutes per night, whereas baseline sleep allowed women an 8-hour sleep opportunity, and SE depends on TST. Therefore, these results also confirm that the sleep fragmentation protocol was successful in my study.

The main group effects were not significant for TST ($F_{(1,17)}$ = 1.63, p = 0.22) and SE ($F_{(1,17)}$ = 2.14, p = 0.16). There were however, significant group x night interactions for both TST ($F_{(2,34)}$ = 4.44, p = 0.02) and SE ($F_{(2,34)}$ = 4.73, p = 0.01)(Table3.3), such that compared with the controls, the women with dysmenorrhoea had a higher SE (SNK: p < 0.01) and longer TST (SNK: p <0.01) during the first sleep fragmentation night, however, no differences in TST (SNK: p = 0.57) and SE (SNK: p = 0.64) were observed between the two groups of women during the second sleep fragmentation night. In contrast, the control group slept significantly better (i.e. improved SE: SNK: p < 0.001) and for a significantly longer period of time (TST, SNK: p < 0.001) during the second sleep fragmentation night compared to the first sleep fragmentation night.

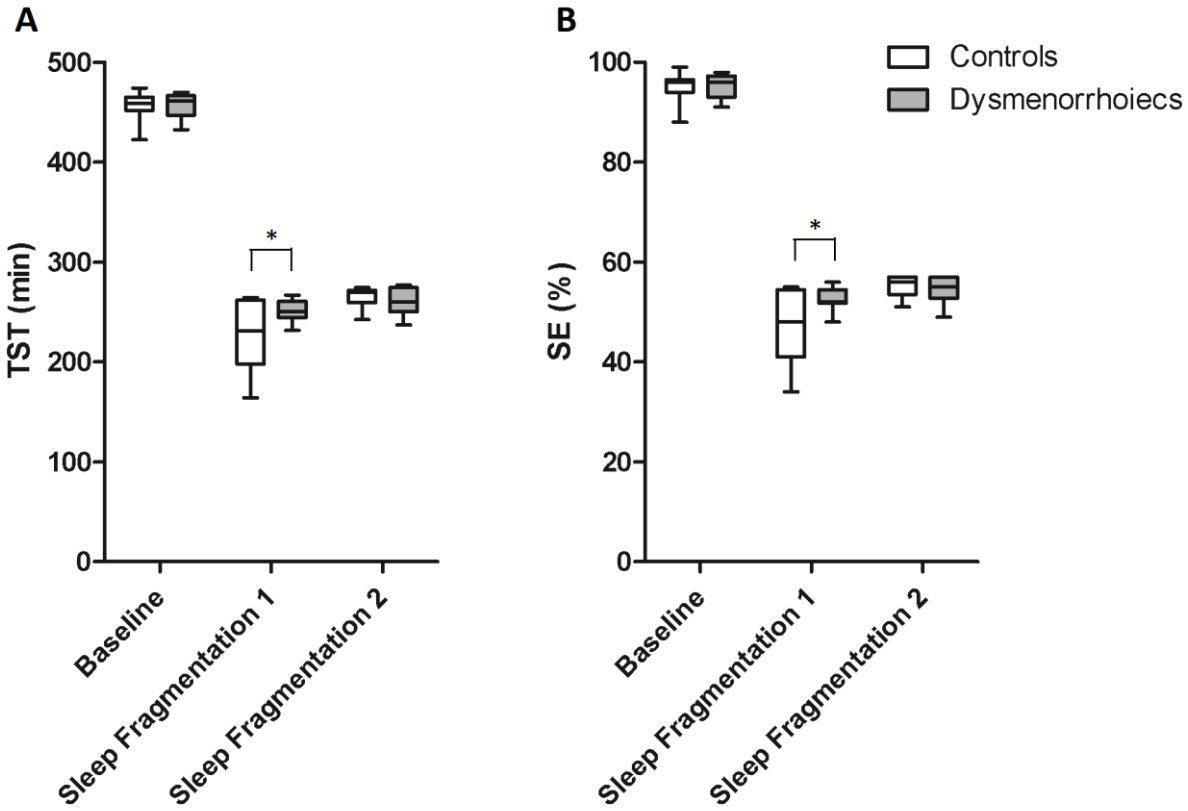


Figure 3.1: Box-and-whisker plots showing A) the total sleep time (TST) and B) sleep efficiency (SE) of women with primary dysmenorrhoea (n = 10) and healthy controls (n = 9) across the baseline (8-hour sleep period) and two consecutive sleep fragmentation nights. Compared to the baseline night, TST and SE were significantly reduced by both sleep fragmentation nights in both groups of women. *p<0.05, significant difference between groups.

3.2.2.2 Percentage of Time Spent in each Phase of Sleep

Due to the nature of the study, where sleep time is purposefully reduced during the sleep fragmentation nights (8 hour vs. 4 hour 40 minute sleeping opportunity), it is expected that the absolute amount of time (i.e. minutes) spent in each sleep stage is significantly reduced. Therefore, the following data, demonstrating the effect of sleep fragmentation on the different sleep stages is only represented as the percentage of time spent in each sleep stage (time spent in sleep stage/TSTx100). All data are displayed in Table 3.3 and in Figure 3.2.

As displayed in table 3.3 for all the experimental nights, the percentage of time spent in stage 1, stage 2, SWS and REM sleep did not differ between the two groups of women. Similarly, the group x night interactions were not significant for the percentage of time spent in stage 1, stage 2, SWS and REM sleep. However, a significant night effect was found for the percentage of time spent in stage 1, stage 2 and SWS, but not for REM sleep. Compared with the baseline night, both groups of women spent a significantly greater percentage of time in stage 1 and in SWS during the first (SNK: $p < 0.001$ and $p = 0.02$, respectively) and second (SNK: $p = 0.01$ and $p = 0.01$, respectively) sleep fragmentation nights. There was no significant difference between the two sleep fragmentation nights for either stage 1 or SWS percentages (SNK: $p = 0.10$ and $p = 0.54$, respectively). In contrast, the percentage of time spent in stage 2 sleep was significantly reduced during the first and second sleep fragmentation nights, compared to baseline (SNK: $p < 0.001$ and $p < 0.001$, respectively); with no significant difference between the two sleep fragmentation nights (SNK: $p = 0.15$). REM sleep did not differ across any of the experimental nights.

Table 3.3: Polysomnographic (PSG) sleep parameters in women with primary dysmenorrhoea and healthy controls across a night of uninterrupted sleep (baseline) and two consecutive nights of sleep fragmentation (fragmentation 1 and 2)

	Women with Dysmenorrhoea (n = 10)			**Control Women** (n =9)			**p-value** (2-way ANOVA)		
	Baseline	**Fragmentation 1**	**Fragmentation 2**	**Baseline**	**Fragmentation 1**	**Fragmentation 2**	**Group Effect**	**Night Effect**	**NightxGroup Interaction**
TST (min)	457 ± 12	251 ± 11	260 ± 13	456 ± 14	226 ± 36	265 ± 10	0.22	<0.001*	0.02*
SE[a] (%)	95 ± 2	53 ± 2	54 ± 3	95 ± 3	47 ± 7	55 ± 2	0.16	<0.001*	0.01*
Stage 1 (%)	4 ± 3	7 ± 3	6 ± 3	4 ± 2	11 ± 6	8 ± 5	0.10	<0.001*	0.11
Stage 2 (%)	51 ± 6	45 ± 9	43 ± 10	48 ± 4	41 ± 7	39 ± 8	0.31	<0.001*	0.95
SWS (%)	22 ± 8	24 ± 10	25 ± 9	21 ± 4	27 ± 9	28 ± 11	0.71	0.01*	0.45
REM (%)	25 ± 4	24 ± 9	26 ± 11	27 ± 4	20 ± 7	25 ± 5	0.72	0.13	0.23

Data expressed as mean ± standard deviation

[a]Sleep efficiency (SE) = total sleep time (TST) /time in bed (TIB) x 100

**Statistical significance*

REM = rapid eye movement; SE = sleep efficiency; SWS = slow wave sleep; TST = total sleep time

For the purpose of this dissertation, I ran some exploratory post hoc analyses, using a SNK posthoc test on the group x night interaction for stage 1 sleep, since the interaction effect was a trend (p = 0.11), even though not significant. The control group spent a significantly higher percentage of time in stage 1 sleep compared to the women with dysmenorrhoea during the first sleep fragmentation night (SNK: p= 0.03), but no differences were observed between the two groups of women during the second sleep fragmentation night (Fig. 3.2a).

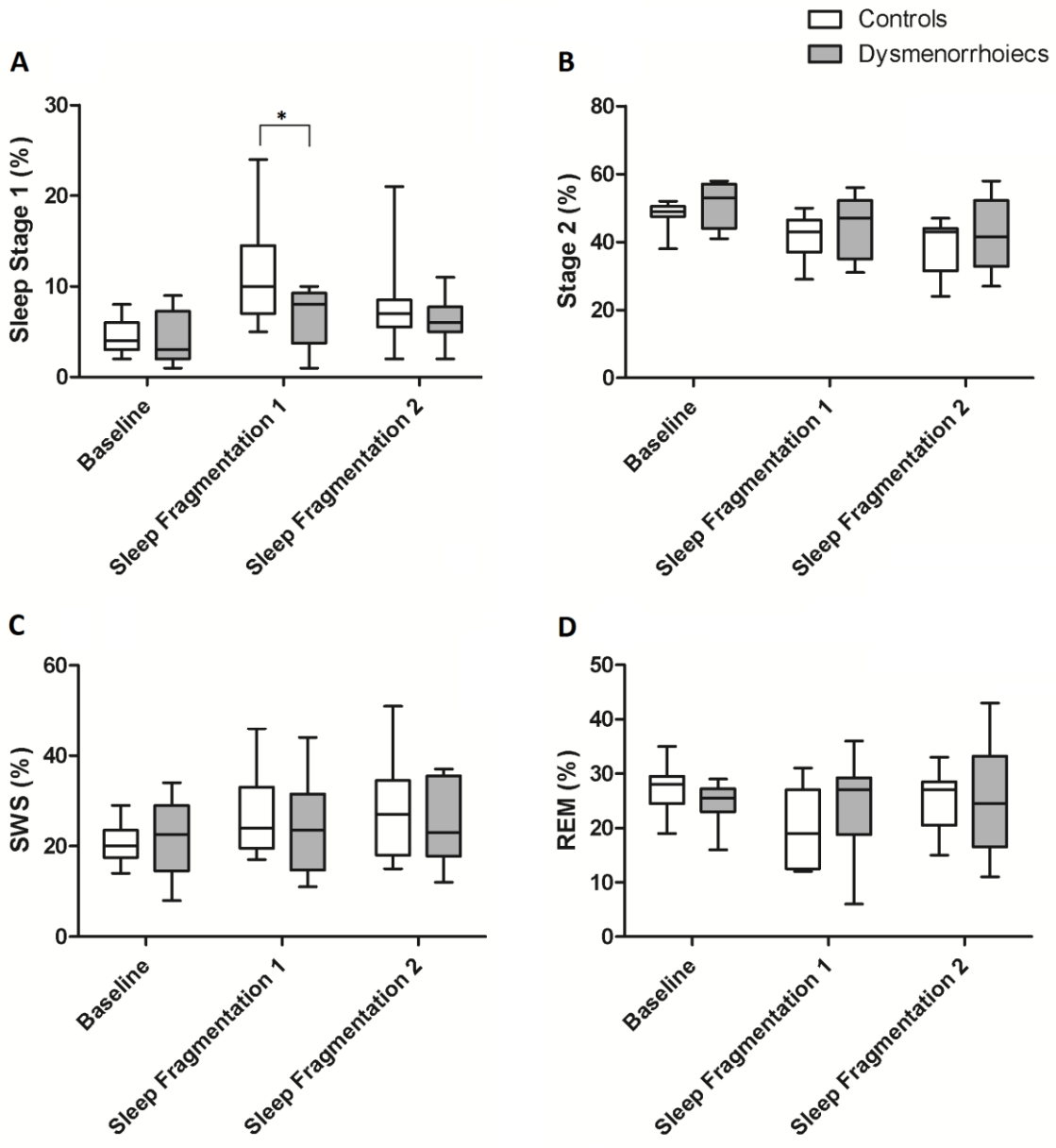


Figure 3.2: Box-and-whisker plots showing the percentage of time women with primary dysmenorrhoea (n = 10) and healthy controls (n = 9) spent in A) stage 1, B) stage 2, C) slow wave sleep (SWS), and D) rapid eye movement (REM) sleep across the baseline (8-hour sleep period) and two sleep fragmentation nights (280-min sleep period per night). *p < 0.05, significant difference between groups.

3.3 Sleep Quality (SQ) and Morning Vigilance

For details on the statistical models for the analyses of SQ and morning vigilance, see Appendix J.

As expected, subjective ratings of SQ were significantly reduced following one and two nights of sleep fragmentation, compared with the baseline night in both groups ($p < 0.001$, Fig. 3.3a). A significant group effect was found ($p < 0.01$), such that the women with dysmenorrhoea rated their SQ as significantly worse than the controls after the baseline night, but controls rated their SQ as significantly worse than the women with dysmenorrhoea after the first night of sleep fragmentation, with no differences in SQ between the two groups of women after the second night of sleep fragmentation.

Morning vigilance VAS scores did not differ between the two groups of women ($p = 0.33$). As expected, both groups of women rated their morning vigilance as significantly lower after one and two nights of sleep fragmentation compared to that following their uninterrupted baseline sleep ($p < 0.001$, Fig. 3.3b).

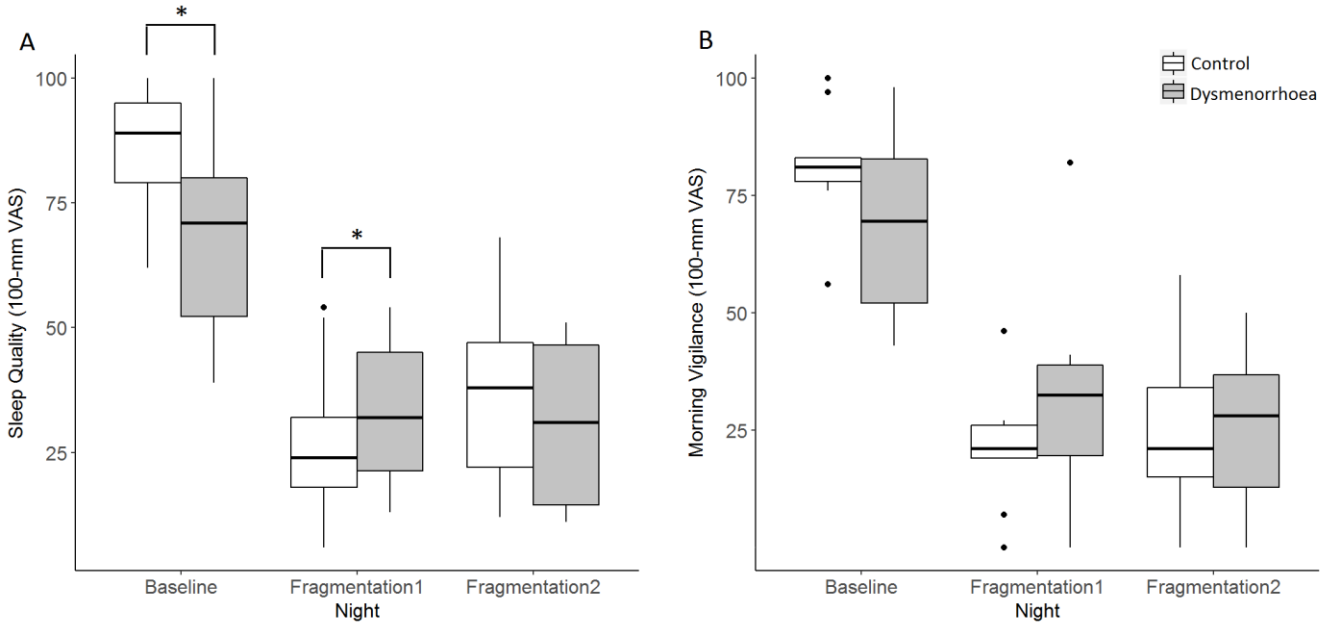


Figure 3.3: Box-and-whisker plots showing the subjective ratings of A) sleep quality (SQ) and B) morning vigilance. Both SQ and morning vigilance were assessed using separate 100-mm visual analogue scales (VASs), where higher scores indicate better SQ, and increased freshness and alertness, respectively. Compared to baseline, SQ and morning vigilance were significantly reduced after both the first and second night of sleep fragmentation in both groups of women. *$p < 0.05$, significant difference between groups. *The dots represent outliers.*

3.4 Mood - POMS

Mood did not differ between the two groups of women ($F_{(1,17)} = 0.78$, $p = 0.39$) across any of the experimental nights or sessions (morning vs. evening)(Fig. 3.4), therefore the night, session and night x session interaction was assessed by collapsing the two groups into one. A significant night ($F_{(2,90)} = 25.15$, $p < 0.001$), session ($F_{(1,90)} = 8.42$, $p < 0.01$) and night x session ($F_{(2,90)} = 3.71$, $p = 0.03$) interaction was found, whereby, compared to baseline, morning mood scores were significantly higher, and therefore considered worse, after one ($p = 0.03$) and two ($p < 0.01$) nights of sleep fragmentation. Compared to evening mood scores, morning mood scores were also significantly higher, reflecting poorer mood, after the first night of sleep fragmentation ($p < 0.01$), but not after the second night ($p = 0.11$).

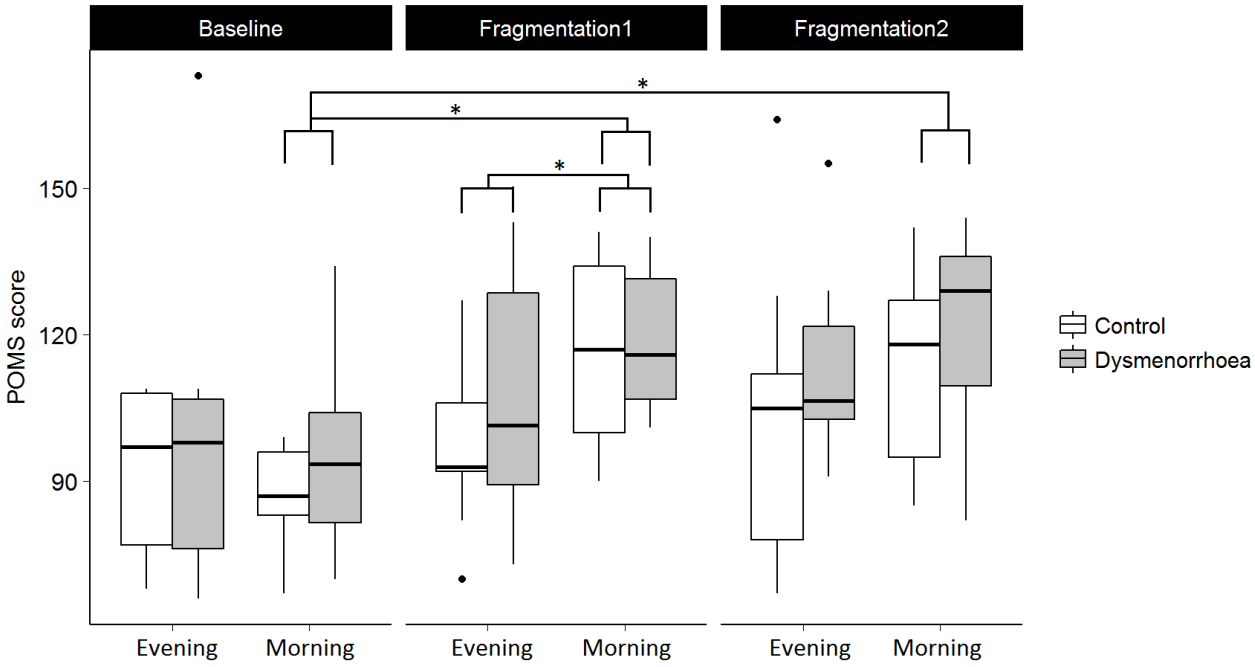


Figure 3.4: Box-and-whisker plots showing the difference in the Profile of Mood States (POMS) scores across experimental nights: habitual sleep (Baseline) and two consecutive nights of sleep fragmentation (Fragmentation 1 and Fragmentation 2) for both groups of women. The POMS was filled out in the evening and morning of each experimental night. Higher scores represent worse moods. *$p < 0.05$. *The dots represent outliers.*

3.5 Touch Thresholds – von Frey Hairs

Touch threshold was unaffected by either sleep fragmentation nights ($F_{(2,85)} = 0.19$, $p = 0.83$), and did not differ between the two groups of women ($F_{(1,17)} = 0.13$, $p = 0.72$) or between evening and morning ($F_{(1,85)} = 0.96$, $p = 0.33$).

3.6 Pain Assessments

3.6.1 Ischaemic Pain

Following the submaximal effort tourniquet test, forearm ischaemia was achieved: the Wilcoxon signed rank test showed that touch thresholds, compared with pre-cuff inflation, were significantly increased after the submaximal effort tourniquet test, in both groups of women and after all experimental nights (baseline: $p = 0.03$; sleep fragmentation night 1: $p = 0.03$; sleep fragmentation night 2: $p = 0.01$)

No significant difference was observed between the two groups of women, across any of the experimental nights ($p = 0.92$)(Fig. 3.5a). Therefore, all statistical analyses conducted thus forward were conducted by collapsing the groups into one ($n = 19$). Ischaemic pain intensity increased over the 10-minute cuff inflation period after all the experimental nights ($p < 0.001$, Table 3.4 and Fig. 3.5b). The extent of this increase was greater after one night of sleep fragmentation compared with baseline ($p = 0.05$) but returned to baseline levels after the second night of sleep fragmentation ($p = 0.40$).

Table 3.4: Summary of the final general additive mixed model of ischaemic pain (n = 19).

	Coefficients			
	Estimate	**Standard Error**	**T**	**P**
Mean (μ) subscale (location)				
Intercept	-0.916	0.141	-6.52	<0.001
Time	0.138	0.022	6.16	<0.001
Fragmentation Night 1 vs baseline	0.382	0.195	1.96	0.05
Fragmentation night 2 vs baseline	0.165	0.197	0.84	0.40
Variance (δ) subscale (scale)				
Intercept	-0.411	0.039	-10.41	<0.001
Shape (η) subscale (skewness)				
Intercept	-3.114	0.213	-14.62	<0.001
Shape (t) subscale (kurtosis)				
Intercept	-2.883	0.191	-15.11	<0.001

NOTE. Model: pain intensity = time + intervention night + (random effects = approximate time per participant), family = inflated β distribution, link = logit. Dependant variable: pain intensity; fixed effects: time and intervention night (baseline night as a reference); random effects: participant (intercept) and time (slope); family: inflated β distribution; link function: logit.

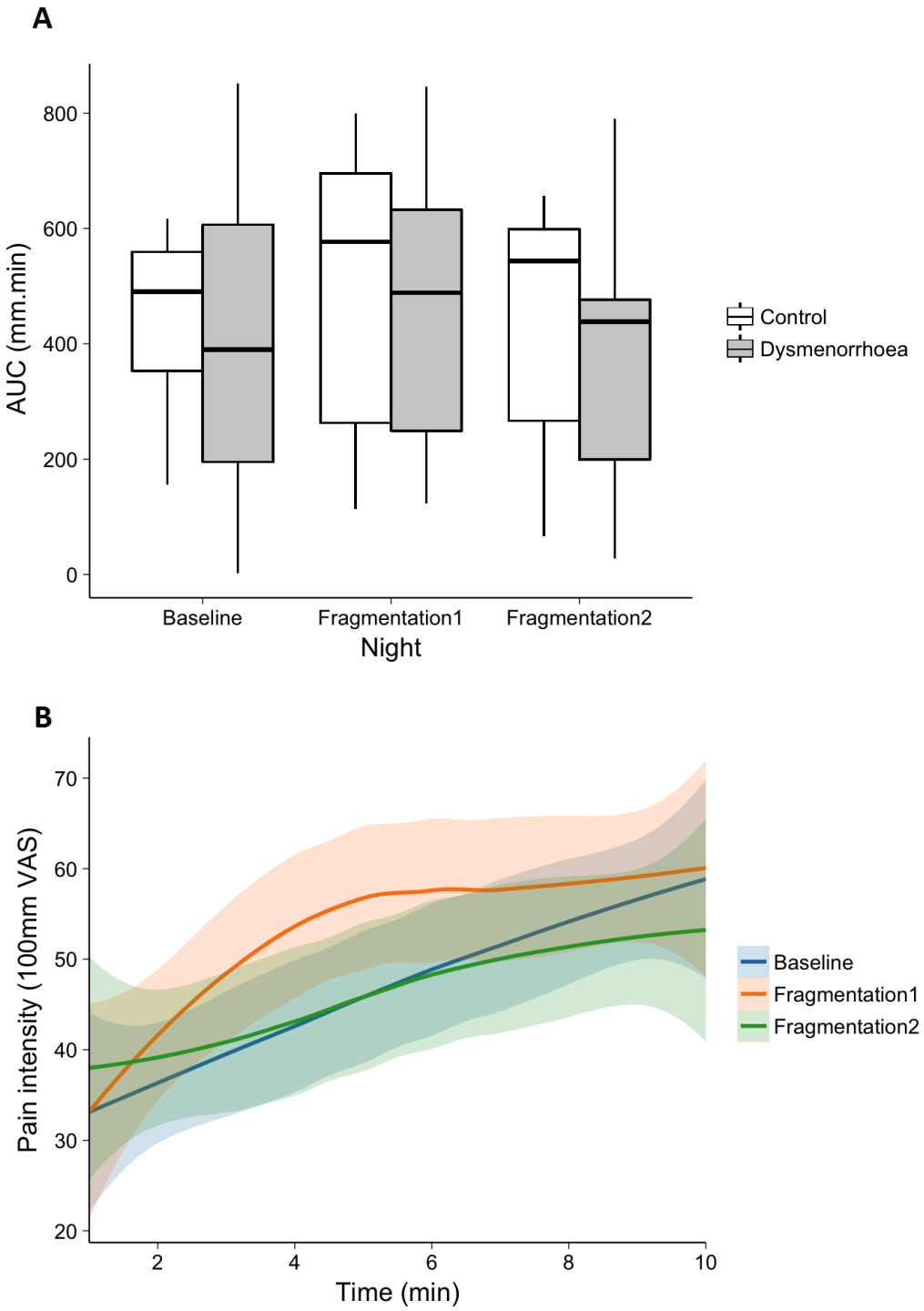


Figure 3.5: A) Box-and-whisker plots, as a representation of all the ischaemic pain data; in particular, the data displayed are the area under the VAS-time curve (AUC, mm.min^{-1}) data in the women with primary dysmenorrhoea and healthy controls, across the experimental nights: habitual sleep (Baseline) and two nights of sleep fragmentation (Fragmentation 1 and Fragmentation 2). The two groups of women did not differ, in terms of pain sensitivity, over any of the experimental nights. B) The general additive mixed model regression (n = 19) depicting an increased pain perception (in both groups of women) over the 10-minute submaximal effort tourniquet test, following one night of sleep fragmentation, when compared to baseline. The figure (B) shows locally weighted smoothing scatterplot (95% confidence interval) of pain intensity (rated on a 100-mm VAS reported by participants at 1-minute intervals over a 10-minute period during forearm ischaemia). Pain intensity increased over the 10-minute cuff inflation period after all the experimental nights.

3.6.2 Hypertonic Saline Injections

3.6.2.1 Arm

AUC, which incorporates muscle-pain intensity over time (i.e. time from injection until pain completely subsides), did not differ by night ($F_{(2,34)}$ = 0.95, p = 0.40) or group ($F_{(1, 17)}$ = 0.89, p = 0.36). However, a significant group x night interaction was found ($F_{(2,34)}$ = 3.61, p = 0.04), such that after the first night of sleep fragmentation, the AUC for the women with dysmenorrhoea was significantly greater, reflecting greater pain intensity over time, than the controls (p = 0.01, Fig. 3.6a).

3.6.2.2 Back

Muscle pain in the lower back over time (AUC) did not differ significantly between the experimental nights ($F_{(2,34)}$ = 0.89, p = 0.42), or between the groups of women ($F_{(1,17)}$ = 0.12, p = 0.74) and the group x night interaction effect was also not significant ($F_{(2,34)}$ = 0.42, p = 0.66). Data are represented in Figure 3.6b.

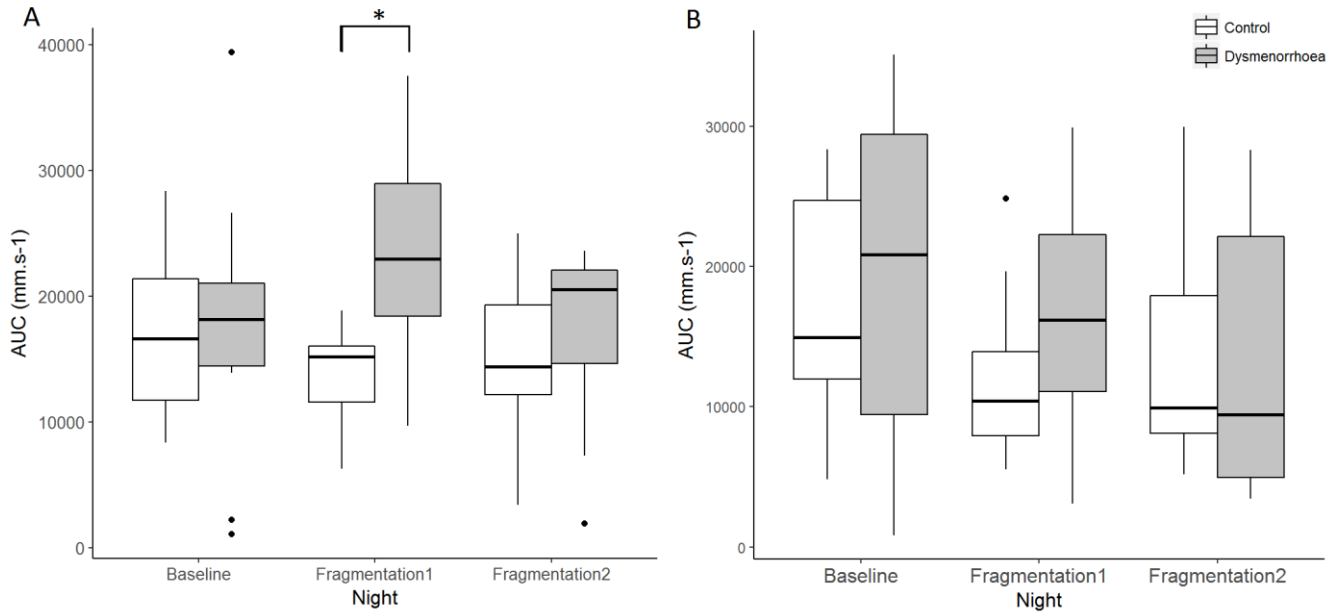


Figure 3.6: Box-and-whisker plots showing the area under the visual analogue scale (VAS)-time curve (AUC, $mm.s^{-1}$), which incorporates both pain intensity and pain duration, after the injection of hypertonic saline into A) the extensor muscles of the forearm and B) the erector spinae muscles in the lower back, in women with primary dysmenorrhoea and healthy controls across experimental nights (Baseline, Fragmentation 1 and Fragmentation 2). *p < 0.05. *The dots represent outliers.*

3.6.3 Spontaneous Pain - PILL

The presence of spontaneous pain did not differ between the two groups of women ($p = 0.21$), nor did it change after one ($p = 0.24$), or two ($p = 1.0$) nights of sleep fragmentation, compared to baseline.

CHAPTER 4 – DISCUSSION

The present study has, for the first time, assessed the effect of sleep fragmentation during the pain-free follicular phase, on the perception of experimentally-induced deep-muscle pain in women with primary dysmenorrhoea compared with young healthy age- and BMI-matched controls. After a night of uninterrupted (baseline) sleep, no differences were observed between the two groups of women in the ratings of both ischaemic and hypertonic saline-induced pain, within and outside an area of menstrual pain referral. One night of sleep fragmentation altered the perception of deep-muscle pain in both groups of young healthy women. In particular, they had an increased ischaemic pain sensitivity following one night of sleep fragmentation, compared to baseline. It appeared that one night of sleep fragmentation also affected the perception of deep-muscle forearm pain in women with dysmenorrhoea but not controls, since their pain intensity ratings significantly diverged after sleep fragmentation. Interestingly, a second, consecutive night of sleep fragmentation did not worsen pain perception for either ischaemic pain or deep-muscle pain in either group; in fact, pain ratings were no longer different following a second night of sleep fragmentation compared to after baseline sleep. No changes in pain perception were observed in either group of women within an area of menstrual pain referral (lower back) across any of the nights. Spontaneous pains, such as headaches, were also unaffected by both nights of sleep fragmentation and did not differ between the two groups of women. These findings suggest mixed effects of sleep fragmentation on pain sensitivity in young women, dependent on duration of sleep fragmentation, pain modality, and whether women have primary dysmenorrhoea, a repetitive clinical pain linked with menstruation.

The sleep fragmentation protocol was considered a success due to the fact that both groups of women experienced a significant decrease in their TST and thus, SE. Subjective SQ, morning vigilance and mood also worsened after both sleep fragmentation nights for both the women with dysmenorrhoea and healthy controls. In terms of the sleep parameters, differences were observed between the two groups of women whereby the women with dysmenorrhoea had a shorter SOL although they rated their SQ as being worse than the controls after a night of uninterrupted sleep (baseline night) during the follicular phase of their menstrual cycle. However, compared to the women with dysmenorrhoea, the controls appeared more affected by the first night of sleep fragmentation in that they obtained less

sleep, rated their SQ as being worse, had a lower SE and experienced more stage 1 sleep during the first night of sleep fragmentation. No differences were observed between the two groups of women during the second, consecutive night of sleep fragmentation, however, compared to the first night of sleep fragmentation, the control women's SE and TST improved during the second night.

4.1 Sleep Fragmentation and Pain Perception

4.1.1 Deep-muscle Pain in Women with Primary Dysmenorrhoea vs. Healthy Controls

After a night of uninterrupted sleep, the women with primary dysmenorrhoea in my study were no different to the control women, in terms of pain sensitivity. The finding that women with dysmenorrhoea are no different to women without any menstrual-related complaints, in terms of pain sensitivity are consistent with the findings reported by some[125, 126] but not all[117, 120-124, 127, 148] studies; with the latter showing that women with dysmenorrhoea are hyperalgesic compared to pain-free women across the menstrual cycle. As discussed in the introduction, these differences are, in part, due to different methodology. Consistent with the methods used in this study, Aberger and colleagues (1983)[125] and Amodei and colleagues (1989)[126] used the submaximal effort tourniquet test to assess ischaemic pain sensitivity across the menstrual cycle, in women with and without dysmenorrhoea. However, in both these studies[125, 126], ischaemia was not confirmed during or after the occlusion of blood flow to the forearm and, importantly, the type of dysmenorrhoea (primary or secondary) was not distinguished. A recent study conducted by Iacovides and colleagues (2015)[124] confirmed ischaemia after using the submaximal effort tourniquet test and found that women with primary dysmenorrhoea were more sensitive to ischaemic pain than their healthy pain-free counterparts, both during menstruation and during the pain-free follicular phase of the menstrual cycle. The findings reported by Iacovides and colleagues (2015)[124] contribute to the growing literature which report that women with dysmenorrhoea are hyperalgesic to various forms of pain, such as, heat[120, 122, 123, 127], electrical stimulation[121], laser stimuli[127], pressure[122] and experimentally-induced deep-muscle pain[117]. The likely explanation as to why my findings do not completely agree with the majority of studies suggesting that women with dysmenorrhoea are more sensitive to experimentally-induced pain compared

with controls[117, 120-124, 127, 148], is possibly due to the small sample size of my study, further explanations may be found in chapter 4.3 Study Strengths, Challenges/Limitations and Future Directions.

Nevertheless, even with my sample, I was able to detect a difference between the two groups of women following one night of sleep deprivation: compared with controls, women with primary dysmenorrhoea rated their pain as more intense over time following a hypertonic-saline injection to the forearm muscle after the first night of sleep fragmentation. Given the relationship between sleep and pain, in which sleep disruption increases sensitivity to pain[23, 27-34], it may be that implementing the sleep fragmentation protocol resulted in a difference between the two groups of women not evident under baseline conditions. These findings partially support my hypothesis that sleep fragmentation would exacerbate the perceived level of deep-muscle pain more so in women with dysmenorrhoea, and suggest that they are more sensitive to pain than healthy women under challenging conditions like sleep fragmentation. I only found a group difference for the hypertonic saline injection in the forearm (an area outside of menstrual pain referral), and not in the lower back, which matches findings of Iacovides and colleagues (2013)[117], who found that during the menstruation, follicular and luteal phase of the menstrual cycle, the effects of injecting hypertonic saline into the extensor muscles of the forearm appear to be more pronounced than those in the erector spinae muscle of the lower back. It is possible that had I injected a larger volume of hypertonic saline into the larger muscle group of the lower back (vs. the forearm), the effect would have been sensitive enough to detect differences between the women with dysmenorrhoea and control women after the first night of sleep fragmentation, just as with pain sensitivity in the forearm. After the second night of sleep fragmentation, there was no longer a group difference in pain intensity ratings in the forearm muscle. Reasons for this finding are unclear, and are discussed in the next section about effects of sleep fragmentation on pain.

4.1.2 The Effect of Sleep Fragmentation on the Perception of Pain in Women with Primary Dysmenorrhoea and Healthy Controls

In both groups of women, I found that one night of sleep fragmentation resulted in increased sensitivity to the submaximal effort tourniquet test (ischaemic pain). This result is in agreement with the findings of multiple studies[28-30, 34, 91, 92] which also assessed the effect

of sleep disruption on pain in healthy volunteers and found that one night of sleep deprivation increases sensitivity to pain. The majority of these studies used TSD to assess the effect of sleep deprivation on experimentally-induced pain and found that one night of TSD increases sensitivity to pressure[28, 34], heat[29, 30, 34], cold[34], spontaneous[91] and cutaneous[34] pain. Iacovides and colleagues (2017)[92] used the submaximal effort tourniquet test to assess ischaemic pain over the course of two sleep fragmentation nights during the mid-to-late follicular phase of the menstrual cycle, just as with the current study, and found that one night of sleep fragmentation increased deep-muscle pain in young healthy women. Smith and colleagues (2007)[31], however, found that one night of sleep fragmentation had no effect on pressure pain thresholds in young healthy women. Both Iacovides and colleagues (2017)[92] and my protocol employed the same sleep fragmentation protocol as Smith and colleagues (2007)[31], however, we used different pain modalities, which could explain the different results between studies. Handheld algometers, such as that used by Smith and colleagues (2007)[31], may be influenced by the rate and angle at which the investigator is pressing[149-151], and are therefore more dependent on external sources than a standardised procedure like the submaximal effort tourniquet test. Additionally, Smith and colleagues (2007)[31] did not measure the women during the same menstrual cycle phase, but rather controlled for menstrual cycle phase by ensuring the different groups of women did not differ with regards to the menstrual cycle phase they were in during the time of the study.

On the basis of the findings of Iacovides and colleagues (2017)[92], where a second night of sleep fragmentation had a cumulative negative effect on ischaemic pain, I hypothesised that I would find the same results. However, on the contrary, I found no difference in pain perception between baseline values and those observed after the second consecutive night of sleep fragmentation. Moreover, when hyperalgesia was evident following one night of sleep fragmentation (i.e. in ischaemic pain for both groups of women, and in deep-forearm muscle pain in women with dysmenorrhoea), pain sensitivity returned to baseline levels after the second sleep fragmentation night. To my knowledge, this finding has not previously been reported in the literature. In fact, studies assessing the effect of sleep disruption on pain sensitivity indicate that any changes to pain sensitivity observed after one night of sleep disruption carry over to, or are exacerbated by, subsequent nights of sleep disruption[30, 90-92].

Reasons for different findings in my study, compared to others, are unclear but could reflect a form of adaptation. Remarkably, Smith and colleagues (2007)[31] found that one night of

TSD, following three nights of sleep fragmentation, returned pain-inhibition functions back to normal, and suggest that an adaptive compensatory response may override the short-term effects of sleep loss, however, how this compensatory response works, and why, is unknown and warrants further research. Furthermore, whether or not this compensatory response continues over multiple nights of sleep disruption, or is lost due to recurrent sleep deprivation, is unknown and requires further investigation.

To my knowledge, the injection of hypertonic saline has yet to be used as a pain-inducing method in studies investigating the relationship between sleep deprivation and pain. The finding that neither one, nor two, nights of sleep fragmentation had a significant effect on the perception of pain, induced by means of hypertonic saline injections, in the healthy pain-free women suggests that this form of pain is not sensitive to sleep fragmentation in young, healthy women. In contrast, women with dysmenorrhoea reported an increase in pain intensity and duration in an area outside of menstrual pain referral compared to the controls after one night of sleep fragmentation, suggesting that women with dysmenorrhoea are more sensitive to the effects of sleep disruption, or more specifically, sleep fragmentation, at least over one night.

4.1.3 Proposed Physiological Mechanisms for the Effect of Sleep Fragmentation on the Perception of Pain

In my study, I used two different techniques to induce deep muscle pain: injection of hypertonic saline and ischaemia. These techniques were chosen because research has shown that muscles, rather than subcutaneous and cutaneous tissue, are more likely to become hyperalgesic due to recurrent visceral pain conditions, such as dysmenorrhoea[121, 152]. Pain induced by the injection of 5% hypertonic saline is an exogenous muscle pain-producing technique which has commonly been described as a dull, cramping, achy, tight sensation that spreads or radiates from the site of injection, and is a clinically relevant method for inducing deep muscle pain, as it mimics musculoskeletal pain[147, 153, 154]. Ischaemic pain is an endogenous muscle pain-producing technique that produces a deep, aching pain that is also relevant to the study of primary dysmenorrhoea given that the pain experienced by women with dysmenorrhoea originates from uterine ischaemia[103, 153].

The injection of hypertonic saline is thought to activate thinly myelinated Aδ- and unmyelinated C-nociceptors within the muscle, leading to the sensation of local and referred deep-muscle pain[154-158]. The transmission of pain during an ischaemic event is complex and not fully understood as various substances are involved, such as bradykinin, histamine, serotonin, acetylcholine, potassium ions and adenosine[159]. However, C-nociceptors are also thought to be involved in the transmission of pain during an ischaemic event[160, 161], such as that experienced by performing the submaximal effort tourniquet test. Therefore, the results from the present study, whereby both the women with primary dysmenorrhoea and controls were more sensitive to pain induced by ischaemia after one night of sleep fragmentation, suggest that C-nociceptors may be involved in the heightened pain sensitivity brought on by sleep disruption. On the other hand, the combination of both Aδ- and C-nociceptors may be involved in the heightened pain sensitivity brought on by sleep disruption in women with primary dysmenorrhoea, as the women with primary dysmenorrhoea in the present study were found to be hyperalgesic to pain induced by the injection of hypertonic saline following a single night of sleep fragmentation. These notions are speculative as 1) I did not measure pain transmission in the various types of fibres, and 2) may explain the mechanisms of sleep restriction rather than sleep fragmentation alone, therefore, further research is warranted as to the exact physiological mechanisms that lead to increased pain sensitivity in women with primary dysmenorrhoea after a night of sleep fragmentation. In young pain-free women, it has been demonstrated that the endogenous pain inhibitory system is disrupted even after just one night of sleep fragmentation[31]. Therefore, future studies assessing the endogenous pain inhibitory system in both women with and without dysmenorrhoea may further explain the differences, and similarities, in the physiological mechanisms involved in the effect of sleep disruption on pain between these two groups of women. As emphasised above, there is also a need to further investigate potential physiological mechanisms for altered pain sensitivity after multiple nights of sleep fragmentation, given my findings of a return to baseline levels after a second night of sleep fragmentation.

4.1.4 The Effect of Sleep Fragmentation on Spontaneous Pain

The presence of spontaneous pains such as headaches, sore muscles and joint pain did not differ between the women with dysmenorrhoea and healthy controls over any of the

experimental nights. This finding is not surprising given that women with dysmenorrhoea tend to report an increase in spontaneous pain only during menstruation due to menstrual pain[123, 127], and the current study was conducted during the mid-to-late follicular phase, in the absence of menstrual pain.

There have been mixed findings with regards to the effect of sleep deprivation or fragmentation on the reporting of spontaneous pain. Studies using TSD have reported that neither one[29, 34] nor two[29] nights of TSD increase spontaneous pain, however, another study[91] reported that even just one night of TSD increases spontaneous pain. These differences may be attributed to the use of different methods to assess spontaneous pain; with one study using 100-mm VASs to assess generalised body discomfort and spontaneous pains such as headaches and back pain[91]; another study made use of two schematic body diagrams, front and back, along with a 100-mm VAS for each bodily complaint marked on the diagrams[29]; and another made use of the number of self-reported bodily complaints specific to pain[34]. However, in general, it has been reported that spontaneous pain usually develops after the accumulation of sleep deficit[91, 162, 163].

The present study found that neither one nor two nights of sleep fragmentation increased the presence of spontaneous pain in either the women with dysmenorrhoea or the controls. Similar findings were reported by Iacovides and colleagues (2017)[92], who used the short version of the PILL questionnaire just as with the present study, whereby neither one nor two nights of sleep fragmentation increased the presence of spontaneous pain in healthy pain-free women during the mid-to-late follicular phase of the menstrual cycle. In contrast, Smith and colleagues (2007)[31], who also used the short version of the PILL questionnaire, reported that just two nights of sleep fragmentation increased spontaneous pain in young pain-free women and continued to do so over a third, consecutive night of sleep fragmentation. Due to the small sample size in the present study, and the study conducted by Iacovides and colleagues (2017)[92], we could not look at individual symptoms, nor did we analyse across the full 5-point likert scale. Instead, given the small sample size, we arranged our data into binary outcome variables, in which scores of 0 were considered as “no pain” and any scores greater than 0 as “the presence of spontaneous pain”. On the other hand, Smith and colleagues (2007)[31] calculated a total spontaneous pain score by adding the scores of the 5-point likert scale for each painful somatic symptom. These differences in the way the scale was assessed, along with the small sample size and different means of controlling

for menstrual cycle phase, may have impacted our ability to detect a change in spontaneous pain following sleep fragmentation. Duration of sleep fragmentation is also important as more than two consecutive nights of sleep fragmentation may induce a larger effect on spontaneous pain.

4.2 Sleep Parameters of Women with Dysmenorrhoea vs. Healthy Controls – Objective and Subjective Data

During my study, PSG was used to confirm the sleep fragmentation protocol, i.e. that I did in fact, successfully induce sleep fragmentation. Collection of these data also allowed the investigation of a secondary outcome: sleep architecture of women with dysmenorrhoea compared with controls. Importantly, both groups of women were free from sleep disorders, reported good sleep quality, regular sleep-wake cycles and regular menstrual cycles. Further, timing of assessments were controlled for, all occurring during the pain-free follicular phase of the menstrual cycle after uninterrupted sleep, and following two consecutive nights of sleep fragmentation. These data are discussed in the following sections.

4.2.1 Sleep Architecture of Women with Dysmenorrhoea vs. Controls after a Night of Uninterrupted (Baseline) Sleep

On the baseline night, which took place between days 6 - 11 of all women's menstrual cycles, sleep architecture of both groups of women was similar over the assigned 8-hour period. These findings contribute to the growing body of evidence that objective measures of sleep, using PSG, are similar in women with and without dysmenorrhoea during the pain-free phases of the menstrual cycle[116, 118, 164]. On the other hand, when women are experiencing menstrual pain, their sleep is disturbed, with more time spent moving, awake and in light stage 1 sleep[116, 118]. Together, the data from these studies[116, 118] show that it is most likely menstrual pain that is disrupting the sleep of these otherwise healthy young women, and when these women are not experiencing pain, their sleep is normal. One study did, however, report that during the pain-free phases of the menstrual cycle, women with dysmenorrhoea tend to obtain less REM sleep than healthy controls[118], a finding not replicated in my or another study[164]. Sample sizes have been small in all these studies, possibly leading to Type 1 errors.

In my study, I surprisingly found that the women with dysmenorrhoea had a shorter SOL than the control women on the baseline night, although it should be noted that both groups of women had very short SOLs (on average, less than 12 minutes) such that the statistical difference in SOL between the groups is unlikely to be clinically meaningful. Both groups also had high SEs (95%, on average) on the baseline night, reflecting that they were adequately adapted to the laboratory environment, and slept well. One study investigated self-reported sleep measures in a large group of women with dysmenorrhoea and found that women with severe dysmenorrhoea took a longer time to fall asleep than women with mild dysmenorrhoea[137]. However, this survey did not distinguish between primary or secondary dysmenorrhoea, and women with insomnia were included in the study[137] which likely impacted the results as insomniacs would naturally report more sleep disturbances.

Although both groups of women experienced similar sleep architecture on the baseline night (a night of seemingly uninterrupted sleep), the women with dysmenorrhoea reported a poorer SQ compared with controls, however, subjective measures of morning mood and morning vigilance after the baseline night did not differ between groups. Given the small sample size for my study, my finding of a poorer SQ in women with dysmenorrhoea needs to be interpreted with caution. Few studies have investigated subjective measures of sleep in women with dysmenorrhoea with which to compare my results[118, 137, 164]. It was initially reported that women with dysmenorrhoea rated their SQ as being worse than healthy controls during menstruation due to the presence of menstrual pain[118] and SQ did not differ between these two groups of women during the pain-free mid-luteal and mid-follicular phases of the menstrual cycle[118, 164]. A more recent study has indicated that women suffering from severe dysmenorrhoea report a worse SQ compared to those suffering with mild dysmenorrhoea and that this phenomenon occurs throughout the menstrual cycle, suggesting that subjective ratings of sleep are negatively affected across the entire menstrual cycle and not only during menstruation, specifically in women with severe dysmenorrhoea[137]. There clearly is a need for further studies to investigate sleep – using both self-reported and PSG measures – in women with primary dysmenorrhoea, to establish whether sleep disturbances are apparent even when these women are not in pain.

4.2.2 The Effect of Sleep Fragmentation on Sleep Architecture in Women with Dysmenorrhoea and Healthy Controls

The sleep fragmentation protocol had an effect on TST, SE, as well as, the portion of time spent in each sleep stage for both the women with dysmenorrhoea and the controls. As expected, TST and SE were significantly decreased by the sleep fragmentation protocol as the women were only allowed the opportunity to sleep for a maximum of 4 hours 40 minutes while spending a total of eight hours in bed. The portion of time spent in stage 1 and SWS increased during the sleep fragmentation nights, compared to baseline, while the portion of time spent in stage 2 decreased, with no change occurring for the portion of time spent in REM sleep across the nights, for both the women with primary dysmenorrhoea and healthy controls. Stage 1 sleep is expected to increase during the sleep fragmentation nights as the women are woken up multiple times in the night, therefore, each time they fall back asleep after being awoken, they would initially transition through stage 1 sleep. SWS is likely increased as it reflects homeostatic pressure for sleep[165], and since the women's sleep is being interrupted, when they return to sleep, they would quickly return to SWS to satisfy the homeostatic drive. Stage 2 sleep is likely decreased as the women transition into deep sleep quickly. Smith and colleagues (2007)[31] reported that sleep fragmentation increases the time spent in stage 1 sleep and decreases the time spent in stage 2 sleep, in young healthy women. They also reported a decrease in the time spent in REM sleep and an initial decrease in the time spent in SWS with the time returning back to baseline values after subsequent nights[31]. In order to compare the results of my study to that of Smith and colleagues (2007)[31], from which the sleep fragmentation protocol was adapted, the amount of time spent in each sleep stage (minutes) was calculated, whereby it was found by both studies that sleep fragmentation decreases the amount of time spent in stage 2 and REM sleep, however, differences appear when comparing stage 1 and SWS. Smith and colleagues (2007)[31] found that sleep fragmentation increased the amount of time spent in stage 1 sleep while decreasing the amount of time spent in SWS on the first night of sleep fragmentation, with SWS showing a rebound back to baseline after a second, consecutive night of sleep fragmentation. The present study, however, found that sleep fragmentation had no effect on the amount of time spent in stage 1 sleep, and while SWS decreased on the first night of sleep fragmentation, there was no rebound back to baseline after a second, consecutive night of sleep fragmentation. These differences may be due to the small sample size of both

studies, and/or randomisation of the sleep fragmentation protocol, whereby every night disrupts different stages of sleep for a different period of time.

There were some small differences in responses to sleep fragmentation between the two groups of women, in the present study. During the first night of sleep fragmentation, the women with dysmenorrhoea slept longer and had more efficient sleep than the healthy controls. Also, while the overall interaction effect was not significant in this small sample, a post-hoc analysis showed that the control women spent a larger portion of time in stage 1 sleep than the women with dysmenorrhoea on the first night of sleep fragmentation. These small group differences in response to sleep fragmentation are unlikely to have impacted pain responses: sensitivity to ischaemic pain was no different between the groups, and the sensitivity to pain induced by the injection of hypertonic saline in the arm was reduced in controls compared to the women with dysmenorrhoea. Therefore, the women with dysmenorrhoea may have slept better than the controls during the first sleep fragmentation night, however, the effect of sleep disruption on their sensitivity to pain was the same, or greater than, the controls.

Reasons for the group of women with dysmenorrhoea apparently adapting more easily to the first night of sleep fragmentation are unclear, although I could speculate that because they likely experience monthly sleep disruptions during menstruation[116, 118], they may have developed coping strategies to maximise their sleep-opportunity. On the second night of sleep fragmentation, there was no longer any difference in SE and TST between the two groups of women, with the controls managing to obtain longer and more efficient sleep compared to the first night, possibly because of increased homeostatic pressure due to insufficient sleep the night before.

Unlike the objective and subjective mismatch reported between sleep measures during the baseline night for the women with dysmenorrhoea, such that they reported poor subjective SQ while demonstrating good SE, the subjective findings of sleep during fragmentation are mirrored by the objective results in both groups of women. Morning mood, morning vigilance and the subjective perception of SQ deteriorated due to sleep deprivation caused by sleep fragmentation, in both the women with dysmenorrhoea and healthy controls, such that the women felt they had unrefreshing, bad quality of sleep, and were in a worse mood, after having their sleep fragmented. The finding that the women with dysmenorrhoea rated

their SQ as being better than the controls after one night of sleep fragmentation matches with the objective data, showing that controls had a poorer SE. Ratings of SQ for the women with dysmenorrhoea, therefore, appear to be better aligned with their actual sleep on the sleep fragmentation nights than on the baseline night.

4.3 Study Strengths, Challenges/Limitations and Future Directions

There are many methodological strengths and limitations in this study that need to be acknowledged. This was a highly controlled study whereby volunteers underwent various screening procedures to ensure that many confounding variables, such as ill health, comorbidities, poor sleep and chronic pain etc., were removed. All women were required to have regular menstrual cycle lengths and were only tested in a small window (days 6 - 11) of the pain-free follicular phase to ensure that the study's results were not confounded by the presence of background menstrual pain (in the dysmenorrhoeic women) and hormonal, and/or sleep, variations across the menstrual cycle (in both groups). Furthermore, a possible circadian variation in pain was also accounted for by testing all women within 2 hours of waking, which always occurred between 07h00 and 09h30. Only women with severe dysmenorrhoea, who had been experiencing recurrent monthly pain within two years of menarche, with no pelvic pain outside of menstruation, were included in the study to ensure the presence of primary, and not secondary, dysmenorrhoea. The screening phase of the study ensured that all the women in the study had regular sleep-wake cycles, and the absence of bodily pains. Successful implementation of the sleep fragmentation protocol according to Smith and colleagues (2007)[31], was confirmed by means of recording and scoring of sleep using PSG. Such data also confirmed the absence of clinically significant sleep disruptions in all participants. As with all well-designed laboratory sleep studies, an adaptation night was included to familiarise the women with the sleeping environment and procedures. Lastly, the study also employed two different pain modalities, particularly relevant to menstrual pain, one of which was conducted both within and outside an area of referred menstrual pain, as an increase in sensitivity to pain both within and outside an area of menstrual pain referral would indicate central sensitisation[121].

The study is not without challenges and limitations. The protocol was challenging, with four nights in the sleep laboratory, two of which were consecutive and involved cripplingly interrupted sleep (further crippling daytime functionality), and three mornings of pain

measurements using ischaemia and hypertonic saline injections; thus volunteers were not always forthcoming. Nevertheless, initially 34 healthy women were recruited, and willing, to undergo the challenging protocol, however, a significant number of these women were unable to complete the study, hence, the study's total sample size was small (n = 19; 10 women with primary dysmenorrhoea and 9 control women) such that there was low statistical power for some of the analyses. The results of a power analysis I subsequently ran for the ischaemic pain data (AUC) demonstrated that an n of 12 per group would have given me 80% power to detect a 5% change in AUC, but based on the confidence intervals, an n of 14 would have been optimal for the study.

The main reason for the women not being able to complete participation in my study was due to erratic sleep-wake cycles during the screening phase of the study. Volunteers were from a student population, which was ideal for the age- and health- requirements of the study, but challenging because of the typical erratic sleeping habits of students. Had the study been extended to the general public, this challenge may have been eliminated, though other limitations such as time constraints would likely surface. Furthermore, although we accounted for circadian variation in pain by only assessing pain in the morning, given that it has been reported that pain may be increased in the afternoon[30], compared with the morning, I may have missed any changes in pain sensitivity in the morning period. However, others have shown no effect of time-of-day on pain sensitivity[28, 31]and further work is needed to determine whether sleep fragmentation or sleep deprivation impacts any possible rhythm in pain sensitivity across the day. Furthermore, due to limited resources, primary dysmenorrhoea was not confirmed via gynaecological examination and therefore it is possible, although unlikely, that the dysmenorrhoeic group may have included women with secondary dysmenorrhoea.

Future studies which could address the questions raised in the current study include the following: 1) a similar study to the present study with a larger sample size, as well as confirmation of primary dysmenorrhoea by gynaecological examination; 2) studies assessing the effect of different types of sleep disruption (selective SWS or REM sleep disruption, sleep restriction and sleep fragmentation) on pain in women with primary dysmenorrhoea compared to controls; 3) studies assessing the effect of sleep disruption on pain in women with primary dysmenorrhoea, using different pain measurement techniques, including those where women with dysmenorrhoea have been found to be hyperalgesic compared with

healthy controls, such as electrical stimulation[121], pressure[122] and heat[120, 122, 123]; 4) a longitudinal study to further assesses the sleep-pain interaction – this can be done in adolescents prior to and throughout their years of menstruation in order to determine cause and effect, i.e. are women with dysmenorrhoea more sensitive to pain prior to menstruation, and hence they experience increased sensitivity to menstrual pain? Or does intense, recurrent menstrual pain lead to hyperalgesia? Which lead on to more questions: Does the recurrent pain affect their sleep enough to be considered as a risk factor for the development of chronic pain syndromes and/or central sensitivity? If so, would the management of the sleep disruption experienced by women with dysmenorrhoea reduce their menstrual pain and/or reduce their risk of developing central sensitisation, and possibly chronic pain later in life?; and lastly, 5) more research is needed to understand the underlying physiological mechanisms of the sleep-pain cycle, which may help researchers better manage it, or even better, break it.

CHAPTER 5 – CONCLUSION

The present study demonstrates, for the first time, that sleep disruption has an effect on the perception of deep-muscle pain after one night of sleep fragmentation, but that this hyperalgesic effect is resolved following a second, consecutive night of sleep fragmentation, in women with and without primary dysmenorrhoea. As such, findings support the literature that sleep disruption plays a role in increasing pain sensitivity. There were some differences in the findings for the different deep-muscle pain modalities: the perception of ischaemic pain (an endogenous muscle-pain-producing technique) was increased to a similar extent in both women with and without dysmenorrhoea after one night of sleep fragmentation. However, perception of pain associated with an injection of hypertonic saline (an exogenous muscle pain producing technique) to an area within menstrual pain referral (lower back) was insensitive to effects of sleep fragmentation in both groups. There was evidence of a divergence between groups in pain sensitivity following sleep fragmentation for pain outside an area of referred menstrual pain (extensor muscles of the forearm): women with dysmenorrhoea had greater pain sensitivity following the injection of hypertonic saline in the forearm after one night of sleep fragmentation, suggesting heightened pain sensitivity in these conditions, compared with controls.

While my findings do not suggest that women with dysmenorrhoea have an increased central sensitivity to pain, due to the fact that they did not differ from the controls in terms of pain sensitivity during baseline, other studies have shown that they do[117, 120-124, 127, 148]. Furthermore, although not determined by the current study, the structural and metabolic CNS changes observed in women with primary dysmenorrhoea collectively support that these women have increased central sensitivity to pain[123, 132-135]. The women with dysmenorrhoea in the present study were young (22 ± 3 years old [mean ± SD]) and had been experiencing menstruation and menstrual pain for a relatively short period (9 ± 2 years and 8 ± 3 years, respectively). Whereas the women in the studies assessing CNS changes were older[123, 132-135] (mean age ranges from 23 – 30 years) and had experienced menstruation[123, 134] (mean length ranges from 11 – 17 years) and menstrual pain[132-134] (mean length ranges from 9 – 10 years) for a longer period of time. Given that prolonged or chronic pain leads to central sensitisation[166], central sensitivity to pain may take a little longer to appear when it is a consequence of recurrent (in this case, monthly), as opposed to

prolonged pain. Although not evident at baseline, some evidence of heightened pain sensitivity in women with dysmenorrhoea emerged following one night of sleep fragmentation, at least for one pain modality (hypertonic saline injection), suggesting that women with dysmenorrhoea are hyperalgesic to deep-muscle pain, which becomes apparent under challenging conditions such as sleep fragmentation. Further, sleep disruption may play a role in the development of central sensitisation as women with dysmenorrhoea may undergo central changes not only due to recurrent visceral pain but also recurrent sleep disruption while experiencing pain, specifically menstrual pain. Therefore, after many years of painful menstruation and disrupted sleep women with dysmenorrhoea may develop central sensitisation and become hyperalgesic within and outside areas of referred menstrual pain even after a night of habitual sleep. Hence, as the young women with dysmenorrhoea in my study get older and experience menstrual pain and sleep disruption for a longer period of time, they may become hyperalgesic to pain both within and outside an area of menstrual pain, across all pain modalities. However, these notions are speculative and require further investigation with longitudinal studies.

In conclusion, the results from the current study suggest that women with primary dysmenorrhoea may be more adapted to sleep disruptions (i.e. they subjectively rate a night of disturbed sleep better than controls), due to their experience of pain-related sleep complaints during menstruation, but are still more sensitive to experimental deep-muscle pain after one night of disrupted sleep. My findings, along with the findings of others, suggest that women with dysmenorrhoea are affected by the bidirectional sleep-pain relationship (Fig. 5.1), and that by treating and maintaining both sleep and pain in women with primary dysmenorrhoea this vicious sleep-pain cycle may improve such that these women no longer experience pain-related sleep complaints or a central sensitivity to pain brought on by disrupted sleep. I also demonstrate, for the first time, that sensitivity to pain either remains unaffected or returns to baseline after a second night of sleep fragmentation, possibly indicating a short-term adaptive compensatory response to sleep deprivation.

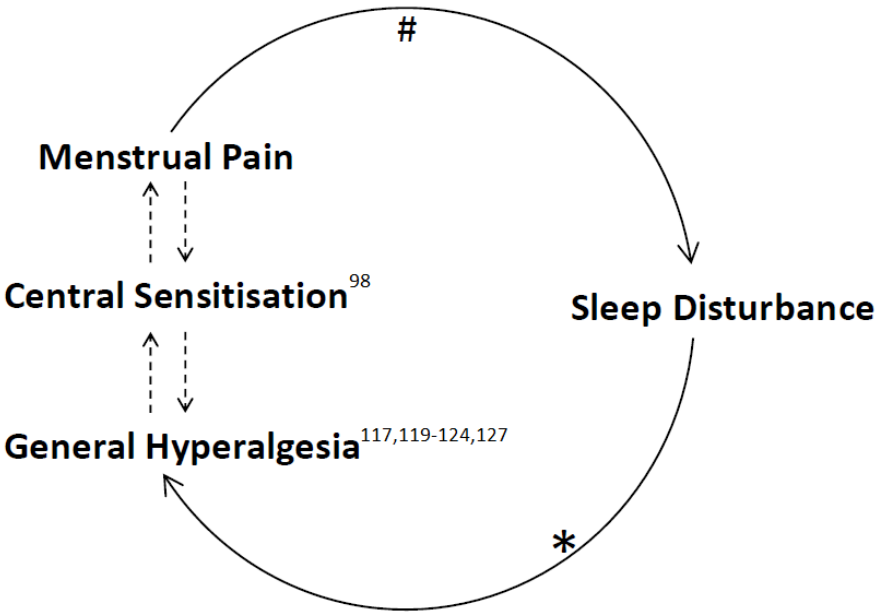


Figure 5.1: Bidirectional relationship of sleep and pain in women with primary dysmenorrhoea, whereby #sleep is disrupted by pain during menstruation[116,118,137] and *disrupted sleep leads to hyperalgesia during the pain-free phase of the menstrual cycle, as demonstrated in the present study.

The numbers denote the studies indicating that women with primary dysmenorrhoea are hyperalgesic[117,119-124,127] and prone to central sensitisation[98].

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Appendices

Appendix A – Ethical Approval Letter

R14/49 Dr Stella Lacovides et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150211

NAME: (Principal Investigator)	Dr Stella Lacovides et al
DEPARTMENT:	Physiology University of the Witwatersrand
PROJECT TITLE:	The Effect of Sleep Fragmentation on the Perception of Experimentally-Induced Deep Muscle in Women with Primary Dysmenorrhoea and Healthy Controls
DATE CONSIDERED:	27/02/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/04/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B – Screening Questionnaire

UNIVERSITY OF THE WITWATERSRAND

SCHOOL OF PHYSIOLOGY

SCREENING QUESTIONNAIRE

Subject profile

Date:.....

Subject name:.....

Subject code:..... *(Not to be completed by subject)*

*D*ate of birth:..... / ..... / 19 ..... Age:.....

Height:.....(m) Bodymass:.....(kg)

What is your first language? .....

What language did you get your education in? .....

Contact details

Home tel:.....

Work tel:.....

Cell no. :.....

E-mail:.....

----------to be cut and kept separate.

Subject code:

Other details

1. Do you smoke? **YES / NO**
2. Have you ever been pregnant, and if so, do you have children?
   .....
3. Are you generally a physically healthy person? **YES / NO**
4. Have you suffered from any recent illness (within the last three months), and if so, what was the illness? .....
5. Are you an emotionally and psychologically healthy person? **YES / NO**
6. Do you have an intrauterine device (IUD) fitted? **YES / NO**
7. Are you taking, or have you ever taken, any form of hormonal therapy (e.g. oral contraceptive, injected contraceptive, Diane for skin)? **YES / NO**. If yes, please give details.....
   .....

Your menstrual cycle

1. When did you have your very first menstrual period? ..... (year) .....(age)

2. If you have a regular menstrual cycle (ie. you never miss your period AND is it roughly the same length), what is its usual length? ..... days

3. If you do have an irregular menstrual cycle, please estimate the maximum and minimum number of days between your menstrual periods, in a typical year:
   Max: ..... days
   Min: ..... days

4. What was the date of the first day of your last period? ..... / ..... / 20.....

5. Usual length of menstruation (bleeding) .....days

6. What should be the approximate date of your next period? ..... / ..... /20....

7. Do you suffer from menstrual pain - on the day before menstruation?
YES / NO
- during menstruation?
YES / NO

8. When did you start suffering from menstrual pain? ..... (year)

9. Have you always suffered from menstrual pain since your teenage years? **YES / NO**
If NO, when have you not had menstrual pain?
.....
.....

10. Do you suffer from any complaints, such as irritability, tearfulness or bloatedness, before or during menstruation? **YES / NO**

Please specify:
.....
.....
.....

11. Do you have any complaints, apart from pain, during menstruation e.g. irritability, back-ache etc.?

.....
.....

12. Do you ever experience pain at the same place where you experience menstrual pain during the non-bleeding phase of your monthly cycle? **YES / NO**

If yes, please specify:.....

.....

.....

13. Have you ever consulted a medical professional about your period pain?

YES/NO

..... (year)

14. Have you ever been diagnosed by a doctor with any of the following conditions:

(a)	pelvic inflammatory disease	**YES / NO**
(b)	endometriosis	**YES / NO**
(c)	adenomyosis	**YES / NO**
(d)	uterine polyps	**YES / NO**
(e)	ovarian cysts	**YES / NO**
(f)	cervical strictures or stenosis	**YES / NO**
(g)	pelvic congestion syndrome	**YES / NO**

Severity of your menstrual pain

1. How does your menstrual pain affect your working ability?

Unaffected **Rarely affected** **Moderately affected** **Severely affected**

2. How often do you take pain-killers for your menstrual pain?

Not required **Rarely required** **Often required** **Always required**

3. Do you ever suffer from nausea, headaches, diarrhoea or any other symptoms associated with your period pain?

Never **Seldom** **Frequently** **Always**

Explain:.....

4. Mark on the scale below how severe the pain is during menstruation. (Past 6 months)

Worst pain I have ever felt

No pain at all ——————————

5. At what time of the day is your period pain the worst?

Morning **Afternoon** **Evening** **Night**

6. List all the medications you have ever tried for your period pain in the past

.....

.....

.....

7. Is the medication effective at relieving your pain? Explain.

.....

.....

General health

1. Are you aware that you are suffering from any of the following complaints?

Diabetes	**Yes**	**No**
Hypertension	**Yes**	**No**
Muscular disorders	**Yes**	**No**
Bone disorders	**Yes**	**No**
Joint disorders	**Yes**	**No**
Gastrointestinal disorders	**Yes**	**No**
Asthma	**Yes**	**No**
Epilepsy	**Yes**	**No**
Kidney disorders	**Yes**	**No**
Liver disorders	**Yes**	**No**
Autoimmune diseases	**Yes**	**No**
Sensory disorders	**Yes**	**No**
Anxiety or Depression	**Yes**	**No**

2. Are you currently on any regular medication (i.e. that you take at least once a day)? **YES / NO**

Name of medication:.....

Taken for what?.....

Since when?.....

3. Do you take anti-inflammatories or painkillers more than once a week?

Yes **No**

5. Do you typically sleep well? **Yes** **No**

If "No", what sort of problems do you have? (tick the problem)

..... Difficulty falling asleep?

..... Waking up in the middle of the night/difficulty falling asleep again?

..... Waking up too early in the morning?

..... Disruptive leg movements/ leg discomfort?

..... Disruptive snoring/ gasping for air?

6. Do you have a regular bedtime and wake up times? **Yes** **No**

If yes; normal bedtime? ..... : .....

normal wake-up time? ..... : .....

7. How many hours do you generally sleep at night?

.....

8. Do you nap; if so, how often?

.....

Previous Pain Intensity

What is the most painful experience you have ever had?

.....

.....

People agree that the following five words represent pain in increasing intensity:

a) Mild
b) Discomforting
c) Distressing
d) Horrible
e) Excruciating

To answer the questions below, write the letter of the most appropriate word in the space provided:

Which word best describes the worst pain you have ever felt?.....

Which word best describes the worst toothache you have ever had?.....

Which word best describes the worst headache you have ever had?.....

Which word best describes the worst stomach-ache you have ever felt?.....

Which word best describes your menstrual pain?.....

Appendix C – General Health Questionnaire

GENERAL HEALTH QUESTIONNAIRE

Subject code: ..... Date: .....

Subject name: ..... Study day: .....

We should like to know if you have any medical complaints, and how your health has been in general **over the past few weeks**.
Please answer ALL the questions by simply circling the answer which you think most nearly applies to you.
Remember that we need to know about present and recent complaints, not those that you had in the past.
It is important that you try to answer ALL the questions

Thank you very much for your co-operation

HAVE YOU RECENTLY:

1. – been able to concentrate on whatever you are doing?	Better than usual	Same as usual	Less than usual	Much less than usual
2. – lost much sleep over worry?	Not at all	No more than usual	Rather more than usual	Much more than usual
3. – been having restless, disturbed nights?	Not at all	No more than usual	Rather more than usual	Much more than usual
4. – been managing to keep yourself busy and occupied?	More so than usual	Same as usual	Rather less than usual	Much less than usual
5. – been getting out of the house as much as usual?	More so than usual	Same as usual	Less than usual	Much less than usual
6. – been managing as well as most people would in your shoes?	Better than most	About the same	Rather less well	Much less well
7. – felt on the whole you were doing things well?	Better than most	About the same	Less well than usual	Much less well
8. – been satisfied with the way you've carried out your task?	More satisfied	About same as usual	Less satisfied than usual	Much less satisfied
9. – been able to feel warmth and affection for those near to you?	Better than usual	About same as usual	Less well than usual	Much less well
10. – been finding it easy to get on with other people	Better than usual	About same as usual	Less well than usual	Much less well
11. – spent much time chatting with people	More time than usual	About same as usual	Less well than usual	Much less well
12. – felt that you are playing a useful part in things?	More so than usual	Same as usual	Less well than usual	Much less well
13. – felt capable of making decisions about things?	More so than usual	Same as usual	Less well than usual	Much less well

14. – felt constantly under strain?	Not at all	No more than usual	Rather more than usual	Much more than usual
15. – felt you couldn't overcome your difficulties?	Not at all	No more than usual	Rather more than usual	Much more than usual
16. – been finding life a struggle all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual
17. – been able to enjoy your normal day-to-day activities?	More so than usual	Same as usual	Less so than usual	Much less than usual
18. – been taking things hard?	Not at all	No more than usual	Rather more than usual	Much more than usual
19. – been getting scared or panicky for no good reason?	Not at all	No more than usual	Rather more than usual	Much more than usual
20. – been able to face up to your problems?	More so than usual	Same as usual	Less able than usual	Much less able
21. – found everything getting on top of you?	Not at all	No more than usual	Rather more than usual	Much more than usual
22. – been feeling unhappy and depressed?	Not at all	No more than usual	Rather more than usual	Much more than usual
23. – been losing confidence in yourself?	Not at all	No more than usual	Rather more than usual	Much more than usual
24. – been thinking of yourself as a worthless person?	Not at all	No more than usual	Rather more than usual	Much more than usual
25. – felt that life is entirely helpless?	Not at all	No more than usual	Rather more than usual	Much more than usual
26. – been feeling hopeful about your own future?	More so than usual	About same as usual	Less so than usual	Much less hopeful
27. – been feeling reasonably happy, all things considered?	More so than usual	About same as usual	Less so than usual	Much less than usual
28. – been feeling nervous and strung-up all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual
29. – felt like life isn't worth living?	Not at all	No more than usual	Rather more than usual	Much more than usual
30. – found at times you couldn't do anything because your nerves were too bad?	Not at all	No more than usual	Rather more than usual	Much more than usual

Appendix D – Pittsburgh Sleep Quality Index

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

Name:..... ID No.:.....

Date:..... Age:.....

Instructions:
The following questions relate to your usual sleep habits during the past month *only*. Your answers should indicate the most accurate reply for the *majority* of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

   USUAL BED TIME _____

2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, when have you usually gotten up in the morning?

   USUAL GETTING UP TIME _____

4. During the past month, how many hours of *actual sleep* did you get at night? (This may be different than the number of hours you spend in bed).

   HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer *all* questions.

5. During the past month, how often have you had trouble sleeping because you

   a) Cannot get to sleep within 30 minutes

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

   b) Wake up in the middle of the night or early morning

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

   c) Have to get up to use the bathroom

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

d) Cannot breathe comfortable

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

e) Cough or snore loudly

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

f) Feel too cold

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

g) Feel too hot

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

h) Had bad dreams

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

i) Have pain

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

j) Other reason(s), please describe:.....

.....

How often during the past month have you had trouble sleeping because of this?

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

6. During the past month, how would you rate your sleep quality overall?

Very good _____
Fairly good _____
Fairly bad _____
Very bad _____

7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month	Less than once a week	Once or twice a week	Three or more times a week .....

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all _____
Only a very slight problem _____
Somewhat of a problem _____
A very big problem _____

10. Do you have a bed partner or roommate?

No bed partner or roommate _____
Partner/roommate in other room _____
Partner in same room, but not same bed _____
Partner in same bed _____

Appendix E – Morning and Evening Questionnaire

Evening McGill Pain – Part 1 and Visual Analogue Scale

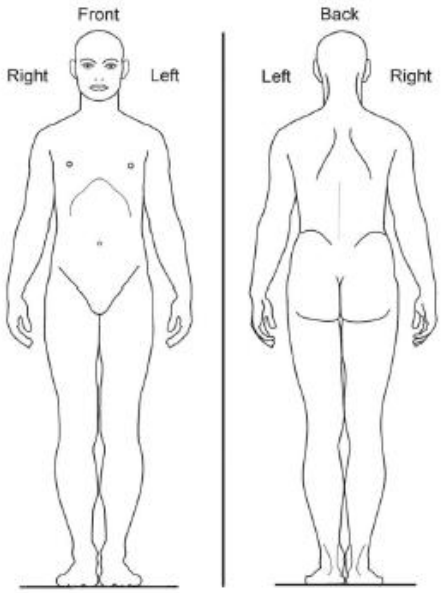
Subject code: ..... Date: .....

Subject name: ..... Time: .....

To be filled out **BEFORE** you go to bed

McGill pain questionnaire – Part 1

On the body chart below please indicate where you experienced pain during the day. Put E if external or I if internal.



SEVERITY OF DYSMENORRHOEA

Please make a mark on the line to indicate the degree of menstrual-associated pain you felt during the day.

No pain ——————————————— **Worst pain I have ever felt**

OTHER COMMENTS

Please make a brief note of any special unpleasant features of the day, such as accidents, rows, weeping, family troubles, or anything else you would like to mention:

.....

.....

Morning McGill Pain – Part 1 and Visual Analogue Scale

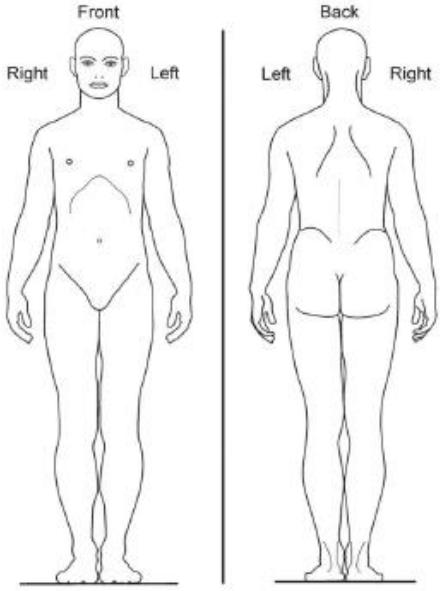
Subject code: ..... Date: .....

Subject name: ..... Time: .....

To be filled out AS SOON AS you wake up; along with the sleep diary.

McGill pain questionnaire – Part 1

On the body chart below please indicate where you are currently experiencing pain (if any). Put E if external or I if internal.



SEVERITY OF DYSMENORRHOEA

Please make a mark on the line to indicate the degree of menstrual-associated pain you felt during the night and/or currently.

No pain ——————————————— **Worst pain I have ever felt**

OTHER COMMENTS

Please make a brief note of any special unpleasant features of the day, such as accidents, rows, weeping, family troubles, or anything else you would like to mention:

.....

.....

SLEEP DIARY

To be filled in first thing in the morning:

Subject code: ……. Date: …….

Subject name: ……. Study day: ……..

Answer the following questions about your sleep last night:

1. What time did you go to bed last night? …… : ……
2. What time did you fall asleep last night? …… : ……
3. What time did you wake up for the day today? …….. : ……..
4. How long do you think it took you to fall asleep last night? …… min
5. Did you take anything to help you sleep last night; if so, what did you take?

YES / NO;……

6. How many times did you wake up last night? ……times
7. If you woke up, how long were you awake for IN TOTAL (include all awakenings)? ……min

SLEEP QUALITY

Please make a mark on the line to indicate how well you slept last night.

No sleep ——————————— Best sleep I have ever had

MORNING VIGILANCE

Please make a mark on the line to indicate how bright, fresh and alert you feel this morning.

Not at all alert and fresh ——————————— Most alert and fresh I have ever felt

COMMENTS:

Please make a note on whether you were disturbed during the night, were uncomfortable, or whether there was anything unusual about your sleep.

.....

.....

.....

During the course of **YESTERDAY**, did you:

1.Take a nap? **Yes** **No**

If yes, what time?.....

Duration of nap? .....

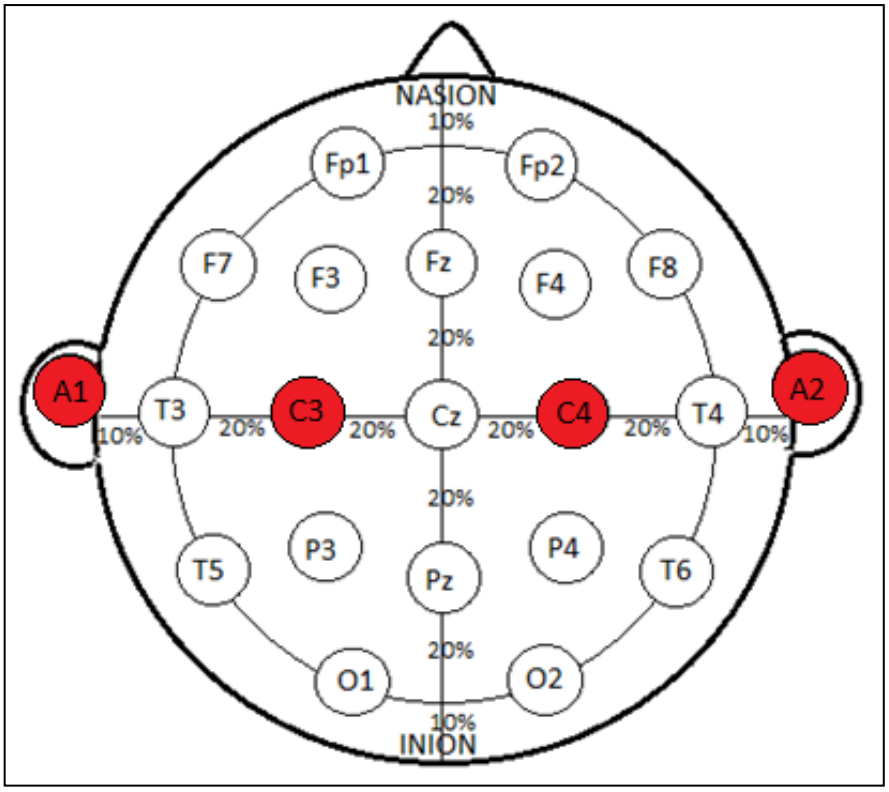
2. Remove the Wrist Actigraph? **Yes** **No**

If yes, what time?.....

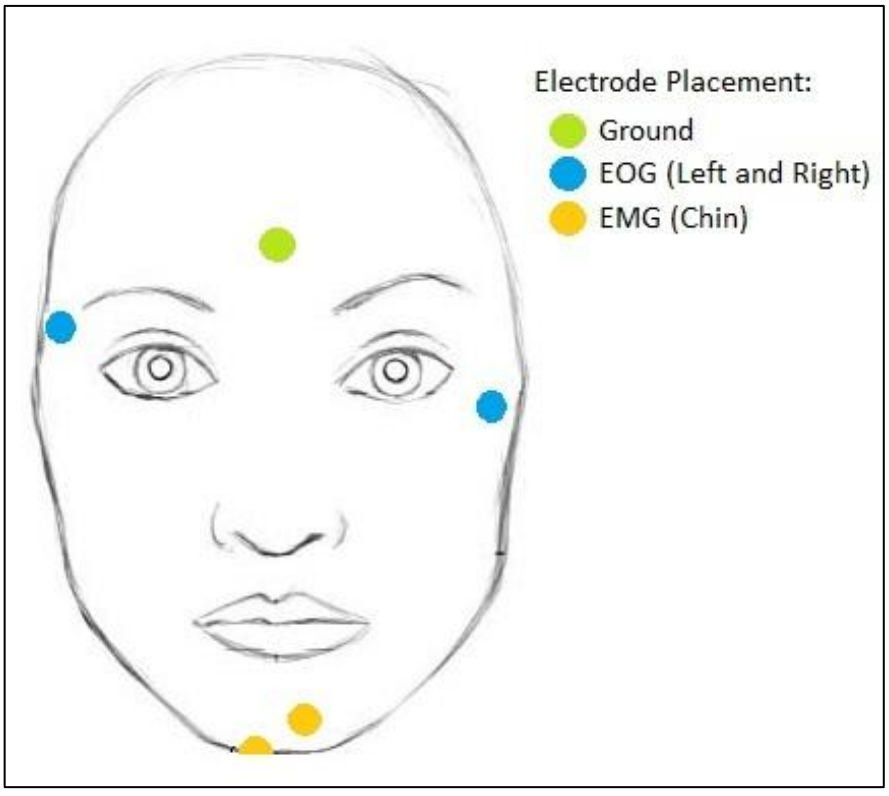
For how long?.....

Appendix F – Electrode Placements

Electroencephalogram (EEG)



Electromyogram (EMG) and Electro-oculogram (EOG)

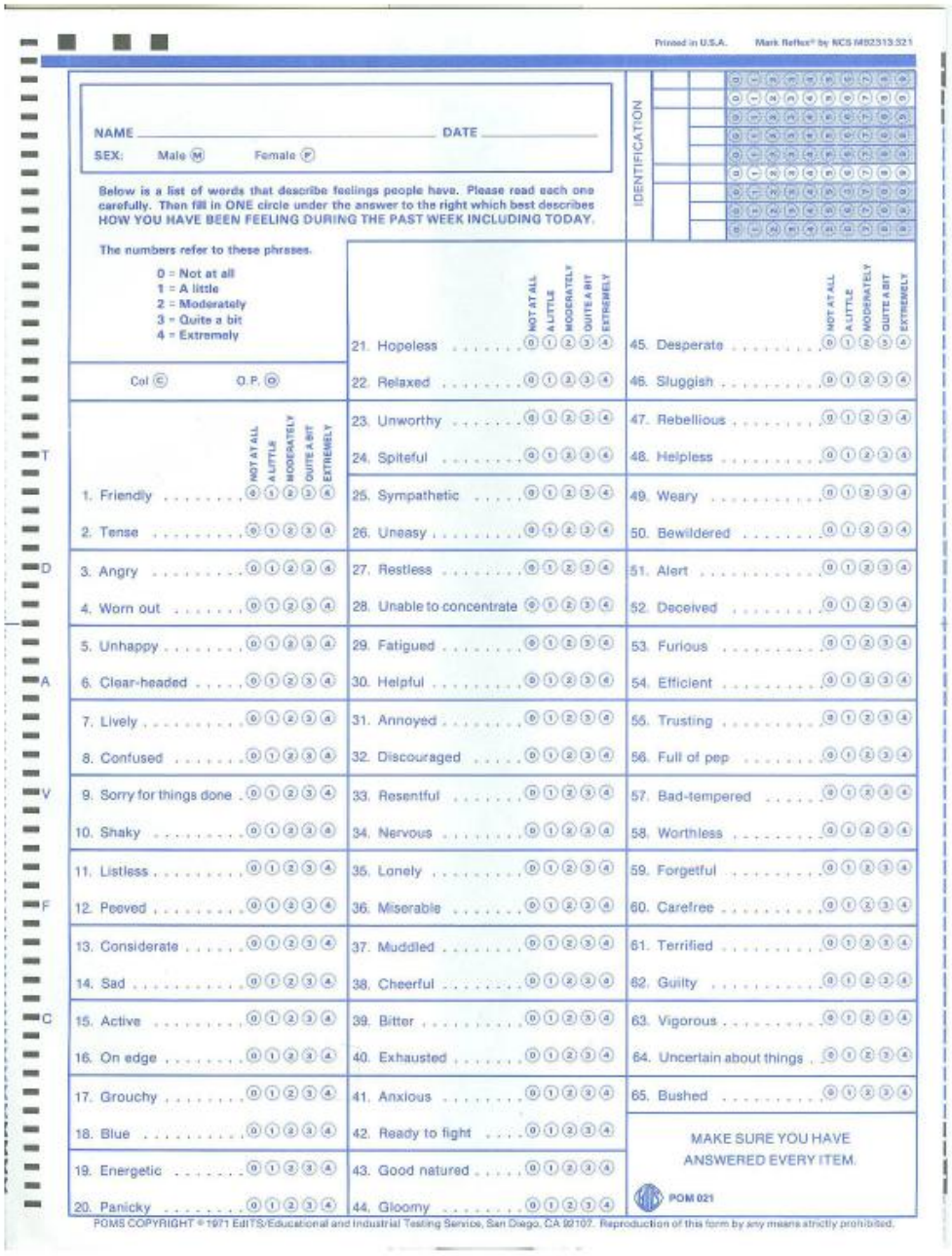


Appendix G – Profile of Mood States

Profile of Mood States Questionnaire

Subject code: ..... Date: .....

Subject name: ..... Time: .....



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NAME _____ DATE _____

SEX: Male (M) Female (F)

IDENTIFICATION

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY.

The numbers refer to these phrases.

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

Col (C) O.P. (O)

	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
1. Friendly	0	1	2	3	4
2. Tense	0	1	2	3	4
3. Angry	0	1	2	3	4
4. Worn out	0	1	2	3	4
5. Unhappy	0	1	2	3	4
6. Clear-headed	0	1	2	3	4
7. Lively	0	1	2	3	4
8. Confused	0	1	2	3	4
9. Sorry for things done	0	1	2	3	4
10. Shaky	0	1	2	3	4
11. Listless	0	1	2	3	4
12. Peeved	0	1	2	3	4
13. Considerate	0	1	2	3	4
14. Sad	0	1	2	3	4
15. Active	0	1	2	3	4
16. On edge	0	1	2	3	4
17. Grouchy	0	1	2	3	4
18. Blue	0	1	2	3	4
19. Energetic	0	1	2	3	4
20. Panicky	0	1	2	3	4
21. Hopeless	0	1	2	3	4
22. Relaxed	0	1	2	3	4
23. Unworthy	0	1	2	3	4
24. Spiteful	0	1	2	3	4
25. Sympathetic	0	1	2	3	4
26. Uneasy	0	1	2	3	4
27. Restless	0	1	2	3	4
28. Unable to concentrate	0	1	2	3	4
29. Fatigued	0	1	2	3	4
30. Helpful	0	1	2	3	4
31. Annoyed	0	1	2	3	4
32. Discouraged	0	1	2	3	4
33. Resentful	0	1	2	3	4
34. Nervous	0	1	2	3	4
35. Lonely	0	1	2	3	4
36. Miserable	0	1	2	3	4
37. Muddled	0	1	2	3	4
38. Cheerful	0	1	2	3	4
39. Bitter	0	1	2	3	4
40. Exhausted	0	1	2	3	4
41. Anxious	0	1	2	3	4
42. Ready to fight	0	1	2	3	4
43. Good natured	0	1	2	3	4
44. Gloomy	0	1	2	3	4
45. Desperate	0	1	2	3	4
46. Sluggish	0	1	2	3	4
47. Rebellious	0	1	2	3	4
48. Helpless	0	1	2	3	4
49. Weary	0	1	2	3	4
50. Bewildered	0	1	2	3	4
51. Alert	0	1	2	3	4
52. Deceived	0	1	2	3	4
53. Furious	0	1	2	3	4
54. Efficient	0	1	2	3	4
55. Trusting	0	1	2	3	4
56. Full of pep	0	1	2	3	4
57. Bad-tempered	0	1	2	3	4
58. Worthless	0	1	2	3	4
59. Forgetful	0	1	2	3	4
60. Carefree	0	1	2	3	4
61. Terrified	0	1	2	3	4
62. Guilty	0	1	2	3	4
63. Vigorous	0	1	2	3	4
64. Uncertain about things	0	1	2	3	4
65. Bushed	0	1	2	3	4

MAKE SURE YOU HAVE ANSWERED EVERY ITEM.

POM 021

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Appendix H – von Frey Hairs





Appendix I – The Pennebaker Inventory of Limbic Languidness

The Pennebaker Inventory of Limbic Languidness (PILL)

Subject code: ..... Date: .....

Subject name: ..... Time: .....

Using the scale below, circle items 1 - 10 to indicate the severity of the symptoms described.

0 Not at all	1 A little	2 Somewhat	3 Moderately	4 Very much

1. Back pain	0	1	2	3	4
2. Headache	0	1	2	3	4
3. Chest pain	0	1	2	3	4
4. Cramps	0	1	2	3	4
5. Toothache	0	1	2	3	4
6. Heartburn	0	1	2	3	4
7. Severe pains or cramps in the stomach	0	1	2	3	4
8. Joint pain	0	1	2	3	4
9. Sore muscles	0	1	2	3	4
10. Sore throat	0	1	2	3	4

Appendix J – Summary of the Final General Additive Mixed Model for Sleep Quality (SQ) and Morning Vigilance

	Coefficients			
	Estimate	**Standard Error**	**T**	**P**
SQ				
Mean (μ) subscale (location)				
Intercept	1.647	0.276	5.97	<0.001
Group	-0.967	0.350	-2.77	0.008
Fragmentation Night				
1 vs baseline	-2.621	0.360	-7.28	<0.001
Fragmentation night				
2 vs baseline	-2.150	0.349	-6.16	<0.001
Group: Fragmentation night				
1 vs baseline	1.232	0.465	2.65	0.01
Group: Fragmentation night				
2 vs baseline	0.641	0.459	1.40	0.17
Variance (δ) subscale (scale)				
Intercept	-0.779	0.121	-6.45	<0.001
Shape (η) subscale (skewness)				
Intercept	-17.54	867.53	-0.02	0.98
Shape (t) subscale (kurtosis)				
Intercept	-3.314	0.720	-4.60	<0.001
Morning Vigilance				
Mean (μ) subscale (location)				
Intercept	1.276	0.275	4.64	<0.001
Group	-0.351	0.356	-0.98	0.33
Fragmentation night				
1 vs baseline	-2.333	0.384	-6.08	<0.001
Fragmentation night				
2 vs baseline	-2.300	0.381	-5.94	<0.001
Group: Fragmentation night				
1 vs baseline	0.878	0.499	1.76	0.08
Group: Fragmentation night				
2 vs baseline	0.403	0.502	0.80	0.43
Variance (δ) subscale (scale)				
Intercept	-0.657	0.126	-5.22	<0.001
Shape (η) subscale (skewness)				
Intercept	-2.565	0.519	-4.94	<0.001
Shape (t) subscale (kurtosis)				
Intercept	-3.951	1.010	-3.91	<0.001