The Effect of a short nap following 4h Sleep Restriction on Pain Sensitivity in Young Healthy Males

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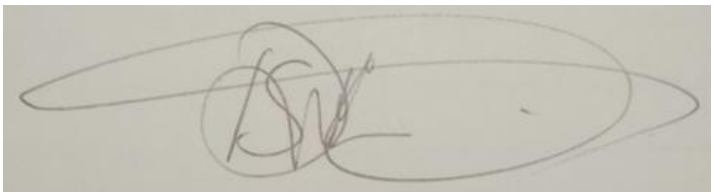
DECLARATION

I, Siphesihle Dakile, declare that this Dissertation is my own, unaided work. It is being submitted for the 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.



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Abstract

Introduction: Sleep is a very important biological process and has been linked to memory cementing, recuperation and regeneration of cells and other bodily systems such as the immune system. Sleep disruption (restriction, deprivation and fragmentation) results in decreased cognitive function, higher reaction times, and increased risk of cardiometabolic dysfunction. A bidirectional relationship between sleep and pain perception has been well documented. Curtailed sleep results in higher pain sensitivity and lower pain thresholds; on the other hand people who are in pain (especially chronic pain syndromes) have been shown to sleep worse than those who are not. Thus, a lot of interest has emerged on the possible recuperative effects of recovery sleep, particularly in the form of a daytime/afternoon nap. There is, at the time of this study, only one other paper that has investigated afternoon naps in relation to pain perception. There is still conflicting evidence as to the length of nap that gives maximum physiological benefits and recuperation (pain, cognitive and immune function). This study aimed to investigate the effects of two kinds of sleep restriction, restricting the first four hours (SR-F4H) and the last four hours (SR-L4H) of sleep, on sleep architecture and pain perception in healthy young males. Furthermore, to grow the literature on naps and pain, I aimed to investigate the potentially recuperative effect of a short afternoon nap following the nights of sleep restriction as well as the relationship between nap sleep architecture and subsequent pain.

Methods: 11 healthy young men (Median [IQR], 21 [20 - 21] years old) were recruited to participate in the study using flyers and word of mouth. They had healthy-slightly overweight Body Mass Index (BMI's) (22.3 [20.7-25.3] $kg.m^2$) and healthy 7-9 hour sleep/wake cycles. They were screened for a week before commencement of the study using the General Health Questionnaire, Pittsburgh Sleep Quality Index, Sleep Diaries and Actiwatches. They then entered the experimental portion of the study. They had one adaptation night followed by five

condition nights (Baseline, SR-F4Hnap, SR-F4Hnonap, SR-L4Hnap and SR-L4Hnonap) that were in random order. The participants would also have afternoon procedures where they either came in for a 30-minute nap or they came into the laboratory for a 30-minute lie-in (where they lay down for 30 minutes in a dark room). Following the night and afternoon polysomnography, they underwent a 10 minute ischeamic pain test, induced using a tourniquet. The pain was scored on a 0-100mm visual analogue scale every minute until the 10 minute mark. I used mixed models in SAS 9.4 ® to investigate 1) the relationship between experimental conditions and sleep composition, 2) the relationship between pain perception and experimental conditions when adjusting for order of conditions, time of day, presence of a nap and time during the test 3) the relationship between pain perception and sleep stage composition during the experimental night preceding the pain tests, when adjusting for the same variables 4) the relationship between pain perception in the afternoon and sleep stage composition during the nap.

Results: Total Sleep Time (TST), N2, REM (in minutes and proportions) were significantly decreased during sleep restriction as per the design of the study. Proportions of TST, N2 and REM showed a protective relationship with pain, whereby an increase in these variables resulted in a decrease in pain perception ($p = 0.006$, $p = 0.003$ and $p = 0.007$ respectively). On the other hand, N3 showed a positive association with pain, whereby increases in N3 resulted in increased pain scores ($p = 0.006$). The proportion of N2 was the most reliable predictor of pain the next day, where increases in N2 were directly associated with decreased pain perception upon pain testing ($p = 0.003$). Pain scores were lower in the afternoon compared to the morning ($p<.0001$). The nap condition was associated with increased pain perception ($p<.0001$). Pain perception decreased throughout the study (order effect $p <.0001$), potentially a result of habituation of the participants to the procedures. The minutes spent in N3 sleep and the longer time awake between waking in the morning and the afternoon nap were

significant predictors of pain, where longer times resulted in higher pain reported by the participants ($p < .0001$ for both).

Conclusions: Sleep restriction, whether SR-F4H or SR-L4H, did not result in higher pain perception in these participants however pain perception was increased with higher N3 and lower N2, TST and REM. Furthermore, the 30-minute afternoon naps seemed to increase the pain perception, mainly through time spent in N3 sleep. The relationship between specific sleep stages and pain perception and the effect of recovery sleep still needs to be further elucidated.

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List of Abbreviations

Abbreviation	Meaning
SR-F4H	Sleep restriction of the first 4 hours (i.e late sleep)
SR-L4H	Sleep restriction of the last 4 hours (i.e early sleep)
BMI	Body Mass Index
TST	Total Sleep Time
TiB	Time in Bed
REM	Rapid Eye Movement
NREM	Non-rapid Eye Movement
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
Hz	Hertz
µV	Micro volts
OSA	Obstructive Sleep Apnoea
PSG	Polysomnography
PVT	Psychomotor Vigilance Test
MSLT	Multiple Sleep Latency Test
HRV	Heart Rate Variability
NSAID	Non-steroidal anti-inflammatory drugs

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CHAPTER 1

Introduction

Sleep is characterized as a state of unconsciousness (that we can be reversed) that interrupts the wake state for about 7 to 9 hours in human adults (Hirshkowitz et al., 2015) and is a complex and organized period of recuperation (Roehrs, 2000). Sleep is, however, not just a resting period. It also is a period where memory is consolidated, cells and protein regenerate and bodily systems recuperate (Wesesten et al., 1999; Fox, 1999). Sleep disruption is the interruption of the sleep process whether it is trouble falling asleep, multiple awakenings during the night (sleep fragmentation), or early awakenings, or all of the above. Sleep disorders, such as obstructive sleep apnoea and insomnia, lead to various forms of sleep disruption, and affect more than a third of people worldwide (Saldatos et al., 2005). Sleep disruption, therefore, is linked to imbalances in daily function such as decreased cognitive performance (Koo et al., 2017), lowered pain threshold and tolerance (Onen et al., 2001) as well as increased cardiometabolic risk (Troxel et al., 2010). The focus of my thesis is sleep and pain; hence this literature review will cover physiological aspects of sleep, physiological aspects of pain, and the interaction between sleep and pain, with a particular emphasis on sleep deprivation and experimental pain.

Sleep Architecture

Sleep is the interruption of the wake state lasting between 7 and 9 hours in adults (Hirshkowitz et al., 2015). There are two main states of sleep which alternate throughout the sleep process in approximately 90-minute cycles. These are 1) rapid eye movement (REM) and 2) non-rapid eye movement (NREM). NREM is further divided into stage 1 sleep (N1), stage 2 sleep (N2) and slow-wave sleep (N3) (Deatherage et al., 2009). Each of these stages has different characteristics on polysomnograms, which includes electroencephalography (EEG), electromyography (EMG) and electro-oculography (EOG). The lighter stages of NREM sleep (N1 and N2) start at alpha frequencies of 8-12 Hz and a voltage of 20-50 μV and slow into theta (4-7 Hz) and then delta (0.5-4) frequencies as sleep progresses. N1 also is characterised by rolling eye movements and occasional vortex waves. In N2 we see the appearance of K complexes as well as spindles. Reaching deeper slow wave sleep (N3), we observe delta waves of a frequency of 0.5-4 Hz and a voltage higher than 75 μV. During NREM sleep, except for the rolling eye movements during drowsy sleep, the EOG is mostly quiet The EMG exhibits some motor activity during NREM sleep, although it decreases as sleep deepens. REM sleep in contrast, is characterized by a low voltage EEG, coupled with paralysis of most major muscle groups (atonia) and rapid bursts of eye movements (Kilkerny & Grenard, 2000; Roerhs, 2000). Figure 1.1 below shows a hypnogram depicting the normal sleep cycle throughout the night. Shown are N1 (1), N2 (2) and N3 (3 and 4), and in red are the REM portions of sleep.

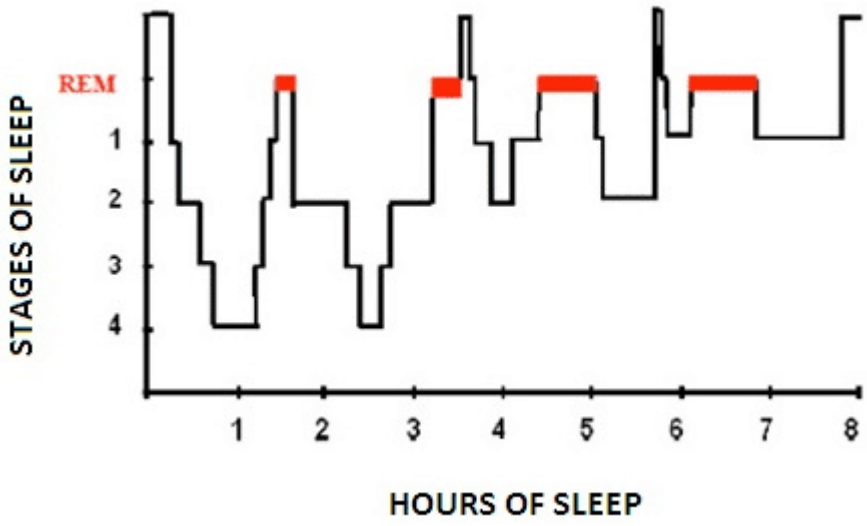

Figure 1.1 A hypnogram illustrating the different sleep stages and the cycle throughout a normal night of sleep in healthy individuals. (Adapted from *Goril & Shapiro, 2011. Patterns of Sleep Disorders and Circadian Rhythm Disruptions in Children and Adolescents with Fetal Alcohol Spectrum Disorders.*)

Sleep Regulation

The sleep process is modulated through various mechanisms, including circadian and homeostatic processes (Vladyslav, 2015; Saper et al., 2001; Roehrs, 2000). As part of the homeostatic mechanism, the length and quality of sleep tends to be determined by the amount of sleep obtained the previous night (Vladyslav, 2015). Following a night of restricted, fragmented or disturbed sleep, there is a homeostatic build-up of sleep pressure which leads to increased amount of subsequent sleep and increased slow wave sleep to compensate for the previous night (Deboer, 2018; Roehrs, 2000). Secondly, there are a few structures (suprachiasmatic nucleus, hypothalamus, tuberomammillary nucleus, pons and ventrolateral preoptic) that maintain the circadian pattern of sleep based on the light patterns and wake/sleep cycles, thus regulating when we feel sleepy and when we wake up (Deboer, 2018). A pacemaker located in the suprachiasmatic nucleus (SCN) works through light patterns and an endogenous cycle, generating circadian rhythms (El Cheick et al., 2019;

Deboer, 2018). The hypothalamus (tuberomammillary nucleus (TMN) and Raphe Nuclei), periaqueductal grey (PAG) and pons (Locus Coeruleus (LC)) are wake centres which regulate sleep/wakefulness using upward pathways that maintain the wake state through relating information to the cortex (Deboer, 2018). Firstly, the pons activates wake-stimulating acetylcholine releasing neurons in the thalamus that relay information to the cortex. Secondly, the TMN, LC, PAG and Raphe nuclei (through monoaminergic, serotonergic, histaminergic and dopaminergic neurons) project to the hypothalamus and the cortex and are wake-stimulating. Lastly, the ventrolateral preoptic (VLPO) is mostly responsible for maintaining the sleep state. The VLPO releases the inhibitory neurotransmitters, galanin and GABA, to the above mentioned wake-stimulating cells and thus inhibits wake and induces and maintains sleep (Deboer, 2018; Saper et al., 2001). The VLPO also receives information from the monoaminergic neurons and this feedback loop helps dictate which state is experienced by the individual at which time.

Additional to the above mentioned structures, we have orexins/hypocretins and adenosine which have influence on the sleep/wake cycle (Deboer, 2018). Orexin is a hormone strongly associated with appetite and hunger (Kim et al., 2004; Samson & Resch, 2000), and is also linked to sleep regulation (Deboer, 2018; Scammell et al., 2017; Mileykovskiy et al., 2005). Orexin is an excitatory neuropeptide produced and released from the lateral hypothalamus (Scammell et al., 2017). Orexin acts by exciting wake-active neurons in the cortex and thalamus to stimulate and maintain the wake state (Deboer, 2018; Mileykovskiy et al., 2005). Increased levels of Orexin have been shown to not only maintain wake but decrease/eliminate REM sleep (Scammell et al., 2017).

Adenosine is produced as a by-product of the energy (ATP) breakdown process and released in the central nervous system (Deboer, 2018; Bjorness & Greene, 2009). Adenosine has four known receptors (A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R), although only two (A_1R, $A_{2A}R$) have received

attention in sleep moderation (Bjorness & Greene, 2009). The $A_{2A}R$ are expressed in the striatum and the A_1R are distributed wider in the brain (thalamus, hippocampus and cortex). Adenosine works both by exciting and inhibiting neurons in the sleep/wake moderation (Franziska et al., 2016; Bjorness & Greene, 2009). Firstly, via the $A_{2A}R$ receptors, it excites the sleep-inducing neurons in the VLPO and modulates N3 and REM expression by modulating the acetylcholine release in the reticular formation (Franziska et al., 2016;). Secondly, via the A_1R receptors, it inhibits the wake-active neurons such as orexin in the hypothalamus and cells in the basal forebrain (Franziska et al., 2016;). Adenosine levels increase and accumulate throughout the wake state and increase their excitation of the sleep-inducing agents and depresses glutamate release, and adenosine starts to decrease during sleep thus decreasing the excitation of the sleep-inducing agents which eventually results in wake (Deboer, 2018). High levels of circulating adenosine, or the use of agonists, have also been linked to deeper sleep through increasing Slow Wave Activity through $A_{2A}R$ (Franziska et al., 2016; Bjorness & Greene, 2009). Figure 1.2 shows the 'on-off' switch of sleep and some of the major players modulating this system.

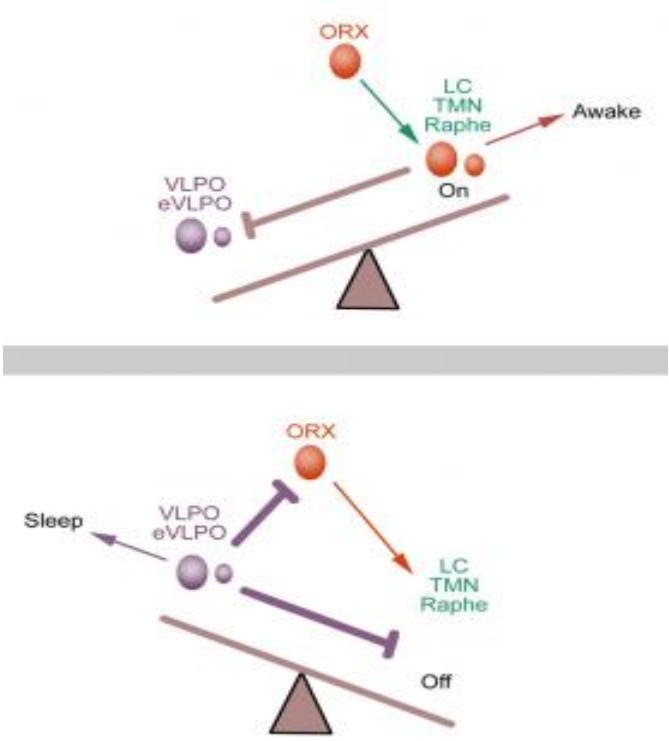

Figure 1.2 Sleep regulating substances and structures (including VLPO, Orexin (ORX) and components of the hypothalamus (Locus Coeruleus: LC, tuberomammillary nucleus: TMN and Raphe)) and how they interact to facilitate the sleep-wakefulness cycle. The 'on' figure shows the awake time where the VLPO is inhibited and the LC, TMN and Raphe Nuclei are maintaining wake. The 'off' indicates the sleep state where the VLPO is active and inhibits the wake-stimulating structures. (Adapted from *Chawla et al., 2020. What Is The Flip-Flop Switch Model Of Sleep Regulation*).

Pathophysiology: Sleep Disorders

Several sleep disorders interrupt sleep, shorten sleep or impair sleep maintenance. The most common being insomnia, about a third of people are reported to suffer from, or have suffered from, insomnia in the general population (Ohayon, 2011). Insomnia is characterized by

difficulty initiating and maintaining sleep, resulting in sleep periods that may be less than 6 hours and usually accompanied by daytime sleepiness. Chronic insomnia is defined as when these symptoms have persisted 3 times a week for at least three months (Ohayon. 2011; Wallace et al., 2012; Sateia, 2014). Insomnia has been linked to increased pain perception (Haack et al., 2012), increased cardiometabolic risks (Wu et al., 2014), decreased cognitive function (Koo et al., 2017), and increased fatigue and daytime sleepiness (Wallace et al., 2012). Obstructive sleep apnoea (OSA) is a sleep disorder where airflow is reduced and restricted to no air-flow, with increasing effort to breathe. It is a common sleep disorder that affects up to 4% of the population, usually more males (Ohayon, 2011). The sleep in patients with OSA is described as fragmented as a result of the awakenings due to not being able to breathe during the night (Ohayon, 2011). OSA is commonly associated with daytime sleepiness (Ohayon, 2011) as well as obesity (Gruber et al., 2006), poor cardiac health (Crowley, 2011) and the metabolic syndrome (Wolk & Somers, 2006).

Other, sleep disorders include: restless leg syndrome, which is characterized by uncontrollable urges to move legs, and has a prevalence of up to 19% (Phillips et al., 2000); narcolepsy, characterized by intrusions of sleep during the wake period and affects approximately 0.05% of the population (Ohayon et al., 2002); hypersomnia, described as excessive daytime sleepiness and difficulty staying alert during the waking period, and affects approximately 0.4% in population (Ohayon, 2011); REM sleep behavior disorder, occurring in less than 1% of the population, and involves patients acting out their dreams due to lack of REM-sleep paralysis (Trotti, 2010); sleeping sickness, which occurs due to infections by *Trypanosoma brucei* primarily in sub-Saharan Africa (10 000 cases per year) (Rijo-Ferreira et al., 2018); sleepwalking, which affects 5% children and 1.5% adults (Stallman & Kohler, 2016); and night terrors which manifest in screams and fear behaviour during sleep and is more common in children than adults (affects up to 50% of children and

1-4% of adults (usually affects those with traumatic experiences)) (Boyden et al., 2018; Mazarakis, 2014). Due to the fact that these sleeping disorders are markedly less common, they have not been studied as extensively as insomnia and OSA (Ohayon, 2011; Trotti, 2010). These sleep disorders also result in decreased and fragmented sleep which has been shown to lower pain thresholds and increase pain perception (Irwin et al., 2012; Smith et al., 2007; Onen et al., 2001).

Experimental Sleep Disruption

As a result of the prevalence of these disorders and their general effects on normal physiology, few laboratory models have been designed to better understand the mechanisms underlying the consequences of fragmented/shortened/disrupted sleep. To investigate the effects and mechanisms underlying the effects of sleep disruption on various aspects of health, protocols involving 1) restricted sleep (2-4 hour restrictions, where participants are kept awake for parts of the night (early or late)), 2) fragmented sleep (multiple forced awakenings throughout the night), 3) selective sleep restriction (restriction of specific parts/stages of sleep, for example restriction of N3), and 4) total deprivation of sleep (no sleep at all for 24h or more) are applied to healthy individuals (Medic et al., 2017; Finan et al., 2015; Reynolds & Banks, 2010; Smith et al., 2009).

As will be further discussed in the next chapter, various studies have used one or more of these sleep disruption models to investigate their effects on various pain outcomes. Increased pain perception, intensity has been reported after total sleep deprivation (Faraut et al., 2015; Kunderman et al., 2004), sleep fragmentation (Iacovides et al., 2017; Smith et al., 2007), sleep restriction (Matre et al., 2015; Haack et al., 2007) and selective sleep restriction (Maldofsky & Scarisbrick, 1976). Some studies found no changes (Arima et al., 2001) and even contradictory results (Onen et al., 2001) in pain related outcomes after sleep disruption.

Sleep Measurements

Actigraphy

Actigraphy is a common tool used to monitor human rest and activity cycles based on an actimetry sensor, which can be used for several purposes (Ancoli-Israel et al, 2003). In this study it was used with the purpose of making sure the participants do not stray from their prescribed sleep and wake times. Numerous studies have used these devices, wrist or waist, as a screening device to monitor the subjects before they start the experiment (Faraut et al., 2015; Van Leeuwen et al., 2010) or as a monitoring device during the experimental period (Bell et al., 2013; Brooks & Lack, 2006).

Polysomnography

Polysomnography is the gold-standard technique when doing sleep studies and looking to measure sleep objectively, distinguish the sleep stages, and potentially find abnormalities in a participant's sleep pattern. Numerous studies doing the same work as this study have used polysomnography with some deviation of the software used to capture it; and whether it was used in-lab or at home (Sauvet et al., 2015; Van Leeuwen et al., 2010; Chhangan et al., 2009; Hayashi et al., 2005). Polysomnography uses electrodes that have designated placements on the scalp (EEG), face (EOG and EMG) and sometimes chest (depending on the aim of the researcher) to acquire a reading of brain waves as the participant either sleeps, lies in bed or performs a given task. Figure 1.3 shows the 10-20 electrode placement system, the accepted standard. The recording can then be used to classify sleep vs non-sleep states, distinguish between sleep stages or determine somnolence. There is appreciation for a little deviance in these recordings because of the discomfort of being in the sleep lab as well as having electrodes on. As such, adaptation nights are required for the subject to familiarize themselves with the lab environment. In one study they attempted to eliminate this deviation

by using a PSG belt which is worn on the head and can be used at home (Drewes et al., 1998). However, the recording might still vary as a result of the different environments of the participants' homes, whilst the sleep lab provides a common, controlled environment for all. For this study, I chose to use an adaptation night to have the participants acclimatize to the laboratory settings in order to minimize variation during the experimental nights.

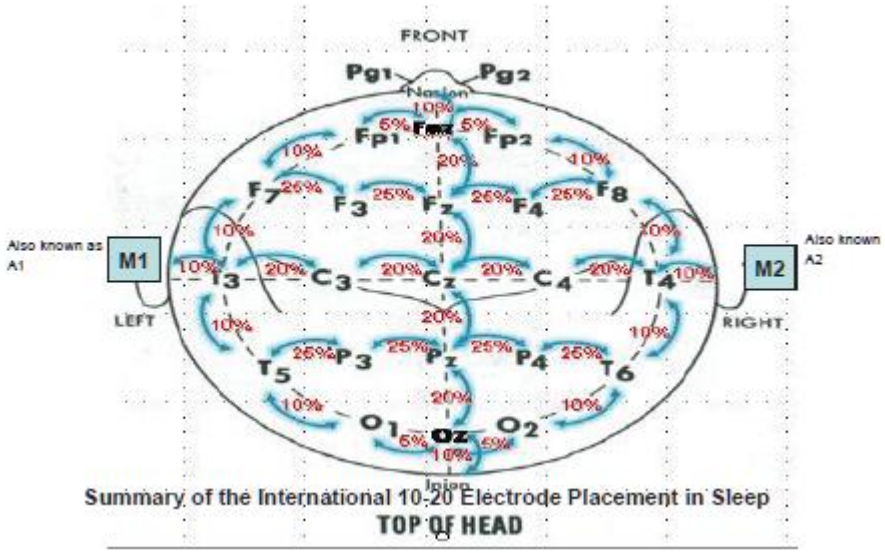

Figure 1.3 Showing the 10-20 electrode placement guidelines for polysomnography readings in sleep studies.

Pain

Pain is an unpleasant sensory and emotional sensation associated with actual or potential tissue damage (Raja et al., 2020). It is an emotional and somatic sensation that one experiences when faced with potentially dangerous stimuli (Verma et al., 2015; Woolf, 2010). Pain ordinarily plays an adaptive role, that is, pain is a protective, survival impulse which helps us move away from danger and towards safety, and alert us of pathology within our bodies (Basbaum et al., 2009). However pain can become maladaptive, with adverse effects on psychological and social well-being and function (Raja et al., 2020), particularly if it persists for longer than three months, termed as chronic pain (Treede et al., 2019). Pain may be nociceptive (pain arising from the activation of nociceptors in response to actual or potential tissue damage e.g hypoxia/ischaemia, or osteoarthritis, mechanical low back pain), inflammation (usually secondary to injury and promotes aversive behaviour to keep the injured area protected during the healing process), neuropathic (pain arising from a lesion or

disease of the somatosensory nervous system e.g painful diabetic polyneuropathy, post-stroke pain), or nociplastic (altered nociception that is not neuropathic or nociceptive in origin e.g fibromyalgia, chronic widespread pain) (Verma et al., 2015; Kosek et al., 2016; Jensen et al., 2011; Woolf, 2010; Woolf, 1995; Lewis, 1938;). For the purposes of this literature review, I will focus specifically on nociceptive pain from the skin and muscles.

Nociceptive pain from the skin is perceived in three stages, namely 1) activation, 2) transduction and 3) perception (Dubin and Patapoutian, 2010). Activation occurs in the presence of noxious stimuli, which are potentially harmful, and include extreme temperatures (higher than 45◦C, less than 15◦C), chemicals (such as capsaicin and hypertonic saline) and mechanical pressures (including punctate stimuli such as pinpricks). Noxious stimuli activate and lead to depolarization of the excitatory pain receptors, or nociceptors. Transduction involves signalling to second order neurons towards the dorsal horn of the spinal cord, where nociceptors release excitatory neurotransmitters such as glutamate and substance P (Yen & Lu, 2013; Millan, 1999). The second order neurons are activated at the synapse, sending the impulse to the brain through the spinothalamic tract. Lastly, the perception of pain occurs following an additional synapse at the thalamus to third order neurons. The signals are projected to the thalamus, mesencephalon and medulla, and subsequently to the somatosensory cortex and anterior cingulate cortex (Millan, 1999), where the pain is perceived and located (Yen & Lu, 2013). Usually, following activation of these nociceptive processes, a protective behavior, such as moving away from the stimulus, will be elicited (Dubin and Patapoutian, 2010, Almeida et al., 2004). Nociception from the muscle follows a similar path, producing dull, aching and less localized sensation compared to nociception from cutaneous stimuli, which induces more of a sharp, piercing sensation (Almeida et al., 2004).

Noxious stimuli activate two different types of nociceptors, namely type A-delta (Aδ) fibers and type C-fibers. Aδ fibers are thin (diameter 1-5μm) and have myelinated axons that support fast conduction (velocities of 5-30m/s) and predominantly respond to intense heat, cold and mechanical stimuli. The pain felt from the Aδ sensory fibers (usually referred to a 'first pain') is described as sharp, stabbing or pricking (Dubin and Patapoutian, 2010). In contrast, the type C fibers have unmyelinated axons and smaller diameters (0.2-1.5μm), which support conduction of 0.4-1.4m/s velocities and they respond to thermal (heat) and mechanical stimuli (Dubin and Patapoutian, 2010; Djouhri et al., 2004; Cain et al., 2001). Of interest for this study is that C fibers are also activated by tissue ischemia (as from a tourniquet cuff); hypoxia and glucose substitution (occurs during ischemia) have been shown to stimulate these fibers (MacIver & Tanelian, 1992). Pain arising from the activation of C fibers ('second pain') is described as burning, aching and throbbing pain (Dubin & Patapoutian, 2010). Anatomically, C fibers are more loosely and broadly distributed whilst the A fibers cluster in small groups, making them more precise in localizing stimuli (Cain et al., 2001).

Pain is modulated by the descending pain pathway. After nociception has reached the thalamus, mesencephalon and medulla, and has been relayed to the cortices, the signals are redirected to the periaqueductal grey (PAG). Excitation of the PAG leads to excitation of the rostroventromedial medulla (RVM) which then elicits the Raphe Nuclei, pontine noradrenergic nuclei and Locus Coeruleus to release inhibitory neurotransmitters, serotonin and noradrenaline, that inhibit nociception at the level of the spinal cord (Ossipov et al., 2014; Lau & Vaughan, 2014). Serotonin, released from the Raphe Nuclei, can either act by inhibiting or exciting at the spinal root ganglion depending on the receptors it binds to. In the presence of noxious stimuli, serotonin acts by inhibiting the upward transmission of pain (Ossipov et al., 2014; Lau & Vaughan, 2014). Noradrenaline, released from the pontine

noradrenergic nuclei and Locus Coeruleus, is released into the spinal cerebrospinal fluid and also acts at the dorsal horn resulting in decreased transmission of pain impulses and therefore antinociception (Ossipov et al., 2014; Lau & Vaughan, 2014).

Pain and Sleep

The relationship between pain and sleep is believed to be reciprocal. A large body of literature demonstrates how pain, both clinical (Bonvanie et al., 2016; Lautenbacher et al., 2006; Moldofsky, 2001) and experimental (Sivertsen et al., 2015; Matre et al., 2015; Haack et al., 2007), disrupts sleep. On the other hand, sleep disruption has been shown to decrease pain thresholds leading to hyperalgesia (where a painful stimulus becomes more painful) and/or allodynia (where a non-noxious becomes painful) (Lee et al., 2004; Smith & Haythornthwaite, 2004). A review which discussed the bi-directionality of the sleep-pain relationship by critically examining longitudinal and experimental studies, highlights the notion that sleep disturbance may better predict pain than pain predicts sleep disturbance (Finan et al., 2013). Indeed, longitudinal studies have remarkably shown that insomnia is an independent risk factor for the development of chronic pain following acute injury, as well as the spreading of pain from a regional disorder to a more widespread condition (Mikkelsson et al., 1999; Smith et al., 2008).

To eliminate the confounding effects of clinical pain disorders and their associated comorbidities, including anxiety (Zhuo, 2016), depression (Humo et al., 2020) and mood disorders (Campbell et al., 2017), experimental studies have used various sleep disruption models to mimic sleep disorders in order to ascertain the effect of sleep disruption on experimentally-induced pain in healthy individuals. A study using small electronic pulses (delivered through an electrode attached to the skin) to induce pain investigated the effect of 50 % sleep restriction (based on the subjects' habitual sleep duration) on 13 and 8 healthy females and males respectively, and reported increased pain sensitivity after two consecutive nights of restricted sleep (Matre et al., 2015). Another study on 18 healthy individuals (6 females, 12 males) investigating the effect of 4 hour sleep restriction over 12 days on pain

sensitivity, found that pain sensitivity and general bodily discomfort was 19 increased after 12 days of sleeping for only 4 hours per night (Haack et al., 2007).). Other studies using thermal, pin prick and pressure painful stimuli in healthy individuals (Faraut et al., 2015; Smith et al., 2007; Kundermann et al., 2004) support that sleep disruption (total sleep deprivation) has hyperalgesic effects.

Interestingly, a study that selectively restricted sleep (REM restriction and N3 restriction) in 9 healthy males, and assessed pain using thermal (heat) and a pressure stimuli, showed that mechanical pain threshold decreased after 2 consecutive nights of the selective sleep restriction (both REM sleep and N3 restriction). However, heat pain thresholds were not affected by either sleep restriction (Onen et al., 2001). This same study also investigated the effects of one night of total sleep deprivation (40 hours) on the same participants and reported decreased mechanical pain thresholds (Onen et al., 2001). Another study on 11 healthy young women, investigating the effect of sleep fragmentation on deep muscle and superficial (pin prick) pain perception, reported increased superficial and deep muscle pain sensitivity after one night of sleep fragmentation, and the hyperalgesic effects were accentuated after a second consecutive night of sleep fragmentation (Iacovides et al., 2017). Similarly, sleep fragmentation led to loss of pain inhibition (diffuse noxious inhibitory control) and an increase in spontaneous pain in healthy women after three consecutive nights (Smith et al., 2007).

A few studies studying the effects of sleep deprivation on pain perception have reported contradictory results, where the findings indicated no relationship between sleep deprivation and pain perception. One such study, mentioned above, demonstrated that sleep fragmentation has more impact on pain perception compared to partial sleep restriction where there were no significant changes in pain perception (Smith et al., 2007). Another, done on thirteen healthy participants (one female), investigating the effects of N3 restriction and REM

restriction reported significant changes only in pain perception in the SWS group and not the REM group (Maldofsky & Scarisbrick, 1976). Another study done on ten healthy men found that after 3 nights of N3 sleep restriction did not produce any changes in the pain perception in the jaw muscles (Arima et al., 2001).

Table 1.1 *A summary of all the papers used pages 27-29 on sleep restriction protocols and pain outcomes*.

Reference	**Participants**	**Sleep Restriction Protocol**	**Pain outcomes**
Matre et al., 2015	21 healthy participants (8 males, 13 females)	50% sleep restriction based on participant's sleep cycle -2 consecutive nights	Increased pain sensitivity after sleep restriction
Haack et al., 2007	18 healthy participants (12 males, 6 females)	4 hour sleep restriction for 12 days	Increased pain sensitivity and general body discomfort
Onen et al., 2001	9 healthy males	Selective (REM/N3) sleep restriction for 2 consecutive nights	-Decreased pressure/mechanical pain after both REM and N3 restrictions. -Heat pain not affected.
Onen et al., 2001	9 healthy males	Total sleep deprivation	Decreased pressure/mechanical pain after restriction
Iacovides et al., 2017	11 healthy males	Sleep fragmentation for 2 consecutive nights	Increased superficial and deep muscle pain
Smith et al., 2007	32 healthy females	Partial sleep loss (fragmented sleep) compared to 50% restricted sleep	Increased spontaneous pain after fragmented sleep. Sleep restriction had no effect on sleep.
Maldofsky and Scarisbrick, 1976	13 healthy participants (12 males, 1 female)	Selective (REM/N3) sleep restriction	Increased pain reported after N3 restriction. REM restriction had no effect.
Arima et al., 2001	10 healthy males	SWS sleep restriction for 3 nights	No changes in pain perception
Faraut et al., 2015		Total sleep deprivation for	Increased pain sensitivity
Kundermann et al., 2004	Review		

The exact physiological/biochemical mechanisms underlying the proposed bidirectional relationship between pain and sleep remain unclear. Some authors propose it to be as a result

of sleep and pain regulation using common pathways, possibly through either the mesencephalic periaqueductal grey or the thalamus or both (Smith & Haythornthwaite, 2004). A paper that reported that an injection of formalin into the tibialis anterior in cats caused both sleep disturbances and pain (Carli et al., 1987), hypothesized the results to be due to a common modulation pathway between sleep and pain, perhaps through serotonin and noradrenaline as they are integral in both pathways. A few studies have used opioids to attempt to demonstrate the existence of common pathways between sleep and pain regulation. One study found that sleep disruption and lack of continuity of sleep decreases the analgesic properties of the opioids (Ukponmwan et al., 1986), another reported a decreased production of opioids (Shapiro et al., 1981) and lastly another described decreased affinity and binding of opioids, thus increasing pain sensitivity (Fadda et al., 1991) In another study, dopamine (which is integral in the sleep-wake regulation and also in the ascending reticular pathway and parts of the raphe nuclei) has also been implicated as a possible common pathway by which sleep and pain interact (Finan et al., 2013).

Recovery Sleep

Given the detrimental effect of sleep restriction on various body systems, researchers have recently become interested in the idea of whether recovery sleep following sleep deprivation can reverse any detrimental effects. Recovery sleep may take on various forms, including extended sleep time following sleep loss (either the next night, or sleep-ins on the weekends following sleep restriction during the week), or naps (short daytime sleeps) (Vladyslav, 2015).

'Banking Sleep'

The concept of "banking sleep", refers to extending the sleep period prior to sleep deprivation. Interestingly, one study on alertness investigated whether sleeping longer the night before the sleep restriction night ('banking sleep') would have the same effect as taking a nap after the sleep restriction night on cognitive performance as measured by PVT (Psychomotor Vigilance Test). They reported that banking sleep was beneficial in building resilience to the effects of sleep restriction and in facilitating recovery after the sleep restriction (Rupp et al., 2009). Another more recent study investigated the effect of 'banked' sleep on MSLT (Multiple Sleep Latency Test) (measures sleep latency) and PVT (measures cognitive performance) after a night of total sleep deprivation. In this crossover study, the 14 male participants either slept for 6 nights extended (EXT) or 6 nights habitual (HAB), after which they had a night of total sleep deprivation and subsequent recovery (Arnal et al., 2015). The authors reported that the extended nights were able to shield the participants from bad MSLT and PVT performances and allowed them to recover faster as compared to when they did the HAB protocol (Arnal et al., 2015). Furthermore, a military study investigated the effects of banking sleep in the field. They allowed platoon members (soldiers) a week of extended sleep (8.9 hours instead of their habitual 5-6 hours of sleep per night) after which

they had a training test to assess improvements. Compared to the results they had the year before, the test scores improved from '756 out of a possible 1,000' to '919 out of 1,000' points after sleep banking (Thompson et al., 2017).

Recovery Sleep after Restriction or Deprivation

Recovery sleep in the form of extended sleep following sleep loss has been shown to reverse several negative physiological effects induced by sleep deprivation. One study demonstrated that following four consecutive nights of sleep restriction (allowed 4.5 h in bed), two consecutive nights of recovery (with 12 h in bed on the first night and 10 h in bed on the second night) restored sleep-loss-induced insulin sensitivity (Broussard et al., 2016). More specifically, compared with normal sleep, following the four nights of sleep restriction, insulin sensitivity was reduced by 23% and disposition index (which is the insulin sensitivity x amount of insulin released into the blood) was reduced by 16%, indicating increased risk of diabetes. However, both measures were reverted to normal following sleep recovery (Boussard et al, 2016). A study investigated cardiovascular risk, using heart rate variability (HRV) and endothelial function, after 30.5h sleep deprivation and subsequently recovery sleep on 11 shift workers and 13 controls (Wehrens et al., 2012). The authors reported that there was a higher HRV (Heart Rate Variability; which indicates higher cardiovascular risk) and sympathetic activation after total sleep deprivation, which declined after recovery sleep (4 hour recovery nap immediately followed by a full night of sleep) (Wehrens et al., 2012). The study also showed that the risk of cardiovascular events was greater in the shift workers compared to the controls, probably due to a misaligned circadian rhythm (Wehrens et al., 2012), thus highlighting the importance of not only sleeping the required amounts but also in alignment with the natural circadian rhythm. A study on fifteen healthy participants investigating the effects of 24h sleep deprivation on muscle pain sensitivity (in craniofacial muscles) reported higher sensitivity after the sleep deprivation and that this sensitivity was

restored to baseline levels after recovery sleep (Kristiansen et al., 2017). Another study which reported loss of pain inhibition and an increase in spontaneous pain in healthy women following sleep fragmentation, also remarkably reported that the hyperalgesia was reversed after recovery sleep (Smith et al., 2007). Similarly, another study reported that recovery sleep, following sleep deprivation, generates an analgesic effect that is comparable to that achieved using level 1 (WHO) analgesic drugs, such as acetaminophen or NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) in healthy subjects (Onen et al., 2001).

Short Daytime Naps

Sleep recovery in the form of extended sleep before or following sleep loss is not always possible. Hence, naps (short daytime sleeps) are another, potentially more practical, form of sleep recovery. Indeed, naps are recommended for the management of disrupted sleep and have been shown to combat many of the negative effects of sleep deprivation. There are data showing nap-induced improvements in memory, cognitive function, reaction times, alertness and mood (Milner& Cote, 2009; Rupp et al., 2009). However, little is known about the potential restorative effect that napping may have on pain. To our knowledge, only one study to-date has investigated the interaction between napping and pain perception, following one night of severe sleep restriction (only 2hrs of sleep) (Faraut et al., 2015). The study found that short sleeps following a night of sleep restriction, in particular, two 30-minute naps (one in the morning and one on the afternoon), can reverse sleep-restriction-induced increases in mechanical and thermal (heat) pain sensitivity in healthy males (Faraut et al., 2015).

Given the practical application of recovery sleep in the form of short daytime sleep (naps), it is insightful to know what length of time, or time of day, of the naps will offer maximum physiological restoration. Such details remain unclear, as research in this field is limited. One investigated cognitive performance in 24 participants who slept for only 5 hours for one night

and then napped the next afternoon. The study found that, although both the 10-minute and 30-minute naps had produced full recovery in terms of subjective sleepiness, fatigue and cognitive performance; ultimately, the authors concluded that 10-minute naps were the more beneficial because the effects were more immediate (occurred sooner) compared to those of the 30-minute nap due to less sleep inertia (impaired cognitive and motor performance immediately after wake) (Brooks & Lack, 2006).

The length of recovery sleep will arguably determine the order and time spent in the various sleep stages as well as the sleep stage one would be awakened from. However, the mechanisms underlying how sleep recovery may improve physiological function, and hence which sleep stage plays the biggest role in this recuperative function, also remains largely unclear. A cross-over study using 10 healthy participants who were allowed to nap for either only 3 minutes (N1), or woken up 5 minutes after first spindle or k-complex (N2), reported that a nap with stage N2 decreased fatigue and improved performance whilst those who only had stage N1 sleep did not differ much from those who had not taken the nap (Hayashi et al., 2005). Similarly, a study investigating sleep spindles (which occur in N2) and their role in memory consolidation on 24 healthy volunteers, reported that more spindle activity and was correlated to better performance on recall of 160 words learnt the previous night (Schabus et al., 2004). However, it is not known whether it is the sleep stage or the length of the nap that is responsible for the observed improvements. It is also possible that the interaction of both time spent napping and the sleep stage reached (or time spent in different sleep stages) during the nap may affect the outcome, and may have different effects on various outcomes tested.

Figure 1.3 summarizes the bi-directional relationship between sleep and pain as studied above. It also leads into the aims and objectives of the study, investigating whether afternoon

recovery sleep in the form of short naps would decrease pain sensitivity following sleep restriction.

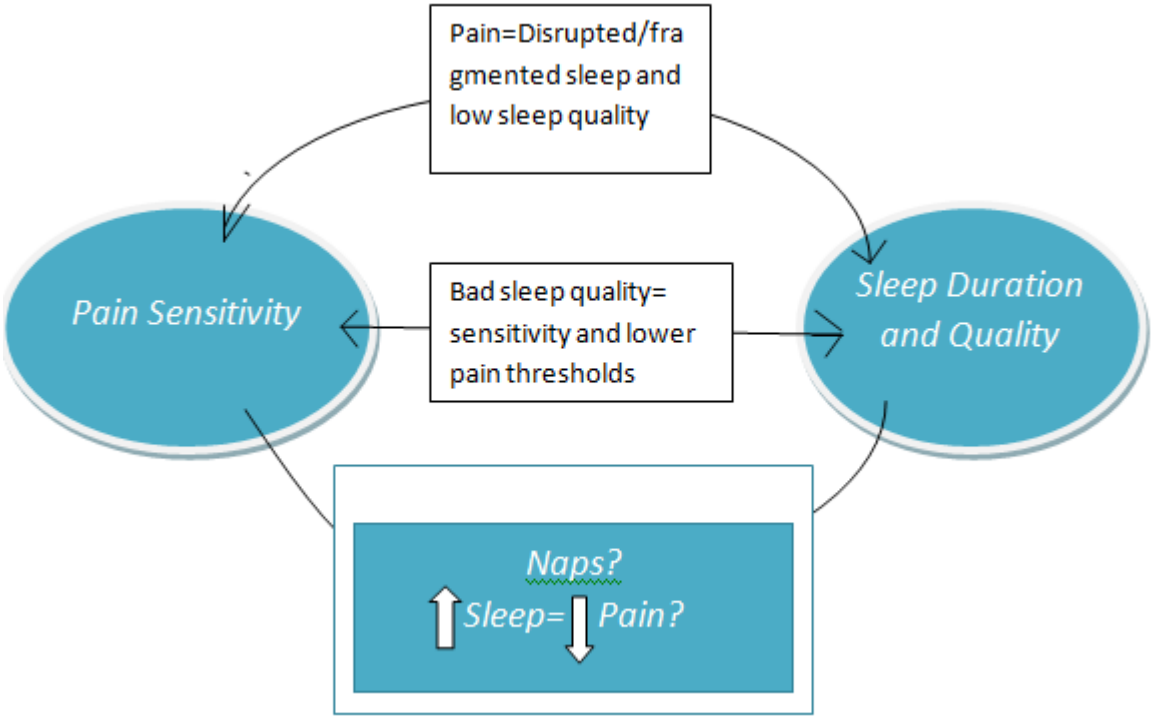

Figure 1.4 Describing the relationship between sleep and pain discussed above, as well as the role that afternoon naps might play in mitigating the vicious cycle between sleep and pain.

Aim

The aim of study was to investigate the effects of sleep restriction on the perception of experimentally-induced pain in healthy young males. Additionally, following a night of sleep restriction, I aimed to determine the effect of recovery sleep, in the form of a short afternoon nap, on the perception of experimental pain.

Objectives

In a randomised order, I used two sleep restriction protocols: restricting the first four hours of sleep (SR-F4H) and restricting the last four hours of sleep (SR-L4H) during an eight-hour night. Using both sleep restriction protocols in healthy, young adult male participants, I aimed to assess:

1. The effect of sleep restriction on sleep architecture during the sleep episode and during the afternoon nap
2. whether there was a difference in pain perception of ischaemia between the two sleep restriction protocols.
3. whether any of the changes in sleep architecture had a direct effect on and a relationship with pain perception
4. whether recovery sleep (following sleep restriction), in the form of a 30-minute afternoon nap had an effect on experimental ischaemia.
5. Whether the naps' sleep architecture had an effect on the subsequent pain perception.

Hypotheses

1. I expected that the nights of four-hour sleep restriction would increase pain perception in the participants.

2. I expected the afternoon nap to bring the variables closer to baseline readings, assuming that pain scores increased following sleep restriction.

CHAPTER 2

Methods

Ethics approval

This study was approved by the Human Research Ethics Committee (HREC) at the University of the Witwatersrand (Clearance number M170283) and conducted at the University of the Witwatersrand's Sleep Laboratory. I explained all procedures to the participants and they signed their informed consent form at the time of recruitment before starting the screening phase.

Participant recruitment

I recruited healthy men aged between 19-23 using flyers from the University of the Witwatersrand and the University of Johannesburg in Johannesburg, South Africa.

Screening Phase

After the recruitment, the participants were screened using 2 questionnaires assessing their sleep quality and general health; and a monitoring of habitual wake times and bedtimes 7 days prior to final enrolment using a sleep diary and rest-activity recording using an Actiwatch Spectrum or an Actiwatch 2 (Sleep; Mini Mitter Division of Respironics Inc., Bend, OR, USA).

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Screening Tools

General Health Questionnaire

This questionnaire (GHQ) was used to assess general psychological status in the participants. Participants had to score less than 6 in the questionnaire, indicating healthy psychological status (Goldberg et al., 1976).

Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index is a validated questionnaire (Buysee et al., 1989) for assessing sleep quality and is commonly used as a screening tool. The PSQI is made of 19 questions which contribute to 7 components (sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, daytime consequences, and sleep medications). It is validated in English speaking populations and in South Africa in people using English as a second language (Redman et al , 2018).

Body Mass Index (BMI)

I assessed baseline BMI as part of the screening procedure to ensure that the participants had a healthy BMI (between 18.5 and 25 $kg.m^{-2}$). For one participant whose BMI was 32, I checked the Berlin Questionnaire to ensure they were not at high risk of sleep apnoea. This participant scored low risk of sleep apnoea on the Berlin Questionnaire (Chung et al., 2008) and was therefore included in the study. Additionally, we tried to maintain the BMI throughout the study by serving standard breakfast and lunch meals and asking the participants to continue their normal eating and activity levels.

Actiwatch and Sleep Diaries

Before starting the laboratory component of the study, the participants were asked to select a preferred wake time and bed time (8h apart). They were then asked to use these times to go to bed and wake up on the week preceding the first in-laboratory experimental condition, the adaptation night. If the participant was not able to stick to those self-selected bedtimes and wake times (within 1h of the selected time), I excluded them from the study.

Actigraphy is used to monitor activity with the purpose of making sure the participants do not stray from their prescribed sleep and wake times (Ancoli-Israel et al, 2003). In this study, we asked our participants to wear an Actiwatch 2 (Sleep; Mini Mitter Division of Respironics Inc., Bend, OR, USA) during the screening, and later in the in-laboratory experimental conditions to monitor whether the participants did keep to those regular wake times and bedtimes in the days separating the different experimental conditions of the study. Sleep diaries have also been widely used for the same purposes as the actiwatch (Matre et al., 2015; Brondel et al., 2010, Brooks & Lack, 2006). The interpretation of rest-activity cycles recorded by actigraphy is indeed limited by the fact that it underestimates wake (for example when a person is sitting quietly watching television in the evening), however it is able to give objective sleep measures such as sleep onset latency, number of arousals and wake after sleep onset (WASO). A sleep diary allows participants to subjectively indicate their bedtimes, wake times and number of conscious arousals during the sleep episode (which then can be checked against actigraphy data).

Together, the actiwatch and sleep diary gave a full picture of the participants' nights, in particular whether they were able to respect the selected 8h bedtimes and wake times within

1h. If a participant was not able to respect their self selected regular bedtimes and wake times during the 7-day screening period, they were excluded from the study.

Because the actigraphy recordings and the sleep diaries were only used for my research project to ascertain regularity of the sleep-wake schedules throughout the study, I will not show analyses associated with those measures in this dissertation.

Using the actigraphy data to confirm the bedtimes and wake times from the sleep diary, I subsequently used the seven bedtimes recorded in the sleep diary (and checked against actigraphy data) to calculate each participant's average bedtime. I also used the seven wake times recorded in the sleep diary to calculate each participant's average wake time.

Those average bedtimes and wake times were used throughout the study for the participant's adaptation and baseline bedtime and wake time. They were also used for the sleep restriction night with the bedtime of the conditions where their sleep was restricted the last four hours of the night corresponding to habitual bedtime; and the wake time of the conditions where their sleep was restricted the first four hours of the night corresponding to their habitual wake time (see the details in the paragraphs below).

Experimental Phase

After screening, the subjects received a date as to when they would be expected back at the sleep lab in order to start with the study. On the given date, they had their one night of adaptation at the lab, basically getting familiar with sleeping in the lab environment with electrodes on. There was then a 3 day (minimum) break after the adaptation night after which the participants had to come back for one of the remaining 5 experimental conditions (which were in random order). Each participant was housed in an individual suite without windows and lights were turned off to start each overnight experimental condition and nap. The light

exposure levels in the suites are below 0.1 lux when the lights are switched off. The laboratory is sound insulated and once the doors of the suites are closed, the participants' rooms are quiet also during the day.

The experimental nights included:

Baseline night- On this night the subjects slept for a full night (8 hours) in the sleep laboratory, without any restriction. The bedtime and wake times were determined during the screening period as described in section above. The day following that night they did all the tests that they would do on the experimental nights in order to obtain baseline readings.

Sleep restriction nights-

There were four sleep restriction nights:

1) SR-L4HNN: sleep restriction (forced wakefulness) last four hours of the night, no nap the following day

2) SR- L4HN: sleep restriction (forced wakefulness) last four hours of the night, with a nap the following day

3) SR-F4HNN: sleep restriction (forced wakefulness) first four hours of the night, no nap the following day

4) SR-F4HN: sleep restriction (forced wakefulness) first four hours of the night, with a nap the following day

- For these sleep restriction nights, the participants only slept for four of the eight hours. They either slept for the first four hours or the last four hours of their habitual 8h-sleep episode. For example, a participant who normally slept from 10pm until 6am (full hours), on the restriction nights would sleep either between 10pm and 2am (corresponding to the conditions where they were sleep restricted the last 4 hours of the night, SR-L4HNN and SR-L4HN) or between 02:00 am and 06:00 am (corresponding to the conditions where they were sleep

restricted the first 4 hours of the night, SR-F4HNN and SR-F4HN). After their habitual waking time (06:00 am) the participant would undergo the ischaemic pain test evaluation about an hour after their wake time, have breakfast approximately an hour and half after their wake time, and be free to leave. They would have to come back 6 hours after their habitual waking time (12:00 pm) for afternoon procedures.

Afternoon procedures:

In the afternoon, following Baseline and the restriction nights they would either have a 30 minute nap (on nap days) or lie down in a dark room (on no nap days) for 30 minutes. After the 30 minutes, they would then undergo the same procedures as in the mornings. The afternoon nap/lie in always took place 6 hours after the habitual wake time. The afternoon pain procedures would also take place approximately an hour after waking from the nap/lie in.

Nap procedure:

The participants returned 6 hours after their habitual wake time after the SR-F4HN and SR-L4HN experimental nights. After preparing the participants for polysomnography, they were asked to go into their suite and try to nap for 30 minutes. Lights were switched off and therefore light levels were below 0.1 lux. I monitored the start of the nap which was the first epoch where there was a spindle. I chose N2 sleep as a marker of the first epoch of sleep as during nap protocols, participants tend to cycle between wake and N1 several times before starting their actual sleep. Therefore the nap lasted 30 minutes from the first epoch of N2 determined by the visualization of the first spindle.

Lie-in procedure:

The participants came 6 hours after their habitual wake time after the SR-F4HNN and SR-L4HNN experimental nights. After preparing the participants for polysomnography, they were asked to go into their suite and try to nap for 30 minutes. Lights were switched off. Even though we asked the participants not to fall asleep, I did monitor whether they did by checking the live polysomnography recording. I went inside the suite when they were falling asleep to keep them awake. Figure 2.1 shows the overall structure of the protocol.

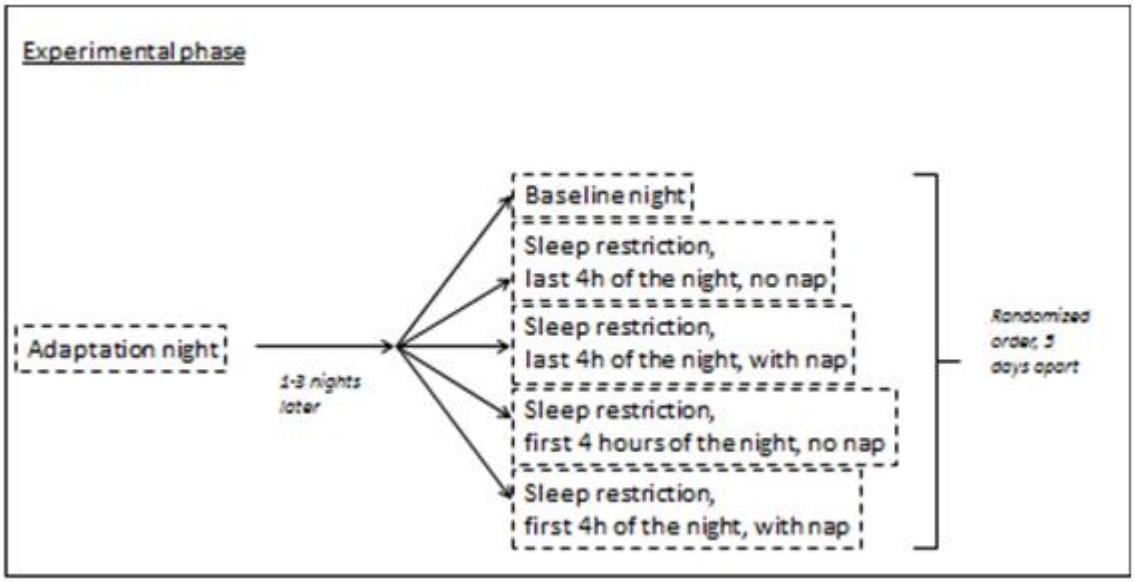


Figure 2.1 The structure of the study protocol.

Randomization Method

I used an online randomizing algorithm (https://www.randomizer.org). I numbered the conditions 1-5 (1 Baseline, 2 SR-F4HN, 3 SR-F4HNN, 4 SR-L4HN and 5 SR-L4HNN). The algorithm then computed 20 different combinations of these numbers (conditions) and the night sequence that the participant's would follow would be based on those random computations. For example, participant 1's night sequence was 1, 2, 3, 4, 5 and participant 2's sequence was 1, 3, 2, 5, and 4. Table 2.1 shows the order of nights for the 11 participants who completed the study. Lastly, Figure 2.2 Shows the structure of the nights, with shaded

block representing sleep (night and nap) and the non-shaded blocks representing awake lie-in periods (sleep restriction nights and no-nap afternoons).

Table 2.1 *The randomized order of the conditions that each of the participants did (N=11).*

	Order of condition				
Participant	1	2	3	4	5
1	Baseline	SR-L4HNN	SR-L4HN	SR-F4HN	SR-F4HNN
2	SR-L4HNN	SR-F4HNN	Baseline	SR-F4HN	SR-L4HN
3	Baseline	SR-L4HN	SR-L4HNN	SR-F4HN	SR-F4HNN
4	SR-L4HNN	SR-F4HNN	Baseline	SR-L4HN	SR-F4HN
5	Baseline	SR-F4HNN	SR-F4HN	SR-L4HNN	SR-L4HN
6	SR-F4HNN	SR-L4HNN	SR-L4HN	Baseline	SR-F4HN
7	SR-F4HNN	SR-F4HN	SR-L4HN	Baseline	SR-L4HNN
8	SR-L4HN	Baseline	SR-L4HNN	SR-F4HNN	SR-F4HN
9	Baseline	SR-L4HNN	SR-F4HNN	SR-F4HN	SR-L4HN
10	SR-F4HNN	SR-L4HNN	SR-F4HN	SR-L4HN	Baseline
11	Baseline	SR-L4HNN	SR-L4HN	SR-F4HN	SR-F4HNN

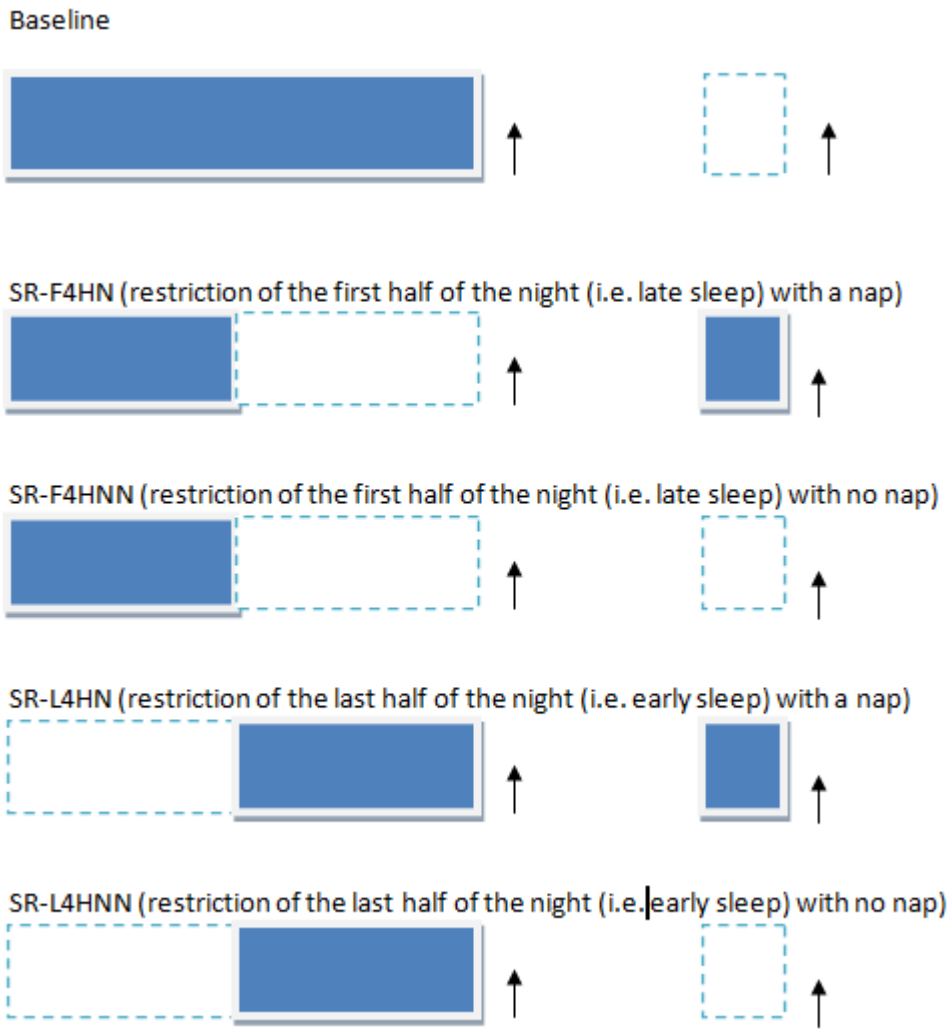


Figure 2.2 Showing the sleep (shaded) and wake (not shaded) periods, the naps (shaded) and lie-ins (not shaded). The arrows represent the pain tests.

Experimental Period Tools

Polysomnography

I used the 10-20 system to determine electrode placement. In this study, I recorded C3, C4, the oculogram, and the chin electromyogram. All even numbered electrodes were referenced to A1 and all odd numbered electrodes were referenced to A2. Chin electrodes were referenced to each other. For all scalp derivations, the high pass filter was set at 35 Hz while

the low pass filter was set at 0.5 Hz. All the input was recorded on a computerized system (CadwellEasy EEG, version 2.0.2, Cadwell Laboratories Inc, Kennewick WA) which has a 200/second (cycles per second) sampling rate and scored using the Rechtschaffen and Kales scoring manual (Rechtschaffen and Kales, 1968) and the updated rules from the AASM (Iber et al., 2007). The sleep records were then scored by myself and a sleep scoring expert outside of the study who had not been a part of, or around the laboratory, during the data collection phase. The scoring was checked for 80% agreement before being admitted into the analyses.

Sleep variables

For the sleep restriction experimental nights, I realized during scoring that when I had to leave the suite during the sleep restriction times, the participants sometimes fell asleep/ dozed off during that time. Since my study had an explicit aim to test the global effect of total sleep duration but also of specific sleep stages on ischaemic pain perception, I exported the sleep restriction nights twice:

The first export used actual LIGHTS OFF and LIGHTS ON used during the sleep restriction nights, based on a 4h-sleep export. From that export, I extracted sleep onset latency for the SR-F4HNN/SR-F4HN and the wake after sleep onset for the SR-L4HNN /SR-L4HN. Even though the participants may have had episodes of dosing during the time I was trying to maintain them awake the first 4h of the night in the SR-F4HN/SR-F4HNN, I still wanted to gauge the actual time it took them to fall asleep (sleep onset latency) once I had actually switched the lights off. Therefore I used the **Sleep Onset Latency (SOL)** from those 4h-sleep exports. Similarly, although the participants may have had episodes of dosing during the time I was trying to keep them awake the last 4h of the night in the SR-L4HN/SR-L4HNN

conditions; I still wanted to gauge the actual **wake after sleep onset (WASO)** before I had actually switched the lights ON. Therefore I extracted WASO from those 4h-sleep exports.

The second export used the lights OFF time and the light ON time close to that of the baseline night, allowing me to score those dosing episodes which occurred during the time when they were supposed to be awake. This way, I was able to have a true reflection of the actual amount of sleep and stages of sleep that had occurred before the pain testing performed the following day.

From this 8h-sleep export, I extracted therefore all the other sleep variables:

- Time in bed (TiB, in minutes, defined as time elapsed between Lights OFF and Lights ON)
- Total sleep time (TST, in minutes, defined as total amount of time spent asleep)
- Sleep Efficiency (SE, (total sleep time/time in bed) x100, in percent)
- Non Rapid Eye Movement Sleep stages 1, 2 and 3 (N1, N2, N3, minutes and percentage of total sleep time)
- Rapid Eye Movement Sleep (REM, minutes and percentage of total sleep time)
- Number of arousals per hour (Arousal Index).

Pain Assessments

Ischaemic Pain

Ischeamia is a clinically relevant model of pain and mimics a variety of conditions such as angina (Deedwania & Carbajal, 1990), fibromyalgia (Bongi et al., 2015), dysmenorrhoea (Liedman et al., 2006) and complex regional pain syndrome (CRPS) (Millecamps & Coderre, 2008). The standardized submaximal effort tourniquet test was used to induce forearm ischaemia in the participant's randomly chosen arm (the arm was chosen by the researcher).

A tourniquet cuff (200mmHg) was positioned above the cubital fossa (elbow level) and inflated to occlude blood flow. The participants then had to do 20 hand grip exercises at 30% of their hand grip strength using a hand-grip dynamometer. Each squeeze lasted for two seconds, with a two second rest in between. After the 20 hand-grip exercises, the cuff remained inflated for 10 minutes and the participants were required to rate their pain intensity on a 100mm visual analogue scale ranging from 'no pain' to 'worst pain ever felt' every minute for the 10 minute duration.

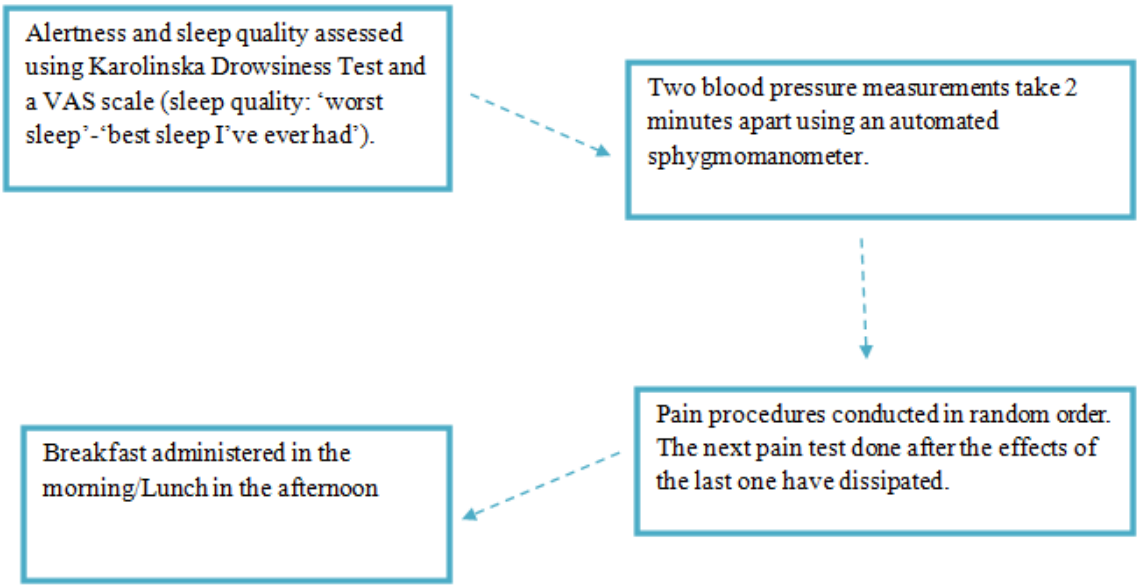


Figure 2.3 Flow diagram showing all procedures carried out in the mornings after wake and in the afternoons after the nap or lie in conditions. For this dissertation, I will only show the results from the ischaemic pain procedure

Statistical Analysis

Comparison of the sleep architecture between the different experimental night conditions

I first tested all continuous sleep variables recorded during the nights (Baseline, SR-F4H and SR-L4H) for normality (Proc Univariate in SAS 9.4®). All the variables (N1, N2, N3, REM, TST, Sleep Efficiency, WASO, Sleep Latency and Arousal Index) were normally distributed. As explained in the Methods section above, most of the variables (N1, N2, N3, REM, TST, TiB, Sleep Efficiency and Arousal Index) were better represented using the 8H reports as they represented the minutes and percentages from the whole 8h sleep episode. However some variables (Wake after Sleep Onset (WASO) and Sleep Onset Latency) were better represented using the 4h sleep reports which had the experimental lights OFF and lights ON. Therefore, I extracted Sleep Onset Latency and WASO from the 4h reports and all other sleep variables from the 8h report

I hypothesized that the sleep architecture of the two SR-F4H experimental nights (SR-F4HN and SR-F4HNN) would not be significantly different; I also hypothesized that the sleep architecture of the two SR-L4H experimental nights (SR-L4HN and SR-L4HNN) would not be significantly different. To test this hypothesis, I used paired t-tests on all sleep variables to investigate whether there were any differences in sleep architecture between SR-F4HN and SR-F4HNN. I also used paired t-tests on all sleep variables to investigate whether there were any differences in sleep architecture between SR-L4HN and SR-L4HNN. There were no significant differences between SR-F4HNN and SR-F4HN (see Table 3.2.1 in Results) and the SR-F4HNN and SR-F4HN nights were therefore collapsed into one SR-F4H condition. There were also no significant differences in sleep architecture composition between SR-L4HNN and SR-L4HN (See Table 3.2.2 in Results) and therefore the SR-L4HNN and SR-L4HN nights were collapsed into one SR-L4H for the analyses. I then used mixed model

analysis, PROC MIXED in SAS 9.4® to compare sleep variables between the Baseline, SR-F4H and SR-L4H experimental conditions. The mixed models analysis takes into account both fixed and random effects in longitudinal analyses, it accounts for repeated measures by specifying a specific covariance/correlation structure to model the repeated measures within the same individual. In the mixed model analysis, I specified a restricted maximum likelihood approach, REML, a CLASS statement for all categorical variables and a RANDOM intercept. I did not specify a random slope as this led to non-convergence issues of the models. For the structure of the covariance/correlation matrix, I specified 'compound symmetry' most of the time but a few times had to choose 'unstructured' due to non-convergence issues in the model. I sometimes had missing data in one participant in one condition due to an unreadable sleep record (See Tables 2.2 and 2.3 below). Those missing data were missing due to technical issues, with either a ground electrode being lost early in the sleep episode, or connectivity issues within the electrode plugging box, and not due to an intrinsic characteristic of the participant which made me lose their data. The mixed models analysis is able to run the analysis even with those missing data, as long as it is missing at random (Fitzmaurice et al., 2011). Descriptive statistics on all sleep variables are presented as Median [Q1-Q3]. I ran a simple mixed model with just the main effect of COND (as Baseline, SR-F4H, and SR-L4H). If I found a significant main effect of COND, I then ran post-hoc analyses with a Tukey adjustment for multiple comparisons to compare Baseline to SR-F4H, SR-F4H to SR-L4H and Baseline of SR-L4H (LSMEANS statement on the main effect of COND, with an adjust = Tukey specification in PROC MIXED). Significance was determined by p values lower than 0.05.

Table 2.2 *The condition with missing information due to unreadable sleep reports from the night polysomnography.*

	Condition with missing/unreadable data				
Participant	Baseline	SR-F4HN	SR-F4HNN	SR-L4HN	SR-L4HNN
1					
2					
3		X			
4				X	
5		X	X		
6	X				
7					
8					X
9					
10	X			X	
11		X			

Table 2.3 *Missing data per participant due to unreadable sleep reports from the 30-minute afternoon nap polysomnography.*

	Condition with missing/unreadable data	
Participant	SR-F4HN	SR-L4HN
1		
2		
3		
4		
5		X
6		
7		
8		
9		

10	X
11	

Naps Analyses

I investigated the sleep architecture of the naps after the SR-F4H and SR-L4H experimental nights. I used paired t-tests to determine differences in sleep architecture between the nap which occurred after the SR-F4H experimental condition and the nap which occurred after the SR-L4H experimental condition.

Pain Analyses

Comparison of pain perception in the morning following the two identical experimental conditions, SR-L4HNN and SR-L4HN, then SR-F4HNN and SR-F4HN

I tested VAS for normality using both traditional normality tests (Shapiro Wilkes, Kolmogorov Smirnov etc) and also just checking for normality through median ~ mean, skew and kurtosis diagnostics. Although VAS did not pass the traditional normality tests, the mean and median were close to each other (47.3 and 49 respectively) while both the skew and kurtosis were between -1 and 1 (0.016 and -0.87, respectively). I therefore estimated the VAS distribution to be meeting the criteria for the normal distribution. I hypothesized that pain perception would not be statistically significantly different between the pain perception measured in the morning after the same types of sleep restriction; ie I hypothesized that pain perception measured in the morning would not be statistically different between SR-F4HN and SR-F4HNN, or between SR-L4HN and SR-L4HNN, respectively. To test those hypotheses, I first determined the average pain score for each participant on the morning of each of those conditions (SR-F4HN, SR-F4HNN, SR-L4HN and SR-L4HNN). I used the trapezoid method to calculate the Area Under the Curve (AUC) for each participant in each

condition in the morning. I also calculated the peak pain as well as pain at the halfway point (50% pain; 5 minutes into the 10-minute pain test). I then used paired t-tests to compare the average pain scores, peak pain, 50% pain and the AUCs (area under the curve) in the morning tests to test for differences between the SR-F4HN vs SR-F4HNN and SR-L4HN vs SR-L4HNN, respectively (see Table 3.4.1 in Results). There were no significant differences between the pain scores in the morning reported for the two first 4 hour restriction nights (SR-F4HN and SR-F4HNN) so I collapsed/averaged them together to make one 'SR-F4H' condition (See Table 3.4.1 in Results section). The same was true for the two last 4 hour restriction nights (SR-L4HN and SR-L4HNN) so they were collapsed/averaged to make one single 'SR-L4H' condition. Similarly to the comparison of sleep architecture between the different experimental conditions, I therefore used the variable COND, which took the values of Baseline, SR-F4H and SR-L4H in the univariate and then multivariable analysis with pain perception as the outcome variable.

Univariate analyses

I identified 5 possible variables associated with pain:

1) Time elapsed during the ischaemic pain test (in minutes, as I evaluated their pain perception every minute for 10 minutes). I labelled this variable TIME.

2) Order in which the different conditions had been randomized to. This variable was labeled ORDER.

3) Experimental night condition, labeled COND, taking the values Baseline, SR-F4H and SR-L4H

4) Time of Day the ischaemic pain test was run at, a variable labeled TOD: either morning (AM) or afternoon (PM).

5) the presence of a nap in the **afternoon** or not, a variable labeled NAP: it took the value 1 if there had been a nap before the pain test, it took the value 0 if there had been no nap before the pain test (ie all morning tests were associated with a NAP=0 , the afternoon lie-ins were associated with NAP=0 and the afternoon naps were associated with NAP=1).

Parsimony helps with the stability of a model so I first ran univariate analyses on each of those variables separately. Again I used mixed model analysis with the same specifications as described above. The outcome variable was VAS, the measurement of pain each minute during the ischaemic pain test. I present the unadjusted estimates and standard errors for each independent variable.

The mixed model would then include only the variables that were found to be significant in the univariate analyses. Once I had run those univariate analyses, I selected the significant variables from the univariate analysis results; and any other variable of interest (such as NAP) for my multivariable analysis. My outcome variable was VAS again. My independent variables were TIME, COND, TOD, ORDER and NAP. I present the adjusted estimates and standard errors for each variable in the model.

Sleep variables and pain

To better understand the effect of condition on pain perception as measured by VAS during the ischaemic pain tests, I ran univariate analyses of the individual sleep variables with pain, and each were significantly related to pain in the individual analyses. The sleep variables were run individually to avoid multicollinearity. I then proceeded to investigate the adjusted association of the sleep variables with pain perception using mixed models including all the significant variables from the first multivariable analysis. By design the sleep variables changed according to COND (Baseline, SR-F4H and SR-L4H). When testing in simple two-variable models the association between any sleep variable and COND, I found that they

were collinear with each other therefore I removed COND from the final models with each sleep variable. The results are presented as Median [Q_1-Q_3].

Thus the pain models with each sleep variable included *time* (vas measurements every minute (0-10)), *ToD* (AM or PM), *order* (the randomized succession of the nights) and *naps* (nap or no-nap afternoon). I then ran the sleep variables (*Awake, N1, N2, N3, REM, TST, Sleep Efficiency, WASO, Sleep Latency and Arousal Index*) in this model. The results are presented as an estimate. A negative estimate signifies a negative relationship to pain, where increases (minutes/%) in the independent variable studied result in decreases in pain sensitivity (dependent variable) perceived by the participants. Likewise, a positive estimate signifies a positive relationship with pain, where increases in the independent variable (minutes/%) results in increased pain perceived by the participants. Significance is classified by a p value less than 0.05.

From those pain models with each sleep variable, I identified the sleep variables which were significantly associated with pain perception in these adjusted analyses. I then created new models where those identified sleep variables were then run together in order to investigate which of them was the most important predictor of pain. I also ran a Pearson Correlation between all the significant sleep variables ($p < 0.05$) to understand how a modification in a sleep variable changed the other sleep variable.

Naps and pain perception

I also investigated whether there were any relationships with pain perception following the afternoon nap/lie in.

I used mixed models to investigate relationships with pain, as well as a Pearson Correlation to investigate the correlations between the significant sleep variables. Results are presented as Median [Q1-Q3]. Significance was determined by p values lower than 0.05.

I used mixed models to further investigate the role of each sleep stage during the afternoon nap in predicting pain perception in the pain tests that follow. I ran mixed model analyses, adjusted for 'time', on N2 and N3, and 'TimetoNT' (a variable that represented the time between when the participants had been awakened from their experimental night condition and the lights off (L_{off}) of the afternoon nap).

Lastly, I wanted to know if the sleep stage that the participants were awoken in from the nap had any effects on the pain perception that came after. I ran t-tests and a chi-square to compare the two SR-L4H and SR-F4H groups and the frequency of being awakened from the N2 and N3 sleeping stages.

Main effects studied during this analysis are represented in capitalized letter and are detailed below:

- The variable CONDITION: this variable took 5 values: Baseline, SR-F4HNN, SR-F4HN, SR-L4HNN and SR-L4HN. I used this variable in the final model investigating the effects of my intervention on overall perception of pain.
- The variable COND: I hypothesized there would be no difference in the sleep architectures of a) SR-F4HNN and SR-F4HN, and b) SR-L4HNN and SR-L4HN, respectively. I also hypothesized there would be no difference in the **morning pain** of SR-F4HNN and SR-F4HN and SR-L4HNN and SR-L4HN, respectively. Both hypotheses were subsequently confirmed (see results section). Therefore, for the descriptive statistics and the analysis of sleep architecture differences, and for the descriptive statistics and the analysis of **morning pain** differences during the different conditions, I collapsed SR-F4HNN and SR-F4HN into one unique condition, SR-F4H and I collapsed SR-L4HNN and SR-L4HN into one unique condition, SR-L4H. I could not do this for the afternoon pain as we had the nap and the lie-in to contrast.
- The variable ORDER: this variable reflected the order in which each condition had been done for each participant. I had 5 randomized order conditions: SR-F4HNN, SR-F4HN, SR-L4HNN, SR-L4HN and Baseline. Those conditions could be in any order as long as it did not repeat the previous participant's randomized order. For example: SR-F4HN= 1st condition completed by the participant; SR-L4HNN= 2nd condition completed by the participant; Baseline = 3rd condition completed by the participant; SR-F4HNN= 4th condition and SR-L4HN= 5th condition completed by the participant. Therefore ORDER could take 5 values: 1, 2, 3, 4 and 5. I hypothesized that due to the habituation of the participants to pain, they may be less sensitive to the pain test across time and therefore used ORDER as a linear variable (ie it was not

included in the CLASS statement in Proc Mixed, a statement for categorical variables).

- The variable TIME: as described before, the variable TIME took the values 0 to 10 in increments of 1, reflecting the time at which I asked the participant to evaluate their pain on the VAS Scale during the ischaemic pain test. Again I assumed a linear effect of time on pain perception and used TIME as a linear variable in the model.
- The variable time of day (ToD): I tested pain the morning and in the afternoon after each experimental night condition therefore the variable ToD took 2 values: AM and PM.
- The variable NAP: this variable took the value 0 when there was no nap and the value 1 when there was a nap. Hence, for all **morning** pain measurements, NAP took the value 0. For all pain measurements after **afternoon lie-ins**, NAP also took the value 0. For all pain measurements after **afternoon naps**, NAP took the value 1.
- Finally, to better tease the role of N3 on pain sensitivity in my analysis of the naps, whether it was a direct role or a reflection of the increased homeostatic sleep pressure build-up before the nap, I also created a variable "TimetoWT' to represent time elapsed between wake time from the main sleep episode (time of lights ON) and nap time (time of lights OFF for the nap).

CHAPTER 3

Results

Participant characteristics

A total of 25 male participants volunteered and were screened for the study. The screening consisted of males between the ages of 19 and 23. Of the 25, only 12 completed the study. Three of the volunteers only did one night of the study before they dropped out. The remaining 10 volunteers dropped out and were excluded during the screening phase either due to inability to adhere to the regular sleep wake schedule requirement of the study, or their busy schedules. Of the 12 participants who completed the study, one had unreadable sleep data and was excluded from the entire analysis. Thus the final cohort consisted of 11 male participants (See Figure 3.1); ten of African and one of European ancestry. Table 3.1 shows all the demographic (age, weight, height, BMI) and screening characteristics (PSQI, Habitual Sleep and Wake Times and GHQ) of our 11 final participants. Ten participants had healthy weights (ie their BMI was comprised between 18.5 and 24.9 kg/m^2), good sleep quality (indicated by PSQI scores of less than 5 (Buysse et al., 1989)) and good physiological health (indicated by scores of less than 6 on the GHQ (Goldberg et al., 1976)). One participant, who had a high BMI ($32kg/m^2$) was asked to complete the Berlin Questionnaire (BQ) as an assessment of risk of sleep apnoea (Chung et al., 2008). He scored Low risk on the BQ and was therefore able to participate in the study. Participants' habitual sleeping times ranged from 22:30 to 00:00 and the wake times from 06:45 to 09:00 with an average total sleep time of 7 hours and 42 minutes.

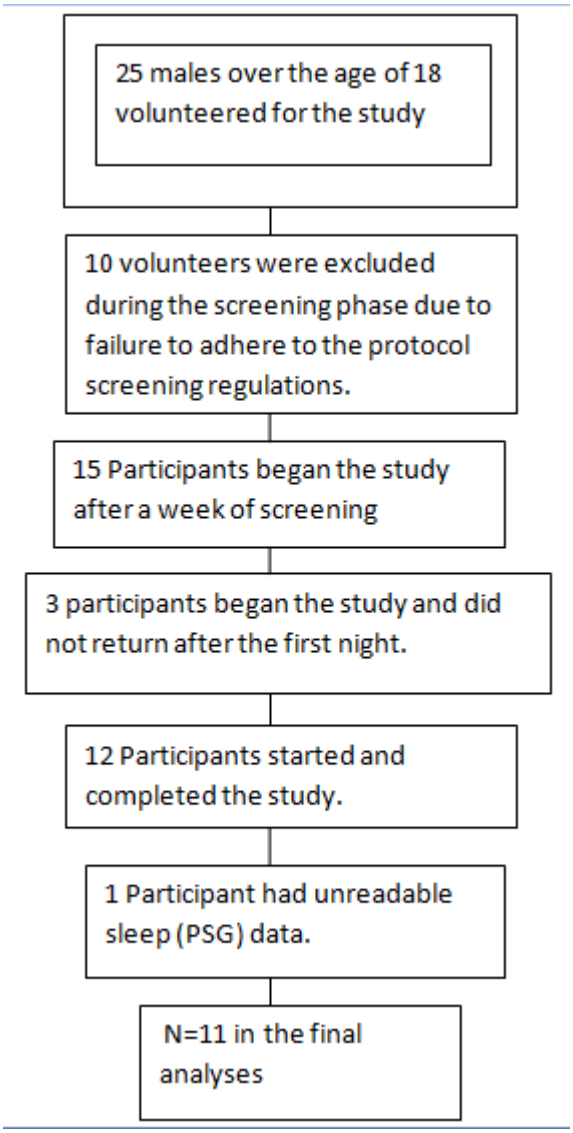


Figure 3.1 Flow diagram representing the recruitment and screening phase of the study and the final number of participants included in the analyses.

Table 3.1 *Participants' characteristics and habitual sleep- and wake-time in the final cohort of 11 males.*

Participant characteristics (N=11)	**Median [Q1-Q3]**
Age (years)	21 [20 - 21]
Weight (kg)	69.9 [65 - 78]
Height (m)	1.77 [1.75-1.81]
BMI (kg/m^2)	22.3 [20.7-25.3]
Total PSQI score	3 [2 - 4]
Habitual sleep time (hour:min)	23:00 [23:00 – 23:37]
Habitual wake time (hour:min)	06:52 [6:26 -7.37]
GHQ score	0 [0-2]

BMI (Body mass Index); PSQI (Pittsburgh Sleep Quality Index); GHQ (general Health Questionnaire)

Overnight sleep architecture between the sleep conditions

Using the sleep data obtained from the polysomnographic (PSG) recordings, I ran univariate analyses using paired t-tests to compare the sleep variables on the nights with the same sleep restriction protocol. The data are displayed in Tables 3.2.1 and 3.2.2. There were no significant differences in the sleep stages between the two first 4 hour restriction nights (SR-F4HN and SR-F4HNN) (Table 3.2.1), therefore I collapsed them into one SR-F4H for further analyses. The same was true for the last 4 hour restriction nights (SR-L4HN and SR-L4HNN) where there were no significant differences in the sleep stages between the two nights (shown in Table 3.2.2), so I averaged them into one SR-L4H variable. Therefore, the final mixed model analyses included the variable 'COND' which comprised Baseline, SR-F4H and SR-L4H experimental conditions.

Table 3.2.1 *Comparison using paired t-tests of the polysomnographic sleep variables (total minutes during the night (min), as well as percentage (%) of total sleep) between the experimental night conditions of the first 4 hour restriction with a nap condition (SR-F4HN) and the first 4 hour restriction without a nap condition (SR-F4HNN). Sleep reports were exported using the habitual bedtimes and wake times reported by the participants in the screening phase, therefore the data below include the period of imposed sleep restriction where I kept the participants awake. However, we did want to analyse time awake when they were in their 4h experimental sleep episode and therefore WASO and sleep latency are calculated from the exported report with lights on and lights off corresponding to those of the experimental procedures (export on the 4h of allowed sleep).*

	SR-F4HN	**SR-F4HNN**	**P**
From 8h-sleep report			
TiB (min)	493 [483-504]	485 [480-501]	0.63
TST (min)	278 [234-355]	270 [229-362]	1.00
Sleep Efficiency (%)	56 [48 - 73]	62 [53 - 83]	0.91
Awake min	114 [41 - 224]	174 [50 - 230]	0.93
Awake %	27 [13-45]	39 [17-45]	0.95
N1 min	18 [11-37]	27 [13-31]	0.99
N1 %	7 [5-10]	9 [6-10]	0.97
N2 min	108 [91-157]	113 [103-179]	0.98
N2 %	42 [36-46]	49 [42-52]	0.86
N3 min	80 [75-91]	70 [62-84]	0.79
N3 %	33 [25-37]	27 [20-33]	0.88
REM min	54 [47-88]	54 [37-76]	0.95
REM %	24 [12-27]	20 [14-25]	0.83
Arousal Index	5.1 [3.7 - 5.8]	5.1 [3.9 - 7.7]	0.98
From 4h-sleep report			
Sleep Onset Latency (min)	6 [3-13]	6 [3-10]	0.85
WASO (min)	5 [1-11]	9 [6-12]	0.90

Data expressed as median [interquartile range]. TST: Total sleep Time; WASO: Wake-time After Sleep Onset; TiB: Time in bed. Sleep Efficiency was calculated by dividing the total sleep time by the total time in bed, and the Arousal Index calculated using the number of awakenings/arousals per hour of sleep.

Table 3.2.2 *Comparison using paired t-tests of the polysomnographic sleep variables (total minutes during the night (min), as well as percentage (%) of total sleep) between the night-time sleep conditions of the last 4 hour restriction with a nap condition (SR-L4HN) and the last 4 hour restriction without a nap condition (SR-L4HNN). Sleep reports were exported using the habitual bedtimes and wake times reported by the participants in the screening phase, therefore the data below include the period of imposed sleep restriction where I kept the participants awake. However, we did want to analyse time awake when they were in their 4h experimental sleep episode and therefore WASO and sleep latency are calculated from the exported report with lights on and lights off corresponding to those of the experimental procedures (export on the 4h of allowed sleep).*

	SR-L4HN	**SR-L4HNN**	**P**
From 8h-sleep report			
TiB (min)	481 [478-503]	490 [487-504]	0.64
TST (min)	303 [230-342]	249 [172-377]	0.96
Sleep Efficiency (%)	64 [48-71]	55 [45-75]	0.98
Awake min	138 [61-173]	127 [67-253]	0.99
Awake %	29 [11-36]	28 [16-53]	0.98
N1 min	34 [19-38]	12 [10-41]	0.99
N1 %	9 [8-11]	7 [5-10]	0.99
N2 min	127 [123-206]	135 [74-155]	0.92
N2 %	54 [46-54]	43 [35-57]	0.82
N3 min	79 [55-91]	70 [51-89]	0.99
N3 %	24 [21-30]	22 [20-48]	0.64
REM min	50 [33-52]	27 [23-52]	0.99
REM %	14 [10-16]	11 [10-15]	0.88
Arousal Index	6.1 [4.9-9.9]	6.9 [5.3-9.0]	0.99
From 4h-sleep report			
Sleep Onset Latency (min)	15 [8-25]	18 [11-25]	1.00
WASO (min)	4 [3-8]	5 [2-7]	1.00

Data expressed as median [interquartile range]. TST: Total sleep Time; WASO: Wake-time After Sleep Onset; TiB: Time in bed. Sleep Efficiency was calculated by dividing the total sleep time by the total time in bed, and the Arousal Index calculated using the number of awakenings/arousals per hour of sleep.

Univariate analysis of the association between condition (as COND=Baseline, SR-F4H, SR-L4H) and all sleep variables

After running the univariate analysis of the association between sleep variables and condition (COND), I found that, all the PSG sleep variables *(absolute time (minutes) and percentage spent awake during the time in bed; absolute time (minutes) and percentage of total sleep episode spent in N1, N2, SWS, REM; Arousal Index, Sleep Efficiency and Total Sleep Time (*

TST)) were significantly associated with condition (COND), $F_{3.17} = 41.11$, (all $p < .0001$). If I found a main effect of condition (COND), I further ran post hoc tests with a Tukey adjustment for multiple comparisons. These results are presented in Table 3.2.3.

Table 3.2.3 *Median [Q1-Q3] of the different sleep stages and awake, in minutes (min) and percentages (%), as well as total sleep time by sleep (TST), arousal index (total arousals/TST in hours), sleep efficiency (TST/total time in bed) by sleep intervention (Baseline (undisturbed) sleep, sleep restriction in first 4 hours (SR-F4H) and sleep restriction in the last 4 hours (SR-L4H)).*

	Baseline	**SR-L4H**	**SR-F4H**	**P**
TiB (min)	484[a] [478-485]	486[a] [493-497]	494[a] [481-497]	<.0001
TST (min)	454[a] [433-459]	277[b] [225-364]	271[b] [229-355]	<.0001
Sleep Efficiency %	94[a] [91-95]	54[b] [48-75]	60[b] [49-74]	<.0001
Awake min	30[a] [25-45]	127[b] [61-226]	143[b] [68-230]	<.0001
Awake %	6[a] [5-9]	28[b] [11-45]	32.3[b] [17-45]	<.0001
N1min	26[a] [22-35]	21[a] [12-38]	25[a] [12-31]	<.0001
N1 %	6[a] [5-8]	8[a] [5-11]	7[a] [5-10]	<.0001
N2 min	215[a] [192-223]	145[b] [99-206]	113[b] [98-168]	<.0001
N2 %	50[a] [44-56]	50[a] [41-57]	44[a] [41-52]	<.0001
N3 min	99[a] [87-125]	72 [b] [55-91]	75[b] [66-84]	<.0001
N3 %	21[a] [20-27]	25[a] [20-36]	30[a] [22-35]	<.0001
REM min	97[a] [83-109]	37[b] [24-52]	54[c] [40-76]	<.0001
REM %	22[a] [18-24]	13[b] [10-16]	22[c] [14-26]	<.0001
Arousal Index	7.8[a] [5.6-8.3]	6.8[a] [4.9-9.9]	5.1[a] [3.9-6]	<.0001
Sleep Onset Latency (min)	16[a] [10-23]	17[a] [11-25]	6[b] [3-11]	<.0001
WASO (min)	12[a] [9-19]	4[b] [3-7]	7[a] [2-12]	<.0001

Superscript letters of the same value show absence of differences in post hoc analyses. Letters of different values show statistically significant differences ($p<0.05$) in the post hoc analyses.) TST: Total sleep Time; WASO: Wake-time After Sleep Onset.

As expected from the study design, compared with SR-F4H and SR-L4H, Baseline (uninterrupted) sleep, participants spent more absolute time (minutes) asleep (TST: 454 [433-459] minutes) compared to the sleep restriction nights (median [IQR] SR-L4H=277 [225-364] minutes and SR-F4H=270 [229-355] minutes, post hoc test with Tukey adjustment, p=0.0003 and p=0.0002, respectively). There was no significant difference in TST between SR-F4H and SR-L4H (p = 0.97). Sleep Efficiency, defined as TST divided by time in bed, was also statistically better in the Baseline condition than in SR-L4H (p = 0.001) and in SR-F4H (p = 0.002). There were no differences between the sleep restriction nights (SR-L4H vs. SR-F4H) for Sleep Efficiency (p = 0.97). Sleep onset latency was lowest in the SR-F4H restriction night when participants were allowed to sleep the last 4 hours of the night. The difference between SR-F4H and the other conditions was significant (post hoc test with Tukey adjustment; Baseline vs SR-F4H p = 0.01 and SR-F4H vs SR-L4H p = 0.002, see Table 3.2.3) however sleep onset latency was not significantly different between Baseline and SR-L4H conditions (post hoc test with Tukey adjustment; p = 0.94). For Wake after sleep onset (WASO) there were significant difference between Baseline and the restriction nights (Ba vs SR-L4H p = 0.01 and Ba vs SR-F4H p =0.04). I did not find significant differences in post hoc analyses between SR-F4H vs SR-L4H (p = 0.98). Overall, participants had their lowest sleep onset latency in the SR-F4H condition, and had more awakenings during their sleep compared to SR-L4H and Baseline nights.

Of all the sleep variables, only N1 (minutes and %), and arousal index were not different across the night conditions. Overall throughout each condition, our participants spent a non significantly different median of 21 to 26 minutes in N1 sleep (post hoc test with Tukey adjustment Ba vs SR-F4H p = 0.58, Ba vs SR-L4H p = 0.79, SR-F4H vs SR-L4H p = 0.89), which corresponded to non significant differences in respective medians of N1 percentage of total sleep time, ranging from 6 to 8%, respectively (post hoc test with Tukey adjustment; Ba

vs SR-F4H p = 0.79, Ba vs SR-L4H p = 0.58, SR-F4H vs SR-L4H p = 0.91, see Table 3.2.3. Lastly, For Arousal Index the post hoc results were Ba vs SR-F4H p = 0.87, Ba vs SR-L4H p = 0.96, SR-F4H vs SR-L4H p = 0.64).

Time spent awake and time spent in REM sleep, as well as the proportional (%) time awake and in REM were significantly different across the experimental conditions. As expected from the experimental design, the percentage of time spent awake was significantly larger during SR-F4H and SR-L4H, compared with baseline (post hoc test with Tukey adjustment; p = 0.01 and p = 0.02, respectively). Similarly, participants spent more absolute time awake (minutes) during SR-F4H and during SR-L4H, compared with Baseline awake time (post hoc test with Tukey adjustment; p = 0.02, and p= 0.03, respectively). Time spent in REM was different across all night conditions, whereby REM sleep was significantly reduced during sleep restriction, compared with baseline (Ba vs SR-F4H: p = 0.002, and Ba vs SR-L4H: p < 0.0001). Absolute time (minutes) spent in REM sleep also differed significantly between the two sleep restriction interventions, with significantly more REM during SR-F4H compared with SR-L4H (p = 0.03). Similarly, as a proportion of total sleep (% REM), baseline sleep and SR-F4H had significantly more REM sleep than SR-L4H (Ba vs SR-L4H: p = 0.01 and SR-L4H vs SR-F4H: p = 0.01).

Significant differences in N2 and N3 were observed across sleep conditions only when comparing the total minutes (not percentages) spent in these sleep stages. Participants spent significantly more minutes in N2 sleep during Baseline compared with SR-L4H and SR-F4H (p = 0.004, and p = 0.001, respectively). Participants spent significantly less time in deep sleep (N3) during SR-F4H compared with Baseline sleep (P = 0.005).

Naps sleep architecture between the conditions

I ran univariate analyses on the afternoon PSG recordings of the naps to investigate the sleep composition of these naps. The results (shown in Table 3.3.1) are separated by the sleep restriction condition (SR-F4H and SR-L4H) experienced the night before the nap. I ran paired t-tests across these two groups. The results are presented as Median [Q1-Q3].

Table 3.3.1 *Median [Q1-Q3], paired t-test results comparing the sleep variables during the afternoon polysomnography of the naps following the 2 different types of sleep restrictions (SR-L4H and SR-F4H).*

	SR-L4H	**SR-F4H**	**T**	**P**
TiB (minutes)	60 [52-61]	47 [43-49]	-2.09	0.19
TST	43 [41 - 43]	40 [38 - 43]	0.91	0.36
Sleep Efficiency (%)	82 [66 - 88]	87 [73 - 92]	5.22	<.0001
Awake min	16 [7 - 20]	6 [4 -14]	-6.08	<.0001
Awake %	18 [12 - 34]	13 [8 - 27]	-5.22	<.0001
N1 min	4 [2 - 6]	3 [2 - 7]	0.95	0.34
N1 %	7 [3 - 18]	7 [4 - 17]	1.48	0.14
N2 min	15 [18 - 24]	20 [14 - 30]	1.37	0.17
N2 %	39 [35 - 53]	42 [38 - 73]	3.47	0.0006
N3 min	18 [11 - 26]	12 [9 - 25]	-0.52	0.60
N3 %	39 [30 - 61]	36 [23 - 54]	0.28	0.78
REM min	0	0	-	-
REM %	0	0	-	-
Arousal Index	2.9 [1.8-4.4]	5.1 [2.5-7.6]	2.02	0.04
Sleep Time L_{off} (hh:mm)	13:26 [13:07-13:42]	13:53 [13:08-14:01]	4.34	<.0001
Wake Time L_{on}(hh:mm)	14:43 [14:05-14:48]	14:32 [14:10-14:56]	1.72	0.09
Sleep Onset Latency (minutes)	8 [3-11]	6 [6-12]	-0.35	0.08
WASO	4 [1-7]	1 [0-2]	-2.24	0.04

Data are shown as median [Q1-Q3]. TST (Total Sleep Time). LOff means the time when lights were switched off and Lon is the time when the lights were switched on.

Lights out during the afternoon naps occurred between 12:39 and 14:36; and lights on between 13:26 and 15:33. In general, participants who napped after the SR-L4H condition spent more time in bed (~60 minutes) compared to when they napped after the SR-F4H condition (~47 minutes) but this was not statistically significant. Participants who napped

after the SR-L4H condition also tended to have a slightly longer sleep latency (t-test $p = 0.08$) and more awakenings during their sleep compared to when they napped after the SR-F4H (WASO t-test $p = 0.04$). The afternoon nap compositions differed significantly in awake time, N2 %, Arousal Index and Sleep Efficiency between the two sleep restriction conditions the night before. When participants napped after being restricted during the last 4H of the night (SR-L4H), they spent significantly more time awake (percentage of total nap time and total number of minutes), when compared to when they napped after being restricted during the first 4h of the night (SR-F4H) ($p < 0.0001$). Sleep Efficiency and Arousal Index were also significantly higher in naps following SR-F4H compared with SR-L4H.

When participants had experienced SR-F4H sleep restriction the night before, 42% of their afternoon nap was light N2 sleep, which was significantly higher than the 39% of N2 seen after the SR-L4H sleep restriction ($p < 0.001$). However, when N2 was analysed in minutes, this difference was not significant.

Effects of Sleep Restriction on Ischaemic Pain

Main Sleep Nights and Pain in the morning

First, I ran paired t tests of the average VAS scores and the AUC (Area Under the Curve) values between the two SR-F4H restriction nights and the two SR-L4H restriction nights to investigate whether they were comparable (no significant differences) and therefore, whether it would be viable to collapse (average) the data of the identical sleep interventions, as I had done for the analyses of the main nights. The results are shown in Table 3.4.1, the average scores, estimated difference between the scores, the t values and the associated p values. In general, I found that pain intensity following Baseline (uninterrupted sleep) tended to be higher (average of 54 mm on the VAS) compared with SR-L4H (47 mm, p = 0.73 and compared with SR-F4H (42 mm, p = 0.08). Between the two restriction nights, pain was higher during the morning following the SR-L4H nights compared to pain scores following SR-F4H nights (p = 0.0006).

Table 3.4.1 *Differences in pain (average VAS scores over 10 minutes of testing, area under the curve, average 50% pain (5 minute mark) and average peak pain scores) between SR-F4HN and SR-F4HNN, and SR-L4HN and SR-L4HNN in the morning tests. Average (SD)*

	SR-L4HN	**SR-L4HNN**	**Estimated Difference**	**T**	**P**
Average VAS	49.48 (16.34)	44.67 (16.20)	4.81	1.43	0.45
VAS AUC	495 (163.5)	447 (162)	48	0.78	0.45
50% VAS	50.83 (21.41)	44.75 (17.31)	6.08	0.66	0.52
Peak Pain	75 (18.15)	69 (24.30)	6	1.21	0.25
	SR-F4HN	**SR-F4HNN**	**Estimated Difference**	**T**	**P**

Average VAS	43.86 (19.96)	40.86 (17.60)	3	0.78	0.47
VAS AUC	428 (191.9)	411 (168.5)	17	0.42	0.68
50% VAS	44.83 (24.93)	44.17 (24.33)	0.66	0.11	0.91
Peak Pain	66 (26.02)	60 (24.15)	6	1.37	0.19

VAS: visual analogue scale 0-100mm.

As displayed in table 3.4.1 above, I found no significant differences in the average pain, average 50% pain, average peak pain (maximum pain score over the ten minute pain experiment), and AUC (which accounts for pain intensity over time). I therefore deduced that it was appropriate to run all further tests on the collapsed (average) data – i.e. I averaged the pain data for SR-F4HNN and SR-F4HN, as well as the data for SR-L4HNN and SR-L4HN. All further analyses to determine the effect of night-time sleep on pain were then run by comparing the Pain scores across the three COND variables (Baseline, SR-F4H and SR-L4H). The raw data on pain intensity (as measured on a VAS every minute for 10 minutes) during the morning experimental pain tests are shown in Figure 3.4.1. Also displayed in the figure, and highlighted in red, is the average for the entire cohort (N=11).

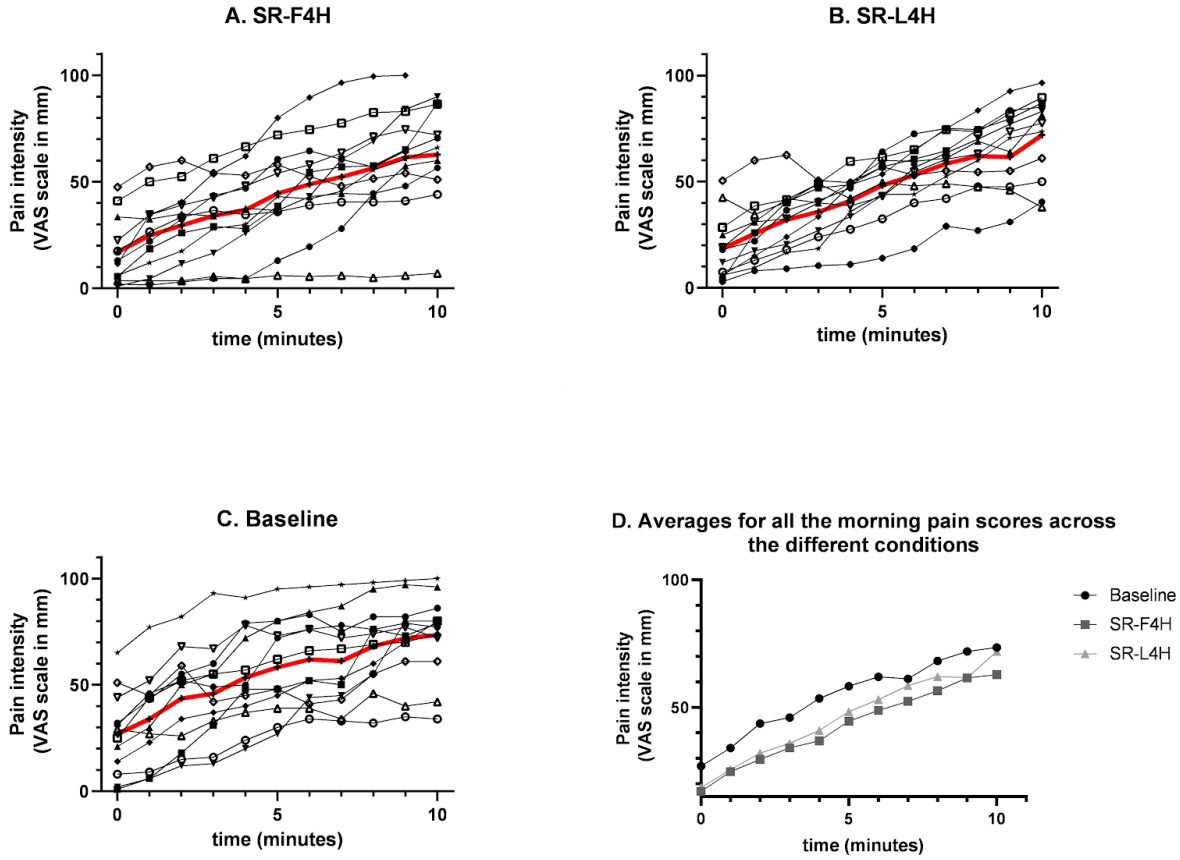


Figure 3.4.1 The raw pain data (VAS 0-100mm) recorded for each participant (each black line represents an individual) during the **morning** after sleep was restricted for the first half of the night (SR-F4H, panel A), when sleep was restricted during the last 4 hours of the night (SR-L4H, panel B), and after baseline sleep (panel C). The red line on panels A-C demonstrates the average for all participants. Panel D shows the trajectory of the averages from panels A-C.

Univariate analysis of the Main Effects on pain perception as measured by VAS score during the ischaemic pain test

I then individually tested all the variables that might have effects on our outcome variable VAS. I tested for the effect of order, investigating whether the order in which the nights were done (1-5) had any association on a higher or lower experience of pain in the participants. Similarly, I tested for the effect of time, investigating whether the 60 second increments in

time during the 10 minute pain tests correlated with increases in the pain scores. I then tested for the effect of Time of Day, testing for a relationship between the pain scores in the morning and pain scores in the afternoon. Lastly, I tested for a nap effect; if the pain scores differed during the nap afternoons and the no nap afternoons. The results are shown in Table 3.4.2.

Table 3.4.2 *Showing the univariate analyses investigating the association between pain (as measured on a VAS scale from 0 to 100 mm over 10 minutes) and each of the main effects we tested (Conditions collapsed into baseline, SR-F4H and SR-L4H, Order of the different conditions (1 to 5), time during the pain tests, the Time of Day and the afternoon nap).*

Effect	Estimate	Standard Error	DF Num, Den	F value	P value
Order	-5.0504	0.4447	1,1188	128.98	<.0001
Time	4.9890	0.1553	1,1188	1032.59	<.0001
ToD (AM vs. PM)	1.64	3.6748	2,1188	86.67	<.0001
Cond			3,1187	64.10	<.0001
Baseline	50.2887	3.8477			
SR-F4H	43.7290	3.7038			
SR-L4H	49.5730	3.7042			
Nap	0.04721	1.7050	1,1188	0.00	0.9779

ToD: Time of day. Num: numerator degrees of freedom; Den: denominator degrees of freedom.

The final pain model included *time, ToD* (time of day), *nap, cond (Baseline, SR-F4H and SR-L4H)* and *order*. The *time* variable represents the pain measurements per minute and the results show that with every minute, there was a 5 mm increase in the pain measurements ($F_{1,1183}$, = 1262.71, p <.0001). For *ToD*, there were, in general, higher pain scores during the morning tests (by 4 mm) than during the afternoon tests ($F_{1,1183}$, = 16.70, p <.0001). *Nap* afternoons had pain scores higher by 6 mm compared to no nap afternoons ($F_{1,1183}$, = 22.59, p <.0001). For *cond*, I found that the pain levels were always lowest following the SR-F4H compared to Baseline (p = 0.001) and SR-L4H (p = 0.007); $F_{2,1183}$ = 7.08, p <.0001). Lastly, with every increase in the variable *order*, there was a 5 mm decrease in the pain scored by the participants ($F_{1,1183}$, = 259.47, p <.0001). In summary, pain levels were higher i) after Baseline 8-

hr sleep (p = 0.001) and early sleep (SR-L4H) compared to the late sleep (SR-F4H) (p = 0.007); F2,1183 = 7.08, p <.0001); ii) during the morning tests (by 4 mm) than the afternoon (F1,1183, = 16.70, p <.0001); iii) on nap afternoons (by 6 mm) compared to no nap afternoons (F1,1183, = 22.59, p<.0001).

Table 3.4.3 *The final pain model including all the main effects that were found to be significant for our outcome variable, pain VAS scores. These are TIME, COND, ToD, ORDER AND NAP.*

Effect	**values taken by each variable**	**Estimate**	**Standard error**	**Num DF**	**Den DF**	**F**	**P**
Time	0,1,2,3,4,5,6,7,8,9,10 (linear)	4.9383	0.139	1	1183	1262.71	<.0001
Cond	Baseline, F4H, L4H			2	1183	7.08	0.0009
	Baseline	42.16	4.08				
	F4H	39.39	4.01				
	L4H	43.06	3.98				
ToD	AM compared to PM	4.1503	1.0156	1	1183	16.70	<.0001
Order	1,2,3,4,5 (linear)	-5.209	0.3234	1	1183	259.47	<.0001
Nap	Nap compared to no nap	6.4164	1.35	1	1183	22.59	<.0001

ToD: Time of Day.

Table 3.4.4 shows the relationship (depicted by the estimate) between each of the sleep variables and the VAS scores after adjusting for all of the main effects I investigated (*TIME, ToD, NAP and ORDER*). A positive estimate signals an increase in pain associated with an

increase in the sleep variable (minutes/%). A negative estimate signals a decrease in pain associated with an increase in the sleep variable (minutes/%).

Table 3.4.4 *Adjusted effects of each sleep variable (regardless of condition) on pain as measured with the VAS scale during 10 minutes of induced ischaemia, each sleep variable being included in a separate model, each model including just ONE individual sleep variable. The main effect for each sleep variable in each of those models was adjusted for the main effects of order (ORDER), time of day (ToD, ie AM vs. PM), time elapsed during the pain test (TIME) and whether the participant had napped or not (NAP). All those main effects were statistically significant in all the adjusted analyses (see detailed table in the appendix B).'*

	Estimate	Standard Error	P
TST (min)	**-0.01**	**0.01**	**0.006**
Sleep Efficiency %	**-0.06**	**0.03**	**0.02**
Awake min	0.002	0.01	0.68
Awake %	0.02	0.03	0.52
N1 min	0.07	0.12	0.05
N1 %	**0.47**	**0.04**	**0.0001**
N2 min	**-0.02**	**0.01**	**0.009**
N2 %	**-0.17**	**0.06**	**0.003**
N3 min	-0.0006	0.02	0.98
N3 %	**0.12**	**0.05**	**0.006**
REM min	**-0.06**	**0.02**	**0.002**
REM %	**-0.21**	**0.08**	**0.007**
Arousal Index	0.12	0.17	0.49
Sleep Onset Latency (min)	0.01	0.01	0.17
WASO (min)	-0.004	0.01	0.68

TST (Total Sleep Time), WASO (Wake-time after sleep onset).N1 min, N2 min, N3 min, REM min: minutes of sleep spent in N1, N2 N3 and REM, respectively.

After adjusting for all the main effects, an increase in N1% was associated with higher pain perception as measured by the VAS. The pain scores across conditions were not associated

by the time spent awake, whether in absolute time (minutes) or in percentage (%) of the Time in Bed ($p = 0.68$ and $p = 0.52$ respectively). Similarly, the sleep onset latency and arousal index were not associated with pain perception. Lastly, higher slow wave sleep percentage (N3) associated with more pain ($p = 0.009$), whereby a one-point increase in the percentage time spent in N3 was associated with a 0.12 mm increase in the pain scores rated by the participants. There was no association between N3 in absolute time (minutes) and pain ($p = 0.98$).

N2%, N2 min, REM% , REM min, TST and Sleep Efficiency were negatively associated with VAS. Wake after Sleep Onset (WASO) was not associated with pain sensitivity measured the next day ($p = 0.68$). The more time spent in N2 (in absolute time (minutes) and in proportion (%)) resulted in lower pain sensitivity during testing time and this relationship was significant ($p = 0.009$ and $p = 0.03$ respectively). Increased REM minutes and % also resulted in lower pain sensitivity ($p = 0.02$ and $p = 0.005$ respectively). Higher total sleep time (TST) and Sleep Efficiency were associated with lower pain sensitivity ($p = 0.006$ and $p = 0.02$, respectively). The more time spent sleeping, the higher the sleep efficiency, the more time spent in N2 and REM all resulted in less pain sensitivity in the participants reported by the VAS scores.

Further, we investigated which sleep variable had more impact on pain perception. Using the results in Table 3.4.4, we singled out N2%, N2min, TST, REM% and N3%. Using our final mixed model, we ran N2% with N3%, N2min with TST, N2min with TST and REM% with N3%. In the model with N2% adjusted for N3%, we found that these two variables were collinear with each other, as shown by an inflation of the standard error by more than 10% for both the variable, and both were no longer significant predictors of pain perception (N2% $p = 0.12$ and N3% $p = 0.70$). In the model with N2% adjusted for TST, TST became non

significant as a predictor of pain perception but N2% remained significant (p = 0.32 and p = 0.02, respectively) and maintained a negative relationship with pain sensitivity. In the model with N2min adjusted for TST, TST was a negative confounder and both these variables were no longer significant predictors of pain perception (N2 min p = 0.15 and TST p = 0.69). Lastly, in the model with REM% adjusted for N3%, these two variables were collinear with each other (standard error inflated >10%) and N3 had a negative confounding effect on REM. These two variables cannot be used to adjust for each other. From these results, we can extrapolate that N2% is the more important sleep variable in predicting pain in the morning as it is the only one that remained significant during these additional tests, even more important than overnight TST. Table 3.4.5 shows the Pearson Correlation results between N2%, N3%, REM% and TST.

Table 3.4.5 *Showing the results from the Pearson Correlation between N2%, N3%, REM% and TST. The first line shows the correlation coefficients and the second line p values.*

	N3 %	**N2 %**	**REM %**	**TST (minutes)**
N3 %	1.00000	-0.74631	-0.57203	-0.67760
		P <.0001	P <.0001	P <.0001
N2 %	-0.74631	1.00000	-0.01481	0.45709
	P <.0001		P=0.6376	P <.0001
REM %	-0.57203	-0.01481	1.00000	0.44214
	P <.0001	0.6376		P <.0001
TST (minutes)	-0.67760	0.45709	0.44214	1.00000
	P <.0001	P <.0001	P <.0001	

TST: Total Sleep Time.

N3 had a negative correlation with all the other sleep variables considered. Increases in percentage total sleep spent in N3 was associated with decreases in all the protective variables for pain (N2%, REM% and TST). N2 and REM were positively associated with TST.

Naps and Pain

In the afternoon pain tests, in general, the nap conditions had higher pain scores compared to the nonap conditions. Pain was highest after the SR-L4H condition with a nap (average 78mm) and lowest after the SR-F4H condition without a nap (average 66mm). Figure 3.4.3 shows the results with individual scores as well as averages for SR-F4H nap and no nap, and SR-L4H nap and no nap. We also show the averages across the three COND variables separated by whether there was a nap that afternoon or not. The Baseline averages are the same as Baseline always did not have a nap.

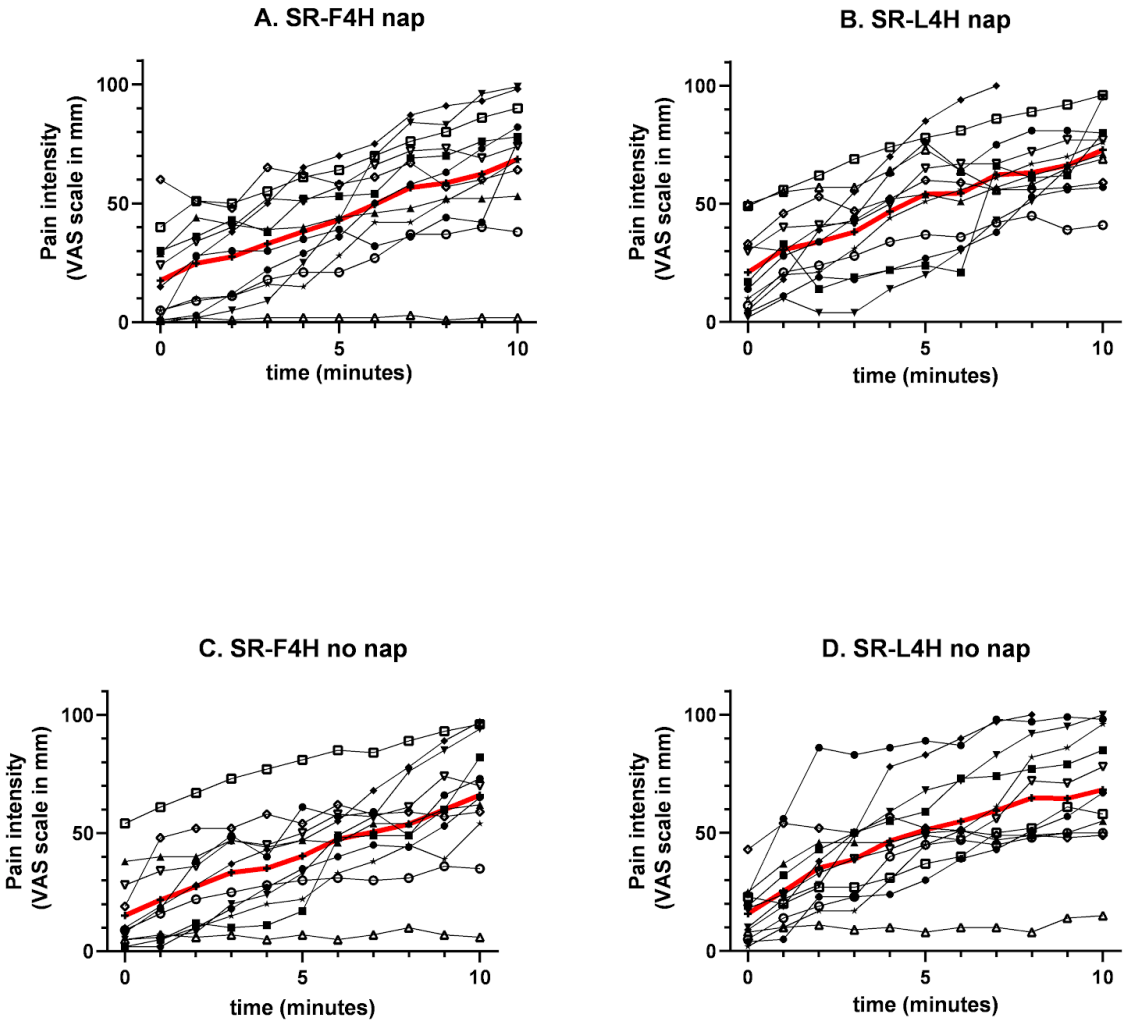


Figure 3.4.2 The minute by minute pain scores (VAS 0-100mm) recorded during the **afternoon** pain tests after SR-F4H nap and nonap and SR-L4H nap and nonap nights. A and B show SR-F4H and SR-L4H pain trajectories after the afternoon nap condition. C and D show SR-F4H and SR-L4H pain trajectories after the afternoon lie-in condition.

Sleep architecture and pain analyses

I used mixed models to further investigate the role that each sleep stage during the afternoon nap plays in predicting pain perception in the pain tests that follow. I ran analyses on TST, N1, N2 and N3, and 'TimetoNT' (a variable that represents the time between the participants

being awakened from the night sleep and the sleep onset time of the afternoon nap). Table 3.4.6 shows the relationship between the sleep variable and pain following the afternoon naps.

Table 3.4.6 *The sleep variables during the afternoon naps and their estimates showing the relationship with pain perception. Positive estimates show a positive relationship with pain, where an increase in the variable results in increased pain. Negative estimates show a negative relationship with pain, where an increase in the variable results in decreased pain.*

	Estimate	**Standard Error**	**P**
TST(min)	**-2.74**	**1.07**	**0.01**
N1 (min)	-0.33	0.52	0.53
N2 (min)	**3.44**	**1.16**	**0.004**
N3 (min)	**3.62**	**1.16**	**0.002**
TimetoNT	**2.32**	**0.30**	**<.0001**
Arousal Index	1.29	1.08	0.22

TST: Total Sleep Time. Arousal Index is the number of arousals per hour.

During the analyses, I found an effect of time and condition order. A minute increase in *time* elapsed during the pain test (0-10 minutes) resulted in higher pain by 5mm ($F_{1, 156}F = 268$, $p < .0001$). Similar to the main night analyses, increasing *order* of nights resulted in decreased pain perception by 3mm ($F_{1, 156}F = 4.27$, $p = 0.04$). Thus, the analyses adjusted for order and time. Total sleep time was significantly related to pain perception and had a protective effect, increased TST was associated with decreased pain scores ($p = 0.01$). The minutes in N2 and N3 had a significant and positive relationship with pain perception after the naps ($p = 0.004$ and $p = 0.002$). Every 1 minute increase in N2 minutes resulted in an increase in the pain

scores by 3mm. Similarly, more time (by 1 minute) spent in N3 resulted in a 4mm increase in the pain scores. 'TimetoNT' was a significant predictor of pain scores in the model. The more time elapsed between wake time from the main sleep episode and nap sleep onset, the higher pain scores recorded by 2mm ($p < .0001$). SR-L4H restriction nights required participants to sleep the first 4 hours and be awake the rest of the night and they therefore had a higher/longer 'TimetoNT'. The 'TimetoNT' findings might play a role in the L4 having higher pain than SR-F4H who slept the last 4 hours of the night.

In the mixed model with' TimetoNT' adjusted by N2 minutes during the nap and time, both 'TimetoNT' and N2 remained significant ($p < .0001$ for both) and maintained their positive relationship with pain, where increases in these variables resulted in increased pain perceived by the participants. In the mixed model with 'TimetoNT' adjusted by N3 min and time, only N3 remained significant ($p < .0001$) and TimetoNT did not ($p = 0.69$). In the mixed model with N2 adjusted for N3, only N3 remained significant ($p < .0001$) and N2 did not ($p = 0.18$). The final model I ran had all three variables (N2, N3 and TimetoNT) adjusted for time. In this model, N3 and TimetoNT remained significant predictors of pain with more N3 and TimetoNT resulting in higher pain scored by 1mm ($p < .0001$) and 2mm ($p < .0001$) respectively. N2 was no longer associated with pain sensitivity ($p = 0.21$).

According to these results, even though all three of these variables are good predictors of pain perception in our model for afternoon naps, N3 was the more significant variable and higher time spent in N3 during the nap was associated with higher pain scores. Table 3.4.7 shows the Pearson Correlation results between these three sleep variables that we considered. N2 and N3 were positively correlated to TimetoNT, and N2 and N3 were negatively correlated with each other.

Table 3.4.7 *Showing the results from the Pearson Correlation between nap N2 in minutes, N3 in minutes and TimetoNT. The first line shows the correlation coefficients, the second line p values.*

	TimetoNT	**Nap N2 min**	**Nap N3 min**
TimetoNT	1.00000	0.02775	0.14189
		P = 0.9250	P = 0.6285
Nap N2 min	0.02775	1.00000	-0.25914
	P = 0.9250		P = 0.2567
Nap N3 min	0.14189	-0.25914	1.00000
	P = 0.6285	P = 0.2567	

TimetoNT: time, in minutes, between when participants woke up and when they had their first epoch of nap sleep).

Lastly, I speculated that my unexpected results showing increased pain following a nap, may be the result of the various sleep stages that the participants were awoken from during the nap. I therefore created a variable 'Woken' which depicts the sleep stages that participants were awoken from when napping. I added my "woken" variable into my final pain model, and adjusted for Order, Time, ToD and Cond and nap. Overall there was no difference in the frequency of N2 and N3 in our variable 'Woken' across all the nap conditions. SR-L4H had a higher frequency of being awoken from N3 than from N2 (5 woke up from N3 and 3 from N2). SR-F4H had a higher frequency of being awoken from N2 than from N3 (6 woke up from N2 and 3 from N3) however this was not statistically significant (chi-square $\chi 2 = 1.4462$; $p = 0.22$). Participants woken from N2 had appeared to have higher pain scores (4mm more), than those woken up in N3). However, this difference was not significant (mixed model analysis, F value = 0.81; p = 0.37).

CHAPTER 4

Discussion

This study investigated the effects of four hour sleep restriction (first half and last half of the night), compared with 8h of uninterrupted sleep (baseline), on the perception of experimentally-induced ischaemic pain in healthy young men. The study tested for acute effects (over one night followed by 5 days recovery) and not chronic effects (over consecutive nights of sleep restriction). I found that a 4 hour sleep restriction (whether first four hours or last four hours) resulted in significant changes in sleep architecture, particularly a decrease in TST, N2 minutes, N3 minutes and REM minutes and proportion. The sleep architecture between restriction nights was similar, however when they slept the last four hours of the night (SR-F4H condition), they had higher REM content (minutes and as percentage of TST) in their sleep than when they slept the first four hours of the night . Upon waking, pain perception did not differ across the different night conditions (baseline and both sleep restriction nights) before adjusting for the main effects. Following the sleep restrictions, the participants were asked either to have a 30 minute lie-in or an afternoon nap. Comparing the naps happening after the SR-L4H (where they slept the first four hours of the night) and the SR-F4H (where they slept the last four hours of the night), respectively, I found that in the naps following both experimental nights, the participants slept the same amount of time, had the same amount of N3 and no REM. When they slept the first part of the night, they went to sleep for their nap earlier, had lower percentage N2 sleep, higher wake after sleep onset during the naps and lower sleep efficiency than when they had slept the last four hours of the night the night before.

Overall, after adjusting for the main effects in multivariable analyses, I found that pain perception measured by a Visual Analog Scale during an experimental 10-minute ischaemic

pain test was higher in the condition when they slept the first four hours of the night (SR-L4H, compared to SR-F4H and Baseline). Pain perception increased with each additional minute during the test and decreased across the study (order effect). Additionally, pain perception was decreased in the afternoons compared to the mornings (time of day effect), and was higher in the afternoon when the participants woke up from a nap following their sleep restriction compared to if they had just had a 30-minute rest.

To better understand how changes in sleep architecture may explain why the highest pain perception was after the L4H nights, I investigated how each sleep variable was associated with pain perception in adjusted analyses. I found that higher amounts of N1 and N3 sleep during the experimental nights associated with higher pain perception the following day and that higher total sleep time, higher sleep efficiency and higher time/ percentage of total sleep time spent in N2 sleep and REM associated with lower pain perception. In addition, higher N3 sleep during an experimental night was associated with a lower total sleep time, lower time in N2 and lower time in REM. The study had sought to investigate the effect of a 30-minute afternoon nap following the sleep restriction protocols on experimental pain. I found that a 30-minute afternoon nap surprisingly worsened pain perception, instead of improving it (as originally hypothesized) following the sleep restriction. I therefore investigated how the sleep composition during the naps or how the cumulative wakefulness/increased homeostatic sleep pressure preceding the naps may affect pain perception: I found in a multivariable analysis that increased pain perception after a nap was associated with higher amount of N2, higher amount of N3 during the nap, and higher cumulative wakefulness preceding the nap. Conversely, higher total sleep time during the nap lowered pain perception.

In this discussion, I will further discuss those findings and their implications for our knowledge about how sleep disruption and recovery sleep influence pain perception.

Sleep

Sleep Architecture during the Main Night Sleep

Sleep Architecture across all conditions

The sleep architecture between the uninterrupted 8h nights (Baseline) and the nights restricted to 4 hours of sleep (SR-F4H and SR-L4H) was, as expected, different. The TST in the sleep restricted nights was reduced to half (protocol-induced) and the total awake time considerably increased; four-fold compared with Baseline awake time). Consequently, Sleep Efficiency (defined as TST/time in bed) was significantly lower during the restriction night. WASO (wake time after sleep onset) was also significantly lower during the restriction nights. This may be just as a result of a shorter night and therefore even the wake after sleep onset is reduced. It could also be for the SR-L4H (where they slept the first part of the night) that WASO is lower during the first four hours of sleep in general as this is where deep sleep (N3) typically predominates. For SR-F4H (where they slept the last part of the night), it is likely that the higher homeostatic sleep pressure accumulated before sleep was allowed led to deeper sleep and lower awakenings during this part of the night where usually REM and N2 predominate but not N3. My participants did not have significantly different amounts of N3 across the three main experimental conditions, implying that there was more N3 in the SR-F4H condition than was expected. REM sleep usually dominates the last part of the sleep night, thus I expected N3 to be similar for Baseline and SR-L4H, but significantly decreased in SR-F4H. This is consistent with the known effects of higher wakefulness before sleep which is dissipated by N3 (Akerstedt et al., 2009). Although some differences were seen in

the time (in minutes) spent in some sleep stages (N2, N3) across the three interventions, when assessing each sleep stage as a proportion of TST, most sleep stages, with the exception of REM sleep, were the same across the three sleep conditions. REM sleep was significantly reduced (both in proportion of TST and in total time spent in REM sleep) during the SR-L4H condition compared with baseline but only differed to SR-F4H in minutes and not proportion. A reduction in REM sleep when restricting the first four hours of sleep, with no change in REM sleep when restricting for the last four hours, has been previously reported (Wu et al., 2010), although these results were after four consecutive nights of restriction.

In contrast to my study, Wu et al. (2010) reported decreased N1 following sleep restriction of the last part of the night and unchanged values following restriction of the first half of the night. I found no change in light N1 across the three different sleep conditions, nor did I find a difference in arousal index (defined as number of arousals per hour) across the three sleep conditions. Another study that did 5 days of sleep restriction between 03:00 and 07:00 reported decreases in all sleep variables except N3, which gradually increased throughout the restrictions period (Akerstedt et al., 2009). An increase in N3 after consecutive nights of sleep restriction has also been previously reported (Wu et al,. (2010). In my study, time spent in N3 was reduced during both SR nights. However, N3, as a proportion of TST, was the same across all sleep conditions. It is possible that with consecutive nights of sleep deprivation, I may have seen a gradual increase in N3, in accordance with the other studies but this was deliberately a study of acute sleep restriction.

Sleep Architecture between the two types of sleep restriction

There were differences in sleep architecture between the two types of restriction (SR-F4H vs SR-L4H). Those who slept during the first half of the night had less REM (in minutes and percentage of TST); a somewhat expected finding since REM is naturally predominant in the

second part of the night (Deatherage et al., 2009), as shown in Figure 1.1 (Chapter 1). A study with a similar study design investigating the effect on sleep restriction on appetite and food intake also found the same, where REM was lowest in the restriction of the last part of the night compared to the control (normal sleep) and restriction of the first part of the night (McNeil et al., 2017). Similarly, another study reported that during 5 consecutive nights of sleep restriction, the subjects experienced lower REM when sleep restricted for the last half of the night compared to when sleep restricted for the first half of the night (Akerstedt et al., 2009).

Compared with restricting the first 4h of sleep, SR-L4H also had the least number of awakenings during sleep (WASO) but also longest sleep onset latency. Although the randomization also had the purpose of blinding our participants to the condition they were about to do, sometimes the participants could infer the condition and therefore may have anticipated the coming sleep deprivation. Increased sleep latency may therefore be as a result of the effects of acute sleep deprivation on cortisol (stress hormone) levels, where cortisol levels are increased and subsequently increase the sympathetic nervous system (SNS) activity making it harder to fall asleep (Wright et al., 2015). Another possible explanation for this finding might be increased noradrenaline levels during sleep deprivation (Irwin et al., 1999), which are also associated with higher sleep latency (Zhang et al., 2011).

TST, Sleep Efficiency, Arousal Index, Awake (minutes and percentage), N2 (minutes and percentage) and N1 (minutes and percentage) were all the same across the two restriction groups.

Sleep Architecture during the Afternoon Nap Sleep

The afternoons following Baseline were always a no-sleep, 30-min lie-in, thus I only have nap conditions following the restriction nights (SR-L4H, SR-F4H). Sleep architecture of the naps following restriction of the first half of the night, and the naps following the last 4h of the night, was different in some aspects. In particular, following SR-L4H the afternoon naps comprised more time awake, had less Sleep Efficiency (defined as TST/TIB), lower Arousal Index (defined as number of arousals per hour) and a lower proportion of N2, compared to SR-F4H.

None of the participants (after neither SR-L4H nor SR-F4H) experienced REM sleep during the afternoon naps, and the N3 (total minutes and proportion of total nap time) was not significantly different between both conditions. In general, the sleep architecture (Sleep Efficiency, Sleep Latency, N3, N2) I observed during the afternoon naps is in line with the recent literature on nap sleep architecture (Jiang et al., 2018; Lee et al., 2017), with the exception of REM sleep (absent in the 30-min naps in my study) and N1 which was very low in this study compared to the ~30min N1 reported in these studies (Jiang et al., 2018; Lee et al., 2017). The naps in the previous studies were longer (91 minutes (Jiang et al., 2018) and 59 minutes (Lee et al., 2017)) than in the present study. The longer length of these studies could account for a presence of REM and higher N1 proportions in their results compared to the findings of my study.

Pain

The adjusted ischaemic pain levels in this cohort of healthy young males were significantly different across the three sleep conditions, the highest pain being experience following SR-L4H. My findings are in agreement to the accumulating evidence on the reciprocal relationship between sleep disruption and pain; such that sleep disruption increases the perception of experimental pain (Matre et al., 2015; Smith & Haythornthwaite, 2004). A

study done on eighteen healthy individuals (6 females, 12 males) investigated the effect of 4 hour sleep restriction over 12 days on pain sensitivity, and found that pain sensitivity and general bodily discomfort was increased after 12 days of sleeping for only 4 hours per night (Haack et al., 2007). However, another study done on ten healthy men found contradictory results, reporting that after 3 nights of N3 sleep restriction did not produce any changes in the pain perception in the jaw muscles (Arima et al., 2001).

I used a standardized and validated method to induce experimental ischaemia (Maixner, 1990) and was surprised to find an "order" effect, regardless of sleep condition. In particular, pain perception was consistently lower with subsequent exposure to the pain procedure. As such, I found there to be a habituation to the painful ischaemia. Habituation to pain has been previously reported in pain perception induced by electrical stimulation (Condes-Lara et al., 1981) and laser evoked potentials (Valeriani et al., 2003). Of particular interest, a study using repeated exposure to a pressure cuff inducing ischaemia in the non-dominant leg of twenty healthy young men also reported habituation with repeated testing (Hoegh et al., 2018). A study of similar design to mine (but using thermal pain tests), investigating the effect of sleep restriction on pain perception, that had randomized nights also found a habituation effect to thermal (heat and cold pressor) and spontaneous pain over the testing period (Simpson et al., 2018). These findings suggest a reduced sensitization to experimental pain, with repeated exposure to the same test, despite several days (on average 5 days in my study) in between experimental exposure. It is important to note that this is somewhat contradictory to what appears to occur in the clinical setting, particularly with regards to repeated exposure to noxious stimuli leading to central sensitization (Woolf, 2011). Central sensitisation is a response to tissue injury and repair and can also be defined as an abnormal augmentation of pain by mechanisms within the central nervous system (CNS), and therefore represents a state

where the response to normal peripheral inputs is greatly enhanced, and hence, increased sensitivity to pain (Woolf, 2011; Nijs et al., 2010).

Furthermore, I found a main effect of *time*, defined as the 1-minute intervals during the 10-minute ischaemic test period. As time increased from time 0 to time 10 (minutes) during the submaximal effort tourniquet test, the pain intensity reported by the participants on the visual analogue scale increased. A study using two different kinds of tourniquets (narrow and wide) reported the same trajectory, where pain increased over the testing time although the pain increased more rapidly in the group using the wider cuff (Estebe et al., 2000). Others, using the identical experimental ischaemia test in healthy women, have reported the opposite pattern in the pain trajectory, where pain intensity decreased over time, and hence, participants became habituated to the ischaemia (Iacovides et al., 2017). Theories on the mechanism underlying tourniquet-induced ischaemic pain support the role of C-fibres in the transmission of pain (slow response), while A-fibre conduction is believed to be abolished during an ischaemic event (Loram et al., 2007; Chabel et al., 1990). It could be that the ischaemic pain intensifies with the prolongation of the ischaemic event and maybe more C fibers are recruited with time during the test. Alternatively, it may also be caused by temporary central sensitization. Central sensitization is defined by changes in neuronal activity which can be temporary or permanent; it is induced after intense, repeated and sustained nociceptive stimuli (Bliddal & Danneskiod-Samsoe, 2007). These neuronal changes are triggered by dorsal horn neurons activity in response to the nociceptor stimuli relayed by C fibers. During central sensitization, density and activity of specific receptors increases, leading to post-synaptic hyperexcitability. Additionally, C fibers are excited by metabolites (such as lactic acid and tricarboxylic acid) that accumulate during ischaemia and over time, the build up of these metabolites may result in increased pain perception as more C fibers are recruited and excited (Queme et al., 2017).

My analyses also showed an effect of *time of day*, such that pain perception later in the day (during afternoon procedures) was lower in intensity than that reported during the morning tests. This finding may be as a result of circadian variation in the perception of, and sensitivity to, pain. Circadian variation is defined as the circadian rhythm regulating when a specific kind of pain is most painful depending on the pathophysiology and tends to peak in the evening in humans (Sondoval et al., 2017; Hagenauer et al., 2017). Circadian variation has been reported previously in various different conditions, cancer (Citron et al., 1992), rheumatoid arthritis (Bellamy et al., 1991) and in postoperative patients (Sondoval et al., 2017).

Surprisingly, contrary to my hypothesis, I found a main effect of *nap*, whereby following an afternoon nap, participants reported higher pain scores compared with the no-nap lie-in afternoon. This was contrary to the limited existing evidence which points to decreased pain perception following two 30-minute naps after severe sleep restriction (Faraut et al., 2015). In particular, Faraut et al (2015) found increased sensitivity to thermal pain following a night of severe sleep restriction, where participants were only allowed a 2h sleep opportunity. Following sleep restriction, participants were allowed to nap for 30 minutes in the morning and 30 minutes in the afternoon. The results showed that the naps attenuated the pain perception, bringing pain perception back in line with those reported following an unrestricted 8h night. It is possible that a single night of 4h sleep restriction in healthy young participants is not enough to increase sensitivity to experimental pain the next morning.

My findings of increased sensitivity to pain following the nap, may be due to the relationship between N3 and adenosine to be discussed thoroughly for the main sleep. It may also be as a result of a lack of REM, as REM has been shown to be protective from pain sensitivity (Onen et al., 2001).

To better tease apart the mechanisms why a nap would increase pain sensitivity, I tested the association between sleep architecture during the nap and pain. I found that N3 and N2 sleep during the nap were associated with higher pain sensitivity. There is large body of literature pointing at adenosine being the mediator of increased sleep pressure; with increased wakefulness leading to increased adenosine in the wake nuclei in the brainstem and sleep decreasing adenosine (for a comprehensive review, see Basheer et al., 2004). However adenosine has been used as an analgesic. I speculated that the decrease in adenosine during recovery sleep of the nap could lead to a lower regulation of pain perception and hence increase pain sensitivity after the nap.

Main Sleep Night Architecture and Pain

Because I found a main effect of condition on pain whereby SR-F4H had lower levels of pain than Baseline or SR-L4H, I further analysed how sleep architecture in itself may be associated with pain perception.

The mixed model pain analysis between sleep variables and pain showed a significant relationship between ischaemic pain and N2 (minutes and proportion), REM (minutes and proportion), TST (minutes) and Sleep Efficiency (percentage). N2, REM, TST and Sleep Efficiency had negative relationships, where increases in these variables resulted in decreased perception of pain reported by the participants the following day. I also found a significant, positive relationship between N3 and pain; such that the greater the proportion of N3 of TST, the greater the perceived pain the next day.

These findings led me to investigate further to answer the question ‘Which sleep variable is the most reliable in determining daytime pain perception after a night of restricted sleep?’ I found N2 to be the most reliable indicator, being a stronger predictor than TST, REM and N3

in the follow up models; N2 remained significant in all the models and always predicted lower pain perception. This finding was interesting seeing as most literature has focused on the effects of TST, REM and N3 on pain perception (Roehrs et al., 2006; Onen et al., 2001; Onen et al., 2001), and not on N2. One such study done using total sleep deprivation and selective sleep restriction (REM restriction, N3 restriction) showed that a decreased TST corresponded with increased mechanical pain perception in healthy subjects, although neither REM nor N3 were significantly associated with that increase (Onen et al., 2001). It seems, from the results of my study, that perhaps N2 has been overlooked in the literature, as an important factor in sleep's hypoalgesic effects. I found that when TST was reduced by 50% during the sleep restriction protocols, % N2 was also reduced by 50%, suggesting that % N2 is directly associated with total sleep time. It is, therefore, possible that where previous studies reported associations between TST and pain (Onen et al., 2001), that it was in fact the proportion of N2 that drove those findings and decreased pain sensitivity in the healthy young men. However, this hypothesis needs to be further investigated.

Studies have shown that increased N3 is associated with decreased pain perception, and hence slow wave sleep is believed to have analgesic properties (Weingarten et al., 2016; Azevedo et al., 2011). These findings are not in agreement with my results. Even after adjusting the mixed model in my study, a positive relationship between pain perception and N3 was maintained, such that increases in pain were associated with increased night time N3 % of TST. The SR-F4H nights had a few more minutes and proportion (non-significant) of N3 compared to SR-L4H and subsequently reported higher pain scores. One could speculate on whether this effect of N3 is stronger than the protective effect of REM on pain, or whether the higher pain reported is as a result of the decreased REM proportions. Future work should investigate further on this and perhaps explore pain perception in participants that have the same amounts of REM and N3.

Another possible explanation for the results I found concerning the relationship between N3 and pain in this study could be the known relationship between N3 and the analgesic adenosine. Adenosine is believed to have anti-nociceptive properties, and is thought to decrease the perception of pain primarily through its A_1R receptors (Sowynok et al., 2016; Sollevi, 1997). Adenosine is affected by sleep, N3 specifically (Basheer et al, 2004) whereby increased slow wave sleep is associated with decreases in adenosine in the central nervous system. Adenosine has been shown to regulate the wake/sleep cycle by interacting with the main sleep homeostatic agents. Specifically, adenosine inhibits, via the A_1R receptors, the wake-inducing agents (for example, orexin and cells in the basal forebrain) and excites, via the $A_{2A}R$ receptors, the sleep-inducing agents (for example, VLPO (ventrolateral preoptic nucleus) in the hypothalamus) (Bjorness & Greene, 2009). More time spent awake also results in increased build-up of adenosine in the circulation and therefore contributes to accumulation of sleep pressure (Franziska et al., 2016; Bjorness & Greene, 2009). Interestingly, and relevant to this study, adenosine levels are reduced in the basal forebrain, hypothalamus and cortex during sleep (specifically N3), and increased time spent sleeping (and in N3) results in smaller amounts of circulating adenosine thereafter (Bjorness & Greene, 2009). There is little research on this but it could serve as a possible explanation for the relationship we see in our results, I am proposing the possibility that a more deep N3 (minutes and proportion) may decrease adenosine and subsequently result in more pain sensitivity and reduced pain thresholds (Bjorness & Greene, 2009). However, it must be noted that adenosine is also known to exhibit pro-nociceptive properties (Sawynok, 2016) and hence, more research is needed to unravel the complex interactions between adenosine, pain and sleep.

Another possibility is not a direct effect of N3 but maybe the effect a higher amount of N3 has on decreasing N2, the sleep stage that seemed the most associated with decreased pain

perception in my study. I found indeed that higher N3 during the experimental night was associated with lower N2, lower TST and lower REM, which were all variables associated with lower pain perception.

Recovery Sleep

My results on napping and pain did not support my hypothesis, that naps would reduce pain perception. On the contrary, I found that the afternoons with the 30-minute naps had higher pain scores compared to the no-nap, 30-minute lie-in afternoons. This was true for nap afternoons of both sleep restriction types.

My hypothesis was based on the only study, that I am aware of, that has investigated the effects of napping on pain perception following sleep restriction in healthy individuals (Faraut et al., 2015). Following one night of severe sleep restriction (only 2 hours of sleep), the study found that short sleeps following a night of sleep restriction, in particular, two 30-minute naps (one in the morning and one on the afternoon), can reverse sleep-restriction-induced increases in mechanical and thermal (heat) pain sensitivity in healthy males (Faraut et al., 2015). The different protocols employed may explain our contradictory results. In particular, I allowed participants to sleep for 4h, not 2h, I only used one afternoon nap, as opposed to a morning and afternoon nap, and I assessed experimental ischaemia over 10 minutes, whilst they assessed heat, cold and pressure pain sensitivity (Faraut et al., 2015). Also, my protocol did not induce sleep-restricted hyperalgesia to begin with.

Total sleep time (TST), time spent (in minutes) in N2, N3 during the nap, and TimetoNT (defined as the time between the participants being awakened from the night sleep and the sleep onset time of the afternoon nap) were all predictors of pain perception following the

naps. In particular, higher TST, similar to the main nights' analyses, resulted in decreased pain perception. The more time (in minutes) spent in N2 and N3, and the longer the time between morning wake and nap onset, the higher the perceived pain intensity. As such, the association between N3 and pain matches what I found after overnight sleep and morning pain testing. However, my findings of time (in minutes) of N2 during the afternoon nap and afternoon pain testing, contradict what I reported after overnight sleep and next morning pain. TimetoNT was higher during the SR-L4H; during this protocol participants slept the first half of the night and woke up approximately 10 hours before the afternoon nap, whilst TimetoNT was lower in the SR-F4H because they slept the last half of the night and woke up approximately 6 hours before the afternoon nap would take place. The SR-L4h nap afternoons provided more time to build sleep pressure, which has been shown to increase N3/deep sleep ((Dinges, 1986) which may later result in higher sleep inertia upon awakening (Signal et al., 2012). Sleep inertia has previously been linked to impaired performance in task-related and memory tests (Hilditch et al., 2016; Fabbri et al., 2014), and could be linked to changes in pain perception as well. However, the effect of sleep inertia on pain is still insufficiently investigated. One study showed that waking up from REM was associated with decreased thermal pain perception compared to waking up from N2 or N3 (Daya et al, 2010).

Following the results above, I further investigated which sleep variable was a reliable predictor of pain perception in the afternoon conditions. After adjusting these three variables (N2, N3 and TimetoNT) for each other, similar to the morning findings, I found that N3 and TimetoNT were the more significant predictors of pain after the afternoon 30-minute naps. This might be as a result of adenosine as discussed above (Franziska et al., 2016; Bjorness & Greene, 2009). It could also be the result of sleep inertia which, as mentioned, has been linked to N3/deep sleep (Hilditch et al., 2016).

N3 was positively correlated to TimetoNT, such that the longer the time between morning wake and nap onset, the higher the minutes spent in N3 sleep during the nap. In contrast, the longer the time between morning wake and nap onset, the lower the minutes spent in N2 sleep during the nap. These findings are in line with the literature on sleep architecture during afternoon naps, where there is N3 rebound following sleep restriction and a higher N3 compared to N2 in the naps (Jiang et al., 2018; Lee et al., 2017). It is possible, considering the N2 and pain results from the main nights' sleep, that the reason for higher pain scores after the naps is because N2 was sacrificed in the naps to allow for more time for recovery N3 sleep. As such, the protective effect of N2 on pain perception was lost.

Strengths and Limitations

I ran a randomized, cross-over-controlled study, which eliminated inter-person variation in sleep and the subjective perception of pain; as each participant was their own control. Another strength is that I used healthy participants who were thoroughly screened using validated questionnaires for physical and psychological health, minimizing any confounding effects on sleep and pain. In addition to the screening interview, all participants went through a very rigorous week-long screening period, during which they were requested to keep sleep diaries and wear actiwatches. I therefore ensured that each participant had regular sleep/wake cycles throughout the study. The main study limitation is the small sample size, and hence lack of the statistical power.

There were some protocol violations during the screening phase, and sometimes during the 5 days in between the experimental nights. If a participant could not adhere to the restrictions during the screening phase, they were excluded from the study. If they deviated during the 5

day breaks, their break period was extended to normalise their sleeping. The only deviation that occurred in the laboratory during the experimental nights was when participants fell asleep during the restriction periods. This would occur during my bathroom break when I had to leave the participant's room.

Conclusion

I found that a 4 hour sleep restriction (whether first four hours or last four hours) results in significant changes in sleep architecture, particularly in TST, N2 proportion and REM minutes and proportion. The sleep architecture between restriction nights was also similar, except for REM and N3. Sleep restriction in this study did not produce a global effect of increased pain perception compared to baseline levels however the different modalities of sleep restriction allowed me to better understand the association between sleep stages and pain, in particular finding that increased N3 during the night was associated with higher pain perception while increased N2, TST and REM were associated with lower pain perception. The role of N2 in decreasing pain perception is a novel finding. The mechanism through which N3 may increase pain perception however still needs to be further investigated. Furthermore, 30-minute afternoon naps in the afternoon increased pain perception following the sleep restriction protocols. My results directly contradict the only other study to-date, investigating the effects of napping on experimental pain following extreme sleep restriction. However, with further statistical analyses using mixed-models I did establish that the presence of N3 in the naps was directly associated with the pain perception, perhaps shorter naps without N3 would show decreased pain perception. Clearly more work is needed in this field in order to establish any potential restorative effects that napping may have on pain and the length of naps necessary to reap the restorative benefit.

References

Abad V, Sarinas P, Guilleminault C (2008).Sleep and rheumatologic disorders. *Sleep Medicine Reviews* 12, 211–228.

Åkerstedt T, Kecklund G, Ingre M, Lekander M, Axelsson J (2009). Sleep Homeostasis During Repeated Sleep Restriction and Recovery: Support from EEG Dynamics. *Sleep* 32, 217-222.

Almeida T, Roizenblatt S, Tufik S (2004). Afferent pain pathways: a neuroanatomical review. *Brain Research* 1000, 40–56.

Arima T, Svensson P, Rasmussen C, Nielsen K, Drewes A, Arendt-Nielsen L (2001). The relationship between selective sleep deprivation, nocturnal jaw-muscle activity and pain in healthy men. *Journal of Oral Rehabilitation* 28, 140-148.

Arnal P, Sauvet F, Leger D, van Beers P, Bayon V,Bougard C, Rabat A, Millet GY, Chennaoui M (2015). Benefits of sleep extension on sustained attention and sleep pressure before and during total sleep deprivation and recovery. *Sleep* 38 (12), 1935–1943.

Basbaum A, Bautista D, Scherrer G, Julius D (2009). Cellular and Molecular Mechanisms of Pain. *Cell* 139, 267-284.

Basheer R, Strecker R, Thakkar M & McCarley R (2004). Adenosine and sleep–wake regulation. *Progress in Neurobiology* 73, 379-396.

Bellamy N, Sothern R, Campbell J, Buchanan W (1991). Circadian rhythm in pain, stiffness, and manual dexterity in rheumatoid arthritis: relation between discomfort and disability. *Annals of the Rheumatic Diseases* 50, 243-248.

Bjorness T & Greene R (2009). Adenosine and Sleep. *Current Neuropharmacology* 7, 238-245.

Bongi S, Rosso A, Lisa D, Orlandi M & Scisciolo G (2015). Ischemia-hyperpnea test is useful to detect patients with fibromyalgia syndrome. *European Journal of Rheumatology* 2, 89-95.

Bliddal H & Danneskiold-Samsøe B (2007). Chronic widespread pain in the spectrum of rheumatological diseases. *Best Practice & Research Clinical Rheumatology* 21, 391-402

Bonvanie I, Oldehinkel A, Rosmalen J, Janssens K (2016). Sleep problems and pain: a longitudinal cohort study in emerging adults. *Pain* 157, 957–963.

Boulton A, Vinik A, Arezzo J, Bril V, Feldman E, Freeman R, Malik R, Maser R, Sosenko J, Ziegler D (2005). Diabetic Neuropathies: A statement by the American Diabetes Association. *Diabetes Care* 28, 956-962.

Boyden S, Pott M, Starks P (2018). An evolutionary perspective on night terrors. *Evolution, Medicine, and Public Health*, 100-105.

Brooks A & Lack L (2006). A Brief Afternoon Nap Following Nocturnal Sleep Restriction: Which Nap Duration is Most Recuperative? *Sleep* 29, 831-840.

Broussard J, Wroblewski K, Kilkus J, Tasali E (2016). Two Nights of Recovery Sleep Reverses the Effects of Short-term Sleep Restriction on Diabetes Risk. *Diabetes Care* 39, e40-e41.

Buysse D, Reynolds C, Monk T, Berman S, Kupfer D (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research* 28, 193-213.

Cain DM, Khasabov SG, Simone DA (2001). Response properties of mechanoreceptors and nociceptors in mouse glabrous skin: an in vivo study. *Journal of Neurophysiology* 85(4), 1561–1574.

Campbell E, Hynynen J, Ala-Ruona E (2017). Vibroacoustic treatment for chronic pain and mood disorders in a specialized healthcare setting. *Music and medicine* 9, 187-197.

Carli G, Montesano A, Rapezzi S, Paluffi G (1987). Differential effects of persistent nociceptive stimulation on sleep stages. *Behavioural Brain Research* 26, 89-98.

Chabel C, Russell L & Lee R (1990). Tourniquet-Induced Limb Ischemia. *Anesthesiology* 72, 1038-1044.

Chawla J, Park Y & Passaro E (2020). What is the flip-flop switch model of sleep regulation? *Medscapecom*. Available at: https://www.medscape.com/answers/1187829-70502/what-is-the-flip-flop-switch-model-of-sleep-regulation

Chung F, Yegneswaran B, Liao P, Chung S, Vairavanathan S, Islam S, Khajehdehi A & Shapiro C (2008). Validation of the Berlin Questionnaire and American Society of Anesthesiologists Checklist as Screening Tools for Obstructive Sleep Apnea in Surgical Patients. *Anesthesiology* 108, 822-830.

Citron M, Kalra J, Seltzer V, Chen S, Hoffman M, Walczak M (1992). Patient-Controlled Analgesia for Cancer Pain: A Long-Term Study of Inpatient and Outpatient Use. *Cancer Investigation* 10, 335-341.

Condes-Lara M, Calvo J, Fernandez-Guardiola A (1981). Habituation to bearable experimental pain elicited by tooth pulp electrical stimulation. *Pain* 11, 185-200.

Crowley K (2011). Sleep and Sleep Disorders in Older Adults. *Neuropsychology Review* 21, 41-53.

Daya V & Bentley A (2010). Perception of experimental pain is reduced after provoked waking from REM sleep. *Sleep Medicine* 10, S45.

Deatherage J, Roden R, Zouhary K (2009). Normal Sleep Architecture. *Seminars in Orthodontics* 15, 86-87.

Deboer T (2018). Sleep homeostasis and the circadian clock: Do the circadian pacemaker and the sleep homeostat influence each other's functioning? *Neurobiology of Sleep and Circadian Rhythms* 5, 68-77.

Deedwania P & Carbajal E (1990). Silent ischemia during daily life is an independent predictor of mortality in stable angina. *Circulation* 81, 748-756.

Dinges D (1986). Differential effects of prior wakefulness and circadian phase on nap sleep. *Electroencephalography and Clinical Neurophysiology* 64, 224-227.

Dubin A, Patapoutian A (2010). Nociceptors: the sensors of the pain pathway. *Journal of Clinical Investestigations* 120(11), 3760- 3772.

Djouhri L, Lawson SN (2004). A[beta]-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain Research Reviews* 46(2), 131–145.

El Cheikh Hussein L, Mollard P, Bonnefont X (2019). Molecular and Cellular Networks in The Suprachiasmatic Nuclei. *International Journal of Molecular Sciences* 20, 2052.

Estebe J, Le Naoures A, Chemaly L, Ecoffey C (2000). Tourniquet pain in a volunteer study: effect of changes in cuff width and pressure. *Anaesthesia* 55, 21-26.

Fabbri M, Tonetti L, Martoni M, Natale V (2014). Remember to Do: Insomnia Versus Control Groups in a Prospective Memory Task. *Behavioral Sleep Medicine* 13, 231-240.

Fadda P, Tortorella A, Fratta W (1991). Sleep deprivation decreases μ and δ opioid receptor binding in the rat limbic system. *Neuroscience Letters* 129, 315-317.

Faraut B, Léger D, Medkour T, Dubois A, Bayon V, Chennaoui M, Perrot S (2015). Napping Reverses Increased Pain Sensitivity Due to Sleep Restriction. *Plos One* 10, e0117425.

Finan P, Goodin B, Smith M (2013). The Association of Sleep and Pain: An Update and a Path Forward. *The Journal of Pain* 14(12), 1539-1552.

Finan P, Quartana P & Smith M (2015). The Effects of Sleep Continuity Disruption on Positive Mood and Sleep Architecture in Healthy Adults. *Sleep* 38, 1735-1742.

Fitzmaurice G, Laird N & Ware J (2011). Applied Longitudinal Analysis. Wiley, Hoboken.

Fox M (1999). The importance of sleep. *Nursing Standard* 13, 44-47.

Gebhart G (2000). Pathobiology of Visceral Pain: Molecular Mechanisms and Therapeutic Implications IV. Visceral afferent contributions to the pathobiology of visceral pain. *American Journal of Physiology Gastrointestinal Liver Physiology* 278, 834–838.

Goril S & Shapiro C (2011). M-B-044 The prevalence and patterns of sleep disorders and circadian rhythm disruptions in children and adolescents with fetal alcohol spectrum disorders (FASD) . *Sleep Medicine* 12, S34.

Gruber A, Horwood F, Sithole J, Ali N, Idris I (2006). Obstructive sleep apnoea is independently associated with the metabolic syndrome but not insulin resistance state. *Cardiovascular Diabetology* 5(22).

Haack M, Sanchez E &Mullington J (2007). Elevated Inflammatory Markers in Response to Prolonged Sleep Restriction Are Associated With Increased Pain Experience in Healthy Volunteers. *Sleep* 30, 1145-1152.

Haack M, Scott-Sutherland J, Santangelo G, Simpson N, Sethna N &Mullington J (2012). Pain sensitivity and modulation in primary insomnia. *European Journal of Pain* 16, 522-533.

Hayashi M, Motoyoshi N & Hori T (2005). Recuperative Power of a Short Daytime Nap With or Without Stage 2 Sleep. *Sleep* 28, 829-836.

Hirshkowitz M, Whiton K, Albert S, Alessi C, Bruni O,. et al (2015). National Sleep Foundation's sleep time duration recommendations: methodology and results summary. *Sleep Health* 1, 40–43.

Hilditch C, Centofanti S, Dorrian J & Banks S (2016). A 30-Minute, but Not a 10-Minute Nighttime Nap is Associated with Sleep Inertia. *Sleep* 39, 675-685.

Hoegh M, Petersen K & Graven-Nielsen T (2018). Effects of repeated conditioning pain modulation in healthy volunteers. *European Journal of Pain* 22, 1833-1843.

Humo M, Ayazgök B, Becker L, Waltisperger E, Rantamäki T & Yalcin I (2020). Ketamine induces rapid and sustained antidepressant-like effects in chronic pain induced depression: Role of MAPK signaling pathway. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 100, 109898.

Iacovides S, George K, Kamerman P, Baker F (2017). Sleep Fragmentation Hypersensitizes Healthy Young Women to Deep and Superficial Experimental Pain. *Journal of Pain* 18(7), 844-854.

Irwin M, Thompson J, Miller C, Gillin J & Ziegler M (1999). Effects of Sleep and Sleep Deprivation on Catecholamine And Interleukin-2 Levels in Humans: Clinical Implications. *Journal of Clinical Endocrinology & Metabolism* 84, 1979-1985.

Irwin M, Wang M, Campomayor C, Collado-Hidalgo A, Cole S (2006). Sleep Deprivation and Activation of Morning Levels of Cellular and Genomic Markers of Inflammation.*Arch Intern Med* 166, 1756-1762.

Jackson C (2007). The General Health Questionnaire. *Occupational Medicine* 57, 79-79.

Jensen T, Baron R, Haanpää M, Kalso E, Loeser J, Rice A & Treede R (2011). A new definition of neuropathic pain. *Pain* 152, 2204-2205.

Jiang F, Kobayashi T, Ichihashi T & Nomura S (2018). Effect of a relatively long afternoon nap on autonomous nervous activity, sleep architecture, and subjective sleep quality. *IEEJ Transactions on Electrical and Electronic Engineering* 13, 1357-1361.

Kalmbach D, Pillai V, Arnedt J, Drake C (2016). DSM-5 insomnia and short sleep: comorbidity landscape and racial disparities. *Sleep* 39(12), 2101–2111.

Kilkenny T & Grenard S (2020). Sleep Staging and Respiratory Scoring of Polysomnograms. *RT: For Decision Makers in Respiratory Care*. Available at: https://www.rtmagazine.com/disorders-diseases/sleep-medicine/sleep-staging-and-respiratory-scoring-of-polysomnograms

Kim H, Hong E, Kim J & Lee W (2004). Solution Structure of Human Orexin-A: Regulator of Appetite and Wakefulness. *BMB Reports* 37, 565-573.

Koo D, Shin J, Lim J, Seong J & Joo E (2017). Changes in subcortical shape and cognitive function in patients with chronic insomnia. *Sleep Medicine* 35, 23-26.

Kosek E, Cohen M, Baron R, Gerbhart G, Mico J, Rice A, Rief W & Sluka K (2016). Do we need a third mechanistic descriptor for chronic pain states?. *Pain* 157, 1832-1838.

Kristiansen E, Nielsen L, Christensen S, Botvid S, Nørgaard Poulsen J & Gazerani P (2017). Sleep deprivation sensitizes human craniofacial muscles. *Somatosensory & Motor Research* 34, 116-122.

Kundermann B, Krieg J-C, Schreiber W, Lautenbacher S (2004). The effect of sleep deprivation on pain. *Pain Research Management* 9(1), 25-32.

Lau B & Vaughan C (2014). Descending modulation of pain: the GABA disinhibition hypothesis of analgesia. *Current Opinion in Neurobiology* 29, 159-164.

Lautenbacher S, Kundermann B, Krieg J (2006). Sleep deprivation and pain perception. *Sleep Medicine Reviews* 10, 357-369.

Lee T, Chang P, Tseng I, Chung M (2017). Nocturnal sleep mediates the relationship between morningness–eveningness preference and the sleep architecture of afternoon naps in university students. *Plos One* 12, e0185616.

Lewis T (1938). Study of Somatic Pain. *BMJ* 1, 321-325.

Li Y, Vgontzas A, Fernandez-Mendoza J, Bixler E, Sun Y, Zhou J, Ren R, Li J, Tang X (2015). Insomnia With Physiological Hyperarousal Is Associated With Hypertension. *Hypertension* 65, 644-650.

Liedman R, Grant L, Igidbashian S, James I, Mcleod A, Skillern L & Åkerlund M (2006). Intrauterine pressure, ischemia markers, and experienced pain during administration of a

vasopressin V1a receptor antagonist in spontaneous and vasopressin-induced dysmenorrhea. *Acta Obstetricia et Gynecologica Scandinavica* 85, 207-211.

Loram L, Mitchell D, Skosana M, Fick L (2007). Tramadol is more effective than morphine and amitriptyline against ischaemic pain but not thermal pain in rats. *Pharmacological Research* 56, 80-85.

Maclver M & Tanelian D (1992). Activation of C Fibers by Metabolic Perturbations Associated with Tourniquet Ischemia. *Anesthesiology* 76, 617-623.

Maixner W, Gracely RH, Zuniga JR, Humphrey CB & Bloodworth GR (1990). Cardiovascular and sensory responses to forearm ischemia and dynamic hand exercise. *American Journal of Physiology* 259, 1165-1163.

Matre D, Hu L, Viken L, Hjelle I, Wigemyr M, Knardahl S, Sand T & Nilsen K (2015). Experimental Sleep Restriction Facilitates Pain and Electrically Induced Cortical Responses. *Sleep* 38, 1607-1617.

Mazarakis T (2014). A case of adult night terrors. *Tzu Chi Medical Journal* 26, 138-140.

McMahon S, Dmitrieva N, Koltzenburg M (1995). Visceral pain. *British Journal of Anaesthesia* 75, 132–144.

McNeil J, Forest G, Hintze L, Brunet J, Finlayson G, Blundell J, Doucet É (2017). The effects of partial sleep restriction and altered sleep timing on appetite and food reward. *Appetite* 109, 48-56.

Medic G, Wille M & Hemels M (2017). Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep* Volume 9, 151-161.

Mikkelsson M, Sourander A, Salminen J, Kautiainen H, Piha J (1999). Widespread pain and neck pain in schoolchildren. A prospective one-year follow-up study. *Acta Paediatrica* 88, 1119-1124.

Mileykovskiy B, Kiyashchenko L & Siegel J (2005). Behavioral Correlates of Activity in Identified Hypocretin/Orexin Neurons. *Neuron* 46, 787-798.

Millan MJ (1999). The induction of pain: an integrative review. *Progress in Neurobiology* 57(1), 1–164.

Millecamps M & Coderre T (2008). Rats with chronic post-ischemia pain exhibit an analgesic sensitivity profile similar to human patients with complex regional pain syndrome — type I. *European Journal of Pharmacology* 583, 97-102.

Milner CE, Cote KA (2009). Benefits of napping in healthy adults: impact of nap length, time of day, age, and experience with napping. *Journal of sleep research* 18, 272-281.

Moldofsky H & Scarisbrick P (1976). Induction of Neurasthenic Musculoskeletal Pain Syndrome by Selective Sleep Stage Deprivation. *Psychosomatic Medicine* 38, 35-44.

Moldofsky H (2001). Sleep and pain. *Sleep Medicine Reviews* 5, 385-396.

Nijs J, Van Houdenhove B & Oostendorp R (2010). Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. *Manual Therapy* 15, 135-141.

Ohayon M, Priest R, Zulley J, Smirne S, Paiva T (2002). Prevalence of narcolepsy symptomatology and diagnosis in the European general population. *Neurology* 58, 1826-1833

Ohayon M (2011). Epidemiological Overview of Sleep Disorders in the General Population. *Sleep Medical Research* 2, 1-9.

Onen S, Alloui A, Jourdan D, Eschalier A & Dubray C (2001). Effects of rapid eye movement (REM) sleep deprivation on pain sensitivity in the rat. *Brain Research* 900, 261-267.

Onen S, Alloui A, Gross A, Eschallier A, Dubray C (2001). The effects of total sleep deprivation, selective sleep interruption and sleep recovery on pain tolerance thresholds in healthy subjects. *Journal of Sleep Research* 10, 35-42.

Ossipov M, Morimura K & Porreca F (2014). Descending pain modulation and chronification of pain. *Current Opinions Support Palliative Care* 8, 143–151.

Phillips B, Young T, Finn L, Asher K, Hening W & Purvis C (2000). Epidemiology of Restless Legs Symptoms in Adults. *Archives of Internal Medicine* 160, 2137.

Queme L, Ross J & Jankowski M (2017). Peripheral Mechanisms of Ischemic Myalgia. *Frontiers in Cellular Neuroscience*; DOI: 10.3389/fncel.2017.00419.

Raja S, Carr D, Cohen M, Finnerup N, Flor H, Gibson S, Keefe F, Mogil J, Ringkamp M, Sluka K, Song X, Stevens B, Sullivan M, Tutelman P, Ushida T & Vader K (2020). The revised International Association for the Study of Pain definition of pain. *Pain* 161, 1976-1982.

Reynolds A & Banks S (2010). Total sleep deprivation, chronic sleep restriction and sleep disruption. *Progress in Brain Research* 185, 91-101.

Ricardo S, David B, Randall S, Roman C (2017). Circadian Variation of Pain as a Measure of the Analgesia Requirements during the First 24-Postoperative Hours in Patients Using an Opioid Patient Controlled Analgesia Delivery System. *Chronic Pain Management*, J103.

Rijo-Ferreira F, Carvalho T, Afonso C, Sanches-Vaz M, Costa R, Figueiredo L & Takahashi J (2018). Sleeping sickness is a circadian disorder. *Nature Communications*; DOI: 10.1038/s41467-017-02484-2.

Roehrs T (2000). Sleep physiology and pathophysiology. *Clinical Cornerstone* 2, 1-12.

Roehrs T, Hyde M, Blaisdell B, Greenwald M & Roth T (2006). Sleep Loss and REM Sleep Loss are Hyperalgesic. *Sleep* 29, 145-151.

Rupp T, Wesensten N, Bliese P, Balkin T (2009). Banking Sleep: Realization of Benefits During Subsequent Sleep Restriction and Recovery. *Sleep* 32, 311-321.

Samson W & Resch Z (2000). The Hypocretin/Orexin Story. *Trends in Endocrinology & Metabolism* 11, 257-262.

Saper C, Chou T & Scammell T (2001). The sleep switch: hypothalamic control of sleep and wakefulness. *Trends in Neurosciences* 24, 726-731.

Sateia M (2014). International Classification of Sleep Disorders-Third Edition. *Chest* 146, 1387-1394.

Sawynok J (2016). Adenosine receptor targets for pain. *Neuroscience* 338, 1-18.

Scammell T, Arrigoni E & Lipton J (2017). Neural Circuitry of Wakefulness and Sleep. *Neuron* 93, 747-765.

Schabus M, Gruber G, Parapatics S, Sauter C, Klösch G, Anderer P, Klimesch W, Saletu B, Zeitlhofer J (2004). Sleep Spindles and Their Significance for Declarative Memory Consolidation. *Sleep* 27, 1479-1485.

Shapiro C, Bortz R, Mitchell D, Bartel P, Jooste P (1981). Slow-wave sleep: a recovery period after exercise. *Science* 214, 1253-1254.

Signal T, van den Berg M, Mulrine H,. Gander P (2012). Duration of Sleep Inertia after Napping during Simulated Night Work and in Extended Operations. *Chronobiology International* 29 (6), 769-779.

Simpson N, Scott-Sutherland J, Gautam S, Sethna N, Haack M (2018). Chronic exposure to insufficient sleep alters processes of pain habituation and sensitization. *Pain* 159, 33-40.

Sivertsen B, Lallukkad T, Petrie K, Steingrımsdottir O, Stubhaug A, Nielsen C (2015). Sleep and pain sensitivity in adults. *Pain* 156, 1433–1439.

Smith M & Haythornthwaite J (2004). How do sleep disturbance and chronic pain inter-relate? Insights from the longitudinal and cognitive-behavioral clinical trials literature. *Sleep Medicine Reviews* 8, 119-132.

Smith M, Edwards R, McCann U, Haythornthwaite J (2007). The effects of sleep deprivation on pain inhibition and spontaneous pain in women. *Sleep* 30(4), 494-505.

Smith M, Klick B, Kozachik S, Edwards R, Holavanahalli R, Wiechman S, Blakeney P, Lezotte D, Fauerbach J (2008). Sleep onset insomnia symptoms during hospitalization for major burn injury predict chronic pain. *Pain* 138, 497-506.

Smith M, Quartana P, Okonkwo R & Nasir A (2009). Mechanisms by which sleep disturbance contributes to osteoarthritis pain: A conceptual model. *Current Pain and Headache Reports* 13, 447-454.

Soldatos C, Allaert F, Ohta T, Dikeos D (2005). How do individuals sleep around the world? Results from a single-day survey in ten countries. *Sleep Medicine* 6, 5-13.

Sollevi A (1997). Adenosine for pain control. *Acta Anaesthesiologica Scandinavica* 41, 135-136.

Stallman H & Kohler M (2016). Prevalence of Sleepwalking: A Systematic Review and Meta-Analysis. *Plos One* 11, e0164769.

Thompson A, Jones B, Thornburg J (2017). *U.S. Army Military Review.*

Treede R., Rief W, Barke A, Aziz Q, Bennett M, Benoliel R, Cohen M, Evers S, Finnerup N, First M, Giamberardino M, Kaasa S, Korwisi B, Kosek E, Lavand'homme P, Nicholas M, Perrot A, Schug S, Smith B, Svensson P, Vlaeyen J & Wang S (2019). Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain* 160, 19-27.

Trotti L (2010). REM Sleep Behaviour Disorder in Older Individuals: Epidemiology, Pathophysiology, and Management. *Drugs Aging* 27(6), 457–470.

Troxel W, Buysee D, Matthews K, Kip K, Strollo P, Hall M, Drumheller O, Reis S (2010). Sleep symptoms predict the development of the metabolic syndrome. *Sleep* 33(12), 1633-1640.

Ukponmwan O, Rupreht J & Dzoljic M (1986). An analgesic effect of enkephalinase inhibition is modulated by monoamine oxidase-B and REM sleep deprivations. *Naunyn-Schmiedeberg's Archives of Pharmacology* 332, 376-379.

Valeriani M, de Tommaso M, Restuccia D, Le Pera D, Guido M, Iannetti D, Libro G, Truini A, Di Trapani G, Puca F, Tonali P & Cruccu G (2003). Reduced habituation to experimental pain in migraine patients: a CO2 laser evoked potential study. *Pain* 105, 57-64.

Verma V, Sheikh Z & Ahmed A (2015). Nociception and role of immune system in pain. *Acta Neurologica Belgica* 115, 213-220.

Vladyslav V (2015). Sleep, recovery, and metaregulation: explaining the benefits of sleep. *Nature and Science of Sleep* 7, 171–184.

Wallace D, Ramos A, Rundek T (2012). Sleep disorders and stroke. *International Journal of Stroke* 7, 231–242.

Wehrens S, Hampton S, Skene D (2012). Heart rate variability and endothelial function after sleep deprivation and recovery sleep among male shift ad non-shift workers. *Scandanavian Journal of Work Environmental Health* 38(2), 171-181.

Weingarten J, Dubrovsky B, Basner R, Redline S, George L & Lederer D (2016). Polysomnographic Measurement of Sleep Duration and Bodily Pain Perception in the Sleep Heart Health Study. *Sleep* 39, 1583-1589.

Wesesten N, Balkin J, Belenky G (1999). Does sleep fragmentation impact recuperation? A review and reanalysis. *Journal of Sleep Research* 8, 237–245.

Wolk R, Somers V (2006). Sleep and the metabolic syndrome. *Experimental Physiology* 92(1), 67-78.

Woolf C (1995). Somatic pain—pathogenesis and prevention. *British Journal of Anaesthesia* 75, 169–176

Woolf C (2010). What is this thing called pain? *Journal of Clinical Investigations* 120(11), 3742-3744.

Woolf C (2011). Central sensitization: Implications for the diagnosis and treatment of pain. *Pain* 152, S2-S15.

Wright K, Drake A, Frey D, Fleshner M, Desouza C, Gronfier C, Czeisler C (2015). Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. *Brain, Behavior, and Immunity* 47, 24-34

Wu H, Stone W, Hsi X, Zhang J, Huang L, Yin Y, Zhang L, Zhao Z (2010). Effects of Different Sleep Restriction Protocols on Sleep Architecture and Daytime Vigilance in Healthy Men. *Physiological Research* 59, 821-829.

Wu M, Lin H, Weng S, Ho C, Wang J, Hsu Y (2014). Insomnia Subtypes and the Subsequent Risks of Stroke Report From a Nationally Representative Cohort. *Stroke* 45, 1349-1354.

Yen C, Lu P (2013). Thalamus and pain. *Acta Anaesthesiologica Taiwanica* 51, 73-80.

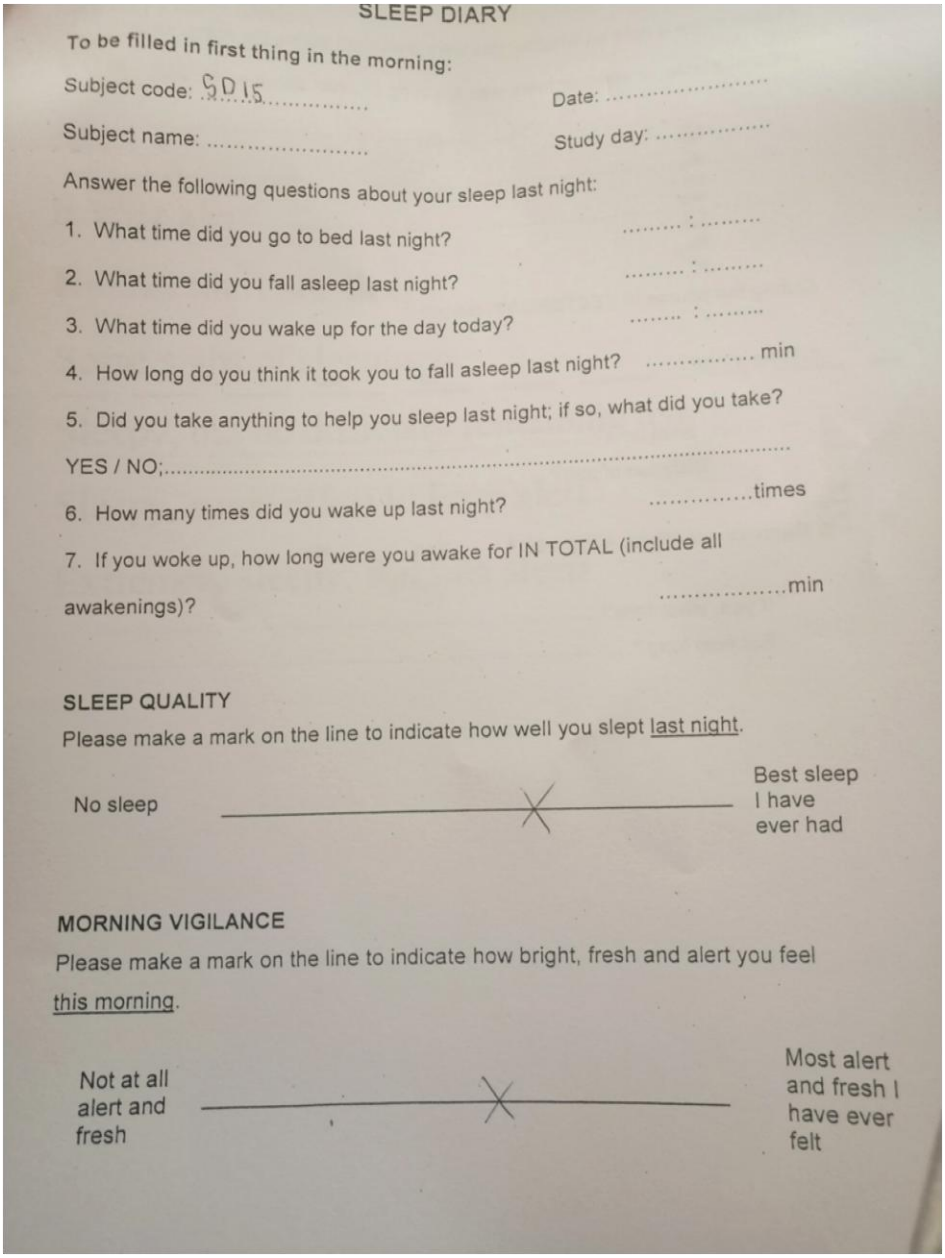
Zhang J, Ma R, Kong A, So W, Li A, Lam S, Li S, Yu M, Ho C, Chan M, Zhang B, Wing Y (2011). Relationship of Sleep Quantity and Quality with 24-Hour Urinary Catecholamines and Salivary Awakening Cortisol in Healthy Middle-Aged Adults. *Sleep* 34, 225-233.

Zhuo M (2016). Neural Mechanisms Underlying Anxiety–Chronic Pain Interactions. *Trends in Neurosciences* 39, 136-145.

APPENDICES

Appendix A: Screening

Sleep Diary



SLEEP DIARY

To be filled in first thing in the morning:

Subject code: SD15 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

Figure 1 An example of the sleep diary I used during screening. The participants were expected to fill in their study code (as seen above), the study day and date. They also had to fill in and answer 1-7 describing their sleep.

Actigraphy Data

Table 1 *Showing the average bed time, wake time, total sleep time and sleep efficiency as collected from the actiwatches during the length of the study.*

Participant	Average Sleep Time-Wake Time (hh:mm)	Average Time In Bed (minutes)	Average Total Sleep Time (minutes)	Average Sleep Efficiency (%)
1 Actigraphy	00:00-08:30	515	450	80
Sleep Diary				-
2 Actigraphy	-	-	-	-
Sleep Diary				
3 Actigraphy	23:50-07:38	449	398	87
Sleep Diary				-
4 Actigraphy	23:36-07:08	451	346	76
Sleep Diary				-
5 Actigraphy	00:30-06:51	535	396	74
Sleep Diary				-
6 Actigraphy	00:16-10:19	602	522	84
Sleep Diary				-
7 Actigraphy	22:47-06:43	476	351	73
Sleep Diary				-
8 Actigraphy	00:25-07:08	403	291	70
Sleep Diary				-
9 Actigraphy	00:30-09:35	521	400	75
Sleep Diary				-
10 Actigraphy	22:02-06:58	535	436	81
Sleep Diary				-
11 Actigraphy	22:36-06:36	480	381	79
Sleep Diary				-

Appendix B: Sleep reports

4h Sleep report

Patient: F4HNap **ID:** SD15 **DOB:**
Study Date: 2018/08/22 **Technician:**
Report created: 2019/11/01 **Scored by:** Sihle Dakile

Sleep Staging Data

Recording Start (4.2 hrs.)	10:06 PM	Total Sleep Time	251.0 min.
Lights Out	02:33 AM	Wake Before Sleep	2.0 min.
Sleep Onset	02:35 AM	Wake During Sleep	4.5 min.
Lights On	06:51 AM	Wake After Sleep	0.0 min.
Recording End	06:59 AM	Total Wake Time	6.5 min.
Recording Time	533.5 min. (8.9 hrs.)	REM Time	94.0 min.
Time In Bed (2.6 hrs.)	258.0 min. (4.3 hrs.)	NREM Time	157.0 min.
Sleep Period Time	256.0 min. (4.3 hrs.)	Slow Wave Sleep Time	70.5 min.
		Movement Time	0.0 min.
Sleep Onset	2.0 min.	Epochs In Bed	516
REM Latency	48.5 min.	Epochs of Total Sleep Time	502

Latency to Stage 1	30.0 min. (from Sleep Onset)
Latency to Stage 2	0.0 min. (from Sleep Onset)
Latency to Stage 3	0.5 min. (from Sleep Onset)
Latency to Stage 4	--- (from Sleep Onset)

	Time (min.)	% TST	% TIB
Stage 1	7.0	3	3
Stage 2	79.5	32	31
Stage 3	70.5	28	27
Stage 4	0.0	0	0
REM	94.0	37	36
Awake	6.5	---	3
Movement Time	0.0	---	0

Sleep Efficiency 1	97% (100 x TST/TIB)
Sleep Efficiency 2	98% (100 x TST/SPT)
Total Stage Changes	49
Stage Changes per Hour of Sleep	11.7

8h Sleep report

Patient: F4HNap **ID:** SD15 **DOB:**
Study Date: 2018/08/22 **Technician:**
Report created: 2019/09/10 **Scored by:** Sihle Dakile

Sleep Staging Data

Recording Start (4.9 hrs.)	10:06 PM	Total Sleep Time	295.5 min.
Lights Out	10:32 PM	Wake Before Sleep	71.5 min.
Sleep Onset (2.1 hrs.)	11:45 PM	Wake During Sleep	126.0 min.
Lights On	06:52 AM	Wake After Sleep	0.5 min.
Recording End (3.3 hrs.)	06:59 AM	Total Wake Time	198.0 min.
Recording Time	533.5 min. (8.9 hrs.)	REM Time	94.0 min.
Time In Bed (3.4 hrs.)	494.0 min. (8.2 hrs.)	NREM Time	201.5 min.
Sleep Period Time	421.0 min. (7.0 hrs.)	Slow Wave Sleep Time	80.0 min.
		Movement Time	0.0 min.
Sleep Onset	72.5 min.	Epochs In Bed	988
REM Latency	213.5 min. (3.6 hrs.)	Epochs of Total Sleep Time	591

Latency to Stage 1	6.0 min. (from Sleep Onset)
Latency to Stage 2	0.0 min. (from Sleep Onset)
Latency to Stage 3	64.0 min. (from Sleep Onset)
Latency to Stage 4	--- (from Sleep Onset)

	Time (min.)	% TST	% TIB
Stage 1	20.0	7	4
Stage 2	101.5	34	21
Stage 3	80.0	27	16
Stage 4	0.0	0	0
REM	94.0	32	19
Awake	198.0	---	40
Movement Time	0.0	---	0

Sleep Efficiency 1	60% (100 x TST/TIB)
Sleep Efficiency 2	70% (100 x TST/SPT)
Total Stage Changes	88
Stage Changes per Hour of Sleep	17.9

Appendix C: Results

Table 1 *Illustrating the results in* Table 3.2.3 *in more detail, including the posthoc p values showing the differences between Baseline, SR-L4H and SR-F4H.*

	Baseline	L4H	F4H	P	Adj P (Ba*L)	Adj P (Ba*F)	Adj P (L*F)
Awake %	6[a] [5-9]	28[b] [11-45]	32.3[b] [17-45]	<.0001	0.02	0.01	0.97
TST (min)	454[a] [433-459]	277[b] [225-364]	271[b] [229-355]	<.0001	0.0003	0.0002	0.97
Sleep Efficiency %	94[a] [91-95]	54[b] [48-75]	60[b] [49-74]	<.0001	0.001	0.002	0.97
Awake min	30[a] [25-45]	127[b] [61-226]	143[b] [68-230]	<.0001	0.03	0.02	0.98
N1 %	6[a] [5-8]	8[a] [5-11]	7[a] [5-10]	<.0001	0.58	0.79	0.91
N1min	26[a] [22-35]	21[a] [12-38]	25[a] [12-31]	<.0001	0.78	0.58	0.89
N2 %	50[a] [44-56]	50[a] [41-57]	44[a] [41-52]	<.0001	0.95	0.48	0.52
N2 min	215[a] [192-223]	145[b] [99-206]	113[b] [98-168]	<.0001	0.004	0.001	0.84
SWS %	21[a] [20-27]	25[a] [20-36]	30[a] [22-35]	<.0001	0.21	0.36	0.91
SWS min	99[a] [87-125]	72[a] [55-91]	75[b] [66-84]	<.0001	0.07	0.05	0.98
REM %	22[a] [18-24]	13[b] [10-16]	22[c] [14-26]	<.0001	0.01	0.89	0.01
REM min	97[a] [83-109]	37[b] [24-52]	54[c] [40-76]	<.0001	<0.0001	0.002	0.03
Arousal Index	7.8 (5.6-8.3)	6.8 (4.9-9.9)	5.1 (3.9-6)	<.0001	0.96	0.87	0.64
WASO	12[a] [9-19]	4[b] [3-7]	7[a] [2-12]	<.0001	0.01	0.04	0.82

Sleep Latency	16[a] [10-23]	17[a] [11-25]	6[b] [3-11]	<.0001	0.94	0.01	0.002

Table 2 *Illustrating the results in* Table 3.4.4 *in more detail, including the main effects adjusted for during the analyses.*

	Time	**ToD**	**Order**	**Nap**	**Estimate**	**Standard Error**	**P**
Awake min	<.0001	0.003	<.0001	<.0001	0.002	0.01	0.68
Awake %	<.0001	0.003	<.0001	<.0001	0.02	0.03	0.52
N1 min	<.0001	0.003	<.0001	<.0001	0.07	0.12	0.05
N1 %	<.0001	0.005	<.0001	<.0001	0.47	0.04	0.0001
N2 min	<.0001	0.004	<.0001	<.0001	-0.02	0.01	0.009
N2 %	<.0001	0.003	<.0001	<.0001	-0.17	0.06	0.003
SWS min	<.0001	0.003	<.0001	<.0001	-0.0006	0.02	0.98
SWS %	<.0001	0.003	<.0001	<.0001	0.12	0.05	0.009
REM min	<.0001	0.003	<.0001	<.0001	-0.05	0.02	0.002
REM %	<.0001	0.002	<.0001	<.0001	-0.21	0.08	0.005
TST	<.0001	0.003	<.0001	<.0001	-0.01	0.01	0.03
Sleep Onset Latency	<.0001	0.002	<.0001	<.0001	0.01	0.01	0.17
WASO	<.0001	0.002	<.0001	<.0001	-0.004	0.01	0.68
Sleep Efficiency	<.0001	0.004	<.0001	<.0001	-0.05	0.03	0.07
Arousal Index	<.0001	0.003	<.0001	<.0001	0.12	0.17	0.49