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THE EFFECT OF HYPERTONIC SALINE INFUSION ON SLEEP ARCHITECTURE IN HUMANS

by

Dyana Siebert

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science

Johannesburg, 2010

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in the Faculty of Science at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other University.

_____

Dyana Siebert

Signed in Johannesburg on this the _____day of _____2010

ABSTRACT

Many patients with chronic pain complain of sleep problems. However, the relationship between sleep and pain in these patients is still not fully understood. Experimental models are used to understand the interaction between pain and sleep, but up until now all such models have been of short duration and assessed different end-points of sleep disruption. The aim of the study is to develop a longer-acting model to mimic the muscle pain seen in patients with chronic pain, assess whether the end-point of sleep disruption is constant over subjects and assess whether the disruption is dependent on length of stimulation and/or the sleep stage involved. Twelve healthy male subjects participated in the study. They were exposed to multiple hypertonic and isotonic saline infusions, for a duration of ten minutes, both while awake and during all stages of sleep. The muscle pain intensity and quality during wakefulness were assessed using the Visual Analogue Scale (VAS) and McGill Pain Questionnaire. Polysomnographic signals were recorded to score sleep changes during all sleep stages. During wakefulness hypertonic saline infusions produce significantly greater VAS Scores than isotonic saline infusions. Differences between the VAS scores from evening to morning were non-significant, therefore implying that there was no overnight hyperalgesia after any of the experimental stimulations. When compared to the isotonic saline infusions, the noxious hypertonic saline infusions triggered significantly more microarousals during REM (67% of subjects); more sleep stage shifts in SWS (42% of subjects) and more full arousals during stage 2 (83% of subjects), SWS (67% of subjects) and REM (67% of subjects) sleep. The data suggests that pain during sleep triggers multiple different end-points of sleep

disruption during sleep and the specific end-points may be determined by the sleep stage involved. The sleep disturbances found in our model of experimental pain may be similar to those found in patients with chronic muscle pain.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor, Alison Bentley for all of her encouragement and help throughout the project. I would also like to thank my parents, Mark and Rafia Davids, for their unconditional support especially during my masters. Thank you to the National Research Foundation and The Wits Dial-a-Bed Sleep Laboratory for funding my project. Lastly, to Dean, for constantly motivating me and always being there for me.

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CONFERENCE PROCEEDINGS

I presented part of my masters project in a presentation titled, “The effect of hypertonic saline infusion on sleep architecture in humans”, at the 2006 Physiological Society of Southern Africa Conference in Durban.

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DEFINITIONS

α	alpha intrusion
CNS	central nervous system
DOMS	delayed onset muscle soreness
EEG	electroencephalography
EMG	electromyography
EOG	electro-oculography
FA	full arousal
FMS	fibromyalgia syndrome
GHQ	General Health Questionnaire
HGH	human growth hormone
HS	hypertonic saline
MA	microarousal
MPQ	McGill Pain Questionnaire
NREM	non-rapid eye movement
PRI	Pain Rating Index
PSQI	Pittsburg Sleep Quality index
REM	rapid eye movement
SSS	sleep stage shift
SWS	slow wave sleep
VAS	Visual Analogue Scale

Chapter 1

Literature Review

1.1 Introduction

Pain, by definition, is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Merskey and Bogduk 1994). In other words, pain is not only described as being noxious, but rather it is also an emotional experience. Pain is so important to survival that it's processing in the brain does not occur in one distinct area (Ranney 1996). Pain affects multiple areas and pathways in the brain, such as the brainstem, cortical areas and limbic system (Nofzinger and Derbyshire 2007). Emotion is permanently involved in all pain because the limbic system, which influences and creates emotion, is always affected in pain. Hence, pain is a subjective phenomenon – the same painful stimulus experienced can be interpreted and expressed in many ways by different people.

Pain, whether acute or chronic, can also be classified according to the location of the pain, the organ system affected, the cause of the pain and how long the pain has persisted (Sessle 2007). In this literature review I will be concentrating on musculoskeletal pain. The increasing prevalence of musculoskeletal pain has been described as an epidemic and insight is needed to understand the condition (Main and Williams 2007).

1.2 Musculoskeletal pain

Musculoskeletal pain is a common complaint among patients with chronic pain and originates in skeletal muscle, tendons and surrounding fascia (Mense 1993). Acute pain exists as a protective measure to warn against damage, whereas some types of chronic pain serves no known function and becomes a disease which affects many aspects of the lives of patients that experience or live with chronic pain (Sessle 2007). A literature review on the prevalence of pain conducted in 2002 revealed the prevalence rates of chronic muscle pain varying from 11.5% to 55.2% (Ospina and Harstall 2002).

Despite extensive research done in the field of musculoskeletal pain, chronic myalgia (muscle pain) remains a problem due to the lack of knowledge of the prevention and treatment of peripheral and more so the central sensitisation observed in patients with chronic muscle pain (Arendt-Nielsen and Svensson 2001).

Musculoskeletal pain is often characterized by the local and referred pain as well as hyperalgesia (Graven-Nielsen et al. 2002). The local muscle pain is the area of muscle where the nociceptors are activated, while the referred pain occurs in an area discontinuous or away from the local pain area (Graven-Nielsen et al. 1997b). An example of referred pain is the model of tibialis anterior direct pain and referred pain, where hypertonic saline injected into the muscle induces a pain in the anteromedial aspect of the ankle (see figure 1) (Travell and Simons 1993).

The referral patterns from the muscles stimulated are characteristic of that muscle and are fairly consistent and reproducible (Travell and Simons 1993) Hyperalgesia is defined as the increased sensitivity to a normally noxious stimulus that an organism may experience after inflammation, injury, or other physiological change that lowers the threshold to pain (Kehl and Fairbanks 2003).

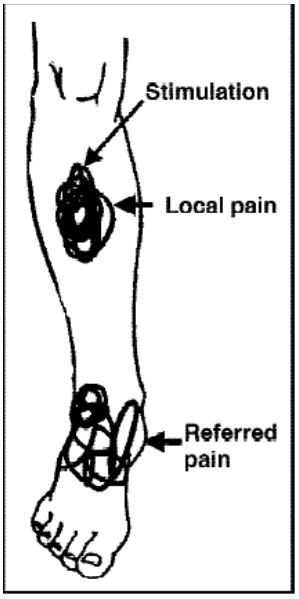


Figure 1. The distribution of local and referred muscle pain after stimulation of the anterior tibilais muscle.

(Arendt-Nielsen and Svensson 2001)

Pain is complex and subjective, and therefore becomes very difficult to assess objectively. When assessing muscle pain, it is preferred that the tests are multimodal and are able to evaluate both the intensity and type of pain (Graven-Nielsen and Arendt-Nielsen 2003). Multimodal assessments on pain result in a more conclusive and thorough result as opposed to a single input assessment which would generate a very limited part of pain perception. Questionnaires using numeric scales and words as descriptors are typically used to assess pain in adults

(Lavigne et al. 2007). A Visual Analogue Scale (VAS) is a numeric scale which consists of a 0 - 100mm line where 0mm indicates “no pain” and 100mm indicates “worst pain ever felt” (see Figure 2). The McGill Pain Questionnaire (MPQ) consists primarily of three major classes of word descriptors: sensory; affective; and evaluative (Melzack 1975). The sensory words describe the sensory qualities of the experience, the affective words describe the tension and fear that are part of the painful experience, and the evaluative words describe the subjective overall intensity of the total pain experience (Melzack 1975).



Figure 2. The Visual Analogue Scale for measurement of pain

1.2.1 Mechanisms of musculoskeletal pain

The sensation of local muscle pain is a result of the activation of group III and group IV muscle nociceptors (Mense 2003). Group III afferents are thin myelinated fibres with conduction velocities between 2.5 and 30 m/s, while group IV affrents are unmyelinated and have conduction velocities less than 2.5 m/s (Mense and Simons 2001). The muscle nociceptors are free-nerve endings and it is here where the neuropeptides are synthesized and are released when there is

stimulation of the receptor (Graven-Nielsen and Mense 2001). The nociceptors then generate an electrical impulse to the dorsal horn in the spinal cord via the group III and IV fibres, where it gets transmitted to the brain where the pain is perceived (Ranney 1992; Graven-Nielsen and Arendt-Nielsen 2007).

When a muscle is pathologically altered, there is a peripheral release of neuropeptides which increases nociceptor activity – this is referred to as peripheral sensitisation (Ranney 1992, Mense 2003). It is this peripheral sensitization that causes the effects of hyperalgesia (Graven-Nielsen and Mense 2001). Although peripheral mechanisms are mainly responsible for hyperalgesia, evidence also shows that central sensitization, in part, mediates hyperalgesia.

Sensitisation of the dorsal horn is referred to as central sensitisation, which is one of the first steps in the shift from acute to chronic muscle pain (Ranney 1992). Central sensitisation may also be involved in referred pain (Arendt-Nielsen et al. 1998). The mechanisms behind the processes of referred pain are still not clearly understood although according to Feinstein et al. (1954) as cited by Arendt-Nielsen et al. (1998), it seems that central mechanisms probably generate the referred pain since the pain can still be induced after sensory loss i.e. after an anaesthetic block. Delayed development of referred pain and hyperalgesia provide evidence for central input given that sensitisation of central neurons take time to develop (Coderre and Katz 1997). In addition, referred pain and hyperalgesia spread to areas from different dermatomes, indicating a dependency on Central

Nervous System (CNS) changes (Coderre and Katz 1997). This central hyperexcitability may be responsible for the hyperalgesia, expansion of the receptor fields which enlarges the referred pain areas in patients with muscle pain and allodynia (Arendt-Nielsen et al. 1998, Graven-Nielsen and Arendt-Nielsen 2002). Allodynia, a painful response to non-painful stimuli, is a clinical feature of many conditions associated with musculoskeletal pain, such as fibromyalgia syndrome (FMS) (Oshinsky 2006). However, it is not specific to the muscles and can occur as cutaneous allodynia which is commonly associated with migraines (Oshinsky 2006).

Central hyperexcitability can be shown in patients with chronic muscle pain, where muscular hyperalgesia exists in a muscle with no spontaneous pain (Sorensen et al. 1998). In patients with FMS, hyperalgesia and referred pain are observed as symptoms of widespread chronic muscle pain.

Although central sensitisation is associated with pain, it is not specific to the condition and is present in other syndromes collectively referred to as central sensitivity syndromes (CSS) (Yunus 2008). The central sensitivity syndromes, which include circulatory, nervous, digestive, urinary and reproductive systems, have overlapping clinical features and are bound by the common pathophysiology of central sensitisation (Cassisi et al. 2008, Yunus 2008).

There are many confounding factors which could affect the perception of pain in patients with chronic myalgia, thus the need to use experimental pain models. Experimental pain models such as hypertonic saline infusion has been shown to successfully produce a muscular pain model with local and referred pain and hyperalgesia, which are both characteristics of clinical chronic and acute muscle pain (Graven-Nielsen et al. 1997a, Graven-Nielsen et al. 2001, Capra and Ro 2004). However, experimental pain models will never be able to be identical to the clinical condition of chronic pain conditions because of the other variables besides pain which have an effect on the disorder. However, isolating and treating one variable, pain, would improve knowledge about the peripheral and central mechanisms causing pain, which would aid in improved development of drugs and therapy for muscle pain, as well as a better understanding of the underlying mechanisms involved in pain transmission, transduction and perception (Graven-Nielsen et al. 1998, Arendt-Nielsen and Sumikura 2002).

1.3 Experimental muscle pain

Experimental pain models have been used in human and animal studies to experimentally induce pain and analyse the responses, whether behavioural, psychological or physiological (Staahl and Drewes 2004). There are various types of experimental pain models each inducing a different kind of pain. Cutaneous pain can be induced via mechanical stimulation, thermal stimulation, electrical stimulation and chemical stimulation (Staahl and Drewes 2004). Muscle pain, the focus of this paper, can be induced by ischaemic stimulation, electrical

stimulation, mechanical stimulation, exercise and chemical stimulation. Ischaemic and mechanical stimulation induce pain but are non-specific as the skin and other deeper tissues are also activated and contribute to the perception of pain, while electrical stimulation activates nerve fibres directly and is not nociceptive specific (Staahl and Drewes 2004). Exercise and chemical stimulation are the better models for muscle pain since they activate specific nociceptors in the muscle (Staahl and Drewes 2004).

1.3.1 Delayed onset muscle soreness

Delayed onset muscle soreness (DOMS) is the most common form of sports injury (Cheung et al. 2003). DOMS is a sensation of pain, discomfort and loss of range of movement which peaks between 24-72 hours after unaccustomed, eccentric exercise (Armstrong 1990; Cheung 2003; Weekerody et al. 2003). Eccentric exercise occurs when the muscle is lengthening during a muscle contraction (Cheung et al. 2003). Typically there is no pain unless the area is touched or contracted or stretched (Weekarody et al. 2003).

1.3.1.2 Mechanisms of muscle pain

Although the underlying mechanisms of DOMS have not yet been fully elucidated, it has been proposed to be a result of a combination of inflammation and muscle damage. It is believed that the structural damage of the muscle causes altered muscle function, which may lead to decreased muscle strength for up to 10

days after the onset of DOMS (Armstrong 1990, Cheung 2003). Eccentric exercise can cause high tensile forces and disruptions in the cross-bridges in the muscle, which ultimately leads to muscle fibre damage (Cheung 2003). The muscle damage occurs almost immediately after eccentric exercise, but gets worse two or three days after exercise – this explains why the pain peaks after two or three days (Allen 2001).

Muscle damage triggers a local inflammatory response which is divided into the acute phase response and the chronic phase response (Macintyre et al. 1995, Proske and Morgan 2001). The acute phase response occurs immediately after injury and is characterised by the influx of neutrophils into the injured tissue, whereas chronic inflammation is the phase involving the removal of neutrophils by macrophages and monocytes (Macintyre et al. 1995). It is evident that the inflammatory response occurs during exercise, since various inflammatory markers have been identified in eccentrically exercised muscle (Macintyre et al. 1995).

In summary, DOMS characteristically produces a muscle pain only when contracted stretched or touched in the area of the muscle affected i.e. hyperalgesia. This model would not be a suitable experimental pain model for chronic pain since the pain is only felt when pressure is applied, and no referred pain is induced. Therefore, a better model is needed to fulfil the requirements of chronic muscle pain.

1.3.2 Hypertonic saline infusion

The "Hypertonic Saline Pain Model" was first introduced by Kellgren and Lewis in 1938. The model involves the infusion of hypertonic saline (HS), a 5% NaCl solution, which is an algogenic substance, into a muscle. It usually involves an infusion into only one muscle and the quality of pain induced using this model elicits local as well as referred pain and hyperalgesia (Graven-Nielsen and Mense 2001). In addition to hypertonic saline producing a good model of muscle pain, there are other advantages to its use: namely; the pain is short-lasting with no reported long term side effects (Graven-Nielsen and Arendt-Nielsen 2003). . This model has become a popular method of inducing muscle pain since information on both the sensory and motor effects can be attained (Arendt-Nielsen et al. 1997).

A local pain, defined as the "pain around the injection site" is induced by hypertonic saline infusion (Graven-Nielsen et al. 1997b). After infusion of hypertonic saline in normal, healthy subjects, it has been shown that VAS scores increased significantly from 0 cm to a peak rating within a range of 3.6 - 7.2cm (Graven-Nielsen et al. 1997a; Graven-Nielsen et al. 1997b; Graven-Nielsen et al. 1998; Johansen et al 1999; Graven-Nielsen et al. 2000; Graven-Nielsen et al. 2003). The increase in the pain rating has been found to be dependent on the rate, duration and the cumulative volume of the infusion.

When assessing pain using the MPQ during an infusion of hypertonic saline, the most frequently chosen word during the infusions, at a higher rate of pain on the visual analogue scale (approximately 72mm) is “cramping” (Graven-Nielsen et al. 2003). When the peak pain on the VAS is approximately 40 – 50mm, the words most frequently chosen by the subjects are “taut”, “radiating”, “tight” and “drilling” (Graven-Nielsen et al. 1997a). Since the words selected from the MPQ differ with changing VAS pain ratings, it seems that there is a link between the pain intensity and pain quality in this model.

Referred pain is also elicited by hypertonic saline infusion, and the size of the area of the referred pain is dependent on the intensity and duration of the infusion of hypertonic saline (Graven-Nielsen et al. 1998), although continuous infusion produces and maintains referred pain with decreasing referred pain intensity over time (Graven-Nielsen et al. 1997a). Referred pain appears approximately 20 seconds after the perception of local pain (Arendt-Nielsen and Svensson 2001). Hypertonic saline infused into the tibialis anterior muscle, a muscle frequently used in hypertonic saline induced pain experiments, induces referred pain at the ventral part of the ankle discontinuous with the local pain area, and to the frontal aspect of the ankle (Graven-Nielsen et al 1997a; Graven-Nielsen et al 1998) (See Figure 2).

1.3.2.1 Mechanisms of muscle pain

Although the hypertonic saline method has been extensively used to induce muscle pain, it is not fully understood how the pain is initiated (Graven-Nielsen et al. 1997b). Graven-Nielsen and colleagues (1997b) have suggested that hypertonic saline infusion into a muscle increases the intramuscular sodium concentration, which causes depolarization resulting in action potentials. The action potentials result in activation of muscle nociceptors, particularly group III and group IV afferents which transmit the impulse to the dorsal horn in the spinal cord where the information is processed (Graven-Nielsen and Arendt-Nielsen 2002).

The pain intensity following hypertonic saline is different when administering it as a bolus or infusing it continually. In a study conducted by Graven-Nielsen and colleagues (2003), a 0.5ml/min rate of infusion over a period of 6 minutes produced a peak pain rating of approximately 72mm on the Visual Analogue Scale. This high pain rating is due to the constant infusion of saline over 6 minutes – the cumulative volume of the saline caused a high pain rating. Four separate 0.5ml injections of saline given at five minute intervals produced a peak pain rating after the first infusion (40mm) which decreased after the subsequent infusions (Graven-Nielsen et al. 1997a). In this case the cumulative volume of hypertonic saline did not cause an increase in the pain rating because the interstimulus interval was too long. However, when the interstimulus interval was

short the peak pain rating had a positive correlation to the cumulative infusion volume (Graven-Nielsen et al. 1997a).

The “Hypertonic Saline Pain Model” of inducing pain mimics the sensation of muscle pain seen in patients with chronic pain. For people with chronic pain, the combination of pain and sleep disturbance is a double-edged sword: the sleep disturbance has been implicated in an exacerbation of pain perception the following day, and an increase in the daytime intensity of pain is presumed to increase the sleep disruption (Ağargün et al. 1999, Lautenbacher et al. 2005). Conducting research in the field of sleep and pain is very difficult since there are many contributing factors involved in poor sleep and pain perception: negative mood and depression, chronic insomnia, sleep medication effects and different levels of pain perceived by patients (Lavigne et al. 2004). Research shows that disturbances in the central nervous system is the final pathway that causes the non-restorative sleep syndrome (Moldofsky 1993). In order to understand how experimental pain may interfere with sleep some knowledge of the basic construct of sleep is essential. Therefore, the next part of my discussion will focus on sleep and how it is affected by pain..

1.4 Sleep Physiology

One of the early misconceptions about sleep was that it is an interruption of the waking state and a passive and relatively unchanging process (Kleitman 1939). In fact, sleep is a very active state and some sleep processes involve a greater amount

of brain activity than during wakefulness (Hirshkowitz 2004). Sleep is an essential physiological process and does not take a single form - several stages of sleep exist, each with specific characteristics (Hirshkowitz 2004).

1.4.1 Characteristics of sleep

Polysomnography (PSG) allows for the objective assessment of sleep by measuring brain activity, eye activity and muscle activity (Carskadon and Dement 2005). Three fundamental measures are used as a basis for defining the different stages of sleep. Brain activity is measured using electroencephalography (EEG); muscle tone is measured using electromyography (EMG) and eye activity is measured using electro-oculography (EOG).

Rechtschaffen and Kales (1968) developed a sleep stage classification which characterises sleep into two different types; non-rapid eye movement sleep (NREM), and rapid eye movement sleep (REM). Non-rapid eye movement sleep can be further divided into four sleep stages namely; stages 1, 2, 3 and 4 (Carskadon and Dement 2005, Rechtschaffen and Kales 1968). Wakefulness, a period when one is drowsy and has the eyes closed, is called stage 0 and is characterized by alpha waves, normally in the range of 8-11 Hz (Rechtschaffen and Kales 1968).

Stage 1 is a transition phase from wakefulness to sleep (stage 2 sleep or deeper) (Horne 1990). This first stage of sleep is characterized by low voltage, mixed frequency EEG, reduction of alpha activity and appearance of 'vertex sharp waves'. There is also rolling of the eyes, which can be seen when observing the electro-oculogram (Hirschkowitz 2004, Rechtschaffen and Kales 1968).

Stage 2 sleep contains a mixture of theta wave activity (within a frequency range of 3.5-7.5 Hz), and two unusual wave phenomena. These wave phenomena, which occur periodically, and are defining characteristics of stage 2 sleep, are termed sleep spindles and K complexes. The former comprises a sudden increase in wave frequency, while the latter shows as a sudden increase in wave amplitude (Hirschkowitz 2004, Rechtschaffen and Kales 1968).

Stage 3 and 4 sleep are often collectively referred to as slow wave sleep (SWS) or delta sleep. This stage is characterised by the presence of high amplitude, low frequency delta waves, which make up between 20 and 100% of the wave patterns during these sleep stages (Hirshkowitz 2004, Rechtschaffen and Kales 1968).

REM sleep is characterised by the concomitant appearance of relatively low voltage, mixed frequency EEG, muscle atonia in the EMG and episodic bursts of rapid eye movement (Rechtschaffen and Kales 1963). Saw-tooth theta waves may

also be present during REM sleep (Hirshkowitz 2004, Rechtschaffen and Kales 1963).

During a normal sleep period, NREM sleep and REM sleep alternate in 3-5 cycles, each lasting approximately 90 to 120 minutes (Hirshkowitz 2004) (Figure 3). The pattern or progression of the sleep stages throughout a sleep period is referred to as sleep architecture (Spriggs 2002). In general, SWS predominates in the first half of the night, while REM sleep is the dominant sleep state during the last half of the sleep period (Carskadon and Dement 2005, Hirshkowitz 2004).

Stage 1 usually comprises 2-5% of total sleep time (TST), stage 2 occupies 45-55% of TST, SWS constitutes 13-23% of TST and REM sleep constitutes 20-25% of TST (Figure 4) (Hirshkowitz 2004). Patients who suffer from chronic pain do not follow the same patterns as people who have normal, undisturbed sleep. Their sleep will be comprised of frequently fragmented sleep and a decreased amount of REM and SWS (Drewes et al. 1997, Call-Schmidt and Richardson 2003).

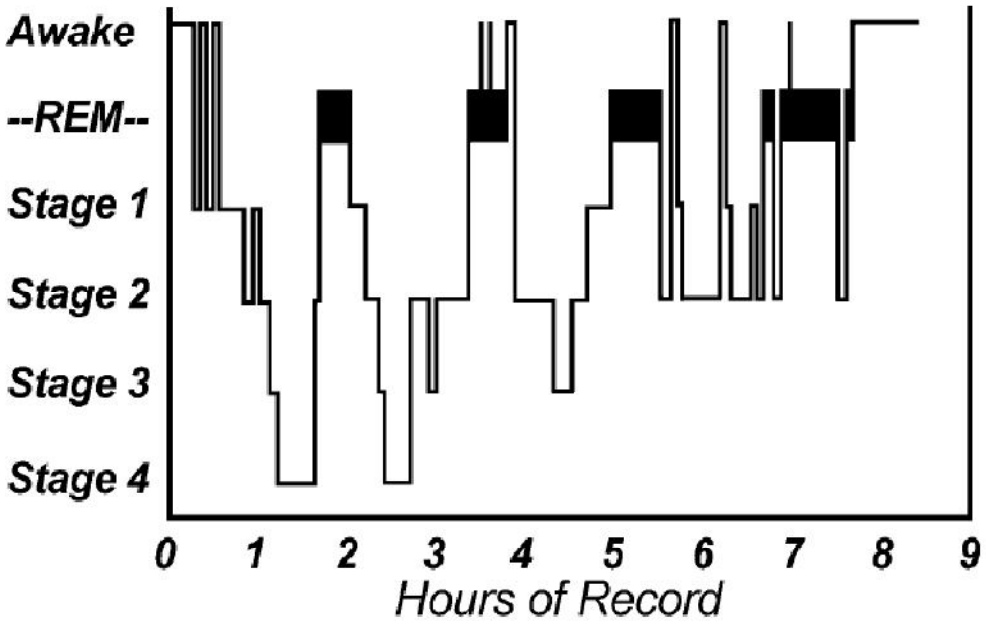


Figure 3. Progression of sleep stages during a complete night of sleep in a normal, healthy adult (Hirschkowitz 2004)

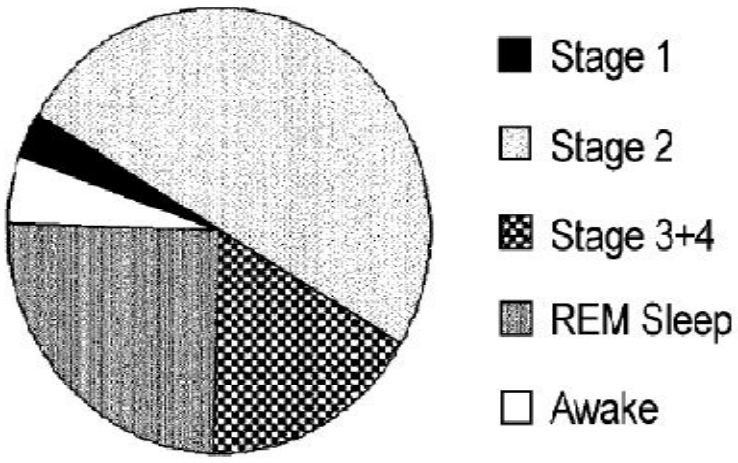


Figure 4. Composition of sleep in a normal healthy adult (Hirschkowitz 2004)

Just as the different stages of sleep are defined by their specific characteristics, sleep also has specific functions.

1.4.2 Function of sleep

The function of sleep is still highly debated, with various theories trying to answer the question, "Why do we sleep?" Sleep is a physiological process essential to survival and brings about a conservation of energy due to a reduced body temperature and metabolic rate – this energy conserving state is beneficial for recuperation (Peever and McGinty 2007).

In addition to the previously mentioned physiological functions of sleep, it has been found that sleep deprivation can lead to increased infections, thus playing a role in immune function (Horne 1988, Peever and McGinty 2007). The clinical features of chronic pain conditions such as FMS, are related to altered biological rhythmical functions (Moldofsky 1993). Features such as altered immune function and serotonin metabolism are related to the disturbances in the sleep of FMS patients (Moldofsky 1993). Sleep is a state of healing and growth with the greatest amount of human growth hormone (HGH) being naturally released during the first three hours of sleep (typically SWS) (Horne 1988). This release of HGH is not dependant on time of day as it is absent in the sleep deprived (Horne 1988). A study conducted on FMS patients showed reduced somatomedin C, which is considered to be an indication of low levels of growth hormone (Bennett et al. 1992). Approximately 80% of the daily production of growth hormone is released during SWS (Bennett et al. 1992). Patients with FMS have decreased SWS which

would account for the low levels of somatomedin C. Sleep may also be linked to memory consolidation and learning by maintaining neural networks and enhancing synaptic plasticity (Peever and McGinty 2007).

It still remains unclear why we sleep, but the general view is that sleep is an anabolic state of growth and rejuvenation while wakefulness is catabolic. The anabolic state of growth of sleep is so important that it needs to be conserved by limiting the amount of sensory input from the environment.

1.4.3 Sensory perceptions during sleep

"...sleep erects a perceptual wall between the conscious mind and the outside world." (Dement 1999)

Awareness can be defined as the ability to receive and integrate all incoming sensory information, both from the external surroundings and from within the body (Evans 2003). When asleep, one is not necessarily aware of auditory, visual, somatosensory and pain stimuli, since all but the most relevant, threatening or significant sensory input should be inhibited so that the sleep can be preserved (Campbell 2000, Kakigi 2003). For example, when music is played loudly, one could sleep without being disturbed, but if one's name is whispered, one would immediately awake.

During sleep there is little cortical activation in response to noxious stimulation (Kakigi et al. 2007). Since there is little cortical activation, sleep is preserved even

in the presence of pain, which indicates that sleep is essential and the brain needs to cut itself off from the outside world. This however, does not mean that the sleeping brain is incapable of awakening if sensory inputs are potentially harmful. The conscious perception of pain is triggered by a pre-frontal cortex arousal response (Bastuji et al. 2007). The perception of pain is conveyed along the spinoreticular and trigeminothalamic tract neurons to the thalamus and brainstem, which act as a gating mechanism to sort through information, allowing important information to get through and causing an awakening response (Lavigne et al. 2005, Soja 2007).

Research on sensory perception during sleep has suggested that the threshold required to induce an awakening is lower in light sleep (Stage 2), and higher during the deeper stages of sleep (SWS) (Bentley et al. 2003, Lavigne et al. 2000, Muzet 2007).

The perception of pain during sleep is also dependant on the type of noxious stimulation applied (Lavigne et al. 2007). Thermal pain stimulations, whereby a thermode was applied to the skin of the leg, caused microacrousals with more arousals observed in stage 2 than in SWS (Bentley 2007, Lavigne et al. 2000). Hypertonic saline infusion, which mimics muscle pain, was more likely to cause awakenings than microarousals during a 26 second infusion across all sleep stages (Lavigne et al. 2004).

Awakenings and arousals are not the only effects of sensory input on sleep. More subtle changes to sensory input can also be seen – in vasoconstrictions and heart rate changes (Muzet 2007). Heart rate increases after experimental nociceptive stimulation, which clearly shows that the sleeper still perceives external sensory input even if there is no arousal response (Lavigne et al. 2001, Muzet 2007).

During sleep the brain reacts to all information, whether the stimulus is environmental or physiological – choosing to activate an arousal response or simply ignoring the stimulus and preserving sleep.

1.4.4 Disruptions during sleep

Sleep can be disturbed in various ways – by a number of awakenings or arousals during the evenings, number of sleep stage changes and changes in the sleep cycle. There are many causes for sleep disturbances, most caused by external factors.

The external factors may be non-noxious or noxious. It has been found that noxious stimuli are more likely to cause awakenings across all sleep stages, whereas non-noxious stimuli are more likely to cause microarousals. Noise is a non-noxious external stimulus which could result in fragmentation of sleep. Research has shown that noise induces awakenings, at a peak noise level of 55 dB and above. The awakening threshold of noise is determined by the sleep stage involved (Muzet 2007). The time to fall asleep can also be prolonged when noise levels increase to 45 dB and above (Muzet 2007). The auditory stimuli are

processed centrally by the cortex, so the brain can act swiftly to the signals (Kakigi et al 2007). This probably serves as a protective function.

Chronic pain, as experienced in patients with fibromyalgia and rheumatoid arthritis, is a noxious stimulus, and has been associated with sleep disturbance (Drewes et al. 1997, Call-Schmidt and Richardson 2003). EEG measurements in patients with chronic musculoskeletal pain show changes in sleep in various ways such as: more superficial and frequently fragmented sleep, a longer sleep onset latency, decreased overall quality of sleep, reduction in SWS and REM sleep, and an increased frequency of alpha waves (also known as alpha intrusion) (Branco et al. 1994, Call-Schmidt et al 2003, Drewes and Arendt-Nielsen 2001, Nicassio et al. 2002, , Moldofsky 1993, Moldofsky 2001).

Whether a stimulus is noxious or non-noxious, the sleeping brain adapts to the interference by conveying a response i.e. a sleep disruption (Parrino et al. 2007). K-complexes are brief responses typically seen during normal sleep, but can also appear when there are environmental interruptions (Smith and Buenaver 2007). Another brief response commonly occurring during sleep is microarousals, which has an increased frequency in the presence of non-noxious stimuli (Lavigne et al. 2004). These brief responses to a stimulus allow for sleep continuity, unless further arousal is needed in response to additional incoming environmental information (Smith and Buenaver 2007). The longest responses are awakenings (Bentley 2007). The greater the need for one to be aware of a stimulus, the greater the degree of disruption.

As discussed before, many factors not related to the pain may contribute to sleep disturbance in chronic pain patients. Isolating and then treating one variable, pain, may make it easier to detect if that variable is responsible for the poor sleep. An experimental model, which mimics the pain observed in patients with chronic pain , would assist in defining the interaction between muscle pain and sleep.

1.5 Experimental pain and sleep

Sleep disturbance is a major complaint among patients with chronic pain with extensive research been carried out to achieve greater insight into the relationship between sleep and pain (Sessle 2007).

Many studies have been conducted investigating other types of pain (such as joint, cutaneous thermal and muscle pain) during sleep (Drewes et al. 1997, Lavigne et al. 2001, Bentley et al. 2003, Lavigne et al. 2004). These studies have shown that although the noxious stimuli produces a disruption in sleep, the degree of noxious stimulus required to produce the disruption observed is not always the same across the stimuli. Also, the studies assessed different end-points of sleep disruption and therefore, they are difficult to compare (Bentley 2007).

1.5.1 Joint and cutaneous thermal pain

Joint pain has been studied using a pressure device to cause pain (Drewes et al. 1997). Such pressure applied was found to decrease the frequency of waves

observed in deeper stages of sleep (delta waves) and to increase frequencies of waves found in lighter stages of sleep (alpha and beta waves). In the same study the effects of cutaneous pain, using an argon laser was investigated. There was no significant difference found in quality of sleep between baseline results compared to results when cutaneous pain was induced. The researchers speculated that there could possibly be a mechanism ensuring continuous sleep despite cutaneous stimuli during sleep (Drewes et al. 1997).

Studies investigating thermal pain showed that stage 2 sleep is more sensitive to changes than other sleep stages (Lavigne et al. 2001). A higher intensity of pain is also needed to cause an arousal from SWS and REM sleep when compared to stage 2 (Bentley 2003).

1.5.2 Delayed onset muscle soreness

In the case of pain caused by DOMS, surprisingly only one study has been performed assessing polysomnographic sleep changes following eccentric exercise (Breus et al. 2000). The main finding was that DOMS did not cause widespread sleep disruption i.e. only one disturbance was documented – a significant decrease in stage one sleep (Breus et al 2000). The data showed that DOMS improved sleep to some extent or had no effect at all (Breus et al 2000). The researchers believe that their findings are important since their results show that not all people suffering from pain experience sleep disturbances.

1.5.3 Hypertonic saline infusion

The hypertonic saline model has been previously used as a method to induce muscle pain during sleep in two separate studies. Hypertonic saline was infused, as at a rate of 0.5ml over 40 seconds, into the anterior tibialis muscle during SWS (Drewes et al. 1997). The post-injection percentage of the delta waves decreased while the percentage of alpha and beta waves increased which indicated that there was an arousal effect caused by muscle pain. The increase in the percentage of the alpha wave band, also known as the alpha wave anomaly, mimics that seen in patients with rheumatic pain (a chronic muscular pain condition (Drewes et al. 1997, Lavigne et al. 2004).

In a second study conducted by Lavigne and colleagues (2004), hypertonic saline was infused at a rate of 0.3ml over 26 seconds into the deltoid muscle. This procedure was performed 8 times throughout the sleep period. The infusion was performed in sleep stages 2, SWS and REM. The aim of the study was to assess the effect of hypertonic saline over all sleep stages using sleep arousal responses as a marker of sleep disruption. Their results showed that after the infusion, the amount of time spent in stage 2 increased while the time spent in SWS decreased, implying that the sleep quality was poorer. The infusion of hypertonic saline also evoked similar sleep awakening responses over all of the sleep stages. As useful as the current technique is, the study suggested that experimental pain stimuli of longer durations may be needed to mimic sleep findings observed in the sleep of

patients with chronic pain. However, hypertonic saline does cause sleep disruption and thus is a good model to mimic musculoskeletal pain.

Unlike the muscle pain induced in the two studies using hypertonic saline described above, patients with chronic muscle pain do not experience sudden bursts of pain only lasting for 20 to 40 seconds throughout the night. The pain is likely to be of lower intensity but lasting for a longer period of time. Therefore, in order to mimic the condition more effectively, the time over which the noxious stimulus is applied should be increased.

There are various ways of measuring how sleep is affected (Bentley 2007). Researchers have used EEG power spectra, heart rate response, EMG muscle response, microarousals, awakenings, sleep stage shifts and magnetic fields of the brain as end point measures of sleep disruption (Bentley 2007). However, usually only one or two end-point measures were assessed during each study, and not all of the studies involved every stage of sleep. Hence, an experimental model for longer duration acute pain during sleep should assess multiple end-points of sleep disruption during all stages of sleep.

1.6 Aims

1. The aim of the study is to investigate the effect of intramuscular hypertonic saline infusion on sleep architecture when infused during sleep for a duration of ten minutes

2. To assess whether the end-points of sleep disruption is constant over subjects

3. To assess whether the type of sleep disruption is dependent on stage of sleep involved

Study hypothesis: If all possible responses/end-points are assessed, then multiple responses will be observed depending on the time and individuality of subjects.

Chapter 2

Materials and Methods

2.1 Subject Recruitment

Twelve healthy males between the ages of 19 and 29 years from a university student population volunteered to participate in the study. Ethical clearance was obtained from the University of the Witwatersrand's Committee for Research on Human Subjects, which adheres to the principles of the Declaration of Helsinki (Clearance no. M060408). All subjects gave written informed consent before participation. The study was conducted at the Wits Dial-a-Bed Sleep Laboratory.

All volunteers presented with regular sleep-wake cycles and showed no signs of psychological disorders. The Pittsburg Sleep Quality Index (PSQI) (Appendix A) was used to screen for any sleep disorders (Buysee at al. 1989). A global PSQI score greater than 5 was taken to indicate poor sleep quality.

The 30-item version of the General Health Questionnaire (GHQ) (Appendix B) was used for psychological and general health screening. A score of less than 6 was used to indicate a normal psychological status (Goldberg 1972).

During the study subjects were advised to continue with their normal daily activities. Subjects were requested not to consume any form of alcohol during the study period and no coffee or tea in the evenings. Subjects were also asked not to smoke from 6pm on the day when sleep recordings were made. The restrictions

were made due to the fact that smoking and changes in caffeine can affect the integrity of sleep.

2.2 Nociceptive Stimulus

On arrival at the sleep laboratory, an anaesthetic cream, Emla ™ (lignocaine 2.5% and prilocaine 2.5% Astrazeneca Pharmaceuticals, South Africa), was used to anaesthetize the area of the skin where the catheter was to be inserted. An hour after application of the anaesthetic cream, an indwelling 22 gauge, 25mm catheter was inserted into the tibialis anterior muscle by a medical doctor. The needle was inserted into the muscle at a distance of 120mm below the patella to a depth of about 20 mm (Graven-Nielsen 1997a). The catheter was attached to a 20ml syringe loaded with either 5% sterile hypertonic saline or 0.9% sterile isotonic saline. The loaded syringe was then placed in an infusion pump, which controlled the infusion of the saline at a rate of 0.3ml/min, for a period of 10 minutes. During sleep, the infusion either lasted for a period of 10 minutes, or until a full arousal was noticed. After full arousal the infusion was stopped and the subject was left to go back to sleep to continue the experiment.

2.3 Experimental Protocol

Each subject was required to undergo one adaptation and two experimental nights in the Wits Sleep Laboratory. During the second and third sessions, either

hypertonic or isotonic saline infusions were given during sleep in random order. The experiment was double-blind.

2.3.1 Adaptation Night

The first of the three nights was the adaptation night. The purpose of the adaptation night was to familiarize the subjects with all of the experimental procedures as well as sleeping in the environment of the sleep laboratory. The adaptation night always took place two to five nights before the first recording night.

Subjects arrived at the sleep lab at least two hours before bed-time. Bed-time was determined by the participant. Immediately after arrival, the catheter was inserted as described in section 2.2 and subjects received one infusion of both hypertonic and isotonic saline at 0.3ml/min for 10 minutes whilst awake. The order of the infusions was randomized, and infusions were administered at least 10 minutes apart to allow for the pain to subside. If the pain had not completely disappeared after the ten minutes, the second infusion was delayed until the subject was completely pain free.

During the infusion subjects scored their pain on two different scales:

1) A 100mm Visual Analogue Scale (VAS), where the anchor at 0mm represented “no pain” and the anchor at 100mm represented “worst pain ever felt”, was used to assess the intensity of the pain. The VAS was given to the subject every 30 seconds throughout the infusion, and the subject marked on the line the point which they felt best represented their pain.

2) A McGill Pain Questionnaire (MPQ) (Appendix C) was used to assess the quality of pain. Subjects were then asked to complete the MPQ once the infusion had stopped.

The subjects were then prepared for overnight polysomnography which included: electroencephalography (EEG), electro-oculography (EOG), and electromyography (EMG). Recordings were made on a computerized EEG system (Easy EEG version 2.0.2, Cadwell Laboratories Inc, Kennewick WA). The placement of the electrodes on each subject’s head and face was identical to that of the successive recording nights. Electrodes were placed on the scalp at C3 and C4 sites, according to the international 10/20 system, and were cross-referenced to A1 or A2. To record muscle activity (EMG), two electrodes were placed over the submentalis muscle, and eye movements (EOG) were recorded by placing electrodes next to each eye. Both EOG electrodes were placed approximately 1cm from the outer corner of the eye; one approximately 1cm up from the middle of the eye, and the other approximately 1cm down from the middle of the eye (Rechtschaffen and Kales 1968). One ground electrode was placed on the

forehead. All electrodes were filled with conducting paste and secured with micropore tape.

No actual polysomnographic recordings were made on the adaptation night. The purpose therefore, of the preparation for overnight polysomnography on the adaptation night, was merely to get the subjects accustomed to sleeping with the electrodes attached to their head and face. Subjects went to bed at their usual bedtimes, slept through the night in a sound-attenuated bedroom with no further nociceptive input applied.

In the morning, subjects were asked to fill out the Morning Questionnaire to assess the preceding night's sleep (Appendix D). A repeat 10 minute infusion during wakefulness was done after waking with a repeat VAS and McGill Pain Questionnaire, identical to that described above.

2.3.2 Experimental Nights

For the two experimental nights the subjects had the catheter inserted, syringes loaded and the sleep recording electrodes attached as in the adaptation night. No infusions during wakefulness were performed.

The order of isotonic and hypertonic infusions was randomized and subjects were not informed as to which solution was going to be infused during the night (only one solution was infused during each night). The infusions commenced once the subject was asleep and had spent at least two minutes in a specific sleep stage. The infusions were run during three sleep stages (stage two, SWS and REM) and at least 15 minutes elapsed between each infusion. The time interval between infusions was given so that one infusion would not impact on subsequent infusions. Not more than six infusions took place during any particular night. Once the subject was asleep and had been in either stage two, SWS and REM sleep for a minimum of two minutes, the infusion of hypertonic saline or isotonic saline was initiated.

The study was concluded when all the subjects had completed both trials, with both isotonic and hypertonic saline on separate occasions, thus, each subject acted as his own control.

Following each experimental night, subjects were asked to fill out the Morning Questionnaire. In addition they were asked if they remembered receiving noxious stimuli during the night. To assess for hyperalgesia, a repeat 10 minute infusion during wakefulness was done after waking with a repeat VAS and McGill Pain Questionnaire, identical to that described in 2.3.1. The morning infusion was performed with the same solution as the previous evenings' infusions.

The following sleep variables were considered endpoints in the study: 1) micro-arousals, 2) awakenings, 3) sleep stage shifts and. These variables are defined as follows:

1) Micro-arousal: the sleep of the subject is stable for at least 1 minute before the stimulus, after which an abrupt shift in the EEG waves lasting between 3 and 10 seconds is observed. (American Sleep Disorders Association, American Academy of Sleep Medicine)

2) Full arousal: scored for responses lasting more than 10 seconds (American Sleep Disorders Association)

3) Sleep stage shifts: scored when sleep stage changes from a deeper to a lighter stage of sleep (such as in an increased frequency of alpha waves) (American Sleep Disorders Association).

The presence of each variable was noted as well as time since the start of the infusion. If multiple events occurred, the type and time delay for each subsequent event was noted and called second and third responses. A minimum of 10 continuous seconds of intervening sleep was necessary to score a second arousal.

A frequency shift had to be three seconds or greater in duration to be scored an arousal.

2.4 Statistical Analysis

All data were analysed using the Instat statistical package (GraphPad Software Inc., version 3, 1997). A two-tailed probability of $P < 0.05$ was considered to be statistically significant. All parametric values are expressed as mean ± SD, while all non-parametric values are expressed as medians (lower, upper 95% confidence limits).

All VAS measurements (in mm), used to describe both pain and sleep subjectively, were normalized before statistical analysis using the arcsine transformation. However, in the results (text and graphs) all the VAS data are reported as back transformed values.

The area under the curve for the VAS-time curve was analysed using the Students T-test and One-way ANOVA to test for the differences between the hypertonic saline and isotonic saline infusions, and to compare the three hypertonic saline infusions. The area under the curve is a statistical means of summarizing information from a series of measurements across time on one individual.

The Friedman Statistic Test will be used to test for differences between the evening and morning infusion data (the pain rating index (PRI) and number of words chosen in the MPQ).

The percentage of responses during sleep was analysed using the The Chi-Square Test Statistic. T-tests were used to evaluate differences in the time to a response. The remaining sleep data was analysed using descriptive statistics.

Chapter 3

Results

3. Results

3.1 Subject characteristics

The age range of the subjects was between the ages of 19 and 29 years (mean 22.2, SD ± 3.1). The mean score for the PSQI was 2.82 ± 0.91, and the mean score for the GHQ was 3.35 ± 1.0, with both values being within the normal range.

3.2 Awake Data

3.2.1 Pain Intensity

The area under the curve for the VAS pain scores obtained during the hypertonic saline infusion and isotonic saline infusion for the adaptation night, adaptation morning and experimental morning were calculated. All hypertonic saline infusions were significantly more painful than the isotonic saline infusions on the adaptation night ($p<0.0001$, $t=6.174$), the adaptation morning ($p<0.0001$, $t=6.625$) and the experimental morning ($p<0.0001$, $t=7.282$) (figure 5). No significant difference was found between the infusions of hypertonic saline at either the adaptation night, adaptation morning or experimental morning ($p=0.5437$, $F_{(1.09)}=0.6265$).

The pain induced by the hypertonic saline becomes significantly different at 150 seconds compared to baseline (30seconds) on the adaptation night ($p < 0.01$, $n=12$, $F=7.701$), on the adaptation morning ($p<0.01$, $n=12$, $F=7.255$) and on the

experimental morning ($p<0.01$, n=12, F=12.27). The pain then remained significantly higher than baseline for the rest of the infusion, indicating a 7½ minute long painful experience (Figure 6).

No significant difference was found in the median PRI (Pain Rating Index) and number of words chosen when comparing the hypertonic saline infusions at the end of the infusion on the adaptation night (PRI 19, words 8), the adaptation morning (PRI 19, words 9) and the experimental morning (PRI 17, words 8) ($p=0.8255$ for PRI, $p=0.8769$ for words).

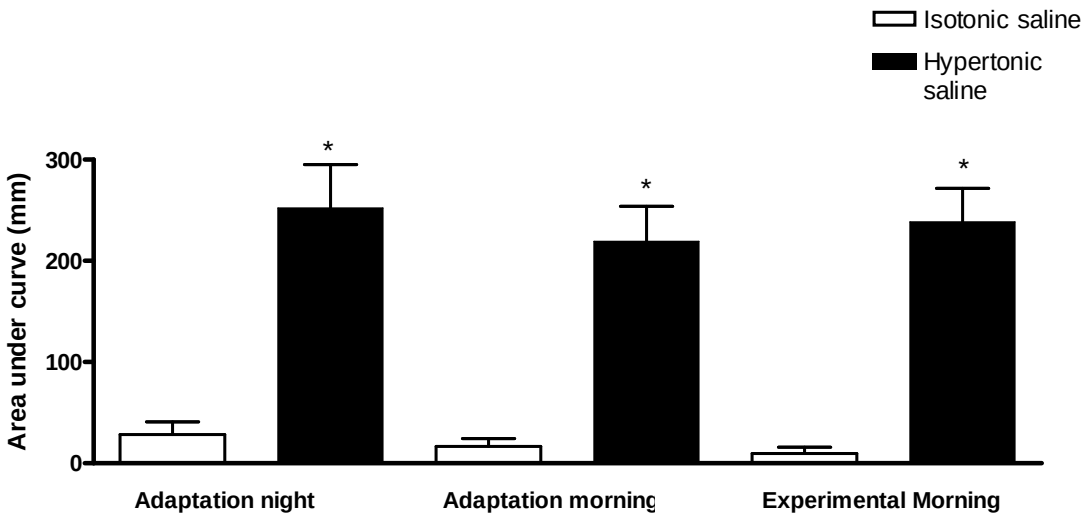


Figure 5: Bar graph of area under curve for visual analogue scale pain scores during hypertonic and isotonic saline infusions. *$p<0.0001$

3.2.2 Pain Quality

Table 1: Words from the McGill Pain Questionnaire used to describe pain after hypertonic saline infusions

Adaptation night	Adaptation morning	Experimental morning
Pricking (67%)	Itching (42%)	Sharp (42%)
Tight (50%)	Tight (42%)	Sore (67%)
Itching (42%)		
Sore (42%)		
Numb (42%)		

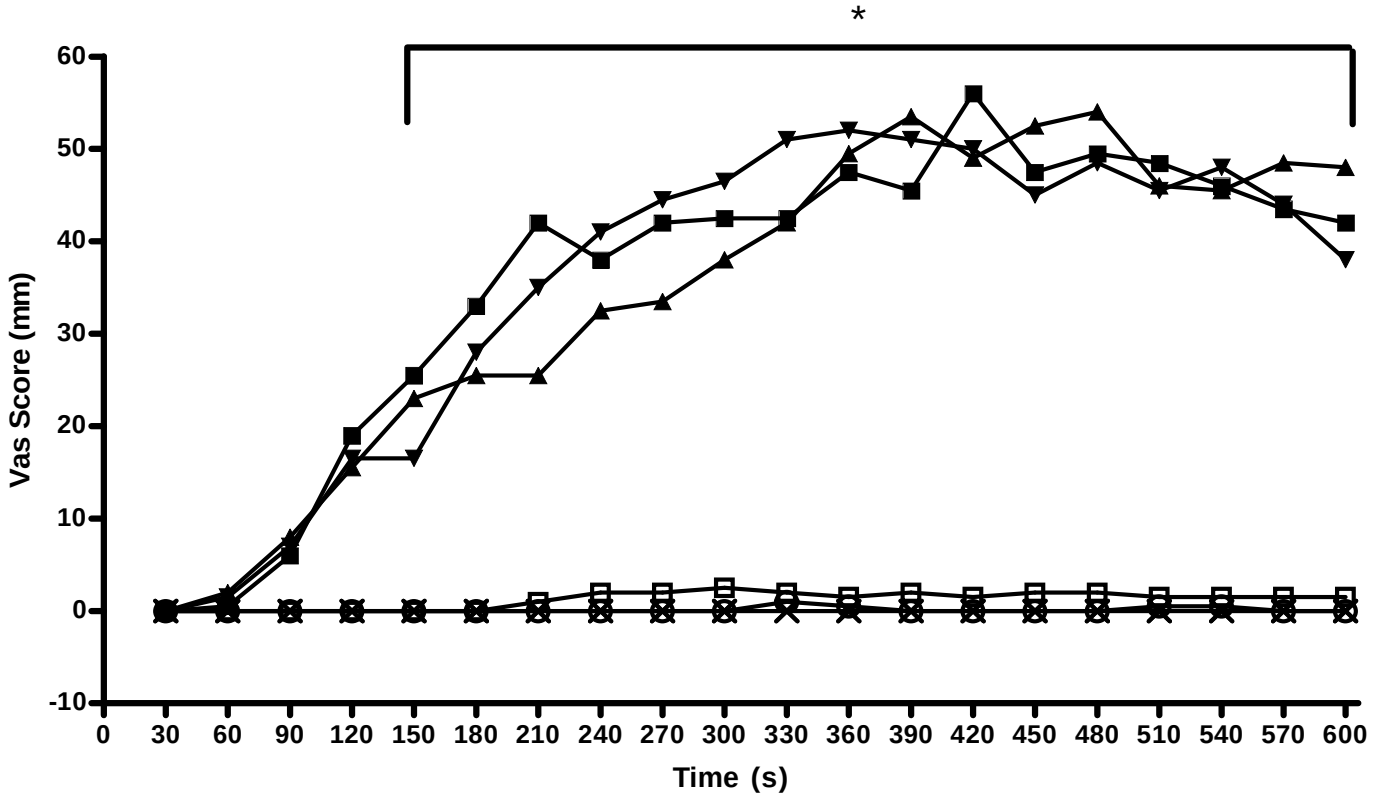


Figure 6: Median VAS-time curve after infusion of hypertonic saline and isotonic saline over ten minutes during wakefulness (▼=hypertonic saline infusion on experimental morning, ■=hypertonic saline infusion on the adaptation night, ▲=hypertonic saline infusion on the adaptation morning, ●=isotonic saline infusion on the experimental morning, □=isotonic saline on the adaptation night, X=isotonic saline on the adaptation morning). * $p<0.01$

3.3. Sleep Data

3.3.1 Isotonic saline infusions

During all sleep stages, only two subjects had responses to the isotonic saline infusions. The subjects both had microarousals as a response, and only during stage 2 (figure 7).

3.3.2 Hypertonic saline infusions (refer to table 2)

Stage 2

During stage 2, nine subjects received one infusion, while three subjects received more than one infusion. Subjects received more than one infusion when their sleep remained stable enough to do more infusions during that particular sleep stage. Ten of the subjects had full arousals as a first response. The mean time for first responses during stage 2 was 182.1 seconds (SD ± 129.6). All responses to the infusions are indicated in Table 2.

Of the subjects who had first responses, other than full arousals as their first response, four had second responses. The mean time between the first and second response, irrespective of the type of response was 47 seconds (SD ± 30.73). The second and third responses to the infusions are indicated in Table 2.

SWS

During SWS, 11 subjects received one infusion, while one subject was given more than one infusion. Of these infusions, four had a full arousal and four had a sleep stage shift as a first response. The mean time for first responses during SWS was 174.5 seconds (SD ± 133.8). All responses to the infusions are indicated in Table 2.

Of the subjects who had first responses, other than full arousals as their first response, seven had second responses. The mean time between the first and second response, irrespective of the type of response was 86.4 seconds (SD ± 57.78). One subject had a full arousal 55 seconds after the second response. The second and third responses to the infusions are indicated in Table 2.

REM

During REM sleep, six subjects had one infusion, and the other six subjects had more than one infusion. Of these infusions, seven had a full arousal as a first response. The mean time for first responses during REM sleep was 163.1 seconds (SD ± 107.2). All responses to the infusions are indicated in Table 2.

Of the subjects who had first responses, but did not have full arousals as their first response, eight had second responses. The mean time between the first and second

responses, irrespective of the type of response was 88 seconds (SD ± 54.6). One subject had a full arousal during REM sleep, 370 seconds after the second response. The second and third responses to the infusions are indicated in Table 2.

3.3.3 Time to responses

The first time a response occurred, regardless of what type of response or which sleep stage it occurred in, was at a mean time of 172.2 seconds (SD ± 119.1). The corresponding VAS score was at approximately 32-42mm (read off graph from figure 6). The second time a response occurred was at a mean time of 230 seconds (SD ± 111.7). The corresponding VAS score was at approximately 39-45mm (read off graph from figure 6). A significant difference was found between the time of the third responses and the time of the first and second responses ($p=0.0004$, $t=3.824$ and $p=0.0079$, $t=2.909$). There was no significant difference between the time of the first responses and the time of the second responses ($p=0.0623$, $t=1.897$).

The time to responses, irrespective of whether the responses are first, second or third, are as follows: microarousals: mean 189.6 seconds, SD ± 162.4; sleep stage shifts: mean 210 seconds, SD ± 162.9; and full arousals: mean 173.7, SD ± 85.71. There was no significant difference in time between the responses ($p=0.8900$, $F=0.2086$). When comparing the different sleep stages, no significant difference was found in the time in which the responses occurred ($p=0.6141$, $F=0.4913$).

3.3.4 Validity of responses

Within a subject there is no validity in the type of response. Only two subjects were consistent in that they had full arousals only across all stages. There is also no consistent response in subjects who had multiple infusions during a stage of sleep. However, there seems to be a trend within each stage.

3.3.5 Subjective morning assessment

None of the subjects reported poor sleep quality the morning after the hypertonic saline trial night (figure 8). All subjects were able to differentiate whether they had received isotonic or hypertonic saline infusions the following morning.

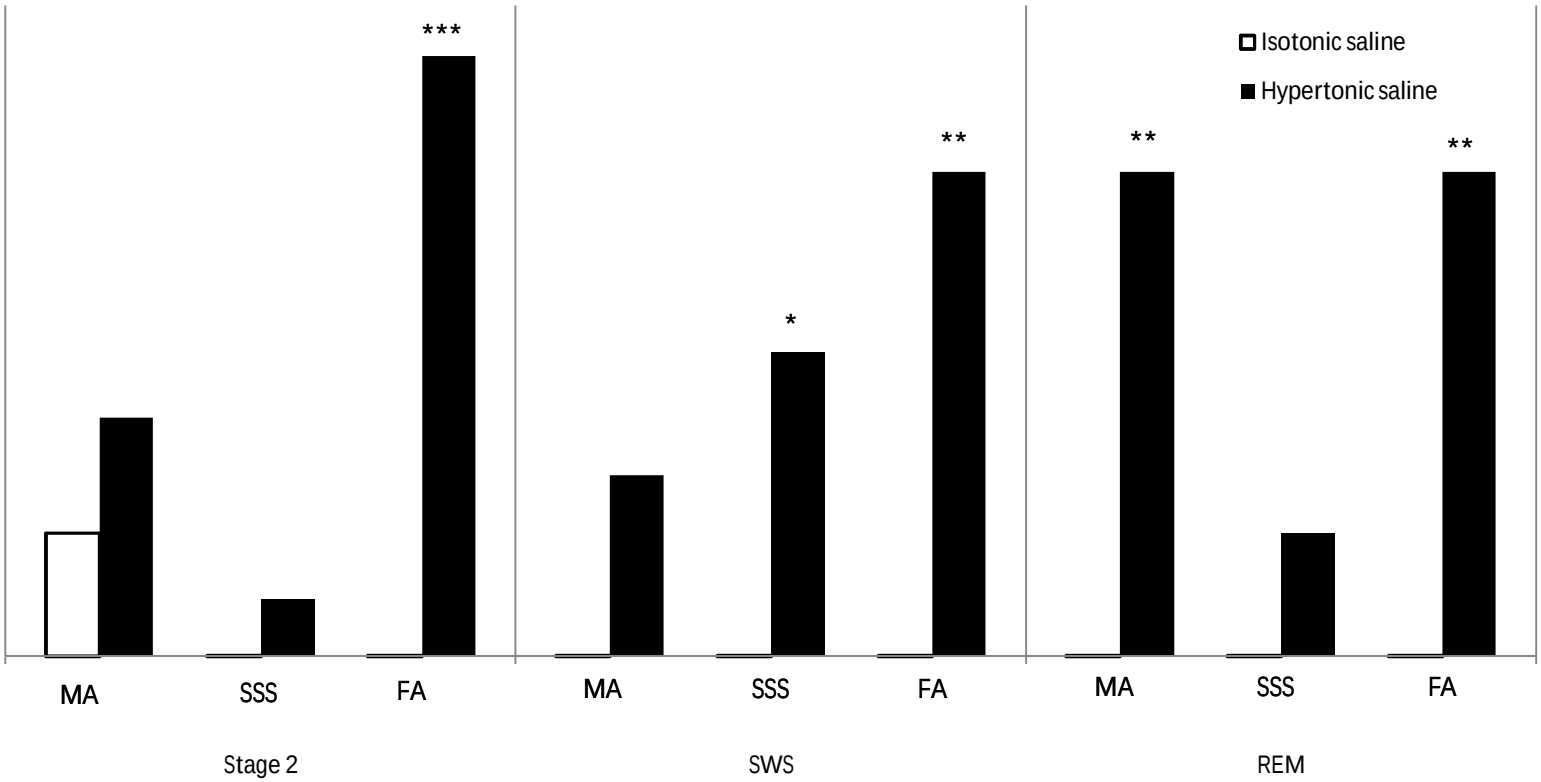


Figure 7: Percentage of subjects with microarousals (MA), sleep stage shifts (SSS) and full arousals (FA) following isotonic and hypertonic saline over all stages. *p<0.05, **p<0.01, ***p<0.001 between isotonic and hypertonic saline infusions

Table 2: Responses and variability within and between subjects observed during Stage 2, SWS and REM sleep to hypertonic saline infusions. Fa – full arousal; sss – sleep stage shift; ma – microarousal. The responses to multiple infusions are indicated on separate lines. Commas (,) distinguish between first, second and third responses.

Subject	Stage2	SWS	REM
1	fa	sss, ma	ma, fa fa
2	ma, fa sss, fa fa	ma, fa fa	ma
3	fa fa	No response	ma
4	fa	No response	fa
5	fa	fa	fa ma, fa fa
6	fa	sss, fa	ma
7	ma, fa	fa	fa fa
8	ma, fa	sss, fa	ma, fa fa
9	fa	fa	fa ma, fa
10	ma	sss, fa	fa
11	No response	sss, ma	ma, sss
12	fa	fa	fa sss

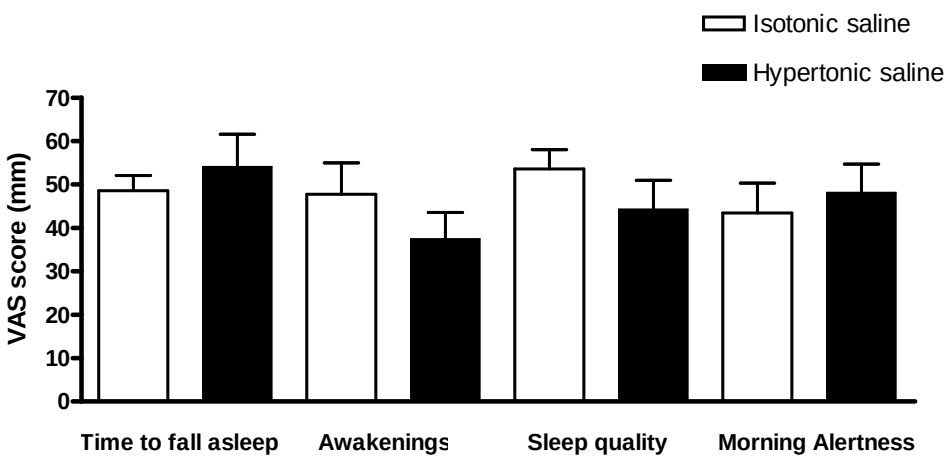


Figure 8: Subjective morning assessment of sleep quality from visual analogue scales for the hypertonic saline and isotonic saline experimental nights

Chapter 4

Discussion

We successfully induced muscle pain in the tibialis anterior muscle after infusion of hypertonic saline. The subjects muscle soreness was identified by an increased subjective pain score, as measured by the VAS and PRI. The area under the curve for all three hypertonic saline infusions were significantly greater than their corresponding isotonic saline infusions indicating that hypertonic saline causes pain. The intensity of pain increased significantly until maximum pain had been reached, plateaued between 330 and 480 seconds then slowly began to decrease and did not change in intensity once maximum pain had been reached. This decrease is similar to findings of Zhang and colleagues (1993), who applied a continuous infusion of hypertonic saline, and to that of the study by Graven-Nielsen and colleagues (1997b), where they showed a decrease in pain with subsequent infusions (of 20 seconds) of hypertonic saline.

Future studies could use our results as a base for testing changes in pain during a chronic infusion of hypertonic saline and increase the time of infusion to 20 or 30 minutes in order to ascertain whether the pain continues to decrease after ten minutes.

The lack of a significant difference in VAS scores or PRI or number of words between three hypertonic saline infusions performed on different days and at different times showed both a lack of circadian rhythm and a lack of hyperalgesia after the experimental night, establishing that all the data is valid. The type of pain was also uniform across all hypertonic saline infusions since the words used to

describe the pain were similar. Even though the words chosen from the MPQ were different from the words used to describe the muscle pain in the tibialis anterior in previous studies (Graven-Nielsen et al. 1997a, Graven-Nielsen et al. 1997b, Graven-Nielsen et al. 1998, Graven-Nielsen et al. 2003), most of the words chosen were from same word groups. The reason for this could be a result of differing volumes and rates changing the severity of pain, therefore influencing the type word chosen.

Our study has shown that hypertonic saline can be safely infused during sleep, with no adverse effects, such as prolonged muscle pain. The muscle pain induced by the hypertonic saline caused a disturbance in all sleep stages. The time at which the disturbances occurred during the different sleep stages was not significant, indicating that the reactions to the stimuli are independent of the sleep stage involved i.e. no matter what stage is affected, a reaction will occur. When comparing the time at which the disturbances occurred during sleep and the time at which a peak pain was felt during the awake infusions, the result is that the disturbance appears at a much lower corresponding VAS score. This indicates that pain is perceived at a low threshold during sleep. The difference in pain perception during sleep and when awake could be due to the fact that a sleeping brain needs to become aware of a painful stimulus and thus needs to allow information to get through at a lower level to ensure safety and survival. An awake brain, however, is already aware of the environment and is required to react at a much higher level of pain.

Patients with rheumatic diseases such as fibromyalgia and rheumatoid arthritis usually complain of decreased sleep quality, which can be seen in the specific changes in the macrostructure and microstructure of their sleep. These disturbances have been shown to be significant in the appearance of the symptoms of these conditions, and have been assessed in four different kinds of experiments (Moldofsky and MacFarlane 2001).

The first type of experiment evaluates sleep disturbances in patients who are diagnosed with FMS. The studies showed a poor quality of sleep with longer sleep latencies, decreased sleep efficiency, frequently fragmented sleep, reduction in SWS and REM sleep and an increased incidence of alpha EEG during NREM sleep (Branco et al. 1994, Call-Schmidt and Richardson 2003, Drewes and Arendt-Nielsen 2001, Nicassio et al. 2002, Mahowald et al. 1989, Moldofsky 1993, Moldofsky 2001, Moldofsky and MacFarlane 2005).

The second type of experiment used to show the importance of sleep disturbances on symptoms of pain utilizes noise to disrupt SWS in healthy subjects (Older et al. 1998, Lentz et al. 1999). These experiments artificially induced diffuse muscle and fatigue similar to the symptoms indicating a link between SWS disruption and pain symptoms.

Sleep deprivation studies are also used to show the correlation between sleep disturbance and pain. In a study conducted by Onen and colleagues (2001), 40 hours of sleep deprivation showed a reduction in pain thresholds. The pain threshold was restored to its normal value after SWS had fully recovered.

The experimental pain model of using hypertonic saline to mimic the pain felt by patients with chronic muscle pain is often used during sleep to observe various disturbances. Previous studies using the method have produced an acute pain during sleep resulting in an increased percentage of alpha and decreased SWS (Drewes et al. 1997, Lavigne et al. 2004). Our study used a longer duration acting model to generate more changes in the sleep macrostructure and succeeded in disturbing sleep across all stages. The most common response to an infusion of hypertonic saline in all sleep stages was a full arousal. Previous research has shown that hypertonic saline was more likely to cause full arousals than microarousals across all sleep stages (Lavigne et al. 2005). In the study conducted by Lavigne and colleagues (2005), the percentage of sleep-evoked microarousals and full arousals following hypertonic saline was 0% and 40%, 0% and 26.7%, and 0% and 45.8% for stage 2, SWS, and REM sleep, respectively. Our study, however, showed that microarousals did occur during all sleep stages. This discrepancy could be due to the methodology. A bolus infusion was given in the previous study, whereas a longer duration infusion was given during our study allowing other sleep disturbances to be observed.

The type of disturbance/arousal observed while infusing hypertonic saline during sleep was dependant on the sleep stage involved, i.e. the infusion affected the sleep stages differently. During stage two, full arousals were significantly more prevalent than any other sleep disturbance. In addition to full arousals, sleep stage shifts were significantly more common than microarousals during SWS, while microarousals were significantly more prevalent during REM. Our data suggest that the threshold for pain increases from stage two to SWS, and from SWS to REM, which is similar to the findings in previous studies (Lavigne et al. 2000, Bentley et al. 2003). This fits in with the basic physiology of sleep where: stage two is the lightest stage of sleep, SWS being a deeper form of sleep and REM being the deepest.

Previous studies assessing the relationship between sleep and pain have not assessed all sleep disturbances as an end-point measure, and did not apply the infusions beyond one minute. A study conducted by Drewes and colleagues in 1997, observed the effect of hypertonic saline infusion, argon laser and joint pressure on sleep microstructure. The study was only performed during SWS and the infusions lasted 40 seconds, joint pressure was applied for 10 seconds and the laser was transmitted to the skin for a maximum of 45 seconds. The results showed a decrease in delta waves with no changes observed during cutaneous pain (Drewes et al. 1997).

Two thermal studies tested the effect of increasing temperatures on the different stages of sleep with a full arousal being the end-point measure (Lavigne et al. 2000, Bentley et al. 2003). Both studies concluded that the subject is more sensitive to pain during stage 2 than during SWS or REM sleep (Lavigne et al. 2000, Bentley et al. 2003).

Only one study looked at microarousals, awakenings, and sleep stage shifts as end-point measures. The study observed changes induced by hypertonic saline in all stages of sleep, but the infusions only lasted 26 seconds at a time (Lavigne et al. 2004). They found that there were more awakenings than microarousals – similar to our findings (Lavigne et al. 2004).

Our study showed that in order to get a more accurate understanding of what kind of changes occur during each sleep stage, all possibilities of sleep disturbances and arousals need to be observed. The pain stimulus should also be long enough to see an effect – our data showed that responses occurred at around three minutes, a time which was not included in previous studies.

The number of responses and the sequence of responses following hypertonic saline infusions showed no trend and is completely dependent on the individual. These results may help explain some of the conflicting results obtained in

previous experimental pain studies especially where their percentage of responses were analysed.

When asked to score their sleep quality on a 100mm scale, subjects reported no significant difference in the time to fall asleep, the number of awakenings, sleep quality and morning alertness between the isotonic and the hypertonic trial. However, all subjects remembered receiving a painful stimulus during the night. Despite having sleep disturbances throughout the night, the subjects perceived their sleep quality to be normal since they had their normal hours of sleep. It would have been interesting to do an afternoon study on the subjects to assess their daytime sleepiness. This information could have helped to evaluate whether the sleep disturbances affected their sleep quality and daytime alertness.

A limitation of the study is that not all subjects had more than one infusion in a particular stage of sleep during the night, thus it was difficult to establish whether there were any trends within or across individuals. Future studies could build on from our experience and include the method of doing a minimum of 3 infusions per sleep stage. Further input would be to assess the beginning of the evening's stage two sleep separately from stage two at the end of the evening as it is possible that the brain responds differently to stimuli during the same stage at various parts of the evening. A second limitation of the study is that the time at which a sleep arousal occurred was compared to the time at which it corresponded

to the pain intensity during wakefulness. Such direct comparison needs further validation.

Our study showed that experimental pain will eventually result in a full arousal, depending on how intense the pain gets. Mild pain causes other sleep disruptions like microarousals and sleep stage shifts to occur before the full arousal can take place, depending on the sleep stage involved. However, not all subjects have the same sleep disruptions. Consequently, if a sleep study is performed with the aim of observing one sleep disruption, one might not even find the disruption in any given subject. Therefore, future sleep studies need to assess multiple sleep disturbances across all sleep stages.

In conclusion, we have successfully developed a longer duration acute model for muscle pain during sleep using a ten minute infusion of hypertonic saline. The data suggests that pain during sleep, if continued for an extended period, may trigger multiple sleep disturbances during sleep and the specific effects may be determined by the individual variation and by the sleep stage involved. The sleep disturbances found in our model of experimental pain may be similar to those found in patients with chronic muscle pain and may also explain differing results between previous experimental pain and sleep studies. These significant findings need to be further assessed with more infusions during the evening and daytime function and reaction tests during the day.

Chapter 5

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Chapter 6

Appendix

APPENDIX A

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

Name: _____ ID No: _____

Date: _____ Age: _____

Instructions:

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

1. During the past month, when have you usually gone to bed at night?
   USUAL BED TIME _____
2. During the past month, how long (in minutes) has it taken you to fall asleep each night?
   NUMBER OF MINUTES _____
3. During the past month, when have you usually gotten up in the morning?
   USUAL WAKE UP TIME_____
4. During the past month, how many hours of actual sleep did you get at night? (This may be different from the number of hours you spend in bed)
   HOURS OF SLEEP PER NIGHT_____

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

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

a) Cannot get to sleep within 30 minutes

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

b) Wake up in the middle of the night

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

c) Have to get up to use the bathroom

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

d) Cannot breathe comfortably

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

e) Cough or snore

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

f) Feel too cold

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

g) Feel too hot

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

h) Had bad dreams

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

i) Have pain

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

j) Other reason(s), please describe:

_____

_____

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

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

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

Very good	Fairly good	Fairly bad	Very bad

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

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

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

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

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

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

10. Do you have a bed partner or roommate?

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

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

a) Loud snoring

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

b) Long pauses between breaths while asleep

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

c) Legs twitching or jerking while asleep

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

d) Episodes of disorientation or confusion during sleep

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

e) Other restlessness while you sleep; please describe:

_____

_____

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

APPENDIX B

GENERAL HEALTH QUESTIONNAIRE

Please read carefully:

We would like to know if you have any medical complaints, and how your health has been in general over the last few weeks. Please answer ALL the questions on the following pages simply by underlining the answer that you think most applies to you. Remember that we want to know about present and recent complaints, not those that you had in the past.

It is important that you try and answer ALL 30 questions.

Thank you very much for your co-operation.

HAVE YOU RECENTLY:

1	Been able to concentrate on whatever you're doing?	Better than usual	Same as usual	Less than usual	Much less than usual
2	Lost much sleep over worry?	Not at all	No more than usual	Rather more than usual	Much more than usual
3	Been having restless, disturbed nights?	Not at all	No more than usual	Rather more than usual	Much more than usual
4	Been managing to keep yourself busy and occupied?	More than usual	Same as usual	Less than usual	Much less than usual
5	Been getting out of the house as much as usual?	More than usual	Same as usual	Less than usual	Much less than usual
6	Been managing as well as most people would in your shoes?	Better than most	Same as usual	Less than most	Much less than usual
7	Felt on the whole you were doing things well?	Better than most	Same as usual	Less than usual	Much less than usual

8	Been satisfied with the way you've carried out your tasks?	Most satisfied	Same as usual	Less satisfied than usual	Much less than usual
9	Been able to feel warmth and affection for those close to you?	Better than usual	Same as usual	Less than usual	Much less than usual
10	Been finding it easy to get on with other people?	Better than usual	Same as usual	Less than usual	Much less than usual
11	Spent much time chatting with friends?	More than usual	Same as usual	Less than usual	Much less than usual
12	Felt that you are playing a useful part in things?	More than usual	Same as usual	Less than usual	Much less than usual
13	Felt capable of making decisions about things?	More than usual	Same as usual	Less than usual	Much less than usual
14	Felt constantly under strain?	Not at all	No more than usual	Rather more than usual	Much more than usual
15	Felt you couldn't overcome your difficulties?	Not at all	No more than usual	Rather more than usual	Much more than usual
16	Been finding life a struggle all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual
17	Been able to enjoy your normal day-today activities?	More than usual	Same as usual	Less than usual	Much less than usual
18	Been taking things hard?	Not at all	No more than usual	Rather more than usual	Much more than usual
19	Been getting scared or panicky for no good reason?	Not at all	No more than usual	Rather more than usual	Much more than usual
20	Been able to face up to your problems?	More than usual	Same as usual	Less than usual	Much less than usual
21	Found everything getting too much for you?	Not at all	No more than usual	Rather more than usual	Much more than usual
22	Been feeling unhappy	Not at all	No more	Rather more	Much more

	and depressed?		than usual	than usual	than usual
23	Been losing confidence in yourself?	Not at all	No more than usual	Rather more than usual	Much more than usual
24	Been thinking of yourself as a worthless person?	Not at all	No more than usual	Rather more than usual	Much more than usual
25	Felt that life is entirely hopeless?	Not at all	No more than usual	Rather more than usual	Much more than usual
26	Been feeling hopeful about your own future?	More than usual	Same as usual	Less than usual	Much less than usual
27	Been feeling reasonably happy, all things considered?	More than usual	Same as usual	Less than usual	Much less than usual
28	Been feeling nervous and strung-up all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual
29	Felt that life isn't worth living?	Not at all	No more than usual	Rather more than usual	Much more than usual
30	Felt at times you couldn't do anything because your nerves were too bad?	Not at all	No more than usual	Rather more than usual	Much more than usual

APPENDIX C

McGILL PAIN QUESTIONNAIRE

SUBJECT CODE: _____ DATE: _____ TIME: _____

PRI: S _____ A _____ E _____ M _____ PRI (Total) _____ PPI _____
(1-10) (11-15) (16) (17-20) (1-20)

1	2	3	4
1. Flickering 2. Quivering 3. Pulsing 4. Throbbing 5. Beating 6. Pounding	1. Jumping 2. Flashing 3. Shooting	1. Pricking 2. Boring 3. Drilling 4. Stabbing 5. Lancinating	1. Sharp 2. Cutting 3. Lacerating

5	6	7	8
1. Pinching 2. Pressing 3. Gnawing 4. Cramping 5. Crushing	1. Tugging 2. Pulling 3. Wrenching	1. Hot 2. Burning 3. Scalding 4. Searing	1. Tingling 2. Itching 3. Smarting 4. Stinging

9	10	11	12
1. Dull 2. Sore 3. Hurting 4. Aching 5. Heavy	1. Tender 2. Taut 3. Rasping 4. Splitting	1. Tiring 2. Exhausting	1. Sickening 2 Suffocating

13	14	15	16
1. Fearful 2. Frightful 3. Terrifying	1. Punishing 2. Gruelling 3. Cruel 4. Vicious 5. Killing	1. Wretched 2. Blinding	1. Annoying 2. Troublesome 3. Miserable 4. Intense 5. Unbearable

17	18	19	20
1. Spreading 2. Radiating 3. Penetrating 4. Piercing	1. Tight 2. Numb 3. Drawing 4. Squeezing	1. Cool 2. Cold 3. Freezing	1. Nagging 2. Nauseating 3. Agonising 4. Dreadful 5. Torturing

APPENDIX D

WITS DIAL.A.BED SLEEP LABORATORY

MORNING FORM

DATE: _____ CODE: _____

TITLE OF STUDY: _____

What time did you switch off the light to go to sleep last night? _____

How long do you think it took you to fall asleep last night? _____

How many times did you wake up last night? _____

If you did wake up during the night, how long were you awake for? _____

Do you remember having a dream last night? _____

What time did you wake up this morning? _____

Do you remember having any pain last night? _____

On the scales below, please make a mark to indicate how your night was different, if at all, from your usual sleep at home.

Time to fall asleep

The shortest it has ever been _____ The longest it has ever been

Number of times woken up

Much more than normal _____ Much less than normal

Sleep Quality

Worst possible _____ Best ever

Morning Alertness

Awfully sleepy and tired _____ Marvellously alert and energetic