Evaluation and Treatment of Opioid-Induced Hyperalgesia

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

May 2007
The worst pain a man can suffer: to have insight into much and power over nothing.

The Histories (Book 9, Chapter 16)
Herodotus of Halicarnassus (484 BCE – 425 BCE)
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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Justin Luke Hay

21 May 2007
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ABSTRACT

Methadone is effective as an opioid substitution therapy, yet its use results in adverse effects such as tolerance and increased pain sensitivity to certain modalities of pain. Recent research has indicated that opioids have the potential to increase pain sensitivity following both acute and chronic dosing. While the pain sensitivity profile of methadone maintained patients is well established, no studies have compared the similarities and differences of chronic opioid users with different aetiologies, namely, chronic pain patients with moderate to severe pain and patients with a substance use disorder on a methadone maintenance treatment program.

Furthermore, limited guidelines exist for the treatment of acute pain in opioid-tolerant patients. Recent animal research and human studies in opioid-naïve patients have shown that the addition of ultra-low doses of an opioid antagonist can enhance the antinociceptive effectiveness and reduce adverse effects of opioid agonists. Moreover, recently developed, high potency opioid agonists such as remifentanil have shown to be antinociceptive in both experimental pain models and for acute pain following surgery. The antinociceptive effectiveness and safety of these novel and emerging pharmacotherapies have not been previously determined in methadone maintained patients. Additionally, animal models exist for the increased pain sensitivity observed following continuous opioid dosing of morphine. However, no comparable model exists for methadone.

The central aims of the studies contained herein were to investigate the effect of chronic methadone administration on pain sensitivity in both humans and rats, and investigate novel, acute pharmacological strategies for modifying nociception in opioid tolerant populations. The first two studies primarily evaluated the pain sensitivity of methadone maintained patients, chronic pain patients managed with methadone or morphine, former opioid users and opioid-naïve healthy subjects. The subsequent two studies investigated two unique approaches in providing antinociception in methadone maintained patients. While the administration of ultra-low doses of naloxone to these patients indicated limited success in providing antinociception, relatively high doses of remifentanil demonstrated that these patients are cross-tolerant to the physiological effects of other opioids. The subsequent study investigated the effect of a range of subcutaneous, continuously administered doses of methadone on pain sensitivity in the Sprague-Dawley rat and established a dose of methadone that can induce hyperalgesia. The current findings indicate that further investigations of this drug are justified especially with regard to its impact on nociception.
PUBLICATIONS AND PRESENTATIONS RELATED TO THIS THESIS

**International Conference Presentations**


**National Conference Presentations**


WINNER ASCEPT CLINICAL PHARMACOLOGY PRIZE

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1 INTRODUCTION

1.1 Prologue

Pain delivered by the nociceptive system acts as a protective mechanism that helps to maintain the homeostasis of the body’s physiological systems (Sherwood, 1997). Pain is the only somatosensory modality that is associated with motivated behavioural response and is subjectively influenced by emotions and experiences. While pain is beneficial in the sense that it is protective, untreated and unnecessary pain negatively impacts on all of the systems and this is especially observed in unrelieved pain. The presence of pain affects all major systems including the endocrine and metabolic systems and can lead to increased heart rate, increased blood pressure, decreased gastrointestinal motility, pulmonary complications and immunosuppression (NHMRC, 2005). Unrelieved acute pain can also have affective impacts such as anxiety, insomnia, feelings of helplessness and loss of control (Cousins et al., 2004). Effective analgesia can improve the recovery process following surgery (Kehlet and Dahl, 2003).

Opioids are the analgesics most commonly used for the treatment of moderate to severe pain. However, a growing number of studies are showing that the use of opioids both acutely and chronically can alter the pain sensitivity of individuals so that they experience increased sensitivity to noxious stimuli (hyperalgesia) (Sjøgren et al., 1994; Compton et al., 2000; Doverty et al., 2001b; Luginbühl et al., 2003) and pain elicited by normal innocuous stimulation (allodynia) (Sjøgren et al., 1993). Much of the evidence regarding this change in pain sensitivity is based on studies with methadone maintained subjects, patients undergoing surgery and experimental studies in opioid-naïve subjects (Angst and Clark, 2006), yet very little is known regarding chronic pain patients managed with opioids (Mao, 2002a). The opioid-induced hyperalgesia has implications for all patients treated with opioids. Hyperalgesia results in the patient feeling more pain and therefore is prone to the negative consequences associated with pain (Wilder-Smith and Arendt-Nielsen, 2006). The concept of opioid-induced hyperalgesia has been suggested as one of the potential reasons (Eisenach, 2000) that there is limited benefit afforded by pre-emptive analgesia evidenced in clinical studies (Katz and McCartney, 2002). Furthermore, evidence suggests that persistent nervous system sensitisation, observed as hyperalgesia, may be one of the mechanisms responsible for the development of chronic pain (Perkins and Kehlet, 2000).
The treatment of moderate to severe acute pain in opioid tolerant populations continues to be challenging (Scimeca et al., 2000; Mitra and Sinatra, 2004) and confounded by lack of guidelines. Heroin addicts are more likely to suffer injury (Cameron, 1964) and medical complaints (Sapira, 1968) than their non-addicted counterparts. The presence of increased pain sensitivity associated with opioids may contribute to the difficulty in treating pain in long-term opioid users. While the incidence of moderate to severe pain in the general population following major surgery is 41% (Dolin et al., 2002), inadequate treatment of pain is more likely to occur in patients with a history of substance abuse, including methadone maintained subjects (Portenoy et al., 1997). While the treatment of acute pain in opioid-tolerant, including opioid-addicted patients requires a multi-disciplinary approach (Jage and Bey, 2000; Mehta and Langford, 2006), pharmacological approaches utilising opioids can be limited due to cross-tolerance to other opioids (Jage and Bey, 2000; Doverty et al., 2001a). Research investigating the pain management requirements in opioid-tolerant patients, as well as patients with a substance use disorder, has been identified as requiring specific attention (NHMRC, 2005).
1.2 Nociception and pain

Several attempts have been made to define pain (for example Sternbach, 1968; Mountcastle, 1980) however, the definition adopted by the International Association for the Study of Pain (IASP) is ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage’ (Merskey, 1986). It has to be stressed that pain is subjective and a psychological state. The IASP definition of pain allows for it to be differentiated from the terms of nociception. Nociception (from Latin nocere: to harm or damage) refers to noxious stimuli and the subsequent activity within the nociceptor and nociceptive pathways. As a consequence, nociception can occur without pain and pain can occur without nociception. Further terminology associated with pain and nociception is detailed in Table 1.1.

Table 1.1 Definitions of pain and nociception terminology

<table>
<thead>
<tr>
<th>Term</th>
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<tr>
<td>Allodynia</td>
<td>Pain due to a stimulus which does not normally provoke pain.</td>
</tr>
<tr>
<td>Analgesia</td>
<td>Absence of pain in response to stimulation which would normally be painful.</td>
</tr>
<tr>
<td>Anesthesia dolorosa</td>
<td>Pain in an area or region which is anestheti.</td>
</tr>
<tr>
<td>Causalgia</td>
<td>A syndrome of sustained burning pain, allodynia, and hyperpathia after a traumatic nerve lesion, often combined with vasomotor and sudomotor dysfunction and later trophic changes.</td>
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<tr>
<td>Central pain</td>
<td>Pain initiated or caused by a primary lesion or dysfunction in the central nervous system.</td>
</tr>
<tr>
<td>Hyperalgnesia</td>
<td>An increased response to a stimulus which is normally painful.</td>
</tr>
<tr>
<td>Hyperesthesia</td>
<td>Increased sensitivity to stimulation, excluding the special senses.</td>
</tr>
<tr>
<td>Hypoalgesia</td>
<td>Diminished pain in response to a normally painful stimulus.</td>
</tr>
<tr>
<td>Hypoesthesia</td>
<td>Decreased sensitivity to stimulation, excluding the special senses.</td>
</tr>
<tr>
<td>Neuralgia</td>
<td>Pain in the distribution of a nerve or nerves.</td>
</tr>
<tr>
<td>Neuritis</td>
<td>Inflammation of a nerve or nerves.</td>
</tr>
<tr>
<td>Neurogenic pain</td>
<td>Pain initiated or caused by a primary lesion, dysfunction, or transitory perturbation in the peripheral or central nervous system.</td>
</tr>
<tr>
<td>Neuropathic pain</td>
<td>Pain initiated or caused by a primary lesion or dysfunction in the nervous system.</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>A disturbance of function or pathological change in a nerve: in one nerve, mononeuropathy; in several nerves, mononeuropathy multiplex; if diffuse and bilateral, polyneuropathy.</td>
</tr>
<tr>
<td>Nociceptor</td>
<td>A receptor preferentially sensitive to a noxious stimulus or to a stimulus which would become noxious if prolonged.</td>
</tr>
<tr>
<td>Noxious stimulus</td>
<td>A noxious stimulus is one which is damaging to normal tissues.</td>
</tr>
<tr>
<td>Pain threshold</td>
<td>The least experience of pain which a subject can recognize.</td>
</tr>
<tr>
<td>Pain tolerance level</td>
<td>The greatest level of pain which a subject is prepared to tolerate.</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>An abnormal sensation, whether spontaneous or evoked.</td>
</tr>
<tr>
<td>Peripheral neurogenic pain</td>
<td>Pain initiated or caused by a primary lesion or dysfunction in the peripheral nervous system.</td>
</tr>
<tr>
<td>Peripheral neuropathic pain</td>
<td>Pain initiated or caused by a primary lesion or dysfunction in the peripheral nervous system.</td>
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Adapted from Mersley & Bogduk (1994)
1.2.1 Concepts and theories of pain perception

Pain is believed to permeate though all life linked to consciousness (Fülop-Miller in Bonica, 1991). Humankind, regardless of era or location, has endeavoured to understand pain and some might say, more importantly, its relief (Bonica, 1991). As with all research, an understanding of where we have come from helps understand where we are going.

Ancient, Medieval and Renaissance Periods

Each of the ancient civilisations including those of Egypt, China, Greece and Rome had their own documentation that reflected their own concepts of life, medicine, sensation and pain. It was Aristotle’s original postulate, that the heart was the centre for emotions and sensations, that prevailed in the western intellectual environment until the Middle Ages (for brief review see Bonica (1991)). Illustrations during this time combining ancient and medieval ideas were the first to suggest that all sensory organs were connected to the brain, albeit via the heart (Figure 1.1). Importantly this illustration was one of the first to exemplify that pain (as illustrated by fire and the snake bite) are part of the sensory systems.

Specificity theory

It was not for another 150 years that René Descartes introduced a mechanistic view of the nervous system and brain (Descartes, 1644) (Figure 1.2). This work by Descartes suggested that external stimuli activated nerves in the periphery, which transmitted their signals to the brain whereby the pineal gland “reflected” the signal, thereby activating motor nerves and consequently moving the appropriate muscles away from the stimulus. He thereby described what is known today as the reflex arc. While not directly pertaining to pain, he was the first to hint that there were nociceptors in the periphery and nociceptive pathways to and within the brain (Merskey et al., 2005).
With the development of experimental physiology, Müller (1840) in Bonica (1991) stated that sensory nerves pass information to the specific centres of the brain regarding the external milieu, and that each sensation had its own specific form of energy that was transmitted from the particular sensory organ straight through to the brain centre.

**Pattern theory**
To explain inadequacies of the specificity theory, other alternative theories were put forward that could be collectively grouped as ‘pattern theory’. Goldscheider (1891; 1894) proposed that the input patterns related to pain are centrally summated in the dorsal horns of the spinal cord. This idea was further developed by Sinclair (1955) who postulated that excessive peripheral stimulation of non-specific receptors results in patterns of nerve impulses which are translated centrally. Unfortunately, this theory ignored the facts of physiological specialisation.

Livingston (1943) had a more central approach and suggested that external stimulation actioned closed-circuit loops within the dorsal horn of the spinal cord with this hypothesis borne from observations of phantom limb pain. Noordenbos (1959) suggested that under normal physiological circumstances, large fibres inhibited smaller fibres that carried nociceptive information; yet a ratio shift, with the domination of small fibres, would result in transmission, summation and pain.

Despite the advances and contributions that were made with these theories, each theory had its own weaknesses. While, specificity theory was limited by its straight-through, skin-to-brain ideology; pattern theory largely ignored the concept of peripheral specificity.

**Gate-control theory**
Melzack and Wall (1965) suggested that the concepts of specificity and pattern theory be re-appraised. They suggested that sensation in the periphery was relayed by large (L) myelinated mechanoreceptive fibers (A-α and A-β fibres) and small (S) myelinated and unmyelinated
nociceptive fibers (A-δ and C fibres) (Figure 1.3). This information (excitation(+) and inhibition(-)) was transmitted to three systems: cells in the substantia gelatinosa (SG cells), the projections to the brain (dorsal fibres) and cells within the spinal cord (T cells) that mediated information to the brain, whereby it potentially elicited a pain response. The theory further proposed that: information from afferent fibres was ‘gated’ in the dorsal horn before transmission to the spinal cord cells; the gating mechanism was inhibited by large diameter fibres, facilitated by small diameter fibres and mediated by nerve impulses descending from the brain; and that a central control, mediated by specialised large diameter fibres, activated cognitive processes that further modulated ‘gating’ via descending pathways. This theory was later expanded to include roles for cognitive, motivational and affective characteristics of pain (Melzack and Casey, 1968). However, the theory was unable to incorporate the observation of plasticity within the nervous system.

*Neuromatrix theory*

Developments since the postulation of the gate-control theory indicate that inflammation, learning, repeated stimulation, the ability to experience pain in the absence of nociceptive input (for example, phantom limb pain) and environmental impacts all can influence the experience of pain (Loeser and Melzack, 1999). To further develop the concept of pain and incorporate these features of pain, it was proposed that a neuromatrix must exist within the brain (Melzack, 1990). It was purported, and later substantiated by functional imaging techniques (Derbyshire, 2000), that the matrix could be partitioned into two indistinct systems associated with nociception and pain: the lateral nociceptive system, responsible for the sensory, discriminatory and motor co-ordination; and the medial nociceptive system, accountable for affect, attention, cognition, memory and response selection (Melzack, 1990). The way each of the physiological structures underlying these systems interacts and consequently nociceptive information is processed determines our reaction to pain.
1.2.2 Physiology of pain

The perception of tissue damage as pain requires many steps: activation of peripheral receptors, transmission of the signal to the spinal cord, processing of this information at the dorsal horn, with facilitatory and inhibitory controls, transmission to higher processing centres and finally perception of pain within the cortex (Figure 1.4).

Figure 1.4 The primary ascending and descending nociceptive pathways

Peripheral receptors

Nociceptors, sometimes incorrectly referred to as ‘pain receptors’, are found throughout the body and respond to different modalities such as mechanical, thermal or chemical stimuli; they can be differentiated from other receptors in that they have a relatively high threshold for their respective stimuli (Wall and Melzack, 1999). Following tissue injury caused by disease, inflammation, injury or surgery, there is cellular damage that leads to the release of algesic ligands from cells (Table 1.2). Furthermore, inflammation can cause extravasation of histamine, serotonin and bradykinin from blood vessels. These ligands not only bind to their
respective receptors causing cellular depolarisation, but also cause sensitisation of the
nociceptors. This sensitisation results in lowering of the threshold of the receptor, such that a
smaller stimulus can cause action potential transmission. This sensitisation is the cause of
primary hyperalgesia and can also lead to pain due to a non-painful stimulus (allodynia).
While peripheral receptors are mainly distributed in the skin and internal surfaces, deep
tissues are weakly supplied. This leads to vague localisation of deep tissue and visceral pain
while cutaneous pain is generally distinct and well-localised.

Table 1.2 Examples of receptors and ligands within the nociceptive pathways

<table>
<thead>
<tr>
<th>Receptor - Ionotropic</th>
<th>Subtype</th>
<th>Example ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient receptor potential (TRP) channel</td>
<td>TRPV1</td>
<td>Heat (&gt;42 °C), capsaicin, H⁺</td>
</tr>
<tr>
<td></td>
<td>TRPV2</td>
<td>Heat (&gt;53 °C)</td>
</tr>
<tr>
<td></td>
<td>TRPV3</td>
<td>Noxious cold (&lt;17 °C)</td>
</tr>
<tr>
<td>Acid sensing ion channel (ASIC)</td>
<td></td>
<td>Protons</td>
</tr>
<tr>
<td>Purine</td>
<td>P2X3</td>
<td>Adenosine triphosphate (ATP)</td>
</tr>
<tr>
<td>Serotonin (5-hydroxytryptamine)</td>
<td>5HT3</td>
<td>5-hydroxytryptamine (5HT)</td>
</tr>
<tr>
<td>NMDA</td>
<td>NR1</td>
<td>Glutamate</td>
</tr>
<tr>
<td>AMPA</td>
<td>iGluR1</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Kainate</td>
<td>iGluR5</td>
<td>Glutamate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Receptor - Metabotropic</th>
<th>Subtype</th>
<th>Example ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabotropic glutamate</td>
<td>mGluR1,2,3,5</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Prostanoids</td>
<td>EP1-4</td>
<td>Prostaglandin (PGE)</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Prostacyclin (PGI2)</td>
</tr>
<tr>
<td>Histamine</td>
<td>H1</td>
<td>Histamine</td>
</tr>
<tr>
<td>Serotonin (5-hydroxytryptamine)</td>
<td>5HT1A,</td>
<td>5-HT</td>
</tr>
<tr>
<td></td>
<td>5HT4,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5HT2A</td>
<td></td>
</tr>
<tr>
<td>Bradykinin (BK)</td>
<td>B1, B2</td>
<td>BK</td>
</tr>
<tr>
<td>Cannabinoid</td>
<td>CB1-2</td>
<td>Anandamide</td>
</tr>
<tr>
<td>Tachykinin</td>
<td>Neurokinin-1 (NK1)</td>
<td>Substance P, neurokinin A</td>
</tr>
<tr>
<td>Opioid</td>
<td>OP1 (δ), OP2 (κ), OP3 (μ)</td>
<td>Enkephalin, dynorphin, β-endorphin</td>
</tr>
</tbody>
</table>

Adapted from NHMRC (2005)

Peripheral mechanisms

Resulting electrical impulses travel along primary afferent fibres, whose cell bodies are
situated in either the dorsal root ganglia (innervating the trunk, limbs and viscera) or the
trigeminal ganglion (afferents innervating the head, oral cavity and neck). These impulses, or
action potentials, are transmitted through the primary afferent nerves and are dependent on
voltage-gated sodium channels. These nerve fibres can be differentiated into three types of
fibres according to the type of action potential they are associated with: Aβ-fibres are
connected to specialised, highly sensitive mechanoreceptors used for the senses of
proprioception and touch. C-fibres and Aδ-fibres encode other sensory qualities such as
thermal stimuli as well as nociceptive information. Aδ-fibres are thinly myelinated fibres that
conduct signals at a velocity of 5-30 m/s usually resulting in sharp, acute prickling sensation.
C-fibres constitute approximately 90% of all the fibres contained in a typical skin nerve. In
contrast to Aδ-fibres, C-fibres are unmyelinated and have a slower signal transmission rate of
0.5-2 m/s and result in aching, throbbing or burning pain. These fibres enter the dorsal horn of the spinal cord either via the ventrolateral bundle of the dorsal root or the ventral root. Primary nociceptive afferent fibres terminate in the dorsal horn of the spinal cord (Wall and Melzack, 1999). Consequent depolarisation of the primary afferent terminal results in release of either peptide or excitatory amino acid neurotransmitters.

**Dorsal horn**
The dorsal horn is divided into laminae due to their histological appearance. C-fibres terminate in lamina II, otherwise known as the substantia gelatinosa. Aδ-fibres project principally to laminae I and V. Aβ-fibres, which transmit signals related to touch and vibration, terminate in laminae III-VI and can synapse with terminals of the C-fibres in lamina II. The extent of nociceptive transmission through the dorsal horn from C-fibres depends on two mechanisms. Firstly, the activity of large myelinated afferent (Aβ) fibres can suppress signal transmission of C-fibres and secondly, descending inhibition from higher centres can also ‘close the gate’ to onward transmission. Central sensitisation leads to secondary hyperalgesia that generally encompasses a greater area of pain.

**Ascending signal transmission**
From the dorsal horn, nociceptive neurons ascend to the ventral posterior lateral nucleus of the thalamus by means of spinothalamic and spinoreticular tracts of the anterolateral white matter of the spinal cord. Some of the secondary afferents ascend to the thalamus, with collateral projections to the periaqueductal grey and then to the post-central gyrus directly via the spinothalamic tract. The majority of fibres, however, ascend via the spinoreticular tract where they synapse in the medulla and then continue to the thalamus. From this point, signals are distributed to the limbic system and cerebral cortex. It is here that the perception of pain is conceptualised.

**Descending modulation**
Projections from the cortex, thalamus, hypothalamus and collaterals from the spinothalamic tract enter the periaqueductal grey in the mid-brain. These projections synapse with neurons which descend to the nucleus raphe magnus in the medulla. Furthermore, axons descend from here to the dorsal horn by means of the dorsolateral funiculus. Modulation can result in either descending facilitation or descending inhibition by means of neuronal ‘on-cells’ and ‘off-cells’, respectively.
1.2.3 Types of pain

Pain can be divided into a taxonomy of three types of pain (transient, acute, chronic) that considers the temporal and causative quantities. However, pain is increasingly being recognised as more of a continuum with no clear-cut definitions of time (Loeser and Melzack, 1999).

Transient

This ubiquitous type of pain is normally characterised by its rapid onset and offset. Transient pain is thought to be protective in nature as it is not associated with substantial tissue damage. Clinically, transient pain is akin to venepuncture (Loeser and Melzack, 1999).

Acute

Acute pain is the result of substantial tissue damage instigating nociceptor activation. Nevertheless, the body has the ability to heal itself and this pain is usually measured in the context of days to weeks. Clinically, surgery and trauma are prime examples of this pain. As a caveat to this though, longer lasting acute pain can be classified as continuous, such as with pain related to malignancy or cancer (Loeser and Melzack, 1999).

Chronic

The nature of chronic or persistent pain is reflected by the body’s inability to heal itself and therefore pain such as back pain, post-herpetic neuralgia, long-term post-surgical pain and fibromyalgia, is related to the timeframe of months to years. Chronic pain is clinically considered the most difficult to treat due to its unrelenting nature and that most treatments are for the pain rather than the nociceptive cause (Loeser and Melzack, 1999).

1.2.4 Experimental pain

Pain is subjective in its nature; therefore, there can be no objective measure of pain perception. Nevertheless, experimental pain models exist that allow for the determination of nociceptive processing and pain perception. As a result of an early controversy, Beecher (1959) postulated that experimental pain models should be: non-invasive, specific (to pain), sensitive, show analgesic dose relation, reproducible, and applicable to various tissues. These criteria were later expanded by Gracely (1999), who suggested that pain models should have the following additional requirements: rapid onset, rapid termination, be natural, be repeatable with minimal temporal effects, be objective and they should excite a restricted group of primary afferents. One simple experimental pain model cannot replicate the multidimensional nature of clinical pain (Petersen-Felix and Arendt-Nielsen, 2002). Indeed, many studies
observe changes in nociception induced with one modality of pain induction but not with others (for example Doverty et al., 2001a; Angst et al., 2003; Luginbühl et al., 2003; Athanasos et al., 2006). It is therefore preferable that studies that quantify nociception using pain models use more than one method of pain induction.

**Thermal**
Thermal pain can be elicited via a variety of ways: heat pain can be induced using either a thermodome, as radiant heat or using a laser. Furthermore thermodomes can be used to induce cold pain which can also be induced using a cold pressor test. The cold pressor test generally involves placing a limb in very cold water; subjects then indicate pain threshold, pain tolerance and in some circumstances pain decay. Other studies using the cold pain test measure the pain intensity caused by the test. The cold pressor test is the principal pain test used in the clinical research of this thesis and is further described in the Chapter 2.

**Electrical**
Electrical pain can be evoked transcutaneously, intracutaneously, intramuscularly and within the viscera. Pain induced by electrical stimulation has the advantage of producing stimulation that is easily controlled with rapid onset and offset. However, its main criticism arises from its ability in directly stimulating a wide range of afferent axons. As a consequence, the sensation it produces is considered unnatural in its nature and cannot be used to infer analgesic effect at the receptor level (Chapman et al., 1985). Some models have been developed that can be used to stimulate subsets of afferent fibres by altering the stimulation frequency or intensity of the electrical stimulation (Arendt-Nielsen et al., 2000).

**Mechanical**
Again, several methods exist for producing mechanical pain in humans. These include the use of Von Frey hairs, pressure algometry, impact forces and distension of the viscera. Many of the nociceptive tests based on mechanical stimuli are used for the assessment of hyperalgesia and naturally, different mechanisms underlie different nociceptive tests. For instance, dynamic hyperalgesia can be assessed by gently stroking the skin with cotton wool; Von Frey hairs can be used for the assessment of punctuate hyperalgesia; furthermore, tonic pain can be measured by applying blunt probes (pressure).

**Ischemic**
The placement of a tourniquet around an arm and exercising the hand produces a severe, continuous pain that rapidly escalates in intensity. Experimentally-induced ischemic pain is
limited by its ability to produce fatigue, as a result temporal summation becomes an issue with repeated testing.

**Chemical**

The application of chemical stimuli can produce pain. For example, the application of carbon dioxide to the nasal mucosa produces a stinging pain (Hummel et al., 1998), while the injection of isotonic saline into the muscle mimics that of muscular pain (Graven-Nielsen et al., 1997). The topical, intradermal or intramuscular application of capsaicin stimulates vanilloid TRPV1 receptors and produces a stinging or burning pain.

One previous study elegantly demonstrated that the analgesic properties of an opioid analgesic can be detected differently by various experimental pain modals. Luginbühl and colleagues (2003) tested the antinociceptive effects of alfentanil using electrical, pressure, heat, cold-water and ischemic pain tests. Results indicated that of these tests, electrical, pressure and cold water, but not the other tests, could detect the antinociceptive properties of the opioid. This study highlighted that nociceptive changes not only need to be assessed using the appropriate pain model, but using more than one pain model allows for more comprehensive quantification of changes in nociception.

**1.2.5 Indices of pain assessment & pain response - stimulus-dependent methods**

Experimental pain models that utilise the stimulus dependent model adjust the stimulus until a pre-defined threshold, such as pain detection or pain tolerance are reached. Being subjective in nature, they are subject to psycho-physiological bias (Gracely, 1999).

*Perception threshold*

This is the point at which a subject can first perceive a stimulus. While not by definition a measure of pain or nociception, it is useful as a measure of sensation sensitivity.

*Pain detection threshold*

Pain detection is the point which a subject distinguishes a stimulus as no longer being non painful but becoming painful. Pain detection is subject to large ‘response bias’, with this confounder amplified when there is a large range of pre-pain intensities (Chapman et al., 1985). Measurements of pain threshold has also been criticised as being sensitive to placebo effects, instruction and expectancy and therefore inappropriate as an endpoint measure in analgesic studies (Chapman et al., 1985).
**Pain tolerance threshold**
Pain tolerance threshold is the point at which a subject recognises the highest pain intensity they are able to tolerate. This threshold is subject to less behavioural influences and is therefore less variable, especially with paradigms that employ repeated pain tests.

The use of stimulus dependent measures and their limitations were the basis of early criticism. The use of pain detection as a primary end point was utilised in early experimental pain models (for example, Hardy et al., 1940). Furthermore, Beecher and colleagues (1959) failed to notice any increase in thermal pain threshold following morphine administration and openly criticised experimental pain models as they lacked the emotional components of clinical pain, including anxiety, fear and distress. Later experimental pain models that employed more severe, continuous pain and thus evoking a ‘reaction component’ were used; consequently, the antinociceptive properties of narcotics were able to be detected by measuring pain tolerance thresholds (Smith et al., 1966). This highlighted the need to utilise appropriate endpoints when designing experimental pain based research.

1.2.6 Indices of pain assessment & pain response - response-dependent method
Experimental pain models that use response dependent methods usually present the subject with a fixed stimulus and the perceived intensity is then measured as the independent variable.

**Categorical verbal descriptor scale**
These scales rely on the subject or patient describing their pain in terms of a verbal description scale (none, mild moderate, severe or other variation) or as a discrete number usually within the range of 0 (no pain) to 10 (worst pain imaginable). This type of scale has the advantage of being simple and easy to administer.

**Visual analogue scale**
The visual analogue scale (VAS) consists of a 100 mm line with labels at each end indicating ‘no pain’ and ‘worst pain imaginable’, or similar description. The subject then indicates their pain by marking their pain intensity on the line appropriately. Studies indicate that using a VAS for the rating of pain intensity can be useful for measuring and monitoring experimental, surgical and retrospective experiences of pain (Chapman et al., 1985; Rosier et al., 2002).

**Multi-dimensional assessment of pain**
Uni-dimensional assessment of pain using tools such as pain intensity using a VAS have been criticised as being too reductionalist and overly simplistic. One of the first to assess pain
multidimensionally was by Melzack and colleagues who developed the McGill Pain Questionnaire (Melzack, 1975; Melzack, 1987). The questionnaire assesses the sensory, affective, evaluative and miscellaneous dimensions of pain. Due to its complexity, the questionnaire is infrequently used for the assessment of experimental pain whereas many clinical pain conditions have been quantified by it. Despite the fact that pain is described as multi-dimensional, the use of related measures in practice is generally not utilised.

**Electrophysiological methods**

A nociceptive withdrawal reflex can be induced by stimulation of the sural nerve and this can be measured by the reflex response in the biceps femoris muscle. The threshold of the reflex corresponds to the pain detection threshold (Willer, 1977). Following nociceptive stimulation the latency and amplitude of evoked potentials can be used to measure pain intensity. Reductions in amplitude and increases in latency reflect analgesia. The main disadvantage of this pain model is that it disregards any modulation that occurs at the dorsal horn or within higher centres. This however, becomes an advantage when investigating the properties of nociceptors associated with A and C fibres (Torebjörk and Ochoa, 1990).

**Brain imagining**

Brain imaging has allowed the assessment of the ‘working’ brain. Measures such as electroencephalography (EEG) tend to be relatively insensitive to analgesic effects. The way the brain reacts to pain and anti-nociceptive therapies can be imaged and quantified using imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). With these methods, the activation of certain sections of the brain can be revealed in PET studies as increases in the regional cerebral blood flow (rCBF), while in fMRI studies the blood oxygen level dependent (BOLD) signal indicates changes in brain activity (Peyron et al., 2000). As mentioned before, these methods have been pivotal in identifying mechanisms underlying nociception and pain (Derbyshire, 2000). However, their expense and reliance on trained staff limits their broad utilisation.

**Micro-dialysis**

By implanting a probe subcutaneously or intramuscularly, perfusing the tissue with saline and sampling the dialysate, tissue concentrations of substances involved with nociception can be measured (Müller et al., 1995). This can allow for the quantification of algesic and analgesic substances within particular tissues and provides insights into what is occurring locally within specific tissues.
1.2.7 Situational and individual influences of pain perception

It is widely known that situational and individual factors influence pain perception and these have been taken into account when designing the studies contained within this thesis. Pain sensitivity is strongly influenced by cultural influences, ethnicity and race (Price and Harkins in Turk and Melzack, 1992; Melzack and Wall, 1996). Psychological factors such as attention, distraction, ability to cope, control over situation, past experiences and situational context can partly explain why pain can be experienced in some cases and not in others. Additionally, these factors indicate why pain can be disproportionate to the severity of injury (Melzack and Wall, 1996; Haythornthwaite et al., 1998; Turk, 1999). Again, this emphasises the subjective nature of pain and provides some explanation for the large variation usually seen in pain assessments observed both in the clinic and the laboratory. The following is a brief selection of other major variables that need to be taken into account by investigators.

Sex

Firstly, it is important to clarify that the term ‘sex’ refers to the biological classification based on chromosomes and reproductive function whereas ‘gender’ refers to anthropological aspects of social and cultural categorisation. It is common for these terms to be interchanged and the distinction between the two to be overlooked. In this thesis, the designations of ‘male’ and ‘female’ refer to the sex of the subject and not their gender.

Experimental and clinical studies have generally demonstrated that females are more sensitive to pain compared with males. This difference is not only attributable to psychosocial factors and social conditioning but more recent studies indicate that there is a biological component as well. Sociocultural factors that influence pain sensitivity depend on elements such as age, ethnicity and family history. Females may be more sensitive to pain compared with men due psychosocial factors such as greater tendency to hypervigilence, body monitoring and higher prevalence of anxiety and depression (Rollman et al., 2004). Biological differences between the sexes can be attributable to dissimilarities in sex hormones; furthermore pain sensitivity has been reported to change according to menstrual cycle (Wiesenfeld-Hallin, 2005).

Age

Pain perception in children differs significantly to that of adults – disparity between the two populations with regards to maturity, cognitive and neurophysiological development, ability to express emotions and past experiences of pain can influence experience and interpretation of pain (Schechter, 1985; Stevens et al., 1994b; Gibson and Farrell, 2004). Similarly, changes
in cognitive function, physiology, the higher incidence of disease, and medication administration in the elderly differentiates pain perception in this population from younger individuals (Macintyre et al., 2003; Gibson and Farrell, 2004).

**Genetics**
There is increasing evidence that genetic factors influence the perception of pain (Mogil, 1999). Heritability studies indicate that genes may have a role in migraine pain (Ziegler et al., 1998), back pain (Bengtsson and Thorson, 1991) and menstrual pain (Treloar et al., 1998).

**Experimenter**
As discussed before, experimental pain is a useful tool for investigating mechanisms of nociception and establishing the potency of analgesics. Yet a caveat exists regarding the psychosocial milieu in which experimental pain tests are performed. Studies have shown that the experimental pain scores of subjects depend on the gender of the experimenter and the subject. Males being administered a cold pressor test by a female experimenter reported lower pain scores than when administered by a male experimenter; no significant differences were observed in female subjects when tested by either a male or a female (Levine and De Simone, 1991; Gijsbers and Nicholson, 2005). Furthermore, one study found that higher ‘professional’ appearance of the experimenter is related to higher pain tolerance in subjects. Moreover, one study has shown that ‘interpersonal transactions’ (talking to the subject) can influence cold pressor tolerance times in females but not males (Jackson et al., 2005). These studies highlight pertinent factors that need to be considered when designing and administering experimental pain tests.

**Psychosocial influences**
Psychosocial factors influence pain sensitivity, as pain perception is influenced by emotional status and past experiences. Factors such as anxiety, depression, fear and coping ability impact on pain perception (Raja in Wall and Melzack, 1999) and can be assessed, at least in part, by global scales of mood such as the profile of mood states (POMS) questionnaire (McNair et al., 1971). One previous study showed that mood disturbance, when measured by the POMS questionnaire, directly correlated with pain intensity scores in chronic pain patients indicating that greater pain intensity was associated with increased mood disturbance (Lin et al., 2003).
1.3 Opioids

1.3.1 Historical perspective

The euphoric, sedative and analgesic properties of opium, derived from the unripe seed pods of the opium poppy (*Papaver somniferum*), have been referenced as far back as 4000 BCE with Sumerian ideograms describing opium as the ‘plant of joy’ (Stefano et al., 2000). Opium contains a mixture of alkaloids including morphine, codeine and thebaine, with most of its analgesic and sedative properties attributable to morphine. It was Sertürner (1806) who first isolated and later purified (Sertürner, 1817) morphine and who named the compound after the Greek god of dreams, Morpheus. It took a further century for the chemical structure of morphine to be identified. The term ‘opiate’ refers to the naturally or semi-synthetically derived compounds found within opium whereas ‘opioid’ is the term used to describe natural and synthetic compounds that have morphine-like actions or compounds to bind to opioid receptors.

1.3.2 Opioid receptors and ligands

Structure-activity analysis of synthetic opioids led to the proposal that a receptor for opioids may exist (Beckett and Casy, 1954); further, more detailed analysis suggested that more than one type of receptor may exist (Portoghese, 1965). In 1973, several research groups simultaneously identified the brain binding sites for opioid drugs (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973). The presence of specific receptors led to the search and eventual discovery of the endogenous ligands: enkephalins (Hughes et al., 1975), β-endorphin (Cox et al., 1976) and dynorphins (Goldstein et al., 1979). This subsequently led to the classification and naming according to the prototypic drug/tissue used in the studies of the opioid receptor, namely, the μ- (morphine), κ- (ketocyclazocine) and δ- (deferens) opioid receptors. More recently, these opioid receptors have been cloned and the recombinant receptors have binding affinities and function analogous to the endogenous receptors (Satoh and Minami, 1995). Sub-types of each of the receptors have been proposed Nonetheless, the basis of this hypothesis may be due to splice variants, post-translational regulation, receptor oligomerisation or interactions with accessory proteins (Williams et al., 2001).
Research using homology cloning led to the G-protein coupled receptor initially designated ORL1 or LC132. With little binding affinity with the known endogenous opioids ligands, research by two independent groups soon identified the endogenous peptide as nociceptin (Meunier et al., 1995) or orphanin FQ (Reinscheid et al., 1995) (referred to as N/OFQ). The N/OFQ receptor has little or no affinity for classic opioid agonists or antagonists. However, the $\mu$-, $\kappa$- and $\delta$-opioid receptors with regard to sequence, structure, distribution and function are comparable to the N/OFQ receptor.

Nomenclature for the opioid receptors has been and continues to be a contentious issue. Originally, it was proposed that the terms $\mu$, $\kappa$, $\delta$ and N/OFQ be replaced with OP$_3$, OP$_2$, OP$_1$ and OP$_4$, respectively (Dhawan et al., 1996). Rejection of this nomenclature by the general research community has lead to the acceptance of the original terms as well as the terms MOP, KOP, DOP and NOP, respectively (Foord et al., 2005). The original terms are used throughout this thesis.

<table>
<thead>
<tr>
<th>Opioid receptor</th>
<th>Locations</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>Neocortex, Thalamus, Nucleus accumbens, Hippocampus, Amygdala, Periaqueductal grey, Hypothalamus, Dorsal horn (spinal cord)</td>
<td>Analgesia, Respiratory depression, Addictive properties, Miosis, Nausea and vomiting, Constipation, Sedation, Euphoria, Cardiovascular effects, Thermoregulation</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Nucleus accumbens</td>
<td>Analgesia, Miosis, Sedation, Dysphoria</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Olfactory bulb, Neocortex, Caudate putamen, Nucleus accumbens</td>
<td>Analgesia, Respiratory depression, Olfaction, Nausea and vomiting, Constipation, Sedation, Euphoria, Hallucinations</td>
</tr>
</tbody>
</table>

Opioid receptors are found throughout the body. However, they are mainly concentrated within the central nervous system (CNS). Distribution of opioid receptors in the CNS and their density reflect functional importance of opioids in those regions (Mao, 1999). Opioid receptors are found on the primary and secondary afferents with $\mu$-, $\kappa$- and $\delta$-opioid receptors
found pre-synaptically and µ-opioid receptors found post-synaptically (Table 1.3) (Satoh and Minami, 1995).

Drugs that interact with these opioid receptors are classified as full agonists, partial agonists, or antagonists depending on the cellular activity they elicit (Table 1.4). A mixed agonist-antagonist drug has the ability to produce agonist activity at one receptor and antagonist activity at another. For instance, pentazocine is an agonist at κ-opioid receptors and has weak antagonist activity at the µ-opioid receptor.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Example opioid ligand</th>
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<tbody>
<tr>
<td>Full agonist</td>
<td>When the receptor stimulus induced by an agonist reaches the maximal response capability of the system (tissue), then it will produce the system maximal response and be a full agonist in that system.</td>
<td>Morphine</td>
</tr>
<tr>
<td>Antagonist</td>
<td>A drug that reduces the action of another drug, generally an agonist. Many antagonists act at the same receptor macromolecule as the agonist.</td>
<td>Naloxone</td>
</tr>
<tr>
<td>Partial agonist</td>
<td>An agonist that in a given tissue, under specified conditions, cannot elicit as large an effect (even when applied at high concentration, so that all the receptors should be occupied) as can another agonist acting through the same receptors in the same tissue.</td>
<td>Buprenorphine</td>
</tr>
</tbody>
</table>

Adapted from Neubig (2003)

1.3.3 Cellular messenger cascades and effectors

Opioids belong to the family of G-protein coupled receptors; G-proteins are characterised by seven transmembrane domains and are coupled to guanosine diphosphate binding proteins. Binding of opioid agonists to all receptors types (µ-, κ- and δ-) causes similar messenger cascades. Following agonist binding the inhibition of adenylyl cyclase and consequently cyclic adenosine monophosphate (cAMP) results in the opening of K⁺ channels, inhibition of voltage gated Ca²⁺ channels, inhibition of neurotransmitter release, activation of protein kinase C (PKC), release of calcium from intracellular stores, activation of mitogen-activated protein kinase (MAPK) cascade, receptor trafficking (eg internalisation) and nuclear signalling. This cascade results in cellular hyperpolarisation and therefore either inhibition of neurotransmitter release (pre-synaptic) or inhibition of action potentials (post-synaptic). While the primary effects are inhibitory, the inhibition of neurotransmitter can also cause excitatory effects in certain neural pathways (Williams et al., 2001).
1.3.4 Binding, intrinsic efficacy and potency

The clinical profile of an opioid agonist depends on many pharmacodynamic factors including its ability to bind to opioid receptors and its ability to activate G-coupled proteins (intrinsic efficacy). The binding affinity of an opioid relates to how well an opioid ligand binds to a receptor (Borgland et al., 2003). While pure opioid agonists generally have similar maximal effects (generally measured in terms of analgesia) and can be titrated to equianalgesic effect (McQuay, 1991), they differ with regards to efficacy; that is, the dose of drug required to elicit an effect. Accordingly, the intrinsic efficacy of an opioid can be linked to its analgesic potency (Traynor, 2004) (Table 1.5).

<table>
<thead>
<tr>
<th>Opioid ligand</th>
<th>Mean (SEM) whole brain $K_i$ (nM)</th>
<th>Relative efficacies determined for G protein coupling$^\wedge$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAMGO</td>
<td>0.6 (0.02)</td>
<td>1</td>
</tr>
<tr>
<td>Morphine</td>
<td>17.0 (1.0)</td>
<td>0.58</td>
</tr>
<tr>
<td>Remifentanil</td>
<td>21.1 (1.2)</td>
<td>NA</td>
</tr>
<tr>
<td>Naloxone</td>
<td>1.7 (0.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Naltrindole</td>
<td>76.0 (3.8)</td>
<td>NA</td>
</tr>
<tr>
<td>U50.488</td>
<td>9703.6 (498.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Methadone</td>
<td>NA</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Adapted from *Poisnel and colleagues (2006) and ^Borgland and colleagues (2003). *Binding affinities ($K_i$, nM) of compounds tested for $\mu$-opioid receptor ([3H]-DAMGO binding displacement) in rat brain and ^the ability of ligands to activate G proteins using inhibition of calcium channel currents as a reporter before and after inactivation of a fraction of receptors by $\beta$-chloromaltrexamine in AtT20 cells stably expressing a low density of FLAG-tagged $\mu$-opioid receptor.
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1.4 General opioid pharmacodynamics
All opioids exert similar effects on the body. Due to diverse binding affinities, intrinsic efficacies and pharmacokinetics, different drugs have different effect profiles. Additionally, due to pharmacokinetic variables, effect profiles also differ depending on route of administration (McQuay, 1991).

1.4.1 Analgesia
The antinociceptive effects of opioids are mediated in several ways. While opioids act mainly within the CNS, peripheral opioid receptors modulate the primary afferents by depressing the membrane potentials and therefore quantitatively reducing action potentials (Stein, 1991). Within the spinal cord, opioids act directly at the synaptic junctions between the primary and secondary afferents in the dorsal horn. Opioid agonists activate descending inhibitory pathways, specifically the neurons that originate within the PAG and project to the nucleus raphe magnus. These neurons in turn project to laminae I, II and V of the dorsal horn consequently inhibiting them. Under normal circumstances on- and off-cell activity is balanced, in that opioid administration alters the balance such that off-cells become continuously active while on-cells lose activity (Fields in Wall and Melzack, 1999). Moreover, analgesic effects of opioids are also mediated by higher central effects such as modifying perception and being anxiolytic in nature (Lasagna et al., 1955; Kaiko et al., 1981).

1.4.2 Respiration
The most severe adverse effect of opioids is respiratory depression and is typically the cause of death due to accidental or suicidal overdose. Opioids primarily mediate this effect via the respiratory centres in the medulla and at the chemoreceptors (White and Irvine, 1999). Equianalgesic doses of opioids result in similar respiratory depressant effects (Schug et al., 2003). However, the presence of pain is thought to counter the respiratory depressant effects of opioids (McQuay, 1999; Schug et al., 2003).

1.4.3 Sedation
The sedative properties of opioids manifest as drowsiness and mental clouding without any associated amnesia. The sedative properties of opioids are relatively dose-dependent and usually precede respiratory depression; consequently, clinically relevant opioid-induced respiratory depression is best assessed by measuring sedation in combination with measuring respiratory rate (Ready et al., 1988; NHMRC, 2005).
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1.4.4 Immune system

It has long been known that opioids are associated with immunosupression (Friedman et al., 2003). More recently, evidence shows that opioid receptors on immune cells impact on their function (McCarthy et al., 2001). Adding to the intricate nature of pain, recent studies have suggested that nociceptive pathways may be modulated via immunological mechanisms and opioid-related phenomenon such as tolerance, analgesia and increased pain sensitivity may be mediated through cells considered to be involved primarily with immunity (Hutchinson and Somogyi, 2004; Shavit et al., 2005; Watkins et al., 2005).

1.4.5 Other effects

Nausea and vomiting are the most common adverse effects following the administration of opioids and occur due to activation of opioid receptors within the chemoreceptor trigger zone (Schug et al., 1992). Furthermore, opioids cause constipation, especially following chronic use, due to delayed gastric emptying, increases in intestinal tone and decreases in intestinal motility (Schug et al., 1992); little to no tolerance develops to this effect. Opioids are also known to inhibit micturition (Schug et al., 2003).

Miosis is caused by the μ- and κ-opioid agonists exerting excitatory action on the parasympathetic nerve innervating the pupil. Opioids can have cardiovascular effects such as hypotension and bradycardia by reducing sympathetic discharge. Furthermore, pulmonary oedema can develop following opioid administration due to increased capillary permeability (Pugsley, 2002). Puritus can be mediated by the activation of mast cells by μ-opioids, initiating histamine-induced itch; itch can also be mediated by independent mechanisms within the CNS (Schmelz, 2002). The opioid agonists methadone and remifentanil do not induce the release of histamine (Schug et al., 2003). Opioid administration may cause CNS excitation and can potentially cause convulsions. Furthermore, opioids can alter the endocrine system and morphine-like drugs can also depress the cough reflex.
1.4.6 Inter-individual variation in response

Individuals respond to opioids differently. Accordingly there are large inter-individual variations in opioid requirements (Lehmann et al., 1990; Macintyre and Jarvis, 1996) and therefore opioids doses need to be titrated for each patient (NHMRC, 2005).

Sex

Recently, research has focused on the impact that sex has on opioid efficacy (Fillingim and Gear, 2004). Studies indicate that females may receive greater analgesia from µ-opioid agonists compared with men. The mechanisms underlying this difference may include effects from hormones, genetic influences, pharmacokinetics, pharmacodynamics and psychological factors (section 1.2.7) involved with pain sensations (Fillingim and Gear, 2004).

Age

With age come changes in physiology. These changes have the ability to affect the pharmacodynamics and pharmacokinetics of medications. Opioids are no exception, with elderly patients being more sensitive to the central effects of opioids (Scott and Stanski, 1987; Minto et al., 1997). Additionally, physiological changes may affect pharmacokinetics. Reduced renal clearance may affect the clearance of some opioids. Consequently, opioid requirements in older individuals may be significantly reduced (Macintyre and Jarvis, 1996).

Genetics

The opioid analgesia has been shown to be dependent on genes responsible for receptors, the metabolism and transport of opioids (Lötsch and Geisslinger, 2006). Single nucleotide polymorphisms (SNPs) in the gene responsible for the µ-opioid receptor (OPRM1) affect the potency of opioids (Lötsch et al., 2002; Skarke et al., 2003), while SNPs associated with the genes responsible for metabolising enzyme cytochrome P450 2D6 (CYP2D6) can render prodrugs such as codeine ineffective (Sindrup et al., 1990; Caraco et al., 1996). Variant alleles in the gene of the catechol-O-methyl transferase (COMT) enzyme may affect its functionality. This may impact on dopamine degradation, which in turn may influence endogenous opioid levels and this consequently could impact on opioid receptor expression (Zubieta et al., 2003). The activation of the melanocortin-1 receptor negatively impacts on opioid activity. Therefore variant alleles in the gene responsible (MCIR) may impact on the potency of opioids (Mogil et al., 2003; Mogil et al., 2005). SNPs in the gene responsible for the P-glycoprotein transporter (ABCB1) may also play a role in opioid efficacy (Hoffmeyer et al., 2000; Coller et al., 2006). The impact of genotype on opioid analgesia is yet to be fully elucidated.
1.5 Opioid tolerance

Following the administration of a drug, tolerance is the phenomenon of diminished effect, or the need for a higher dose to maintain the same effect. With regard to opioids, tolerance occurs at different rates depending on the physiological system affected (Ling et al., 1989). For example, tolerance develops to respiratory depression, sedation and nausea relatively quickly, whereas modest to insignificant tolerance develops to constipation and miosis. Tolerance to one opioid agonist often infers tolerance to other opioids of the same class, termed cross-tolerance. Tolerance can manifest in different forms: innate, learned (behavioural and conditioned) and acquired (pharmacokinetic and pharmacodynamic) (Goodman et al., 2006). Tolerance is likely to be one of the greatest influences impacting on achieving adequate acute pain relief in the methadone maintained individual.

1.5.1 Innate

Innate tolerance refers to tolerance afforded by genetically determined sensitivity and is apparent following first exposure. For example, individuals who are phenotypically poor metabolisers due to a non-functional cytochrome P-450 enzyme CYP2D6, cannot metabolise codeine to morphine and therefore this drug provides them with little analgesic effects (section 1.4.6) (Eckhardt et al., 1998).

1.5.2 Learned

Individual can learn to counter the effects of drugs, such that there is an apparent reduction in the effects of the drug. This is termed behavioural tolerance. Another type of learned tolerance is conditioned tolerance, such that if a drug is administered with the same cues, an adaptive response can be learned to oppose the drugs effects.

1.5.3 Acquired

**Pharmacokinetic**

Pharmacokinetic tolerance involves a change in the distribution or metabolism (usually presented as altered clearance) (Goodman et al., 2006). This may be caused by alterations in absorption, hepatic or renal clearance or altered plasma protein binding (Rang, 2001).

**Pharmacodynamic**

The mechanisms of acquired tolerance have not been fully elucidated. There are thought to be several main mechanisms underlying pharmacodynamic opioid tolerance: receptor down-regulation, receptor desensitisation by decoupling, receptor internalisation, alternative
coupling and functional antagonism (Raith and Hochhaus, 2004). The mechanisms relating to opioid tolerance also appear to impact on the development of opioid-induced hyperalgesia and dependence.

The idea of receptor down-regulation instigated by chronic drug administration was originally postulated by Himmelsbach (1941) and Collier (1965). While in vitro experiments support this concept of tolerance, in vivo experiments have been less conclusive (Raith and Hochhaus, 2004).

Receptor desensitisation involves increases of phosphorylation of the G-protein-induced receptor kinases and the cAMP-induced kinases. Furthermore, receptor desensitisation is mediated by the phosphorylation of the G-protein coupled receptors and the subsequent binding of the regulatory protein, β-arrestin. This binding of β-arrestin can also promote receptor internalisation (Zuo, 2005).

The internalisation, or endocytosis, of opioid receptors from the surface of the cell is one further mechanism of tolerance. The binding of an opioid agonist to the opioid receptor promotes the endocytosis of receptors, mediated by clathrin-coated pits on the cell surface. Following this internalisation, receptors can either be degraded or later recycled back to the cell surface. The degree of internalisation is related to the intrinsic activity of the agonist binding to the opioid receptor. Opioid receptor agonists such as DAMGO and fentanyl have higher intrinsic activity compared with the prototypic agonist morphine, and consequently cause greater degrees of receptor internalisation (Borgland et al., 2003).

Normally, opioid receptors preferentially bind to the inhibitory G-proteins: G_i and G_0-linked proteins; however, G_s-linked opioid receptors are also possible targets (Cruciani et al., 1993). Up-regulation of the G_s-linked opioid receptors can also mediate tolerance. This is reviewed in greater detail in Chapter 3 of this thesis.

Functional antagonism is considered one of the more important mechanisms of opioid tolerance. As described before, the agonist activity at opioid receptors causes the activation of intracellular PKC. This in turn promotes translocation of the NMDA receptor from the cytosol to the membrane where it becomes activated thus causing increased Ca^{2+} influx (Mao et al.,
1995b; Mayer et al., 1995). The antagonism of the NMDA receptor by MK-801, ketamine and Mg$^{2+}$ all show that they have the potential to reduce the development of tolerance.

Other mechanisms underlying tolerance have been hypothesised. It appears that cholecystokinin (CCK) and nitric oxide (NO) also are involved in the development of tolerance (King et al., 2005). In addition, evidence exists that opioid receptors can form homo- and heterodimers; these oligomers may have an influence on opioid pharmacodynamics and cellular surface trafficking (Gomes et al., 2002; Gomes et al., 2003). However, the significance and detailed mechanisms underlying these events are not fully understood.

The mechanisms associated with tolerance are also related to those of hyperalgesia (Wilder-Smith and Arendt-Nielsen, 2006). Understanding the molecular systems involved in these systems will help research into finding pharmacotherapies that may help prevent, if not at least limit, the development of tolerance and/or hyperalgesia. This is further discussed in section 1.10.
1.6 Opioid addiction, dependence and withdrawal

Addiction is considered to be a chronic disease and as such, the psychological, social and physical problems that develop do not necessarily disappear following detoxification (Meyer, 1996). However, it is becoming apparent that neuropharmacological and neuroadaptive mechanisms play an important role in the evolution of occasional, controlled drug use to abuse that defines drug addiction by being uncontrolled and displaying drug-seeking behaviour (Koob and Le Moal, 1997).

Addiction can be referred to in the literature as substance dependence and consequently the terms of dependence and addiction are sometimes used interchangeably. However, substance dependence (addiction) is distinct from physiological dependence (Table 1.1); therefore within this thesis the term dependence refers to the physiological adaptation and not to the disease state of addiction.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependence</td>
<td>Physiological adaptation to a drug whereby abrupt cessation, dose reduction or reversal of the drug causes a withdrawal (abstinence) syndrome.</td>
</tr>
<tr>
<td>Tolerance</td>
<td>A need to increase the amount of drug to achieve the original effect (cross-tolerance: tolerance to other drugs within the same class).</td>
</tr>
<tr>
<td>Addiction (Substance abuse disorder or substance dependence)</td>
<td>A disease that is characterised by loss of control over drug use, compulsive drug use and craving despite the risk of psychological, social and physical harm.</td>
</tr>
<tr>
<td>Pseudo-addiction</td>
<td>Behavioural changes that seem similar to drug-seeking but are secondary to the under-treatment of pain.</td>
</tr>
</tbody>
</table>


Heroin (3,6-diacetylmorphine) is rapidly de-acetylated to 6-monoacetylmorphine and then further de-acetylated to morphine. Binding studies show that activity of heroin is dependent on its metabolites and that it is essentially a pro-drug with limited binding or opioid activity itself (Inturrisi et al., 1983). Intoxication with an opioid has been described as four overlapping components including the short-lived ‘rush’ characterised by intense euphoria; the ‘high’ associated with feelings of general well being; the ‘nod’ which can range from sleepiness to unconsciousness followed by ‘being straight’ which is described as feeling calm and relaxed yet no experiences of withdrawal (Agar (1973) in Koob, 2006).

If however, following chronic opioid administration, opioids are ceased, an acute withdrawal syndrome ensues and manifests in symptoms such as yawning, perspiration, anxiety, craving,
lacrimation, piloerection, hot and cold flushes, muscle aches, nausea, vomiting, diarrhoea and insomnia. Furthermore, less severe symptoms can occur for months following opioid detoxification termed ‘protracted abstinence’ (Himmelsbach, 1942; Himmelsbach, 1943). Opioid withdrawal is not only physiological in nature but has a psychological component such that its effects can be conditioned to environmental stimuli: withdrawal effects can be attenuated by self-injection of placebo saline (Koob, 2006) or exacerbated by environmental cues (O'Brien et al., 1977).

Conceptually, addiction can be thought of as a disease that is initially characterised by impulsivity and perpetuated by positive reinforcement (such as euphoria and the absence of pain) but develops into a compulsive disorder that is mediated by negative reinforcement (avoiding withdrawal syndrome) (Koob, 2004 in Koob, 2006). Early models of addiction suggested that the acute effects of drugs are counteracted by homeostatic modifications within systems that cause the primary effect of the drug. Alternatively, addiction can be conceptualised as an opponent-process whereby positive euphoric effects such as euphoria are followed by negative effects such as craving, but over time (and repeated drug use) tolerance develops to the positive effects and these effects diminish; yet the negative effects become more substantial. Consequently, a new allostatic state is achieved, albeit with a homeostatic anchor that is lower than normal (Koob and Le Moal, 2001; White, 2004). Complementary to this idea is that addiction can be described as a process of ‘spiralling distress’: repeated drug usage results in the increased amplitude of the three main components of addiction: preoccupation/anticipation, withdrawal/negative affect and binge/intoxication which thereby results in the addiction state (Koob and Le Moal, 1997). These concepts are demonstrated by studies that show that opioid administration initially produces feelings of euphoria and elevated mood but following chronic usage, opioids are associated with brief euphoria accompanied by increasing dysphoria and negative affect (Price et al., 1975; Babor et al., 1976; Mirin et al., 1976a; Mirin et al., 1976b).

1.6.1 Neurobiology of dependence and addiction

Acute drug administration, including that of opioid use, involves the activation of the mesolimbic reward system. Classically, it is considered that following opioid administration, the activation of opioid receptors in the ventral tegmental area, nucleus accumbens and the amygdala of the brain facilitated the release of dopamine in the nucleus accumbens. This consequently activates the reward (mesolimbic or ventral tegmental area-nucleus accumbens-
prefrontal cortex) pathway within the brain. It is further hypothesised that opioids can activate dopamine-independent pathways, namely pathways that link the amygdala, bed nucleus of the stria terminalis and the nucleus accumbens and this can further reinforce the pleasurable effects of opioids (Koob, 2006).

1.6.2 Methadone maintenance treatment
Methadone is a synthetic opioid agonist developed during World War II and is structurally dissimilar to morphine-like compounds. For this reason its analgesic properties were unexpected. However, its utilisation as an analgesic was unrealised, probably as high initial doses resulted in intolerable opioid side effects (Chen, 1948). Nonetheless, its use as a medication for the treatment of opioid withdrawal was first shown in the late 1940s (Isbell and Vogel, 1949). Methadone was first used as a maintenance pharmacotherapy in Vancouver in 1959, with research on its use in the United States first published by Dole and Nyswander (1965). It was introduced to Australia in 1969, although its endorsement in this country as a treatment for heroin dependence was not made until 1985 (Henry-Edwards et al., 2003). Since the introduction of methadone to Australia, other opioid maintenance pharmacotherapies have been trialled for the treatment of heroin dependence, including: buprenorphine (registered by the Australian Register for Therapeutic Goods for the treatment of opioid dependence in October 2000), levo-α-acetyl-methadol (LAAM, discontinued by its manufacturers in August 2003 (as cited in Longshore et al., 2005) and most recently the buprenorphine/naloxone combination (Suboxone™) (registered by the Australian Register for Therapeutic Goods for the treatment of opioid dependence in July 2005). The opioid antagonist naltrexone is used as a maintenance pharmacotherapy in detoxified patients but low treatment retention rates limit its use. Internationally, some countries allow the prescription of heroin, intravenous (IV) methadone, and slow release oral morphine (SROM) for the treatment of heroin dependence (Wodak, 2001).

The fundamental goals of opioid maintenance treatments are to relieve opioid craving, suppress opioid abstinence syndrome (opioid withdrawal) and reduce or eliminate the use of additional opioids (Joseph et al., 2000; Henry-Edwards et al., 2003). Further aims of opioid maintenance treatment include to: improve the health and well-being of those in treatment, facilitate the social rehabilitation of those in treatment, reduce the spread of blood borne diseases associated with injecting opioid use, reduce the risk of death associated with opioid use, and reduce level of involvement in crime associated with opioid use (Henry-Edwards et al., 2003).
Randomised controlled trials have shown that methadone maintenance treatment is more effective than either no-treatment, drug-free counselling and rehabilitation, placebo medication, and detoxification/withdrawal. Furthermore, methadone maintenance has been shown to achieve one of the highest rates of retention when compared with other pharmacotherapies, namely, buprenorphine, LAAM and naltrexone (Mattick et al., 2001; Mattick et al., 2004). However, it is easier to become abstinent from opioids with buprenorphine compared with methadone (Mattick et al., 2004).

In Australia, it is estimated that in 2003 there were 26 000 and 9 000 individuals registered in methadone and buprenorphine maintenance treatments, respectively; more specifically, in South Australia there were 2 846 individuals registered for pharmacotherapy treatment (either methadone or buprenorphine) on 30 June 2003 (Australian Government Department of Health and Ageing, 2005b). With regard to illicit drug use, the 2004 National Drug Strategy Household Survey (2005a) reported that in Australia 0.3% of the population had used heroin, illicit methadone or other illicit opioids in the previous 12 months. Of those individuals, 45% of them had used heroin or illicit methadone as frequently as weekly or daily. This would have represented approximately 11 000 individuals in Australia. Without including chronic pain patients managed with opioids, this represents a significant proportion of the population who could be considered to be opioid dependent, if not show some degree of opioid tolerance.

### 1.6.3 Chemistry

Methadone (systematic name: 3-heptanone, 6-(dimethylamino)-4,4-diphenyl-) has an asymmetric carbon atom (Figure 1.6). Therefore it can exist as two isomers: R-(−)- and S-(+)-methadone and it is administered as a racemate mixture of equal proportions of each enantiomer. Methadone has a pKa of 9.0, is highly lipid soluble with an octanol-to-water partition coefficient of 120 at pH 7.2 (Eap et al., 2002; Foster et al., 2004).

![Figure 1.6 Structural formula of methadone](source: chemIDplus)

### 1.6.4 Pharmacodynamics

Most of the μ-opioid receptor activity of methadone is attributable to the (R)-methadone enantiomer, with it having a 10-fold higher affinity than (S)-methadone for μ-opioid receptors.
(Kristensen et al., 1995). (R)-methadone has up to 50 times more analgesic activity than (S)-methadone (Scott, 1948). Furthermore, substitution of (R)-methadone with (S)-methadone can cause withdrawal symptoms in opioid maintained patients (Dole and Nyswander, 1966) and administration of 15-90 mg (S)-methadone fails to produce subjective or objective opioid effects in former opioid users (Isbell and Eisenman, 1948); this suggests that most, if not all, opioid effects are attributable to (R)-methadone (Eap et al., 2002).

Methadone is an atypical opioid not only in its structure but also receptor binding affinities. In contrast to other opioids, methadone has opioid antagonist properties at the NMDA receptor. Both isomers of methadone have the ability to bind to the non-competitive-, but not the competitive-site of the NMDA receptor (Gorman et al., 1997). Furthermore, (S)-methadone has the ability to displace the NMDA receptor antagonist MK-801 at the non-competitive NMDA receptor site (Ebert et al., 1995). In addition, both enantiomers of methadone are strong inhibitors of serotonin uptake and noradrenaline (norepinephrine) uptake and therefore, this may also be a factor in its antinociceptive activity (Codd et al., 1995).

1.6.5 Pharmacokinetics

The bioavailability of methadone is approximately 85-95% (Nilsson et al., 1982a). The incomplete bioavailability of methadone is mainly attributable to intermediate permeability across the intestinal wall of the gut, due to the presence of the active transporter P-glycoprotein, as well as some hepatic first-pass metabolism (Kristensen et al., 1996; Bouër et al., 1999). Following oral administration, the time to reach peak plasma concentrations is between 2 to 4 hours (Meresaar et al., 1981; de Vos et al., 1995; Wolff et al., 1997). Methadone has a distribution phase half-life of 3 to 6 hours (Meresaar et al., 1981; Wolff et al., 1997). In the blood, methadone is highly bound to plasma proteins, mainly α1-acid glycoprotein and to a lesser extent albumin and lipoproteins, resulting in a free fraction of approximately 10% (Romach et al., 1981). The volume of distribution of methadone is about 3-5 L/kg (Meresaar et al., 1981; Nilsson et al., 1982a; Wolff et al., 1997).

Methadone is metabolised primarily by the liver enzyme cytochrome P450 (CYP) isoform 3A4 (Foster et al., 1999). The major metabolic pathway involves N-demethylation and subsequent cyclisation into 1,5-demethyl-3,3-diphenyl-2-ethyldene-pyrrolidine (EDDP). Methadone can also be metabolised through two secondary pathways to methadol or it can be hydroxylated to parahydroxymethadone. The resulting metabolites of methadone do not contribute to the opioid agonist activity of the parent compound. While the principal enzyme
involved in the metabolism of methadone is CYP3A4, the isoforms CYP2C9, CYP2C19, CYP2B6 and CYP2D6 are also implicated in the metabolism of methadone (Foster et al., 1999). The total body clearance of methadone can vary between 0.02 and 2 L/min (Plummer et al., 1988). The mean elimination (terminal) phase half-life is 22 hours (Olsen et al., 1977). However, this value can range between 15 and 60 hours (Säwe, 1986; Foster et al., 2000b). To a lesser extent, methadone is cleared by renal excretion; the rate of renal excretion is dependent on urine pH with alkalinisation of the urine reducing the renal clearance rate of methadone and EDDP (Inturrisi and Verebely, 1972; Nilsson et al., 1982b). Even though methadone is administered as a racemic mixture, its metabolism is stereospecific, with large inter-individual variability observed in enantiomer ratios at both peak (Foster et al., 2000a) and trough (Eap et al., 2002) plasma methadone concentrations.

1.6.6 Chronic pain management

Opioids are increasingly being used for the treatment of chronic non-malignant pain (Bell, 1997). The suitability of opioids for the treatment of non-malignant pain is increasingly being recognised (Collett, 2001). While opioids reduce the subjective discomfort related to pain, their use for increasing an individuals level of functioning, improving lifestyle, decreasing environmental stress may be limited (Brena and Sanders, 1991). The analgesic activity of methadone combined with its relatively long half-life may make it suitable for the management of chronic pain. Furthermore, the combination of NMDA antagonist activity may potentiate the opioid mediated analgesic activity in addition to attenuating tolerance and extending analgesic effects (Hewitt, 2000).
1.7 Opioid-induced hyperalgesia

Opioid-induced hyperalgesia has been reported in many animal experimental models.

**High-dose**

Kayan and colleagues (1971) investigated the ability of morphine to induce hyperalgesia in rats injected with 5 mg/kg s.c. morphine every second day for 21 days. Hot plate latency was measured at 30 minute intervals before and up to 240 minutes after morphine injection. They found that chronic morphine administration produced hyperalgesia between 60 and 120 minutes after drug administration compared with saline treated animals.

Many case reports are available that associate hyperalgesia with the administration of relatively high doses of morphine in humans. Intrathecal morphine given for pelvic carcinoma caused burning sensation (Ali, 1986). Other clinical reports have suggested a relationship between the occurrence of hyperalgesia and allodynia with high doses of morphine (Potter et al., 1989; Parkinson et al., 1990; De Conno et al., 1991; Sjøgren et al., 1993). Furthermore, fentanyl analogues have also been shown to induce hyperalgesia (Devulder, 1997; Mercadante and Arcuri, 2005). Yet, this condition generally resolves quickly with the reduction or substitution of opioid dosage (Sjøgren et al., 1994; Mercadante and Arcuri, 2005).

**Surgery-based pain**

Opioids are commonly used peri-operatively. Several studies have described the post-operative use of opioids and subjective pain scores of surgery patients receiving either high or low systemic doses of opioids during the intra-operative period.

Two studies have shown that surgery patients experience greater subjective post-operative pain after receiving a high dose compared with a low dose of fentanyl (IV 1 vs 22 µg/kg) (Chia et al., 1999) or remifentanil (IV 0.1 vs 0.3 µg/kg/min) (Guignard et al., 2000). In contrast, two studies reported that there was no change in subjective pain sensitivity post-operatively following high or low doses of IT fentanyl (0 vs 25 µg) (Cooper et al., 1997) or IV remifentanil (0.1 vs 0.23 µg/kg/min) (Cortinez et al., 2001). However, when examining the post-operative opioid doses used following surgery, three of the studies demonstrated that high-doses of opioids during the intra-operative period increased the post-operative need of opioid (Cooper et al., 1997; Chia et al., 1999; Guignard et al., 2000). The other study found no difference (Cortinez et al., 2001). In a comparable experimental model, pain tolerance was
quantified using a cold pressor (2 °C) and mechanical (pressure) stimulation tests, before and during a constant infusion of 0.1 µg/kg/min of remifentanil in 8 healthy volunteers (Vinik and Kissin, 1998). Cold pressor tolerance increased significantly 90 minutes into the remifentanil infusion compared with baseline; yet when cold pain was measured at 120, 180 and 240 minutes into the remifentanil infusion, cold pressor tolerance values were significantly lower than the peak score obtained at 90 minutes.

The results of these studies can be interpreted in two ways. Either opioid-induced hyperalgesia developed in the patients receiving the higher doses of the opioid and/or acute tolerance to the opioids occurred in the patients. Without testing pain sensitivity directly in the surgical patients, it is difficult to differentiate between tolerance and opioid-induced hyperalgesia (Carroll et al., 2004). This highlights an important caveat in this field of research – the difficult nature of investigating pain sensitivity in surgical patients. Consequently, much of the clinical research that has a focus on investigating the impact of opioid on pain sensitivity utilises opioid maintained patients.

1.7.1 Opioid maintained patient

Early studies by Ho and Dole (1979) compared drug-free ex-addicts, methadone maintained ex-addicts and their non-addicted siblings, with 10 subjects per group. Using a cold pressor test that consisted of 30 °C water and subsequent 1 °C water, subjects’ pain tolerance and pain threshold was measured. Results demonstrated that the pain threshold of both the drug-free and methadone maintained ex-addicts was significantly lower than the non-addicted siblings. There were no significant differences between the groups with regard to pain tolerance.

Compton and colleagues (1994) compared the cold pressor tolerance of current and abstinent cocaine users and opioid users. Compton found that methadone maintained patients were pain intolerant when compared with current or abstinent cocaine abusers. Further evidence that methadone maintenance alters pain sensitivity was shown by Compton and colleagues (2000) Methadone maintained patients were significantly less tolerant of cold pressor pain than control subjects, again supporting previous work.

Schall and colleagues (1996) investigated the pain perception of methadone maintained patients compared with healthy drug-free controls. The nociceptive stimuli used in this experimental protocol consisted of applying various discrete pressures to the middle finger
and measuring pain detection and tolerance. Nociceptive testing was done before, 1, 2 and 4 hours after methadone dosing. While the analgesic effect of the methadone was observed, there were no significant differences between the methadone maintained patients and the control subjects. These results were confirmed by Dyer and colleagues (1999) who recruited 18 methadone maintained subjects, half of whom experienced withdrawal during the intra-dosing period (‘non-holding’ subjects); ten healthy subjects served as controls. Pain threshold was measured by means of electrical stimulation of the ear lobe. The pain threshold was significantly increased in the maintained subjects compared with control subjects at all times (except just before dosing), reached a peak between 1 and 2 hours after dosing, and lasted about 6 hours after dosing.

In an attempt to clarify the apparent differences seen in the previous studies Doverty and colleagues (2001b) tested both pain tolerance and pain threshold using two different pain stimuli, the cold pressor test and electrical stimulation test in 16 methadone maintained subjects compared with 16 matched controls. The pain tests were performed prior to (0 hours) and three hours after their daily methadone dose. Methadone maintained patients demonstrated an analgesic response to their methadone dose with significant increases in pain detection and pain tolerance for both pain tests. Compared with control subjects, it was found that methadone maintained patients had lower pain tolerance but not pain detection values at 0 hours. With regard to pain induced by the cold pressor test, methadone maintained patients were significantly less pain tolerant and detected pain significantly earlier than controls at 0 hours. This demonstrated that chronic methadone dosing is associated with hyperalgesia especially with regard to the cold pressor test. Furthermore, the study highlighted the need to measure both pain threshold and pain tolerance, and the need to measure nociception using different pain stimuli.

While quite extensive research has focused on the pain sensitivity of methadone maintained patients, studies have also provided evidence that patients maintained on other opioids may experience hyperalgesia. Such patients have shown similar degrees of hyperalgesia when using the cold pressor test; including buprenorphine maintained patients (Compton et al., 2001) and patients maintained on slow release oral morphine (Mitchell et al., 2006). Similar findings have been reported in heroin addicts entering treatment (Ling et al., 2003) and in heroin and methadone opioid addicts entering a detoxification program with no previous history of being in a methadone maintenance program (Pud et al., 2006).
1.7.2 Chronic pain in opioid maintained patients

The occurrence of chronic pain in opioid maintained patients in methadone maintenance programs may well be another indicator with regard to the occurrence of hyperalgesia. The incidence of moderate to severe chronic pain in methadone maintained patients was estimated to be 37% (Rosenblum et al., 2003), which corresponds to the upper range of chronic pain prevalence in the general population (Verhaak et al., 1998). Of 248 methadone maintained patients interviewed, it was found that over 60% of patients stated they suffered from chronic pain (Jamison et al., 2000). It was noted by Brands and colleagues (2004) that of the methadone maintenance patients who reported chronic pain, a high proportion of them reported the onset of this pain prior to commencing methadone maintenance treatment. Furthermore, Jamison and colleagues (2000) found an association between time in treatment and incidence of chronic severe pain, implying that chronic opioid administration may further exacerbate hyperalgesia. Most importantly, these studies suggest that the prevalence of chronic pain is disproportionately higher in methadone maintained populations compared with the general population.
1.8 Opioid-abstinence hyperalgesia: acute studies

1.8.1 Animal studies

Increased pain sensitivity associated with the abrupt reduction or cessation of opioid dosage has been well characterised. Increased pain sensitivity following opioid withdrawal was used as a measure of the degree of opioid dependence (Tilson et al., 1973). Additional studies demonstrated that the degree of hyperalgesia was indeed associated with the degree of opioid abstinence syndrome following either precipitated (Kayan et al., 1971) or spontaneous (Tilson et al., 1973; VonVoigtlander and Lewis, 1983) opioid withdrawal.

Investigations by Li and colleagues showed that following the establishment of morphine-induced hyperalgesia in rats (Li et al., 2001b) and mice (Li et al., 2001a), nociceptive thresholds returned to baseline values within two to three days, depending on the nociceptive test. Célèrier and colleagues (2001) showed that the time-course for the development of heroin-induced hyperalgesia was mirrored by its resolution once heroin administration ceased. Lastly, Davies and colleagues (2003) demonstrated that morphine-induced hyperalgesia largely resolved by 72 hours following morphine discontinuation. Common to all these studies was that the time-course of the resolution of opioid-induced hyperalgesia was comparable to its development (Angst and Clark, 2006).

Li and colleagues (2001b) chronically administered morphine, by implantation of subcutaneous morphine pellets in mice. Mechanical allodynia was assessed using Von Frey filaments applied to the hind paw and thermal hyperalgesia was assessed by focusing a beam of light on the hind paw. Results showed that upon removal of the morphine pellet after 6 days, mice exhibited thermal hyperalgesia and mechanical allodynia, which was maximal 24 hours after removal and slowly resolved during the following days. Naloxone treatment on days 2, 4 and 6 caused a more profound and longer-lived period of opioid-induced hyperalgesia than in animals receiving morphine alone.

Periodic withdrawal has been shown to enhance nociception (Dunbar and Pulai, 1998). Rats were administered spinal morphine for 4 days with nociceptive thermal latencies measured just prior to a daily dose of either subcutaneous saline or naloxone. The morphine/naloxone treated rats had significantly shorter paw withdrawal latencies than the morphine/saline
treated rats and demonstrated thermal hyperalgesia 24 hours following cessation of the morphine infusion.

After the induction of analgesia by heroin, an injection of naloxone (30 min after heroin administration) not only reversed the analgesia but induced a significant lowering of the nociceptive threshold below basal level (Devillers et al., 1995a). This is indicative of activation of the pain facilitatory system.

1.8.2 Case-reports

Hyperalgesia is a well recognised characteristic of opioid withdrawal or abstinence syndrome. This phenomenon is not only limited to that of opioid addicted subjects undergoing opioid withdrawal but there are reports of thermal hyperalgesia and allodynia in humans after reduction in dose or abrupt cessation of therapeutic opioids.

One small study investigated the heat pain tolerance of chronic pain patients. Subjects experiencing opioid withdrawal at baseline had reduced pain tolerance values compared with normal volunteers, whereas chronic pain patients not undergoing opiate withdrawal evidenced increased baseline pain tolerance latencies compared with normal volunteers (Lipman and Blumenkopf, 1989). Following IT or IV administration of morphine, increases in cutaneous pain tolerance were observed. This study highlighted that thermal hyperalgesia is associated with opioid withdrawal yet opioid antinociception is still possible.

Miser and colleagues (1986) reported on five young adults with malignant neoplasms whose opioid therapy was abruptly discontinued after 6 to 21 days of administration. This report stated that withdrawal effects included hyperalgesia among other typical withdrawal symptoms, all of which resolved after reinstating opioid therapy. Furthermore, one case report described a patient whose sudden discontinuation of intrathecal morphine due to catheter removal caused allodynia to mechanical stimuli that resolved after recommencement of opioid administration (Devulder et al., 1996).

1.8.3 Experimental pain models

Limited studies exist regarding the observation of hyperalgesia associated with opioid cessation in opioid-naive humans. Several researchers have investigated the effect of an opioid infusion on an experimental skin lesion made hyperalgesic prior to the infusion. These authors observed that pre-existing mechanical hyperalgesia can be aggravated following a
short term (30-90 minute) infusion of remifentanil. These studies consistently showed that the area of pre-existing mechanical hyperalgesia increased significantly following the cessation of the remifentanil infusion (Angst et al., 2003; Hood et al., 2003). In contrast, heat pain sensitivity did not change, either during or after the cessation of the remifentanil infusion (Angst et al., 2003; Hood et al., 2003). In one of the studies, the aggravation of hyperalgesia lasted for up to 6 hours following the termination of the remifentanil infusion, however, resolution of mechanical hyperalgesia occurred by 24 hours (Hood et al., 2003). Many of these authors conclude that hyperalgesia is associated with ‘withdrawal’; however, in none of the studies were opioid withdrawal symptoms measured other than pain sensitivity (Angst et al., 2003; Hood et al., 2003).

Two of these studies demonstrated that co-administration of ketamine during opioid administration could eliminate any remifentanil-induced aggravation of the pre-existing hyperalgesia (Angst et al., 2003; Koppert et al., 2003). In contrast, co-administration of ketamine had no effect on hyperalgesia associated with the cessation of remifentanil as measured by pressure pain (Luginbühl et al., 2003). This highlights that altered pain sensitivity associated with opioids is mediated, at least partially, by NMDA receptors.

Compton and colleagues (2003; 2004) investigated the pain tolerance of healthy male subjects following the administration of a bolus dose of morphine, hydromorphone or placebo followed by the administration of naloxone 2 to 6 hours later. They found that naloxone precipitated both objective and subjective opioid withdrawal symptoms (Compton et al., 2004) in addition to decreasing cold pressor pain threshold and tolerance values (Compton et al., 2003) 5 and 15 minutes following naloxone dosing.
1.9 Pain sensitivity in former opioid users

Cross-sectional studies investigating former opioid users can shed some light on whether the discontinuation of opioids is associated with resolution of opioid-induced hyperalgesia. An early study by Andrews (1943) measured the heat pain threshold in former opiate addicted men. This study found that baseline pain thresholds of former addicts “were quite comparable with those obtained with non-addicts”.

Martin and Inglis (1965) studied two groups of 12 female prisoners, one group formerly addicted and the other non-addicted. They investigated pain sensitivity using a cold pressor test (5 °C) and found that there were significant differences in mean pain tolerance times between addicts (73.2 seconds, n=12) and non-addicts (404.4 seconds, n=12). There was no correlation between years since first use of the drugs and cold pressor test scores. Time exposed to drug could only be estimated and so no correlation could be calculated between cold pressor values and previous drug exposure.

Ho and Dole (1979), using the cold pressor test (1 °C), investigated pain threshold and tolerance times in ex-addicts and methadone maintained subjects. They found that ex-addicted subjects had significantly lower pain threshold times than non-addicted subjects. However, there were no differences between groups for tolerance values. There were no differences in cold pressor times, either threshold or tolerance, between methadone maintained subjects and ex-addicts.

A series of studies investigated the cold pain sensitivity of former opioid users. An initial study compared the cold pressor (5 °C) threshold and tolerance scores in a group of 40 detoxified former opioid users to those of 40 age-matched non-dependent volunteers (Liebmann et al., 1994). The subjects who were former users (‘ex-addicts’) were recruited if they had not used opioids for at least a month and had a mean (± SD) abstinence period of 8.1 (± 4.7) months. This study reported that the subjects who were former users had significantly longer pain threshold times but there was no difference with regards to pain tolerance times. These results were substantiated by a similar study in that cold pressor (5 °C) pain thresholds were significantly lower for a group of drug-free subjects (n=31) compared with a group of detoxified opioid users undergoing rehabilitation (n=31) (Liebmann et al., 1997). A comparable trend was seen for pain tolerance, but statistical significance was not reached.
An associated study assessed the retrospective ratings for the pain, cold and warmth sensitivity before and during addiction, during detoxification and during rehabilitation in 63 opioid detoxified subjects and 63 matched control subjects (Lehofer et al., 1997). Results indicated that detoxified opioid users were less sensitive to pain and cold, but not warmth during each period of assessment except during detoxification, compared with control subjects. As an explanation, the authors proposed that opioid addiction comes from a physiological predisposition related to pre-existing reduced sensitivity to pain in the opioid using group (Lehofer et al., 1997).

One study compared pain tolerance times for the cold pressor test (1 °C) in current and former opioid and cocaine abusers (Compton, 1994). Former users were at least 6 weeks abstinent and the current opioid users were a sample of methadone maintained subjects. While the study found no significant differences between the individual groups with regard to pain tolerance scores, drug using status did significantly relate to pain tolerance scores with former users tolerating the cold pressor test twice as long as those subjects still using. The pain tolerance time for all subjects ‘did not appear to differ’ from that of a historical control group of healthy males.

The only prospective study in the field compared the pain sensitivity of 60 opioid addicted subjects entering a 28-day detoxification program compared with 70 healthy controls (Pud et al., 2006). Opioid addicts were defined as a cohort of subjects who presented a positive opioid urine result at entry and had a past history of either heroin or methadone abuse. Pain threshold (latency) time, tolerance time and resulting pain intensity (as measure using a VAS) to the cold pressor test (1 °C) were measured upon entry to the program and at 7 and 28 days following cessation of opioid consumption. Results showed that the opioid addicts had longer pain threshold times, shorter pain tolerance times and lower VAS pain intensity scores compared with the healthy controls. There were no significant changes in any of the measured parameters at 7 or 28 days following opioid cessation compared with baseline values.

The results of these studies suggest that in the short to medium term, former opioid users may continue to be in a hyperalgesic state (Martin and Inglis, 1965; Ho and Dole, 1979; Pud et al., 2006), yet in certain circumstances, pain sensitivity may return to ‘normal’ levels (Andrews, 1943; Liebmann et al., 1997). Results of these studies may also reflect particular behavioural
and psychosocial differences between opioid addicts and the general population. It has been suggested that opioid addicts may be prone to over-reaction (Martin et al., 1973) and this may be reflected in lower pain tolerance scores (Pud et al., 2006). While Hajek (1998) in response to the results of Lehofer and colleagues (1997) suggest that the higher pain tolerance of detoxified heroin users may actually be related to an individual’s improved ability to achieve and maintain abstinence from opioid addiction. The underlying mechanisms are further discussed in Chapter 4.

Comparison of the aforesaid studies is difficult because of dissimilar methods. As mentioned before, pain detection threshold is a highly variable measure (section 1.2.5) and this is especially reflected in this cohort of presented studies. Furthermore, conclusions derived from these studies have mainly depended on cold pressor threshold times and consequently have concluded that former opioid users have pain sensitivity either the same, higher or lower than that of healthy controls. This paradox can partially be explained by methodological differences between studies, especially with regard to cold pressor water temperature, which prohibits any direct comparison between the studies. Moreover, these conclusions have been based on a single modality of pain; as emphasised in earlier chapters, the multi-dimensional nature of pain necessitates the need for its assessment by tools that represent different modalities of pain. One further confounding feature of the studies is the heterogeneity of the populations, both within and between studies. This is particularly true for the former opioid users with regard to their prior opioid use and time since opioid use.

Many of these studies have relied solely on variants of the cold pressor test whereas the proposed study (Chapter 4) aimed to compare responses of formerly opioid-dependent patients to normal healthy controls using a range of nociceptive tests. In order to further establish whether hyperalgesia is associated with opioid use, the objective of the proposed study presented in Chapter 4 was to investigate whether opioid-induced hyperalgesia resolves after opioid abstinence and the extent to which pain responses return to normal levels. This would demonstrate similarities and differences in pain thresholds and pain tolerance levels between patients formerly dependent on opioids and current opioid users.
1.10 Mechanisms of opioid-induced hyperalgesia

1.10.1 Concepts
It has been postulated that following opioid administration, the body counters the direct response of the drug with adaptational responses (Solomon and Corbit, 1974; White, 2004). These adaptational, or drug-opposite responses, result in a reduced effect and is expressed as tolerance. Following cessation of the opioid, opioid effects diminish, yet adaptational responses take longer to cease and hence a temporary withdrawal state is caused. With regard to nociception (and potentially mood), the drug-opposite response is larger than the direct antinociceptive effect of the opioid. Consequently, the direct antinociceptive effect of an opioid is countered by a hyperadaptational response, thus producing a hyperalgesic state. Furthermore, this hyperalgesic state is potentiated following cessation of the opioid until resolution of the drug-opposite response.

Complementary to this idea is that of Célèrier and colleagues (2001), who present a model of balance between pro-nociceptive and anti-nociceptive neuronal systems. Prior to opioid exposure, both systems balance each other with low level activity. However, upon opioid exposure, the pronociceptive system is upregulated and results in a hyperalgesic state. Once opioid use ceases, pronociceptive systems maintain their high level of activity, but the antinociceptive systems are upregulated countering the effects of the pronociceptive systems. As a consequence, a new allostatic equilibrium is achieved, which may be susceptible to permutation such that an individual may be susceptible to future pain or quicker onset of hyperalgesia following subsequent exposure to opioids.

Clinically, hyperalgesia and tolerance are indistinguishable as both result in lowered opioid effect. Tolerance results in a rightward shift in the dose-response curve. Hyperalgesia on the other hand, can be considered as either a down-ward shift in the dose response curve (Carroll et al., 2004) or an increase in the slope of the dose response curve (Ling et al., 2003). Tolerance represents a decrease in the sensitivity of antinociceptive pathways to opioids while hyperalgesia is characterised by an increase in the sensitivity of the pronociceptive pathways (Angst and Clark, 2006). Regardless of whether tolerance, hyperalgesia or both mechanisms are applicable within an individual consuming opioids, both cause the same clinical effect – decreased opioid effectiveness.
1.10.2 Physiological mechanisms of hyperalgesia

The mechanisms underlying hyperalgesia suggest that pronociceptive systems oppose the antinociceptive effects of opioids, representing a sensitisation to painful stimuli.

Peripheral mechanisms

The administration of opioid in the rodent hind paw tissue of rodents has found that precipitation with naloxone caused mechanical hyperalgesia. This suggests that the peripheral endings of primary sensory nerves may become sensitised to the effects of opioids. Furthermore, this effect appears to be mediated, at least in part, by PKC (Aley and Levine, 1997) and GTP binding proteins (Khasar et al., 1995).

Central mechanisms

Central mechanisms play a primary role in the pronociceptive mechanisms underlying opioid-induced hyperalgesia. Several mechanisms are thought to contribute to the propagation of opioid-induced hyperalgesia within the dorsal horn of the spinal cord. These include enhanced excitatory amino acid activity, descending facilitation of nociceptive pathways mediated by dynorphin, sensitisation of ascending neurons as well as other putative mechanisms.

The results of many experiments have implicated the importance of excitatory amino acid neurotransmission in the pronociceptive state caused by opioid administration. Tolerance and hyperalgesia states involve activation of excitatory glutamate receptors of the N-methyl-D-aspartate (NMDA) type in the central nervous system. Studies have shown that opioid administration leads to downregulation of spinal glutamate transporters, potentially contributing to opioid-induced hyperalgesia (Mao et al., 2002). Glutamate receptor activation leads to an intracellular cascade which principally consists of increased intracellular calcium concentrations. This leads to the activation of PKC and calcium-calmodulin-mediated production of nitric oxide (NO). NO is a highly diffusible molecule that is important in signal transduction pathways, especially in enhancing nociception transmission in the central nervous system (CNS). It has been shown that NO synthase inhibitors reverse hyperalgesia (Li et al., 2001a) and prevent/re retard the development of opioid tolerance (Kolesnikov et al., 1993). NO is up-regulated after painful stimuli and NO release contributes to secondary hyperalgesia and allodynia. Opioid receptor activation results in PKC stimulation, which leads to phosphorylation of NMDA receptors. This causes a release of the Mg$^{2+}$ block in the NMDA receptor allowing Ca$^{2+}$ to enter the cell. This results in cascade activation, which
leads to opioid receptor down-regulation (tolerance) and the hyper-responsiveness that underlies hyperalgesia.

In addition to experiments showing that intrathecal administration of opioids induces hyperalgesia, other studies point to the role of the spinal cord. Vanderah and colleagues (2001b) showed that tonic descending spinal cord facilitation from the rostral ventromedial medulla (RMV) during chronic opioid exposure may be partly responsible for opioid-induced hyperalgesia which may be mediated by spinal dynorphin. Morphine-induced hyperalgesia can be blocked by lidocaine microinjections in the RMV and can be attenuated by lesions of the dorsolateral funiculus (DLF) (Vanderah et al., 2001b). After chronic exposure to an infusion of morphine, raised spinal dynorphin levels suggest that opioids regulate expression of spinal dynorphin, probably as a result of opioid receptor occupation (Vanderah et al., 2000; Vanderah et al., 2001a). Over-expression of spinal dynorphin is also likely to cause the increased release of excitatory neurotransmitters such as substance P (Arcaya et al., 1999). Furthermore, studies indicate that long-term opioid administration leads to alterations in on- and off-cell firing within the RMV such that the pro-nociceptive on-cells increase firing while off-cell firing is diminished (Fields, 2000).

In addition to these aforementioned systems there is growing evidence for a model that suggests that the CNS synthesises neuropeptides that attenuate the analgesic effects of endogenous and exogenous opioids. Studies show that morphine administration leads to increased spinal extracellular levels of CCK (Bourgoin et al., 1994), NPFF (Devillers et al., 1995b) and orphanin FQ / nociceptin (Yuan et al., 1999). While these peptides are only considered anti-analgesic, it has been shown that i.c.v. injection of NPFF can produce temporary hyperalgesia in rats (Yang et al., 1985), which may indicate that this peptide may be a component of pro-nociceptive pathways.

One other component of hyperalgesia that may be important in its understanding is the suggestion that opioids may cause an excitatory effect by binding to Gs-coupled opioid receptors. Established pharmacology implicates that opioid antinociception is mediated by activation of Gi/Go-coupled opioid receptors, which in turn causes inhibitory effects via the adenylate cyclase (AC) / cyclic AMP (cAMP) / protein kinase A mediated transduction system. The experiments of Shen and Crain (Shen and Crain, 1989; Crain and Shen, 1992; Crain and Shen, 1995; Shen and Crain, 1997; Crain and Shen, 1998; Crain and Shen, 2000a;
Crain and Shen, 2000b; Crain and Shen, 2001; Shen and Crain, 2001) propose that at ultra-low doses opioid agonists potentially cause pronociceptive effects and cause hyperalgesia.

The importance of the opioid receptors, and more specifically the \( \mu \)-opioid receptor, in opioid-induced hyperalgesia has been demonstrated by studies that block morphine-induced hyperalgesia by naloxone administration (Mao et al., 2002) and studies that show that hyperalgesia does not develop in \( \mu \)-opioid receptor gene knock-out mice (Li et al., 2001a).

Building on the idea that the immune system is involved in modulation of nociceptive pathways (section 1.2.7), researchers have implicated that spinal cytokines and chemokines may be involved in the development of hyperalgesia (Watkins et al., 2005).

It is worth noting that much of the animal research investigating the mechanisms underlying opioid-induced hyperalgesia involves the use of morphine. Yet much of the clinical evidence for opioid-induced hyperalgesia in patients comes from subjects maintained with methadone. While certain pharmacological similarities exist between morphine and methadone, these two opioids are particularly divergent in other aspects; this disparity if further discussed and explored in Chapter 3 and 7.
1.11 Chronic pain patients managed with opioids

Chronic pain patients who are managed with opioid medication are a population in whom little is known about changes in pain sensitivity. While studies exist that have investigated pain sensitivity in chronic pain patients, very few have investigated the impact of opioid treatment on pain sensitivity.

Studies that investigated pain sensitivity in chronic pain patients have yielded conflicting results. One early study reported that the heat pain threshold was higher in 11 chronic low back pain patients compared with that of a control group (Cohen et al., 1983). Another study reported the results of administering the cold pressor test (1 °C) to a group of 24 chronic low back pain patients compared with 24 age- and sex-matched healthy controls (Schmidt and Brands, 1986). The results indicated that the chronic pain patients had significantly shorter cold pressor tolerance times, pain decay (extinction) times and significantly higher pain intensity scores resulting from the cold pressor test. There were no data on medication administration in any of the subjects. Moreover, one study compared 500 healthy, drug- and pain-free volunteers to 113 chronic pain patients, matched for age, sex and race. The authors found no statistically significant differences between the two sample populations with regard to cold pain (Walsh et al., 1989). This sample of studies highlights that the results of nociceptive testing may depend not only on the way pain sensitivity is measured but also on the aetiology of the chronic pain the subject is experiencing.

Other studies have investigated the impact that opioid dosing has on pain sensitivity. Brodner and Taub (1978), based on 4 case reports, suggested that chronic non-malignant pain could be exacerbated by the administration of narcotics (opioids) and that this pain could be relieved by the cessation of ‘narcotic’ use. The authors suggested that the manifestation of a subtle opioid withdrawal syndrome during the administration of opioid may be the reason for the exacerbation of the chronic pain; therefore removing opioids from the treatment regimen eliminates any chance of a withdrawal syndrome and the subsequent maintenance of pain.

One study of 240 patients with chronic back pain suggested that subjects prescribed opioids compared with subjects prescribed non-opioids showed no difference between the groups with regard to ischemic pain tolerance (Fillingim et al., 2003).
One recent study compared the thresholds for punctuate pain, mechanical pressure pain, heat pain and the intensity of heat pain in 142 opioid treated compared with 82 non-opioid treated patients (Reznikov et al., 2005). The sample included patients who had cancer-related or non-malignant chronic pain. No significant differences in pain threshold or pain intensity between the two groups were reported. Furthermore, no significant differences in these parameters were reported between the groups when stratified by those receiving either ‘weak’ or ‘strong’ opioids. However, this study was limited by its use of pain threshold as a primary end point, as this stimulus dependent measure has previously been shown to be inappropriate for pain studies (Beecher, 1959; Smith et al., 1966) because of its large variability and lack of relevance for the patient. Furthermore, the use of a heterogeneous sample of patients with and without malignant pain would have further confounded the results of this study.

Investigating the pain sensitivity of chronic pain patients managed with opioids may provide insights into if hyperalgesia is associated with opioids. Furthermore, by differentiating between chronic pain patients managed with either morphine or methadone may highlight if opioid-induced hyperalgesia is a drug specific phenomenon in humans, that is, only caused by methadone, or a class-specific function of opioids.

Chronic opioid use in humans is generally limited to two indications: as an illicit substance or as a maintenance therapy for addiction or in chronic pain patients. Studying opioid-induced hyperalgesia is limited by confounding factors in both populations, with the co-administration of additional drugs, both medicinal and/or illicit, being virtually universal. Furthermore, irregular opioid administration and the presence of chronic pain confound the interpretation of opioid-induced hyperalgesia in these populations. Theoretically the hypothesis that long-term opioid administration leads to hyperalgesia could be determined by the chronic administration of opioids to a cohort of healthy controls. However, ethical issues restrict such a paradigm (White, 2004). Furthermore, it is unrealistic to attempt prospective studies investigating alterations in nociception in individuals going from pre-addiction to opioid addiction (Liebmann et al., 1997).

Another approach to assess what impact opioid administration has on pain sensitivity would be to investigate pain patients before and after induction onto an opioid pain management plan. One preliminary study has reported that chronic pain patients became hyperalgesic following the commencement of opioid treatment. However, not all subjects in this study
were opioid-naïve, thus limiting the conclusions that can be made with regard to these results (Chu et al., 2006). This study accentuates the difficulty in recruiting opioid-naïve subjects prior to the commencement of opioid management. In the Australian context, opioid prescription is relatively liberal and this is highlighted by one study that has shown that 83% of patients with chronic benign and malignant pain were already prescribed opioids by their general practitioner at time of referral to a specialised pain centre (Nissen et al., 2001).
1.12 Pain management needs in opioid tolerant patients

There is a paucity of clinically-based, scientific evidence for the pain management needs of methadone maintained patients. Most recommendations for severe pain management in methadone maintained patients are based on theoretical and/or empirical findings.

1.12.1 Prevention of opioid withdrawal

One of the main foci when managing acute pain in opioid tolerant patients is the prevention of opioid abstinence syndrome (Jage and Bey, 2000). This highlights two main treatment strategies: continuing or substituting the opioid maintenance dose and avoiding the administration of medications that may precipitate withdrawal. Opioid-withdrawal is generally associated with motor and autonomic effects contrary to the action of the substance (Handelsman et al., 1987).

Cushman (1972) suggested that in preparation for surgery, methadone maintained patients should have their methadone dose tapered to 10 mg given every 6 hours. On the day of surgery and for the following 4 days the patient’s methadone dose should be discontinued. During this interval, analgesic and maintenance needs could be achieved with conventional doses of analgesic such as pethidine. Such a dosing regimen would clearly precipitate withdrawal (Mitra and Sinatra, 2004). It is now well recognised that opioid withdrawal should be prevented, and if that is not possible, at least treated. This generally means that the maintenance dose should be continued or substituted (Jage and Bey, 2000). Substitution may be required when oral methadone cannot be tolerated (i.e. when nil by mouth), therefore administering the equivalent methadone dose by an intravenous, subcutaneous, intramuscular or rectal route may be appropriate (Jage and Bey, 2000; Peng et al., 2005).

Furthermore, the use of drugs that can displace methadone from the µ-opioid receptor should be avoided. Therefore, opioid antagonists (naloxone or naltrexone) (Manfredi et al., 1996) and mixed agonist-antagonists (buprenorphine, pentazocine or nalbuphine) should also be avoided in methadone maintained patients (Mitra and Sinatra, 2004).

1.12.2 Opioid analgesia

Several researchers and clinicians have suggested pharmacological strategies for treating severe acute pain in methadone maintained patients.
Clinical approaches

One of the earliest recommendations regarding the pain management needs in methadone maintained patients admitted for emergency and elective surgery came from Rubenstein and colleagues (1976). They investigated the post-operative analgesic needs of a cohort of 100 methadone maintained patients (average daily methadone dose 83 mg). They reported that meperidine (pethidine) 50 to 100 mg administered every three hours would adequately control post-operative pain. In support of these findings, a retrospective study by Kantor and colleagues (1980) evaluated 25 surgical patients on a methadone maintenance program (average daily methadone dose 70 mg) and recommended that ‘standard doses of narcotic drugs provide adequate analgesia’. In spite of these recommendations, both studies were retrospective and both failed to assess actual pain relief.

Kreek (1978) was one of the first to recognise tolerance in methadone maintained patients and documented that these patients required higher and more frequent opioid doses for acute pain management. This observation was confirmed by Tucker (1990) who, based on three retrospective cases studies, stated that chemically dependent (not just opioid dependent) patients require higher doses of opioids for adequate pain relief compared with opioid naïve patients. Moreover, Hicks (1989) suggested that, compared with opioid naïve patients undergoing similar surgical procedures, 1.5 times the dose of opioids may be required in opioid dependent patients.

Weinrieb and colleagues (2004) reported various outcomes of liver transplant patients. They compared the analgesic needs of a group of 10 methadone maintained patients (mean daily methadone dose pre-operatively 65 mg, range 30-120 mg) compared with a group of 19 control subjects not dependent on opioids. Post-operatively, the methadone maintained patients required higher average daily doses of IV morphine (68 ± 39 mg/d) compared with the control patients (12 ± 10 mg/d).

Mitra & Sinatra (2004) suggested that opioid doses 30 to 100% higher than the requirements of opioid naïve patients may be needed; with the rationale being that opioid tolerance (receptor down regulation) needed to be recognised. Nonetheless, this counsel was based on other case reviews and expert opinions (Collett, 2001; May et al., 2001).
Experimental approaches

There are several experimental approaches to investigate appropriate opioid analgesia in methadone maintained patients. The approach of using experimental pain for the determination of appropriate analgesia in methadone maintained patients provides many advantages. Firstly, extraneous and compounding factors, such as disease co-morbidity, treatment anxiety and other negatively influencing issues (see section 1.12.5) can be eliminated as confounding factors. Secondly, the nociceptive response to an analgesic can be viewed in a reasonably objective manner.

Doverty and colleagues (2001a) compared the antinociceptive properties of IV morphine in methadone maintained patients and healthy controls. They used the cold pressor and electrical stimulation tests, and achieved two clinically relevant pseudo steady-state plasma morphine concentrations: up to 55 ng/mL in methadone maintained subjects and 33 ng/mL in the controls. Results indicated that methadone maintained patients were cross-tolerant to the antinociceptive effects of conventional doses of morphine with minimal mean increases in pain tolerance scores at the end of the morphine infusion. In comparison, control subjects had significant increases in pain tolerance from baseline in both the cold pressor and electrical stimulation tests.

To extend this further, Athanasos and colleagues (2006) utilised similar methods and achieved a mean pseudo steady-state plasma morphine concentrations of 173 ng/mL in methadone maintained patients. At peak morphine concentrations there were no significant antinociceptive responses between morphine or saline administration periods. The results from this study suggest that, in methadone patients, very high plasma morphine concentrations did not provide antinociception. Intrinsic activity and efficacy have been linked to opioid potency (Traynor, 2004) and as a consequence, the decreased ability of morphine compared with methadone to activate µ-opioid receptors (Borgland et al., 2003) may go partway to explaining the results of Athanasos and colleagues (2006).

Methadone

Dyer and colleagues (Dyer et al., 1999) showed that pain threshold related to the electrical stimulation test changed in relation to plasma methadone concentrations in maintained patients. Similarly, Schall and colleagues (1996) showed that sensitivity to mechanical pain decreased as plasma methadone concentrations increased. These results suggest that
methadone maintained patients are not completely tolerant to the analgesic effects of their methadone dose. An attractive inference might be that the patient’s daily methadone may be useful as an analgesic. This has been suggested by several researchers, including Rogers (1989) who, based on a case study, suggested that methadone maintained patients experiencing pain respond better to methadone than morphine.

A retrospective case review of 6 methadone maintained patients who were also being treated for early stage cancer pain recommended the use of ‘divided doses’ of methadone (Manfredi et al., 2001). The recommendation suggested that the maintenance dose of methadone be given every 6 to 12 hours. Manfredi and colleagues (2001) went on to propose that if another opioid medication is chosen for analgesia, then the methadone maintenance dose should be continued and the dose of the co-administered opioid rapidly titrated up to achieve analgesia.

The use of methadone as an analgesic may be appropriate in patients refractory to the analgesic effects of morphine. Rapid and effective pain control through the use of methadone (50 mg every 6 hours) has been reported in a heroin-dependent patient and in whom morphine and ketamine were ineffective (Sartain and Mitchell, 2002). The effectiveness of methadone in certain opioid-dependent patients has been attributed to its unique receptor binding profile at the opioid receptors as well as its ability to be an antagonist at NMDA receptors (Sartain and Mitchell, 2002).

Methadone may be an appropriate analgesic for the treatment of acute pain in patients dependent on an opioid other than methadone. However, it has been advocated that using the same drug for acute pain management and addiction therapy may complicate issues especially when dose tapering is intended following the resolution of acute pain (Savage, 1996). It has been recommended that methadone maintained patients’ analgesic needs should be treated with another analgesic rather than the maintenance drug itself, especially as methadone is generally inappropriate for the management of acute pain due to its long time to analgesic onset (Scimeca et al., 2000).

**Opioids with high intrinsic activity**

Morphine is considered the gold standard for acute pain. Nonetheless, other opioids have been considered for their potential in treating pain in the opioid tolerant patient, especially opioids with high intrinsic activity (Inturrisi, 2002; Mitra and Sinatra, 2004).
A case review described the use of fentanyl in a single patient undergoing cardiac surgery and who had opioid tolerance and past history of severe post-operative pain (Davis et al., 2003). The authors proposed that a high-dose fentanyl infusion could be given pre-operatively to determine the level of opioid tolerance and then used intra- and post-operatively for pain management. The outcome of the study suggested that 50 to 70 times the plasma fentanyl concentration normally associated with analgesia in the opioid-naïve patients may be needed. Furthermore, de Leon-Casasola and colleagues (1994) presented a case review where successful pain control in an opioid tolerant patient was achieved by switching to sufentanil; this highly potent opioid agonist was given in conjunction with bupivacaine and administered as an epidural infusion.

This approach to acute pain management may be advantageous. When compared with morphine, methadone has a relatively high affinity for and intrinsic efficacy at the µ-opioid receptor (Table 1.5) (Kristensen et al., 1995; Selley et al., 1998; Borgland et al., 2003; Poisnel et al., 2006) and, therefore, morphine may have reduced efficacy in the presence of methadone. An alternative strategy is to use a µ-opioid receptor agonist that has both high potency as well as high intrinsic opioid agonist activity at least as great as that of methadone. The clinically available fentanyl analogues generally satisfy these criteria (Adams et al., 1990; Stevens et al., 1994a; Barrett et al., 2003).

Following the synthesis in the 1950s of fentanyl, several analogues have been developed such as the short acting alfentanil, the highly potent sufentanil, the large-animal anaesthetic carfentanil and the ultra-short acting remifentanil. Of these, remifentanil, has the advantage that it is a potent opioid agonist and is routinely used for analgesia in the intra-operative setting (Glass, 1995; Duthie, 1998; Beers and Camporesi, 2004). While the ultra-short half-life limits the utility of remifentanil in the post-operative setting (section 6.1.1), this feature, in addition to its very short equilibration half-life between plasma and effect site (Glass et al., 1993; Lötsch, 2005), makes it an attractive and useful clinical research tool as doses can be quickly titrated to effect and adverse effects generally resolve rapidly following infusion cessation. As a consequence, the use of remifentanil was the basis of the study presented in Chapter 4.
1.12.3 Non-opioid analgesia

It has been recommended that opioid tolerant patients can benefit from a multimodal approach to pain treatment, for example the use of NSAIDS, paracetamol, NMDA antagonists such as ketamine, mixed opioid/non opioids such as tramadol or regional analgesia) (NHMRC, 2005). As with patients not dependent on opioids, the use of non-opioid analgesics continue to be useful for the treatment of mild to moderate pain and as an adjuvant to opioids for severe pain (Mehta and Langford, 2006), as the combination of analgesics with the additive and synergistic effects may prove to be beneficial (Jage and Bey, 2000).

The use of epidural ketamine for the successful treatment of an opioid dependent patient following surgery has been advocated by de Leon-Casasola and Lema (1992). This suggestion concur with another case report that demonstrated the use of ketamine as an adjuvant to morphine being successful for post-operative pain in a methadone maintained patient (Haller et al., 2002). The mechanism underlying analgesic effects are largely attributed to its NMDA antagonist properties while other mechanisms, for example, antagonist-agonist action μ-opioid and agonist action at noradrenergic α₂ and 5-HT receptors have also been implicated in its analgesic activity (Hirota and Lambert, 1996). Nonetheless, studies of experimental pain in methadone and buprenorphine maintained patients suggest that conventional doses of the NSAID ketorolac, the mixed opioid/non opioid tramadol or the NMDA antagonist (S)-ketamine either used alone or in combination with high dose morphine provide limited antinociception (Athanasos et al., 2004).

Pharmacotherapies that exhibit either opioid dose-sparing or synergistic effects when combined with opioids may also be beneficial (Mitra and Sinatra, 2004), especially as these approaches to acute pain management may be relevant to both opioid tolerant and opioid-naïve patients. Potential examples include the anticonvulsant gabapentin (Seib and Paul, 2006), the NMDA antagonist dextromethorphan (Weinbroum and Ben-Abraham, 2001), α₂-adrenergic receptor antagonist dexmedotomidine (Weinbroum and Ben-Abraham, 2001) or the cyclooxygenase (COX) 2 inhibitors (Rømsing et al., 2005).

One novel approach is the addition of an ultra-low dose of opioid antagonist to the regular opioid dosing regimen. This concept has arisen from research that has shown that the administration of ultra-low doses of opioid agonists has the potential to be algesic, yet at regular doses they are analgesic; this is suggestive of a bi-modal opioid receptor model of
opioid agonists (Shen and Crain, 1989; Shen and Crain, 1997; Crain and Shen, 2000b; Crain and Shen, 2001). It follows that the addition of an ultra-low dose of opioid antagonist may be able to potentiate the analgesic effects of a regular dose of opioid agonist. Animal studies support this concept (Holmes and Fujimoto, 1993; Powell et al., 2002) and clinical studies suggest that adverse effects and opioid consumption may be reduced by application of this concept (Gan et al., 1997; Cepeda et al., 2004). The addition of an ultra-low dose of opioid antagonist to the opioid regimen of methadone maintained individuals may potentiate the analgesic effects of the agonist and thereby may reduce the degree of hyperalgesia in this population. This concept is further discussed and utilised in a research study presented in Chapter 5.

### 1.12.4 Other opioid-tolerant populations

Further perspective and direction can be gained from studies investigating the management of acute pain in other populations who are opioid-tolerant. One review of two case reports of opioid dependent and addicted patients with cancer pain, suggested that adequate pain control could be achieved with a dosing regimen of 20 mg of methadone every 6 hours in addition to being given 100 tablets of 4 mg hydromorphone per week (Hoffman et al., 1991).

The research of de Leon-Casasola and colleagues (1993) compared the post-operative, analgesic needs of a group of 17 opioid tolerant cancer pain patients compared with those of a group of 99 opioid-naïve patients given epidural analgesia. The opioid-tolerant group had been taking oral morphine for at least 3 months previously and had a mean pre-operative morphine dose of 183 mg. Following the provision of adequate analgesia it was reported that the opioid tolerant group required higher epidural doses (137 vs 44 mg) and higher IV (48 vs 10 mg) doses of morphine than the control group.

As with opioid maintained patients, limited evidence exists for the use of ketamine in opioid tolerant patients with malignancy-related pain. Several researchers have demonstrated that ketamine can reduce opioid requirements and increase pain relief in opioid tolerant patients (Bell, 1999; Eilers et al., 2001; Sator-Katzenschlager et al., 2001)

In a retrospective case review by Rapp and colleagues (1995) compared 180 chronic pain, opioid consuming (CPOC) patients with control subjects who did not use opioids or have pain pre-operatively; subjects were matched for age, gender and surgery type. The study found that
CPOC patients had higher post-operative pain scores than the previously opioid-naïve patients despite the fact that the CPOC patients were administered more opioids (136 ± 69 mg morphine equivalent) within the first 24 hours postoperatively than the control patients (47 ± 33 mg morphine equivalent). This suggests that the CPOC patients were more pain sensitive and (cross) tolerant to opioid analgesia.

Kaplan and colleagues (2000) investigated the analgesic needs of patients with acquired immunodeficiency syndrome (AIDS) related pain. Sustained release oral morphine was titrated to pain relief in a cohort of 11 former drug users (75% of whom where using opioids at enrolment) and 29 non-drug users (25% of whom were using opioids at enrolment). The study reported that at the end of the titration period the former users and non users required a mean of 177 mg/d and 85 mg/d morphine, respectively.

While differences exist between chronic pain patients managed with opioids and opioid maintained patients, factors such as opioid dependence and tolerance remain the same and, as such, need to be treated similarly. A review suggests that where opioids are indicated for the successful treatment of acute pain management needs in chronic pain patients managed with opioids, that 2-4 times the opioid dose may be required compared with opioid-naïve patients (Kopf et al., 2005).

1.12.5 Factors limiting effective acute pain management in the methadone maintained patient.

Early recommendations (for example Rubenstein et al., 1976) that ignored the concepts of tolerance and dependence and therefore potentially causing under-medication may reflect clinicians’ fear of causing adverse effects such as respiratory depression, drug diversion and iatrogenic drug addiction (Lander, 1990; Alford et al., 2006). The term ‘opiophobia’ has been used to describe the irrational and undocumented fear of prescribing opioids even though their use is indicated (Morgan, 1985). A review of pain management requirements in opioid-dependent patients would not be complete without brief reference to other aspects of analgesic treatment in these patients.

Addiction

Clinicians’ fears of causing drug addiction following the prescribing of opioids have generally proved unfounded. In the general community it has been estimated that the occurrence of addiction following acute opioid administration is relatively rare (Porter and Jick, 1980). In
chronically treated patients, its incidence may be between 3% and 19% (Fishbain et al., 1992) and in some ‘high risk’ patient populations, as high as 30% (Chabal et al., 1997). With regards to patients in methadone maintenance programs, several reports highlight that the relapse rate of patients given opioids for surgery is the same as matched non-surgical controls in the same program (Kantor et al., 1980). In fact, in methadone maintained patients the incidence of pain is considered to be one of the main reasons for the initiation and continued use of opioids (Karasz et al., 2004).

**CNS depression**
The concern that the use of opioids may cause respiratory depression in methadone maintenance patients has never been clinically validated (Alford et al., 2006). In fact, methadone maintained patients not only show tolerance to the antinociceptive effects of opioids but also to the respiratory depressant effects as well (Athanasos et al., 2006). Furthermore, the respiratory depressant effects following the acute administration of opioids may be more pronounced in volunteers without pain compared with patients with pain (McQuay, 1999). The presence of acute pain is thought to counter the respiratory depressant effects of opioids. In addition to the development of tolerance, this may be one of the reasons that there appears to be no ceiling opioid dose in chronic pain patients.

**Communication**
Recent review articles (for example Jage and Bey, 2000; Mitra and Sinatra, 2004; Peng et al., 2005; Mehta and Langford, 2006), including the guidelines recently endorsed by the Australia’s NHMRC (2005) have placed a great emphasis on open communication between the treating physician and the opioid dependent patient. Highlighted in most reviews is the need to firstly establish that the patient is in an opioid substitution program and that they are administering the substitution doses as prescribed (Peng et al., 2005). Secondly, most reviews stress the importance of clearly communicating what expectations both parties should have with regard to pain management. Ideally, this should be done before treatment commences and should encompass details relating to length of analgesic treatment, a discontinuation plan and the dispensing of out-patient medications in some cases done explicitly via a documented plan (Peng et al., 2005).

**Co-morbidity**
Opioid dependent patients especially those with a history of a substance abuse disorder, may present with other disorders and diseases such as human immunodeficiency virus (HIV) or
hepatitis infection (May et al., 2001). Psychiatric and behavioural disorders are also prevalent in the opioid dependent population. Moreover, anxiety and fear related to lack of communication (see previous section) can also be common (Mitra and Sinatra, 2004). The treatment of these co-morbidities is also of high priority if successful healthcare is to be implemented (Jage and Bey, 2000).

1.12.6 Summary
As indicated, acute pain management in methadone maintained patients is a complex and challenging problem. Drug-specific adaptations related to maintenance opioids such as methadone include opioid tolerance, dependence and withdrawal. Overcoming these issues as well as other illnesses and diseases may make the holistic care of such a patient more involved and complicated; the lack of evidence-based guidelines further obscures the ability of the clinician to provide adequate treatment. This indicates that further research is necessary to provide evidence that will support high quality guidelines for the successful treatment of acute pain in opioid maintained patients as well as other opioid tolerant patients.
1.13 Overview and aims of the present research

Past research and expert opinion have resulted in several recommendations for the treatment of acute pain in opioid tolerant individuals. However, there are few guidelines and many uncertainties surround pain management in opioid tolerant individuals. Focused research investigating acute pain management issues is required so that guidelines can be evidence-based. This should include the study of underlying mechanisms associated with opioid dependence and tolerance, including opioid-induced hyperalgesia. Furthermore, research investigating apposite opioid dosing regimens is required. Strategies that may potentiate the analgesic effect of opioid may be useful in methadone maintained patients such that they may reverse the hyperalgesia observed in this population.

Therefore, the broad aims of the research presented in this thesis were: to evaluate pain sensitivity in a diverse group of opioid users and former opioid users; if there are pharmacological strategies for reversing hyperalgesia in methadone maintained subjects; and to develop an animal model of methadone-induced hyperalgesia. This thesis describes five studies, which are organised by chapter.

1.13.1 Nociception and chronic opioid use (Chapter 3)

The response to nociceptive tests has been relatively well described in opioid-naïve subjects and patients taking opioids for substitution therapy. This previous research has indicated that methadone and buprenorphine maintained patients are hyperalgesic when assessed using the cold pressor test, and to a lesser degree, the electrical stimulation test. While this implies that chronic opioid use is associated with increased pain sensitivity, it does not exclude other factors, such as genetics, current perceptions to pain or affective state. The purpose of this study was to investigate if there were similarities or differences between other populations who chronically administer opioids, namely, chronic pain patients. The primary aim of this study was to compare and contrast the magnitude of opioid-induced hyperalgesia in methadone maintained patients, chronic pain patients treated with methadone or morphine, and normal controls. Secondary aims of this study were to compare and contrast the psychosocial, opioid- and pain-related characteristics in the aforementioned sample populations. It is hypothesised that opioid managed, chronic pain patients will demonstrate a similar nociceptive, opioid effect and psychosocial profile to methadone maintained patients.
Accordingly, subjects taking opioids will demonstrate hyperalgesia, greater opioid effects and increased mood disturbance compared with healthy controls.

1.13.2 Nociception and former opioid use (Chapter 4)

To further explore the impact of chronic opioid dosing and pain sensitivity, this study sought to investigate pain sensitivity and other factors in a cohort of subjects who formerly used opioids. The primary aim of this study was to compare and contrast the magnitude of opioid-induced hyperalgesia in former opioid users and normal healthy controls. Secondary aims of this study were to compare and contrast the psychosocial, opioid- and pain-related characteristics in the aforesaid sample populations. It is hypothesised that formerly-opioid dependent subjects will demonstrate hyperalgesia and greater mood disturbance compared with healthy control subjects and that these parameters are related to the time since last opioid use in the formerly-dependent opioid users.

1.13.3 Antinociceptive effect of naloxone in methadone maintained subjects (Chapter 5)

An increasing body of research indicates that the antinociceptive properties of opioids may be potentiated by the addition of ultra-low doses of opioid antagonist. This study investigated the effects of ultra-low doses of naloxone administered to methadone maintained patients. The primary aim of this study was to investigate if ultra-low doses of naloxone alter the cold pressor tolerance time of methadone maintained patients. It is hypothesised that there will be a significant increase from baseline in antinociception, but no adverse effects, with the administration of ultra-low doses of naloxone in methadone maintained subjects.

1.13.4 Antinociceptive effect of remifentanil in methadone maintained subjects (Chapter 6)

There is limited evidence of effective acute pain management guidelines in opioid tolerant patients. Recent research indicates that morphine may provide limited antinociception in methadone maintained patients. Therefore, the use of highly potent opioid agonists is worth exploration. The primary aim of this study was to investigate the antinociceptive effects of remifentanil in a cohort of methadone maintained patients. It is hypothesised that a dose of remifentanil can be found that will reduce hyperalgesia in methadone maintained patients without causing adverse effects.
1.13.5 Development of an animal model of methadone-induced hyperalgesia (Chapter 7)

Research utilising animals is crucial for providing insights into mechanisms of drug actions and strategies for the treatment of disease. Animal-based research allows for the study of the induction of opioid-induced hyperalgesia without exposing humans to drugs that may cause addiction and dependence. In addition, it eliminates other confounding factors such as genetics, socioeconomic circumstances and concurrent drug use. While an animal model exists for morphine-induced hyperalgesia, no such model exists for methadone. The primary aim of this study was to develop a model of methadone-induced hyperalgesia in Sprague-Dawley rats. It is hypothesised that there is a dose of chronically administered methadone that significantly decreases paw withdrawal latency in the male, SD rat when compared with baseline values.
Chapter 2 – General methods

The purpose of this chapter is to justify and describe methods that are common to some or all of the studies.

2.1 Cold pressor test

2.1.1 Rationale
The cold pressor (CP) test was originally used in, and derived its name from, experiments that cooled the forearm using iced water; thereby increasing the subject’s blood pressure by inducing vasoconstriction (Hines and Brown, 1933). In terms of sensation, the test initially produces an initial cold feeling followed by a crushing/aching pain which quickly increases in intensity until maximum tolerance is reached (Hines and Brown, 1933; Posner et al., 1985). Once this maximum is reached, the sensation plateaus and then subsides until no more pain is perceived 3-5 minutes after immersion. The intensity of pain induced by the cold water and the duration of immersion is inversely related to the temperature of the water (Hilgard, 1969; Mitchell et al., 2004a). Pain associated with the test rapidly disappears following removal of the limb from the cold water (Posner et al., 1985; Blasco and Bayés, 1988; Walsh et al., 1989). Cold pressor pain is not subject to spatial summation as pain intensity caused by immersion of one finger is the same as that of the whole hand (Wolff and Hardy, 1941).

While the cold pressor test has been used widely for the assessment of analgesic treatments, studies indicate that it may be subject to substantial inter-individual variation (Blasco and Bayés, 1988). However, these concerns have been addressed in the doctoral thesis by La Vincente (2005) and previous studies suggest that opioid maintained subjects have lower degrees of variation associated with the cold pressor test (Doverty et al., 2001b; Athanasos et al., 2006).

The mechanism by which the cold pressor test induces pain has been shown to be due to cold pain receptors within the hand and arm. Cold pain activates nociceptors associated with deep skin structures and with large veins (Kreh et al., 1984; Arndt and Klement, 1991; Klement and Arndt, 1991). Selective partial nerve blocks have shown that cold pain is then transmitted via small, unmyelinated C fibres. This pain, and consequent adaptation, can be explained by a physiological response whereby if a hand or arm is exposed to cold this initially induces vasoconstriction followed by vasodilatation (Lewis, 1930) and subsequent oscillation between these two effects (Lewis, 1931). This oscillation (termed the Lewis effect/wave) can be
controlled for by restricting blood flow in the arm using a sphygmomanometer and adapting the arm to warm water prior to immersion in the cold water.

The cold pressor test has effectively been used to quantify pain responses in healthy individuals, opioid dependent, formerly opioid dependent and chronic pain patients. Furthermore, the cold pressor test has been used to test the effectiveness of opioid analgesia in opioid dependent patients as well as healthy subjects (Doverty et al., 2001a; Doverty et al., 2001b; Athanasos et al., 2006). However, it has been shown that the test has limited ability in distinguishing the analgesic activity of the non-steroidal anti-inflammatory drug, ibuprofen from placebo (Jones et al., 1988).

The following method (Doverty et al., 2001b) was adapted from Eckhardt and colleagues (1998).

### 2.1.2 Setup

Two containers (each were a 20 L plastic cylindrical container 38 cm in depth and 30 cm in diameter) were placed on a trolley 10 cm apart with the container for the warm water placed on the left side, relative to where the subject would stand. One container was filled with warm tap water up to 5 cm from the top of the container; the second container was half filled with crushed ice and filled with cold tap water up to 5 cm from the top of the container. A thermo-regulator (Unistat 110, Thermoline Scientific, Sydney, Australia) was immersed in the warm water, set to 35 °C and switched on. A water pump (Brolga MV 1500, Brolga Australia Pty. Ltd., Haberfield, NSW, Australia) was adjusted such that all of the green lever was visible (no red) and the yellow lever half way across. The pump was placed in the cold water on the far side of the container (distal from where the subject stands) with the water jet facing upwards. The digital thermometer (Checktemp1, Hanna Instruments, Keysborough, VIC, Australia) was placed in the cold water and water or ice added/removed to achieve a water temperature of between 0.5 and 1.0 °C. Subsequent to use, the water/ice was emptied from the buckets and equipment cleaned with 70% ethanol in water.

### 2.1.3 Test administration

The cold pressor test was described to the subject as follows:

*This is the cold pressor test. It is a test of your tolerance to cold pain. Here are two water containers, one filled with warm water, one filled with cold water. You will place your non-*
dominant arm into the warm water for two minutes, then take it out and put it immediately into the cold water container. When your arm is in the cold water container, there are two things I will ask you to tell me when you first feel pain, then leave your arm in the cold water as long as you can possibly tolerate the pain. Tell me when you feel you can longer tolerate the pain and remove your arm from the water. I will pass you a towel, which you may use to dry your arm. Tell me when the sensation in your arm ceases to be painful. While you are completing the test you will be blindfolded, and I will inflate a blood pressure cuff on your arm just before you transfer your arm to the cold water container. This is to control for other factors that may interfere with the results. There is a water pump in the cold water container to keep the water circulating and keep the ice from clumping together. When you put your arm in each water container, immerse your arm quickly but carefully. As you will be blindfolded, I will help you transfer your arm from the warm water to the cold water. Keep your fingers straight and spread apart. Do not touch the sides or the bottom of the container and try not to move your arm too much in the water. I will not speak to you during the test except to give you reminder instructions. You should not speak during the test unless you have an urgent question or concern. The pain you experience from the test disappears quickly after removing your arm from the cold water, and there is no risk of permanent damage. Every person is different in terms of his or her pain sensitivity. It is very important that we obtain an accurate and honest assessment of your pain tolerance. There is no reward for setting a record time, but please try to perform the test honestly and leave your arm in the cold water as long as you can tolerate the pain. Do you have any questions?

If not already done, the subject’s blood pressure was taken and record of the subject’s non-dominant arm was made. The subject was then stood in front of the containers such that the non-dominant arm could be fully immersed in the containers. The temperature of each container was then checked and adjusted to ensure that temperature is within the required range (warm water: 34.5-35.5 °C; cold water: 0.5-1 °C). The subject was asked if they were ready to start. A blood pressure cuff was attached to the non-dominant arm and a blind fold placed over the subject’s eyes. With the assistance of the experimenter, the non-dominant arm of the subject was immersed in the warm water. The subject was reminded to spread their immersed fingers comfortably and hold their arm such that there was no contact with the side or bottom of the container. A generic electronic timer/stopwatch was started as soon as the arm was immersed. At 1 minute and 45 seconds after the arm was immersed the blood pressure cuff was inflated to 20 mmHg below the subject’s diastolic blood pressure and
remained inflated for the duration of the test. After two minutes of the subject’s arm being immersed in the warm water the subject was assisted in transferring their arm to the cold water. As soon as the subject’s arm was immersed in the cold water the timer was re-started. The subject was reminded to state when they first felt pain. The time was recorded, in seconds, from when the subject’s arm was immersed in the cold water to the point where they verbally indicated onset of pain (CPTHTR). The subject was instructed to leave their arm in the water as long as they could tolerate the pain. The time was recorded, in seconds, from when the subject’s arm was immersed in the cold water to the point where they verbally indicated they can not tolerate pain (CPTOL). The subject was instructed to take their arm out of the container. A towel was wrapped around the forearm, the blood pressure cuff deflated and the subject instructed to indicate when the pain sensation had disappeared. The time from the subject removing their arm to verbally indicating no more pain was recorded in seconds and termed decay (CPDEC). The blind-fold and blood pressure cuff was then removed from the subject. In the case of a subject keeping their arm in the cold water for 180 seconds without indicating limit of tolerance, the subject was asked to remove their arm and informed that numbness of the arm would prevent the test from continuing. In this circumstance cold pressor tolerance time was recorded as Did Not Respond (DNR).

2.2 Electrical stimulation test

2.2.1 Rationale

Electrical pain is widely been used as a pain induction technique. One of the general advantages of an electrical stimulation pain test is that it involves the easy application of an electrical current transcutaneously on an area of the body such as the finger, hand, leg or earlobe. This elicits a sharp, localised pain caused by the activation of Aδ fibres. However, this phasic pain model has been described as being unnatural (Gracely, 1999). More complex models of application exist involving the application of electrical pain in either a percutaneous, intracutaneous or intramuscular manner (Petersen-Felix and Arendt-Nielsen, 2002). However, the advantage of applying the electrical current through the earlobe is that it minimises differences between subjects, especially with regard to cutaneous thickness (for example, differences in hand texture caused by manual labour).

Pain induced by the electrical stimulation (ES) test has been used in a wide variety of experimental settings. The test has been used to test the analgesic effect of methadone in
methadone maintained populations (Dyer et al., 1999). Furthermore, the test has been utilised to determine the antinociceptive activity of opioids and other non-opioid analgesics in both healthy and opioid dependent subjects (Doverty et al., 2001a; Athanasos et al., 2006).

2.2.2 Setup

The delivery of electric pulses through one earlobe was achieved using a model S6C Grass stimulator (Grass Instrument Co., Quincy, MA, USA) and delivered via an attached electrode (ear clip). The Grass stimulator was adjusted initially to the following settings outlined in Table 2.1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Setting (Multiplier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
<td>7 (x 0.1)</td>
</tr>
<tr>
<td>Delay</td>
<td>7 (x 10)</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>14 (x 1)</td>
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<td>Volts (V)</td>
<td>0 (x10)</td>
</tr>
<tr>
<td>Output</td>
<td>MONO</td>
</tr>
<tr>
<td>Polarity</td>
<td>NORMAL</td>
</tr>
<tr>
<td>Mode</td>
<td>REPEAT</td>
</tr>
<tr>
<td>Stimulus</td>
<td>REGULAR</td>
</tr>
</tbody>
</table>

2.2.3 Test administration

The electrical stimulation test was described to the subject as follows:

This is the electrical stimulation test. It is a test of your tolerance to electrical pain. I will smear some gel on your earlobe and then attach this ear clip [indicate], the ear clip is an electrode connected to this machine [indicate] which delivers electrical current in pulses. When you are ready to commence, I will put a blindfold over your eyes. I will start sending a very low voltage current through the electrode. At first you will not feel anything. Then I will slowly increase the voltage of the current. I want you to tell me three things: when you first feel the pulse, when the pulse becomes painful and then when you can no longer tolerate the pain, at which time I will switch off the machine and remove the ear clip and blind fold. Aside from indicating these things, do not speak during the test unless you have an urgent question or concern. I will not speak to you during the test except to give you reminder instructions. The pain you experience from the test disappears quickly after switching off the machine, and there is no risk of permanent damage. Every person is different in terms of his or her pain sensitivity. It is very important that we obtain an accurate and honest assessment of your pain tolerance. There is no reward for setting a record voltage on this test, but please try to perform the test honestly and for as long as you can tolerate the pain. Do you have any questions?
The subject was seated comfortably in a quiet room; if the subject had earrings then these were removed. A small amount of electrical conduction gel (Livingstone International Pty Ltd, Sydney, NSW, Australia) was applied to the earlobe and to the ear contact points of the ear clip. The ear clip was then placed on the subject’s ear lobe. A blindfold was then placed over the subject’s eyes until the end of the test to minimise distraction and cues for time. The subject was asked if they were ready to start. Electrical pulses were delivered using the Grass stimulator at a frequency of 0.7 Hz of 14 milliseconds duration. Pulses were started at 0 V and were increased by 2 V every 1.4 seconds, up to a maximum of 100 V. Subjects were asked to indicate when they: felt a sensation, recorded as detection and measured in volts (ESDET); when they could detect pain, recorded as threshold and measured in volts (ESTHR); and when they could no longer tolerate the pain, again measured in volts and recorded as tolerance (ESTOL). Once pain tolerance was reached electrical current was terminated. The blindfold and ear clip was then removed from the subject. A tissue was given to the subject to wipe off remaining conduction gel. The electrode was cleaned with 70% ethanol in water. If the subject could tolerate the maximal voltage of 100 V then the Grass Stimulator was turned off and electrical tolerance time was recorded as Did Not Respond (DNR).

2.3 Von Frey hair test

2.3.1 Rationale

In 1898, Von Frey developed a clinical method for assessing the skin’s sensitivity to touch (Von Frey, 1922). Later the equipment was refined using modern materials and labelled as the Weinstein-Semmes pressure aesthesiometer (Weinstein, 1962). A Von Frey hair is a calibrated, single nylon monofilament applied to the skin for the assessment of cutaneous sensation and allodynia. While Von Frey hairs are primarily used by neurologists for the objective evaluation of sensory loss, the hairs can be used as an objective test for static allodynia. Physiologically, the test stimulates mechanoreceptors which transmit sensation centrally via the large, myelinated Aβ afferent fibres (Johansson et al., 1980).

Allodynia is associated with disease states that cause central sensitisation. Central sensitisation can be caused by intense nociceptive input, herpes zoster infection, inflammation, diabetic neuropathy and nerve injury (Wall and Melzack, 1999). Allodynia is distinct from hyperalgesia in that pain is caused by a low intensity stimulus mediated by low
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Intensity afferents (Aβ fibres); this is distinct from hyperalgesia whereby pain is caused by high intensity stimuli albeit at a lower threshold (Wall and Melzack, 1999). However, punctuate hyperalgesia is probably mediated in part by C-fibre sensitisation as well (Kilo et al., 1994).

Tactile sensory threshold is not dependent on sex and increases slowly with age (Halar et al., 1987). The monofilaments come with normal ranges as suggested by Semmes and colleagues (Semmes et al., 1960). However, these values have been contested with suggestions that the 2.83 labelled filament be used as a screening device for sensory abnormality (Bell-Krotoski et al., 1995) while others have suggested more rigid filament such as 4.08 be used (Voerman et al., 1999).

2.3.2 Setup

The static mechanical allodynia and touch sensations were determined by application of the Semmes Weinstein Von Frey Aesthesiometers (Touch-Test Sensory Evaluator Kit/Von Frey Hairs, Stoelting, Wood Dale, IL, USA) and was based on the methods of Burnstein and colleagues (2000). The set of Von Frey Hairs consists of 20 nylon ‘hairs’ of the following numbered filaments: 1.65, 2.36, 2.44, 2.83, 3.22, 3.61, 3.84, 4.08, 4.17, 4.31, 4.56, 4.74, 4.93, 5.07, 5.18, 5.46, 5.88, 6.10, 6.45, 6.65. These numbers refer to the calculated/theoretical log scale of the actual peak force the filaments can apply i.e. Filament number = log_{10}(10 \times \text{force (mg)}). The filaments were periodically cleaned with 70% ethanol in water.

2.3.3 Test administration

The Von Frey Hairs test was described to the subject as follows:

This is the Von Frey Hair test. It is a test of your tolerance to touch pain. These are the Von Frey Hairs [indicate], they are nylon plastic) filaments, similar to fishing line. They range from thin filaments [indicate – not the smallest, though] to thick [indicate – not the thickest, though]. When you are ready to commence, I will put a blindfold over your eyes. I will start by applying the thinnest Von Frey Hair to your forearm [indicate region]. At first you may not feel anything. I will then apply the Von Frey Hairs, starting with the thinnest through to the thickest. I want you to tell me three things: when you first feel the hairs, when the sensation becomes painful and when you can no longer tolerate the pain, at which time I will not apply any more hairs and remove blind fold. Aside from indicating these things, do not speak during the test unless you have an urgent question or concern. I will not speak to you during the test except to give you reminder instructions. The pain you experience from the test...
disappears quickly after applying the Von Frey Hairs, and there is no risk of permanent
damage. Every person is different in terms of his or her pain sensitivity. It is very important
that we obtain an accurate and honest assessment of your pain tolerance. There is no reward
for setting a record tolerance on this test, but please try to perform the test honestly and for
as long as you can tolerate the pain. Do you have any questions?

The tactile pain threshold was determined using calibrated Von Frey filaments. The subject
was seated comfortably in a quiet room and was asked to place a blindfold over their eyes to
avoid distraction and visual cues. The subject was asked to place their exposed arm on the
chair’s armrest with their palm facing up. In this manner the subject would not to see the
filament being applied. Each monofilament was applied three times in succession to the inside
forearm (volar aspect) approximately 5 cm distal the antecubital crease, of the dominant arm,
each for a duration of 2 seconds with at least 2 seconds interval between filament
applications. Once a sensation was elicited, the remaining of the three applications were made
and the subject asked how many times they felt that sensation. The smallest filament capable
of inducing sensation (VFDET)/pain threshold (VFTHR)/pain tolerance (VFTOL) in at least
two out of three applications was considered the endpoint. Once pain tolerance was reached
no more filaments were applied. If the subject did not consider any of the filament capable of
inducing sensation/pain threshold/pain tolerance the response was recorded as Did Not
Respond (DNR).

2.4 Physiologic parameters

Vital signs (blood pressure, heart rate, and oxygen saturation) were measured using an
Agilent A3 Patient Monitor (Philips Electronics, Eindhoven, Netherlands). Oxygen saturation
of the blood and heart rate were measured by an attached pulse oximetry finger-clip and were
calculated using the spectrophotometry technique (for review see Middleton and Henry,
2000). Blood pressure was calculated using an oscillometric technique using an attached
sphygmomanometer cuff (for general review on blood pressure measurements see Pickering,
2002). On other occasions, blood pressure was measured using standard procedures, that is,
with a stethoscope and manual sphygmomanometer cuff. Respiratory rate was monitored
visually for one minute unbeknownst to the subject.
2.5 Nausea and sedation

Nausea was measured according to Del Favero (1992) (Table 2.2). Sedation was measured using the Royal Adelaide Hospital Sedation Scores (Ready et al., 1988) (Table 2.3). If a subject complained of severe nausea and or vomiting, there was permission for 10-20 mg IV metoclopramide to be administered.

<table>
<thead>
<tr>
<th>Score</th>
<th>Level of Nausea</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No nausea</td>
</tr>
<tr>
<td>1</td>
<td>Slight nausea</td>
</tr>
<tr>
<td>2</td>
<td>Mild nausea</td>
</tr>
<tr>
<td>3</td>
<td>Severe nausea</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score</th>
<th>Level of Sedation</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Awake</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>Occasionally drowsy, easy to rouse, and can stay awake once woken</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Constantly drowsy, still easy to rouse, unable to stay awake once woken</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>Somnolent, difficult to rouse, severe respiratory depression</td>
</tr>
<tr>
<td>S</td>
<td>Normally Asleep</td>
<td>Easy to rouse</td>
</tr>
</tbody>
</table>

2.6 Adverse effect and serious adverse effect monitoring

As a consistent method of soliciting adverse events, the subjects were asked a non-leading question such as “How do you feel?” or “Do you feel any different to when I last asked you?” During drug administration studies, this assessment was made prior to every nociceptive test.

2.7 Questionnaires

2.7.1 Profile of mood states

The Profile of Mood States (POMS) (McNair et al., 1971) questionnaire assesses the mood and feelings of a subject. The questionnaire consists of 65 adjectives that are rated on a scale from 0 (not at all) to 4 (extremely), according to how subjects feel. These items yield scores on subscales measuring six distinct affective states (ranges in parentheses): Tension (0-36), Anger (0-48), Depression (0-60), Vigour (0-32), Fatigue (0-28), and Confusion (0-28). Except for vigour, higher scores indicate greater negative affective state. The POMS-Total Mood Disturbance (POMS-TMD) scale provides a global assessment of affective state and is calculated by adding the subscales scores with vigour weighted negatively. POMS-TMD ranges from –32 to 200 such that high scores indicate more negative mood states.
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2.7.2 Fear of Pain - III
The FPQ-III is a 30 item questionnaire that assesses fear related to a variety of situations associated with pain. Subjects rate how much they fear the pain associated with a certain situation by indicating on a 5 point Likert-type scale whether they would fear the pain associated with a certain situation as ‘not at all’, ‘a little’, ‘a fair amount’, ‘a lot’, or ‘extreme’. The scale consists of 3 sub-scales relating to medical pain, severe pain and minor pain; each with 10 items. Scores can be summed to produce a total fear of pain score with a possible range of 30 – 150. The FPQ-III was developed, validated and shown to be highly reliable by McNeil and Rainwater (1998).

2.7.3 Morphine-Benzodrine Group scale
The Morphine-Benzodrine Group (MBG) scale consists of two subscales that rate euphoric drug effects and is from the Addiction Research Centre Inventory (Haertzen, 1966; Haertzen and Hickey, 1987). The questionnaire consists of 16 items rated as true or false by the subject. A total score ranges from 0 to 16 with higher scores indicating a greater extent of euphoria.

2.7.4 Opiate withdrawal scales
Characteristics of opioid abstinence syndrome can be quantified using both subjective and objective scales. The Subjective Opiate Withdrawal Scale (SOWS) is a self report questionnaire of 16 common symptoms related to opioid-withdrawal (Handelsman et al., 1987). Subjects indicate on a Likert-type scale the degree they are feeling a particular symptom from ‘not at all’, ‘a little’, ‘moderately’, ‘quite a bit’ to ‘extremely’. Summation of scores yields a score between 0 and 64, with higher scores indicating more opioid withdrawal.

The Objective Opiate Withdrawal Scale (OOWS) is a clinician/researcher rated questionnaire of 13 physically observable signs reflecting manifestations of opioid-withdrawal during a 5 minute observation period (Handelsman et al., 1987). Symptoms are rated as present (scored 1) or absent (0); with a total score ranging from 0 to 13 with higher scored indicating a greater degree of opioid withdrawal.

The Clinical Opiate Withdrawal Scale (COWS) objectively rates 11 symptoms related to opioid withdrawal in subjects(Wesson et al., 1999; Wesson and Ling, 2003). Each symptom is given a score between 0 and 4 or 5 with summation yielding a total score between 0 and 48.
Total scores can be interpreted as either mild (5-12), moderate (13-24), moderately severe (25-36) or severe (36-48) withdrawal.

Intensity of opioid withdrawal was also assessed using a visual analogue scale (VAS) (for further review see sections 0 and 2.7.6) anchored with ‘no withdrawal’ (scored 0) and ‘worst imaginable withdrawal’ (scored 100).

2.7.5 Severity of Opiate Dependence

The Severity of Opiate Dependence Questionnaire (SODQ) measures the degree of opioid dependence in subjects administering opioids (Sutherland et al., 1986). Subjects rate the applicability of 21 statements relating to quantity and pattern of opiate use, physical and affective symptoms of withdrawal, withdrawal related drug taking behaviour. Items are scored on a scale ranging from ‘never or almost never’ (scored 0), ‘sometimes’ (1), ‘often’ (2), or ‘always or nearly always’ (3). A total score is calculated by summing all individual scores ranging from 0 to 63 with higher scores indicating a greater severity of opioid dependence. The questionnaire has been validated in the Australian context (Burgess et al., 1989).

2.7.6 Visual Analogue Scale

A Visual Analogue Scale (VAS) is one of the most widely used forms of rating scale due to its ease of administration and scoring. A VAS generally consists of a 100 mm line anchored at each end with two extreme measures such as ‘no pain’ and ‘worst pain imaginable’. Subjects then indicate on the line how they feel in relation to the two statements. Scoring consists of measuring the distance from the negative statement to the point the subject indicated. VASs measuring the sensory intensity of pain induced by experimental pain have successfully been used for the assessment of both pharmacological and non-pharmacological interventions (Price, 1988). Interpretation of VAS (pain intensity) scores as follows have been suggested: 0-5 mm ‘no pain’, 5-44 mm ‘mild pain’, 45-74 mm ‘moderate pain’ and 75-100 ‘severe pain’ (Aubrun et al., 2003).

2.8 Plasma drug concentrations

2.8.1 Plasma methadone concentrations

Human plasma (R)- and (S)-methadone concentrations were assayed using a Liquid Chromatography – Mass Spectroscopy (LCMS) method developed by Foster and colleagues (2006).
The LCMS system comprised of a Cyclobond I 2000 RSP (150x2.1mm) (Astec) HPLC column with in-line pre-filter (2um). Mobile phase consisted of all of HPLC grade (HiPerSolv, BDH), except Acetic Acid (AlanAR) solvent with a Final pH of 4.4 ± 0.1 (unadjusted) and the flow rate set at 0.175 mL/min. The Mass Spectroscopy detector consisted of an APCI probe. Conditions are as per tuning except for the following: APCI Probe 400C; Corona Needle Volt 4.5 kV; CDL voltage 40V; CDL temp 250C; Block temp 200C; Qarray voltage DC 10V; RF 150; Detector gain voltage 1.5 kV; Run time 11.0 min; Nebuliser gas (N2) 2.5L/min and drying gas (N2) 2L/min. Ions were monitored in SIM positive ion mode on channel 1 (quantification): 1 – 310.15 amu, d0-methadone and 2 – 313.15 amu, d3-methadone (internal standard); and channel 2 (identification only): 1 – 265.15 amu, d0-methadone fragment and 2 – 268.15amu, d3-methadone fragment (internal standard). Microscan was set at 0.15 amu in all channels. Retention times were 7 and 8 minutes for (R)- and (S)- methadone, respectively.

Patient samples were quantified with calibration curves consisting of eight standards over the concentration range 50–3000 ng/mL of (R)- and (S)-methadone prepared in blank plasma. Plasma samples (0.5 mL) and internal standard (50 µL 5 µg/mL rac-methadone d3 in water) were aliquoted into 10 mL tapered bottom plastic tubes, alkalinized (0.4 mL, 0. 1 M, Na2CO3, pH 10) and extracted with 6 mL of 30 : 70 (v/v) diethyl ether: hexane for 20 min on a rotary mixer. Samples were then centrifuged (2000 g, 10 min) and the organic phase transferred to a clean 10 mL tapered bottom plastic tube containing 0.2 mL 5 mM HCl and vortexed for 1 min. Samples were then centrifuged (2000 g, 10 min), the organic phase aspirated to waste and 100 µL of the acid phase was injected onto the chromatography system.

Inter-assay variability was monitored with quality control (QC) samples prepared in duplicate at three concentrations; low (LQC, 150 ng/mL), medium (MQC, 350 ng/mL) and high (HQC, 1500 ng/mL) of (R)- and (S)-methadone. Inter-day accuracy and precision (mean ± %coefficient of variation (%CV); n = 3 assays on separate days) was 99 ± 3% (LQC), 102 ± 2% (MQC) and 100 ± 1% (HQC) for (R)-methadone, and 95 ± 3% (LQC), 96 ± 4% (MQC) and 99 ± 2% (HQC) for (S)-methadone. Similarly, intra-assay accuracy and precision (n = 6 replicate samples) was 95 ± 1% (LQC), 96 ± 5% (MQC) and 101 ± 1% (HQC) for (R)-methadone, and 94 ± 4% (LQC), 89 ± 3% (MQC) and 100 ± 1% (HQC) for (S)-methadone. The assay was both precise and accurate at the limit of quantification (5 ng/mL), with
interassay accuracy and precision ($n = 3$ assays on separate days) being $98 \pm 5\%$ and $98 \pm 2\%$ for (R)- and (S)-methadone, respectively.

### 2.8.2 Plasma morphine concentrations

Plasma morphine concentrations were determined using a high performance liquid chromatography (HPLC) assay based on the method described by Doverty and colleagues (2001a).

The HPLC system consisted of a LC1110 pump (GBC Scientific Equipment, Victoria, Australia), pulse damper, Model 5020 guard cell (ESA, MA, USA) set at 700 mV, SIL-9A auto injector (Shimadzu), C18 cartridge pre-column (Alltech), Waters COSMOSIL C18 150 mm x 4.60 mm stainless steel column, Coulochem II Detector (ESA), ESA Model 5010 analytical cell (settings Table 2.4) and integrated with a C-R6A chromatopac (Shimadzu).

Mobile phase consisted of 4% acetonitrile and NaH$_2$PO$_4$ (50 mM final concentration) adjusted to pH 3.00 with 85% orthophosphoric acid. Mobile phase was filtered through 0.2 micron paper and sonicated for 15 minutes prior to use. Mobile phase was recycled throughout sample testing. These conditions allowed for baseline resolution of morphine and hydromorphone, which had retention times of 7 and 13 minutes respectively.

<table>
<thead>
<tr>
<th>Cell potential (mV)</th>
<th>Range (nA)</th>
<th>Filter (sec)</th>
<th>Output (V)</th>
<th>Offset (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode 1</td>
<td>+ 250</td>
<td>1000</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Electrode 2</td>
<td>+ 500</td>
<td>200</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Plasma samples (1 mL), 50 µL internal standard (10 µg/mL hydromorphone), 500 µL sodium bicarbonate buffer (500 mM; pH 9.6) were aliquoted into 10 mL flat bottomed tubes and extracted with 6 mL of acid washed chloroform extraction solvent for 15 min on a rotary mixer. Samples were then centrifuged at 3250 rpm for 10 min and the aqueous layer aspirated off. A further 500 µL sodium bicarbonate buffer was added to the chloroform extract and vortexed for a minimum of 10 seconds to wash any residual aqueous plasma. The sample was then re-centrifuged at 3250 rpm for 10 min and the upper aqueous layer was aspirated off. The remaining organic solvent (approximately 5 mL chloroform) was transferred into correspondingly numbered 10 mL flat plastic tubes containing 200 µl sodium di-hydrogen phosphate (NaH$_2$PO$_4$) (50 mM; pH 2) and then gently mixed on rotary mixer for 10 min.
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After centrifugation at 3250 rpm for 10 min, 150 µL of the upper acid was pipetted into injection vials and 100 µL injected onto the column.

Patient samples were quantified using calibration curves that consisted of 6 standards over the concentration range of 1.0 – 50 ng/mL morphine in drug free blank plasma. Inter- and intra assay variability was monitored with quality control (QC) samples prepared in duplicate at two concentrations; low (LQC, 2 ng/mL) and high (HQC, 20 ng/mL) morphine and were both within 9% of the nominal concentrations.

2.9 Urinalysis and external laboratory results

On the spot urinalysis for morphine was assayed using InstaCheck® MOR 300 Drug Screen Test (ABI / Forefront Inc, San Diego, CA, USA). The test has a limit of detection of 300 ng/mL.

External diagnostic tests used for medical assessment/screening including complete blood examination, biochemistry and urine illicit drug screens were performed by the Institute of Medical and Veterinary Science (IMVS, Adelaide, SA, Australia).

2.10 Genotyping

It is well recognised that one of the important determinants of variability in opioid response relates to genetic polymorphisms regulating the pharmacodynamics and pharmacokinetics of opioids and pain sensitivity (Lötsch and Geisslinger, 2006). Subjects in the following studies gave consent for a blood sample to taken and for the DNA to be genotyped for opioid-receptor, -transporter and -metabolism genetic polymorphisms. The results of these studies are not presented in this thesis as they fall outside the scope of the general aims and hypotheses.

2.11 Ethical and regulatory issues

All studies were performed in accordance to The Declaration of Helsinki first adopted in 1964 (Helsinki, Finland) and revised in 1975 (Tokyo, Japan), 1983 (Venice, Italy), 1989 (Hong Kong), 1996 (Somerset-West, South Africa) and 2000 (Edinburgh, Scotland); and clarified in 2002 (Washington) and 2004 (Tokyo). The principals of set by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) for Good Clinical Practice (GCP) and those adopted by the Therapeutic Goods
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Administration (TGA), Department of Health and Aging, Australian Government were adhered to throughout all studies in addition to the principals of Good Laboratory Practice. Clinical trials involving the administration of a pharmaceutical product were conducted following informing the TGA by the Clinical Trial Notification (CTN) Scheme

2.12 Data collection and statistical analyses

All clinical data was initially recorded using pen-and-paper case report forms (CRFs). Relevant data were then collated using Microsoft Office Excel 2003 (Microsoft Corporation, WA, USA), transformed as needed and exported to be analysed. All data in this thesis were analysed using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA, USA). While reducing the alpha (α) level for multiple comparisons reduces the chance of obtaining a type I error (false positives) (Tukey, 1977), this method can prove too conservative and can increase the chance of a type II error (false negatives) (Perneger, 1999). The adjustment of an α level thus has important ethical and practical consequences. Therefore, an α level of P>0.05 was used for all studies. Furthermore, a priori power analysis utilised a beta (β) level of 0.8. This approach was used to limit both type I and type II errors. Further details regarding statistical analyses are included under relevant headings in subsequent chapters.
3  NOCICEPTION IN CHRONIC OPIOID USERS

3.1  Prologue

Increased pain sensitivity caused by both short- and long-term opioid use has been extensively studied in animals. These studies have provided informative insights into the mechanisms underlying opioid-induced hyperalgesia. Evidence for opioid-induced hyperalgesia in clinical studies is restricted to two main populations, patients treated for heroin dependence with an opioid maintenance treatment program and opioid-naïve patients receiving opioids during surgery. There is one other population that receives opioids on a long term basis, yet very little information regarding their pain sensitivity exists; this is chronic pain patients treated with opioids. This population has been identified as a population in which studies are required (Mao, 2002a). Previous studies have suggested that the pain sensitivity of chronic pain patients managed with opioids does not significantly differ when compared with chronic pain patients managed with non-opioid analgesics (Fillingim et al., 2003; Reznikov et al., 2005) (Section 1.11). However, no studies have compared chronic pain patients, either managed with morphine or methadone, to methadone maintained patients or healthy opioid-naïve controls. Therefore, the primary aim of this study was to investigate similarities and differences between two populations that were chronically receiving opioids: chronic pain patients and methadone maintained patients, particularly with regard to pain sensitivity and psychosocial factors such as mood and fear of pain. Healthy drug-free subjects acted as controls.

3.1.1  Mood

Several authors have shown that the oral administration of methadone to maintained subjects continues to result in mood fluctuations during the inter-dosing period (Dyer et al., 2001; Mitchell et al., 2004b; Mitchell et al., 2006) and that these changes correlate with changes in plasma methadone concentrations. Dyer and colleagues (2001) examined changes relating to mood during a 24-hour inter-dosing period in 18 methadone maintained patients, half of whom reported inadequate withdrawal suppression (‘non-holders’) between dosing. Mood was assessed using the POMS on 11 occasions during the 24 hours following methadone administration. Negative mood states were at a maximum just prior to dosing and at a minimum at the time of peak plasma methadone concentrations (2-4 hour post dose). Conversely, the lowest and highest levels of positive mood state measured by the POMS-Vigor were associated with trough and peak plasma methadone concentrations, respectively.
In comparison, a group of drug-free healthy controls showed no significant changes during a 24 hour observation period. Similar intra-dosing changes have been reported by Mitchell and colleagues (2006), who investigated the mood status of 14 opioid maintained subjects switched between and stabilised on methadone or morphine maintenance treatment. The study found that during a 24 hour observation period, methadone maintenance subjects experienced inter-dosing interval mood disturbances for POMS-TDM, POMS-Tension and POMS-Vigor.

Mitchell and colleagues (2004b) collated data relating to the mood of 55 methadone maintained subjects and related these to plasma methadone enantiomer concentrations. Plasma methadone concentrations were calculated as a ratio of (S)/(R)-methadone, both as an average area under the curve (AUC) ratio during a 23 hour inter-dosing period, or as a trough concentration. Results indicated negligible relationships between plasma methadone concentration ratios and mood in the cohort as a whole. Yet, a subset of the maintained population (n=17) whose methadone dose was ≥ 100 mg/day demonstrated positive correlations between the (S)/(R)-methadone AUC ratio and the POMS-TMD, POMS-Fatigue, POMS-Confusion and POMS-Depression. In the same subset, trough ratios of (S)-methadone to (R)-methadone were only positively correlated with POMS-Tension and POMS-Fatigue. These results support previous studies that indicate that (S)-methadone is associated with negative effects distinctive from (R)-methadone (Fraser and Isbell, 1962).

It is well recognised that the effects of chronic pain negatively impact on the physical, physiological and social dimensions of the patient. Cancer patients with pain report higher levels of mood disturbance including significantly more anger, fatigue, depression, confusion, and lethargy when compared with cancer patients without pain (Lin et al., 2003). One year of multi-disciplinary treatment, including opioid pharmacotherapy, in 477 chronic pain patients resulted in clinically significant decreases in pain intensity, yet there was no change in POMS-TMD compared with baseline measurements (Sator-Katzenschlager et al., 2003). This suggests that chronic pain patients experience negative moods associated with the nociception they experience, but it is not known if opioids further exacerbate this or not. However, there is a paucity of information relating to the effects of opioids for the management of chronic pain to mood status. Therefore, this study aimed to investigate the relationships between pain, opioids and psychosocial factors such as mood and fear of pain.
3.1.2 Opioid PK-PD relationships

Guidelines for the use of methadone are limited by the lack of knowledge regarding pharmacokinetic-pharmacodynamic (PK-PD) relationships. Studies commonly investigate relationships between the pharmacokinetic parameters of drugs such as dose, plasma drug concentration and pharmacodynamic measures, for instance, analgesia and mood (see previous section). Athanasos and colleagues (2006) stratified a cohort of methadone maintained patients according to daily methadone dose and found that the respiratory depressant effects of a very high dose of morphine was significant in the highest dose methadone group (81-115 mg/day) but not the lower dosed groups. Due to inter-individual pharmacokinetic differences, dose may not be as appropriate in determining pharmacodynamic relationships compared with plasma drug concentration; yet dose is an easily obtained measure and therefore makes it clinically relevant.

PK-PD relationships can be further investigated by analysing relationships between plasma drug concentration and effect. The studies of Dyer and colleagues (1999) demonstrated that in methadone maintained patients there were significant relationships between the plasma concentration of racemic methadone and pharmacodynamic parameters such as euphoria, opioid-withdrawal symptoms, pupil diameter and ESTHR. However, this study also highlighted a caveat regarding the inference of concentration-effect relationships with drugs that have long distribution phases (Birkett, 2002). Due to the relatively long distribution half-life of methadone (Wolff et al., 1997), measuring plasma methadone concentrations at times of ‘trough’ concentrations, that is, just prior to the next scheduled dosing may be more appropriate (Dyer et al., 1999).

Furthermore, special consideration also needs to be made for enantiomeric drugs, such as methadone, whereby the different enantiomers possess different pharmacokinetic and pharmacodynamic properties; thus both enantiomers need to be studied separately (Birkett, 2002). Athanasos and colleagues (2006) suggested that there was a trend (P <0.06) toward a significant positive relationship between the trough plasma (R)-methadone concentration in methadone maintained patients and CPTOL. The results of the study may indicate that trough plasma (R)-methadone concentration relates directly with the primary outcome measure of the CP test.
3.2 Aims

The aims of the study were to: determine whether chronic pain patients managed with opioids were hyperalgesic; to compare and contrast the magnitude of opioid-induced hyperalgesia in methadone maintained patients, chronic pain patients treated with methadone or morphine; and to compare the results of the opioid administering patients with drug-free, healthy controls. Furthermore, this study aimed to investigate psychosocial differences between opioid administering populations and healthy control subjects.

3.3 Hypothesis

It is hypothesised that chronic pain patients managed with methadone or morphine will demonstrate a similar nociceptive, opioid effect and psychosocial profile to methadone maintained patients. Accordingly, subjects taking opioids will demonstrate hyperalgesia, greater opioid effects and increased mood disturbance compared with healthy controls.

3.4 Study design

This study was an observational, cross-sectional study.

3.5 Methods

3.5.1 Ethics and consent

This study was conducted with the approval of the Research Ethics Committee of the Royal Adelaide Hospital, South Australia (RAH Protocol 030509). Written informed consent was obtained from all participants prior to screening.

3.5.2 Subjects

Eligibility for the study was subject to several restrictions. Sufficient volunteers were screened such that 10 subjects per cohort were enrolled into the study. Each potential subject needed to meet the following inclusion and exclusion criteria in order to qualify for admission into the study.

3.5.3 Inclusion criteria

A subject was eligible for inclusion in this study if all of the following criteria applied:

All Subjects
- Agreed to and capable of signing an informed consent form.
- Males and females between 18 and 65 years of age.
- In reasonable health, with a World Health Organisation Performance Status Scale Score of 0, 1 or 2.
- In the case of a medical condition needing ongoing treatment, be in the care of a physician who was willing to take responsibility for such treatment.
- Have reasonable venous access.
- Completion of pre-study screening to the satisfaction of the principal investigator or delegate.

**Chronic Pain Patients Administered Opioids.**
- Patients who experience clinically-assessed, non-cancer, chronic pain and who had already received oral methadone or oral morphine for chronic pain management.
- Patients who had received methadone or morphine for at least one month whose dose had been stable for one month.

**Current Methadone Maintained Patients**
- Patients who had received methadone for at least one month for the treatment of dependence and whose dose had been stable for one month.

### 3.5.4 Patient exclusion criteria
- Be a university student who is being or will be assessed by the investigators.
- Be a pregnant or lactating female.
- Be currently participating in another research project, which may interfere with the present study.
- Considered unwilling, unable or unlikely to comply with the study protocol.
- Be dependent on alcohol, benzodiazepines or other drugs of abuse (except opioids related to this study and tobacco).
- Alcohol consumption exceeding National Health & Medical Research Council (Australia) guidelines.
- Be acutely psychotic, severely depressed and in need of inpatient treatment, or an immediate suicide risk.
- Have a neurological or psychiatric illness that would affect pain responses.
- Have a current unstable or untreated medical condition such as acute hepatitis, cardiovascular disease, blood pressure greater than 140/90, liver or renal disease.
Have existing conditions that would affect sensitivity to cold (such as atherosclerosis, Raynaud’s disease, urticaria, hypothyroidism)
- Taking antiretroviral drugs.
- Suffer or have suffered chronic pain (unless as part of inclusion criteria i.e. chronic pain patients).
- Had taken any analgesics in past 3 days (this exclusion criterion applied only to normal controls and methadone maintained patients (‘analgesics’ did not include methadone taken for the treatment of dependence)).

3.5.5 Patient Withdrawal Criteria
- Subjects could withdraw at any time for any reason without having to divulge their reason to the investigators.
- Non co-operation with the study staff and/or non-compliance with the study protocol.
- Unacceptable adverse events

3.5.6 Testing protocol
Subjects accepted into the study were tested on a single occasion. Approximately one hour before the subject’s next scheduled opioid dose (or when convenient for control subjects), subjects attended the clinical testing rooms of the Department of Clinical Pharmacology at the Royal Adelaide Hospital.

3.5.7 Medical history and medication review
All subjects completed a structured interview to determine their:
- Medical history detailing past medical conditions and current diagnosis.
- Survey of concomitant medications, complementary medications, over-the-counter medications and recreational drug use.

3.5.8 Opioid effects questionnaires
Subjects taking opioids were asked to complete the following questionnaires to characterise the effects of opioids in the different groups:
- Clinical Opiate Withdrawal Scale (COWS)
- Subjective Opiate Withdrawal Scale (SOWS)
- Visual Analogue Scale (VAS) – Withdrawal Severity
- Severity of Opioid Dependence Questionnaire (SODQ)
3.5.9 **Psychosocial questionnaires**
To examine psychosocial differences between groups all subjects were asked to complete a Profile of Mood States (POMS) questionnaire and a Fear of Pain Questionnaire III (FPQ-III) before nociceptive testing.

3.5.10 **Chronic pain intensity**
Subjects completed a visual analogue scale rating the intensity of their current chronic pain as well as the worst and best the chronic pain had been in the previous 24 hours.

3.5.11 **Physiologic and Adverse Event Measures**
Prior to the CP test, vital signs were tested and adverse events monitored.

3.5.12 **Blood sampling**
A blood sample of 10 mL was collected by venipuncture for the measure of plasma opioid concentrations and for genotyping.

3.5.13 **Quantitative sensory assessment**
Each subject underwent the cold pressor, electrical stimulation and Von Frey hair tests as described previously. Measures for determining the resultant pain sensitivity were:

- stimulus detection, when the subject first feels a sensation (VFH and ES tests),
- pain threshold, when the subject first feels pain (CP, ES and MA tests),
- pain tolerance, when the subject can no longer tolerate the stimulus (CP and ES tests) and
- pain decay, when the pain sensation is no longer present (CP test only).

Following each pain test, participants completed a visual analogue scale rating pain intensity caused by the tests.

3.5.14 **Reimbursement**
Subjects received AUD50 for a testing visit as compensation for their inconvenience. If a subject withdrew for personal reasons before finishing, they were not paid for their involvement. However, if they had to withdraw because of the study itself, they were paid for the involvement on a pro rata basis. In addition, travel costs were covered (e.g. car parking fee, taxi fare or bus ticket).
3.5.15 Statistical and other analysis

Qualitative data were analysed descriptively. Data from each experimental group were tested for normality and, where appropriate, analysed using Analysis of Variance (ANOVA). Where data existed for only two groups (i.e. chronic pain patients) data were analysed using unpaired, two-tailed t-tests; non-parametric data were analysed using two-tailed Mann-Whitney tests. The non-parametric data generated by the VFH test were analysed by the Mantel-Haenszel (logrank) test due to the non-parametric data generated by the test. Data were presented as mean ± SEM (with 95% confidence intervals (CI)).
Chapter 3 – Nociception in chronic opioid users

3.6 Results

3.6.1 Demographics

Table 3.1 Demographic details.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MMP</th>
<th>CPP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>ANOVA (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>8/2</td>
<td>-</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45.6 ± 2.5 (29-57)</td>
<td>43.3 ± 2.2 (31-48)</td>
<td>40.5 ± 2.1 (31-54)</td>
<td>46.5 ± 3.0 (33-65)</td>
<td>0.332</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.6 ± 6.1 (51-112)</td>
<td>71.9 ± 6.0 (50-105)</td>
<td>93.5 ± 10.0 (54-142)</td>
<td>91.0 ± 9.1 (46-155)</td>
<td>0.204</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 1.3 (20-35)</td>
<td>23.1 ± 1.0 (19-28)</td>
<td>30.5 ± 2.6 (20-46)</td>
<td>29.3 ± 2.3 (19-47)</td>
<td>0.051</td>
</tr>
</tbody>
</table>

Presented as mean ± SEM (range) and analysed with ANOVA. No significant differences were observed between data. MMP – methadone maintained patients, CPP – chronic pain patients.

3.6.2 Chronic pain location / diagnosis

Table 3.2 Chronic pain patient primary diagnosis / location of pain.

<table>
<thead>
<tr>
<th></th>
<th>Chronic Pain Patients (Methadone) (n)</th>
<th>Chronic Pain Patients (Morphine) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary pain diagnosis / location</td>
<td>Lower back (6)</td>
<td>Lower back (5)</td>
</tr>
<tr>
<td></td>
<td>Crushed pelvis (1)</td>
<td>Rheumatoid Arthritis (1)</td>
</tr>
<tr>
<td></td>
<td>Upper and lower back (1)</td>
<td>Knee pain (1)</td>
</tr>
<tr>
<td></td>
<td>Osteoarthritis (1)</td>
<td>Upper back (1)</td>
</tr>
<tr>
<td></td>
<td>Sternal stitching (1)</td>
<td>Gastric bypass adhesions (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Head/Neck cancer removal (1)</td>
</tr>
</tbody>
</table>

3.6.3 Pain history

Table 3.3 Characteristics of chronic pain in subjects.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MMP</th>
<th>CPP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>ANOVA (P) or *t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain Duration (mths)</td>
<td>-</td>
<td>-</td>
<td>89 ± 26</td>
<td>159 ± 36</td>
<td>0.134 *</td>
</tr>
<tr>
<td>Chronic Pain VAS (0-100)</td>
<td></td>
<td></td>
<td>3.2 ± 1.9</td>
<td>44.6 ± 11.3***</td>
<td>48.3 ± 5.3***</td>
</tr>
<tr>
<td>Now</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>6.7 ± 4.6</td>
<td>62.2 ± 6.7***</td>
<td>69.4 ± 8.8***</td>
</tr>
<tr>
<td>Worst in past 24 hr</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>2.7 ± 1.9</td>
<td>26.3 ± 9.6**</td>
<td>43.2 ± 6.0***</td>
</tr>
<tr>
<td>Best in past 24 hr</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed by ANOVA with Tukey’s post hoc test; **P<0.01, ***P<0.001 compared with control subjects. MMP – methadone maintained patients, CPP – chronic pain patients.

3.6.4 Opioid treatment history

Table 3.4 Opioid treatment history.

<table>
<thead>
<tr>
<th></th>
<th>MMP</th>
<th>CPP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>ANOVA (P) or *t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total daily methadone dose (mg)</td>
<td>59.5 ± 6.2</td>
<td>70 ± 21.1</td>
<td>-</td>
<td>0.639 *</td>
</tr>
<tr>
<td>Total daily morphine dose (mg)</td>
<td>-</td>
<td>-</td>
<td>136.5 ± 34.5</td>
<td>-</td>
</tr>
<tr>
<td>Time since last opioid dose (hr)</td>
<td>23.6 ± 0.4</td>
<td>14.1 ± 2.0***</td>
<td>11.0 ± 1.1***</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Opioid Treatment Duration (months) (≥3)</td>
<td>56 ± 12</td>
<td>37 ± 12</td>
<td>16.6 ± 2.8</td>
<td>0.311</td>
</tr>
<tr>
<td>SODQ (-)</td>
<td>19.1 ± 3.3</td>
<td>17.1 ± 2.6</td>
<td>16.6 ± 2.8</td>
<td>0.818</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and results of ANOVA with Tukey’s post hoc test ***P<0.001 compared with maintained patients; or *t-test–methadone dose (maintained vs pain management patients); *t-test–opioid treatment duration in pain patients (morphine vs methadone). Severity of dependence questionnaire (SODQ). MMP – methadone maintained patients, CPP – chronic pain patients.
3.6.5 Concomitant medication use

Table 3.5 Chronic pain patient concomitant medication use.

<table>
<thead>
<tr>
<th>Primary Opioid</th>
<th>Chronic Pain Patients (Methadone)</th>
<th>Chronic Pain Patients (Morphine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug (n) Dose range (mg/day)</td>
<td>Drug (n) Dose range (mg/day)</td>
</tr>
<tr>
<td>Adjuvant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alprazolam</td>
<td>(1) 3</td>
<td>Amitriptyline HCl (1) 200</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>(3) 35-75</td>
<td>Codeine phosphate (1) 300</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>(3) 40-400</td>
<td>Diazepam (5) 5-20</td>
</tr>
<tr>
<td>Codeine</td>
<td>(2) 120-180</td>
<td>Diclofenac potassium (1) 150</td>
</tr>
<tr>
<td>Dihydrocodeine</td>
<td>(3) 5-15</td>
<td>Ketoprofen (1) 200</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>(3) 300-1800</td>
<td>Meloxicam (1) 15</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>(2) 2000-3000</td>
<td>Nitrazepam (2) 5</td>
</tr>
<tr>
<td>Sodium</td>
<td>valproate (2) 400-1000</td>
<td>Paracetamol (1) 5000</td>
</tr>
<tr>
<td>Temazepam</td>
<td>(2) 10-20</td>
<td>Rofecoxib (1) 25</td>
</tr>
<tr>
<td>Supplementary</td>
<td></td>
<td>Sodium valproate (1) 1000</td>
</tr>
<tr>
<td>Acitretin</td>
<td>(1) 25</td>
<td>Temazepam (1) 20</td>
</tr>
<tr>
<td>Amiloride</td>
<td>hydrochloride (1) 5</td>
<td>Tramadol hydrochloride (1) 600</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>trihydrate (1) 500</td>
<td></td>
</tr>
<tr>
<td>Dexamphetamine</td>
<td>sulfate (1) 10</td>
<td></td>
</tr>
<tr>
<td>Hydrochlorothiazide (1) 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>(1) 400</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>HCl (1) 3000</td>
<td></td>
</tr>
<tr>
<td>Oestrogen</td>
<td>(1) 0.625</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>(1) 10</td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td>magnesium (2) 20-40</td>
<td></td>
</tr>
<tr>
<td>Pericyazine</td>
<td>(1) 30</td>
<td></td>
</tr>
<tr>
<td>Perindopril</td>
<td>erbumine (1) 4</td>
<td></td>
</tr>
<tr>
<td>Pindolol</td>
<td>(1) 90</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>fumarate (1) 400</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>(1) IMPLANT</td>
<td></td>
</tr>
<tr>
<td>Trimeprprim</td>
<td>(1) 300</td>
<td></td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>HCl (2) 225-450</td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td>sodium (1) 5</td>
<td></td>
</tr>
<tr>
<td>There was one subject in each of the control and methadone maintained patient cohorts who reported medication use, which was oestradiol (2 mg/day) and acamprosate calcium (666 mg/day), respectively.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.6.6 Recreational drug use history

Data relating to recreational drug use history are presented in Appendix 4.
3.6.7 Withdrawal measures

Opioid withdrawal measures were measured using the SOWS, COWS and a VAS of withdrawal intensity. There were no significant differences except for the SOWS, which indicated that CPP (methadone) subjects had significantly higher scores than the control subjects.

<table>
<thead>
<tr>
<th>Sample Population (Scoring Range)</th>
<th>Controls</th>
<th>MMP</th>
<th>CPP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>ANOVA (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOWS (0-76)</td>
<td>5.9 ± 1.9</td>
<td>8.0 ± 2.2</td>
<td>16.4 ± 2.8*</td>
<td>14.30 ± 2.9</td>
<td>0.015</td>
</tr>
<tr>
<td>COWS (0-64)</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.3</td>
<td>0.1 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.081</td>
</tr>
<tr>
<td>VAS (0-100)</td>
<td>0.0 ± 0.0</td>
<td>11.8 ± 4.7</td>
<td>13.3 ± 6.0</td>
<td>15.1 ± 7.3</td>
<td>0.191</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed by ANOVA with Tukey’s post hoc test, *P<0.05 compared with control subjects. MMP – methadone maintained patients, CPP – chronic pain patients.

3.6.8 Profile of mood states

Results for the POMS-TMD are shown in Figure 3.1. POMS-TMD scores were significantly higher for CPP (morphine) (P < 0.05, 95%CI -64.30 to -3.500) and CPP (methadone) (P < 0.05, 95%CI -63.40 to -2.600) when compared with control subjects. Results for the subscales of the POMS are shown in Table 3.7.

Figure 3.1 Profile of mood states – total mood disturbance (POMS-TMD) scores. Data presented as mean ± SEM; scoring range -32 to 200, and analysed by ANOVA with Tukey’s post hoc test *P<0.05 compared with control subjects.
### Table 3.7 Subscale scores for POMS.

<table>
<thead>
<tr>
<th>Sub-scales (scoring range)</th>
<th>Controls</th>
<th>MMP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>ANOVA (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension-Anxiety (0-36)</td>
<td>4.4 ± 0.8</td>
<td>5.6 ± 1.4</td>
<td>8.6 ± 1.6</td>
<td>8.3 ± 1.5</td>
</tr>
<tr>
<td>Depression-Dejection (0-60)</td>
<td>0.9 ± 0.5</td>
<td>9.1 ± 3.1</td>
<td>9.5 ± 3.2</td>
<td>7.7 ± 2.6</td>
</tr>
<tr>
<td>Vigor-Activity (0-32)</td>
<td>19.3 ± 2.2</td>
<td>8.2 ± 1.7***</td>
<td>10.4 ± 1.6**</td>
<td>8.8 ± 1.6***</td>
</tr>
<tr>
<td>Anger-Hostility (0-48)</td>
<td>1.2 ± 0.7</td>
<td>2.0 ± 1.1</td>
<td>4.8 ± 1.9</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>Fatigue-Inertia (0-28)</td>
<td>3.3 ± 1.1</td>
<td>6.3 ± 2.3</td>
<td>9.6 ± 2.4</td>
<td>10.5 ± 2.7</td>
</tr>
<tr>
<td>Confusion-Bewilderment (0-28)</td>
<td>3.8 ± 0.7</td>
<td>5.5 ± 1.5</td>
<td>5.2 ± 0.8</td>
<td>7.4 ± 1.5</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed by ANOVA with Tukey’s post hoc test. *P<0.05 compared with control subjects. MMP – methadone maintained patients, CPP – chronic pain patients.

### 3.6.9 Fear of pain questionnaire

Results for the FPQ-III are presented in Table 3.8. No significant differences (P>0.05) were observed between any of the groups.

### Table 3.8 Subscale scores for FPQ-III.

<table>
<thead>
<tr>
<th>Sub-scales (scoring range)</th>
<th>Controls</th>
<th>MMP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>ANOVA (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor (10-50)</td>
<td>21.3 ± 2.0</td>
<td>19.5 ± 1.8</td>
<td>21.6 ± 2.2</td>
<td>18.4 ± 1.5</td>
</tr>
<tr>
<td>Severe (10-50)</td>
<td>34.0 ± 2.1</td>
<td>28.5 ± 3.1</td>
<td>36.2 ± 1.8</td>
<td>33.8 ± 1.7</td>
</tr>
<tr>
<td>Medical (10-50)</td>
<td>28.3 ± 2.9</td>
<td>22.0 ± 1.7</td>
<td>27.4 ± 3.3</td>
<td>20.4 ± 3.1</td>
</tr>
<tr>
<td>Total (30-150)</td>
<td>83.8 ± 6.3</td>
<td>70.0 ± 5.3</td>
<td>85.2 ± 5.3</td>
<td>72.6 ± 5.0</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed by ANOVA. No significant differences between groups were observed. MMP – methadone maintained patients, CPP – chronic pain patients.
3.6.10 Von Frey hairs

There were no significant differences in survival curves for each of the cohorts for either VFH detection ($\chi^2=3.94, P=0.268$), threshold ($\chi^2=3.40, P=0.334$) or tolerance ($\chi^2=0.003, P=0.999$) (Figure 3.2). Censoring subjects using medication known to be effective against allodynia (i.e. gabapentin, amitriptyline and sodium valproate) yielded similar results (data not shown): there were no significant differences in survival curves for each of the cohorts for either VFH detection ($\chi^2=6.03, P=0.11$), threshold ($\chi^2=4.82, P=0.19$) or tolerance ($\chi^2=0.003, P=0.999$).

![Figure 3.2 Von Frey Hair (VFH) test. Filament numbers for (A) detection, (B) threshold and (C) tolerance. Data presented as survival curves and analysed by Mantel-Haenszel (logrank) tests. No significant differences between groups were observed.](image-url)
3.6.11 Electrical stimulation test

There were no significant differences for each of the cohorts for either ES detection \((F_{3,36}=0.792, P=0.506)\), threshold \((F_{3,36}=0.616, P=0.609)\) or tolerance \((F_{3,36}=0.668, P=0.577)\).

![Figure 3.3 Electrical stimulation (ES) test. Results for detection, threshold or tolerance. Data presented as mean ± SEM and analysed by ANOVA. No significant differences between groups were observed.](image)

3.6.12 Cold pressor test

There were no significant differences for each of the cohorts for CPTHR \((F_{3,36}=0.993, P=0.407)\). CPTOL times compared with control values were significantly shorter for MMP \((P<0.05; 95\%CI 1.337, 22.34)\), CPP (methadone) \((P<0.05; 95\%CI 0.5572, 21.56)\) and CPP (morphine) \((P<0.05; 95\%CI 2.097, 23.10)\). CPDEC times were significantly longer for CPP (methadone) when compared with MMP values \((P<0.05; 95\%CI 1.337, 22.34)\).

![Figure 3.4 Cold pressor (CP) test. Results for threshold, tolerance and decay times in control and opioid dependent subjects. Data presented as mean ± SEM; ANOVA with Tukey’s post hoc test *P<0.05 compared with control data, #P<0.05 compared with methadone maintained patients (MMP).](image)
3.6.13 Pain intensities

The maximum pain intensity caused by each of the pain tests are shown in Table 3.9. ANOVA of each of the tests suggested significant differences between the groups for the cold pressor test only. However, Tukey’s post hoc test revealed no significant differences between any of the cohorts.

Table 3.9 Pain intensity scores following each nociceptive test.

<table>
<thead>
<tr>
<th>Sample Population</th>
<th>Controls</th>
<th>MMP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>ANOVA (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pain intensity VAS (0-100 mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>58.8 ± 7.7</td>
<td>59.8 ± 5.9</td>
<td>75.2 ± 8.7</td>
<td>84.1 ± 5.3</td>
</tr>
<tr>
<td>ES</td>
<td>51.0 ± 5.3</td>
<td>51.4 ± 6.2</td>
<td>63.6 ± 9.4</td>
<td>68.0 ± 6.5</td>
</tr>
<tr>
<td>VFH</td>
<td>28.6 ± 9.6</td>
<td>18.3 ± 7.9</td>
<td>34.2 ± 10.5</td>
<td>13.1 ± 6.8</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed by ANOVA with Tukey’s post hoc test P>0.05. MMP – methadone maintained patients, CPP – chronic pain patients.

3.6.14 Plasma opioid concentrations

Plasma opioid concentrations are shown in Table 3.10. Plasma methadone concentrations between the two cohorts taking methadone were compared. No significant differences between the cohorts were observed.

Table 3.10 Plasma concentrations for (R)-, (S)-methadone and morphine.

<table>
<thead>
<tr>
<th>Sample Population</th>
<th>Controls</th>
<th>MMP (Methadone)</th>
<th>CPP (Morphine)</th>
<th>M-W or ^t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-methadone</td>
<td>&lt;LOQ</td>
<td>86.0 ± 21.0</td>
<td>160.8 ± 58.2</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>(S)-methadone</td>
<td>&lt;LOQ</td>
<td>82.0 ± 25.2</td>
<td>152.2 ± 56.3</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>(R)/(S)-methadone ratio</td>
<td>-</td>
<td>1.15 ±0.07 (0.73 – 1.69)</td>
<td>1.18 ±0.12 (0.72 – 1.82)</td>
<td>-</td>
</tr>
<tr>
<td>(S)/(R)-methadone ratio</td>
<td>-</td>
<td>0.90 ± 0.06 (0.59 – 1.37)</td>
<td>0.93 ± 0.09 (0.55 – 1.39)</td>
<td>-</td>
</tr>
<tr>
<td>Morphine</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>21.78 ± 5.26</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM (range); plasma methadone concentration data were analysed using Mann-Whitney (M-W) test; (R)/(S)-methadone ratios were analysed using a t-test; for all groups n=10 except for plasma morphine concentrations in morphine managed chronic pain patients where n=6. Methadone and/or morphine were not detected in plasma samples of certain populations (i.e. less than the limit of quantification (LOQ). MMP – methadone maintained patients, CPP – chronic pain patients.

3.6.15 Dose correlations

Spearman or Pearson correlation analyses were performed between subjects’ total daily doses and covariates. No significant correlations were observed except for correlations between total daily dose and plasma methadone concentrations in the CPP (methadone) cohort (Table 3.11).
Table 3.11 Dose correlations.

<table>
<thead>
<tr>
<th>Total daily opioid dose vs covariate</th>
<th>MMP</th>
<th>CPP (Methadone)</th>
<th>CPP (Morphine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COWS</td>
<td>0.009</td>
<td>0.105</td>
<td>-0.072</td>
</tr>
<tr>
<td>SOWS</td>
<td>-0.436</td>
<td>-0.230</td>
<td>0.563</td>
</tr>
<tr>
<td>SODQ</td>
<td>0.118</td>
<td>0.176</td>
<td>0.371</td>
</tr>
<tr>
<td>FPQ-III</td>
<td>0.474</td>
<td>-0.210</td>
<td>0.462</td>
</tr>
<tr>
<td>WITHDRAWAL VAS</td>
<td>0.026</td>
<td>-0.277</td>
<td>0.502</td>
</tr>
<tr>
<td>CPTHHR</td>
<td>0.138</td>
<td>0.034</td>
<td>0.083</td>
</tr>
<tr>
<td>CPTOL</td>
<td>0.686</td>
<td>-0.143</td>
<td>-0.233</td>
</tr>
<tr>
<td>CPDEC</td>
<td>0.350</td>
<td>-0.139</td>
<td>-0.158</td>
</tr>
<tr>
<td>ESDET</td>
<td>0.689</td>
<td>-0.676</td>
<td>-0.062</td>
</tr>
<tr>
<td>ESTHR</td>
<td>0.353</td>
<td>-0.601</td>
<td>0.080</td>
</tr>
<tr>
<td>ESTOL</td>
<td>0.423</td>
<td>-0.045</td>
<td>0.301</td>
</tr>
<tr>
<td>Plasma (R)-methadone conc. †</td>
<td>0.411</td>
<td>0.881***</td>
<td>-</td>
</tr>
<tr>
<td>Plasma (S)-methadone conc. †</td>
<td>0.362</td>
<td>0.850**</td>
<td>-</td>
</tr>
<tr>
<td>(R)/(S)-methadone ratio</td>
<td>0.215</td>
<td>-0.214</td>
<td>-</td>
</tr>
<tr>
<td>Plasma morphine conc. †</td>
<td>-</td>
<td>-</td>
<td>-0.087</td>
</tr>
</tbody>
</table>

Correlation coefficients for relationships between total daily opioid dose and plasma drug concentrations. Values presented as †Spearman r or Pearson r. * P<0.05, **P<0.01, ***P<0.001. MMP – methadone maintained patients, CPP – chronic pain patients.

3.6.16 Plasma methadone concentrations and nociception correlations

Spearman or Pearson correlation analyses were performed between subjects’ plasma methadone concentrations or plasma (S)/(R)-methadone ratio and nociceptive response covariates. No significant correlations were observed except for correlations between (S)/(R)-methadone ratio and ESTOL, CPTHR and CPTOL in the CPP (methadone) cohort (Table 3.12).

Table 3.12 Plasma methadone concentrations and nociception correlations.

<table>
<thead>
<tr>
<th>Plasma concentration vs covariate</th>
<th>Electrical Stimulation</th>
<th>Cold Pressor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detection</td>
<td>Threshold</td>
</tr>
<tr>
<td><strong>(R)-methadone†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP</td>
<td>-0.2494</td>
<td>-0.5045</td>
</tr>
<tr>
<td>CPP (methadone)</td>
<td>-0.5063</td>
<td>-0.4785</td>
</tr>
<tr>
<td><strong>(S)-methadone†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP</td>
<td>-0.3155</td>
<td>-0.4122</td>
</tr>
<tr>
<td>CPP (methadone)</td>
<td>-0.3070</td>
<td>-0.2852</td>
</tr>
<tr>
<td><strong>(S)/(R)-methadone ratio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP</td>
<td>-0.3569</td>
<td>-0.2352</td>
</tr>
<tr>
<td>CPP (methadone)</td>
<td>0.3747</td>
<td>0.5797</td>
</tr>
</tbody>
</table>

Correlation coefficients for relationships between plasma methadone concentrations and nociceptive responses. Values presented as †Spearman r or as Pearson r. * P<0.05, **P<0.01. MMP – methadone maintained patients, CPP – chronic pain patients.
**3.6.17 Mood and opioid administration correlations**

Spearman or Pearson correlation analyses were performed between subjects’ POMS-TMS score and opioid, plasma opioid concentrations and pain intensity VAS score covariates. No significant correlations were observed except for correlations between POMS-TMD scores and pain intensity VAS scores except ‘now’ and ‘best in past 24 hours’ VAS scores in the CPP (morphine) cohort (Table 3.13).

Table 3.13 Mood and opioid administration correlations.

<table>
<thead>
<tr>
<th>POMS-TMD vs Covariate</th>
<th>MMP</th>
<th>CPP (Methadone)</th>
<th>CPP (Morphine)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total daily opioid dose</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>0.2319</td>
<td></td>
<td>0.1128</td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td>-0.2927</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma opioid concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-methadone†</td>
<td>0.09091</td>
<td>-0.1520</td>
<td>-</td>
</tr>
<tr>
<td>(S)-methadone†</td>
<td>-0.07693</td>
<td>0.3615</td>
<td>-</td>
</tr>
<tr>
<td>(S)/(R)-methadone ratio</td>
<td>0.3045</td>
<td>0.1975</td>
<td>0.5440</td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Pain Intensity VAS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best 24 hours</td>
<td>0.0266</td>
<td>0.5990</td>
<td>0.6398*</td>
</tr>
<tr>
<td>Worst 24 hours</td>
<td>0.1479</td>
<td>0.5428</td>
<td>0.4596</td>
</tr>
<tr>
<td>Now</td>
<td>0.0525</td>
<td>0.5128</td>
<td>0.7985**</td>
</tr>
</tbody>
</table>

Correlation coefficients for relationships between Profile of Mood States – Total Mood Disturbance (POMS-TMD) scores and total daily opioid dose, plasma opioid concentrations and pain intensity (PI) VAS scores. Values presented as †Spearman r or Pearson r. * P<0.05, **P<0.01. MMP – methadone maintained patients, CPP – chronic pain patients.

**3.6.18 Adverse effects and vital signs**

No other adverse events were observed in or reported by the subjects. All vital signs (data not presented) were within normal ranges and/or were not clinically significant.
3.7 Discussion

The central aim of this study was to compare the nociceptive profile of two groups of opioid taking patient populations and to determine if there were psychosocial or other factors that distinguished these opioid treatment groups from each other and from non-opioid treated individuals.

3.7.1 Pain

The primary aim of this study was to compare the nociceptive profile of methadone maintained patients and opioid managed chronic pain patients. Results indicated that there were no significant differences with regards to the VFH or ES test yet significant differences were observed with the CP test, in particular CPTOL.

The VF test is a sensitive test for both sensation and allodynic thresholds. The results obtained from the VF test indicated that all subjects, regardless of observational group, were considered to have ‘normal’ VFDET (Bell-Krotoski et al., 1995; Voerman et al., 1999). This indicates that all subjects had ‘normal’ peripheral nerve function. There were no significant differences between groups regarding either VFTHR or VFTOL. This suggests that allodynia and/or increased pain sensitivity to the VFH test were not present in any of the groups. This is not entirely surprising though, for two reasons. Firstly, while allodynia has been reported in patients administered high doses of opioids (Ali, 1986), the presence of allodynia is unpleasant for the patient and usually rapid medical attention is sought to relieve the increased pain sensitivity and is usually resolved by reducing the opioid dose or substituting it for another opioid (Sjøgren et al., 1994; Mercadante and Arcuri, 2005). Moreover, the recruitment of subjects stabilised on opioid doses would have excluded such patients. Secondly, patients with allodynia would probably be less likely to enrol in a trial utilising pain induction techniques.

The nociceptive profile of methadone maintained patients compared with healthy control subjects in the present study has shown that opioid maintained patients are hyperalgesic when assessed with the CP test but not the ES test. Similar results were obtained in the present study, with methadone maintained subjects observed to have shorter CPTOL, yet similar ESTOL compared with healthy control subjects. It has been suggested that the shorter CPTOL latency observed in methadone maintained patients may be as a results of ‘a proneness to overreact’ (Martin et al., 1973; in Doverty et al., 2001b). Similarly, it has been postulated that
opioid addicts may at first deny the pain they are suffering but when this denial cannot be overcome, then they react very quickly (Pud et al., 2006). This suggests that opioid addicts may have an exclusive nociceptive profile: one that confers longer thresholds yet shorter tolerances, compared with drug-free populations (Pud et al., 2006). The addiction liability of subjects was not measured in this study, yet other studies suggest that addiction to opioids occurs at a low rate in chronic pain patients (Fishbain et al., 1992). Nevertheless, chronic pain patients managed with either methadone or morphine demonstrated a comparable nociceptive profile to the methadone maintained populations in the present study. This may suggest that chronic pain patients too are prone to over-reaction; other studies indicate that chronic pain patients are hyper-vigilant (Van Damme et al., 2006) and attentive to pain (Eccleston and Crombez, 1999). As a consequence, these psychological factors may influence pain perception in chronic pain patients and methadone maintained patients alike.

The reporting of CPDEC is not done in studies that utilise the CP test due to its generally large inter-individual variability, and this is reflected in the results of the present study. Using the arguments presented above it could be inferred that hyperalgesic populations may indicate longer CPDEC due to attention to pain. However, there were no significant differences in CPDEC times between the control population and the other populations. Yet, analysis of the results from the CP test did show that chronic pain patients managed with methadone had significantly longer CPDEC times than methadone maintained subjects. This may reflect an important psychological difference between the two populations and may be linked to the reasons just mentioned with regard to CPTOL including hypervigilance and increased focus on pain.

One other study suggests that chronic pain patients may have similar nociceptive profiles to non-opioid taking chronic pain patients, these studies have used mechanical and heat stimuli as well as different indices of pain response (Reznikov et al., 2005) compared with present study. Indeed, the choice of nociceptive stimuli may be an important determinant if hyperalgesia is to be observed or not. Tonic pain induced by the CP test may utilise different neurophysiological pathways compared with the phasic pain induced by the ES test (Chen et al., 1989). Furthermore, phasic pain differs compared with generally more intense and longer-acting tonic pain states that are induced experimentally and which are more directly linked to clinical pain states. This may also explain why hyperalgesia is detectable with the CP test and not other tests, for example, ES and VFH tests.
Importantly, this study has also shown that cold-induced hyperalgesia is associated with morphine administration. While a great deal of human research has focused on hyperalgesia associated with methadone use, this study clearly associates long-term morphine use with cold pressor hyperalgesia. Together with recently published evidence that slow-release oral morphine in maintained subjects (Mitchell et al., 2006) is associated with hyperalgesia, the present study emphasises the animal data that associate morphine administration with increased pain sensitivity (Woolf, 1981; Mao et al., 1994; Li et al., 2001a; Li et al., 2001b; Vanderah et al., 2001b; Li and Clark, 2002; Mao et al., 2002).

3.7.2 Sample populations

While each of the sample populations was similar in terms of basic demographics such as age, weight and BMI, each of the cohorts was unique in terms of other factors.

The two chronic pain groups managed with opioids were similar in terms of their pain, both qualitatively and quantitatively. Both the methadone managed and the morphine managed groups were very similar in terms of the duration of the chronic pain they had experienced. While pain was quantified simplistically using a VAS indicating the intensity of the pain either at the time of testing, and the best and worst it had been in the preceding 24 hours, both chronic pain groups reported similar pain intensity scores. The pain VAS scores of the chronic pain patients suggest that these patients generally experienced mild to moderate pain during the 24 hours prior to testing and during the testing period (Aubrun et al., 2003).

The methadone maintained population was unique in many ways. Selection criteria for this cohort of subjects resulted in many similarities compared with the control group, especially with regard to demographic details, pain history and concomitant medication use apart from oral methadone. Yet, when compared with the chronic pain patients managed with methadone, the methadone maintained patients demonstrated particular differences distinctive of their respective treatment. Differences in treatment regimens demonstrated that methadone maintained patients generally took their methadone dose once a day while chronic pain patients generally took their methadone dose twice a day. This resulted in significantly shorter times since last opioid dose in the chronic pain patients compared with the methadone maintained patients. This reflects the shorter analgesic duration of methadone (4-6 hours) (Beaver et al., 1967) compared with its ability to prevent opioid-withdrawal syndrome (Dole and Nyswander, 1965). There were no significant differences to be observed in the total daily
dose of methadone taken by either group. Interestingly, there were similarities in the degree of opioid dependence between all three opioid taking groups. However, the results of the SODQ must be treated as indicative only. The SODQ primarily been validated only in the context of opioid dependent patients entering a treatment clinic for opioid dependence and abuse. Some of the questions are rather obtuse when asked of a chronic pain patient, while others questions relate more to addiction.

3.7.3 Withdrawal

Generally, there was no evidence of opioid-withdrawal syndrome in any of the subjects. While the SOWS score was significantly higher in the CPP (methadone) cohort compared with control subjects, the mean score signifies that if these subjects were experiencing any withdrawal, it was mild. However, this result may also reflect a lack of the chronic pain patient to discriminate between opioid-withdrawal effects and effects resulting from the pain they experience. This absence of opioid-withdrawal effects in any of the opioid treated groups further suggests that the cohort of subjects were stabilised and taking adequate doses of their respective opioid.

3.7.4 Fear of pain

The present study indicated that there were no significant differences between the groups with regard to their pain-related fear. Similarities between the groups may reflect sampling bias in the recruitment process, with opioid taking subjects selected if they were stabilised on their opioid dose with no changes during the previous month. It has been noted that the morbidity associated with chronic pain may be related to this population’s fear of pain (Vlaeyen and Linton, 2000) and indeed may contribute to the development of chronic pain in these individuals (Keefe et al., 2004). Furthermore, it has been proposed that fear (Asmundson et al., 1999) and anxiety (Greenberg and Burns, 2003) may underlie chronic pain. However, the selection criteria used in the present study may have potentially excluded subjects with heightened anxiety or psychiatric illness. Additionally, the use of an experimental pain model may have excluded subjects vulnerable to higher levels of anxiety, pain-related fear or negative responses to pain. Nonetheless, total and sub-scale scores relating to the FPQ-III indicated that all sample populations were similar in their attitudes to pain.
3.7.5 Mood

The present study intended to investigate similarities and differences in mood between patients taking opioids either for pain management or for methadone maintenance and compare these subjects to a cohort of healthy controls. Several studies have demonstrated that the mood of methadone maintained patients fluctuates during the inter-dosing period with maximal differences, compared with healthy controls, occurring at the time of trough plasma opioid concentration in the methadone maintained patients (Dyer et al., 2001). However, in contrast with the findings of Dyer and colleagues (1999) the present study found no significant difference between healthy control subjects and methadone maintained patients studied when at putative trough plasma methadone concentrations. It must be noted, though, that half of the sample population in the study of Dyer and collegues (1999) were classified as ‘non-holders’, that is, experienced significant opioid-withdrawal at the end of the inter-dosing methadone period. The other half of the methadone maintained subjects in the study of Dyer and collegues (1999) were ‘holders’ and these subjects had comparable POMS-TDM scores to the methadone maintained subjects in the present study.

Interestingly, the POMS-TMD scores obtained in the present study for the control group were markedly lower than scores in normative studies of adults and young college students (McNair et al., 1971; Nyenhuis et al., 1999), yet comparable to scores obtained previously in analogous populations (Dyer et al., 1999).

The POMS-TDM scores obtained from the chronic pain patients, regardless of opioid used for pain management were significantly higher than those of control subjects. This is in-line with previous studies that indicate that cancer patients with pain demonstrate greater mood disturbance than similar patients without pain (Lin et al., 2003). This indicates that the global affective state of the chronic pain patients was approaching levels similar to psychiatric out-patients (McNair et al., 1971). While subjects may note improvements in mood during opioid dose titration (Jamison et al., 1998), it has been shown that longer-term multi-disciplinary (Sator-Katzenschlager et al., 2003) and opioid pharmacotherapeutic (Lorenz et al., 1997) approaches for the treatment of chronic pain generally have minimal impact on mood, yet improve cognitive function. The concept that mood initially improves but gradually deteriorates following the long-term administration of opioids has been observed in other opioid dependent subjects (Price et al., 1975; Mirin et al., 1976a). Possible explanations why the chronic pain patients had significantly higher POM-TMD scores than controls and
methadone maintained patients may be due to the presence of persistent pain (Glover et al., 1995; Lin et al., 2003) as well as adaptive processes induced by long-term opioid administration (White, 2004). Examination of the POMS subscales suggests that the main contributing factor to POMS-TMD was POMS-Vigour. Mood indicative of lower levels of energy, ebullience, vigour and greater degrees of lethargy and fatigue are routinely reported in studies of chronic pain patients (Covington, 1991) and this was reflected in this sample of chronic pain patients.

3.7.6 PK-PD relationships

One of the secondary aims of this study was to investigate the PK-PD relationships. Differing methadone dosage regimens resulted in significantly different ‘time since the last methadone dose’ in the methadone maintained patients compared with the chronic pain patients. This is reflected by the general lack of correlation between total daily opioid dose and any of the other measured parameters in the present study. As the dosage value is the most readily available variable, it is an attractive basis for PK-PD studies. However, many previous studies have shown that the inter- and intra-individual pharmacokinetics of methadone vary considerably (Inturrisi et al., 1987; Inturrisi et al., 1990; Foster et al., 2004) and therefore dosage may not be a suitable predictor of pharmacodynamic outcomes.

Direct comparison of plasma methadone concentrations between populations in this study at the time of testing yielded a wide range of values. Furthermore, large inter-individual differences in the opioid dosage regimen and the pharmacokinetics of methadone mean that analysis based on these parameters may be uncertain in this study. This can be partially resolved by analysing the trough plasma opioid concentrations as done in the present study and analysing correlations dependent on individual enantiomers rather than racemic totals. Previous studies have suggested that the exposure to (S)-methadone is associated with adverse subjective outcomes (Scott, 1948; Olsen et al., 1977). The present study found no correlation between plasma (R)- or (S)-methadone concentrations and any parameter. The study did observe that the ratio of plasma (S)-/(R)-methadone concentrations did correlate strongly with both ESTOL AND CPTOL in the chronic pain patients managed with methadone but this relationship was not observed in the methadone maintained patients. This result is difficult to interpret especially given that there is no correlation between the tolerance indices and the concentrations of individual enantiomers. Given that (S)-methadone is a NMDA antagonist (Ebert et al., 1995) and other NMDA antagonists have been shown to reduce the development...
of analgesic tolerance (Trujillo and Akil, 1991; Herman et al., 1995; Mao et al., 1998), this correlation may indicate that chronic pain patients who have larger (S)-/ (R)-methadone ratios may receive an advantage in that analgesic tolerance and hyperalgesia may be delayed or inhibited. Furthermore, the reason that the ratio correlates well with nociceptive response may be due to the way methadone is prescribed. While in methadone maintained patients, methadone dose is titrated until craving and illicit opioid use are minimised; in chronic pain patients methadone dose is primarily titrated such that pain is reduced while minimising adverse effects.

The last PK-PD relationship this study sought to investigate was the relationship between mood and other selected parameters. The present study found no significant relationship between either opioid dose or plasma opioid concentration and POMS-TMD in any of the patient groups. This result differs to the results of other studies that have indicated that there is a relationship between plasma methadone concentration and POMS-TMD (Dyer et al., 2001; Mitchell et al., 2004b). However, the present study differs from these studies in that only a trough plasma opioid concentration and corresponding mood measures were obtained; the studies of Dyer and colleagues (2001) and Mitchell and colleagues (2004b) measured both plasma opioid concentrations and affective status over a 24-hour inter-dosing period. The variable nature of mood may not be as easily linked to plasma opioid concentration when measured at a single time point rather than over an extended period of time.

This study also investigated the relationship between mood status and pain intensity in chronic pain patients. The present study observed that pain intensity was significantly and positively correlated with POMS-TMD only in chronic pain patients managed with morphine. This result is in accordance with other studies that have presented similar results with both cancer pain (Poulos et al., 2001; Lin et al., 2003) and non-malignant pain (Sator-Katzenschlager et al., 2003). Neurologically, it is recognised that neurological sites related to pain are also related to emotion (Price, 2000). The clinical implications of these results indicate that chronic pain patients with greater levels of pain, especially during the preceding 24 hours, were more likely to experience negative affect, highlighting the need to treat both the psychological distress as well as alleviate pain in these patients.

3.7.7 Limitations

There are clear limitations to this study that require addressing. As this was an observational study it is more difficult to infer causality compared with a randomised study. Small sample
Concomitant medication use in the chronic pain population further reduces the ability to draw solid conclusions associating opioid use and hyperalgesia. This confounding factor is unavoidable when studying chronic pain populations for two reasons. Firstly, the nature of chronic pain management is usually multi-disciplinary. This not only means that pain is treated from numerous fronts including the use of counselling, psychiatry treatment, acupuncture, physiotherapy and/or trancutaneous electrical nerve stimulation (TENS), but also involves various pharmacotherapeutic strategies. It is well documented that the analgesic effects of opioids can be potentiated with the use of adjuvant pharmacotherapies including NSAIDS, paracetamol and analgesics targeting potential neuropathic pain (including gabapentin and valproate). Furthermore, the concomitant use of analgesic adjuvants and opioids is advocated for the treatment of chronic pain (Aronson, 1997). Secondly, the co-morbidities of chronic pain patients are highlighted in the present study by the diversity of the co-medications.

3.7.8 Further studies
This is the first study to simultaneously investigate the nociceptive profiles of three distinct groups of opioid treated patients. Further studies are required into other groups of long-term opioid users to further elucidate whether cold pressor hyperalgesia is only restricted to methadone and morphine. Appropriate treatment groups would include chronic pain patients being managed with oral opioids such as oxycodone, codeine or drugs with opioid properties such as tramadol.
As mentioned previously, patients maintained on methadone for the treatment of opioid addiction are the most studied population. Other populations have not been as extensively investigated; for instance, subjects using heroin, and subjects maintained on slow release oral morphine, buprenorphine and LAAM. However, further nociceptive profiling of these treatment populations is required before definite conclusions can be made regarding opioid-induced hyperalgesia in these individuals.

3.8 Summary

This study made several important observations. Firstly, it confirmed that methadone maintained patients demonstrate hyperalgesia when nociception is measured using the cold pressor test, but not with electrical stimulation or Von Frey hairs. Secondly, it showed that similar nociceptive profiles are obtainable from chronic pain patients managed with methadone. Thirdly, this study demonstrated that the nociceptive profile associated with methadone administration is also observed in morphine managed chronic pain patients.

This study has demonstrated that the hyperalgesia associated with methadone use is not only a phenomenon associated with a particular population, but reinforces the concept that hyperalgesia is associated with the long-term administration of opioids.
Chapter 4 – Nociception in former opioid users

4 NOCICEPTION IN FORMER OPIOID USERS

4.1 Prologue

Former opioid addicts may have ‘comparable’ nociceptive thresholds when evaluated against healthy controls (Andrews, 1943), yet are less pain sensitive when compared with currently using addicts (Compton, 1994). Opioid-addicted subjects have been found to be just as hyperalgesic 28 days into a detoxification program as they were when they first entered (Pud et al., 2006). Much of this research has been reviewed previously, especially in sections 1.8 and 1.9. The current study proposed to investigate the nociceptive profile of a cross section of subjects who had previously been dependent on opioids, yet had not used opioids for at least 6 months. It has been shown that chronic opioid administration leads to increased pain sensitivity. Therefore, it was the objective of this study to investigate if there are permanent changes associated with opioid use or if cessation of opioid use subsequently leads to normal pain sensitivity. It was decided to investigate a group of formerly-dependent opioid users (former users) with regards to nociceptive and psychosocial factors.

4.1.1 Mood

Secondary aims of this study were to investigate the relationship between opioid cessation and affect. White (2004) proposed that mood may be similar to cold pressor pain in that it is influenced by ‘drug-opposite’ changes following long-term opioid administration. Early studies have indicated that methadone administration eventually leads to greater dysphoria and a propensity toward negative mood states (Martin et al., 1973). These changes have been discussed in the previous chapter. It is thereby postulated that the discontinuation of the administration of opioids may result in a resolution of the ‘drug-opposite’ effect and mood returns to a ‘normal’ state.

4.2 Aims

The aims were to determine whether formerly-opioid dependent subjects were hyperalgesic and to compare and contrast the nociceptive profile of formerly-opioid dependent subjects and healthy controls. Furthermore, this study aimed to investigate psychosocial differences between formerly-opioid dependent subjects and healthy control subjects.
4.3 Hypothesis

It is hypothesised that formerly-opioid dependent subjects will demonstrate hyperalgesia and greater mood disturbance compared with healthy control subjects and that these parameters are related to the time since last opioid use in the formerly-dependent opioid users.

4.4 Study design

This study was similar in design to the previous study investigating nociceptive changes in chronic opioid users, except that subjects were former users of opioids. Due to the similarities between the studies, the control group used in this study and the data obtained from them were the same as that used in the previous study.

4.5 Methods

4.5.1 Ethics and consent

This study was conducted with the approval of the Research Ethics Committee of the Royal Adelaide Hospital, South Australia (RAH Protocol 030509). Written informed consent was obtained from all participants prior to screening.

4.5.2 Subjects

Eligibility for the study was subject to several restrictions. Sufficient volunteers were screened such that 10 subjects were enrolled into the study. Each potential subject needed to meet the following inclusion and exclusion criteria in order to qualify for admission into the study.

4.5.3 Inclusion criteria

A subject was eligible for inclusion in this study if all of the following criteria applied:

All Subjects

- Agree to and capable of signing an informed consent form.
- Males and females between 18 and 65 years of age.
- In reasonable health, with a World Health Organisation Performance Status Scale Score of 0, 1 or 2.
- In the case of a medical condition needing ongoing treatment, be in the care of a physician who is willing to take responsibility for such treatment.
- Have reasonable venous access.
Chapter 4 – Nociception in former opioid users

- Completion of pre-study screening to the satisfaction of the principal investigator or delegate.

**Patients formerly dependent on opioids**

- Patients who were formerly opioid dependent as defined by DSM-IV criteria.
- Patients who have not used or are not using opioid antagonists (naloxone or naltrexone) for long-term therapy.
- Patients who do not currently use opioids and have not for at least six months.

### 4.5.4 Patient exclusion criteria

- Be a university student who is being or will be assessed by the investigators.
- Be a pregnant or lactating female.
- Be currently participating in another research project, which may interfere with the present study.
- Considered unwilling, unable or unlikely to comply with the study protocol.
- Be dependent on alcohol, benzodiazepines or other drugs of abuse (except opioids related to this study and tobacco).
- Alcohol consumption exceeding National Health & Medical Research Council (Australia) guidelines.
- Be acutely psychotic, severely depressed and in need of inpatient treatment, or an immediate suicide risk.
- Have a neurological or psychiatric illness that would affect pain responses.
- Have a current unstable or untreated medical condition such as acute hepatitis, cardiovascular disease, blood pressure greater than 140/90, liver or renal disease.
- Have existing conditions that would affect sensitivity to cold (such as atherosclerosis, Raynaud’s disease, urticaria, hypothyroidism)
- Taking antiretroviral drugs.
- Suffer or have suffered chronic pain.
- Have taken any analgesics in past 3 days.

### 4.5.5 Patient Withdrawal Criteria

- Subjects could withdraw at any time for any reason without having to divulge their reason to the investigators.
- Non-co-operation with the study staff and/or non-compliance with the study protocol.
- Unacceptable adverse events
4.5.6 Testing protocol

The testing protocol was identical to the one presented in Chapter 3.

4.5.7 Statistical and other analysis

Qualitative data were analysed descriptively. Data from each experimental group were tested for normality and where appropriate, analysed using unpaired, two-tailed t-tests; non-parametric data were analysed using two-tailed Mann-Whitney tests. The VFH test was analysed by the Mantel-Haenszel (log rank) test due to the non-parametric data generated by the test.

The data from the control group used in this study is the same as that used in the previous study (chapter 3). This leads to an issue regarding multiple comparisons and adjustment of the $\alpha$ level with the studies (section 2.11). However, the ethical implications associated with testing the nociceptive profile of additional subjects and the negligible chance of altering the number of type I or type II errors far outweighs the need for adjustment of the studies’ $\alpha$ level, especially given that the number of multiple comparisons remains trivial.
4.6 Results

4.6.1 Demographics

Table 4.1 Demographic details.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Former users</th>
<th>t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>5/5</td>
<td>5/5</td>
<td>-</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45.6 ± 2.5</td>
<td>30.9 ± 2.1</td>
<td>0.0003</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.6 ± 6.1</td>
<td>73.1 ± 3.8</td>
<td>0.379</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 1.3</td>
<td>23.5 ± 0.9</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed with t-test.

4.6.2 Drug (recreational/medication) use history

There was one subject in the control cohort who reported medication use, which was oestradiol (2 mg/day). Of the former users, the following medication use was reported: insulin (n=1), ethinyloestradiol 0.030 mg/day / levonorgestrel 0.150 mg/day (n=2), citalopram hydrobromide 20 mg/day (n=1). Data pertaining to recreational drug use history are presented in Appendix 4. The mean ± SEM (median (range)) time since last opioid (TLSO) use in the former opioid users was 20.7 ± 6.0 (12 (6 – 57)) months.

4.6.3 Withdrawal measures

Table 4.2 Opioid withdrawal measures including SOWS, COWS and VAS (intensity).

<table>
<thead>
<tr>
<th>Sample Population (Scoring Range)</th>
<th>Controls</th>
<th>Former users</th>
<th>t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOWS (0-76)</td>
<td>5.9 ± 1.8</td>
<td>4.9 ± 2.3</td>
<td>0.741</td>
</tr>
<tr>
<td>COWS (0-64)</td>
<td>0 ± 0</td>
<td>0.1 ± 0.1</td>
<td>NA</td>
</tr>
<tr>
<td>VAS (0-100)</td>
<td>0 ± 0</td>
<td>0.4 ± 0.2</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed by t-test. Due to lack of distribution, t-test was not applicable (NA) to some data.

4.6.4 Profile of mood states

Results for the subscales of the POMS and the POMS-TMD score are shown in Table 4.3.

Table 4.3 Individual sub-scales for POMS and POMS-TMD.

<table>
<thead>
<tr>
<th>Sample Population</th>
<th>Controls</th>
<th>Former users</th>
<th>t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension-Anxiety (0-36)</td>
<td>4.4 ± 0.8</td>
<td>6.3 ± 1.3</td>
<td>0.3704</td>
</tr>
<tr>
<td>Depression-Dejection (0-60)</td>
<td>0.9 ± 0.5</td>
<td>2.9 ± 0.9</td>
<td>0.1013</td>
</tr>
<tr>
<td>Vigor-Activity (0-32)</td>
<td>19.3 ± 2.2</td>
<td>15.9 ± 2.3</td>
<td>0.3024</td>
</tr>
<tr>
<td>Anger-Hostility (0-48)</td>
<td>1.2 ± 0.7</td>
<td>2.0 ± 1.0</td>
<td>0.5228</td>
</tr>
<tr>
<td>Fatigue-Inertia (0-28)</td>
<td>3.3 ± 1.1</td>
<td>3.4 ± 2.0</td>
<td>0.6023</td>
</tr>
<tr>
<td>Confusion-Bewilderment (0-28)</td>
<td>3.8 ± 0.7</td>
<td>4.2 ± 0.7</td>
<td>1.0000</td>
</tr>
<tr>
<td>Total Mood Disturbance (-32-200)</td>
<td>-3.2 ± 3.9</td>
<td>2.9 ± 6.2</td>
<td>0.4178</td>
</tr>
</tbody>
</table>

Mean ± SEM. Data presented as mean ± SEM and analysed by t-test.
4.6.5 Fear of pain questionnaire

Results for the FPQ-III are presented in Table 4.4.

Table 4.4 Individual sub-scales and total for FPQ-III.

<table>
<thead>
<tr>
<th>Sample Population</th>
<th>Controls</th>
<th>Former users</th>
<th>t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor (10-50)</td>
<td>21.3 ± 2.0</td>
<td>16.6 ± 1.9</td>
<td>0.1046</td>
</tr>
<tr>
<td>Severe (10-50)</td>
<td>34.0 ± 2.1</td>
<td>33.4 ± 4.4</td>
<td>0.9050</td>
</tr>
<tr>
<td>Medical (10-50)</td>
<td>28.3 ± 2.9</td>
<td>21.9 ± 2.3</td>
<td>0.1006</td>
</tr>
<tr>
<td>Total (30-150)</td>
<td>83.8 ± 6.3</td>
<td>71.9 ± 7.0</td>
<td>0.2275</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM and analysed by ANOVA. No significant differences between groups were observed.

4.6.6 Von Frey hairs

Figure 4.1 Von Frey Hair (VFH) test.
Filament numbers for (A) detection, (B) threshold and (C) tolerance.
Data presented as survival curves and analysed by Mantel-Haenszel (logrank) tests. No significant differences between groups were observed.

There were no significant differences in survival curves for either of the cohorts for VFH detection ($\chi^2=0.024$, P=0.878), threshold ($\chi^2=0.713$, P=0.399) or tolerance ($\chi^2=0.001$, P=0.970) (Figure 4.1).
4.6.7 Electrical stimulation test

![Electrical stimulation test graph](image)

Figure 4.2 Electrical stimulation (ES) test.
Results for detection, threshold or tolerance. Data presented as mean ± SEM (volts) and analysed by t-tests. No significant differences between groups were observed.

There were no significant differences between the cohorts for either ESDET (P = 0.661), ESTHR (P = 0.557) or ESTOL (P = 0.164) (Figure 4.2).

4.6.8 Cold pressor test

![Cold pressor test graph](image)

Figure 4.3 Cold pressor (CP) test.
Results for threshold, tolerance and decay times. Data presented as mean ± SEM (seconds) and analysed by t-tests. No significant differences between groups were observed.
There were no significant differences between each of the cohorts for either CPTHR (P=0.661), CPTOL (P=0.557) or CPDEC (P=0.164) (Figure 4.3). However, CPTOL data demonstrated non-parametric distribution especially in the former user group and this was reflected partly by the large coefficient of variation (CV%) seen in the CPTOL of the former users (CV=135%) compared with the controls (40%). Additional analysis indicated that the median CPTOL of former users was not significantly different compared with controls (P=0.063) (Figure 4.4).

Figure 4.4 Cold pressor tolerance (CPTOL) times. Data of control and formerly opioid dependent (former users) subjects. Data presented as median ± IQR (box) and range (whiskers) and analysed by a Mann-Whitney test. No significant differences between groups were observed.

4.6.9 Pain intensities

The maximum pain intensity caused by each of the pain tests are shown in Table 4.5. No significant differences between the groups were observed.

<table>
<thead>
<tr>
<th>Sample Population</th>
<th>Controls</th>
<th>Former users</th>
<th>t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain intensity VAS (0-100 mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>58.8 ± 7.7</td>
<td>71.1 ± 8.7</td>
<td>0.3031</td>
</tr>
<tr>
<td>ES</td>
<td>51.0 ± 5.3</td>
<td>48.5 ± 7.9</td>
<td>0.7953</td>
</tr>
<tr>
<td>VF</td>
<td>28.6 ± 9.6</td>
<td>15.6 ± 7.7</td>
<td>0.3043</td>
</tr>
</tbody>
</table>

4.6.10 Plasma drug concentrations

Plasma samples were analysed for methadone and morphine. Neither morphine nor methadone was detected in any of the subjects’ plasma sample.
4.6.11 Time since last opioid dose correlations

Time since last opioid (TLSO) use was correlated against various study parameters and results presented in Table 4.6. There were significant correlations between TLSO and POMS-Vigour-Activity, POMS-Depression-Dejection and POMS-TMD.

Table 4.6 Time since last opioid dose correlations.
Correlation coefficients for relationships between time since last opioid (TSLO) (months) and other study parameters in former opioid users. Values presented as Spearman r; * P<0.05.

<table>
<thead>
<tr>
<th>TLSO vs Covariate</th>
<th>Withdrawal</th>
<th>Psychological</th>
<th>Nociception</th>
</tr>
</thead>
<tbody>
<tr>
<td>COWS</td>
<td>0.879</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOWS</td>
<td>0.323</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WITHDRAWAL VAS</td>
<td>0.135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPQ-III - Total</td>
<td></td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td>POMS-Tension-Anxiety</td>
<td></td>
<td>-0.186</td>
<td></td>
</tr>
<tr>
<td>POMS-Depression-Dejection</td>
<td></td>
<td>-0.724*</td>
<td></td>
</tr>
<tr>
<td>POMS-Anger-Hostility</td>
<td></td>
<td>-0.578</td>
<td></td>
</tr>
<tr>
<td>POMS-Vigour-Activity</td>
<td></td>
<td>0.692*</td>
<td></td>
</tr>
<tr>
<td>POMS-Fatigue-Inertia</td>
<td></td>
<td>-0.175</td>
<td></td>
</tr>
<tr>
<td>POMS-Confusion-Bewilderment</td>
<td></td>
<td>-0.322</td>
<td></td>
</tr>
<tr>
<td>POMS-TMD</td>
<td></td>
<td>-0.704*</td>
<td></td>
</tr>
<tr>
<td>CPTHR</td>
<td></td>
<td>-0.310</td>
<td></td>
</tr>
<tr>
<td>CPTOL</td>
<td></td>
<td>-0.249</td>
<td></td>
</tr>
<tr>
<td>CPDEC</td>
<td></td>
<td>-0.480</td>
<td></td>
</tr>
<tr>
<td>ESDET</td>
<td></td>
<td>0.480</td>
<td></td>
</tr>
<tr>
<td>ESTHR</td>
<td></td>
<td>-0.432</td>
<td></td>
</tr>
<tr>
<td>ESTOL</td>
<td></td>
<td>-0.061</td>
<td></td>
</tr>
</tbody>
</table>
4.7 Discussion

This study sought to compare the nociceptive and psychosocial profiles of a group of subjects who were formerly dependent on opioids to those of a group of healthy control subjects and to determine if there were any factors that distinguished these groups from each other. Overall, this study observed that there were very few differences in either nociception or psychosocial profile between subjects who had not used opioid for at least six months and healthy, opioid-naïve subjects.

4.7.1 Demographics

The demographics of the subjects demonstrated that there were some differences between groups. While pain sensitively has been shown to change with age (section 1.2.7), these changes have been generally demonstrated between relatively large age differences, such as, between children, adults and the elderly. The actual age difference, of approximately 15 years, between the two population samples in the present study was not considered to be considerable large.

4.7.2 Pain

The primary aim of this study was to investigate the nociceptive profile of subjects who had previously been opioid dependent compared with opioid-naïve control subjects. Results for the cold pressor test demonstrate that the two population samples of this study are relatively similar. This is particularly the case for CPTH and CPDEC. However, interesting results were obtained for primary measure of the study, CPTOL. While analysis of the results indicated that there were no statistical differences between the groups, this is partly explained by the distribution of the CPTOL times for the former user group. While most of the former users had CPTOL times that were closely distributed around 20 seconds, two of the subjects had CPTOL times that were closely distributed around 20 seconds, two of the subjects had CPTOL times of 60 and 180 seconds thus explaining the large IQR in this group (Figure 4.4). This indicates two critical results: firstly, this result signifies that the majority of former users are hyperalgesic following the cessation of opioid use. While the pain sensitivity of these subjects either before or during active opioid addiction is not known, it can be inferred from other studies that they were probably hyperalgesic with regard to cold pain when they used opioids. This may suggest that they continue to be hyperalgesic and that pain sensitivity does not rapidly return to normal for these subjects. Secondly, the results indicate that the cold pressor tolerance times of two subjects were relatively longer than those of the rest of the former users and also the control group. This signifies that, either pain sensitivity can be
restored to ‘normal’ levels (assuming these subjects were pain intolerant during opioid use), or that these subjects have always been pain intolerant and were so at the time of testing.

The results of the study indicated that there were no differences in any of the pain tests between the two groups. This is especially true for the Von Frey Hair test and the electrical stimulation test, indicating no difference between the groups in terms of punctuate pressure thresholds and phasic pain thresholds, respectively. Comparable results were obtained in the previous study investigating the nociceptive profile of opioid using populations. Again, this indicates two important features; it is apparent that opioids have minimal effect on either punctuate pressure or phasic pain. Alternatively, this may indicate that nociceptive tests utilising milder stimuli may not be adequate for the detection of opioid-induced hyperalgesia. This characteristic has been shown in previous studies investigating the nociceptive profile of opioid and non-opioid consuming populations (Reznikov et al., 2005). The results of the Von Frey hair test suggest that none of the subjects were experiencing allodynia. Furthermore, the results of the electrical stimulation test indicate both groups were similar in terms of the pain detection, threshold and tolerance for this test. Such a result further supports the notion that the electrical stimulation test is a less sensitive test than the cold pressor test to the effects of opioids.

Data from this study were further analysed by correlating the TSLO versus the nociceptive measures. These results suggested no significant correlation between these two indices. Previous studies have also failed to note any correlation between opioid use parameters and nociceptive thresholds using similar paradigms (Martin and Inglis, 1965). This may suggest that the resolution of hyperalgesia, if it occurs, pertains to particular individual factors rather than just the cessation of opioids. Other aspects that may be worthy of consideration are genetic factors or other psycho-social factors. This said, the results obtained for the FPQ-III questionnaire suggested that there was neither a difference between the control group and the former user group with regard to this variable, nor was there a relationship between TSLO and the FPQ-III scores. These results indicate that further investigation of pain sensitivity in former users, preferably in a longitudinal study, is required before definite statements considering the impact of opioid cessation has on the nociceptive profile of individuals.
4.7.3 Mood

Data relating to the POMS questionnaire indicated that the affective state of controls and the former users was comparable. The evaluation of the control data to previous studies has already been discussed (section 3.7.5). It can be surmised that the former users demonstrated similar affect to the control subjects. Of interest though is the correlations obtained between TSLO and the subscales of the POMS, namely vigour-activity and depression-dejection, and the global assessment score of POMS-TMD. These scales suggest that the longer the time spent since using opioids the lower the mood disturbance. This is mainly caused by an increase in feelings of ebullience and energy and supplemented with decreased levels of depression and feelings of personal inadequacy. Other feelings relating to tension, anger, fatigue and confusion contributed in a negligible manner. The psycho-social impact of opioid use and its potential cessation is immense. While previous studies have indicated that opioid use initially produces positive mood states, longer-term opioid administration leads to greater mood disturbance (Mirin et al., 1976a). The results of the present study suggest that this negative mood disturbance may continue for a certain period but may gradually diminish over time. Naturally, mood is related to the general milieu that the subject is in and the results may suggest that, as the opioid-addicted subject removes himself/herself both physically and temporally from opioid addiction, affect improves. Furthermore, relationships between the TLSO and POMS may suggest improvements in the socio-economic position of the subjects. However, inadequate data collection with regard to this variable limited its analysis in this study.

4.7.4 Putative underlying mechanisms

Interpretation of the results is challenging especially as modest amounts of information exists regarding pain tolerance in chronic opioid users and less so for former users. The results of Compton and colleagues (1994) suggest that former drug users have increased pain tolerance compared with current drug users (opioid or cocaine) and this is substantiated by the similar results of Martin and Inglis (1965). Other studies suggest that there is little difference between the pain tolerance of former opioid users compared with healthy controls (Liebmann et al., 1994; Liebmann et al., 1997), while other studies suggest that former opioid users have increased pain sensitivity (Pud et al., 2006).

Various explanations have been offered regarding the apparent differences and similarities between former opioid users and healthy controls. It has been suggested that opioid users may
represent a sub-group of the population that initially disregard feelings of pain, but when denial of these feelings becomes impossible, they rapidly respond (Pud et al., 2006). Other researchers have suggested that there may be behavioural differences between individuals such that decreased pain sensitivity may cause a predisposition to opioid addiction (Liebmann et al., 1994; Lehofer et al., 1997; Liebmann et al., 1997). Others have countered this hypothesis and suggested that the decreased pain sensitivity observed in former opioid users may in fact be a characteristic of their ability to successfully tolerate pain and discomfort and therefore overcome such feelings during opioid withdrawal (Hajek, 1998). In support of this concept, it has been shown that subjects who experience greater mood disturbance during opioid-withdrawal are less likely to complete a detoxification regimen (Kanof et al., 1993). Therefore, the former users in this study may actually represent a sub-set of addicted individuals who experience less mood disturbance during detoxification, are better able to cope with any mood disturbance experienced and continue to experience positive affect following opioid detoxification. This is especially pertinent given that mood and subjective experiences are the major reason for the continued use of opioids (Jasinski, 1991).

Other researchers have proposed more of a physiological-based argument underlying opioid-induced hyperalgesia and its resolution. They suggested that individuals may experience opponent-processes in response to long-term opioid administration and therefore experience ‘drug-opposite’ effects (Solomon and Corbit, 1974). These effects are seen particularly in certain physiological systems including those that regulate pain sensitivity and mood (White, 2004). Chronic opioid use may result in negative mood states and increased pain sensitivity that may then be a factor for continuous drug use. Moreover, long-term abstinence from opioids may cause systems to reset such that pain sensitivity may return to normal (White, 2004; Pud et al., 2006). However, resolution of hyperalgesia following chronic opioid administration may not represent a return of nociceptive systems back to pre-drug levels but rather that a new allostatic equilibrium is achieved between pro-nociceptive and nociceptive systems, and is thought to be susceptible to modification (Célèrier et al., 2001).

4.7.5 Limitations

The results of this study need to be considered with regard to certain caveats. The sample size was small. The limitation of recruiting former users who had taken part in a supervised opioid detoxification program maintained a certain level of homogeneity in this cohort. The additional recruitment of former opioid users from a ‘narcotics anonymous’ program may
have included subjects who used different abstinence treatment strategies and thus additional confounding factors would need to be considered. Furthermore, the unusually large variation observed in the CPTOL times of the former users would have significantly reduced the power of the study, therefore reinforcing the need to increase the subject numbers and thus the power of the study.

Caution is required with regards to the conclusion of this study due to its cross-sectional nature. Investigation of the nociceptive and psychological changes associated with the cessation of opioid use is certainly better approached with the use of a prospective longitudinal study. However, this study provides useful preliminary data pertaining to the resolution of the altered affective and hyperalgesic states associated with long-term opioid use.

4.8 Summary

The objective of this study was to compare, against healthy control subjects, the nociceptive and psychosocial profiles of subjects formerly dependent on opioids. This study observed that there were no significant differences between these two population samples for either nociception, fear of pain or mood. This study has given valuable insight into potential temporal changes that may occur following the cessation of long-term opioid use. The large degree of variation in particular with regard to the CPTOL, primary outcome of the study, suggests that some former users normalise while others do not. With previous studies indicating that opioid users have an increased sensitivity to pain this study indicates that pain and mood may resolve back to a normal level following the cessation of opioid use. Moreover this study has provided extra perspective regarding the potential mechanisms underlying opioid-induced hyperalgesia.
5 ULTRA-LOW DOSE NALOXONE IN METHADONE MAINTAINED PATIENTS

While opioids such as morphine and methadone are very effective analgesics, their beneficial effects are limited by adverse outcomes, including respiratory depression, gastro-intestinal symptoms, cognitive impairment and tolerance. Evaluating alternative and novel strategies for pain management in methadone maintained patients is a valuable exercise.

The notion that opioid antagonists can potentiate the nociceptive qualities of opioid agonists seems incongruous if classical opioid pharmacology is assumed. However, recent research has indicated that the antinociceptive qualities of opioid agonists can be enhanced at the same time as reducing the adverse effects associated with them when administered in conjunction with ultra-low doses of opioid antagonists.

5.1 Opioid antagonist pharmacology

The principal therapeutic uses of opioid antagonists are for the reversal of opioid agonist overdose, diagnosing opioid physical dependence and for the treatment of substance use disorders, especially opioid-abuse and alcoholism (Goodman et al., 2006). Opioid activity depends on the activation of endogenous opioid systems or due to the administration of exogenous opioids. In the absence of opioids, opioid antagonist administration generally results in few effects (Goodman et al., 2006).

The opioid antagonists naloxone and naltrexone have antagonistic properties at all opioid receptor types (Goldstein and Naidu, 1989), while other opioid antagonists are more selective; these include β-funaltrexamine (µ), naltrindole (δ) and nor-binaltorphimine (κ) (Goodman et al., 2006). Other agonists, such as nalorphine and levallorphan, have antagonist properties at the µ-opioid receptor but are κ-opioid agonists. Many opioid antagonists have structural similarities to opioids agonists, with substitution of the N-methyl group with a larger structure. For example, naloxone and naltrexone are considered to be structurally substituted oxymorphone (Goodman et al., 2006).

5.1.1 Sensitisation

Human studies have shown that the chronic administration of opioid antagonists such as naloxone or naltrexone alone can increase the analgesic effect of subsequent doses of
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morphine (Bardo et al., 1984; Yoburn et al., 1986; Paronis and Holtzman, 1991). It has been postulated that the mechanism underlying this is caused by the up-regulation in the number of opioid receptors as well as the number of opioid receptors that are in a conformation that allows binding (Unterwald et al., 1998). At a cellular level, this may be a result of decreased internalisation (Daws and White, 1999) or inhibited receptor degradation (Belcheva et al., 1991), but has been found to be not due to changes in \( \mu \)-opioid receptor mRNA transcription (Unterwald et al., 1995; Castelli et al., 1997; Shah et al., 1997). Conversely, studies in rats have shown that changes in opioid receptor number caused by either chronic opioid agonist or antagonist administration do not necessarily result in functional changes, such as changes in nociception (Paronis and Holtzman, 1992).

5.2 Nociceptive qualities of naloxone

5.2.1 Pronociceptive properties

Naloxone, administered in doses as high as 20 mg does not alter experimental pain, whether it is induced by electric shock (El-Sobky et al., 1976), ischemic arm pain (Grevert and Goldstein, 1978) or cold pressor pain (Grevert and Goldstein, 1978; Volavka et al., 1980). In contrast, naloxone has the potential to induce hyperalgesia when experimental pain is induced under stressful conditions (Frid et al., 1979), Schull 1981). Similarly, in clinical situations, where stress is present, similar results can be observed. Lasagna (1965) and Levine and colleagues (1978; Levine et al., 1979) demonstrated that naloxone administration caused increases in post-surgical pain. This has been explained by Grevert and Goldstein (1978) who postulated that, under ordinary situations, endogenous opioids do not tonically inhibit pain, whereas under stressful circumstances, endogenous opioids are released and resulting analgesia is able to be antagonised by naloxone.

5.2.2 Antinociceptive properties

It is generally accepted that opioid antagonists such as naloxone and naltrexone are pure opioid antagonists, in that they do not have any agonistic properties, and it has been consistently shown that administration of opioid antagonists results in either no change or decreased levels of nociception (see above). In spite of this, there are several studies that have indicated that naloxone can induce analgesia.

Lasagna and colleagues (1965) demonstrated that following a 2 mg or an 8-10 mg subcutaneous dose of naloxone, either analgesia or increased pain sensitivity to ischemic pain
could be induced, respectively. Similarly, in a small study, Levine and colleagues (1979) demonstrated that, in dental surgery patients who were ‘placebo responders’ (subjects who did not report an increase in pain intensity following placebo administration, that is, the administration of the placebo induced a potential analgesic response), there was a bi-phasic dose-response to naloxone. In this population low doses of naloxone (0.4 and 2 mg) produced analgesia, while higher doses of naloxone (7.5 and 10 mg) produced hyperalgesia (Levine et al., 1979). A study by Buchsbaum and colleagues (1977) observed that subjects considered ‘pain insensitive’ responded to a 2 mg IV dose of naloxone with hyperalgesia. However, another study utilising the same treatment found that subjects considered ‘pain sensitive’ displayed analgesia (Greeley et al., 1988).

This bi-phasic response has been substantiated in animal studies. Ueda and colleagues (1986) demonstrated that low doses of naloxone were capable of inducing analgesia, while higher doses induced hyperalgesia. Analgesia has also been reported in rats following the implantation of naloxone and naltrexone slow-release pellets (Greeley et al., 1988). The proposed mechanism underlying the paradoxical antinociception caused by low doses of naloxone include the blocking of auto-inhibition of enkephalin release (Ueda et al., 1986). Isolated brain tissue studies suggested that pre-synaptic opioid auto-receptors are inhibited by low doses of naltrexone, thereby facilitating the release of further enkephalins (Ueda et al., 1986). Another mechanism proposed to explain naloxone-induced analgesia suggests that opioid antagonists may be able to block an endogeneous dynorphin ‘anti-analgesia’ system (Holmes and Fujimoto, 1993). This complements research suggesting that hyperalgesia is mediated by mechanisms associated with dynorphin release caused by opioid agonist administration (Vanderah et al., 2000; Vanderah et al., 2001a).

5.3 Attenuation of side effects by opioid antagonists

Naloxone can be titrated to reduce some of the effects of opioid agonists, such as decreased respiratory rate, itching, nausea and vomiting, whilst sparing others, such as analgesia in patients experiencing acute (Gan et al., 1997; Maxwell et al., 2005) or chronic pain (McNicol et al., 2003). Dailey and colleagues (1985) demonstrated that analgesia provided by IT morphine administered for labour pain was not affected by administration of a 0.4 mg bolus of naloxone, followed by a 0.4-0.6 mg/hr IV infusion of naloxone. The administration of continuous (IV 5 µg/kg/hr) (Rawal et al., 1986) naloxone has been shown to reverse respiratory depression while still maintaining analgesia following epidural morphine. Other
studies have revealed that side effects such as pruritus and nausea in hysterectomy patients (Choi et al., 2000) and constipation in gastrectomy patients (Lee et al., 2001) are reduced by epidural naloxone while epidural morphine analgesia was unaffected.

Joshi and colleagues (1999) reported the use of either a 15 µg or 25 µg IV bolus dose of the long acting opioid antagonist nalmefene in 119 women directly following lower abdominal surgery. Following this dose of nalmefene, morphine was titrated to achieve adequate analgesia and then IV patient controlled analgesia (PCA) morphine was commenced. During the first 24 hours postoperative period there was no significant difference in the total morphine consumption. However, there was a reduction in the need for antipruritic or anti-emetic medication in patients receiving nalmefene compared with those receiving placebo. Interestingly, patients who received nalmefene retrospectively described their pain, during the 24 hour postoperative period, as less severe that the placebo treated group.

5.3.1 Peripherally acting drugs
The effect of selectively blocking certain effects of morphine and not the analgesic effects has also been demonstrated in other systems. Drugs that act peripherally can reduce some side effects while not affecting others. For example, the antagonist methylaltrexone has been shown to reverse decreased gastrointestinal motility while having minimal effect on analgesia (Yuan and Foss, 2000; Friedman and Dello Buono, 2001), while the peripherally acting drug, naloxone methiodide has been shown to block respiratory depression without inducing opioid-withdrawal in rats (Lewanowitsch and Irvine, 2002). These effects can be attributed to the fact that these drugs are peripherally acting due to their inability to cross the blood-brain barrier.

5.4 In vivo animal studies
Ultra-low doses of opioid antagonists, which selectively inhibit excitatory effects, have been reported to augment systemic morphine analgesia and inhibit the development of tolerance/physical dependence in the rat. Powell and colleagues (2002) reported that administration of intrathecal (it) (0.05 and 0.1 ng) or systemic (10 ng/kg intraperitoneally (IP)) naltrexone augmented the antinociception produced by an acute sub-maximal dose of spinal (5 µg it) or systemic (7.5 mg/kg IP) morphine in the tail-flick test. Chronic it (0.005 and 0.05 ng) or systemic (10 ng/kg) naltrexone combined with morphine (15 µg it; 15 mg/kg IP) over a 7-day period inhibited the decline in morphine antinociception and prevented the loss of morphine potency. In animals rendered tolerant to intrathecal (15 µg) or systemic (15 mg/kg)
morphine, administration of naltrexone (0.05 ng it; 10 and 50 ng/kg IP) significantly restored the antinociceptive effect and potency of morphine. Holmes and Fujimoto (1993) demonstrated that an ultra-low dose of naloxone (1 ng/kg) administered concurrently with an IP injection of morphine enhanced the analgesic properties of the opioid agonist in a radiant tail flick test.

The administration of an ultra-low dose of an opioid antagonist has also been shown to attenuate analgesic tolerance. The addition of a low dose of naltrexone (10 µg/kg) significantly diminished the development of analgesic tolerance caused by chronic administration of morphine (30-50 mg/kg) (Shen and Crain, 1997). Studies by Powell and colleagues (2002) report that the addition of ultra-low doses of naltrexone (either 0.005 ng IT, 0.05 ng IT or 10 ng/kg IP) with morphine (either 15 µg IT or 15 mg/kg IP) inhibited the development of analgesic tolerance over a 7 day period. Furthermore, studies suggested that similar doses of naltrexone could also reverse established analgesic tolerance and restore the analgesic effect of morphine to between 50 and 70% of its original effect (Powell et al., 2002).

5.5 The bimodal opioid receptor model

At regular doses, opioid agonists activate G_i/G_o coupled µ-opioid receptors resulting in inhibition of adenylyl cyclase activity and decreased neuronal cAMP levels (Uhl et al., 1994; Williams et al., 2001). Pre-synaptically, activation of µ-opioid receptors inhibits voltage-sensitive Ca^{2+} channels, thereby shortening the action potential duration and reducing neurotransmitter release, whilst post-synaptically, opioid activity opens K^{+} and hyperpolarises the neuron (Ikeda et al., 1995). Both these processes result in the inhibition of neuronal activity and cause potent analgesia. Opioid receptor antagonists, such as naloxone and naltrexone, are classically thought to block these effects through competitive binding (Goodman, 2006 #656; Gonzalez, 1988 #411).

More recently, research has emerged that indicates that opioid receptor agonists, at doses below those that induce an inhibitory effect, may produce a stimulatory effect. In vivo tissue studies have shown that opioid receptors have the ability to stimulate adenylyl cyclase and promote calcium influx (Smart, 1996 #822). In cultured dorsal root ganglion neurons, opioid agonists at micromolar concentrations causes decreased action potential duration while at nanomolar concentrations increased the action potential duration (Chen et al., 1988; Shen and...
Crain, 1989). The bimodal opioid receptor model proposed by Crain and Shen (2000a) endeavour to explain this dual action of opioids (Figure 5.1). Electrophysiologic studies on the nociceptive types of dorsal root ganglion neurons have shown that picomolar to nanomolar concentrations of morphine can cause the activation of stimulatory secondary messenger systems via a subset of opioid receptors. These studies have suggested that at these ultra-low concentrations, opioid agonists activate Gs-coupled opioid receptors which then go on to activate adenylyl cyclase, resulting in the prolongation of the action potential duration. This elicits an excitatory effect within the neuron and results in behavioural hyperalgesia (Crain and Shen, 2001). Nevertheless, at higher (micromolar) concentrations, opioid agonists activate Gi/Go-coupled receptors, masking stimulatory effects and, as a consequence, produce classical analgesia.

Conversely, opioid antagonists when administered in ultra-low doses have the potential to block the stimulatory effects of micromolar concentrations of opioid agonist, thus potentiating the analgesic effects of the agonist. This bimodal opioid receptor model also attempts to explain the development of opioid dependence and tolerance. It is proposed that during chronic opioid agonist treatment, the Gs-coupled µ-opioid receptor predominates, opposes the analgesic response elicited by Gi/Go-coupled receptors and therefore attenuates potency and presents clinically as tolerance (Crain and Shen, 1992). Studies in mice, supporting this theory, have revealed that systemic, ultra-low doses of naltrexone, both enhance the analgesic properties of morphine and impede the development of physical dependence and tolerance (Shen and Crain, 1997).

It has been proposed that the mechanism underlying this is mediated by an auto-upregulation of Gs-coupled receptors. GM1 ganglioside, a neuronal glycolipid, readily converts Gi/Go-coupled opioid receptors into the Gs-coupled mode (Wu et al., 1997; Wu et al., 1998). Stimulation of Gs-coupled opioid receptors increases adenylyl cyclase, PKA and cAMP levels, which in turn, activates glycosyltransferase, a regulator of GM1 (Scheideler and Dawson, 1986). Therefore, a positive feedback loop promotes the up-regulation of Gs-coupled receptors (Crain and Shen, 1998) (Figure 5.1).

Furthermore, Gi/Go-coupled opioid receptors are considered to be desensitised following chronic agonist activity (Harris and Williams, 1991; Freedman and Lefkowitz, 1996). This
promotes the activation of G protein coupled kinases (GRK) and PKC, thereby perpetuating further tolerance.

Figure 5.1 Bimodal opioid receptor model.

5.6 Clinical studies

5.6.1 Morphine and ultra-low dose naloxone combinations

Clinically, the potentiation of morphine analgesia by the addition of ultra-low doses of naloxone has shown mixed results. One study reported that naloxone had the potential to both attenuate side effects and reduce postoperative opioid requirements in patients who underwent abdominal hysterectomy. Gan and colleagues (1997) randomised 60 women to receive either a continuous IV naloxone infusion of 0.25 µg/kg/hr, 1 µg/kg/hr or placebo for 24 hours postoperatively. Analgesia was provided by IV morphine administered via a patient controlled analgesia (PCA) machine. Total cumulative morphine dose during the 24 hours postoperatively was significantly lower in the 0.25 µg/kg/hr group compared with the saline placebo group, whilst the 1 µg/kg/hr group was no different from placebo. Verbal rating scores for pain were not different between the groups. With regard to side effects, both naloxone doses significantly diminished the incidence of nausea, vomiting and puritus compared with placebo (Gan et al., 1997).
A series of blinded, randomised trials by Cepeda and colleagues highlighted the importance of different doses of opioid antagonists in attenuating certain properties of opioid agonists. In the first of a series of two studies, they investigated the direct combination of a low dose of naloxone with morphine in a PCA solution evaluated against morphine alone (Cepeda et al., 2002). The sample population consisted of 166 patients undergoing a variety of surgical procedures, all under 3 hours in length. Patients either received morphine (1mg/mL) alone or morphine (1 mg/mL) plus naloxone (6 µg/mL) by PCA post-operatively. Patients received less mean ± SD morphine in the morphine only (13.4 ± 8.9 mg) group compared with the naloxone plus morphine (15.6 ± 9.1 mg) group during the 24 hours postoperatively. Furthermore, there were more treatment failures, higher pain intensities and lower levels of patient pain treatment satisfaction in the combination cohort when compared with the morphine only cohort. There were no significant differences between the treatment groups with regard to the number of side effect.

The second in the series employed a similar protocol. However, a lower ratio of naloxone was given with the morphine PCA (Cepeda et al., 2004). Patients either received PCA morphine (1 mg/mL) alone or morphine (1 mg/mL) in combination with naloxone (0.6 µg/mL). Total cumulative morphine dose, pain intensity, and the incidence of vomiting did not differ between the two treatment groups. In accord with other studies, the addition of ultra-low dose naloxone to the morphine PCA caused a significant attenuation of nausea and pruritus compared with the morphine group (Cepeda et al., 2004).

Bijur and colleagues (2006) investigated the effect of adding ultra-low doses of naloxone to morphine in 156 emergency department patients with severe pain. The protocol specified that following a standard bolus dose of IV morphine (0.1 mg/kg), one of 3 different IV, bolus doses of naloxone (0.1 ng/kg, 0.01 ng/kg or 0.001 ng/kg) or saline placebo was administered. The authors concluded that the addition of naloxone had neither a significant effect on pain scores over a 3 hour period, nor did it alter the need for additional analgesia (morphine, other opioids or non-opioid analgesics) during this time. A similar protocol utilising higher doses of naloxone (0.25 µg/kg) also failed to yield any improvement in nausea, pruritus or vomiting while similar decreases in pain intensity compared with placebo were observed (Greenwald et al., 2005).
5.6.2 Other opioid agonist/opioid antagonist combinations

A case report by Cruciani (2003) presented a patient suffering from diabetic polyneuropathy and being treated unsuccessfully with an analgesic regimen of oral methadone (60 mg, 4 times daily) and methylphenidate (30 mg, twice daily). The addition of ultra-low dose naltrexone (1 µg twice daily, orally) to this regimen was reported to reduce the patients’ pain score and incidence of nausea.

Chindalore and colleagues (2005) investigated the combination of oxycodone and ultra-low dose naltrexone (Oxytrex®) in 360 patients with moderate to severe chronic osteoarthritic pain of the hip or knee. Subjects either received placebo, oxycodone 4 times a day, or Oxytrex® either twice or four times daily. Subjects administered the oxycodone doses were started at 10 mg/day and after three weeks ended on 40 mg/day. Naltrexone doses were given in conjunction with the oxycodone such that the total daily dose of naltrexone was 2 µg/day for the Oxytrex® given twice a day, while for subjects administered Oxytrex® 4 times a day, naltrexone doses were 4 µg/day. Oxytrex® given twice a day resulted in close to a 40% reduction in pain intensity, which was significantly more than either that of the placebo, oxycodone or 4 times daily Oxytrex® groups. Furthermore, Oxytrex® was superior with regards to quality of analgesia and duration of pain control when compared with placebo, but not other active treatments. The incidence of adverse side effects was similar between each of the active treatment groups. Other researchers have investigated the addition of naltrexone to an opioid dosing regimen and have measured outcomes that have been based on subjective reports. Neither have reported whether pain sensitivity, when measured with a nociceptive test, changes following the addition of the opioid antagonist (Cruciani et al., 2003; Chindalore et al., 2005).

Other studies have investigated the combination of naloxone in addition to opioid agonists acting via opioid receptors other than the µ-opioid receptor. One of the first studies to investigate the potential of an opioid antagonist to enhance the analgesic potency of an opioid agonist was by Levine and colleagues (1988). They investigated the administration of either morphine (8 or 15 mg) or pentazocine (60 mg), both alone and in combination with low dose naloxone (0.4 mg), for the treatment of pain caused by the extraction of impacted third molars. Analgesia produced by the κ-opioid receptor partial agonist, pentazocine, was potentiated by the addition of naloxone while the antinociceptive effects of morphine were attenuated by the addition of low dose naloxone. As the administration of diazepam to the
patients was considered to be a confounding factor in the interpretation of these results, further studies were performed in an analogous rat model of the experiment; the findings of this subsequent study confirmed the results of the clinical trial (Levine et al., 1988).

Gear and colleagues (2003), investigated the properties of the κ-opioid receptor partial agonist nalbuphine in patients with postoperative dental pain. The authors reported that the addition of naloxone (0.4 mg) enhanced nalbuphine (5 mg) analgesia (Gear et al., 2000); but the same dose of naloxone did not potentiate a 2.5 mg dose of nalbuphine (Gear et al., 2003). The earlier effect was conformed in similar studies investigating lower doses but maintaining the dosing ratio (i.e. 2.5 mg nalbuphine: 0.2 mg naloxone) (Gear et al., 2003).

5.6.3 Limitations of the above research

The clinical studies investigating the impact of low and ultra-low doses of naloxone on morphine efficacy are difficult to compare owing to differences in methodologies, especially with regard to different regimens of both opioid agonist and antagonist, different ratios of opioid agonist:antagonists, and different outcome measures. Mehlisch (2003) highlighted several differences in methodology that may have contributed to differences in results between groups. The comments of Mehlisch (2003) are equally applicable to the other studies in this field. Discrepancies in the dose administered of either the opioid antagonist or agonist have been discussed by Mehlisch (2003) and indeed addressed by other researchers (Gear et al., 2000; Cepeda et al., 2002; Gear et al., 2003; Cepeda et al., 2004; Greenwald et al., 2005; Bijur et al., 2006). Additionally, the importance of administering the opioid agonist such that it does not precipitate hyperalgesia (for example, by intermittent dosing) has also been considered to be an important factor (Mehlisch, 2003) and this may explain the disappointing results obtained by other groups who have administered naloxone as a bolus dose (Cepeda et al., 2002; Cepeda et al., 2004; Greenwald et al., 2005; Bijur et al., 2006).

5.6.4 Naloxone and methadone maintained patients

Special consideration needs to be given to opioid antagonist dose when combining with an opioid agonist. In acute dosing studies and studies involving opioid-naïve subjects, the dose of opioid antagonist needs to be low enough to not block the analgesic effects of the primary opioid agonist (Kendrick et al., 1996). Additionally, consideration needs to be given to the dose of opioid antagonist such that it does not induce opioid-withdrawal. As mentioned previously, the potential of opioid-withdrawal changes the focus of dose-finding studies in this population: not only is it important to investigate if there is a dose of opioid antagonist
that may potentiate the analgesic effect of the methadone, but also a dose that does not result in opioid-withdrawal.

The highest IV bolus dose of naloxone given to 5 maintained patients stabilised on 24 mg/day of methadone that subjects could not differentiate from saline placebo with regard to either subjective or objective withdrawal scores was 0.1 mg of naloxone (Kanof et al., 1992). In the same study the lowest naloxone dose considered to be significantly different to saline with regard to producing withdrawal effects was 0.15 mg (Kanof et al., 1992). Another group reported that 6 methadone maintained (mean daily methadone dose 46.5mg) subjects could distinguish 0.2 mg IV bolus dose of naloxone from saline placebo with regards to withdrawal and ‘bad effects’ but not a 0.1 mg bolus dose of naloxone (Lamas et al., 1994). Neither of these studies (Kanof et al., 1992; Lamas et al., 1994) investigated the impact of naloxone on pain sensitivity.

5.7 Naloxone pharmacology

Naloxone (systematic name: morphinan-6-one, 4,5-alpha-epoxy-3,14-dihydroxy-17-(2-propenyl)- ) is a pure (no agonist activity) opioid receptor antagonist at all three classic opioid receptor subtypes (Martin, 1976).

Naloxone is extensively absorbed by the gastrointestinal tract (91%) however it undergoes extensive first pass metabolism by the liver(Fishman et al., 1973), consequently it has a very low oral bioavailability of 2%. Naloxone is rapidly distributed within the body and has an onset of action following IV administration of 1-2 minutes. It is metabolised by the liver primarily by glucuronide conjugation and has a half-life of approximately 1 hour (Ngai et al., 1976).

5.7.1 Naloxone pharmacodynamics

The pharmacodynamic effects of naloxone depend on the presence of exogenous or endogenous opioid agonists. A dose of 12 mg of naloxone administered subcutaneously in healthy, opioid-naïve humans resulted in no apparent subjective effect, with doses of 24 mg causing slight drowsiness only (Goodman et al., 2006). Naloxone administration causes little effect on endogeneous opioid systems, most likely due to low tonic activity of this system.
(Goodman et al., 2006). However, naloxone can antagonise the analgesic effects afforded by placebos (Levine et al., 1978).

Naloxone doses of 0.4-0.8 mg either given subcutaneously or intravenousy can either prevent or reverse effects of opioid agonists. It is indicated for the reversal of effects such as respiratory depression, sedation and hypotension. Reversal of these effects can be observed within 1-2 minutes of dosing, generally peak at 20-30 minutes and subside within 1 hour (Blachly, 1973; Wang et al., 1974; Kanof et al., 1992). Bolus IV doses of 0.15 mg of naloxone can precipitate withdrawal in patients maintained on 24 mg of methadone (Kanof et al., 1992). Both naturalistic and naloxone-precipitated opioid withdrawal produces an opioid withdrawal syndrome that can include sweating, yawning, nausea, vomiting, shivering nervousness, abdominal cramps and restlessness/irritability (Himmelsbach, 1941; Isbell and Eisenman, 1948). The presence or degree of these symptoms provides a basis for scales such as the Subjective Opioid Withdrawal Scale (SOWS), Objective Opioid Withdrawal Scale (OOWS) (Handelsman et al., 1987) or (WOWS) (Haertzen et al., 1970) that measure the severity of opioid-withdrawal. Such scales rate the intensity of gastrointestinal, psychic, musculoskeletal and autonomic symptoms of the opioid withdrawal syndrome. Abrupt naloxone-precipitated opioid withdrawal can also result in hypertension, tachycardia, ventricular arrhythmias (Cuss et al., 1984), and pulmonary oedema (Partridge and Ward, 1986); these effects are due to catecholamine release. Nonetheless, naloxone dosage can be titrated so that the desired effects, such as adequate ventilation, can be achieved without causing significant loss of analgesia or causing discomfort.

It has been suggested that sensitivity to the antagonist properties of naloxone is related to both the time since and the quantity of the last opioid dose (Goodman et al., 2006) and that naloxone sensitivity is related to initial opioid dose (Higgins et al., 1992). However there is a paucity of data relating to whether maintained subjects on higher doses of methadone are more sensitive to the effects of naloxone compared with those on lower doses.
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5.8 Summary

The clinical pharmacology of opioid antagonists may not be restricted to the concept that they simply block the effects of opioid agonists. Pre-clinical studies have shown promising results indicating that ultra-low doses of opioid antagonist can augment the analgesic activity of opioid agonists as well as reverse established tolerance. Indeed, limited clinical studies suggest that low doses of opioid antagonist may be analgesic in certain populations; furthermore, some studies indicate that ultra-low doses of opioid antagonist may be able to potentiate the analgesic activity of opioid agonists. Yet, it must be acknowledged that the clinical studies have yielded contradictory results. While the mechanisms of how antagonists may potentiate the analgesic effects of opioids are yet to be fully elucidated, research has mainly been focused on a bimodal model while other researchers implicated the involvement of endogenous opioids. Undoubtedly, these concepts may be advantageous if applied to a methadone maintained population, as it offers a chance to reverse hyperalgesia.

5.9 Rationale of present research

The present study was designed to determine whether IV, ultra-low dose of naloxone would reduce hyperalgesia in methadone maintained subjects without precipitating opioid-withdrawal. This may add to the scientific knowledge base in understanding the nature and mechanisms of opioid-induced hyperalgesia and provide opportunities to develop more rational and effective management of pain and dependence in methadone maintained patients.
5.10 Pilot Study

5.11 Aim
The aim of this study was to determine whether there was an ultra-low dose of naloxone that would reduce hyperalgesia in methadone maintained patients.

5.12 Hypothesis
It is hypothesised that there will be a significant increase from baseline in antinociception, but no adverse effects, with the administration of ultra-low doses of naloxone in methadone maintained subjects.

5.13 Study design
This study was an open label, unblinded, proof of concept, pilot study.

Due to the lack of data on the antinociceptive effects of naloxone in methadone maintained subjects, an initial pilot study was conducted. While the administration of naloxone, even in relatively high doses in opioid-naïve is not associated with significant adverse effects, the administration of naloxone to methadone maintained subjects presents the risk of precipitating opioid-withdrawal.

5.14 Methods
An overview of the testing schedule for this study is provided in Appendix 1.

5.14.1 Ethics and consent
This study was conducted with the approval of the Research Ethics Committee of the Royal Adelaide hospital, South Australia (RAH Protocol 040210). Written informed consent was obtained from all participants prior to screening.

5.14.2 Subjects
Eligibility for the study was subject to several restrictions. A sufficient number of male volunteers were screened such that 8 subjects were enrolled into the study. The first two subjects participated in the pilot study. Each potential subject needed to meet the following inclusion and exclusion criteria in order to qualify for admission into the study.
5.14.3 Inclusion criteria
A subject was eligible for inclusion in this study if only all of the following criteria applied:

- Subjects who received between 50 and 80 mg methadone per day for at least three months for the treatment of dependence and whose dose has been stable for one month.
- Agree to and capable of signing an informed consent form.
- Be a male aged between 18 and 45 years (to reduce variation in response and subject numbers).
- Be in good physical health.
- Have reasonable forearm venous access.
- Completion of pre-study screening to the satisfaction of the principal investigators or delegate. This included:
  - Medical History
  - Physical Examination
  - Blood Tests for
    - Liver function (AST and ALT not greater than 8 times the upper limit of normal range; serum albumin not less than 33g/l, INR between 0.9-1.2)
    - Kidney function (calculated creatinine clearance greater than 80 mL/min)
    - Bone marrow functions (within normal reference ranges)
  - Urine screen for illicit drugs (for drug monitoring and to help with interpretation of results).
  - Normal electrocardiogram.
- Present with no signs of withdrawal, immediately prior to their methadone dose as indicated by administration of:
  - Subjective Opioid Withdrawal Scale (SOWS),
  - Objective Opioid Withdrawal Scale (OOWS) and a
  - Visual Analogue Scale (VAS) of severity of opioid withdrawal.
- Body Mass Index between 20-30 kg/m².

5.14.4 Exclusion criteria
Subjects who satisfied the following criteria were not enrolled in the study:

- Considered unwilling, unable or unlikely to comply with the study protocol.
- Be dependent on alcohol, benzodiazepines or other drugs of abuse (except opioids related to this study and tobacco).
- Alcohol consumption exceeding NHMRC guidelines (an average of more than 4 standard drinks per day and not more than 28 per week in males).
- Any current, known medical condition, especially heart disease, hypertension, peptic ulcers, any other gastrointestinal disorder, psychiatric disorders, asthma, any other lung disease, any neurological disorder, abnormalities of the blood-forming organs.
- Have existing conditions that would affect sensitivity to cold (such as atherosclerosis, Raynaud’s disease, urticaria, hypothyroidism).
- Taking any prescribed or over-the-counter medications, except methadone.
- Had taken any analgesics in the 3 days prior to testing (‘analgesics’ do not include methadone taken for the treatment of dependence).
- Suffer or have suffered chronic pain.
- Have a known sensitivity to naloxone.
- Have a urine drug screen detecting opiates (not including methadone), cocaine and/or amphetamines.
- Be a university student who is being or will be assessed by the investigators.
- Participation in another clinical trial within the previous three months.

5.14.5 Withdrawal criteria

The following withdrawal criteria applied to this study:
- Subjects could withdraw at any time for any reason without having to divulge their reason to the investigators or clinical staff.
- Non co-operation with the study staff and/or non-compliance with the study protocol.
- Unacceptable adverse events.
- Development of a disease or illness that requires drug therapy.

5.14.6 Testing protocol

Subjects accepted into the study were tested on a single occasion within 21 days of their pre-study screening.

At approximately 0800 on the morning of the testing session subjects attended the clinical testing rooms of the Department of Clinical Pharmacology at the Royal Adelaide Hospital. A urine sample was taken for an illicit drugs screen.
Blood sampling, withdrawal assessment and nociceptive testing occurred prior to the saline infusion (familiarisation), 20 minutes after commencement of each infusion (ie 20 minutes into each 30 minute infusion), and hourly during the 4-hour wash-out period. Thus, subjects underwent nociceptive testing 11 times during the testing day.

5.14.7 Dosing protocol

Testing commenced approximately 20 hours after the subject’s previous methadone dose.

The testing session commenced with a 30 minute saline IV infusion at approximately 0830. Subsequently, naloxone was given at 0.05, 0.1, 0.5, 1.0 and 5.0 µg/min doses, as IV infusions at a rate of 1 mL/min each for 30 minutes. Doses were tested in ascending order to minimise risk of precipitated withdrawal. A total of 199.5 µg of naloxone was given throughout the testing day.

At the conclusion of the last infusion, subjects were administered their usual daily dose of methadone, and observed for a further four (4) hours.

5.14.8 Blood sampling

Subsequent to the urine sample collection, a 18 gauge indwelling venous catheter (Insyte™, Becton Dickinson, Sandy, UT, USA) plus bung (Baxter, IL, USA) and secured with Opsite (Smith & Nephew Medical, Hull, UK) was inserted into the best available forearm vein of both arms. The catheter on the non-dominant arm was used for taking blood samples, while the dominant arm was used for naloxone infusions (see below). The catheter used for blood sampling was flushed with 2 mL of normal saline following each blood sampling. Twelve (12) mL of blood was collected at each sampling time; the first two (2) mL were discarded and ten (10) mL separated and the plasma kept at -18 °C until analysis. Blood sampling times were immediately prior to withdrawal monitoring and nociceptive testing.

5.14.9 Withdrawal measures

Withdrawal, the most likely adverse outcome, was assessed prior to nociceptive testing and continuously during the study day. Withdrawal symptoms were measured every 30 minutes using the:

- Subjective Opioid Withdrawal Scale (SOWS),
- Objective Opioid Withdrawal Scale (OOWS) and a
Chapter 5 - Ultra-low dose naloxone in methadone maintained patients

- Visual Analogue Scale (VAS) of severity of opioid withdrawal.
In addition, subjects were under constant observation for withdrawal symptoms.

5.14.10 Physiologic and adverse event measures

Prior to the cold pressor test, vital signs were tested and adverse events monitored.

5.14.11 Nociceptive testing

The cold pressor test was used to measure changes in nociception, the primary end-point.

5.14.12 Reimbursement

Subjects were reimbursed AUD200 for their time and inconvenience. Subjects who completed
the screening process but did not satisfy the criteria for enrolment were renumerated AUD20.
Subjects were reimbursed parking expenses (screening only) or given taxi vouchers for travel
to and from the hospital on the testing day.

5.14.13 Testing follow-up

Subjects were phoned 24 hours after testing to monitor any adverse events that may have
occurred subsequent to leaving the Royal Adelaide Hospital.

5.15 Statistical analysis

No statistical analyses were performed on the data collected from this pilot study. The
purpose of this pilot study was to investigate the nociceptive and safety properties of low-
doses of naloxone in methadone maintained subjects, and if dose adjustment would be
required for the main study.
5.16 Results

5.16.1 Subject Characteristics

The pilot study was conducted with two male subjects with their characteristics described below.

Table 5.1 Demographic details.

<table>
<thead>
<tr>
<th>Subject Code</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Methadone dose (mg QD)</th>
<th>Time on current dose (months)</th>
<th>Time on methadone (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLX(IV)MMP03</td>
<td>30</td>
<td>60</td>
<td>50</td>
<td>4</td>
<td>58</td>
</tr>
<tr>
<td>NLX(IV)MMP04</td>
<td>35</td>
<td>66</td>
<td>72</td>
<td>6</td>
<td>46</td>
</tr>
</tbody>
</table>
5.16.2 Opioid withdrawal effects

Figure 5.3 Opioid-withdrawal effects.
Data for the Objective Opioid Withdrawal Scale (OOWS – Graph A), Subjective Opiate Withdrawal Scale (SOWS – Graph B) and Opioid Withdrawal Severity Visual Analogue Scale (VAS – Graph C) for subject NLX(IV)MMP03 (■) and subject NLX(IV)MMP04 (▲) at each time point: familiarisation (F), baseline (saline) (B(S)), at the end of each naloxone infusion, and hourly following the cessation of the naloxone infusions.
5.16.3 Physiological effects

Figure 5.4 Physiological measures.
Data for respiration rate (Graph A), blood pressure (Graph B: closed-systolic / open-diastolic), heart rate (Graph C) and arterial oxygen saturation (Graph D) for subject NLX(IV)MMP03 (●) and subject NLX(IV)MMP04 (▲) at each time point: familiarisation (F), baseline (saline) (B(S)), at the end of each naloxone infusion, and hourly following the cessation of the naloxone infusions.
5.16.4 Nociceptive effects

Figure 5.5 Cold pressor (CP) test.
Data for threshold (Graph A), tolerance (Graph B) and decay (Graph C) for subject NLX(IV)MMP03 (■) and subject NLX(IV)MMP04 (▲) at each time point: familiarisation (F), baseline (saline) (B(S)), at the end of each naloxone infusion, and hourly following the cessation of the naloxone infusions.
5.16.5 Adverse events and other drug effects

Neither subject was observed to be sedated at any time point. However, subject NLX(IV)MMP04 did sleep for the hour following the infusion cessation.

The only report of nausea was by subject NLX (IV)MMP03 during the 5.0 mcg/min naloxone infusion who stated it was slight in nature; the feeling disappeared following cessation of the naloxone infusion and administration of the subject’s normal daily methadone dose.

No other reports of adverse effects occurred during testing or up to the 24 hour follow up phone call.
5.17 Discussion

The primary outcome measure for this study, as with other studies presented in this thesis, was the cold pressor tolerance time. The maximal change in cold pressor tolerance time from baseline (saline infusion) values was 22% and -36% for subject NLX(IV)MMP03 and NLX(IV)MMP04 during the naloxone infusion, respectively. Maximal increase, from baseline (saline) values, in cold pressor tolerance values of 26% and 15% occurred 3 and 4 hours following methadone dosing for subject NLX(IV)MMP03 and NLX(IV)MMP04, respectively. This indicates minimal antinociceptive effects afforded by the methadone dose in these two subjects.

The highest dose of naloxone (5.0 µg/min for 30 minutes) caused moderate withdrawal in both subjects. This included peak OOWS scores (maximum score 13) of 8 and 3 and peak SOWS scores (maximum score 64) of 16 and 18 for both patients, respectively. Complete resolution of withdrawal symptoms occurred within 60-90 minutes of discontinuation of the naloxone infusion. Furthermore, there were no clinically significant changes in physiological parameters either during the naloxone infusion or following methadone dosing. It is noteworthy that during the 1.0 µg/min naloxone infusion, respiratory rate decreased to 8 breaths/minute in subject NLX (IV)MMP03. This resolved by the end of the 5.0 µg/min naloxone infusion rate and then dropped again to the same level 3 to 4 hours following methadone dosing.

Other authors have suggested that bolus doses of naloxone of between 0.15 and 0.2 mg could be distinguished from saline in methadone maintained patients (Kanof et al., 1992; Lamas et al., 1994). The total naloxone dose in the present study was just under 0.2 mg administered over a 2 ½ hour period. Review of data pertaining to the use of opioid antagonists in opioid overdoses suggests that a bolus dose titrated to reverse respiratory depression, but not induce opioid withdrawal, and then an infusion rate given at a rate of two-thirds of the initial bolus per hour would be required for overdoses involving long acting opioid agonists (Goldfrank et al., 1986; Clarke and Dargan, 2002). Given this, the final infusion rate of 5.0 µg/min for 30 minutes (equivalent to 0.3 mg/hr), would have been greater than a two-thirds bolus dose required to induce opioid withdrawal in methadone maintained patients (Kanof et al., 1992; Lamas et al., 1994). Nonetheless, the combination of a prolonged infusion of antagonist and
the fact that the subjects were at trough plasma methadone concentrations may have facilitated the mild precipitated withdrawal in these subjects.

5.18 Summary

The higher doses of naloxone, especially the 5.0 µg/min naloxone dose caused mild withdrawal and no significant changes in cold pressor tolerance time in two methadone maintained subjects. It was therefore decided to use the same protocol but with lower naloxone doses in the next 6 subjects.
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5.19 Principal study

5.19.1 Study design

The objective of this study was identical to the pilot study.

5.19.2 Sample size and power analysis

The primary outcome measure used in this study was the cold pressor tolerance time. A clinically significant change in antinociception (cold pressor tolerance time) as measured by this method was considered to be 40% as determined by previous studies (Doverty et al., 2001a; La Vincente et al., 2003; La Vincente, 2005; Athanasos et al., 2006). An a priori power analysis indicated that a minimum of 6 subjects was required to provide approximately 80% power of detecting a cold pressor tolerance increase of 40% with a significance level of 0.05.

5.20 Methods

An overview of the testing schedule for this study is provided in Appendix 1.

5.20.1 Ethics

Ethical approval for the naloxone dosage decrease was given by the Research Ethics Committee of the Royal Adelaide Hospital (Protocol Number 040210A). Six subjects were recruited for the study under this amendment.

5.20.2 Procedures and measures

Assessment and nociceptive testing were the same as previously described.

5.20.3 Dosing protocol

The testing session commenced with a 30 minute saline IV infusion given at a rate of 1 mL/min. Subsequently, naloxone was given at 0.005, 0.01, 0.05, 0.1 and 0.5 µg/min doses as IV infusions at a rate of 1 mL/min each for 30 minutes. Doses were tested in ascending order to minimise risk of precipitated withdrawal. Given this dosing protocol, a total of 19.5 µg of naloxone was given throughout the testing day.

At the conclusion of the last infusion, subjects were administered their usual daily dose of methadone and observed for a further four (4) hours.
5.20.4 Testing follow-up

Subjects were phoned 24 hours after testing to monitor any adverse events that may have occurred subsequent to leaving the Royal Adelaide Hospital.

5.21 Statistical analysis

Statistical analyses were performed using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA, USA). Within-group comparisons were made during and following infusion data (both including baseline (saline)) using one-way, repeated measures ANOVAs and appropriate post-hoc tests with statistical significance defined as \( P < 0.05 \). Data are presented as mean ± SEM (with 95% confidence intervals (95% CI)).
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5.22 Results

5.22.1 Subject characteristics

The six male subjects’ characteristics are described in Table 5.2.

Table 5.2 Demographic details.
<p>| Data presented as mean ± SEM (Range); n=6. |
|---------------------------------------------|-----------------|-------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Methadone dose (mg QD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 ± 3 (24-43)</td>
<td>76 ± 4 (64-94)</td>
<td>68.3 ± 5.4 (50-80)</td>
</tr>
</tbody>
</table>

5.22.2 Subject withdrawal and missing data

Ten subjects were screened for the principal trial. Of these subjects, one subject was not contactable following screening, one was not recruited due to prescription medication use, one not recruited due to poor venous access and one due to poor physical health. All the remaining subjects completed the testing session with no significant protocol deviations.

5.22.3 Familiarisation and baseline measurements

Subjects underwent subjective, physiological and nociceptive testing just prior to the saline infusion so that they were familiar with the testing procedures. There were no statistically significant differences between the familiarisation time-point and the saline-infusion time-point for any of the variables (Table 5.3). Baseline is classified as the value measured during the saline infusion.

Table 5.3 Familiarisation (pre-saline) vs baseline (saline) differences.
| Data presented as mean±SEM. |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|
| Variable | Familiarisation (Pre-saline) | Baseline (Saline) | T-test (P) |
|-----------------|-------------|-----------------|-------------|-----------------|
| OOWS (score) | 1 ± 0 | 1 ± 0 | Same (No) Difference |
| SOWS (score) | 3 ± 2 | 8 ± 5 | 0.16 |
| Withdrawal Severity VAS (mm) | 8 ± 4 | 10 ± 5 | 0.11 |
| Respiration Rate (bpm) | 13 ± 0 | 13 ± 1 | 1.00 |
| Systolic BP (mmHg) | 135 ± 10 | 126 ± 7 | 0.23 |
| Diastolic BP (mmHg) | 80 ± 5 | 74 ± 6 | 0.34 |
| Heart Rate (bpm) | 82 ± 4 | 76 ± 6 | 0.21 |
| SpO2 (%) | 98 ± 0 | 99 ± 1 | 0.47 |
| CP Threshold (sec) | 15.4 ± 5.6 | 15.0 ± 8.9 | 0.91 |
| CP Tolerance (sec) | 27.3 ± 9.2 | 27.3 ± 11.4 | 0.99 |
| CP Decay (sec) | 24.1 ± 5.1 | 18.3 ± 4.2 | < 0.05 |
Results for opioid-withdrawal measures are shown in Figure 5.6. There were no statistically significant differences in mean values either during or after the naloxone infusions for the OOWS (During: $F_{5,5}=1.0$, $P=0.43$; After: $F_{4,5}=1.0$, $P=0.43$) or SOWS (During: $F_{5,5}=1.9$, $P=0.13$; After: $F_{4,5}=2.3$, $P=0.09$).
Subjects’ opioid-withdrawal VAS mean score was significantly higher at the end of the 0.05 µg/min naloxone infusion compared with baseline ($F_{5,5}=3.7$, $P=0.01$; Dunnett’s Post hoc test: $P<0.01$; 95%CI -36.4, -5.6); there were no statistically significant differences in mean opioid-withdrawal scores after the naloxone infusions ($F_{4,5}=1.2$, $P=0.20$).
5.22.5 Physiological measures

![Graphs A, B, C, D showing changes in respiratory rate, blood pressure, heart rate, and oxygen saturation over time following naloxone infusion cessation.]

**Figure 5.7 Physiological measures.**
Data for respiration rate (Graph A), blood pressure (Graph B: closed-systolic / open-diastolic), heart rate (Graph C) and arterial oxygen saturation (Graph D) at baseline (saline) (B(S)), during and following cessation of naloxone infusion in 6 male methadone maintained subjects.
Results for physiological measures are shown in Figure 5.7. There were no statistically significant differences in mean values either during or after the naloxone infusions for systolic blood pressure (During: $F_{5,5}=2.0$, $P=0.12$; After: $F_{4,5}=1.3$, $P=0.32$), diastolic blood pressure (During: $F_{5,5}=0.7$, $P=0.63$; After: $F_{4,5}=2.2$, $P=0.12$), heart rate (During: $F_{5,5}=0.027$, $P=1.0$; After: $F_{4,5}=1.1$, $P=0.43$) or arterial oxygen saturation (During: $F_{5,5}=0.14$, $P=0.98$; After: $F_{4,5}=1.4$, $P=0.32$).

Subjects’ mean respiratory rate was significantly lower 3 hours following the cessation of the naloxone infusion compared with baseline ($F_{4,5}=2.9$, $P=0.048$. Dunnett’s Post hoc test: $P<0.05$; 95%CI 0.26, 3.1); there were no statistically significant differences in mean respiratory rate during the naloxone infusions ($F_{4,5}=1.1$, $P=0.39$).
5.22.6 Nociception

Figure 5.8 Cold pressor (CP) test.
Data for threshold (Graph A), tolerance (Graph B) and decay (Graph C) at baseline (saline) (B(S)), during and following cessation of naloxone infusion in 6 male methadone maintained subjects.
Results for the cold pressor test are shown in Figure 5.8. There were no statistically significant differences in mean values either during or after the naloxone infusions for threshold time (During: $F_{5,5}=0.83$, $P=0.54$; After: $F_{4,5}=0.94$, $P=0.46$), tolerance time (During: $F_{5,5}=1.3$, $P=0.29$; After: $F_{4,5}=2.6$, $P=0.07$) and decay time (During: $F_{5,5}=1.2$, $P=0.33$; After: $F_{4,5}=1.6$, $P=0.21$).

5.22.7 Adverse events and other drug effects

Nausea, of slight intensity, was experienced by two subjects; one subject reported nausea during the final two infusions while the other subject only experienced slight nausea during the final naloxone infusion. The nausea experienced resolved within the following hour.

No sedation was experienced by any of the subjects. No other adverse events were observed or reported by the subjects, either during or within the 24 hours following the subjects’ time in the clinical testing rooms.
5.23 Discussion

The aim of this study was to determine whether there was an ultra-low dose of naloxone that would reduce hyperalgesia in methadone maintained patients. Results indicated that doses of IV naloxone (0.005-0.05 µg/min for 30 minutes) administered to methadone maintained subjects did not significantly change nociceptive thresholds nor induce opioid withdrawal when compared with baseline measurements.

There were no statistically different changes between familiarisation and baseline values for all variables except for cold pressor decay time (Table 5.3). This suggests relative stability and minimal effect of the infusion of saline on all measurements. The significant decrease in pain decay time between familiarisation and baseline is difficult to interpret. It could be due to the large variability of this measure and its lack of inclusion in many studies that use the cold pressor test (Jones et al., 1988; Vinik and Kissin, 1998; Doverty et al., 2001a; Doverty et al., 2001b; La Vincente, 2005; Athanasos et al., 2006). Importantly, the primary outcome measure of mean cold pressor tolerance time remained relatively constant between these two time points. Previous studies using the cold pressor test in methadone maintained patients have shown that values for cold pressor variables do not change significantly between familiarisation and following a saline infusion (personal communication, P. Athanasos).

The infusion of naloxone in this cohort of male methadone maintained patients did not precipitate opioid-withdrawal. During the infusion, neither the objective nor subjective opioid withdrawal scale scores indicated any significant changes in score. On the other hand, the opioid-withdrawal severity VAS indicated that at the end of the final naloxone infusion there was a statistically significant increase in opioid withdrawal severity. However, the magnitude of this increase was relatively small and suggests only slight opioid withdrawal at this dose. This may indicate that the highest naloxone dose was the lowest dose required to precipitate opioid-withdrawal. Conversely, at this time point most subjects were approximately 24 hours following their preceding methadone dose. While subjects were tested for opioid withdrawal using these measures just prior to methadone dosing, none of the subjects indicated opioid withdrawal during screening. This suggests that the upper dose of naloxone used in this study was appropriate and higher doses would probably have caused precipitated opioid-withdrawal. As opioid withdrawal is associated with increased pain sensitivity (Compton et al., 2003), any higher doses of naloxone would have contravened the aims of the study.
The primary outcome measure was the cold pressor tolerance time. Baseline values of cold pressor tolerance time, during saline infusion suggest that subjects were hyperalgesic and compare well with previously published data (Doverty et al., 2001b; Athanasos et al., 2006; Mitchell et al., 2006). The design of this study meant that the protocol could not be tested in an opioid naïve population; therefore, this conclusion can only be tentative. There were no statistically significant changes in cold pressor tolerance times when compared with baseline during naloxone infusion in these subjects. The possible reasons are discussed below. Moreover, there was no significant change in cold pressor tolerance time, compared with baseline, during the period following cessation of the naloxone infusion and administration of the subjects’ methadone doses. This is somewhat surprising, given that previous studies firstly suggest that methadone maintained patients are not completely tolerant to their maintenance opioid and methadone still produces analgesia in this population (Schall et al., 1996; Dyer et al., 1999). Secondly, the analgesic potential of opioid agonists has been shown to be increased following opioid antagonist administration. Again due to limitations within the study, this was not observed in the current study and is discussed in greater detail below.

There were no significant changes in any of the physiological assessments. Neither were there any significant changes in the other nociceptive parameters measured, either during or after the naloxone infusion. This indicates that that the doses of naloxone administered have minimal, if any, impact on physiological or nociceptive systems in male methadone maintained subjects.

There were several limitations to this study. Power calculations performed prior to the study suggested that the number of subjects was sufficient to detect a 40% change in cold pressor tolerance times. However, the inter-individual variability in cold pressor tolerance times at baseline were larger than the data that the power calculation were based on. Consequently, there is the chance that there was a type II error involved. Due to this initial large variability in cold pressor tolerance times, the power of the study to detect any statistically significant differences was reduced. This may be rectified in future studies by increasing the number of subjects. However, other design limitations may have been behind this lack of change in cold pressor tolerance times.
Firstly, the lack of change in cold pressor tolerance time during naloxone infusion may be a result of the combination of the opioid receptor antagonist and agonist. This is the first study to investigate the combination of ultra-low doses of intravenously administered naloxone and orally administered methadone in a human population. The only previously reported study investigating opioid antagonist in combination with methadone was a case study by Cruciani (2003), who investigated the addition of oral naltrexone to the methadone regimen of a patient with diabetic neuropathy. While the case report by Cruciani (2003) indicated that naltrexone might be able to improve the analgesic effectiveness of methadone, this result may have been due to the combination of giving both oral drugs concurrently. Similarly, oral dosing of ultra-low dose of naltrexone and oxycodone was found to lower pain intensities in chronic pain patients administered the combination twice daily (Chindalore et al., 2005). Previous acute dosing studies have focused on the combination of morphine and naloxone given intravenously and have found some advantageous effects from this combination (Gan et al., 1997; Cepeda et al., 2004) while other studies have reported limited benefit (Cepeda et al., 2002; Greenwald et al., 2005; Bijur et al., 2006). The pharmacology of naloxone precludes it from being given orally not only due to its low and variable bioavailability but also due to its potential to cause opioid withdrawal in opioid-tolerant individuals (Friedman and Dello Buono, 2001; McNicol et al., 2003).

Secondly, the importance of opioid antagonist dose ratio has been demonstrated by several studies. The studies by Gear and colleagues (2003) suggested that a dose ratio of 12.5:1 of nalorphine and naloxone was superior in producing analgesia over a ratio of 6.25:1. The importance of dose ratio is further highlighted by the studies presented in the doctoral thesis of La Vincente (2005), who suggest iv buprenorphine:naloxone given in a ratio of 12.5:1 to healthy subjects was efficacious at potentiating the antinociceptive effects of buprenorphine, without enhancing the occurrence of adverse effects. Yet if the 12.5:1 opioid agonist to antagonist ratio were to be used in the current study, based on the average methadone dose of the subjects (68.3 mg), this would equate to a naloxone dose of 8.5 mg. A naloxone dose of this magnitude would definitely precipitate opioid withdrawal in methadone maintained subjects based, not only on the results of this study, but others as well (Kanof et al., 1992; Lamas et al., 1994). Other variables must be taken into consideration with respect to factors involving dose ratio. Acute dosing studies that have indicated enhanced analgesic effect and/or reduction in adverse side effects have generally utilised opioid agonist doses that were either sub-analgesic or titrated to be analgesic. The doses of methadone in the current study
were considered relatively high, mainly due to the tolerance these subjects display. Consequently, this may have an impact on whether or not opioid antagonists may be able to enhance antinociception in maintained subjects. It may be that the beneficial effects of ultra-low doses of opioid antagonists may not be possible when high doses of opioid agonist are co-administered such as with opioid maintained subjects.

Thirdly, the results of this study may also be a consequence of the time of exposure to the opioid antagonist. Longer exposure times to the antagonist may be required for intrinsic mechanisms to adjust and consequent antinociceptive effects to be observed; and given the dosing protocol, steady-state plasma naloxone concentration were probably not achieved. Clinical studies suggest that opioid antagonists administered in ultra-low doses in combination with an opioid agonist may require some period of time before an effect is observed. The opioid agonist dose-sparing effects (Gan et al., 1997) or decreases in nausea and pruritus (Joshi et al., 1999; Cepeda et al., 2004) were only observable at the end of an extended observation period such as 24 hours. Chindalore and colleagues (2005) reported effects observed three weeks following the beginning of medication titration.

The dosing regimen of naloxone in this study was limited to half an hour for each dose. If steady state pharmacokinetics were taken into account, then this would have not been enough time for the naloxone to achieve steady state plasma concentrations within the body. Therefore, as naloxone has an approximate half life of one hour, a minimal infusion time of 5 hours of one infusion rate would have been required to achieve steady state. Therefore this dosing protocol suggests that the plasma concentrations of naloxone were in a constant state of flux. When any drug is given as a continuous IV infusion the steady state concentration will be determined by only two factors: clearance and the infusion rate (Birkett, 2002). Alternatively, a dosing protocol using a bolus (loading dose) and infusion rate could have been employed such that steady state could be implied. This requirement of relatively ‘continuous’ blood opioid antagonist concentrations may be a reason some researchers have noted significant benefits in treatment outcomes (Gan et al., 1997; Joshi et al., 1999) while others have not (Mehlisch, 2003).

Lastly, as this was a proof-of-concept study, the design lacked the inclusion of a placebo control treatment arm. This study would have benefited from inclusion of a saline placebo. Nevertheless, previous studies have shown that conditioning due to repetitive cold pressor
testing does not result in significant changes in pain tolerance, either in healthy subjects (Jones et al., 1988) or methadone-maintained subjects (Athanasos et al., 2006), using a similar protocol as the one used in this study.

The aims and hypotheses of this study may be better tested using other paradigms. While other studies have indicated that combining ultra-low dose opioid antagonist and opioid agonist may increase the duration and increase the analgesic effect of morphine (Joshi et al., 1999), other studies indicated that such combinations may improve the side effect profile of morphine. Longer term administration of naloxone or the administration of longer acting opioid antagonists, such as naltrexone or nalmefene in appropriate doses may improve the side effect profile of methadone in addition to potentiating its analgesic effects as well as preventing or reversing the hyperalgesia that is associated with its administration. This has major implications, not only for methadone maintenance, but also pain management using methadone. This may mean that lower dose of opioid agonist may be required to achieve the same analgesic effects and as a result may precipitate fewer side effects and therefore make treatment with methadone more appropriate for patients.

5.24 Summary

This study aimed to investigate if the administration of IV, ultra-low dose naloxone would increase the antinociceptive properties of methadone in methadone maintained subjects. Using the dosing regimen employed in the study above, naloxone administered in ultra-low doses to methadone maintained subjects had no effect on any parameter measurement.
6 REMIFENTANIL IN METHADONE MAINTAINED SUBJECTS

6.1 Prologue

Research has shown, in methadone maintained patients, that morphine is inefficacious as an antinociceptive, even when plasma concentrations are achieved that are well beyond those used in normal clinical practice in opioid-naïve individuals (Athanasos et al., 2002). This research showed that IV morphine doses of 45.2 mg (over 2 hours) did not alter experimental pain tolerance scores in methadone maintained patients. However, when compared with morphine, methadone has a relatively high affinity for and intrinsic efficacy at the μ-opioid receptor (Codd et al., 1995; Kristensen et al., 1995; Selley et al., 1998; Borgland et al., 2003). An alternative strategy is therefore to use a μ-opioid receptor agonist that has both high potency as well as high intrinsic opioid agonist activity at least as great as that of methadone. The fentanyl analogues, such as remifentanil, generally satisfy this criterion (Adams et al., 1990; Stevens et al., 1994a; Barrett et al., 2003).

6.1.1 Remifentanil pharmacology

Remifentanil (systematic name: 1-piperidinepropanoic acid, 4-(methoxy-carbonyl)-4-((1-oxopropyl)phenylamino)-, methyl ester) or GI 87084B (Figure 6.1) is a synthetic drug purposely designed as a potent, short acting, μ-opioid agonist (Feldman et al., 1991) and has a number of characteristics that make it useful as an anaesthetic, a nalgesic and/or research drug.

Remifentanil is a 4-anilidopiperidene with properties similar to others within this group (eg fentanyl, alfentanil and sufentanil). However, in contrast to these related compounds which depend on hepatic biotransformation and renal excretion for elimination, the structural formula of remifentanil shows that it contains a methyl ester group that makes it susceptible to rapid metabolism by non-specific esterases within the blood and tissues (see Figure 6.1) (Feldman et al., 1991). Furthermore, the pKa of remifentanil (7.26) allows it to circulate within the body in a greater proportion in the non-ionised form and quickly penetrate the lipid blood-brain barrier (Beers and Camporesi, 2004). As a consequence, it has a rapid blood-brain equilibrium half-time (t_{1/2} k_{e0}) of 1±1 minutes (Egan et al., 1993; Glass et al., 1993) resulting in a rapid onset of action. With regards to offset, remifentanil has a very short terminal half-life of 10-20 minutes (Glass et al., 1993; Westmoreland et al., 1993) and the time for a 50%
reduction in blood concentration after discontinuation of an infusion (context sensitive half time) is 3-6 minutes (Egan et al., 1993; Glass et al., 1993; Westmoreland et al., 1993). These properties mean that the dose of remifentanil is very easy to titrate to effect and it can be administered at high doses and for long periods as there is minimal accumulation.

The principal metabolite of remifentanil, GR90291, is primarily eliminated by renal pathways and has a potency 4600 less than its parent compound for producing delta EEG activity in dogs (Hoke et al., 1997) due to its poor binding affinity for the μ-opioid receptor. A negligible component of remifentanil metabolism is also by N-dealkylation (Egan, 1995).

Remifentanil selectively binds to the μ-opioid receptor, with little affinity for the δ- or κ-opioid receptors (James et al., 1991). It lacks activity at the M2 muscarinic receptors, α1 adrenoceptors and has no effect on catecholamine uptake (James et al., 1991). The relative binding affinity for the μ-opioid receptor, as indicated by drug inhibition constants obtained for [3H]-DAMGO binding report that remifentanil (Ki=21 nM) has a similar affinity as morphine (Ki=17 nM) (Poinsel et al., 2006) and agrees with the research of Cox and colleagues (1999). However, when receptor affinity is measured by the agonist dissociation constant (Ka) determined by β-chlornaltrexamine in guinea pig ilium of remifentanil is 220 nM (James et al., 1991); when determined using a congruent model, morphine has a Ka of 1440 nM (Porreca et al., 1990), indicating that remifentanil may have a greater affinity for the μ-opioid receptor.

Compared with other μ-opioid receptor agonists, the analgesic potency (compared with morphine) is: sufentanil (1000X) > remifentanil (300X) > fentanyl (100X) > alfentanil (15X) > morphine (1X) = methadone (1X) (Martin, 1983; Clotz and Nahata, 1991; Glass et al., 1993). The combination of high affinity for the μ-opioid receptor and the greater potency at this receptor would suggest that remifentanil may be able to overcome μ-opioid receptor binding by methadone, resulting in analgesia in methadone maintained patients.

6.1.2 Clinical studies
Many researchers have investigated the use of remifentanil as an adjuvant in anaesthesia and therefore address aims that are beyond the scope of this thesis. Yet, these studies provide valuable information regarding the discontinuation of remifentanil and in particular demonstrating that special attention needs to be made to remifentanil cessation and dealing
Chapter 6 – Remifentanil in methadone maintained subjects

with potential underlying pain, opioid-withdrawal and short-term opioid tolerance (Cortinez et al., 2001; Hansen et al., 2005; Joly et al., 2005). Furthermore, evidence exists suggesting that intra-operative remifentanil may enhance post-operative pain scores (Hansen et al., 2005). However, there are limited studies investigating the use of remifentanil at the lower dose range commonly used for postoperative analgesia. The following studies highlight the use of remifentanil as an analgesic agent for moderate to severe post-operative pain.

Bowdle and colleagues (1996) examined the use of remifentanil as a post-operative analgesic following abdominal, spine, joint replacement or thoracic surgery in 157 patients. Following a 30 minute titration period of remifentanil, they found that adequate analgesia was achieved in 78% of patients with IV infusions of between 0.05 µg/kg/min and 0.15 µg/kg/min. The upper dosing limit in this study was 0.5 µg/kg/min and as a result ‘morphine rescue’ was required by 8% of patients who still had inadequate pain control at this dose. The results of this study were confounded by variations in the infusion rate as well as allowing supplementary boluses of remifentanil. Of note in this study was that respiratory adverse events (SpO2 < 90% or respiratory frequency < 12/min) affected 29% of patients.

Schüttler and colleagues (1997) investigated the use of 0.05 µg/kg/min (n=83) and 0.1 µg/kg/min (n=33) remifentanil infusion as postoperative analgesic in patients following major abdominal surgery that utilised remifentanil-base anaesthesia. The study allowed remifentanil dose-titration, additional bolus dosing of remifentanil and morphine-based analgesia commenced 30 minutes after surgery. When morphine-based analgesia commenced, the mean ± SD infusion rate of remifentanil in the patient group given an initial remifentanil infusion rate of 0.05 µg/kg/min was 0.082 ± 0.045 µg/kg/min while the patient group given an initial remifentanil infusion rate of 0.01 µg/kg/min was 0.098 ± 0.060 µg/kg/min. The authors concluded that 0.05 µg/kg/min was inadequate for post-operative analgesia and that an initial infusion rate of 0.1 µg/kg/min was appropriate in this setting (Schüttler et al., 1997).

In a study of similar design, Yarmush and colleagues (1997) compared remifentanil- and morphine-based analgesia in the 35 minutes post-extubation following various inpatient surgery. For the remifentanil group (n=93), an initial infusion rate of 0.1 µg/kg/min was used whereas for the comparator group (n=98), 2 mg morphine was given every 5 minute as needed. At the end of the 35 minute titration period, the mean ± SD (range) dose of
remifentanil required for analgesia in these patients was $0.125 \pm 0.036$ (0.05 to 0.23) µg/kg/min.

Calderón and colleagues (2001) investigated constant-dose continuous infusions of remifentanil at rates of 0.05 and 0.1 µg/kg/min for post-operative severe pain following abdominal or thoracic surgery with 15 patients in each group. They reported that after 4 hours of the infusion, patients randomised to the 0.1 µg/kg/min dose of remifentanil were 4 times less likely to require rescue analgesia. In addition, adequate analgesia (simple verbal scale (max=4) 0-1, respiratory frequency > 8/min and $\text{SpO}_2 > 90\%$) was achieved in 75% and 78% of patients in the 0.05 µg/kg/min and 0.1 µg/kg/min groups respectively (Calderón et al., 2001).

Two studies have investigated the use of remifentanil as part of patient controlled analgesia (PCA) regimen for post-operative pain. Remifentanil was administered using a PCA regimen (loading dose: 45 µg; maintenance dose 1 µg/min; bolus dose 15 µg, lockout time: 10 minutes) and compared with a morphine PCA regimen (loading dose: 5 mg; maintenance dose: 0.3 mg/hr; bolus dose: 1 mg, lockout time: 15 minutes) for abdominal surgery (Kucukemre et al., 2005). Remifentanil provided comparable analgesia and side effect profile was similar to morphine. The total mean ± SD remifentanil dose for the first 24 hours post-operative was $2.3 \pm 0.2$ mg and equated to a rate of 0.022 µg/kg/min during the first 24 hours post-operatively (Kucukemre et al., 2005). Another study investigating remifentanil PCA, but utilising a regimen based on target plasma remifentanil concentrations, noted that the regimen was effective in providing effective analgesia for 30 orthopaedic-surgery patients within 30 minutes of surgery. Effective analgesia was maintained with a plasma remifentanil concentration of approximately 2 ng/mL, which resulted in a mean approximate cumulative remifentanil dose (over 6 hours) of approximately 2 mg, roughly equating to 0.079 µg/kg/min (Figure 3 in Schraag et al., 1998). However, it was noted that the analgesic dose requirements varied by $>200\%$ (Schraag et al., 1998). While evidence suggests that IV PCA opioid administration provides better analgesia, there is little evidence to suggest that there are any significant advantages over conventional (s.c. or i.m.) opioid analgesia in terms of opioid consumption or the incidence of adverse effects (Walder et al., 2001).

When used as the sole analgesic agent for painful medical procedures (not post-operative pain) in 31 patients the mean ± SD remifentanil dose required was $0.5 \pm 0.3$ µg/kg/min.
However, 10 subjects developed hypoxemia and a further 15 developed apnea (Litman, 2000). This suggests that remifentanil may not be appropriate in this setting.

The results of most of these studies suggest that effective analgesia for moderate to severe pain can be achieved with remifentanil administered intravenously at the rate between 0.1 and 0.5 µg/kg/min while adverse side effects, such respiratory depression need to be closely monitored. While not explicitly noted in any of the above studies, it is presumed that the patients were opioid-naïve or at least non-opioid tolerant, and therefore this may limit the applicability of these studies to the treatment of acute pain in opioid-tolerant methadone maintained patients.

6.1.3 Remifentanil in recreational opioid users

As mentioned previously there is a paucity of information regarding the analgesic doses of opioids required in opioid-dependent subjects. Baylon and colleagues (2000) investigated the abuse potential of remifentanil in opioid-users. The authors examined bolus doses of remifentanil up 3.0 µg/kg compared with fentanyl doses up to 4.5 µg/kg in 12 recreational opioid-using volunteers; the study utilised an escalating-dose design which limited advancement to the next dose if the subject’s haemoglobin SpO₂ dropped to 90% or lower. The authors concluded that both remifentanil and fentanyl may have some abuse potential in recreational users of opioids. This may be limited to individuals who have access to the drug and who prefer briefer, repeated effects. The study presented by Baylon and colleagues (2000) indicated that opioid-dependent subjects, not in treatment, were able to tolerate bolus doses of remifentanil up to 3 µg/kg.

6.2 Study rationale

The present proposal is designed to determine whether there is a dose of remifentanil that will produce analgesia in methadone maintained patients balanced against minimal adverse events. This may add to the scientific knowledge base in understanding the nature of opioid-induced hyperalgesia and provide opportunities to develop more rational and effective management of pain in methadone maintained patients.
Chapter 6 – Remifentanil in methadone maintained subjects

6.3 Pilot study

6.4 Aim
To determine the dose of remifentanil that is effective in reducing the hyperalgesia of methadone maintained patients, without causing adverse effects.

6.5 Hypothesis
It is hypothesised that a dose of remifentanil can be found that will reduce hyperalgesia in methadone maintained patients without causing adverse effects.

6.6 Study design
Due to the lack of data on the antinociceptive activity of remifentanil in methadone maintained subjects using the cold pressor test, an initial pilot study was completed. This study was an open-label, unblinded, cumulative- and escalating-dose study initially completed in two subjects. Subjects attended for one study day with one subject tested per day.

6.7 Methods
An overview of the testing schedule for this study is provided in Appendix 2.

6.7.1 Ethics
Ethical approval for this study was granted by the Research Ethics Committee of the Royal Adelaide Hospital (RAH Protocol 040828).

6.7.2 Subjects
Eligibility for the study was subject to several restrictions. A sufficient number of male volunteers were screened within 21 days of the administration of the study treatment such that 8 subjects (2 in the first instance) were enrolled into the study (see section 7.1). Each potential subject met the following inclusion and exclusion criteria in order to qualify for admission into the study.

6.7.3 Inclusion criteria
A subject was eligible for inclusion in this study only if all of the following criteria applied:
- Patients who had received oral methadone for at least three months for the treatment of opioid-dependence and whose dose had been stable and between 30 and 120 mg/day for one month at screening.
Agree to and be capable of signing an informed consent form.
Be a male aged between 18 and 65 (inclusive)*.
Have reasonable forearm venous access.
 Fluent in the English language.
Be in good physical health. This was determined by completion of a pre-study screening which comprised of:
  - Medical History including
    - a drug history identifying any known drug allergies and
    - any chronic use of medication
  - Physical Examination
  - Clinical Laboratory Tests for:
    - Haematology: Haemoglobin, red blood cell count (RBC), Packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), white cell count, neutrophils, lymphocytes, monocytes, eosinophils and basophils
    - Biochemistry: Sodium, potassium, chloride, bicarbonate, anion gap, glucose, urea, creatinine, creatinine clearance†, cholesterol, urate, phosphate, total calcium, ionised calcium, albumin, globulins, protein, total bilirubin, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LD).
    - Coagulation Test: International Normalised Ratio (INR)
    - Urine Test for Illicit Drugs: Cocaine, amphetamines, opiates, methadone, buprenorphine, benzodiazepines and cannabinoids (for drug monitoring and to help with interpretation of results).
  - Electrocardiogram (ECG).
  - Vital signs, taken after subject had been seated for 3 minutes:
    - Temperature (°C)
    - Respiratory rate (breaths/minute)
    - Radial pulse rate (beats/minute)

* Pharmacodynamic activity of remifentanil increases with increasing age – the EC₅₀ and kₑ₀ for the development of delta waves as measure by EEG was 50% less for both in the age range of 20 to 85 years (Minto et al., 1997)
† Calculated using Cockcroft and Gault formula (Cockcroft and Gault (1976)): CLᵦᵦ = (140 – age(yrs)) x (weight (kg)) / (SCR(mmol/L) x 814).
- Blood pressure (mmHg)
- Body Mass Index between 19-30 kg/m$^2$\(^{\text{†}}\).

### 6.7.4 Exclusion criteria

Subjects who satisfied any of the following criteria were not enrolled in the study:

- Considered unwilling, unable or unlikely to comply with the study protocol.
- Dependent on alcohol, benzodiazepines or other drugs of abuse (except opioids related to this study and tobacco).
- Alcohol consumption exceeding NHMRC guidelines for males (i.e. an average of more than 4 standard drinks (40 gm alcohol) per day and not more than 28 per week).
- Any evidence of organ dysfunction, or any deviation in clinical laboratory values which is confirmed on re-examination to be clinically significant (that is, in the opinion of the Medical Officer would jeopardise the safety if the subject or impact on the validity of the study results) including any of the following laboratory values:
  - Liver function test (LFT):
    - AST and ALT greater than 3 times the upper limit of normal (ULN)
    - Serum albumin less than 33g/L
  - INR greater than 1.2
  - Kidney:
    - Calculated creatinine clearance less than 75 mL/min
  - Bone marrow functions:
    - not clinically significant abnormal results
- Any current, clinically significant, known medical condition, not including opioid dependence, especially cardiovascular disease, hypertension, peptic ulcers, any other gastrointestinal disorder, psychiatric disorders, asthma, any other lung disease, any neurological disorder or abnormalities of the blood-forming organs.
- Have existing conditions that would affect sensitivity to cold (such as atherosclerosis, Raynaud’s disease, urticaria, hypothyroidism).
- Taken any prescribed, over-the-counter or complementary medications except methadone, for 14 days prior to dose administration and for the duration of the study.
- Suffer or have suffered chronic pain.

\(^{\text{†}}\) In obese patients (excluded from this study), ideal, rather than actual, body weight is more clinically useful (Egan et al., 1998).
• Have a known hypersensitivity to remifentanil or fentanyl-analogues, including fentanyl, alfentanil and sufentanil.
• Have a urine drug screen detecting opiates (not including methadone) on the testing day.
• Be a university student who is being or will be assessed by the investigators.
• Previous entry into this study or participation in any other investigational drug study within 3 months of enrolment.

6.7.5 Withdrawal criteria

The following withdrawal criteria applied to this study:

• Subjects may withdraw at any time for any reason without having to divulge their reason to the investigators or clinical staff.
• Non co-operation with the study staff and/or non-compliance with the study protocol.
• Unacceptable adverse events
• Development of a disease or illness that requires drug therapy

6.7.6 Testing protocol

Methadone maintained subjects were tested on a single occasion within 21 days of their pre-study screening.

At approximately 0800 on the morning of the testing session, subjects attended the clinical testing rooms of the Department of Clinical Pharmacology at the Royal Adelaide Hospital. A urine sample was taken for an illicit drugs screen. On-the-spot opioid testing was used using InstaCheck® MOR 300 Drug Screen Test (Aplied Biotch / Forefront, CA, USA). Following an opiate negative result, one 18 gauge indwelling venous catheter (Medex, CA, USA) plus bung (Baxter, IL, USA) was inserted into the subject’s dominant arm for drug administration and secured with Opsite (Smith & Nephew Medical, Hull, UK). Weight was measured and used to calculate the correct remifentanil infusion rate. Dosing commenced at approximately 0830.

Assessment and nociceptive testing (see below) occurred prior to the saline infusion (for familiarisation), 10 minutes after commencement of each infusion (ie 10 minutes into each 20 minute infusion), and hourly during the 4-hour wash-out period.
6.7.7 Subjective measures

Five (5) minutes prior to each cold pressor test, the Morphone-Benzodrine Group (MBG) Scale and the Subjective Opioid Withdrawal Scale (SOWS) were administered.

6.7.8 Physiologic and adverse event measures

Prior to the cold pressor test, vital signs were tested and adverse events monitored.

6.7.9 Nociceptive testing

The cold pressor test was used to measure changes in nociception.

6.7.10 Dosing protocol

Testing commenced approximately 20 hours after the subject’s previous methadone dose.

The testing session commenced with a 20 minute IV saline infusion given at a rate of 10 mL/hour. Subsequently, remifentanil was given at 0.10, 0.15, 0.20, 0.25, 0.30, 0.40 and 0.50 µg/kg/min doses as IV infusions. For safety reasons, doses were administered in ascending order.

Remifentanil was supplied as a 50 µg/mL solution. All infusions were administered by a Graseby Syringe Pump 3100 (SIMS Graseby Ltd., Watford, Herts, UK). The remifentanil was prepared to cover the testing day’s dose plus line priming. Dosage rates were adjusted by adjusting the infusion rate according to body weight, as measured to nearest whole kilogram on the morning of testing.

Dose rates were calculated using the following formula:

\[
\text{Infusion Rate (mL/hour)} = \frac{\text{Weight (kg)} \times \text{Infusion Rate (µg/kg/min)} \times 60 \text{ (min)}}{\text{Supplied Infusion Concentration (µg/mL)}}
\]

Each infusion rate lasted for 20 minutes and given this dosing protocol, a total of 38 µg/kg of remifentanil was given throughout the testing day.
Ten minutes after the conclusion of the last infusion, subjects were administered their usual daily dose of methadone, provided that respiration rate and sedation were at baseline levels. Subjects were observed for a further four (4) hours with testing occurring hourly.

### 6.7.11 Reimbursement

Subjects were reimbursed AUD200 for their time and inconvenience. Subjects who completed the screening process but did not satisfy the criteria for enrolment were renumerated AUD20. Subjects were reimbursed parking expenses (screening only) or given taxi vouchers for travel to and from the hospital on the testing day.

### 6.7.12 Testing follow-up

Subjects were phoned 24 hours after testing to monitor any adverse events that may have occurred subsequent to leaving the Royal Adelaide Hospital.

### 6.8 Statistical analysis

No statistical analyses were performed on the data collected from this pilot study. The purpose of this study was to investigate the nociceptive properties of standard analgesic doses of remifentanil in methadone maintained subjects, and if dose adjustment would be required for the main study.
6.9 Results

6.9.1 Subject characteristics

The pilot study was conducted with two male subjects with their characteristics described below in Table 6.1.

Table 6.1 Demographic details – pilot study.

<table>
<thead>
<tr>
<th>Subject Code</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Methadone dose (mg QD)</th>
<th>Time on current dose (months)</th>
<th>Time on methadone (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REM(IV)MMP01</td>
<td>34</td>
<td>76</td>
<td>75</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>REM(IV)MMP02</td>
<td>36</td>
<td>70</td>
<td>90</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

6.9.2 Subjective Effects

![Subjective effects graph](image)

Figure 6.2 Subjective effects.

Data of the Subjective Opiate Withdrawal Scale (SOWS – Graph A) and Morphine-Benzodrine Group Scale (MBG – Graph B) for subject REM(IV)MMP01 (■) and subject REM(IV)MMP02 (▲) at each time point: familiarisation (F), baseline (saline) (B(S)), at the end of each remifentanil infusion, and hourly following the cessation of the remifentanil infusions.
6.9.3 Physiological

Figure 6.3 Physiological measures.
Data for respiration rate (Graph A), blood pressure (Graph B: closed-systolic / open-diastolic), heart rate (Graph C) and arterial oxygen saturation (Graph D) for subject REM(IV)MMP01 (■) and subject REM(IV)MMP02 (▲) at each time point: familiarisation (F), baseline (saline) (B(S)), at the end of each remifentanil infusion, and hourly following the cessation of the remifentanil infusions.
6.9.4 Nociception

Figure 6.4 Cold pressor (CP) test. Data for threshold (Graph A), tolerance (Graph B) and decay (Graph C) for subject REM(IV)MMP01 (■) and subject REM(IV)MMP02 (▲) at each time point: familiarisation (F), baseline (saline) (B(S)), at the end of each remifentanil infusion, and hourly following the cessation of the remifentanil infusions.
6.9.5 Adverse events and other drug effects

Neither subject was observed to be sedated at any time point; however, subject REM(IV)MMP01 slept between the testing time points, up to 3 hours following taking his methadone dose.

The only report of nausea was by subject REM(IV)MMP02 during the saline infusion who stated it was slight in nature; the feeling disappeared by the end of the first remifentanil infusion.

The only other mentioned adverse effect was by subject REM(IV)MMP02 who stated as he took his methadone dose that he felt “some withdrawal equivalent to if he had waited for his dose”. This feeling resolved within 40 minutes and did not prevent him from eating lunch during this time.

Neither subject reported any adverse events from the time between leaving the hospital and 24 hours later.

6.10 Discussion

The primary outcome measure for this study, as with other studies presented in this thesis, was the cold pressor tolerance time. The maximal increase in cold pressor tolerance time was 14.8% and 9.3% for subject REM(IV)MMP01 and REM(IV)MMP02 at the end of the 0.4 µg/kg/min remifentanil infusion, respectively. No dose-response relationship could be observed between remifentanil doses and nociceptive tolerance. This result supports previous studies that indicate that opioid-tolerant subjects require higher doses of opioid additional to their primary opioid for additional pain relief (Athanasos et al., 2006).

Clinical studies have shown that remifentanil has clear analgesic properties when given to opioid non-tolerant patients at the rate of 0.1 µg/kg/min for postoperative pain (Schüttler et al., 1997; Yarmush et al., 1997; Calderón et al., 2001) and up to 0.5 µg/kg/min for painful medical procedures (Litman, 2000). From the preliminary data presented were, doses up to 0.5 µg/kg/min did not appear to produce analgesia in methadone maintained patients as indicated by minimal increases in cold pressor tolerance scores (Figure 6.4 B).
Following the administration of the subjects’ normal methadone dose, 10 minutes after cessation of the remifentanil infusion, slight hyperalgesia developed in one subject (REM(IV)MMP01) when measured by the cold pressor tolerance (Figure 6.4 B). This increase in pain tolerance time also corresponded to a decrease in pain threshold and increase in pain for the cold pressor pain to decay, all of which resolved to baseline levels by the end of the testing session. This suggests that this subject may have become more sensitive to pain following cessation of the remifentanil, possibly due sub-clinical opioid withdrawal. This observation is confounded by the administration of methadone and the consequent increase in plasma methadone concentrations. No significant changes were observed for the other subject with regard to pain sensitivity and pain tolerance following cessation of the remifentanil infusion.

Respiratory rate was not depressed significantly, with the lowest rate of 10 breaths/minute recorded in REM(IV)MMP02 during the remifentanil infusion. Furthermore, SpO₂ levels did not drop below 96% for either subject during any of the testing sessions. There were no clinically significant changes in blood pressure, with the sharp increase in subject REM(IV)MMP01’s diastolic blood pressure during the 0.1 µg·kg⁻¹·min⁻¹ remifentanil infusion considered to be a sampling error by the automated Agilent machine. Heart rate did not change significantly during the remifentanil infusion in either subject. However, during the four hours following methadone administration, REM(IV)MMP01’s heart rate decreased while REM(IV)MMP02’s increased; these changes were not considered clinically significant.

One subject indicated that he was feeling some mild opioid-withdrawal as indicated by the SOWS at the end of the saline infusion. This feeling was soon ameliorated by the remifentanil infusion. The same subject also indicated an increase in morphine-like effects (MBG) with the remifentanil infusion and these feelings continued throughout the testing day. These observations are consistent with the fact that remifentanil is a µ-opioid receptor agonist.

### 6.11 Conclusion

Remifentanil given at a rate of up to 0.5 µg/kg/minute to methadone maintained patients causes a minimal increase in pain tolerance, with minimal changes in respiratory measures. In conclusion, higher doses of remifentanil should be used to investigate the antinociceptive and adverse event profile of remifentanil in a methadone maintained patient population.
Chapter 6 – Remifentanil in methadone maintained subjects

6.12 Principal study

6.12.1 Study design

The objective of this study was identical to that of the pilot study. As with the pilot study, two subjects were initially recruited and tested to investigate safety and nociceptive aspects of the study. In addition, further subjective and physiological effects were measured for one hour following cessation of the remifentanil infusion.

6.12.2 Sample size and power analysis

The primary outcome measure used in this study was the cold pressor tolerance time. A clinically significant increase in antinociception as measured by this method was considered to be 40% as determined by previous studies (Chapter 5 results, Doverty et al., 2001a; Athanasos et al., 2006). An \textit{a priori} power analysis was performed indicating 8 subjects were required to provide approximately 80% power of detecting a cold pressor tolerance increase of 40% with a significance level of 0.05.

6.13 Methods

An overview of the testing schedule for this study is provided in Appendix 2.

6.13.1 Ethics

Ethical approval for the remifentanil dosage increase was given by the Research Ethics Committee of the Royal Adelaide Hospital (Protocol Number 040828A). Two subjects were recruited for the study under this amendment.

Ethical approval for the increased subjective measurements following remifentanil infusion cessation was given by the Research Ethics Committee of the Royal Adelaide Hospital (Protocol Number 040828B). Twelve subjects were recruited for the study under this amendment.

6.13.2 Procedures and measures

Assessment and nociceptive testing occurred were the same as previously described. In addition to this, the subjective and physiologic measures were taken at 15, 30 and 45 minutes following the subject’s methadone dose.
6.13.3 Dosing protocol

Similar to the pilot study, the testing session began with a 20 minute saline IV infusion given at a rate of 10 mL/hour. Subsequently, remifentanil was given at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 µg/kg/min doses as IV infusions. For safety reasons, doses were administered in ascending order.

Remifentanil was supplied as a 250 µg/mL solution. Each infusion rate lasted for 20 minutes and given this dosing protocol, a total of 280 µg/kg of remifentanil was given throughout the testing day.

6.13.4 Testing follow-up

Subjects were phoned 24 hours after testing to monitor any adverse events that may have occurred subsequent to leaving the Royal Adelaide Hospital.

6.14 Statistical analysis

Within-group comparisons of parametric data were made using one-way, repeated measures ANOVA and Dunnett’s post hoc tests where appropriate. Within-group comparisons of non-parametric data were analysed with Friedman tests with Dunn’s post hoc tests where appropriate. Statistical significance defined as a P-value <0.05. Data are presented as mean ± SEM.
6.15 Pharmacokinetic modelling

An outline of the testing session, indicating nociceptive testing and methadone administration, is shown in Figure 6.5. Plasma remifentanil concentrations were simulated following the study using pharmacokinetic software, STANPUMP (Shafer, 2006), using the subjects’ average parameters, including age, weight and height.

Figure 6.5 Remifentanil study design. Saline (S) and remifentanil (R) were infused intravenously in methadone maintained patients. The plasma remifentanil concentrations illustrated above were simulated using the software program STANPUMP (see methods section). Nociceptive testing occurred on 13 occasions during the testing session as indicated by the arrows. The subject’s daily methadone dose was administered 10 minutes following termination of the remifentanil infusion (as indicated by the solid vertical line).
Chapter 6 – Remifentanil in methadone maintained subjects

6.16 Results

6.16.1 Subject characteristics

The principal study was conducted with eight male subjects with their characteristics described below in Table 6.2.

Table 6.2 Demographic details – principal study. Principal study (n=8). Mean ± SEM (Range).

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Methadone dose (mg OD)</th>
<th>Time on current dose (months)</th>
<th>Time on methadone (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35±2 (29-44)</td>
<td>81±4 (65-98)</td>
<td>82.5±8.2 (50-110)</td>
<td>4±1 (2-7)</td>
<td>23±6 (6-60)</td>
</tr>
</tbody>
</table>

6.16.2 Subject withdrawal and missing data

Fourteen subjects were screened for the principal trial. Of these subjects, one was not recruited due to poor health, one due to poor venous access and two due to testing positive for urine morphine on the morning of the testing days. Two subjects had to be withdrawn during the 2.0 µg/kg/min remifentanil infusion, one due to severe nausea and one due to accidentally pulling out the cannula while using the urine bottle and then feeling nauseated. Both subjects were given 10 mg metoclopramide and observed until the symptoms resolved and vital measurement were normal. One other subject withdrew from the study 15 minutes subsequent to taking his methadone dose, citing severe opioid-withdrawal effects. All the remaining subjects completed the testing session without any protocol deviations.

One subject completed testing under the Protocol Number 040828A; as a result, no subjective or physiological data were obtained for this subject during the hour following methadone administration. Subjective measures were obtained from all other subjects recruited under Protocol Number 040828B excluding the subject who withdrew from the study subsequent to methadone dosing.

6.16.3 Familiarisation and baseline measurements

Subjects underwent subjective, physiological and nociceptive testing just prior to the saline infusion so that they were familiar to the testing procedures. There were no statistically significant differences between the familiarisation time-point and the saline-infusion time-point for any variable (Table 6.3). Baseline was defined as the value measured during the saline infusion.
Table 6.3 Familiarisation (pre-saline) and baseline (saline) differences. 
Data presented as mean±SEM, n=8

<table>
<thead>
<tr>
<th>Variable</th>
<th>Familiarisation (Pre-saline)</th>
<th>Baseline (Saline)</th>
<th>Statistical Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOWS (score)</td>
<td>4±1</td>
<td>2±1</td>
<td>p=0.17</td>
</tr>
<tr>
<td>MBG (score)</td>
<td>4±1</td>
<td>4±1</td>
<td>p=0.76</td>
</tr>
<tr>
<td>Respiration Rate (bpm)</td>
<td>15±1</td>
<td>14±1</td>
<td>p=0.51</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>126±2</td>
<td>127±4</td>
<td>p=0.72</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>75±3</td>
<td>79±4</td>
<td>p=0.44</td>
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<tr>
<td>Heart Rate (bpm)</td>
<td>74±5</td>
<td>72±5</td>
<td>p=0.26</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
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<td>99±0</td>
<td>p=0.20</td>
</tr>
<tr>
<td>CP Threshold (sec)</td>
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<td>7.44±1.46</td>
<td>p=0.38</td>
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<tr>
<td>CP Tolerance (sec)</td>
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<td>15.63±3.46</td>
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<tr>
<td>CP Decay (sec)</td>
<td>17.49±4.21</td>
<td>13.22±3.66</td>
<td>p=0.37</td>
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</tbody>
</table>

6.16.4 Subjective effects

Figure 6.6 Subjective effects. 
Data for the Subjective Opiate Withdrawal Scale (SOW – Graph A) and Morphine-Benzedrine Group Scale (MBG – Graph B) during (n=7) and following cessation (n=6) of remifentanil infusion in male methadone maintained subjects.

Results for the Subjective Opiate Withdrawal Scale and Morphine-Benzedrine Group Scale are shown in Figure 6.6 A and Figure 6.6 B, respectively. There were no statistically significant differences compared with baseline (saline) either during (F7,7=1.60, P=0.72) or after (F7,5=1.28, P=0.29) the remifentanil infusions for the SOWS. For the MBG Scale there were no statistically significant differences compared with baseline (saline) either during (F7,7=0.64, P=0.72) or after (F7,5=0.53, P=0.81) the remifentanil infusions.
6.16.5 Physiological effects

Figure 6.7 Physiological measures.
Data for respiration rate (Graph A), blood pressure (Graph B: closed-systolic / open-diastolic), heart rate (Graph C) and arterial oxygen saturation (Graph D) during (n=7) and following cessation (n=6) of remifentanil infusion in male methadone maintained subjects. ANOVA with Dunnett’s post hoc test *p<0.05, **p<0.01 compared with Baseline (Saline). Arterial oxygen saturation data were analysed with Freidman test with Dunn’s post hoc test *p<0.05, **p<0.01 compared with Baseline (Saline).
Subjects’ respiratory rate was significantly lower than baseline (saline) values at the end of the 1.0 (P<0.01; 95%CI 1.3, 5.2), 1.5 (P<0.01; 95%CI 2.5, 6.3), 2.0 (P<0.01; 95%CI 2.327, 6.2), 2.5 (P<0.01; 95%CI 3.3, 7.2), 3.0 (P<0.01; 95%CI 3.3, 7.2) and 3.5 (P<0.01; 95%CI 4.0, 7.8) µg/kg/minute infusion rates; no significant differences compared with baseline were observed following infusion cessation (F=8.311, P=0.306) (Figure 6.7 A). The lowest respiratory rate was 7 breaths/minutes in 4 of the subjects during the remifentanil infusion. There were no statistically significant differences compared with baseline (saline) either during or after the remifentanil infusions for systolic blood pressure (F=1.17, P=0.36; $F_{7,5}=0.81$, $P=0.53$) or diastolic blood pressure ($F_{7,7}=1.17$, $P=0.10$; $F_{7,5}=0.35$), respectively (Figure 6.7 B). For heart rate there were no statistically significant differences compared with baseline (saline) either during ($F_{7,7}=0.23$, $P=0.98$) or after ($F_{7,5}=1.55$, $P=0.22$) the remifentanil infusions (Figure 6.7 C). Subjects’ arterial oxygen saturation was significantly lower than baseline (saline) at the end of the 2.5 (P<0.05; Rank Sum Difference 31.0) and 3.5 (P<0.01; RSD 40.0) µg/kg/minute infusion rates; no significant differences compared with baseline were observed following infusion cessation (F=8.311, P=0.306) (Figure 6.7 D). Oxygen saturation values remained stable during the remifentanil infusion (range: 93%-100%) with no episodes of hypoxemia (oxygen saturation < 90%) occurring.
6.16.6 Nociception

Figure 6.8 Cold pressor test.
Data for threshold (Graph A), tolerance (Graph B) and decay (Graph C) during (n=8) and following cessation (n=7) of remifentanil infusion in male methadone maintained subjects. ANOVA with Dunnett’s post hoc test *p<0.05, **p<0.01 vs. Baseline (Saline).
Subjects’ pain threshold values for the cold pressor test were significantly higher than baseline (saline) values at the end of the 3.5 (P<0.01; 95%CI -41.72,-11.38) µg/kg/minute infusion rate; no significant differences compared with baseline were observed following infusion cessation (F_{4,6}=1.40, P=0.27) (Figure 6.8 A).

Subjects’ pain tolerance values for the cold pressor test were significantly higher than baseline (saline) values at the end of the 2.0 (P<0.05; 95%CI -82.6, -0.5), 2.5 (P<0.01; 95%CI -95.4, -13.4), 3.0 (P<0.05; 95%CI -88.6, -6.6 ) and 3.5 (P<0.01; 95%CI -102.7, -20.7) µg/kg/minute infusion rates; no significant differences compared with baseline were observed following infusion cessation (F_{4,6}=2.415, P=0.0768) (Figure 6.8 B).

There were no statistically significant differences compared to baseline (saline) either during (F_{7,7}= 1.15, P= 0.35) or after (F_{4,6}= 1.115, P= 0.38) the remifentanil infusions for subjects’ pain decay values for the cold pressor test (Figure 6.8 C).

6.16.7 Adverse events and other drug effects

Slight nausea was reported in 2 subjects who completed the remifentanil infusions. During the 1.0 (n=1) and 2.5 (n=1) µg/kg/minute remifentanil infusions, two subjects became drowsy, although easily rouseable and were able to stand for the cold pressor test; this resolved following infusion cessation. No other adverse events were reported or observed. No subject reported any adverse events from the time between leaving the hospital and 24 hours later.
6.17 Discussion

This study sought to investigate the antinociceptive, physiological and subjective effects of remifentanil administered to a sample of male methadone maintained subjects. Cumulative, ascending dosing of remifentanil, up to 3.5 µg/kg/minute, resulted in a five-fold increase in pain tolerance values at the same time as decreasing respiratory rate from a mean of 14 to 8 breaths per minute.

Previous authors investigating the analgesic properties of remifentanil have indicated that doses of 0.1 µg/kg/minute generally provide adequate post-operative analgesia in otherwise normal subjects (Bowdle et al., 1996; Schüttler et al., 1997; Yarmush et al., 1997). In a study by Vinik and Kissen (1998), a 0.1 µg/kg/minute infusion of remifentanil in healthy subjects resulted in a maximal 3-fold increase in cold pressor tolerance. The doses of remifentanil needed to achieve minimal increases in cold pressor tolerance in this study were substantially higher than analgesic doses in opioid-naive subjects. This implies that methadone maintained subjects are tolerant to opioids other than methadone, confirming previous research that indicated that methadone maintained subjects are cross-tolerant to the analgesic effects of standard and high doses of morphine (Doverty et al., 2001a; Athanasos et al., 2006). While it has been suggested that methadone maintained patients require either ‘normal’ (Kantor et al., 1980) or 3-4 times (Rapp et al., 1995) the dose of opioids for pain compared with non-opioid tolerant patients, in light of the current findings these recommendations may not be appropriate and opioid doses 20 times ‘normal’ may be more appropriate; nonetheless whether such high doses of other opioids are analgesic, or safe in methadone maintained patients is yet to be elucidated. Nevertheless, an antinociceptive response to remifentanil administration is still achievable in this population while adverse side effects such as nausea and emesis, sedation and subjective measures of euphoria and withdrawal were kept to a minimum or with minimum change from baseline.

Respiratory rate decreased in a dose-dependent manner during the remifentanil infusion, in a reciprocal manner to the nociceptive effects discussed above. Cross-tolerance was seen such that high doses of remifentanil were needed to observe effects normally seen at lower doses in opioid-naive subjects. In the present study, methadone maintained subjects showed no evidence of hypoxemia even though mean respiratory rate dropped to mean rate of 8 breaths per minute during the 3.5 µg/kg/minute remifentanil infusion. Indeed, in some subjects
respiratory rate was as low as 7 breaths per minute. This may be of concern as studies suggest that methadone maintained patients have significantly lower respiratory rates compared with normal controls (Gritz et al., 1975). Studies have shown that methadone or slow-release oral morphine maintained patients still experience significant decreases in respiratory rate following maintenance-opioid dosing (Dyer et al., 1999; Mitchell et al., 2003) indicating incomplete tolerance to the respiratory depressant effects of opioids in opioid maintained patients. Previous studies have indicated that remifentanil caused dose-dependent respiratory depression in healthy subjects (Glass et al., 1993) and caused respiratory depression at predicted plasma remifentanil concentrations of between 1 and 4 ng/mL (Black et al., 1999; Hsu et al., 2004); pharmacokinetic simulation of this study using STANPUMP (Shafer, 2006) (Figure 6.5) suggests that plasma remifentanil concentrations many-fold higher (maximal plasma remifentanil concentration of approximately 90 ng/mL) than this may have been achieved in these methadone maintained subjects, highlighting the extent of tolerance these subjects have for other opioids. Regardless, hypoventilation was observed in this population, confirming findings of other clinical studies that recommend that close observation of patients is required when remifentanil is administered, especially with regard to respiration (Bowdle et al., 1996; Schüttler et al., 1997; Kucukemre et al., 2005).

The rapid offset of opioid effects normally seen with remifentanil was observed in this study. This observation was highlighted with respiratory rate returning to baseline levels within 25 minutes of the cessation of the remifentanil infusion. Furthermore, following cessation of the remifentanil infusion, varying degrees of opioid-withdrawal were observed in subjects; while not statistically significant when measured by the subjective opiate withdrawal scale (SOWS) in six of the subjects, it was clinically significant enough for one subject to withdraw from the study. This highlights the need for pre-emptive analgesia or slow dose tapering of remifentanil before discontinuation to allow for both the prevention of opioid-withdrawal and maintenance of effective analgesia, a requirement needed in the acute pain management of opioid-tolerant patients (Jage and Bey, 2000).

The positive subjective effects of remifentanil are of concern when administering it to methadone maintained subjects. In this study, these effects were measured using the MBG scale, which showed no significant changes from baseline values throughout the testing session. This indicates that neither remifentanil nor the subject’s normal daily methadone dose conferred any euphoric effect. While this may be interpreted as insensitivity of this
questionnaire to the positive effects of remifentanil in methadone maintained patients, this scale has been used to show that bolus doses of remifentanil can cause effects comparable to fentanyl in opioid-dependent subjects (Baylon et al., 2000). The lack of effect in the present study may well be a result of opioid tolerance in subjects stabilised on their daily dose of methadone.

The apparent cross-tolerance between methadone and remifentanil observed in this study with regard to analgesia, respiration and euphoria is presumed to be pharmacodynamic in nature. Yet other forms of tolerance, such as pharmacokinetic tolerance, possibly due to increased clearance (section 1.5) caused by increased esterase activity in methadone maintained patients cannot be ruled out without a formal pharmacokinetic study of remifentanil in this population. However, due to the rapid metabolism of remifentanil in blood and tissue, pharmacokinetic studies of this drug can prove difficult and sampling site (i.e. venous versus arterial) may impact on interpretations of tolerance (Hermann et al., 1999; Kabbaj et al., 2005).

Furthermore, previous studies have suggested that maximal analgesic effects occur 90 minutes into an infusion of remifentanil and that acute analgesic tolerance occurs rapidly in volunteers administered a constant infusion of remifentanil (0.1 µg/kg/minute) over a 4 hour period (Vinik and Kissin, 1998). The design of the current study utilised an infusion period of 20 minutes and therefore, the maximal analgesic effects of remifentanil may not have been observed. In addition, effects such as analgesic tolerance following remifentanil infusions have been cited by some authors (Vinik and Kissin, 1998; Guignard et al., 2000) but not others (Cortinez et al., 2001); nonetheless acute analgesic tolerance may have occurred in the present study, however, the cumulative dosing regimen employed by this study may have excluded this observation.

During the four hours subsequent to methadone dosing, nociception did not differ significantly from baseline values. Opioid-withdrawal is associated with hyperalgesia (Mao et al., 1994; Célèrier et al., 2001; Vanderah et al., 2001b; Li and Clark, 2002) and increased pain sensitivity has been observed following a remifentanil infusion (Guignard et al., 2000; Angst et al., 2003; Hood et al., 2003; Hansen et al., 2005). While there was a large variation in the degree of opioid-withdrawal exhibited in the subjects following the cessation of the remifentanil infusion, most of these effects had subsided by the nociceptive testing session one hour following methadone dosing. Nevertheless, hyperalgesia was not observed following
discontinuation of the remifentanil infusion. However, due to methadone administration 10 minutes following cessation of the remifentanil infusion, any opioid-withdrawal and consequent hyperalgesia would have been attenuated.

There are several limitations to this study. Firstly, the study was limited by the lack of the inclusion of a placebo treatment arm. However, placebo treatment would have been of little value as blinding is unrealistic: subjects clearly detected the effects of remifentanil. Nonetheless, previous studies have shown that conditioning due to repetitive cold pressor testing does not result in significant changes in pain tolerance, either in healthy subjects or methadone maintained subjects (Athanasos et al., 2006), using a similar protocol as the one used in this study at one hour interval or as short as 30 minute intervals (Jones et al., 1988).

Secondly, the sample size was small. Power analysis, however, indicated that the number of subjects was appropriate for the determination of the primary outcome measure, namely changes in nociception as measured by cold pressor tolerance time. This small sample size may have excluded the observation of side effects typically observed at these high doses in opioid non-tolerant patients, including bradycardia, muscle rigidity, pruritus or hypotension. These effects were not observed. Lastly, this study utilised an experimental pain paradigm rather than a clinical situation; further clinical research is required before recommendations for treatment of clinical pain can be made.

6.18 Conclusion
In summary, this study shows that antinociception can be achieved using remifentanil in methadone maintained subjects with minimal side effects. Substantially higher doses were required compared with published studies on opioid-naïve patients, but adverse effects were mild to moderate in magnitude. Like antinociception, respiratory depression was dose-related, but there was no evidence of hypoxemia. Remifentanil shows promise as an analgesic for the treatment of acute pain in methadone maintained patients, but the doses required may be as high as 20 fold greater that those used in non-tolerant patients. Typical opioid-like effects dissipate rapidly after abrupt cessation of remifentanil infusion.
Chapter 7 – Animal model of methadone-induced hyperalgesia

7 ANIMAL MODEL OF METHADONE-INDUCED HYPERALGESIA

7.1 Prologue

Developing an animal model of methadone-induced hyperalgesia has the advantage of determining if hyperalgesia is caused by opioids *per se*; other confounding factors can be excluded including the presence of other drugs or pathologies. This approach is advantageous for many reasons. Firstly, if an animal model of methadone-induced hyperalgesia could be established, it would extend the field of research beyond the prototypic µ-opioid receptor agonists to a compound that possesses additional NMDA receptor antagonist properties. Secondly, an animal model of methadone-induced hyperalgesia can be used to investigate mechanisms underlying the nociceptive changes associated with long-term opioid administration. Thirdly, by utilising a rodent model of methadone-induced hyperalgesia, potential pharmacotherapies aimed at reducing hyperalgesia could be tested for effectiveness prior to testing in human subjects. Lastly, the development of an animal model would help link observations made in the clinical setting to basic laboratory research.

7.2 Experimental pain models in animals

In a similar way that Beecher (1959) and Gracely (1999) postulated that human experimental pain models require certain properties, similar characteristics have been detailed with regard to nociceptive models for pain in animals (Le Bars et al., 2001). These qualities include that pain models utilising animals require specificity, sensitivity, validity, reliability and reproducibility.

The antinociceptive properties of opioids can be assessed in rats in a variety of ways. Nonetheless, the majority of tests involve the use of a thermal stimulus with the most commonly used thermal tests being the tail-flick test, the hotplate test and Hargreaves’ test. Other non-thermal, acute nociceptive tests include the use of electrical, mechanical and chemical stimuli applied to various organs or structures. However, these models tend to be more invasive or organ specific (Le Bars et al., 2001). Animal models of chronic pain also exist and usually represent either neuropathic or rheumatic types of pain (Wang and Wang, 2003). However, the description of these is beyond the scope of this thesis.

The tail-flick test has two main variants: using radiant heat applied to the tail and the other by immersion of the tail in (generally) hot water. The radiant heat test involves the application of
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a heat source to the tail of an animal until the withdrawal of the tail (tail-flick) and the time for this to occur is recorded (D’Amour and Smith, 1941). The hotplate test involves placing the animal on a metallic hotplate and the time until the animal exhibits a behavioural component such as paw licking or jumping is measured (Woolfe and MacDonald, 1944). Unfortunately, both tests have limitations: the tail-flick test is susceptible to habituation (Groves and Thompson, 1970), while the hotplate test is prone to producing a conditioned response (learning) (Knoll et al., 1955); in both cases these phenomena can lead to significant reductions in reaction time.

Hargreaves’ test involves applying a focused light (heat) to the plantar surface of the rat’s hind paw. This has the advantage that it can be done in a freely moving animal and therefore minimises stress responses (Hargreaves et al., 1988). Another advantage comes from the fact that the tail is not utilised as it is in the tail-flick tests; the rodent tail is the predominant physiological structure involved in thermoregulation. Hence, changes in body temperature or blood flow may affect nociceptive thresholds (Le Bars et al., 2001). Nonetheless, Hargreaves’ test can be modified such that it becomes a modified version of D’Amour and Smith’s (1941) tail-flick test by applying the radiant heat source to the tail instead of the paw (Kozela et al., 2001). This may be useful in distinguishing mechanistic features of pharmacotherapies as stimulation of the tail and the paw utilise different nociceptive pathways. While not an absolute postulation, the tail-flick tests can be considered to be dependent on spinal reflexes while the paw withdrawal tests are considered to involve peripheral nociceptive pathways (Ramabadran and Bansinath, 1986; Le Bars et al., 2001). Additionally, lower intensity heat activates C fibres, whereas high intensity heat causes rapid temperature increase resulting in Aδ fibre activation (Yeomans et al., 1996).

Hargreaves’ test has certain limitations that require some consideration. Applying heat to the paw and measuring nociceptive response can be a slight disadvantage as the position of the leg can change the background activity of the flexors (Le Bars et al., 2001); this is a confounding factor especially with Hargreaves’ test, where the animal is freely moving. In addition, changes in basal activity can have an impact on outcomes; while opioids have the ability to increase motor activity (Fog, 1970), methadone appears to lack this potential (Lewanowitsch et al., 2004). Furthermore, due to the fact that Hargreaves’ test involves thermal stimulation, physiological factors involving the rat’s ability to thermoregulate need to
be considered. However, in most cases baseline variation can be minimised by controlling cutaneous skin temperature and room temperature (Dirig et al., 1997).

### 7.3 Nociception and opioids in animals

The recent research investigating increased pain sensitivity relating to chronic opioid use has focused on the mechanistic examination of opioid-induced hyperalgesia and has been discussed in earlier chapters of this thesis. Regardless, these studies provide a useful insight into possible models and a potential basis for a model of methadone-induced hyperalgesia.

#### 7.3.1 High-dose opioid-induced hyperalgesia

Early studies reported that opioids had the ability to induce behaviour similar to peripheral nerve injury. Large intrathecal doses of morphine cause pain and hyperalgesia (Woolf, 1981). Similarly, Yaksh and Hardy (1988) reported that high intrathecal doses of morphine could initiate spontaneous aggression and behaviour indicating allodynia in the rat. This observation of hyperalgesia caused by large doses of opioid has also been observed in the clinical setting and is generally resolved with dose reduction (Gillman and Lichtigfeld, 1985; Ali, 1986; De Conno et al., 1991; Devulder, 1997). This type of opioid-induced hyperalgesia may be comparable to the hyperalgesia seen in methadone maintained patients and may be appropriate for the basis of a methadone-induced hyperalgesia animal model.

#### 7.3.2 Opioid-withdrawal hyperalgesia

Numerous studies have shown that hyperalgesia is associated with opioid withdrawal following the cessation of long-term opioids, whether by natural or precipitated means. The degree of hyperalgesia related to opioid withdrawal is considered to relate to the extent of dependence. This has been substantiated in experimental pain models. For example, rats made tolerant to either systemic or spinal morphine showed hyper-reflexia and extreme sensitivity to handling following the administration of naloxone (Yaksh et al., 1977). Comparable effects have been observed with heroin, albeit following a shorter time-course (Larcher et al., 1998). Shortened tail-flick latencies, indicative of hyperalgesia, could be induced by naloxone administered 40 minutes following a bolus dose of heroin in rats (Larcher et al., 1998). This phenomenon of hyperalgesia associated with antagonist precipitated opioid-withdrawal is discussed elsewhere (section 1.8).
Furthermore, other authors have described hyperalgesia after the analgesic effects of a single dose of opioid have diminished. Studies have shown that after a single subcutaneous dose of heroin, antinociception was followed by lowered thresholds to the paw-pressure induced vocalisation (Larcher et al., 1998; Célèrier et al., 1999). These studies suggest that analgesia masks the hyperalgesic effect of opioids, but once analgesia has subsided, increased pain sensitivity is observed.

However, it has been suggested that morphine-induced inhibition of neuronal function is compensated for by neuronal hyperactivity (Gutstein, 1996). Furthermore, this hypersensitivity becomes apparent once the opioid is discontinued, or between repeated injections and may be a result of “mini-withdrawals” (Gutstein, 1996). This is a criticism of many studies that utilise a repeated dosing regimen with nociceptive testing occurring just prior to the subsequent dose or after the termination of the opioid dose (Trujillo and Akil, 1991; Mao et al., 1994; Mao et al., 1995a; Larcher et al., 1998; Laulin et al., 1998; Célèrier et al., 2001).

7.3.3 Opioid-induced hyperalgesia

The administration of opioids in a chronic, continuous manner addresses the concerns relating to increased pain sensitivity due to opioid withdrawal. Few studies have thoroughly investigated nociceptive changes following long-term continuous opioid administration. The continuous spinal administration of morphine or D-Ala²-N-Me-Phe⁴-Gly-ol⁵-enkephalin (DAMGO) for 6 days has resulted in the development of hyperalgesia (Vanderah et al., 2000). Similarly, the spinal administration of the active (-)-oxymorphone, but not the inactive enatiomer (+)-oxymorphone, is associated with hyperalgesia following continued administration for 6 days (Gardell et al., 2006).

Previous research involving animal models of opioid-induced hyperalgesia have focused almost exclusively on morphine, with other pertinent studies limited to µ-opioid receptor agonists such as heroin (Laulin et al., 1998), DAMGO (Vanderah et al., 2000), and fentanyl (Laulin et al., 2002). This generally reflects the methodological need to use relatively selective µ-opioid agonists such that appropriate conclusions regarding opioid-induced hyperalgesia can be made.

However, there is a large discrepancy between the clinical studies that indicate that chronic methadone use is associated with hyperalgesia and most, if not all, of the animal studies that
investigate morphine. While the preceding chapters of this thesis have described strategies for both investigating and resolving methadone-induced hyperalgesia, no animal model exists that investigates methadone-induced hyperalgesia.

An ideal animal model that could be considered analogous to methadone maintenance would require administering methadone systemically, maintaining relatively stable plasma methadone concentrations and preventing opioid-withdrawal. Relatively stable plasma drug concentrations can be achieved using a subcutaneous osmotic pump. This paradigm has already been used by Vanderah and colleagues who gave 200-300 g Sprague Dawley rats continuous, subcutaneous morphine either via an osmotic pump (1.08 mg/day) or as a pellet (150 mg). Nociceptive threshold was tested once daily using two pain models: tactile allodynia was assessed using Von Frey filament and thermal hyperalgesia was assessed using the radiant heat paw flick test (Hargreaves’ test). Administration of morphine or inactive placebo, either by pellet or pump, yielded similar results with both nociceptive tests. Initially analgesia (increased paw withdrawal latency) was seen at 2 and 6 hours following drug implantation. Both allodynia and hyperalgesia developed by day 2 following implantation and lasted the duration of the observation period of 7 days. No physical signs of opioid withdrawal were seen during the observation period. Most importantly by administering opioids continuously this study addressed one of the largest criticisms directed toward this area of research, that of increased neuronal activity caused by ‘mini-withdrawals’. A congruent model using methadone instead of morphine, could take advantage of its simplicity while still being comparable to the clinical situation of methadone maintenance.

### 7.3.4 Resolution of opioid-induced hyperalgesia

Some studies that have investigated opioid-induced hyperalgesia have also investigated its resolution. Earlier chapters of this thesis explore in greater detail the literature and concepts associated with the resolution of hyperalgesia following the discontinuation of opioid administration and the relationship between hyperalgesia and opioid-withdrawal. However, several studies are of note as they relate to an animal model of opioid-induced hyperalgesia.

Investigations by Li and colleagues showed that following the establishment of morphine-induced hyperalgesia in rats (Li et al., 2001b) and mice (Li et al., 2001a), nociceptive thresholds return to baseline values within two-three days depending on the nociceptive test. Célèrier and colleagues (2001) showed that the time-course for the development of heroin-
induced hyperalgesia was mirrored by its resolution once heroin administration ceased. Lastly, Davies and colleagues (2003) demonstrated that morphine-induced hyperalgesia largely resolved by 72 hours following morphine discontinuation. Common to all these studies was that the time-course of the resolution of opioid-induced hyperalgesia was comparable to its development.

### 7.4 Pharmacology of methadone and morphine in SD rats

The pharmacokinetics of methadone in rats differs markedly from those of humans. Whereas the half-life of methadone in humans can range between 5 and 130 hours (Eap et al., 2002), in rats it is between 70 and 90 minutes (Pierce et al., 1996). The IP threshold dose of methadone for analgesic action in rats is 1 mg/kg (Chen, 1948) and the LD$_{50}$ toxicity level is 23 mg/kg (White and Zagon, 1979; Borron et al., 2002). Similarly, morphine has a shorter half-life in rats (45 minutes) (Cicero et al., 1997) than it does in humans (1.9 hours) (Goodman et al., 2006). The ED$_{50}$ of morphine in male SD rats can range between 2.1 and 8.8 mg/kg (Bulka et al., 2004; Peckham and Traynor, 2006). The opioid agonist properties of morphine and methadone are similar although they have different binding affinities for the $\mu$-, $\delta$-, $\kappa$-opioid receptors (Table 7.1).

### Table 7.1 Opioid ligand binding affinities

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$\mu$</th>
<th>$\kappa$</th>
<th>$\delta$</th>
</tr>
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<tr>
<td>Morphine</td>
<td>1.7 (0.5)</td>
<td>65.5 (22.6)</td>
<td>104.6 (27.2)</td>
</tr>
<tr>
<td>Methadone</td>
<td>2.9 (0.9)</td>
<td>1427 (249)</td>
<td>359 (119)</td>
</tr>
</tbody>
</table>

Mean (SEM) affinity values ($K_i$ (nM)) in C6$\mu$, CHO$\kappa$ and C6$\delta$ membranes. Adapted from Peckham & Traynor (2006).

Sex differences in the pharmacokinetics and the analgesic response to morphine (South et al., 2001), methadone (Rodriguez et al., 2002) and other opioid agonists (Bartok and Craft, 1997; Holtman and Wala, 2006; Peckham and Traynor, 2006) have been observed in the SD rat; therefore in the following experiments only male rats were used to reduce variability.

### 7.5 Study rationale

Human-based research indicates that long-term methadone administration induces hyperalgesia, yet this research is limited to observational studies. Animal models that parallel the clinical situation exist, albeit that the prototypic opioid, morphine, is used. Consequently, there is a need for the development of an animal-based model of methadone-induced
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hyperalgesia. Therefore the overall objective of this study was to develop a model of methadone-induced hyperalgesia in the adult, male, SD rat.

7.6 General methods

7.6.1 Ethics
Ethical approval for this study was granted by the Animal Ethics Committee of the University of Adelaide (UA Protocol M67-2002 and UA Protocol M27-2006). All studies were conducted such to comply with the guidelines of the National Health and Medical Research Council of Australia (NHMRC, 2004).

7.6.2 Animals
All rats were obtained from Laboratory Animal Services (University of Adelaide, SA, Australia). Adult, male, Sprague-Dawley (SD) rats were used. Rats were provided with standard rat chow and water ad libitum. Animals were maintained in a standard 12 hour light-dark cycle, changing at 0600 and 1800 hours, and subject to a constant room temperature of 23 ± 2 °C.

7.6.3 Familiarisation
Rats were made familiar with the testing apparatus and the nociceptive testing procedures by going through Hargreaves’ test at least once a day for a minimum of one week preceding the formal study. This also allowed adjustment of Hargreaves’ test such that the correct baseline values could be determined.

7.6.4 Drugs
Methadone hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD, USA). Morphine sulphate was purchased from GlaxoSmithKline (Boronia, VIC, Australia). A weight-based dosing regimen designed around the animal’s initial (baseline) weight was used. However, for simplicity, dosing rates are expressed as mg/day due to the constant delivery rate imposed by the osmotic pumps and the variable nature of the rats’ body weight.

7.6.5 Osmotic pump implantation
Osmotic pumps are implantable devices capable of delivering solutions continuously and at a defined rate over a period of 7-14 days. Prior to implantation, the rat was anaesthetised with a
60 mg/kg IP dose of 60 mg/mL pentobarbital sodium (Rhône Merieux, Pinkenba, QLD, Australia). Prior to the commencement of surgery, rats were tested by means of the pedal reflex and intermittently monitored throughout surgery to ensure an adequate level of anaesthesia was maintained. Pre-loaded ALZET Osmotic pumps (ALZA, Palo Alto, CA, USA) (Table 7.2), were placed in isotonic saline at 37 °C for 4-6 hours prior to implantation. One osmotic pump was placed in a small subcutaneous pocket cut into the dorsum of the rat with the pocket closed with wound clips. Rats recovered on a 37 °C hotplate until a righting-reflex was obtained. A post-operative analgesic was not administered to the rats as this would confound subsequent results. Topical antibiotic powder (neomycin sulphate 2.5 mg/g, sulfacetamide sodium 100 m/g, nitrofurazone 2 mg/g, phenylmercuric nitrate 0.05 mg/g, benzocaine 5 mg/g) (APEX Laboratories, Somersby, NSW, Australia) was applied to the wound site postoperatively and daily for 7 days following surgery. Wound clips were removed after 7 days. When pumps were removed, similar methodology was used.

<table>
<thead>
<tr>
<th>Table 7.2 Technical description of ALZET osmotic pumps</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model (batch)</strong></td>
</tr>
<tr>
<td>Mean ± SEM Pumping Rate (µl/hr)</td>
</tr>
<tr>
<td>Mean ± SEM Fill Volume (µl)</td>
</tr>
<tr>
<td>Lot #</td>
</tr>
</tbody>
</table>

### 7.6.6 Nociceptive testing

Hargreaves’ test (Hargreaves et al., 1988) was used for the evaluation of thermal hyperalgesia in rats and measured response latencies to hind paw thermal stimulation. Thermal sensitivity was measured using the Model 336 Analgesia Meter (IITC Life Science, Woodland Hills, CA USA). Four rats were placed in adjacent, individual, covered, clear plastic chambers (180 mm (H) x 290 mm (D) x 125 mm (W)) with a glass floor (5 mm (H) 250 mm (D) 950 mm (W)). Rats were allowed to adjust to their environment for at least 10 minutes before testing. During this time, rats initially demonstrated exploratory behaviour but subsequently stopped exploring and stood quietly with occasional bouts of grooming. After the adjustment period, the radiant heat source was positioned under the glass floor directly beneath the hind paw. When paw withdrawal latency was measured, the guide light was positioned to include the two most distal pads of the rat hind paw. A trial commenced by a switch which activated the radiant heat source and started an electronic timer. The radiant heat source consisted of a 150 W high intensity projector bulb (Osram, Pennant Hills, NSW, Australia) located below the glass floor and projecting through an aperture in the top of a movable case, with the light focused to the top of the glass, essentially creating a light spot of 4 mm x 6 mm. Withdrawal latency to the nearest 0.1 s was determined by the operator observing the rat moving its paw.
and then pressing a button to turn off the lamp and the electronic clock. The intensity of the light bulb was adjusted such that baseline values were approximately 15-20 s. A cut-off time of 40 s was imposed to prevent tissue damage. Testing occurred at approximately 1500 hours.

### 7.6.7 Adverse event monitoring

Rats were monitored at least once a day with regard to adverse events according to Table 7.3.

Table 7.3 Adverse event monitoring.

<table>
<thead>
<tr>
<th>Symptoms/Signs</th>
<th>Scoring</th>
<th>Total Score</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dull or Ruffled Coat</td>
<td>Presence=1</td>
<td>Absence=0</td>
<td>No Action</td>
</tr>
<tr>
<td>Poor Posture or Hunched</td>
<td></td>
<td></td>
<td>Check Animal 3x/day</td>
</tr>
<tr>
<td>Squealing when Handled</td>
<td></td>
<td>3</td>
<td>Animal Welfare/Veterinary Advice Required</td>
</tr>
<tr>
<td>Reluctance to Move</td>
<td></td>
<td>&gt;3</td>
<td>Euthanasia Required</td>
</tr>
<tr>
<td>Pale or Sunken Eyes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in Behaviour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight Loss</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced Food or Water Intake (where known)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 7.6.8 Opioid withdrawal measures

Where appropriate, the severity of spontaneous abstinence syndrome was evaluated just before and 24 after osmotic pump removal. Animals were observed for 10 minutes and the following signs were measured: ptosis, abnormal posture, irritability/hostility on handling and diarrhoea; these were recorded as 0 (absent) or 1 (present). Weight change (loss) was evaluated by comparing weight just after pump removal and 24 hours later. These signs were considered the most representative of opioid abstinence syndrome in the rat (Bläsig et al., 1973; Pierce et al., 1996).
7.7 Nociceptive effects of morphine

7.7.1 Introduction

Previous research by Vanderah and colleagues (2001b) indicated that morphine given at a rate of 1.08 mg/day to 200-300 g male Sprague Dawley rats could significantly reduce paw withdrawal latency from a pre-infusion baseline of 22 ± 0.6 sec to 16.6 ± 0.3 sec at 7 days after osmotic pump implantation. The objective of this experiment was to replicate this work and to validate the methodology in this laboratory.

7.7.2 Aim

To examine if chronic, continuous morphine administration could significantly lower nociceptive threshold in adult, male Sprague Dawley rats.

7.7.3 Hypothesis

If morphine is administered in a chronic, continuous manner at a rate of 1.2 mg/day, then paw withdrawal latency will initially increase from baseline indicating antinociception and then subsequently decrease from baseline indicating hyperalgesia.

7.7.4 Study design

Eight rats were familiarised with the Hargreaves’ test. Nociceptive threshold was determined by measuring the paw withdrawal latency in the hind left paw on a single occasion at each testing session. In an investigator-blinded, randomised manner, each rat was implanted subcutaneously with a model 2ML2 osmotic pump delivering either morphine sulphate (1.2 mg/day) or normal saline placebo. Rats weighed between 300-350 g and light intensity was set to 2.5 units.

7.7.5 Statistical analysis

Data were analysed using Prism 4.03. Within-saline treatment data were analysed using two-way ANOVA. If an interaction effect was observed then a between group t-test for each time-point was undertaken.
7.7.6 Results

![Paw withdrawal latencies graph](image)

Figure 7.1 Paw withdrawal latencies.
Adult, male Sprague Dawley rats received subcutaneous implantation of osmotic pumps containing saline placebo (●) or morphine (1.2 mg/day) (▲). Paw withdrawal latencies to radiant heat applied to the plantar aspect of the hind paw were determined at baseline (B) and once daily afterward for 7 days. No significant changes in pain sensitivity were observed (n=3 saline placebo, n=4 morphine).

One rat (saline placebo group) had to be excluded from analysis due to the development of an infection around the osmotic pump. No other adverse events occurred during the experiment.

Table 7.4 Two-way repeated measures ANOVA results

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num df</th>
<th>Den df</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>54</td>
<td>0.14</td>
<td>0.72</td>
</tr>
<tr>
<td>Time</td>
<td>9</td>
<td>54</td>
<td>1.52</td>
<td>0.16</td>
</tr>
<tr>
<td>Interaction</td>
<td>9</td>
<td>54</td>
<td>0.90</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Results of the ANOVA are presented in Table 7.4. There were no significant effects with any of the parameters.

7.7.7 Discussion

The aim of this study was to investigate if morphine given at 1.2 mg/day to male Sprague Dawley rats could result in hyperalgesia. While the results indicated that neither the implantation of the osmotic pump nor the repeated nociceptive testing caused any changes in nociceptive threshold, the infusion of morphine via the osmotic pumps did not change the nociceptive threshold in the rats. Consequently, the results of Vanderah and colleagues (2001b) could not be replicated. The absence of any changes may have been due to either equipment malfunction in the current study or due to subtle differences in the experimental protocol between the laboratories.
There are several explanations for the lack of change in nociceptive threshold. Firstly, the osmotic pumps used in this experiment may have failed; consequently little or no morphine (or saline) solution may have been pumped into the subcutaneous region. Subsequent analysis of the recovered pumps showed no significant increases in weight indicative of blockage. Another method for determining if the pumps were working at the end of the infusion period could be by collecting a blood sample and analysing it for morphine to confirm its presence in the systemic circulation. Analysing the blood/plasma would also verify the treatment allocation for each rat. However, this was not done in the current study.

Alternatively, the sensitivity of the testing equipment may have been the reason for any lack of change in observation. To eliminate the equipment as a source of insensitivity, testing the equipment using an acute dosing protocol using doses that are known to cause analgesia in rats is required.

Subtle differences between testing protocols may underlie the contrasting results of this study and the results presented by Vanderah and colleagues (2001b). In terms of equipment, the current study utilised an unheated glass plate on which the rats were placed. As a result, the glass may act as a heat-sink, therefore requiring a greater amount of energy before the rat elicits a response. Furthermore, glass temperature affects cutaneous temperature and therefore paw withdrawal latency (Galbraith et al., 1993; Dirig et al., 1997). However, these effects are negligible as the room temperature and thus glass temperature remained constant throughout the experiment. With regard to baseline latency, Vanderah and colleagues started with a baseline paw withdrawal latency of 22 sec compared with a lower initial baseline used in this study of 16 sec. Even though a lower baseline was set in the current study and the light source was adjusted such that the baseline paw withdrawal latencies were close to maximum, this does not explain why a significant change in paw withdrawal latency was not observed.

Furthermore, the comparator study used osmotic pumps that delivered saline or morphine at a rate of 1 µl/hr compared with the 5 µl/hr used in the current study. However this difference is countered by the fact that a different morphine concentration was used such that there was only a slight difference in the amount of morphine delivered to the rats, with the study of Vanderah and colleagues delivering 1.08 mg/day compared with the 1.2 mg/day in this study. However, this rate becomes inconsequential when the heavier weight of the rats used in the
current study is factored into the dosage regimen. If weight is considered as part of the dosage calculation, the rats used in the present study received a dose of 3.7 mg/kg/day whereas Vanderah and colleagues were dosed at a rate of 4.3 mg/kg/day. While these differences between experimental protocols are subtle they may explain why no significant changes were observed in the above study.

7.7.8 Conclusions

Overall, morphine, given at 1.2 mg/day, did not alter the nociceptive threshold of male SD rats. To ensure the quality use of animals (NHMRC, 2004), replication of this work was not performed. Therefore the remaining studies of this chapter are concerned with developing an animal model of opioid-induced hyperalgesia focusing on the use of methadone.
7.8 Acute nociceptive effects of morphine and methadone

7.8.1 Introduction
The preceding experiment found that morphine administered in a chronic, continuous manner to male SD rats had no effect on nociceptive threshold. This study intended to eliminate the equipment and the procedures as a source of insensitivity. An additional objective was to confirm the quality of the morphine used in the previous experiment. Therefore a study investigating the acute effects of morphine and methadone given at analgesic doses was proposed.

7.8.2 Aim
The aim of this study was to validate the Hargreaves’ test in the laboratory and confirm that it could detect changes in nociception caused by acute opioid administration.

7.8.3 Hypothesis
Methadone and morphine given as a bolus dose of 10 mg/kg and 15 mg/kg, respectively will produce analgesia detectable using the Hargreaves’ test, compared with bolus saline.

7.8.4 Study design
Four male SD rats were familiarised with the Hargreaves’ test. Each rat was administered 10 mg/kg methadone, 15 mg/kg morphine or normal saline placebo (1 mL/kg) on three separate occasions separated by a minimum of 48 hours in an investigator-blinded, randomised, crossover manner. Rats weighed between 280-350 g and light intensity was set to 2.0 units.

7.8.5 Statistical analysis
Paw withdrawal latency data from the saline-treatment day were analysed using one-way repeated measures ANOVA. If no significant difference in mean paw withdrawal latency was observed within the saline treatment group then a between group ANOVA for each time-point was undertaken with Dunnett’s post hoc test done where appropriate.
Chapter 7 – Animal model of methadone-induced hyperalgesia

7.8.6 Results

Figure 7.2 Paw withdrawal latencies.

Adult, male SD rats received an intraperitoneal injection at time 0 of saline placebo (▲), 10 mg/kg methadone (●) or 15 mg/kg morphine (▼). Paw withdrawal latencies to radiant heat applied to the plantar aspect of the hind paw were determined at baseline (Time -5) and subsequently up to 240 minutes following the injection. One-way paired ANOVA indicated significant differences between morphine *p<0.05, **p<0.01 and methadone #p<0.05, ##p<0.01 compared with saline (n=4).

Repeated measures ANOVA indicated there was no overall effect of saline treatment (F₃,₆=2.022, P=0.1154). No differences in mean paw withdrawal latency times were observed at baseline (F₃,₂=0.95, P=0.44). Statistically significant increases in paw withdrawal latency compared with saline were observed at 30 minutes (F₃,₂=18.57, P=0.003; methadone P<0.01; 95%CI -43.67 to -11.38, morphine P<0.01; 95%CI -40.09 to -7.796), 60 minutes (F₃,₂=11.17, P=0.01; methadone P<0.05; 95%CI -44.16 to -7.083, morphine P<0.05; 95%CI -37.72 to -0.6406), 90 minutes (F₃,₂=8.073, P=0.0199; methadone P<0.05; 95%CI -43.70 to -0.8326, morphine P<0.05; 95%CI -44.52 to -1.655), 120 minutes (F₃,₂=8.297, P=0.0187; methadone P>0.05; 95%CI -28.57 to 8.987, morphine P<0.01; 95%CI -41.96 to -4.398), 180 minutes (F₃,₂=9.77, P=0.013; methadone P>0.05; 95%CI -10.40 to 0.0502, morphine P<0.05; 95%CI -11.93 to -1.480) but not 240 minutes (F₃,₂=0.127, P=0.883). No adverse events occurred during the experiment.

7.8.7 Discussion

The aim of this experiment was to investigate whether acute dosing with either methadone or morphine could cause changes in nociception when measured using Hargreaves’ test. Methadone, morphine and saline placebo were given to rats on three separate occasions as bolus doses. Results indicated that methadone and morphine were capable of causing acute analgesia that was detectable using Hargreaves’ paw withdrawal test.
Results showed that there were no significant changes following saline placebo dosing. This indicates that paw withdrawal threshold was not altered by the vehicle. It also shows that baseline results were relatively constant during the testing period and that repetitive testing had minimal effect on paw withdrawal threshold.

Both methadone and morphine caused increases in paw withdrawal latency close to maximal values. Both drugs showed antinociceptive activity within 30 minutes of dosing. For methadone, significant antinociceptive activity continued for 90 minutes following dosing, with paw withdrawal values returning to baseline values for the subsequent testing time points. In contrast, morphine antinociception persisted for a longer period with significantly higher paw withdrawal latencies occurring up to 180 minutes post-dose. Thereafter, latencies returned to baseline levels 240 minutes following bolus morphine dosing. These results compare favourably to similar studies that have measured the antinociceptive properties of acute doses of morphine or methadone in opioid naïve, SD rats using a hotplate test (Liu et al., 1979; Bulka et al., 2004). The reduced antinociceptive effect seen with methadone in contrast to morphine could be a result of more rapid elimination or other PK/PD reasons. Moreover, in the rat morphine has active metabolites (Easterling and Holtzman, 1998) while main metabolites of methadone are pharmacologically inactive (Liu et al., 1983).

Furthermore, these results demonstrate that the morphine used in both the previous experiment and this experiment was active. This eliminates morphine as a reason for lack of response in the previous experiment.

### 7.8.8 Conclusion

In summary, Hargreaves’ test was able to detect changes in nociception (analgesia) caused by either methadone or morphine administration. This resolved two issues that were raised in the previous experiment, namely, the nociceptive activity and potency of opioids used and the sensitivity of Hargreaves’ test in detecting these changes in rats.
7.9 Nociceptive effects of methadone – dose-finding studies

7.9.1 Introduction
The previous study indicated that methadone was able to cause antinociception in the rat and was able to be detected using the Hargreaves’ test. The follow series of experiments were carried out to determine the most appropriate dose of methadone that might induce hyperalgesia.

7.9.2 Aim
The aim was to investigate the most likely dose of chronically administered methadone that may cause hyperalgesia in SD rats detectable using Hargraves test.

7.9.3 Hypothesis
It is hypothesised that there is a dose of chronically administered methadone that significantly decreases paw withdrawal latency in the male, SD rat when compared with baseline values.

7.9.4 Study design
This study consisted of 3 cohorts of rats. Each cohort was familiarised with Hargreaves’ test. Subsequently, each group was tested for changes in nociceptive threshold with a range of chronically administered methadone doses. As the cohorts were part of a series of sequential experiments, there were minor differences in some of the methods used, as improvements were made in experimental techniques. Within each cohort, rats were implanted in an investigator-blindet, randomised manner with an osmotic pump containing different concentrations of methadone. Rats received only one methadone dose.

For cohort one, withdrawal latency was measured on the left hind paw only. For cohorts 2 and 3, paw withdrawal latency for each rat was taken as the average of the paw withdrawal latency of the right and left hind paws to reduce variability.

When tail withdrawal latency was measured, a permanent ink marker was used to indicate the point midway along the tail. When using Hargreaves’ test, the guide-light was positioned on the ventral side within either 2 cm distal or proximal of the middle of the tail. Tail withdrawal latency was the average of one distal and one proximal withdrawal latency times. A variation
of this method has been used before to test nociceptive threshold in rats (Kozela et al., 2001) and was as an additional method suggested by Dr M. Hutchinson (personal communication).

Table 7.5 Summary of experimental variables and testing methods

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Methadone Rate 1 (mg/day (mg/kg/day at baseline))</th>
<th>Methadone Rate 2 (mg/day (mg/kg/day at baseline))</th>
<th>N per treatment</th>
<th>Pump Model</th>
<th>Rat Weight range (g)</th>
<th>Nociceptive Test (T=tail &amp; P=paw)</th>
<th>Light Intensity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.25 (10)</td>
<td>-</td>
<td>4</td>
<td>2ML1</td>
<td>330-400</td>
<td>P</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>1.05 (3)</td>
<td>0.35 (1)</td>
<td>2</td>
<td>2ML1</td>
<td>350-400</td>
<td>P+T</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>0.1125 (0.3)</td>
<td>0.0375 (0.1)</td>
<td>4</td>
<td>2ML1</td>
<td>375-450</td>
<td>P+T</td>
<td>2.5</td>
</tr>
</tbody>
</table>

7.9.5  Statistical analysis

Average paw or tail withdrawal latency times were analysed using Prism 4.03.

7.9.6  Results

Cohort 1

Figure 7.3 Paw withdrawal latencies. Adult, male SD rats received subcutaneous implantation of osmotic pumps delivering methadone at 3 mg/day (*). Paw withdrawal latencies to radiant heat applied to the plantar aspect of the hind paw were determined at baseline (B) and once daily afterwards for 13 days. Pumps were not guaranteed to pump beyond 7 days (grey section). One-way ANOVA with Dunnett’s post-hoc test. *p<0.05, ** p<0.01 vs baseline; n=4 per dose.

Results indicated that there were significant differences in paw withdrawal latency ($F_{13,3} = 11.45$, $P<0.0001$). Paw withdrawal latencies were significantly higher than the baseline value on days 1 ($P<0.01$; 95%CI -29.12 to -8.297), 2 ($P<0.01$; 95%CI -31.07 to -10.24), 3 ($P<0.01$; 95%CI -29.10 to -8.277), 4 ($P<0.01$; 95%CI -32.72 to -11.90), 5 ($P<0.01$; 95%CI -32.72 to -11.90), 6 ($P<0.01$; 95%CI -29.59 to -8.767), 7 ($P<0.01$; 95%CI -28.28 to -7.450), 8 ($P<0.05$; 95%CI -22.34 to -1.510), 9 ($P<0.01$; 95%CI -30.31 to -9.485), 10 ($P<0.05$; 95%CI -21.82 to -0.9971), 11 ($P<0.01$; 95%CI -29.42 to -8.595), 13 ($P<0.01$; 95%CI -32.72 to -11.90); but not
on day 12 (P>0.05; 95%CI -5.063 to 15.76) following osmotic pump implantation (Figure 7.3). No adverse events occurred during the experiment.

**Cohort 2**

**Figure 7.4 Paw withdrawal latencies.**
Adult, male SD rats received subcutaneous implantation of osmotic pumps containing methadone (1.05 mg/day) (■) or methadone (0.35 mg/day) (▲). Paw withdrawal latencies to radiant heat applied to the plantar aspect of the hind paw were determined at baseline (B) and once daily afterwards for 14 days. Pumps were not guaranteed to pump beyond 7 days (grey section). One-way ANOVA with Dunnett’s post-hoc test. *p<0.05 vs baseline; n=2 per dose.

**Figure 7.5 Tail withdrawal latencies.**
Adult, male SD rats received subcutaneous implantation of osmotic pumps containing methadone (1.05 mg/day) (■) or methadone (0.35 mg/day) (▲). Tail withdrawal latencies to radiant heat applied to the plantar aspect of the mid-tail section were determined at baseline (B) and once daily afterwards for 14 days. Pumps were not guaranteed to pump beyond 7 days (grey section). One-way ANOVA with Dunnett’s post-hoc test. *p<0.05; **p<0.01 vs baseline; n=2 per dose.

There were significant differences following 0.35 mg/day methadone administration for both paw (F_{12,1}=2.551, P=0.495) and tail (F_{12,1}=4.487, P=0.0073) withdrawal latencies. Dunnett’s post hoc analysis indicated that paw withdrawal latencies were significantly lower than the
baseline value on days 8 (P<0.05; 95%CI 1.056 to 16.71) and 12 (P<0.05; 95%CI 0.3538 to 16.01) following osmotic pump implantation (Figure 7.4). Dunnett’s pos hoc analysis indicated that tail withdrawal latencies were significantly lower than the baseline value on days 7 (P<0.01; 95%CI 1.684 to 13.18), 8 (P<0.05; 95%CI 0.3087 to 11.81), 9 (P<0.05; 95%CI 1.386 to 12.88), 10 (P<0.05; 95%CI 0.9887 to 12.49), 11 ((P<0.05; 95%CI 1.301 to 12.80), 12 (P<0.05; 95%CI 0.7912 to 12.29) and 14 (P<0.05; 95%CI 0.1412 to 11.64) following osmotic pump implantation (Figure 7.4).

For the 1.05 mg/day methadone treatment group, there were no significant differences in mean paw withdrawal latency times (F12,1=0.9713, P= 0.5197; Figure 7.4). While there was a significant difference in means for the tail withdrawal latency time during the 14 days following osmotic pump implantation (F12,1=3.824, P=0.0139), Dunnett’s post hoc analysis indicated no statistically significant difference in mean values when compared with baseline days 1-14; P>0.05; Figure 7.5). No adverse events occurred during the experiment.

Cohort 3

**Figure 7.6 Paw withdrawal latencies.**

Adult, male SD rats received subcutaneous implantation of osmotic pumps containing methadone (0.11 mg/day) (■) or methadone (0.04 mg/day) (▲). Paw withdrawal latencies to radiant heat applied to the plantar aspect of the hind paw were determined at baseline (B) and once daily afterwards for 14 days. Pumps were not guaranteed to pump beyond 7 days (grey section). One-way ANOVA with Dunnett’s post-hoc test. *p<0.05 vs baseline; n=4 per dose.
Figure 7.7 Tail withdrawal latencies.
Adult, male SD rats received subcutaneous implantation of osmotic pumps containing methadone (0.11 mg/day) (■) or methadone (0.04 mg/day) (▲). Tail withdrawal latencies to radiant heat applied to the plantar aspect of the mid-tail section were determined at baseline (B) and once daily afterwards for 14 days. Pumps were not guaranteed to pump beyond 7 days (grey section). One-way ANOVA with Dunnett’s post-hoc test. *p<0.05; **p<0.01 vs baseline; n=4 per dose.

For paw withdrawal latency times there were no significant differences in mean values in the 0.04 mg/day methadone treated group (F3,9=1.372 P=0.2490). However, there were significant differences in mean values for paw withdrawal latency times in the 0.11 mg/day methadone treated group (F3,9=3.220, P=0.0088); Dunnett’s post hoc analysis showed significant increases in mean paw withdrawal latency times on days 1 (P<0.05; 95%CI -9.512 to -0.091) and 6 (P<0.05; 95%CI -10.08 to -0.657) following osmotic pump implantation.

With regard to tail withdrawal latency times there were significant differences in mean values for both the 0.11 mg/day methadone (F3,9=2.63, P=0.025) and 0.04 mg/day methadone (F3,9=4.65, P=0.0009) treatment groups. On day 8 following osmotic pump implantation, Dunnett’s post hoc analysis indicated significant decreases in tail withdrawal latency for both the 0.11 mg/day methadone (P<0.05; 95%CI 0.535 to 6.002) and 0.04 mg/day methadone (P<0.05; 95%CI 1.911 to 7.499) treatment groups. No adverse events occurred during the experiment.

7.9.7 Discussion

Cohort 1
The rats in this cohort were administered methadone at a rate of 3 mg/day and tested for 13 days following osmotic pump implantation. While the dose used in this experiment was the same as that used in the previous acute dosing experiment, instead of a bolus dose, the dose
was administered over a 24 hour period. The pumps used in this cohort guaranteed continuous methadone infusion for at least 7 days. Results show that for the first 7 days following implantation, rats achieved close to the maximal imposed threshold with the Hargreave’s test. This suggests that methadone delivered at this rate was able to achieve and maintain antinociception throughout this treatment period. In contrast to the study by Vanderah and colleagues (2001b) where morphine initially demonstrated antinociception and subsequently hyperalgesia, the results of this study indicated that no tolerance to the methadone dose developed. Furthermore, no hyperalgesia was observed during the methadone administration period. This suggests that the methadone dose used in this study was too high, and the physiological mechanisms that normally oppose the effects and cause tolerance and/or hyperalgesia were overwhelmed by the large dose of methadone used.

One other explanation for the continued high response could be due to increased muscle tone caused by the relatively high dose of methadone used in this cohort. The paw withdrawal latency time is dependent on a reflex reaction of the paw. It is well documented that large doses of opioids, including methadone, can cause increased muscle tone, muscular rigidity (Barnett et al., 1975) and in its most severe form, catatonia, otherwise known as Straub’s reaction (Bilbey et al., 1960). Despite the fact that muscle rigidity was not, this dose of methadone may have increased paw withdrawal latencies by increased muscle tone rather than it being a reflection of changes in nociception.

The data past day 7 are difficult to interpret due to the variable nature of the pumps. The osmotic pumps used in the study were assured to last at least 7 days; due to the small variation in both pumping rate and pump volume, the time at which the pumps do not continue to pump solution is variable. This is seen in the rats following day 7, with large variation seen in response following this time. Consequently, some rats would have experienced opioid withdrawal at various times during this time.

**Cohort 2**
The rats in this cohort were administered a dose of approximately one-third and one-tenth of the dose used in the previous study. In addition, Hargreaves’ test was performed on the tail of the rats as well as the paw.
No significant differences in either paw or tail withdrawal latency were observed in the rats infused with 1.05 mg/day of methadone. This suggests that the rats may have developed analgesic tolerance to the methadone given at this rate.

There was no significant difference in paw withdrawal latency during the 0.35 mg/day methadone assured infusion period of the first 7 days. There was a significant decrease in paw withdrawal latency on the 8th and 12th day following osmotic pump implantation. While there would have been a high chance that the osmotic pump was still administering methadone by the 8th day and may indicate methadone-induced hyperalgesia this assumption can not be certain; by the 12th day the significant decrease compared with baseline probably indicates hyperalgesia associated with opioid withdrawal. With regard to tail withdrawal latency during the 0.35 mg/day methadone infusion, rats developed hyperalgesia by the 7th day. This hyperalgesia continued during the subsequent 7 days, except for the penultimate testing day. Again, the data during this period are difficult to interpret, due to the variable termination of the infusion pumps. The hyperalgesia during this period could be due to one of three possibilities: long-lasting methadone-induced hyperalgesia, opioid-withdrawal hyperalgesia or a combination of both reasons.

**Cohort 3**

This cohort investigated doses of continuously administered methadone at the rate of 0.11 and 0.03 mg/day in male, SD rats for a week.

The dose of 0.11 mg/day methadone did not cause any statistically significant differences in either tail or paw withdrawal latencies during the definite infusion period. This indicates that this dose does not have the ability to induce either analgesia or hyperalgesia, as detectable by the Hargreaves’ test. As with the other cohorts, some hyperalgesia was observed on the 8th day following osmotic pump implantation indicated by the significant decrease in tail withdrawal latency. However, this data point is beyond the guaranteed time that the pumps may continue to function and therefore no firm conclusions can be made regarding the reasons for this decrease in tail withdrawal latency.

The pain sensitivity data obtained from the rats administered methadone at the rate of 0.03 mg/day show similar results to the rats treated with methadone at a rate of 0.11 mg/day. The only difference was the two significant increases in paw withdrawal latency times at days 1
and 6 following osmotic pump infusion. The increase at day 1 probably indicates that the rats initially experienced a degree of analgesia. The significant increase at day 6 probably indicated an irregularity in data collection at this data point, the reason for this is unexplainable.

7.9.8 Conclusion

Overall the 3 mg/day dose of methadone was considered too high and caused continuously increased paw withdrawal latency times. The remaining doses of methadone, except for the 0.35 mg/day dose, did not show any potential to cause hyperalgesia during the guaranteed administration period. The 0.35 mg/day methadone dose appeared to show the greatest potential to induce hyperalgesia, by decreasing tail withdrawal latency times during the administration period. Therefore, this dose of methadone warrants further investigation with regard to its capability to induce hyperalgesia in the male SD rat.
7.10 Nociceptive effects of methadone

7.10.1 Introduction

The previous dose-finding studies indicated that methadone given at a rate of 0.35 mg/day (or baseline-weight based rate of 1 mg/kg/day) could induce hyperalgesia in rats. The objective of this study was to test if this dose of methadone could induce hyperalgesia using a randomised, blinded, placebo controlled study design.

7.10.2 Aim

The aim of this study was to develop a model of methadone-induced hyperalgesia in the male SD rat.

7.10.3 Hypothesis

Methadone given at the rate of 1 mg/kg/day for 14 days to male SD rats can induce hyperalgesia detectable by Hargreave’s test.

7.10.4 Study design

This study consisted of 2 cohorts of rats. Each cohort of rat was familiarised with Hargreaves’ test. Subsequently, each group was tested for changes in nociceptive threshold. Cohort one were tested for 14 days following osmotic pump implantation; following the final nociceptive test, a blood sample from each rat was taken and qualitatively tested for methadone using LCMS (section 2.8.1). Quantitative assessment of plasma was not possible as published method was validated for human blood. Furthermore, plasma methadone concentrations were not required for this study. A similar protocol was employed for cohort two, however, following testing on day 14, osmotic pumps were retrieved but no blood sample was taken for methadone quantification. Rats were monitored for opioid withdrawal between days 14 and 15 and tested for nociceptive threshold for a further 7 days.

Within each cohort, rats were implanted in an investigator-blinded, randomised manner with an osmotic pump containing either saline placebo or methadone. Methadone was administered at a rate of 1 mg/kg/day based on baseline weight. Study parameters are summarised in Table 7.6.
Table 7.6 Summary of experimental variables and testing methods

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Methadone Rate (mg/day)</th>
<th>Methadone Rate (weight based) (mg/kg/day at baseline)</th>
<th>N per treatment</th>
<th>Pump Model (see Table 7.2)</th>
<th>Rat Weight at baseline (mean) (g)</th>
<th>Light Intensity (units)</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
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<td>1</td>
<td>6</td>
<td>2002 (1)(2)</td>
<td>200</td>
<td>2.0</td>
</tr>
</tbody>
</table>

7.10.5 Statistical analysis

Data were analysed using a repeated measures two-way ANOVA (mixed-model) and where appropriate, post-hoc t-tests compared differences between the treatment groups.

7.10.6 Results

Figure 7.8 Paw withdrawal latencies.

Adult, male SD rats received subcutaneous implantation of osmotic pumps containing saline placebo (■) or methadone (0.25 mg/day) (▲). Paw withdrawal latencies to radiant heat applied to the plantar aspect of the hind paw were determined at baseline (B) and once daily afterward for 14 days. No significant change in pain sensitivity was observed (n=4 per treatment).

Qualitative plasma methadone analysis confirmed the treatment allocation of each rat (methadone or saline). No adverse event occurred during the experiment.

Table 7.7 Two-way repeated measures ANOVA results

<table>
<thead>
<tr>
<th>Effect</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F Value</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>0.90</td>
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<td>Time</td>
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<td>84</td>
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<td>0.05</td>
</tr>
<tr>
<td>Interaction</td>
<td>14</td>
<td>84</td>
<td>0.84</td>
<td>0.62</td>
</tr>
</tbody>
</table>
Results of the two-way repeated measures ANOVA are presented in Table 7.7. There were no significant effects observed with regard to time, treatment or interaction. It was evident that there were large variations in paw withdrawal latencies and there appeared to be a cyclic variation with a minimum that was observed at baseline, day 4, 9 and 13 - approximately every 4 days. Following the conclusion of the experiment, it was discovered that on the same floor but in the adjoining building demolition work occurred during the familiarisation and testing period, mainly after hours. Anecdotally, the rats were also very difficult to settle and usually required longer that the standard 10 minutes acclimatisation time before testing could commence. Research has shown that chronic intermittent broadband noise and vibration such as that caused by building works can be stressful in rats and this can have an effect on a wide variety of physiological systems (McDonald et al., 2002). In fact, exposure to noise can induce endogenous opioid-mediated antinociception (hypoalgesia) in rats (Bellgowan and Helmstetter, 1996).

Accordingly, the protocol was repeated; however this time the experiment was conducted at Waite campus, in the quarantine facility (Animal Biotechnology Centre) of Laboratory Animal Services.

7.10.7 Repeat testing results

Figure 7.9 Paw withdrawal latencies.
Adult, male SD rats received subcutaneous implantation of osmotic pumps containing saline placebo (■) or methadone (0.20 mg/day) (▲) which was removed following testing on day 14 (grey section). Paw withdrawal latencies to radiant heat applied to the plantar aspect of the hind paw were determined at baseline (B) and once daily afterward for 21 days. Significant changes in pain sensitivity were observed (post hoc t-test #P<0.05, ##P<0.01 vs saline baseline post hoc t-test *P<0.05, **P<0.01 vs saline; n=6 per treatment).
Table 7.8 Two-way repeated measures ANOVA results

<table>
<thead>
<tr>
<th>Effect</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>Time</td>
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<td>Interaction</td>
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<td>0.026</td>
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</table>

Table 7.8 presents the results from the two-way repeated measure ANOVA. Data from days 4 and 5 were missing due to an inability to collect data at these time points. The effect of time was significant (P<0.0003), suggesting that for both groups pain levels were not constant at all times. More importantly, there was a significant group by time interaction effect (P=0.026), suggesting that the effect of the treatment depended on the time point at which pain was measured. Post hoc t-test analysis showed that the methadone treated rats had a mean paw withdrawal latency lower than corresponding values saline on days 8, 9, 10, 11, 12, 14 and 17. At all other time points the mean paw withdrawal latency was not significantly different from saline (P>0.05) (Figure 7.9).

Differences in mean paw withdrawal latencies for the saline treated group were analysed using one-way ANOVA and showed significance (F_{19,5}=2.1, P<0.05). Dunnett’s post hoc t-tests indicated that saline treated rats had decreased paw withdrawal latencies on days 1 (P<0.05, 95%CI 0.8 to 13.7) and 3 (P<0.01, 95%CI 2.3 to 15.3) when compared with baseline values.

Measures of opioid withdrawal were made on days 14 and 15. No rat showed any sign of ptosis, abnormal posture, irritability/hostility on handling or diarrhoea on either day 14 or 15. Mean rat weight for the saline (316 vs 320 g, P>0.05) and methadone (298 vs 302 g, P>0.05) treated groups did not significantly change between day 14 and 15, respectively. No adverse events occurred during the experiment.

7.10.8 Discussion

The aim of this study was to formally evaluate if methadone could induce hyperalgesia in a rat model. Data obtained from the previous dose-finding studies indicated that a dose of methadone of approximately 0.2 mg/day (corrected for baseline weight) showed promise for the placebo-controlled study.
Chapter 7 – Animal model of methadone-induced hyperalgesia

The initial study highlighted the importance of maintaining suitable environmental factors when performing behavioural studies in animals. The mean paw withdrawal latency of the saline treated group in the first cohort of this study was quite variable and appeared to have a unique pattern to it. This variation in nociceptive threshold was probably associated to stress induced by noise and vibration related to building works. This invalidated the initial experiment.

The protocol was repeated, with minor variations, in a quieter environment. The effect of time was statistically significant, indicating that pain levels for each group were not constant. However, more importantly, there was an interaction (between time and treatment) effect implying that the effect of the methadone administration on pain threshold was dependent on the time point at which it was measured.

Post hoc analysis showed that during the infusion period, mean paw withdrawal latency was decreased in the methadone treatment group compared with saline treatment group for the second week of the infusion. This demonstrates that methadone, given at a rate of 0.2 mg/day can induce and maintain thermal hyperalgesia in rats. The time of onset of this hyperalgesia was approximately 8 days. This is comparable to the dose-finding study using the same dose rate. However, in the dose-finding study, thermal hyperalgesia in the paw was observed on day 8, a time-point at which it cannot be assured that methadone was continued to be infused. The results of this extended study confirm that methadone-induced hyperalgesia can develop by day 8 of the infusion.

In contrast to the only other study that has investigated the time-course of nociceptive changes induced by continuous opioid infusion, this is a relatively long time. Vanderah and colleagues (2001b) investigated the infusion of morphine at a rate of 1.08 mg/day using a comparable model. However, these researchers found that hyperalgesia could be induced by day 2 of the infusion.

Other studies that have utilised continuous infusions have shown the development of hyperalgesia only at selected time-points. For instance, Vanderah and colleagues (2000) demonstrated that a continuous spinal infusion of DAMGO produced thermal hyperalgesia by day 6. However, no other data were presented; therefore hyperalgesia may have developed before this point. Similar results to those published by Vanderah and colleagues (2000) have
been obtained for spinal administration of (−)-oxymorphone (59.2 nmol/µL/hr) (Gardell et al., 2006). Other studies that have utilised intermittent administration of opioids, either spinally or systemically, have shown the development of hyperalgesia over the course of several days (Mao et al., 1994; Célèrier et al., 2001). In contrast, this study demonstrated that methadone-induced hyperalgesia takes at least a week to develop. This may be due to the unique pharmacodynamic qualities of methadone, as it possesses both opioid agonist and NMDA antagonist properties (Kristensen et al., 1995; Gorman et al., 1997). Previous studies have shown that co-administration of an NMDA antagonist can prevent the development of opioid induced hyperalgesia as well as potentiate the analgesic effects of opioid agonists (Trujillo and Akil, 1991; Mao et al., 1998; Bell, 1999; Allen and Dykstra, 2000; Bulka et al., 2002; Lauin et al., 2002). In fact, it has been shown that the administration of (S)-methadone can attenuate NMDA-induced hyperalgesia as well as block morphine analgesic tolerance (Davis and Inturrisi, 1999). It could therefore be argued that the anti-hyperalgesic properties of methadone (conferred by the (S)-methadone) could cancel out the opioid-induced hyperalgesia. However, this annulment of effect was not observed in this study and suggests that the hyperalgesic properties of racemic methadone are greater than its anti-hyperalgesic qualities.

Of note is the fact that the dosing rate of 0.2 mg/day of methadone did not notably produce any statistically significant antinociception during the initial dosing period. This is not surprising given that acute, bolus doses of methadone as low as 1 mg/kg provide minimal, short-lived analgesia in SD rats when measured using the hotplate test (Bulka et al., 2004; Peckham and Traynor, 2006). The lack of analgesia seen in the present study contrasts with the work of Vanderah and colleagues (2001b), who observed antinociception initially (6 hours following pump/pellet implantation). The protocol of the current study prevented testing so soon after implantation as anaesthesia was induced using pentobarbitone. Consequently rats were still recovering from surgery at the 6 hour time point. In addition to this, it must be noted that during the first two days following the administration of the methadone and saline, there was large variation in the paw withdrawal latency of the saline group. This large initial inter-day variation may have confounded any observation of analgesia in the methadone treated group. While this initial variation was not observed in the original dose-finding studies, the impact of anaesthesia and surgery may have influenced nociceptive pathways and, therefore, caused the large initial variation in thermal threshold values.
The dose of methadone administered in this study did not produce any observable abstinence syndrome (opioid withdrawal), when discontinued. This suggests that dependence did not develop and therefore emphasises the fact that the dose was relatively low. However, nociceptive studies by Athanasos and colleagues (2006) have shown that the degree of opioid-induced hyperalgesia in methadone maintained subjects was not dose-dependent. The initial dose-finding studies presented earlier were limited to a 7 day infusion and this study indicated that methadone-induced hyperalgesia requires at least that length of time to develop in rats. Therefore, with the previously studied methadone doses, if methadone-induced hyperalgesia is not dose-dependent, an observation of hyperalgesia may have been missed due to the brevity of the experiments. Further studies investigating different doses of methadone over longer infusion periods are warranted to investigate this.

While it may be hypothesised that methadone, regardless of the dose, may induce hyperalgesia, the fact that the dose used was relatively small cannot be overlooked. The study of Crain and Shen (2001) have shown that low-doses (sub-analgesic) of morphine have the ability to induce hyperalgesia; this study and previous studies suggest that the Gs-coupled opioid receptor activation may mediate this effect (Crain and Shen, 1992; Crain and Shen, 1998; Crain and Shen, 2000a; Crain and Shen, 2001). It is possible that the dose of methadone used in this study exploited similar mechanisms; however, further studies are required before any definite conclusion regarding mechanisms can be made.

Additionally, other researchers have proposed that descending facilitatory pathways play a critical role in the development of opioid-induced hyperalgesia (Urban and Gebhart, 1999; Vanderah et al., 2001a; Gardell et al., 2002; Porreca et al., 2002; Gardell et al., 2006). While not investigated in the current study, it can be hypothesised that the underlying mechanisms of methadone-induced hyperalgesia are similar to the mechanisms implicated by other opioid agonists. Nevertheless, future studies investigating potential mechanisms are needed before any definite conclusions can be made regarding how methadone induces hyperalgesia.

One of the limitations of this study was that it utilised only one nociceptive test. Without utilising another modality of nociception, it cannot be concluded that modulation of nociceptive pathways are the basis of the changes observed. For instance, as Hargreaves’ test is a thermal test, changes in the ability of rats to thermoregulate may also have a role to play in the results. Opioids have been shown to alter thermoregulation in rodents (Adler et al.,
Chapter 7 – Animal model of methadone-induced hyperalgesia

1988). This may translate to modified cutaneous temperatures, a variable that has been shown to influence the results obtained from Hargreaves’ test (Dirig et al., 1997). To further strengthen the conclusions made by this study, other nociceptive tests should be used to show if methadone-induced hyperalgesia is associated with other modalities of pain such as mechanical pain.

7.10.9 Further studies

This study has provided data that show that systemically administered methadone can produce hyperalgesia in the SD rat. This model of methadone-induced hyperalgesia provides a basis for future studies that can examine mechanisms and treatment strategies for methadone-induced hyperalgesia.

The development of this model has generated several hypotheses and unanswered questions. For instance, the methadone used in this study was administered as a racemate mixture of each enantiomer. Other studies have shown that the active (normally analgesic), but not the inactive, isomer of oxymorphone, can induce hyperalgesia (Gardell et al., 2006). Furthermore, studies that have investigated the analgesic properties of the different enantiomers of methadone have found that (R)-methadone has analgesic activity while (S)-methadone is inactive (Scott, 1948; Ingoglia and Dole, 1970). This stereoselective nature of methadone analgesia may be reflected in its ability to induce hyperalgesia. Future studies could investigate the potential of each of the isomers of methadone to induce hyperalgesia.

As highlighted before, the dose of methadone used in this study was comparatively low. It takes at least 8 days of administration to induce hyperalgesia and does not induce a withdrawal syndrome when discontinued. The dose chosen was based on initial studies that investigated the administration of methadone over 7 days. It would be interesting if higher doses that produced analgesia in the initial dose finding studies (i.e. 3 mg/day) could induce hyperalgesia if a longer dosing regimen were employed. Higher doses administered over longer periods, as in methadone maintained patients, would better mimic the clinical situation where we observe hyperalgesia. Furthermore it would be interesting to investigate if long-term methadone administration that is capable of inducing hyperalgesia also produces opioid tolerance. Several other studies have shown that opioid-induced hyperalgesia develops in parallel with tolerance to analgesic effects (Mao et al., 1994; Mao et al., 1995b; Laulin et al., 1999).
Célerier and colleagues (2001) postulated that following the resolution of opioid-induced hyperalgesia, a new allostatic balance between antinociception and pronociception is achieved. Therefore, naloxone-precipitated hyperalgesia is achievable long after the discontinuation of opioid administration. This hypothesis was based on a paradigm using heroin. It would be worthy then to investigate if this is also the case for methadone. This paradigm has been attempted before in human subjects defined as former opioid users; cold pressor pain threshold and tolerance times were compared 24 hours following either a 50 mg oral dose of naltrexone or inactive placebo (Liebmann et al., 1997). No significant changes were reported. However, the cold pressor test used a relatively high temperature water bath (4-6 °C) and thus approximately half the sample population reached the imposed maximal tolerance time of 7 minutes. Due to these methodological inadequacies, repetition of a similar study protocol using a more robust cold pressor test and/or other pain models would be worthy of consideration.

Several past studies have shown that the spinal administration of opioids can induce hyperalgesia and, in doing so, have eliminated potentially confounding peripheral mechanisms (Vanderah et al., 2000; Gardell et al., 2006). Similarly, future studies utilising a model of methadone-induced hyperalgesia could investigate if the spinal administration of methadone can induce hyperalgesia.

The pharmacotherapies used for maintenance treatment are not only limited to the full opioid agonists such as methadone, morphine and heroin; the partial agonist buprenorphine is gaining popularity as a pharmacotherapy and has also been shown to induce hyperalgesia in an opioid maintained population (Compton et al., 2001). The current animal model could be adapted to utilise these drugs to further investigate the opioid-induced hyperalgesia.
7.11 Summary

The objective of this series of studies was to investigate if methadone could induce hyperalgesia using an animal model. Initial studies attempted to replicate previous work investigating morphine-induced hyperalgesia using the same model. This was then followed by dose-finding studies investigating a range of chronic, continuously administered doses of methadone. The most promising methadone dose was chosen to be used in a blinded, randomised and placebo controlled study to investigate the nociceptive changes induced by methadone in the male SD rat.

This series of experiments demonstrated that methadone given at a continuous rate of 0.2 mg/day for 7 days to male SD rats can increase nociceptive threshold when measured using Hargreaves’ test. In addition it has been shown that nociceptive threshold can resolve to control levels following cessation of the methadone administration.
8 GENERAL DISCUSSION AND SUMMARY

8.1 Summary of major findings

In conclusion, the major contributions and findings of this thesis are as follows. Firstly, the nociceptive profile of chronic pain patients managed with opioids reflects that of methadone maintained patients. This reveals that regardless of the reason for long-term opioid use, opioids have the potential to negatively impact on the pain sensitivity of individuals. Secondly, the cessation of long-term opioid use is associated with a variable nociceptive profile suggesting that opioid use may have nociceptive effects long-after its cessation. Thirdly, the first two studies also provided preliminary evidence suggesting an association between opioid use and negative mood and affect in opioid administering patients and its consequential resolution in former opioid users. Fourthly, the novel use of existing, well-established medications may have the potential to provide effective analgesia to methadone maintained patients. The use of ultra-low dose naloxone in methadone maintained patients requires further investigation regarding its effectiveness in this population. The use of remifentanil demonstrates that methadone maintained patients have a high degree of cross-tolerance to this opioid yet antinociception is possible. Lastly, the animal study demonstrated that methadone induces hyperalgesia in a rat model and that hyperalgesia resolves following opioid cessation. This animal study has provided a link between existing pre-clinical data and clinical evidence for opioid-induced hyperalgesia.

The studies contained in this thesis can be linked by an underlying theme regarding the resolution and/or reversal of opioid-induced hyperalgesia. The nociceptive studies in the former opioid users and the animal study suggest that there is the potential for hyperalgesia to resolve back to ‘normal’ levels or pre-opioid administration levels. This thesis, and in particular the animal study, provides evidence that following the discontinuation of opioids, hyperalgesia is reversible. Furthermore, this thesis has aimed to investigate the active reversal of hyperalgesia using pharmacological methods. As such, one of the objectives has been to investigate potential analgesic approaches in methadone maintained patients. The studies investigating the use of naloxone and remifentanil have suggested that, depending on the approach utilised, hyperalgesia can be reversed, or at least overcome temporarily. The overall results of this thesis demonstrate that increased pain sensitivity occurs in populations chronically exposed to opioids and that this hyperalgesia is reversible, either following the cessation of opioids, or following particular pharmacological interventions.
8.2 Clinical implications of research findings

The findings of this thesis have important implications for the management of acute pain in methadone maintained patients; moreover they offer valuable direction for the treatment of pain in opioid-tolerant patients in general. Finally, the results of this thesis provide critical insights into other disciplines including addiction and dependence.

8.2.1 Acute pain management in methadone maintained patients

In the clinical setting, lowered pain threshold caused by opioid-use may not necessarily be immediately evident; yet what may be a minor inconsequential injury to a non-opioid user, the presence of hyperalgesia potentially results in exacerbated pain intensity. Furthermore, hyperalgesia may increase the possibility that the original pain has the potential to become persistent or chronic pain. This could result in increases of opioid dose and consequently expose the individual to an increased probability to developing a cycle of addiction and potentially exacerbating opioid use.

The potential for the under-treatment of pain in methadone maintained patients is highly likely. The use of experimental pain models allows for the comparison of adequate doses of opioids in both opioid-naïve and opioid tolerant patients. Other authors have suggested that opioid dependent individuals may require higher doses of opioids for acute pain management (section 1.12.2). The remifentanil study indicated that doses 20-30 times those of opioid-naïve patients may be required for effective analgesia in methadone maintained patients. The tolerability of remifentanil in methadone maintained patients suggests that high potency opioids may be effective for the treatment of severe, acute pain in these patients. This study has also demonstrated the degree of opioid cross-tolerance present in methadone maintained patients. This may be translatable to other opioids and other opioid maintained populations. However, further research is required before definite conclusions can be made regarding appropriate opioid dosing regimens in opioid maintained patients.

8.2.2 Chronic pain

Recent research has indicated that chronic pain can be frequently associated with the incidence of an acute pain injury (Blyth et al., 2003), whether it be acute back pain, acute herpes zoster or certain types of surgery (Carey et al., 2000; Perkins and Kehlet, 2000; Macrae, 2001). There is no doubt that different mechanisms underlie the progression from acute pain into persistent chronic pain, including central sensitisation, neuropathy and psychological dysfunction (Perkins and Kehlet, 2000); the presence of opioid-induced
hyperalgesia may have a role to play in this progression. Moreover, the high incidence of chronic pain in the opioid maintained population may also be linked with opioid-induced hyperalgesia. There is the potential that in long-term opioid users, lowered pain thresholds result in the emergence of chronic pain.

In addition, hyperalgesia caused by opioid use may have a role in pseudo-addiction. The development of increased pain sensitivity caused by opioid use may result in the need for increases in opioid dose or longer-term opioid use. Consequently, the drug-seeking behaviour that is normally associated with addiction may become apparent. This situation may be applicable not only to chronic pain patients administering opioids but also opioid maintained patients with developing chronic pain. There is also the possibility this pseudo-addiction, which is potentiated by hyperalgesia, may be observed in the acute pain setting such as post-surgery. However, this behaviour may actually be a result of a genuine need for pain relief caused by opioid-induced hyperalgesia. Further studies are required to investigate the nociceptive impact of opioids, not only in chronic pain patients, but also acute pain patients.

### 8.2.3 Former opioid using populations

The long-term modification of the opioid-nociceptive system has important implications for individuals attempting to cease opioid use. Increased acute pain sensitivity caused by opioid use has repeatedly been shown to be related to the termination of opioid use. It has normally been thought that this increased pain sensitivity is relatively short-lived and generally parallels the opioid-withdrawal syndrome. The studies in this thesis demonstrate that formerly-dependent subjects have variable nociceptive profiles; this may be related to the concept that opioid use results in a nociceptive system that is highly susceptible to alteration (Célèrier et al., 2001). For former opioid users this may mean that they are more sensitive to pain even though they may have ceased opioid use for quite a period of time. Furthermore, these former opioid users may have a ‘vulnerable’ nociceptive system such that even relatively small opioid doses may trigger hyperalgesia more readily in these individuals than in an opioid naïve individual. In terms of addiction, this altered nociceptive system may potentially predispose such individuals to a greater incidence of addiction and may hinder the ability to discontinue opioid use.
8.2.4 Ultra-low dose opioid antagonist and opioid agonist combinations

The use of an adjuvant drug that potentiates the effectiveness and reduces the side effects of an opioid is an extremely attractive idea. Preclinical studies suggest that the use of ultra-low dose opioid antagonist with opioid agonists may be successful; however, clinical studies are yet to conclusively demonstrate such an advantageous combination. If such an opioid antagonist/agonist combination exists, it has the potential to revolutionise opioid therapy. In terms of analgesic management, potentiation of analgesic effects suggests that lower agonist doses would be required and thus lower costs could be involved. Furthermore, lower opioid agonist doses potentially equate to a lower incidence of side-effects. This may mean that fewer resources would be required to monitor and treat adverse consequences such as respiratory depression, constipation, nausea and vomiting. For maintenance treatment, such a combination may mean more effective substitution programs due to better ‘holding’ throughout the inter-dosing period (due to less craving), less illicit drug use, easier dose-reduction and more successful opioid cessation. In general, there is the potential that such a combination may be useful in reducing tolerance, dependence or addiction and this has benefits for all opioid users.

8.2.5 Addiction science

Parallels exist between the neurobiology of addiction and pain research. Addiction has been likened to a type of chronic pain syndrome with similar characteristics such as emotional (affective) pain, dysphoria, stress and anxiety (Koob, 2006). As such, findings from pain research may provide insights to addiction and vice versa. In fact, it has been hypothesised that pain, whether it be affective, persistent or both, may be a driving force behind addiction (Koob, 2006). Recent reports have shown the increased use (and potential abuse) of opioids in the community (Compton and Volkow, 2006). While these data may indicate that there is better or possibly excessive treatment of pain, they do suggest that more individuals are being exposed to opioids. Therefore, the incidence of hyperalgesia will no doubt be higher as well. As indicated previously, opioid use may have long-term effects, even after their cessation; this may undermine the clinician’s ability to treat pain in the future and may have important implications for addiction liability. Furthermore, the overlap in the incidence of chronic pain and addiction (section 8.2.2) indicates that there may be a significant interaction caused by opioid-induced hyperalgesia occurring.
Chapter 8 – General discussion and summary

8.3 Directions for further research

8.3.1 Mechanisms of opioid-induced hyperalgesia

There is a paucity of clinical studies demonstrating opioid-induced hyperalgesia; this is primarily due to the difficulty in differentiating hyperalgesia and opioid-tolerance in the clinical setting. While experimental pain models in humans go partway to addressing this issue, further research investigating the incidence of hyperalgesia and dissecting this from opioid tolerance in relevant populations is required to assess the clinical significance of opioid-induced hyperalgesia.

There are ethical concerns associated with the long-term administration of opioids to otherwise healthy and pain-free individuals. This field of research would benefit considerably if the pain sensitivity of opioid-naïve populations could be assessed before, during and following the administration of opioids. Moreover, this would offer an important insight into the induction, progression and resolution of opioid-induced hyperalgesia. While animal models indicate that the commencement of opioid administration is associated with increased pain sensitivity, this phenomenon is yet to be conclusively demonstrated in human studies. While the results contained in this thesis go partway to demonstrating this, further clinical studies are required to differentiate if opioids induce hyperalgesia or if an inherently increased sensitivity to pain predisposes an individual to opioid use and potentially greater degrees of long-term opioid use and consequential addiction and dependence.

The field of genetics offers exciting prospects allowing researchers to investigate the heritability and innate propensity for the incidence of disease. Even though the field of genetics is still in its infancy in the field of pain, genes have been identified that have been associated with the various responses to opioids and pain. No doubt future genetic research will identify genes that are involved with the prevalence and propensity of opioid-induced hyperalgesia in different individuals and populations.

Former opioid users may well provide a highly beneficial contribution to this field of research. The research contained herein suggests that the physiological disturbances in these individuals may re-adjust to ‘normal’ levels after long-term opioid use, yet other individuals continue to be highly pain sensitive. Studies utilising a longitudinal, prospective design are
required to accurately investigate if physiological mechanisms resolve to ‘normal’ levels following the cessation of long-term opioid use. Moreover, pre-clinical models used to determine alterations in the nociceptive system, such as the administration of a ‘naloxone challenge’ long after opioid use has ceased, resulting in a temporary increase in pain sensitivity (Célèrier et al., 2001) may be translated into clinical trials utilising former opioid users. This type of research would elegantly demonstrate if opioid use has long-term impact on the nociceptive system.

Pharmacokinetic data obtained from the chronic pain patients managed with methadone indicated that enantiomeric ratios of methadone may be related to alterations in pain sensitivity. The unique pharmacodynamic properties of the methadone and especially its individual enantiomers may well be useful in determining mechanisms associated with hyperalgesia. Future studies investigating what contribution the opioid agonist and NMDA antagonist properties of methadone enantiomers contribute to altering pain sensitivity are required.

Clinical studies have also demonstrated that acute peri- and post-operative administration of opioids may be associated with hyperalgesia. However, many of these studies have been confounded by using outcome measures, such as opioid dose, that may also indicate the incidence of tolerance. While it has long been believed that opioid-tolerance is a long-term consequence of opioid use, emerging evidence indicates that opioid-tolerance is a great deal more dynamic than previously thought. For example, the analgesic properties of morphine could be demonstrated in methadone maintained patients when they were at peak plasma methadone concentrations yet not at trough plasma methadone concentrations (Doverty et al., 2001a). Research investigating the plastic nature of the nociceptive system would not only help our understanding of tolerance but also hyperalgesia.

8.3.2 Acute pain management in opioid-tolerant patients

This thesis has demonstrated that significant antinociception can be achieved in methadone maintained patients with remifentanil. However, further research is still required to investigate the safety and efficacy of high doses of opioids in methadone maintained patients as well as other opioid-tolerant populations. Research in this field is so severely lacking that even published retrospective case reviews of what may be effective analgesic regimens would prove useful.
Mechanisms that may be able to turn off or reverse hyperalgesia may be clinically useful for the treatment of acute pain in opioid-maintained patients. Studies have shown that opioid-withdrawal and hyperalgesia are thought to be mediated by the development of central excessive excitation, facilitation of pain pathways and a reduction of inhibition of these pathways (Porreca et al., 2002). Whether related pharmacotherapies, such as NMDA-antagonists, NO synthase inhibitors and NK1-antagonists (Vanderah et al., 2000; Li et al., 2001a), that impede these mechanisms are potentially useful clinically is yet to be elucidated. Further investigations into these pharmacotherapies are required.

8.3.3 Animal model of opioid-hyperalgesia

Recently there has been an emphasis on translational research, that is linking preclinical, basic and clinical research such that findings from the bench can be related to clinic and back again especially in the field of pain (Mao, 2002b). The animal model of methadone-induced hyperalgesia in this thesis goes part way to addressing this issue. This animal study has provided a basis for future studies investigating both mechanisms as well as potential solutions for opioid-induced hyperalgesia. The clinical studies in this thesis have demonstrated that pain is multidimensional. Recent studies have emphasised the need to test more than one modality of pain. Certainly, future studies investigating underlying features of methadone-induced hyperalgesia in the rat may well benefit from using additional pain tests, such as mechanical pain.

The animal model in this thesis used racemic methadone; however, it has been shown that the pharmacology of the individual enantiomers is quite distinct. Reports have demonstrated enantiomeric differences for oxymorphone (Gardell et al., 2006), which may well hold true for the individual enantiomers of methadone. Furthermore, the methadone-induced hyperalgesia study in rats suggested that the induction and resolution time of hyperalgesia tends to be distinct from other opioid drugs. Understanding the reasons underlying why some opioids induce hyperalgesia rapidly whilst others do not will not only provide information regarding underlying mechanisms but could lead to better treatment solutions for both maintenance and pain patients.

This thesis has provided translational research links. The development of an animal model of methadone-induced hyperalgesia may also provide a basis for understanding the limitations of
some of the other studies presented in this thesis. Animal models have demonstrated evidence to suggest that ultra-low doses of opioid antagonist may potentiate the analgesic effects of opioid agonists, yet clinical studies, including the one presented in this thesis, have reported varied results. Utilising the animal model of methadone-induced hyperalgesia and trialling ultra-low doses of naloxone in this model may provide further evidence of whether this combination is clinically viable. Furthermore, the animal model of methadone-induced hyperalgesia provides a perfect opportunity to generate pre-clinical data for the aforementioned proposed studies.
8.4 Conclusion

Opioids have been successfully used for the treatment of pain for thousands of years and will no doubt continue to be effective analgesics in the future. Yet the unwanted effects of opioids including tolerance, dependence and increased pain sensitivity reduce their effectiveness. This thesis provides evidence that opioid use, especially methadone administration, is associated with hyperalgesia in both a broad range of clinical situations and can be demonstrated in pre-clinical studies. Understanding the mechanisms associated with opioid-induced hyperalgesia has the potential to not only benefit the long-term opioid user but also the general community by providing safer, more effective and better designed opioid analgesics. Strategies for acute pain management in opioid-tolerant as well as opioid-dependent populations are severely lacking. Nonetheless, this thesis has provided evidence for potentially useful approaches for treating pain in these challenging patients. These findings have the potential to reduce the costs and impact of pain. This could lead to the development of clinically-useful, evidence-based guidelines for the treatment of acute pain in opioid-dependent patients.
Chapter 9 – References

9 REFERENCES


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## 10 APPENDICES

### 10.1 Appendix 1 – Ultra-low dose naloxone study schedule

Table 10.1 Ultra-low dose naloxone in methadone maintained patients study schedule.

<table>
<thead>
<tr>
<th>PLANNED TIME (24hr)</th>
<th>PILOT STUDY TIME-POINT #</th>
<th>PRINCIPAL STUDY TIME-POINT</th>
<th>BLOOD, W/DL, AE, VITALS &amp; CP TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>~1000</td>
<td>Methadone Dose Previous Day</td>
<td>Methadone Dose Previous Day</td>
<td></td>
</tr>
<tr>
<td>0800</td>
<td>Arrive</td>
<td>Arrive</td>
<td></td>
</tr>
<tr>
<td>0805</td>
<td>Urine</td>
<td>Urine</td>
<td></td>
</tr>
<tr>
<td>0810</td>
<td>Insert Cannula</td>
<td>Insert Cannula</td>
<td></td>
</tr>
<tr>
<td>0820</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>0830</td>
<td>Start Saline IV</td>
<td>Start Saline IV</td>
<td></td>
</tr>
<tr>
<td>0850</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>0900</td>
<td>End Saline IV</td>
<td>End Saline IV</td>
<td></td>
</tr>
<tr>
<td>0900</td>
<td>Start 0.05 µg/min NLX IV</td>
<td>Start 0.005 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>0920</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>0930</td>
<td>End 0.05 µg/min NLX IV</td>
<td>End 0.005 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>0930</td>
<td>Start 0.1 µg/min NLX IV</td>
<td>Start 0.01 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>0950</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1000</td>
<td>End 0.1 µg/min NLX IV</td>
<td>End 0.01 µg/min NLX IV</td>
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</tr>
<tr>
<td>1000</td>
<td>Start 0.5 µg/min NLX IV</td>
<td>Start 0.05 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>1020</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1030</td>
<td>End 0.5 µg/min NLX IV</td>
<td>End 0.05 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>1030</td>
<td>Start 1.0 µg/min NLX IV</td>
<td>Start 0.1 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>1050</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1100</td>
<td>End 1.0 µg/min NLX IV</td>
<td>End 0.1 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>1100</td>
<td>Start 5.0 µg/min NLX IV</td>
<td>Start 0.5 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>1120</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1130</td>
<td>End 5.0 µg/min NLX IV</td>
<td>End 0.5 µg/min NLX IV</td>
<td></td>
</tr>
<tr>
<td>1130</td>
<td>Administer Methadone</td>
<td>Administer Methadone</td>
<td></td>
</tr>
<tr>
<td>1130</td>
<td>Cancel Daily Methadone Dose</td>
<td>Cancel Daily Methadone Dose</td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>Lunch</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>1230</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1330</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1430</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1530</td>
<td>Testing</td>
<td>Testing</td>
<td>✔</td>
</tr>
<tr>
<td>1535</td>
<td>Remove Cannula</td>
<td>Remove Cannula</td>
<td></td>
</tr>
<tr>
<td>1545</td>
<td>Discharge</td>
<td>Discharge</td>
<td></td>
</tr>
<tr>
<td>~1600</td>
<td>Follow-up Phone Call Next Day</td>
<td>Follow-up Phone Call Next Day</td>
<td>✔ AE ONLY</td>
</tr>
</tbody>
</table>

Legend: RAH Ethics committee protocol number #040210 A. NLX-naloxone, IV-intravenous
## 10.2 Appendix 2 – Remifentanil study schedule

Table 10.2 Remifentanil in methadone maintained patients study schedule.

<table>
<thead>
<tr>
<th>PLANNED TIME (24hr)</th>
<th>PILOT STUDY TIME-POINT #</th>
<th>PRINCIPAL STUDY TIME-POINT (*)</th>
<th>AE &amp; VITALS</th>
<th>SOWS &amp; MBG</th>
<th>CP TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>~1000</td>
<td>Methadone Dose Previous Day</td>
<td>Methadone Dose Previous Day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800</td>
<td>Arrival, Urine Test, Weight</td>
<td>Arrival, Urine Test, Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0815</td>
<td>Insert Cannula</td>
<td>Insert Cannula</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0820</td>
<td>Testing (Familiarisation)</td>
<td>Testing (Familiarisation)</td>
<td>✓  ✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>0830</td>
<td>Start Saline IV</td>
<td>Start Saline IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0840</td>
<td>Testing (Baseline(Saline))</td>
<td>Testing (Baseline(Saline))</td>
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<td>✓</td>
<td></td>
</tr>
<tr>
<td>0850</td>
<td>End Saline IV Start 0.10 µg/kg/min REM IV</td>
<td>End Saline IV Start 0.5 µg/kg/min REM IV</td>
<td>✓  ✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>0900</td>
<td>Testing</td>
<td>Testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0910</td>
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<td>End 0.5 µg/kg/min REM IV Start 1.0 µg/kg/min REM IV</td>
<td>✓  ✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>0920</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>End 0.15 µg/kg/min REM IV Start 0.20 µg/kg/min REM IV</td>
<td>End 1.0 µg/kg/min REM IV Start 1.5 µg/kg/min REM IV</td>
<td>✓  ✓</td>
<td>✓</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>End 0.20 µg/kg/min REM IV Start 0.25 µg/kg/min REM IV</td>
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<td>✓</td>
<td></td>
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<tr>
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<td>✓</td>
<td></td>
</tr>
<tr>
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<td>Testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1030</td>
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<td>End 2.5 µg/kg/min REM IV Start 3.0 µg/kg/min REM IV</td>
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<td></td>
</tr>
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<td></td>
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<td>✓</td>
<td></td>
</tr>
<tr>
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<td>Testing</td>
<td>Testing</td>
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<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>1120</td>
<td>Administer Methadone</td>
<td>Administer Methadone</td>
<td></td>
<td></td>
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</tr>
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<td>Testing</td>
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<td>✓*</td>
<td></td>
</tr>
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<tr>
<td>1530</td>
<td>Remove Cannula</td>
<td>Remove Cannula</td>
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<td></td>
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</tr>
<tr>
<td>1545</td>
<td>Discharge</td>
<td>Discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>~1600</td>
<td>Follow-up Phone Call Next Day</td>
<td>Follow-up Phone Call Next Day</td>
<td>✓ AE ONLY</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

Legend: RAH Ethics committee protocol number #040282, ∞040282A, *040282B. REM - remifentanil, IV - intravenous.
### 10.3 Appendix 3 – Recreational drug use history

<table>
<thead>
<tr>
<th></th>
<th>Ever used (%)</th>
<th>Used in previous 28 days (%)</th>
<th>Days of use in previous 28 days (median (range))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>100</td>
<td>90</td>
<td>8 (1–28)</td>
</tr>
<tr>
<td>CPP (Morphine)</td>
<td>100</td>
<td>60</td>
<td>2 (1–25)</td>
</tr>
<tr>
<td>CPP (Methadone)</td>
<td>90</td>
<td>70</td>
<td>1 (1–28)</td>
</tr>
<tr>
<td>MMP</td>
<td>100</td>
<td>50</td>
<td>3 (1–28)</td>
</tr>
<tr>
<td>Former Users</td>
<td>100</td>
<td>0</td>
<td>(–)</td>
</tr>
<tr>
<td><strong>Barbiturates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0</td>
<td>0</td>
<td>(–)</td>
</tr>
<tr>
<td>CPP (Morphine)</td>
<td>0</td>
<td>0</td>
<td>(–)</td>
</tr>
<tr>
<td>CPP (Methadone)</td>
<td>0</td>
<td>0</td>
<td>(–)</td>
</tr>
<tr>
<td>MMP</td>
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</tr>
<tr>
<td>Former Users</td>
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<td>0</td>
<td>(–)</td>
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**Legend:** Subjects enrolled in the chronic opioid use study and the former opioid user study. RAH Ethics committee protocol number 030509. CPP – Chronic pain patients, MMP – methadone maintained patients. VOC – volatile organic compounds. MDMA - 3,4-methylenedioxy-N-methylamphetamine (ecstasy)
11 ABBREVIATIONS

AC   Adenylate cyclase  
AIDS  Acquired immunodeficiency syndrome 
ALP   Alkaline phosphatase 
ALT   Alanine aminotransferase  
ANOVA Analysis of variance 
AST   Aspartate transaminase  
AUC   Area under the curve 
cAMP  Cyclic adenosine monophosphate  
CCK   Cholecystokinin  
CI   Confidence interval  
CNS   Central nervous system  
COWS  Clinical opiate withdrawal scale  
COX   Cyclooxygenase  
CP   Cold pressor (test)  
CPDEC Cold pressor decay 
CPP   Chronic pain patient 
CPTH  Cold pressor threshold  
CPTOL Cold pressor tolerance  
CRF   Case report form  
CTN   Clinical trial notification  
CV   Co-efficient of variation  
CYP   Cytochrome P450  
DAMGO D-Ala²-N-Me-Phe⁴-Gly-ol⁵-enkephalin  
df   Degrees of freedom  
DLF   Dorsolateral funiculus  
DNR   Did not respond  
ECG   Electrocardiogram 
ED⁵₀ Median effective dose  
EDDP  1,5-demethyl-3,3-diphenyl-2-ethylidene-pyrrolidine  
ES   Electrical stimulation (test)  
ESDET Electrical stimulation detection  
ESTHR Electrical stimulation threshold 
ESTOL Electrical stimulation tolerance  
FPQ   Fear of pain questionnaire  
GCP   Good clinical practice  
GGT   Gamma glutamyl transferase  
GRK   G protein-coupled receptor kinase  
GTP   Guanosine 5'-Triphosphate  
HIV   Human immunodeficiency virus  
ICH   International Conference on Harmonisation  
INR   International normalised ratio (coagulation test)  
IP   Intraperitonealy  
IT   Intrathecal  
IV   Intravenous  
Ki   Dissociation constant for binding of inhibitor to enzyme (affinity)  
LCMS  Liquid chromatography mass spectroscopy  
LD   Lactate dehydrogenase  
LD⁵₀ Median lethal dose  
LFT   Liver function test
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The International System of Units (Système international d'unités (SI)) symbols for base units, derived units and prefixes are used throughout this thesis.