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A comparison between methadone and buprenorphine for perioperative analgesia in dogs and cats undergoing ovariohysterectomy

A comparison between methadone and buprenorphine for perioperative analgesia in dogs and cats undergoing ovariohysterectomy

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Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: DATE:

Acknowledgements

Abstract

Opioids are considered to provide effective perioperative analgesia for acute surgical pain of which buprenorphine and methadone are most commonly used in clinical practice. The aim of our research was to compare analgesic efficacy of methadone and buprenorphine in dogs and cats undergoing ovariohysterectomy, to help guide clinicians in their decision making regarding opioid choice for ovariohysterectomy and other moderate to severely painful procedures.

In dogs undergoing ovariohysterectomy, a premedication of methadone or buprenorphine combined with acepromazine or medetomidine was administered intramuscularly and anaesthesia induced with propofol. In cats undergoing ovariohysterectomy, methadone or buprenorphine combined with ketamine, midazolam and medetomidine (QUAD protocol) was administered intramuscularly and no induction agent was required. Anaesthesia was maintained with isoflurane in both cats and dogs. Pain was assessed regularly postoperatively using a species-specific composite pain scale (SF-GCPS in dogs and CMPS-F in cats) and the Dynamic Interactive Visual Analogue Scale (DIVAS). Rescue analgesia (methadone) was administered intramuscularly if indicated by the composite scale pain score.

Both dogs and cats showed that methadone groups required less rescue analgesia ($p = 0.02$ in dogs; $p = 0.04$ in cats). Dogs administered methadone showed lower overall SF-GCPS pain scores ($p < 0.001$) and DIVAS pain scores ($p < 0.01$) compared to buprenorphine groups. Cats administered methadone also showed lower over CMPS-F pain scores for methadone groups compared to buprenorphine groups ($p = 0.04$), however, there was no difference in postoperative DIVAS scores between buprenorphine and methadone groups ($p = 0.06$).

We concluded that overall preoperative methadone provides better postoperative analgesia compared to buprenorphine in the context of ovariohysterectomy and this is likely to be true for other moderate to severely painful procedures in both dogs and cats.

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Table of Abbreviations

μg	Microgram
acp	Acepromazine
acpBUP	Acepromazine and buprenorphine
acpMET	Acepromazine and methadone
ASA	American Society of Anaesthesia
BUP	Buprenorphine
CMPS-F	Feline Composite Measure Pain Scale
DAP	Diastolic arterial pressure
DIVAS	Dynamic Interactive Visual Analogue Scale
$ETCO_2$	End tidal carbon dioxide concentration
f_R	Respiration rate
HR	Heart rate
IM	Intramuscular
ISO	Isoflurane
IV	Intravenous
kg	Kilogram
MAP	Mean arterial pressure
medBUP	Medetomidine and buprenorphine
medMET	Medetomidine and methadone
MET	Methadone
mg	Milligram
MNT	Mechanical Nociceptive Threshold
NIBP	Non-invasive blood pressure
NMDA	N-methyl D-aspartate
PRoD	Pressure onset device
SAP	Systolic arterial pressure
SC	Subcutaneous
SDS	Simple Descriptive Scale
SF-GCPS	Short Form of the Glasgow Composite Pain Scale
SpO2	Arterial haemoglobin saturation with oxygen

1. Introduction

There are an estimated 9.5 million dogs and 8.5 million cats in the UK (Pet population report 2015-2016, Pet Food Manufacturers Association). Most of these animals will undergo at least a neuter surgery in their lifetime, a procedure which is encouraged in the UK to prevent reproduction and reproductive diseases. Surgical procedures produce intense and unavoidable noxious inputs which result in acute postoperative pain, the severity of which is dependent on the procedure. It is now widely accepted that animals sense pain and many experience the affective component of pain similarly to humans (Panksepp 2011). In addition, untreated pain induces the stress response; delays healing and return to function; and can lead to maintained hypersensitivity and chronic pain states (Latremoliere & Woolf 2009). Therefore, preventing and treating pain in animals is fundamental from both a welfare and clinical outcome perspective.

Opioids are considered to be the cornerstone treatment for surgical pain. Of those licensed for use in dogs and cats in the UK, buprenorphine and methadone are the most widely used (Hunt et al. 2015). Until recently, buprenorphine was thought to be a partial mu (μ) receptor agonist. However, recent studies suggest that buprenorphine can achieve full analgesia at less than 100% occupancy of μ opioid receptors, therefore acting as a full μ agonist. However, this is dependent on the intensity of the noxious stimuli and buprenorphine may not provide effective analgesia for higher intensity input (Raffa & Ding 2007; Pergolizzi et al. 2010). Methadone is a full μ opioid receptor agonist and is considered to provide effective analgesia for moderate to severe pain. In support of this hypothesis, methadone has been shown to provide greater analgesia compared to buprenorphine in dogs undergoing orthopaedic surgery (Hunt et al. 2013a). It is considered good practice to stock both methadone and buprenorphine to enable adequate pain management for all procedures carried out in general practice. Despite this, there is a disparity in the use of buprenorphine and methadone in clinical practice in the UK. A recent survey revealed that 98.9% of practices stock buprenorphine, whilst only 57.3 % stock methadone (Hunt et al. 2015). Consequently, a significant proportion of practices may be undertreating moderate to severe pain.

The aim of this research was to formally compare the analgesic efficacy of methadone and buprenorphine in dogs and cats undergoing ovariohysterectomy - a commonly performed procedure familiar to all veterinary surgeons, with the potential to elicit moderate to severe pain (Hardie et al. 1997). We hope that the findings will guide clinicians in their decision making regarding opioid choice and improve overall pain management in general practice, as well as highlight the importance of postoperative pain assessment.

2. Literature review

2.1. Pain

The International Association for the Study of Pain (IASP) describes pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage". There are three dimensions to the pain experience in humans: sensory-discriminative, motivational-affective and cognitive-evaluative (Melzack & Casey 1968). The sensory-discriminative aspect communicates the properties of the noxious stimulus itself. The motivational –affective component conveys unpleasantness and triggers the reflexive escape response. These two components have been the focus of pain assessment in both human and veterinary medicine. The cognitive-evaluative dimension contextualises pain with regard to social factors, previous experiences and prior conditioning and is what determines the 'suffering' associated with pain that extends beyond unpleasantness (Bustan et al. 2015).

The detection of a noxious stimuli (nociception) primarily serves a protective function. It generates a reflex withdrawal and creates an unpleasant sensation leading to complex behavioural strategies for further avoidance of such stimuli (Navratilova & Porreca 2014). Nociception also encourages healing of damaged tissue by promoting immobilisation of the affected area. People with loss of pain function repeatedly succumb to burns, repeat fractures and self-injuries because they do not learn self-awareness necessary to avoid danger. Therefore, pain is fundamentally a survival mechanism, offering an evolutionary advantage and thus has been conserved throughout the animal kingdom (Navratilova & Porreca 2014).

The nociceptive pathways in animals are similar to those in people and result in similar behaviours associated with discomfort, such as a reluctance to move or an aversion to palpation of the wound. What is less known, are the emotional, contextual and cognitive components of pain in animals since this relies heavily on self-reporting. Nevertheless, there is growing evidence supporting complex cognitive processes in many animal species regardless of their inability to communicate emotion (Paul-Murphy et al. 2004; Panksepp 2011). Behaviours such as reduced appetite and changes in temperament e.g. fear, frustration, anxiety and depression shown by animals in pain (Reid et al. 2007) mirror those associated with suffering and negative emotions in humans (Bustan et al. 2015). ISAP specifically note that "the inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment". This statement is aimed at infants and people suffering a disease rendering them unable to speak but it is equally applicable in the context of animal patients.

2.1.a. Pain pathway

Nociception involves detection and transmission of stimuli, with the potential to cause tissue damage, from the peripheral nervous system (PNS) to spinal and supraspinal centres of the CNS. The pathway is composed of four components: transduction, transmission, modulation and perception (Figure 1).
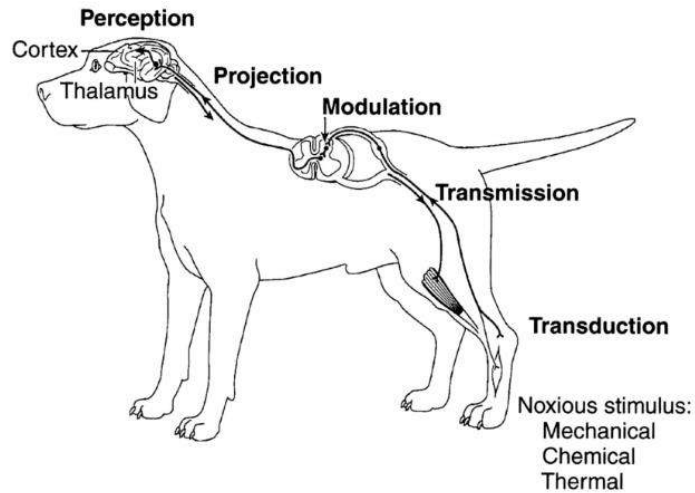
Figure 1. A schematic overview of the pain pathway (Shilo & Pascoe 2013). Noxious stimuli are detected by nociceptors in the periphery. Nociceptors convert the mechanical, thermal or chemical stimuli into an electrical stimulus (transduction), which is transmitted to the dorsal horn of the spinal cord (transmission). Here it is either enhanced or dampened by descending pathways (modulation) and then projected (projection) to the brain and higher centres for processing and contextualising (perception).

Noxious stimuli (mechanical, thermal or chemical) are sensed by free sensory nerve endings (nociceptors) in the skin and other tissues. Nociceptors are high threshold receptors and respond progressively to increasing stimuli intensity. Therefore, they are selective to damaging (noxious) or potentially damaging stimuli and do not respond to harmless stimuli such as touch or warmth (D'Mello & Dickenson 2008). These environmental signals activate voltage-gated ion channels and initiate an action potential which converts the physical stimulus into an electrophysiological neural impulse (transduction). The action potential is transmitted from the PNS to the dorsal horn of the spinal cord by primary afferent sensory neurones (transmission) (D'Mello & Dickenson 2008).

The majority of nociceptive signals are conducted by Aδ or C nerve fibres. Aδ fibres are large in diameter, thinly myelinated, fast conducting and transmit first pain described as sharp or pricking pain. There are two types of Aδ nociceptive ending: Type I and Type II nociceptors. Type I respond to high intensity mechanical and chemical stimuli. Type II respond to noxious thermal

stimuli. C fibres are small in diameter, unmyelinated, slow conducting and transmit second pain described as dull pain. Most C fibre nociceptors respond to mechanical, chemical and thermal stimuli with subgroups highly sensitive to one type of stimuli. Inflammation of tissues can activate additional 'silent' C-fibre nociceptors increasing their sensitivity to mechanical and chemical stimuli, which may contribute to peripheral sensitisation (Farquhar-Smith 2008). Aδ and C fibres are found in both cutaneous and visceral nerves, although in different ratios. The ratio of Aδ to C fibres is 1:1 to 1:2 in cutaneous nerves and 1:8 to 1:10 in visceral nerves (Wiese & Yaksh 2015).

Primary sensory neurones synapse with second order neurones in the dorsal horn of the spinal cord, and are projected along the spinoreticular or spinothalamic tract terminating in the thalamus (Hudspith 2016). Aδ and C fibres release excitatory neurotransmitters such as aspartate, glutamate and substance P into the dorsal horn, which activate glutamate receptors such as N-methyl-D aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4 isoxazoleproprionic acid (AMPA) and kainite on the presynaptic membrane of second order neurones. Activation of these receptors results in depolarisation of the second order neurone. If depolarisation reaches a certain threshold an action potential is triggered. Neuronal relays are sent from the dorsal horn to other areas of the CNS including the sensory cortex, limbic system and medulla (Figure 2) where the pain signals are contextualized by previous experiences and emotions (perception) (Wiese & Yaksh 2015).

Ascending nociceptive signals are modified at the level of the spinal cord by supraspinal descending pathways (modulation). The most studied and important descending pathway is the periaqueductal gray (PAG) – rostral ventromedial medulla (RVM) –dorsal horn pathway (Ottestad & Angst 2013). The PAG is located in the midbrain and receives information from the cortex, amygdala, and hypothalamus. Ascending nociceptive input causes the PAG to release endogenous opioids in the RVM - a group of neurones located on the floor of the medulla. This stimulates the release of serotonin and noradrenalin which activate inhibitory neurones within the spinal cord. This modulation was thought to serve as an endogenous analgesic system, however, it is now evident descending pathways can both facilitate and inhibit nociceptive signals (Ossipov et al. 2010). The RVM has been shown to contain two types of neurones – On cells and Off cells. Off cells are activated by opioids (endogenous and exogenous) and inhibited by nociceptive stimuli and an increase in their activity has shown to reduce nociception. On cells have the opposite response and facilitate ascending pathways (Ossipov et al. 2010). This bidirectional pain modulation means RVM may contribute to endogenous pain inhibitory pathways, as well as enhanced pathological pain states. Collectively, processing by all centres

produces an integrated response that coordinates arousal (visual, olfactory, auditory input), somatosensory input, and autonomic and motor output (Wiese & Yaksh 2015) (Figure 2).
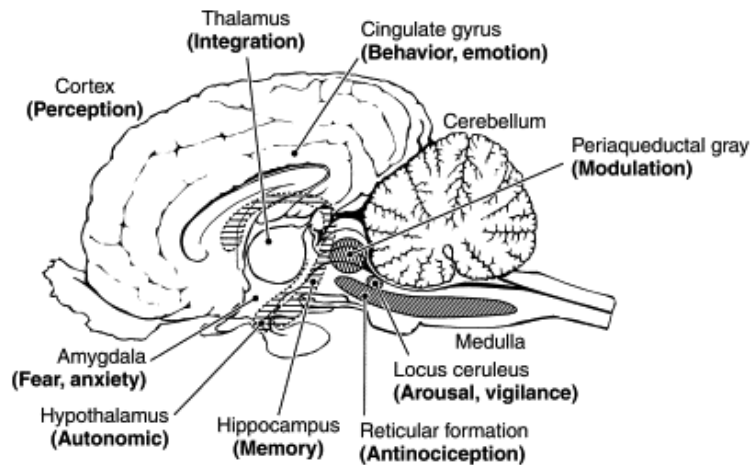
Figure 2. A schematic diagram showing the regions of the brain involved in the affective and evaluative components of pain (Wiese & Yaksh 2015). The thalamus is the central integrating and transmission point.

2.1.b. Sensitisation

Acute pain is generally proportional to the stimulus and as the injury heals pain subsides. In the context of surgical pain, acute pain is usually accompanied by factors such as inflammation and nerve damage resulting in peripheral sensitisation of primary afferent neurones (Gurney 2012). The sensitised nociceptors have a lower threshold and evoke a stronger response to a given stimulus resulting in hypersensitivity at the area of injury. Peripheral sensitisation is long lasting but not permanent. If noxious stimulation is prolonged and intense, ongoing peripheral sensitisation can lead to central sensitisation. Central sensitisation is an adaptive process, which is initially reversible over time and is responsible for radiation of pain to uninjured areas (Hudspith 2016). These processes still have a physiological protective function. However, if the acute postoperative pain is not treated effectively, neuroplastic changes in the CNS can result in chronic pain. At this point, the correlation between pain and injury is lost and pain becomes pathological (Gurney 2012).

2.1.c. Peripheral sensitisation

Peripheral sensitisation was first discovered in the 1970s by Perl and then others (Perl et al. 1976). They found that the activation threshold of nociceptors is dynamic and dependant on the surrounding cellular milieu. Tissue injury and inflammation trigger chemical mediators to be released from the nociceptor itself and nearby non-neuronal cells, such as immune cells and fibroblasts (Woolf 2011). Chemical mediators either directly activate the nociceptor (e.g. ATP, 5-

hydroxytryptamine and hydrogen ions) or decrease the nociceptor's threshold to further stimuli (e.g. bradykinins, leukotrienes, prostaglandins and growth factors). Both are mediated by intracellular signalling pathways such as cyclic AMP, protein kinase A and protein kinase C, which cause phosphorylation of ion channels and receptors reducing activation threshold and increasing excitability. In addition, the inflammatory mediators activate very high threshold and usually silent nociceptors. This results in hypersensitivity of the injured or inflamed area and is termed primary hyperalgesia (Farquhar-Smith 2008).

2.1.d. Central sensitisation

Central sensitisation is the result of changes in the CNS following intense, prolonged and/or repeated nociceptive input, facilitated by peripherally sensitised nociceptors. Unlike peripheral sensitisation, central sensitisation involves novel inputs to the pain pathway, which are not usually activated by noxious stimuli (Woolf 2000). Sensitised peripheral nociceptors generate high frequency inputs resulting in the release of excitatory glutamate and other neuromodulators. This produces slow excitatory post synaptic potentials in dorsal horn neurons generating sufficient depolarisation to activate N-methyl D-aspartate (NMDA) receptors (D'Mello & Dickenson 2008). The NMDA receptor is a glutamate receptor subtype and is gated by both membrane voltage and ligand binding. The receptor is blocked by Mg^{2+} at resting membrane potential and can only be activated if the membrane of the cell is partly depolarised. The NMDA receptor is a Ca^{2+} channel and activation triggers intracellular biochemical processes e.g. membrane protein phosphorylation, protein synthesis upregulation and nitric oxide synthase activation, which collectively result in long term potentiation of the neuronal synapse (Latremoliere & Woolf 2009). Consequently, central sensitisation results in hypersensitivity of non-inflamed tissue (secondary hyperalgesia) and sensitivity in response to non-noxious stimuli (allodynia), as well as maintained sensitivity after termination of initial noxious input. Pain becomes uncoupled from the initial stimulus and shifts from high-threshold nociception to low-threshold hypersensitivity (Woolf 2011). Studies in humans have shown that NMDA receptor antagonists reduce central sensitisation by prevention of temporal summation (increased pain after repeated painful stimuli). There have been limited studies on the role of NMDA and NMDA receptors in veterinary pain but it can be inferred that the neurophysiology is similar in cats and dogs (Eide 2000).

2.2. Perioperative analgesia

The perioperative period comprises the preoperative, intraoperative and postoperative phases (Gurney 2012). It was initially thought that 'pre-emptive' analgesic administration prior to the incision would minimise nociceptive processing and prevent intraoperative amplification of pain pathways, thereby reducing postoperative pain. However, this only focusses on the pre-operative element of the perioperative period. It is now widely accepted that factors in each peri-operative phase can contribute towards the development and severity of postoperative pain. These include genetic predisposition, preoperative noxious inputs, intraoperative tissue injury and inflammation (site, nature and extent), and postoperative inflammatory responses (Katz et al. 2011). Studies in human medicine have shown that the provision of analgesia throughout the perioperative period, termed preventive analgesia, is better than pre-emptive analgesia alone. Preventive analgesia is considered the optimal method of reducing transmission of primary afferent nociceptive signals to the spinal cord, thereby preventing peripheral and central sensitisation and amplification of pain pathways (Vadivelu et al. 2014). Although there have been very few studies investigating pre-emptive vs. preventive analgesia in animals, the evidence from human studies can most likely be extrapolated. These processes begin with the incisional noxious input and are amplified by subsequent inflammatory inputs that continue into the post-operative period. Therefore, it is important that analgesics are given with sufficient time to allow maximum bio-availability so that they are effective at the time of noxious stimulation. It is also important that their action extends into the post-operative period, in which further inflammatory or sensory input is common (Kissin 2000) and that further analgesia is administered within the perioperative period where necessary.

Preoperative analgesia in the form of an opioid in combination with a sedative and usually a non-steroidal anti-inflammatory drug (NSAID) is a common pre-surgical protocol in veterinary medicine in the U.K. Postoperative pain assessments and administration of additional analgesia if required is strongly encouraged in the immediate postoperative period along with short term NSAIDs over. Our studies focused on the preoperative aspect of preventive analgesia and investigated two different opioids administered preoperatively in their ability to provide adequate postoperative analgesia.

2.2.a. Opioids

Opioid analgesics are the cornerstone of analgesia for moderate to severe surgical pain and are considered to produce their analgesic effects by mimicking the action of endogenous opioids through opioid receptors in the brain and spinal cord (Inturrisi 2002). The first opioid compound

to be used for analgesia was morphine, derived from the juice of poppy seeds (opium). Two other alkaloids with analgesic potential were also isolated – codeine, and thebaine. Manipulation of the structure of these alkaloids has led to numerous semi-synthetic (e.g. codeine, hydromorphone and buprenorphine) and synthetic (e.g. methadone and fentanyl) opioid analgesics (Inturrisi 2002).

Opioids are characterised by their interaction with the three major opioid receptors– mu (μ), delta (δ) and kappa (κ). A further fourth receptor, the opioid receptor-like 1 (ORL-1) receptor has more recently been identified (Lehmann 1997). Opioid receptors are found both centrally and peripherally. All opioid receptors are transmembrane proteins and coupled to inhibitory G proteins. Activation of the receptor inhibits adenyl cyclase which reduces intracellular cAMP production and results in hyperpolarisation of the cell by increasing potassium conductance and decreasing calcium conductance. The overall result is a decrease in neurotransmitter release and inhibition of the postsynaptic impulses (Feng et al. 2012), thus decreasing pain from nociceptive stimuli.

The μ opioid receptor is the primary receptor involved in exogenous opioid induced analgesia. Activation of μ receptors results in inhibition of GABAergic neurons and activation of the descending inhibitory pathway, resulting in an analgesic effect (Pasternak & Pan 2011). Opioids also exert direct inhibitory effects on the dorsal horn of the spinal cord and peripheral nociceptive afferent neurones by preventing the release of nociceptive mediators such as glutamate, substance P and nitric oxide and inhibiting impulse transmission. By working at all phases of the pain pathway (peripheral, spinal and supraspinal) opioids prevent pain transmission and peripheral and central sensitisation (Pathan & Williams 2012).

Opioids have a direct effect on respiratory centres in the brain via μ_2 receptors, which results in a decrease in response to carbon dioxide and an increase in arterial carbon dioxide partial pressure. This leads to a dose dependant depression of ventilation (KuKanich & Wiese 2015). Sedative and anaesthetic agents compound this effect meaning respiratory depression and hypercapnia are more likely to occur during anaesthesia than in conscious animals. In general, animals are less sensitive to respiratory depression compared to humans and at recommended clinical doses, respiratory depression is not significant (Maiante et al. 2009). Cardiovascular effects are a result of centrally mediated vagal stimulation (KuKanich & Wiese 2015) and include bradycardia and reduced cardiac index. The clinical significance of this is minimal at clinical doses.

2.2.b. Methadone

Methadone is a synthetic opioid characterised as a full mu opioid receptor agonist (Ingvast-Larsson et al. 2010). The licensed form of methadone in the UK is a racemic mixture of two isomers. The L isomer (levomethadone) competitively binds to μ receptors and is responsible for its analgesic effect. The D isomer (dextromethadone) is a non-competitive N-methyl-d-aspartate (NMDA) receptor antagonist with potential to prevent the development of central sensitisation in nociceptive pain states such as neuropathy or persistent inflammation (Vorobeychik et al. 2015). Since methadone is a full agonist, it produces a maximal response at full saturation of receptor binding sites (Inturrisi 2002) and is effective for moderate to severe pain. It also has a dose-dependent action making it titratable and easy to dose to effect. Methadone generally has little clinical effect on cardiovascular parameters at recommended clinical doses and this has been shown in a number of studies (Monteiro et al. 2008; Bortolami et al. 2013; Hunt et al. 2013a; Slingsby et al. 2015; Amengual et al. 2017). However, high doses (1 mg/kg) have been shown to depress heart rate and cardiac index compared to baseline in conscious (Maiante et al. 2009) and anesthetised (Credie et al. 2010) dogs, although, mean arterial pressure was not effected (Maiante et al. 2009; Credie et al. 2010). Another concern for many clinicians is opioid induced respiratory depression. However, studies have shown that the incidence of respiratory depression is low even at relatively high doses of methadone. A study by Maiante et al. (2009) showed there was no change in partial pressure of carbon dioxide following an intravenous (IV) bolus of 0.5 or 1 mg/kg of methadone in conscious dogs, suggesting minimal respiratory depression. Other studies have also reported no respiratory depression after IV administration of 0.2 mg/kg methadone post spinal surgery in dogs (Amengual et al. 2017) and 0.1, 0.3 and 0.5 mg/kg methadone in cats undergoing OVH (Dobromylskyj 1993).

2.2.c. Buprenorphine

Buprenorphine has been primarily characterised as a partial mu opioid receptor agonist, but is also a kappa and delta receptor antagonist and an ORL-1 receptor agonist (Lutfy & Cowan 2004). However, a study by Raffa and Ding (2007) challenged this classification and suggested that buprenorphine can produce full analgesia dependent on the intensity of the noxious stimuli in rats. A study in humans has also shown that full analgesia can be achieved at less than 100% receptor occupancy - the definition of a full agonist. (Pergolizzi et al. 2010). However, this is dependent on the severity of pain and buprenorphine may be adequate for mild to moderate pain but not for moderate to severe pain (Raffa & Ding 2007). Furthermore, there is some evidence that buprenorphine may produce a ceiling effect resulting in plateauing of the analgesic effect at higher doses (Lutfy & Cowan 2004). There is currently limited evidence that

the ceiling effect occurs in dogs and cats. Slingsby et al. (2011) showed that an increase in buprenorphine dose from 20 μg/kg to 40 μg /kg caused no changes in physiological parameters and did not increase analgesia in dogs that had undergone ovariohysterectomy (Slingsby et al. 2011). However, this may be due to insensitivities in pain scoring. A full dose response curve is needed to confirm the existence of the ceiling effect in dogs and determine whether it is clinically relevant. Slingsby et. al also showed that the cardiovascular and respiratory intraoperative values were within clinical range at both 20 and 40 μg/kg doses and there was no evidence of cardiovascular or respiratory depression. Similar conclusions have been drawn by a number of different studies (Cowan et al. 1977; Stanway et al. 2002; Shih et al. 2008; Hunt et al. 2013b; Morgaz et al. 2013; Steagall et al. 2014; Slingsby et al. 2015) One study has shown buprenorphine administered at a dose of 16 μg/kg (IV) reduces HR, cardiac index and arterial blood pressure in healthy dogs anesthetised with isoflurane, but these changes were not clinically important (Martinez et al. 1997) .

2.2.d. Use of opioids in veterinary practice

The administration of opioids and multimodal analgesia has increased within the veterinary profession over the last 20 years (Lascelles et al. 1999; Hunt et al. 2015). This is a result of a greater range of licensed analgesics available, an increasing understanding of how to treat different forms of pain, an improvement in pain assessment techniques and a shift in client expectation (Hunt et al. 2015). Of the opioids licensed for dogs and cats in the UK and suitable for surgical analgesia, the two most commonly used are buprenorphine and methadone. Both opioids differ in their opioid receptor binding characteristics and are effective against different severities of pain. Buprenorphine is recommended for mild to moderate pain, whereas methadone is recommended for moderate to severe pain. It is therefore seen as good practice to stock both opioids (Murrell 2011b). Possible explanations for the difference in the use of methadone (57.3%) and buprenorphine (98.9%) in veterinary practice (Hunt et al. 2015) are unfamiliarity with dosing and potential side effects of methadone, since it has only been licenced for veterinary use in dogs and cats since 2011. Furthermore, there has been limited research into the analgesic effect of methadone compared to buprenorphine in routine first opinion procedures and practitioners may remain unconvinced about the additional benefits of methadone. In addition, methadone is a Schedule 2 controlled drug with stricter guidelines for storage, record-keeping and disposal compared to buprenorphine (Schedule 3 drug) and this may present logistical concerns for some practices.

2.3. Anaesthetic drugs

2.3.a. Sedatives

Sedatives are used in veterinary medicine for their anxiolytic properties and are often used for preanesthetic medication (Rankin 2015). Reducing fear and anxiety prior to induction of anaesthesia improves the quality of anaesthetic induction and recovery as well as improving the welfare of the patient. It also helps handling of patients and placement of an IV catheter, especially in anxious and nervous patients. In addition, the dose of induction and maintenance agent needed is reduced. Sedative drugs do not typically possess sufficient analgesic activity and are usually combined with an opioid analgesic. The sedative and opioid act synergistically to improve sedation and therefore lower doses of sedative are also required reducing the potential of adverse effects. Commonly used sedatives in general practice in the UK include acepromazine and medetomidine (Murrell 2007).

2.3.b. Acepromazine

Acepromazine (acp) is a phenothiazine and is used primarily as a sedative for premedication prior to anaesthesia. As well as producing sedation, it is also an antipsychotic and can help reduce anxiety. Its sedative and anxiolytic effects are mediated by antagonism of dopamine, primarily D2, receptors and are initially dose dependent (Rankin 2015) . However at doses >0.05mg/kg the degree of sedation plateaus but adverse effects continue to increase and duration of action is prolonged (Murrell 2007). Sedation produced by acp is mild to moderate. and onset of sedation occurs within 15 minutes after intramuscular (IM) administration, with peak effects observed within 30 minutes (Monteiro et al. 2008). In this time animals require a quiet environment and undisturbed. Acepromazine results in good muscle relaxation. However it provides no analgesia and is usually combined with an opioid, which improves the quality of sedation due to synergism between the two drugs (Monteiro et al. 2008).

In addition to D2 antagonism, acepromazine also has antagonist activity at α-1 adrenoreceptors which, results in peripheral vasodilation and a consequent decrease in blood pressure. Studies have shown a 20% to 25% decrease in mean arterial pressure after IV administration of 0.1 mg/kg acepromazine in conscious dogs (Coulter et al. 1981) and by 24% after IM administration of the same dose in dogs anaesthetised with isoflurane (Bostrom et al. 2003). Heart rate does not change compared to baseline. In healthy animals falling into American Society of Anaesthesia categories 1 and 2 i.e. animals with no detectable disease or mild systemic disease these changes in blood pressure are not significant because adequate compensatory mechanisms are in place (Rankin 2015). However, acp should be used with caution in animals in

shock or that have cardiovascular disease. Clinical doses of acp have little effect on the respiratory system (Rankin 2015).

2.3.c. Medetomidine

Medetomidine is an alpha-2 adrenoreceptor agonist (α-2 agonist) and produces profound sedation. α-2 adrenoreceptors are found post-synaptically in peripheral tissues such as blood vessels where they have a physiologic function, and pre-synaptically on sympathetic nerve endings and noradrenergic neurones within the dorsal horn of the spinal cord and the locus coeruleus (LC) in the brain where they inhibit the release of noradrenaline. The LC is a small neuronal nucleus located in the upper brain stem and is important in the regulation of wakefulness. The sedative effects of α-2 agonists are thought to be mediated by their effect on adrenoreceptors in this region (Rankin 2015). The α-2 adrenoreceptor is a transmembrane G-protein coupled receptor. The binding of medetomidine results in activation of potassium ion channels and consequent neuronal hyperpolarisation, and a decrease in calcium ion channel conductance resulting in inhibition of neurotransmitter release (Murrell & Hellebrekers 2005).

Activation of post-synaptic α-2 receptors in vascular smooth muscle causes vasoconstriction and has a profound effect on the cardiovascular system in a biphasic manner. Vasoconstriction causes an initial increase in blood pressure and a reflex decrease in heart rate (phase1). The level of vasoconstriction then decreases and blood pressure falls, but a prolonged bradycardia remains as a result of central reduction in sympathetic tone (phase 2) (Rankin 2015). In addition to bradycardia, cardiac output is also reduced. In healthy animals within American Society of Anaesthesiology (ASA) category 1 or 2 the cardiovascular system compensated for the lower cardiac output. However, in animals with limited cardiovascular reserve a drop in cardiac output can reduce oxygen delivery to organs and have a detrimental effect (Murrell & Hellebrekers 2005). Therefore, medetomidine use should be reserved for healthy animals. Significant cardiovascular effects are present at doses as low as 5 μg/kg and higher doses of medetomidine have been shown to have little additional effect on cardiovascular function. Clinical doses of medetomidine have a minimal effect on the respiratory system of healthy animals (Murrell 2007).

Alpha-2 agonists have also been shown to have antinociceptive properties, although the mechanism is not entirely understood since their sedative effects confound evaluation of analgesia. α-2 receptors are wide spread in the CNS and both spinal and supraspinal sites are likely to be involved (Yaksh 1985). Possible theories include: inhibition of neurotransmitter release from primary to second-order neurones; modulation in the dorsal horn; modulation of

descending pathways from the brainstem; or modulation of ascending signals in the diencephalon and limbic areas (Murrell & Hellebrekers 2005). However, studies in cats evaluating response to inter-digital pad, tail and skin pinch/clamp have shown that although analgesic effects of medetomidine were present they were mild and would only be effective against minor pain, even at high doses of 150 µg/kg (Ansah et al. 1998). Although, it is possible that the methods used to assess nociception may have been confounded by a reflex response to the stimuli.

Another advantage of medetomidine is the ability to antagonise it with an α-2 antagonist such as atipamazole. This means sedation and any potential adverse reactions can be reversed if required. In dogs a dose volume equal to that of medetomidine administered is required and half the dose volume is required in cats. Potential adverse effects of atipamezole administration include vomiting, diarrhoea, hypersalivation. Intramuscular administration provides rapid uneventful recovery from medetomidine (Dechra 2013).

2.3.d. Quad protocol
The QUAD protocol, comprising medetomidine, ketamine, midazolam and buprenorphine, was developed at the RSPCA Greater Manchester Animal Hospital (GMAH) to provide safe anaesthesia and analgesia for neutering in cats, particularly young cats, by dosing on the basis of body surface area (Joyce & Yates 2011). However, the combination can be used effectively for most surgical procedures in any aged cat and is the main anaesthetic protocol used at the GMAH, with popularity increasing in general practice. The combination of drugs has a dose sparing affect and the dose of each component is relatively low. Induction is rapid and atipamezole (10-50% of the medetomidine volume) can be used to quicken recovery. The properties of medetomidine and buprenorphine have been discussed above.

2.3.e. Midazolam
Midazolam is a water-soluble benzodiazepine and binds to the gamma-aminobutyric acid (GABA) receptor complex in the brain. It does not displace GABA but enhances the receptor's response to GABA by increasing the frequency of Cl⁻ channel opening and thereby potentiating neuronal inhibition (Rankin 2015). This produces anxiolytic and sedative effects as well as good muscle relaxation. However, in healthy cats, when administered alone, it does not typically cause the required level of sedation and can induce ataxia, restlessness and abnormal behaviours (Kanda & Hikasa 2008). Therefore, it is usually combined with medetomidine, opioids and/or ketamine to enhance its anxiolytic and sedative effects and reduce the dose of

additional drugs. Midazolam is absorbed well via the IM route and reaches peak plasma concentrations within 15 minutes (Kanda & Hikasa 2008). One of the main advantages of using midazolam is that it has minimal effect on the cardiovascular and respiratory systems. Therefore, it is a relatively safe drug and can be used in cardiovascularly compromised patients (Rankin 2015).

2.3.f. Ketamine

Ketamine is a well-established anaesthetic drug which results in 'dissociative anaesthesia' – an anaesthetic state produced by the disruption of ascending transmission from areas of the brain responsible for consciousness, such as the limbic system and thalamocortical pathway, resulting in a change in awareness (Sleigh et al. 2014). This is different from the generalised depression of all brain centres via interaction with GABA receptors seen with most other injectable anaesthetics (Berry 2015). The neuropharmacology of ketamine is complex but it's main mechanism of action is use-dependant non-competitive antagonism of NMDA receptors, which accounts for most of the hypnotic, analgesic and psychomimetic effects seen in clinical practice. Ketamine binds to the phencyclidine binding site on a previously activated NMDA receptor which subsequently prevents further activation of the receptor by glutamate This results in dose dependant hypnosis producing psychomimetic effects at low concentrations followed by increased sedation and unconsciousness at higher doses (Sleigh et al. 2014).

Antagonism of the NMDA receptor also results in antinociception and prevention of hyperalgesia since the NMDA receptor is activated during tissue trauma and plays a role in wind up and central sensitisation. Therefore, perioperative, especially preoperative, ketamine administration may be useful in attenuating central sensitisation (Slingsby & Waterman-Pearson 2000). However, clinical evidence for this is still controversial. Other proposed mechanisms for analgesia are ketamine's action on opioid receptors. It is thought that analgesia produced by ketamine is greater for somatic pain compared to visceral pain and this has been demonstrated in a study in cats in which doses of ketamine (8mg/kg) resulted in anaesthesia but did not prevent a response to colonic receptor stimulation (Sawyer et al. 1991).

Ketamine is relatively cardiovascularly stable, although it increases heart rate and cardiac output, thus increasing myocardial work and oxygen consumption. In a severely compromised heart this may have a negative effect. Ketamine maintains airway tone unlike most other injectable anaesthetics. It also produces reliable anaesthesia when administered

intramuscularly, a key reason for its widespread use in veterinary medicine. However, in clinical practice it is not often used as a sole agent for anaesthesia due to associated muscle rigidity and incomplete anaesthesia. Therefore, it is usually combined with a drug that produces good muscle relaxation such as benzodiazepines and/or alpha-2 receptor antagonists and used as an induction agent or used with another injectable anaesthetic such as propofol as part of a total intravenous anaesthetic regime (Berry 2015).

2.3.g. Propofol

Propofol is a phenolic compound that induces CNS depression and anaesthesia by enhancing the $GABA_A$ receptor complex and increasing the neuroinhibitory effect of GABA (Concas et al. 1991). Propofol is used as an induction agent for general anaesthesia. It is commonly administered IV as a bolus over 90 seconds and is usually given to affect (Sams et al. 2008). There is rapid uptake into the CNS which results in a rapid onset of action. It is also rapidly redistributed from the brain to other tissues and is metabolised by both hepatic and extra-hepatic tissues resulting in a short duration of action of approximately 10 minutes. Propofol has minimal analgesic properties and must be paired with an analgesic for painful procedures. The most common effect on the cardiovascular system is transient arterial hypotension due to a decrease in systemic vascular resistance and peripheral vasodilation (Sams et al. 2008).

2.4. Pain assessment

Providing good post-surgical analgesia requires reliable and accurate pain assessment. Pain is subjective to each individual and dependent on several factors including previous experience, environment and genetics. In humans, self-reporting of pain is heavily relied upon and allows both quantitative and qualitative measurement (Younger et al. 2009). In animals, pain can only be assessed by observation of pain associated behaviours and this presents a harder challenge. Attempts have been made to correlate objective measures such as heart rate and blood pressure with pain, but these parameters are influenced by many factors and no study has found a consistently reliable measure (Hoglund et al. 2017). Further confounding factors include species differences in pain expression. For example, cats tend to mask signs of pain and pain behaviours are less obvious compared to dogs. This is most likely a result of evolutionary selection since cats are historically a solitary prey species and masking pain would offer an advantage (Merola & Mills 2015). Therefore, pain in some animals may go unnoticed and untreated if not actively assessed. Different types of pain such as acute vs chronic pain can also result in different behaviours (Reid et al. 2013). Therefore, observers must be experienced in

recognising behaviours in different species and in different conditions and pain assessment tools must also reflect this.

2.4.a. Unidimensional scales

Until relatively recently, pain assessment tools in animals have been restricted to unidimensional scales first developed for self-reporting pain in human medicine (Holton et al. 1998). These include the simple descriptive scale (SDS), numeric rating scale (NRS) and visual analogue scale (VAS) (Holton et al. 1998). However, in veterinary medicine self-reporting of pain by animals is impossible and the scales are used by a veterinarian or nurse to make a subjective judgement on severity of pain by observation of behaviour. The SDS uses a series of expressions to describe increasing severity of pain e.g. no pain, mild pain, moderate pain etc. Each expression corresponds to a number which then becomes the pain score (Figure 3a). The NRS uses an ascending number scale e.g. 0-10 which represents increasing severity of pain. The VAS consists of a 100mm line with 0mm representing no pain and 100 mm representing the worst possible pain for a particular procedure and a mark is placed on the scale by the observer corresponding to perceived pain severity (Figure 3b). All three scales are unimodal assessing mainly pain intensity and are unlikely to adequately assess all aspects of pain perception especially in a veterinary setting (Myles et al. 1999). None of these scales have defined behaviours and therefore rely on assessor experience of pain behaviours and worst possible pain for a given procedure. In addition, SDS is unable to detect small differences in pain which may be clinically significant. All scales have been shown to produce significant interobserver variability due to their subjectivity and poor sensitivity (SDS) or specificity (VAS) (Holton et al. 1998).

A

B

Figure 3. Examples of a simple descriptive (a) and visual analogue scale (b) used to assess pain in animals (Reid 2013).

2.4.b. Dynamic scales

The importance of observing both spontaneous behaviours and interactive behaviours in animals has led to the use of more dynamic scales such as the dynamic interactive VAS scale (DIVAS). The DIVAS aims to assess animals inside and outside of the kennel including palpation of the wound, assessment of movement, as well as demeanour and general behaviour providing a more complete assessment of pain (Holton et al. 2001). This is especially important in a hospital setting where animals may be reluctant or unable to show the full extent of their pain behaviours unless stimulated (Hansen and Hardie 1993). Another advantage of the DIVAS is that it is quick and relatively simple to use DIVAS and can easily be incorporated into a clinical setting. However, it still focusses on pain intensity although interaction with the patient allows for inference of affective components of pain. This inference and the perception of worst possible pain is subjective to each assessor and is affected by factors such as age, gender, personal health and clinical experience, which inevitably results in inter-assessor variability (Reid et al. 2013). In addition, it has been shown in human studies that repeated VAS assessments can vary by ±20 mm in the immediate postoperative period even when self-reporting (DeLoach et al. 1998). It is likely that the lack of anchor points in the DIVAS would also result in intra-assessor variability, especially when assessment is by a proxy assessor.

2.4.c. Composite scales

The optimal approach to pain assessment is now considered to be the use of multi-dimensional behaviour based composite scales that endeavour to assess the whole pain experience including the affective and emotional aspects of pain. Several composite scales have been developed for both dogs and cats. However, only a handful are validated including the Glasgow Composite Measure Pain Scale (CMPS) for acute pain in dogs (Reid et al. 2007), the Glasgow CMPS for acute pain in cats (Calvo et al. 2014) and the UNESP Botucatu multidimensional composite scale for postoperative pain in cats (Brondani et al. 2013).

The CMPS scales, produced by the University of Glasgow, are the first to utilise psychometric principles in their design. Psychometric principles are well established in human medicine to measure concepts such as intelligence, pain and quality of life which are difficult to define and quantify (Reid et al. 2007). This approach involves collection and categorisation of relevant words and expressions, allocation of intensity values to expressions so behaviours are weighted according to severity and validation of categories and expressions. The questionnaire must then be constructed and tested for validity, reliability and sensitivity within a clinical setting. Finally, an intervention level for rescue analgesia, at which point it is likely that behaviours shown are a result of pain and not environmental or situational factors is validated (Reid). The CMPS scales

are easy to use questionnaires and have been shown to have minimal interobserver variation (Guillot et al. 2011). However, they do require clinical reasoning so that confounding factors such as sedation or anxiety are taken into account when judging behaviours.

The dog and cat Glasgow CMPS are made of categories including posture, vocalisation, attention to painful area, mobility, demeanour, response to touch. Each category consists of words or expressions to describe behaviours associated with increased severity of pain. The assessor chooses the expression that best fits the animal's behaviour. A newer version of the cat CMPS also analyses facial expression since pain behaviours in cats are very subtle (Reid et al. 2017). Since the expressions remain constant, use between assessors is more consistent. Once all categories have been scored the scores are totalled to give an overall score. This is compared to the intervention level and addition analgesia administered of required. Validation is important when considering the ability of a particular pain scale to produce repeatable and reliable results and to detect clinically significant differences in the pain response. For a scale to be fully validated it is usually assessed against a 'gold standard' (criterion validity) (Reid et al. 2013). However, in veterinary medicine a gold standard does not exist so an established scale such as the SDS or NRS is used (Murrell et al. 2008; Calvo et al. 2014). The scale must also be valid for content and construct to ensure the content assesses pain appropriately and that the general hypotheses regarding pain are supported i.e. the scale supports the hypothesis that pain increases post-surgery and decreases after analgesia administration, as is expected (Holton et al. 2001). Scales must be validated in each language to ensure that meaning and intent of original terms is not lost (Brondani et al. 2013). Reliability is tested by measuring intra and interobserver variability. Ideally a successful pain scale will have minimal variability so that the scores are repeatable and interpretable by different users (Reid et al. 2007). In addition, pain scales must also be user-friendly and quick to carry out in order to be compatible in veterinary establishments, which are often busy.

2.4.d. Mechanical Nociceptive Threshold

Nociceptive thresholds show plasticity and adapt in response to peripheral and central sensitisation after acute or chronic pain. This results in primary hyperalgesia at the site of injury and secondary hyperalgesia in distant uninjured areas (Woolf 2011). The nociceptive threshold can be measured by applying a progressively increasing mechanical stimulus and recording the pressure or force at which an aversive response to the stimulus is shown by the individual (Le Bars et al. 2001). This is termed the mechanical nociceptive threshold (MNT) and provides an objective and quantified measurement of nociception in Newtons. A cut off value is applied

above which point pressure applied is stopped. This is to prevent the underlying tissue from being damaged.

The MNT can be measured using a variety of different tools including hand-held algometers, von Frey filaments or fixed actuators. These tools do not require a Home Office licence and can be used in a clinical setting. Hand held tools are generally easier to use because they do not require complicated set ups and can be used on different parts of the body and at different proximities to the wound. A hand held algometer was the tool of choice in the studies reported in this thesis. The tip size used has been shown to affect the MNT, since the force applied depends on the surface area of the probe tip as well as the pressure of application (force = pressure / area). Recent studies have shown that smaller tip sizes result in less variability (Taylor & Dixon 2012; Harris et al. 2015).

There are many variables that can confound MNT scores including tip size, test site, force application rate (Taylor & Dixon 2012; Harris et al. 2015), position of animal (Harris et al. 2015), environment, age, temperament (Briley et al. 2014) and conditioning to the stimulus (Coleman et al. 2014). This makes it hard to compare MNT results across studies and between individuals. A recent study by Harris et al. 2015 investigating MNT in healthy non-painful dogs showed that variability between individuals was the greatest factor affecting the response rate and repeatability of MNT (Harris et al. 2015). It is important to try and keep the above variables constant throughout the study. Inter-individual variability must also be accounted for when analysing MNT in pain studies. For example, in the case of postoperative pain, raw scores can be converted to percentage change from a baseline value taken for individuals prior to the application of a noxious stimulus such as surgery.

2.5. Surgical Procedures

Neutering is one of the most commonly performed procedures in veterinary practice. It is strongly recommended by the profession for population control and for welfare concerns associated with roaming and reproductive disease (BVA Neutering Policy). Removing the gonads and minimising the production of oestrogen or testosterone reduces sexually motivated behaviours such as roaming and vocalising and prevents diseases associated with the ovaries, uterus, mammary glands, testes or prostate.

2.5.a. Ovariohysterectomy in the dog

Ovariohysterectomy is the complete removal of the ovaries and uterus and has been shown to be moderately to severely painful in dogs and moderately painful in cats (Hardie et al. 1997;

Lascelles et al. 1999; Hellyer et al. 2007). This is consistent with the level of pain reported in women undergoing abdominal hysterectomy (Perniola et al. 2014). In the dog OVH involves a midline incision through the linea alba, a fibrous connective tissue layer between the abdominal muscles. The linea alba is chosen for the point of incision because it contains fewer nerve endings and blood vessels than the surrounding muscle resulting in less pain (Grint et al. 2006). Once within the abdominal cavity, the uterine horns are located using gentle manipulation of the abdominal organs. The ovaries are located by following the uterine horns to their cranial point. The ovaries are connected to the abdominal wall by the suspensory ligament. This ligament must be ruptured in order to gain adequate exposure to the ovaries in order to safely clamp and ligate the ovarian blood vessels. Pulling and tearing the suspensory ligament produces visceral pain and is considered to be the most stimulating aspect of surgery (Hoglund et al. 2011). Once the ovarian vessels, uterine body and associated vessels have been ligated the ovaries and uterus can be removed. The abdominal muscle layer, subcutaneous layer and skin are closed using appropriate suture material.

2.5.b. Ovariohysterectomy in the cat

In the cat, the principles of OVH are the same as for the dog, however the ovaries are slack and the suspensory ligament does not have to be torn. This reduces the associated visceral pain. A second technique termed the 'flank' technique is performed by many UK veterinary surgeons. In this technique, the incision is made through the abdominal muscles of the flank which allows immediate exposure to the uterus. However it is associated with greater pain compared to a midline approach because the muscle has more nerve endings (Grint et al. 2006). In our study the midline approach was performed on all cats. Since we were carrying out OVH on rescued cats many with unknown history, there was a possibility of pregnancy at the time of neutering. The midline approach is best in pregnancy as it allows better visualisation of the whole uterus and the ability to extend the incision length if needed.

2.6. Previous relevant literature

Recent studies investigating buprenorphine have shown to provides adequate analgesia for ovariohysterectomy in dogs (Shih et al. 2008; Slingsby et al. 2011; Hunt et al. 2013b). However, the difference in pharmacology between methadone and buprenorphine suggests that methadone may provide more efficacious analgesia in moderate to severely painful procedures. In addition, there have been limited studies directly comparing the two drugs with respect to analgesia. Recently, Hunt et al. (2013) compared buprenorphine and methadone in dogs undergoing orthopaedic surgery. They found that premedication with methadone in

combination with acepromazine had significantly better analgesic efficacy compared to buprenorphine and acepromazine (Hunt et al. 2013a).

Both methadone and buprenorphine have also been shown to provide adequate analgesia for feline ovariohysterectomy (Stanway et al. 2002; Taylor et al. 2010; Polson et al. 2012; Bortolami et al. 2013; Slingsby et al. 2014). However, there is some controversy over opioid efficacy in cats. Bortolami et al. (2013) and Slingsby et al. (2014) showed no difference between methadone, buprenorphine and butorphanol, when combined with acepromazine and medetomidine respectively, in post-operative analgesia following neutering (OVH and castration). However, castration in cats is thought to be mildly painful and the low pain scores obtained from male cats in this study reduced the power of the study to detect differences between the analgesics. In contrast, both buprenorphine and methadone have been shown to be superior to butorphanol in cats undergoing ovariohysterectomy (Taylor et al. 2010; Warne et al. 2013). To the author's knowledge there have been no studies comparing methadone and buprenorphine in the context of the triple or quad anaesthesia protocols.

A limited number of studies have directly compared the two opioids in their analgesic efficacy. We hypothesised that methadone would provide superior analgesia compared to buprenorphine in both cats and dogs undergoing ovariohysterectomy when incorporated into common premedication protocols in general practice. This would result in fewer animals requiring rescue analgesia and lower overall pain scores   within methadone groups. We also hypothesised that methadone would result in lower MNT scores since its NMDA antagonism should prevent central sensitisation and hyperalgesia.

3. Methodology

3.1. Design

Two, assessor blinded, randomised, prospective clinical trials were conducted. One investigating the analgesic efficacy of methadone and buprenorphine in dogs undergoing ovariohysterectomy and the other in cats undergoing ovariohysterectomy. The study protocols were approved by a local ethical review group (VIN/15/023) and were carried out under an Animal Test Certificate-S issued by the VMD.

3.2. Population

Sample size, inclusion criteria, and randomisation of drug protocols differed between the two studies and have been detailed in Chapters 4 and 5.

3.3. Assessments

Animals were fasted for a minimum of eight hours prior to anaesthesia but were provided with water until time of premedication. Baseline parameters for heart rate (HR), respiratory rate (f_R), sedation, pain and the mechanical nociceptive threshold (MNT) at the site of surgery were measured by the same assessor who was blinded to the treatment group. All assessments were carried out in this same order with a short break (1-2 minutes) between assessments of sedation, physiological parameters, pain and MNT. In the dog OVH study assessments were carried out at baseline, thirty minutes after premedication and postoperatively at 2, 3, 4, 5, 6, 7, and 8 hours after premedication. In the cat OVH study assessments were carried out at baseline, after QUAD induction and 2, 4,6 and 8 hours after QUAD administration.

3.3.a. Physiological parameters

Heart rate and respiration rate were measured manually by auscultation of heart and observation of breathing.

3.3.b. Simple descriptive scale for sedation

Sedation was measured using a simple descriptive scale (SDS) in both cats and dogs, which assigned a number to descriptions of increasing severity of sedation (Table 1).

Table 1. Simple descriptive scale (SDS) used to measure sedation (Hunt et al. 2013a)

SDS	Behaviour indicative of sedation
0	None
1	Mild (dog was relaxed but could be roused and could walk with little or no ataxia)
2	Moderate (dog was in sternal or lateral recumbency, but could be roused and had obvious signs of ataxia)
3	No response to stimulation

3.3.c. Dynamic interactive visual analogue scale (DIVAS) for sedation and pain

The Dynamic interactive visual analogue scale (Figure 4) was used to measure sedation (DIVASsed) and pain (DIVASpain). The DIVAS uses a 100mm scale where 0 represents no sedation or pain and 100 represents maximal sedation or the worst possible pain for the

procedure. Animals were assessed undisturbed from outside the kennel. They were then approached, spoken to and encouraged to stand and walk. The incision and surrounding area of the abdomen were palpated and reaction assessed. A mark corresponding to the intensity of sedation or pain was placed on the scale.

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0 mm                                                                          100 mm
No pain/      ●━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━●       Worst possible pain/
Sedation                                                                     Unarousable
```
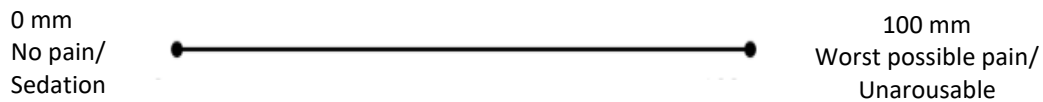
Figure 4. Schematic diagram of the DIVAS scale used to record level of pain (Lascelles et al. 1998).

3.3.d. Glasgow composite pain scales

Pain was also measured using the short form of the Glasgow Composite Pain Scale (SF-GCPS) (Reid et al. 2007) in dogs (Appendix 1), and the Composite Measure Pain Scale- feline (CMPS-F) (Calvo et al. 2014) in cats (Appendix 2). The questionnaire includes a set of behavioural categories each with descriptors which are ranked numerically according to their associated pain severity. The assessor assigns a score based on the descriptors that best match the behaviour of the patient. The total of the scores from each category make up the final pain score. In dogs the overall maximum score for the SF-GCPS is 24 in ambulatory dogs and 20 in non-ambulatory dogs, with an intervention score of 6/24 or 5/20 respectively. In cats the maximum score of the CMPS-F is 16 with an intervention score of 4.

N.B. Since the cat OVH study was conducted an additional category ranking ear and muzzle position has been added to the CMPS-F(Reid et al. 2017). This was not included in pain assessments conducted in our study since it was not available at the time of study.

3.3.e. Mechanical Nociceptive threshold

The MNT was measured as an indicator of secondary mechanical hyperalgesia using a pressure onset device (PRoD) manufactured by Topcat Metrology (Figure 5). A 2mm probe was placed approximately 3 cm away from the incision site and pressure applied at a rate of 2 Newtons per second. Testing was terminated when a positive response was seen such as deliberate movement away from the probe, guarding against the probe, looking around towards the probe, snapping/ hissing or biting. A cut-off of 20 N in dogs and 15 N in cats was applied due to risk of

tissue damage should higher forces be applied. Three measurements were taken and averaged to obtain a baseline value. At all other timepoints a single measurement was taken.

Figure 5. Image of the PRoD algometer (Topcat Metrology ®)

3.4. Anaesthesia protocol

Anaesthesia and analgesia protocol differed between the dog and cat studies and will be detailed in Chapters 4 and 5

3.5. Anaesthesia maintenance

Anaesthesia was maintained and monitored with the same method in both dogs and cats. Anaesthesia was maintained with isoflurane (ISO) vaporised in oxygen delivered with a T-piece breathing system for animals <10 kg bodyweight, and a circle system in animals >10kg bodyweight. Depth of anaesthesia and physiological parameters were monitored continuously by a Registered Veterinary Nurse. The ISO concentration was recorded as the vaporiser dial setting. Physiological parameters were measured using a multi-parameter monitor (PM9000 multiparameter monitor; Burtons) and included heart rate (HR), respiration rate (f_R), non-invasive blood pressure (NIBP), arterial haemoglobin saturation with oxygen (SpO$_2$) and end tidal carbon dioxide concentration (ETCO$_2$). Measurements were recorded prior to the first surgical incision and then at the following important time points: incision, ligation of right and left ovarian pedicles, ligation of cervix, final suture. Additional readings were taken if 5 minutes had passed since the last reading. The duration of surgery was measured from the time of incision to the placement of the last closing suture. All surgeries were carried out by the same experienced veterinary surgeon. Isoflurane administration was stopped at the end of surgery and the animal was taken back to its kennel for recovery.

3.6. Reversal and Recovery

Reversal and recovery differed between the dog and cat studies and have been described in Chapters 4 and 5.

3.7. Postoperative pain management

Postoperative pain management differed between the cat and dog studies and has been detailed in Chapters 4 and 5.

3.8. Adverse events

Any adverse events seen pre, intra or post operatively were noted. Adverse events included hypersalivation, vomiting, sedation and apnoea.

3.9. Statistical methods

Data from the studies were handled differently since the dog study had four treatment groups, whereas the cats study only has two treatment groups. Details of data analysis has been described in Chapters 4 and 5.

4. Dog Ovariohysterectomy Study

A comparison between methadone and buprenorphine for perioperative analgesia in dogs undergoing ovariohysterectomy

4.1. Abstract

Objective

This study investigated whether preoperative methadone provided superior perioperative analgesia compared to buprenorphine in dogs undergoing ovariohysterectomy.

Method

Eighty female dogs were recruited to an assessor-blinded, randomised, clinical trial. Dogs received either 0.05 mg/kg acepromazine (acp) or 10 µg/kg medetomidine (med) combined with 0.3 mg/kg methadone (MET) or 20 µg/kg buprenorphine (BUP) intramuscularly as premedication. Anaesthesia was induced with propofol and maintained with isoflurane. Pain was assessed using a dynamic interactive visual analogue scale (DIVAS) and the Glasgow Composite Pain Scale (SF-GCPS). Assessments were completed prior to premedication, 30 minutes later and every hour for eight hours after premedication. If indicated by the SF-GCPS, rescue analgesia was provided with methadone (0.3 mg/kg). If rescue analgesia was not given within 5 hours of premedication, a second dose of test opioid was administered. Meloxicam was administered after the last assessment. The area under the curve for change in SF-GCPS and DIVAS pain scores over time were compared using a General Linear Model (GLM). Requirement for rescue analgesia was compared using a Chi-squared test. Data are presented as mean ± SD, or median (range) as appropriate. Mean difference [95% CI] has been reported for comparisons between groups.

Results

Buprenorphine groups had significantly higher SF-GCPS and DIVASpain scores over time compared to methadone groups. There was no interaction between opioid and sedative for any outcome measure. Rescue analgesia was required by significantly more dogs premedicated with buprenorphine (45%) compared to methadone (20%) (p = 0.017).

Clinical significance

At the doses investigated methadone produced superior postoperative analgesia compared to buprenorphine in dogs undergoing ovariohysterectomy.

4.2. Introduction

Ovariohysterectomy has been shown to have the potential to cause moderate to severe acute postoperative pain in dogs (Hardie et al. 1997; Lascelles et al. 1999; Coleman & Slingsby 2007) and provision of sufficient analgesia is essential for patient welfare. Perioperative analgesia is considered to be the most effective method of reducing postoperative pain (Katz et al. 2011) and incorporates analgesia throughout the pre-, intra-, and postoperative phases. Opioids are the cornerstone of perioperative analgesia and the two opioids most widely used in UK veterinary practice are buprenorphine and methadone (Hunt et al. 2015). Buprenorphine is characterised as a partial μ receptor agonist, but has recently been shown to have the ability to produce full analgesia at less than 100% receptor occupancy (Raffa & Ding 2007). Methadone is a full μ receptor agonist considered to effectively treat moderate to severe pain (Inturrisi 2005).

Studies investigating buprenorphine have shown it to provide adequate analgesia for ovariohysterectomy in dogs (Shih et al. 2008; Slingsby et al. 2011; Hunt et al. 2013b). However there have been limited studies directly comparing the two drugs with respect to analgesia. Recently, Hunt and colleagues (2013b) compared buprenorphine and methadone in dogs undergoing orthopaedic surgery. They found that premedication with methadone in combination with acepromazine had significantly better analgesic efficacy compared to buprenorphine and acepromazine (Hunt et al. 2013a). We hypothesised that methadone would also provide superior analgesia compared to buprenorphine in dogs undergoing ovariohysterectomy.

A recent survey has shown that 57.3% of practices in the UK stock methadone compared to 98.9% stocking buprenorphine (Hunt *et al*. 2015). A possible explanation for the reduced use of methadone is unfamiliarity with dosing, safety and potential adverse reactions. This study aimed to address these concerns by investigating premedication with methadone and buprenorphine in combination with the common sedative drugs acepromazine and medetomidine to provide evidence for their effectiveness and practicality and in turn aid clinicians in their decision-making regarding opioid choice for moderate to severely painful procedures.

4.3. Materials and methods

4.3.a. Design

An assessor blinded, randomised, prospective clinical trial was conducted. The study protocol was approved by a local ethical review group (VIN/15/023) and was carried out under an Animal Test Certificate-S issued by the Veterinary Medicines Directorate.

4.3.b. Sample size

A formal power calculation was not conducted, but a similar study in dogs undergoing orthopaedic surgery by Hunt and colleagues (2013a) was able to show a difference between methadone and buprenorphine with 18 dogs per group (Hunt et al. 2013a). Similar outcome measures were used in the present study and a similar difference of 3 points in the SF-GCPS score was accepted as being clinically relevant. Given that all ovariohysterectomies would be carried out by the same qualified veterinary surgeon, 20 animals per group was predicted to be sufficient to see a difference in outcome measures in the present study.

4.3.c. Enrolment and inclusion

Eighty dogs undergoing routine ovariohysterectomy were recruited. Written, informed consent for inclusion in the study was obtained for all dogs prior to surgery (Appendix 3). All dogs underwent a pre-anaesthetic examination and only those falling within the American Society of Anaesthesiologists (ASA) physical status classification category 1 or 2 were included. Exclusion criteria included dogs which had received any analgesia, anaesthesia, or sedation within the previous 7 days, or animals that were not amenable to handling.

4.3.d. Randomisation

Dogs were block allocated to receive medetomidine or acepromazine. The first 20 animals received acepromazine, the second 20 received medetomidine and this was repeated. Dogs were randomly allocated to receive either buprenorphine or methadone (random number generator; www.random.org) within the two sedative groups separately to ensure an equal number of animals (n = 20) in each of the four groups: 1. acpBUP (acepromazine and buprenorphine), 2. acpMET (acepromazine and methadone), 3. medBUP (medetomidine and buprenorphine), 4. medMET (medetomidine and methadone). This method of randomisation was chosen for practical reasons because it would have been more difficult to run an efficient surgery list if dogs were completely randomised to the four groups.

4.3.e. Assessments

Methods used to measure sedation, pain and physiological parameters were similar in both studies and have been described in Chapter 3.

4.3.f. Administration of test drugs

Baseline parameters for HR, f_R, sedation, pain and the MNT were recorded prior to administering premedication.

Premedication consisted of either 0.05 mg/kg acepromazine (ACP 2 mg/mL; Novartis Animal Health) or 10 µg/kg medetomidine (Sedator 10 mg/mL; Dechra Veterinary Products) combined with 20 µg/kg buprenorphine (Buprenodale 0.3 mg/mL; Dechra Veterinary Products) or 0.3 mg/kg methadone (10 mg/mL Comfortan; Dechra Veterinary Products). Drugs were drawn up in the same syringe by the surgeon (not blinded to the treatment) and administered intramuscularly into the quadriceps muscles. The assessor was not present when premedication was administered. Thirty minutes were allowed to elapse for the premedication drugs to have an effect before further measurements were taken.

4.3.g. Anaesthesia

Pre-induction parameters for HR, f_R, temperature (T), sedation and the MNT were recorded at the 30-minute time point. An intravenous (IV) catheter was placed into the cephalic vein and anaesthesia induced by IV injection of propofol to effect (Propoflo 10mg/mL; Zoetis). Jaw-tone and palpebral reflexes were monitored until the level of anaesthesia was adequate for orotracheal intubation with a cuffed endotracheal tube. The dose of propofol used was recorded. Maintenance and monitoring of anaesthesia and reversal and recovery have been described in Chapter 3.

4.3.h. Reversal and Recovery

Medetomidine was antagonised with atipamazole (Atipam 5 mg/mL; Dechra Pharmaceuticals). A dose volume equivalent of the volume of medetomidine administered as premedication in was given intramuscularly at the point of extubation. The orotracheal tube was removed when the swallowing reflex returned. Dogs were placed in a kennel and covered with padded kennel liners or reflective blankets. Body temperature was measured until normal (>37°C). Time taken from extubation to head lift, sternal recumbency, and standing was recorded. Quality of recovery was evaluated using a SDS scale (Table 2).

Table 2. Simple descriptive scale (SDS) used to measure the quality of recovery (Hunt et al. 2013a)

SDS	Quality of recovery
0	Poor - animal shows **major** signs of excitement during recovery such as thrashing or moving around rapidly unaware of surroundings, which do not respond to gentle handling.
1	Moderate - animal shows **some** signs of excitement during recovery such as thrashing or moving around rapidly unaware of surroundings, which resolve with gentle handling.
2	Good - **mild** signs of excitement, which resolve quickly so that the animal becomes calm
3	Excellent - animal is **calm** and relaxed during recovery

4.3.i. Postoperative assessments and pain management

Heart rate, f_R, sedation, pain and the MNT were assessed 2, 3, 4, 5, 6, 7 and 8 hours after the administration of premedication in the same order unless the animal was still anaesthetised.

A second dose of the allocated test opioid was scheduled to be administered at 5 hours post premedication in all dogs. This was drawn up by the surgeon carrying out the surgery and given intramuscularly. The assessor remained blinded to the second administration of analgesia. However, a SF-GPCS score of score of ≥ 5/20 in non-ambulatory dogs or ≥ 6/24 in ambulatory dogs indicated requirement of additional (rescue) analgesia (methadone 0.3 mg/kg, IM). The assessor was not blinded to administration of rescue analgesia. Pain was assessed 30 minutes later and if required another dose of 0.3 mg/kg methadone given IM. No dogs needed additional analgesia following a second dose of rescue analgesia, but if additional analgesia had been necessary the assessor would have administered 0.2 mg/kg meloxicam (Metacam; Boehringer-Ingelheim) subcutaneously. Dogs that required rescue analgesia before or at the 5-hour time-point did not receive the scheduled second dose of test opioid. All dogs were administered 0.2 mg/kg meloxicam subcutaneously (Metacam®; Boehringer-Ingelheim) after assessments were completed 8 hours post premedication. Any adverse events seen pre, intra or post operatively were noted. Adverse events included hypersalivation, vomiting, sedation and intraoperative apnoea.

4.3.j. Statistical methods

Data were assessed for normality by visual inspection of histograms and the Shapiro-Wilk test and appropriate parametric and non-parametric techniques used (SPSS Statistics Version 23; IBM Corporation). Parametric data including age, weight, surgery time, pain and MNT scores were analysed using a general linear regression model (GLM) to enable analysis of the factorial effect of sedative and opioid and the interaction between the two. The area under the curve (AUC) was calculated for repeated intraoperative and postoperative pain and MNT

parameters. The SF-GCPS was scored out of 20 in non-ambulatory dogs or 24 in ambulatory dogs. In order to compare all results, scores were converted to a fraction (between 0 and 1) with 20 or 24 as the denominator. Postoperative MNT scores for each individual were converted to percentage change from baseline values to account for the variation in pain threshold between individuals. Baseline values were given a value of 100%. The proportion of dogs in each group requiring rescue analgesia and experiencing adverse events was compared using a Chi-squared test. A Cox-regression survival curve was used to analyse the effect of sedative and opioid on the requirement of rescue analgesia. No parametric data such as sedation scores and recovery data were analysed using a Kruskall-Wallis test as separate time points. A Bonferroni correction was applied when multiple comparisons were carried out. Pain scores were not corrected for rescue analgesia i.e. in dogs receiving rescue analgesia recorded scores were included in the analysis rather than using the last observation carried forward technique. P values of < 0.05 were considered statistically significant unless multiple comparisons were performed when a Bonferroni correction was applied. Data for all four groups are reported as mean ± SD for normal data or median (range) for non-normal data. The mean difference with 95% confidence intervals (95%CI) are reported for comparisons between groups.

4.4. Results

4.4.a. Demographic data

Demographic data were similar between groups (Table 3).

Table 3. Age, weight and surgery time (mean ± SD) in dogs undergoing ovariohysterectomy (n = 20 for all four groups). There was no significant difference between groups for age, weight or surgery time.

Group	Age (months)	Weight (kg)	Surgery duration (mins)
acpMET	22.4 ± 12.3	14.8 ± 12.7	23.9 ± 4.27
acpBUP	28.9 ± 24.9	16.4 ± 10.6	23.1 ± 4.66
medMET	22.3 ± 19.0	14.4 ± 8.3	22.5 ± 3.30
medBUP	25.1 ± 19.8	15.4 ± 8.8	22.8 ± 3.18

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.b. Preoperative assessments

    4.4.b.i. Physiological parameters

Baseline HR and f_R were within normal clinical limits and did not differ among the four groups. Thirty minutes after premedication HR and f_R were significantly lower compared to baseline for all four groups but remained within normal expected clinical limits after administration of the sedative drugs ($p < 0.001$) (Table 4). There was no difference in HR or f_R between methadone and buprenorphine groups 30 minutes after premedication ($p=0.54$ and 0.66 respectively), however, medetomidine groups had significantly lower HR (42 beats/minute [95% CI 32-50]) and f_R (16 breaths/minute [95% CI 8-23]) than acepromazine groups ($p < 0.001$ for both comparisons).

    4.4.b.i. Sedation

Sedation was significantly increased in all groups post premedication ($P < 0.001$) (Table 5). There was no difference in SDS or DIVAS sedation scores post-premedication with respect to opioid administration. However, both SDS and DIVAS scores showed dogs administered medetomidine were significantly more sedated following premedication than those administered acepromazine ($P < 0.0001$).

Table 4. Heart rate and respiration rate (mean ± SD) at baseline and thirty minutes post premedication (sedated) in all four premedication groups (n = 20 for all four groups). There was no difference in baseline HR or f_R value between groups. After premedication both HR and f_R were significantly lower in groups administered medetomidine ($p < 0.001$ for both HR and f_R). Opioid did not affect HR or f_R.

Group	Baseline HR (beats / minute)	Sedated HR (beats / minute)	Baseline RR (breaths / minute)	Sedated RR (breaths / minute)
acpMET	112 ± 18	91 ± 24	35 ± 14	32 ± 16
acpBUP	109 ± 27	99 ± 28	29 ± 5	32 ± 16
medMET	123 ± 24	53 ± 12	32 ± 9	21 ± 6
medBUP	116 ± 22	53 ± 11	30 ± 11	23 ± 13

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

Table 5. SDS sedation scores (median (range)) and DIVASsed scores (mean ± SD) in dogs following premedication (sedated) in all four groups (n = 20 in all four groups). There was significant sedation after premedication compared to baseline sedation scores ($p < 0.001$) in all four groups. Dogs administered medetomidine showed greater sedation than those administered acepromazine after premedication. Opioid choice had no effect in sedation ($p < 0.0001$).

Group	Sedated SDS	Sedated DIVAS (mm)
acpMET	2 (1-3)	36 ± 20
acpBUP	2 (1-3)	27 ± 21
medMET	3 (2-3)	83 ± 19
medBUP	3 (2-3)	79 ± 18

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.b.i. Pain

Pain scores, measured using the DIVAS and SF-GCPS were 0 in all dogs at baseline and following premedication, with no significant differences between groups.

4.4.b.ii. MNT

Baseline MNT values were not significantly different between groups, but significantly increased post premedication in all groups (Table 6) (P < 0.0001).

There was no significant difference in MNT score post premedication with respect to opioid (p=0.28). However, post-premedication MNT scores were significantly higher in medetomidine groups compared to acepromazine groups (158 % [95% CI 123-249], p < 0.001). There was no interaction between opioid and sedative (p=0.439)

Table 6. Mechanical nociceptive threshold (MNT) (mean ± SD) before premedication and thirty minutes after premedication (sedated) in all four groups (n = 20 for all four groups). MNT threshold significantly increased in dogs after premedication compared to baseline (p < 0.0001). Dogs administered medetomidine showed a greater increase in MNT post premedication compared to those administered acepromazine (p < 0.001). Choice of opioid did not affect sedation.

Group	Baseline MNT (Newtons)	Sedated MNT (Newtons)	Sedated MNT (% change)
acpMET	6.47 ± 2.50	11.63 ± 4.11	192 ± 75.6
acpBUP	6.06 ± 2.22	10.1 ± 3.99	182 ± 86.7
medMET	5.48 ± 2.81	17.8 ± 3.45	403 ± 201
medBUP	5.51 ± 2.35	16.6 ± 4.88	344 ± 161

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.b.iii. Propofol requirement

There was no difference in propofol requirement between dogs that received buprenorphine and dogs that received methadone (0.47 mg/kg [95% CI 0.18-1.13 mg/kg] p=0.150). However, dogs premedicated with acepromazine required significantly more propofol than dogs premedicated with medetomidine (2.08 mg/kg [95% CI 1.43-2.74 mg/kg] p < 0.001) (Table 7). There was no significant interaction between sedation and opioid administered (p=0.378).

Table 7. Dose of propofol (mean ± SD) required for induction of anaesthesia in all four groups (n = 20 for all four groups). Choice of opioid did not affect propofol requirement. However, dogs administered medetomidine required less propofol than those administered acepromazine (p < 0.001).

Group	Propofol dose (mg/kg)
acpMET	3.70 ± 1.88
acpBUP	4.10 ± 2.09
medMET	1.55 ± 0.74
medBUP	1.74 ± 0.96

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.c. Intraoperative assessments

4.4.c.i. Isoflurane requirements

There was no significant difference in ISO concentration, measured as the dialled vaporiser setting, between methadone and buprenorphine groups (p=0.510). However, dogs administered acepromazine required more isoflurane over time than the medetomidine group (0.5% [95% CI 0.3-0.8] p < 0.001) (Table 8). There was no significant interaction between sedative and opioid (p=0.604) in terms of required isoflurane concentration.

Table 8. ISO concentration (mean ± SD) over time (dialled vaporiser setting) for all four groups premedication (n = 20 for all four groups). Choice of opioid did not affect propofol requirement. However, dogs administered medetomidine required less propofol than those administered acepromazine (p < 0.001).

Group	ISO concentration (%)
acpMET	2.80 ± 0.55
acpBUP	2.66 ± 0.59
medMET	2.20 ± 0.65
medBUP	2.15 ± 0.57

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.c.ii. Physiological parameters

Physiological parameters (HR, f_R, SBP, DBP, MBP, ETC0$_2$ and SpO$_2$) from six time-points during surgery (baseline, skin incision, ligation of right and left ovarian pedicles, ligation of cervix, last closing sutures) were analysed over time (Table 9). There was no significant difference between methadone and buprenorphine for any parameter over time, however dogs administered medetomidine had lower HRs (23 beats/minute [95% CI 16-30], p < 0.001) and higher SAP (10 mmHg [95% CI 2-19], p = 0.014), DAP (23 mm Hg [95% CI 16–30], p < 0.001) and MAP (16 mmHg [95% 9-22], p < 0.001) compared to dogs the received acepromazine. There was no significant difference between groups with respect to ETCO2 or SpO2.

4.4.d. Recovery

Recovery quality was generally good in all dogs. There was no significant difference in recovery quality with respect to opioid (p=0.643) or sedative (p=0.354). There was also no difference in the time taken between removal of endotracheal tube and head lift, sternal recumbency and standing with respect to opioid (p=0.09, 0.128 and 0.173 respectively), however, dogs in the medetomidine groups had shorter recovery times in all three-time frames (p < 0.001) for each time period (Table 10). There was no significant interaction between sedative and opioid for any time period (p=0.386, 0.782, 0.801 for the three time periods respectively).

Table 10. Median (range) recovery quality and recovery times (minutes) to headlift, sternal recumbency and unaided standing after removal of endotracheal tube for all four groups (n = 20 ifor all 4 groups). There was no difference in recovery quality or time with respect to opioid. Recovery quality was also not affected by choice of sedative, however, recovery time was significantly shorter for dogs administered medetomidine compared to acepromazine (p < 0.001).

	Recovery quality				Recovery time (minutes)		
	Extubation	Head lift	Sternal	Standing	Headlift	Sternal	Standing
acpMET	3 (0-3)	3 (0-3)	3 (1-3)	3 (2-3)	6 (0-23)	20 (5-40)	35 (10 - 70)
acpBUP	3 (2-3)	3 (2-3)	3 (2-3)	3 (3-3)	10.5 (3-35)	23 (8-47)	37.5 (12 - 60)
medMET	3 (2-3)	3 (1-3)	3 (1-3)	3 (2-3)	5 (2-9)	7 (3-18)	17 (5-40)
medBUP	3 (2-3)	3 (2-3)	3 (2-3)	3 (2-3)	5 (0-15)	10 (2-25)	23 (9-45)

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

Table 9. Intraoperative variable from 6 time points during surgery for all four groups. There was no significant difference between methadone and buprenorphine for any parameter over time, however dogs administered medetomidine had lower HRs ($p < 0.001$) and higher SAP ($p = 0.014$), DAP ($p < 0.001$) and MAP ($p < 0.001$) compared to dogs the received acepromazine.

| | | Time points | | | | | | | | | | | |
| | | Baseline | | Incision | | Ligating R pedicle | | Ligating Left pedicle | | Ligating cervix | | Last suture | |
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ISO vapouriser setting (%)	acpMET	3	1	3	1	3	1	3	1	3	1	2	1
	acpBUP	3	1	3	1	3	1	3	1	3	1	3	1
	medMET	2	0	2	1	2	1	2	1	2	1	2	1
	medBUP	2	0	2	0	2	1	2	1	2	1	2	1
Heart rate (beats/min	acpMET	92	17	99	17	103	18	102	25	100	20	96	22
	acpBUP	101	21	103	23	105	24	104	16	101	15	97	12
	medMET	68	18	74	19	74	18	80	16	75	17	79	16
	medBUP	77	22	81	21	85	17	89	17	89	18	82	16
Respiration rate (beats/min)	acpMET	15	8	15	8	14	8	11	9	9	3	10	4
	acpBUP	13	6	12	6	17	18	10	7	10	9	8	2
	medMET	11	5	12	6	15	9	12	8	10	7	12	8
	medBUP	12	8	13	9	19	18	17	15	10	4	11	4
Systolic arterial pressure (mmHg)	acpMET	91	15	92	14	107	25	116	20	104	23	92	22
	acpBUP	90	21	95	22	112	26	107	28	97	23	90	18
	medMET	101	17	101	16	108	18	115	20	107	22	98	16
	medBUP	109	21	114	27	129	19	123	19	116	17	110	22
Diastolic arterial pressure (mmHg)	acpMET	32	9	37	14	56	16	61	20	48	21	40	17
	acpBUP	32	11	42	28	50	19	46	21	40	19	32	12
	medMET	54	14	53	15	62	17	70	12	61	22	52	13
	medBUP	64	20	66	29	84	20	83	18	73	19	69	24
Mean arterial pressure (mmHg)	acpMET	57	9	59	10	78	18	83	19	66	20	62	20
	acpBUP	56	14	62	21	72	18	73	21	63	19	58	13
	medMET	71	14	70	14	82	16	87	16	78	21	73	12
	medBUP	79	17	81	23	99	22	96	20	86	19	80	18
End tidal CO_2 (mmHg)	acpMET	41	6	41	5	41	5	44	6	45	5	42	7
	acpBUP	43	9	41	9	42	11	44	12	45	8	47	7
	medMET	48	5	49	6	46	9	48	7	47	7	47	5
	medBUP	45	7	46	9	43	10	45	8	47	7	44	8
SpO_2 (%)	acpMET	96	2	95	2	94	4	95	3	96	3	95	4
	acpBUP	96	3	96	3	94	3	94	4	95	3	96	2
	medMET	95	3	95	4	95	3	93	5	94	4	95	4
	medBUP	96	2	95	4	92	5	93	5	93	3	94	3

acpMET: acepromazine and methadone; acpBUP: acepromazine and buprenorphine; medMET: medetomidine and methadone; medBUP: medetomidine and buprenorphine.

4.4.e. Postoperative assessments

4.4.e.i. Pain

Rescue analgesia was required by significantly more dogs premedicated with buprenorphine (45%, 18/40 dogs) compared to methadone (20%, 8/40 dogs) (Chi squared test, p = 0.017). Of the dogs that required rescue analgesia, 88% (6 dogs in the methadone group and 16 dogs in the buprenorphine group) required additional analgesia prior to the scheduled second dose of analgesia 5 hours post premedication, and 12% (2 dogs in the methadone group and 2 dogs in the buprenorphine group) required it after 5 hours but before 8 hours post premedication. A Cox regression survival analysis showed methadone resulted in a lower requirement of rescue analgesia compared to buprenorphine, p= 0.02. Choice of sedative (p=0.413) or the interaction between opioid and sedative (p=0.107) did not affect rescue analgesia requirement (Figure 6).
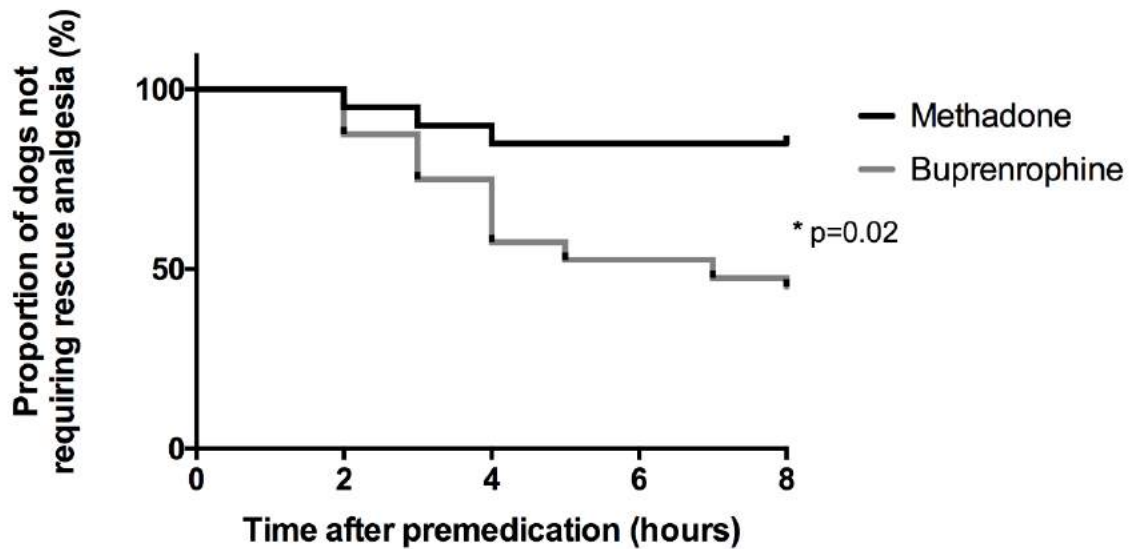
Figure 6. A Cox Regression curve showing survival as the number of dogs not requiring analgesia at each postoperative timepoint between methadone (n = 40) and buprenorphine groups (n = 40). Methadone groups required significantly less rescue analgesia (p = 0.02). Choice of sedation did not affect postoperative rescue analgesia requirement

Methadone resulted in lower overall pain scores compared to buprenorphine for both SF-GCPS and DIVAS pain scores. Mean area under curve for the SF- GCPS pain scores was significantly greater (P < 0.001) in the buprenorphine group than the methadone group (0.31 [95% CI 0.204-0.459]). Choice of sedative (p = 0.729) and the interaction between sedative and opioid (p= 0.370) had no effect on SF-GCPS pain scores. Mean area under curve for the DIVAS pain scores were also significantly greater (P = 0.01) in buprenorphine groups than methadone groups (3.02 [95% CI 1.18-4.86] cm). Choice of sedative (p = 0.579) and the interaction between sedative and opioid (p = 0.593) had no effect on DIVAS pain scores (Figures 7a and 7b).

A

B
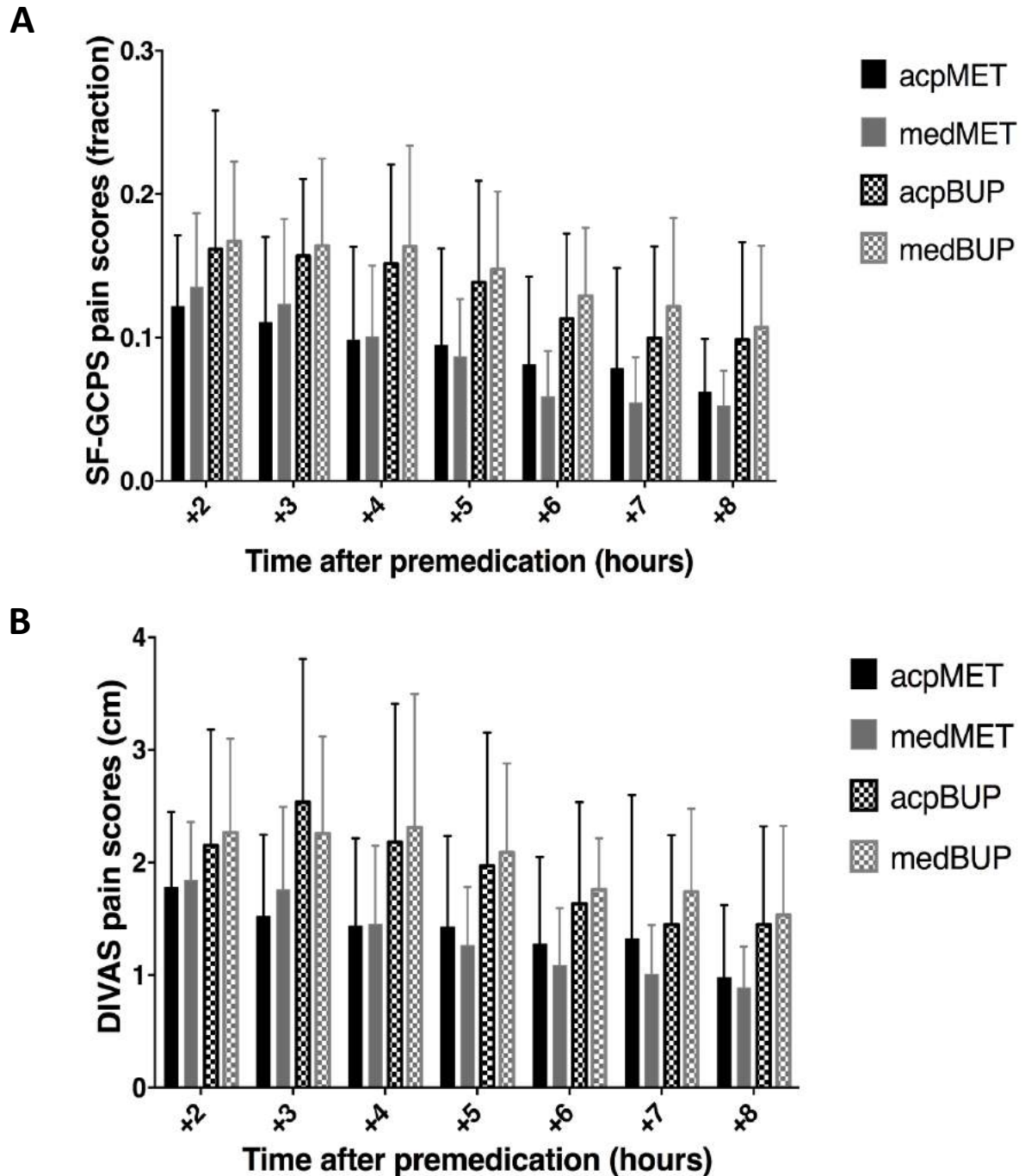
Figure 7. **(A)** Mean postoperative Glasgow Composite Pain Scores (SF-GCPS) over time for all four groups (n = 20 for all four groups). Values are presented as a fraction of the total possible score (20 or 24). Error bars indicate SD. **(B)** Mean postoperative dynamic interactive visual analogue scale scores for pain (DIVAS pain scores) over time for all four groups (n = 20 for all four groups). Error bars indicate SD. Dogs administered buprenorphine had greater overall postoperative SF-GCPS (p < 0.001) and DIVAS (p = 0.01) scores compared to methadone groups. Choice of sedative did not affect postoperative pain scores.
acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.e.ii. Mechanical Nociceptive Threshold

Percentage change was used to calculate the difference in the MNT scores post-operatively compared to baseline to account for individual variation in nociceptive threshold. There was no difference in the overall postoperative MNT scores between dogs receiving methadone and buprenorphine (p = 0.25). Choice of sedative (p = 0.09) and interaction between sedative and opioid (p = 0.9) did not have a statistically significant effect on MNT (Figure 8).
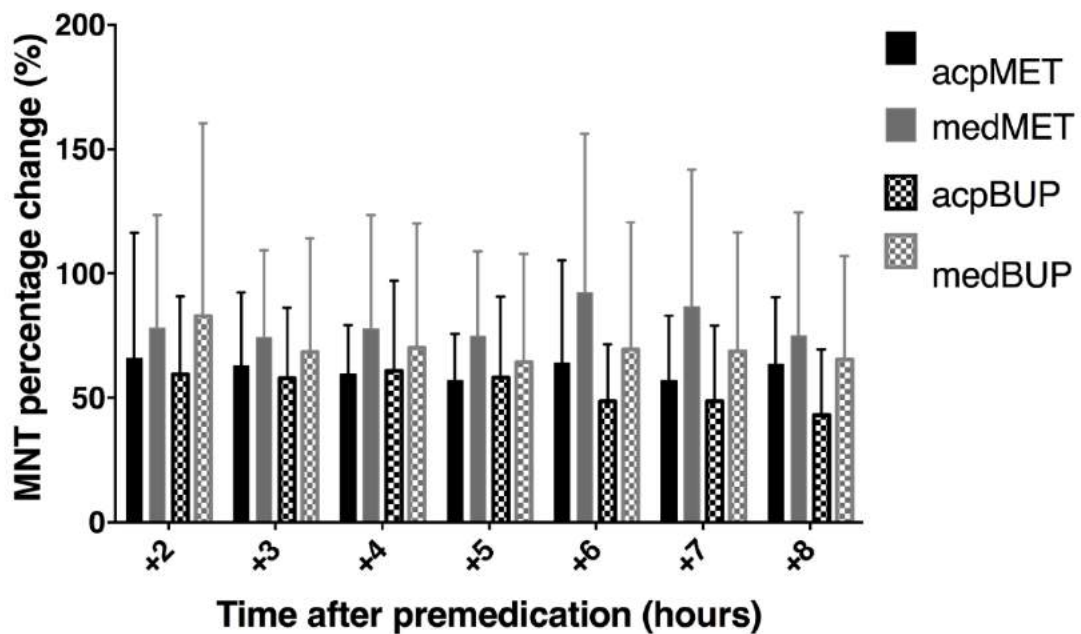
Figure 8. Mean postoperative mechanical nociceptive threshold (MNT) scores over time for all four groups (n = 20 for all four groups). Values are presented as percentage change from baseline. Error bars indicate SD. Postoperative MNT scores did not differ between groups. acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.e.iii. Sedation

There was no statistically significant difference in SDS or DIVAS postoperative sedation scores at any time-point with respect to opioid. However, dogs premedicated with acepromazine showed significantly higher SDS (Figure 9a) and DIVAS sedation (Figure 9b) scores 2, 3 and 4 hours post-premedication compared to the medetomidine groups (p < 0.001 at each timepoint).
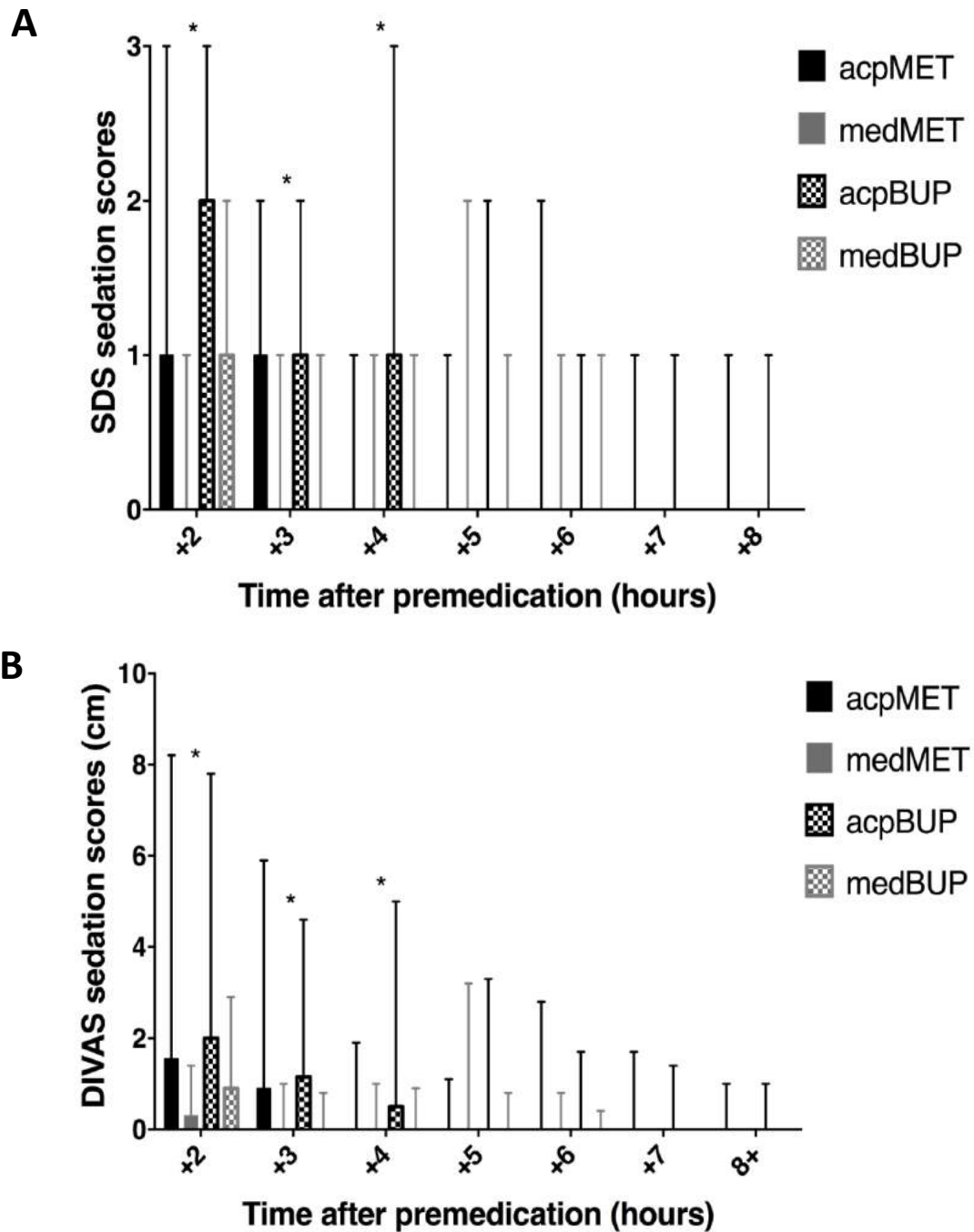
Figure 9. **(A)** Median postoperative simple descriptive scale (SDS) sedation scores over time for all four groups (n = 20 for all four groups). Error bars indicate range. * denotes a significant difference between acepromazine and medetomidine groups at each timepoint (p < 0.001). **(B)** Mean postoperative DIVASsed scores over time for all four groups (n = 20 for all four groups). Error bars indicate SD. * denotes a significant difference between acepromazine and medetomidine groups at each timepoint (p < 0.001). acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.4.e.iv. Adverse effects

The frequency of adverse effects was low overall and those observed during the study included hypersalivation, vomiting, sedation and apnoea during anaesthesia. Each adverse effect was compared among all four groups (Table 11). Only hypersalivation on recovery showed a significant difference between groups (p = 0.03). This difference occurred between medetomidine and acepromazine groups and there was no difference with respect to opioid (p = 1.00) or interaction between sedative and opioid (p = 1.00). There was no significant difference among groups for any other adverse effect.

Table 9. Adverse reactions observed and the number of dogs affected in each group (n = 20 for all four groups). Hypersalivation on recovery was the only noted adverse effect to differ between groups, with dogs administered medetomidine showing significantly more hypersalivation on recovery than those administered acepromazine (p = 0.03).

Adverse reaction	acpMET	acpBUP	medMET	medBUP
Hypersalivation after premedication	0	0	1	0
Hypersalivation after opioid at 5 hours	1	0	1	1
Hypersalivation on recovery	0	0	4	4
Vomiting after premedication	0	1	1	0
Vomiting after opioid at 5 hours post premedication	2	1	1	0
Vomiting on recovery	0	0	1	0
Sedation after rescue	1	0	0	0
Apnoea during anaesthesia	0	0	2	1

acpMET: acepromazine and methadone premedication; acpBUP: acepromazine and buprenorphine premedication; medMET: medetomidine and methadone premedication; medBUP: medetomidine and buprenorphine premedication.

4.5. Discussion

Dogs that received 0.3 mg/kg methadone had significantly lower SF-GCPS and DIVAS pain scores and required significantly less rescue analgesia compared to groups that received 20 µg/kg buprenorphine. This supports our hypothesis that methadone produces superior perioperative analgesia compared to buprenorphine for ovariohysterectomy in dogs.

Previous studies have found buprenorphine produces adequate analgesia in dogs undergoing OVH. Hunt et al. (2013a) showed 20 µg/kg buprenorphine combined with 25 µg/m^2 dexmedetomidine or 0.03 mg/kg acepromazine provided suitable analgesia in dogs and cats undergoing ovariohysterectomy and castration. However, perioperative meloxicam was also administered, likely decreasing postoperative pain scores. In addition, castration is less painful than ovariohysterectomy (Slingsby *et al*. 2011) and inclusion of associated data may have decreased overall pain scores. Slingsby *et al*. (2011) found 20 µg/kg buprenorphine combined with 0.03 mg/kg acepromazine resulted in an overall mean DIVASpain score of 40mm in dogs undergoing OVH, suggesting adequate analgesia since ≥50mm was set as the rescue point. Despite low overall pain scores, 92% (11/12) dogs required rescue analgesia within 6 hours post premedication. Our study set 40mm as the rescue point and found that 40% (16/40) of dogs administered 20 µg/kg buprenorphine required rescue analgesia within 5 hours post premedication. This disparity may be due to a difference in pain scoring tools used to determine requirement of rescue analgesia, different criteria for rescue analgesia or a difference assessor sensitivity to pain. A study by Morgaz et al. (2013) investigating 20 µg/kg buprenorphine combined with 3 µg/kg medetomidine used a composite scale to determine rescue analgesia requirement and similarly showed 43% (10/23) dogs required rescue analgesia. This suggests composite scales are more sensitive to pain behaviours than the DIVAS pain scale. In our study overall pain scores were low with mean SF-GCPS and DIVAS scores for both methadone and buprenorphine groups falling below the cut off scores for rescue analgesia, 0.25 and 40mm respectively. However, many animals still required rescue analgesia highlighting the importance of regular postoperative pain scoring for any surgical procedure.

Despite low overall pain scores, dogs administered methadone had even lower pain scores and required significantly less rescue analgesia compared to buprenorphine groups. This suggests that methadone is the more efficacious analgesic in ovariohysterectomy and this is likely to be true for other moderate to severely painful surgeries. These findings are mirrored by the results of a study by Hunt and colleagues (2013) which show that methadone is superior to buprenorphine in dogs undergoing orthopaedic surgery.

In the population of dogs that required rescue analgesia, the number of additional methadone administrations per rescue did not differ despite initial premedication opioid, suggesting previous administration of buprenorphine did not antagonise the clinical analgesic effect of methadone. Of the dogs that received rescue analgesia, 88% needed treatment before the scheduled second dose of test opioid at 5 hours, i.e. before the expected duration of action of both drugs (Brodbelt et al. 1997, Ingvast-Larsson et al. 2010). This illustrates individual variation in response to opioids and highlights the need for regular postoperative pain assessments. Five hours was chosen as the time for the second dose of test opioid as it incorporated the expected duration of action of both methadone and buprenorphine. Therefore, the difference in requirement of rescue analgesia between methadone and buprenorphine groups is a result of inadequate analgesic efficacy and unlikely to be due to pharmacokinetic differences between the two drugs.

Our results showed no difference in pain scores between medetomidine and acepromazine groups despite medetomidine's antinociceptive activity. This is most likely because the antinociceptive effects of medetomidine are short lived and have been shown to only produce analgesia for mild pain even at high doses (Ansah et al. 1998). In addition, antinociception is dose dependent (Murrell & Hellebrekers 2005) and a relatively low dose of 10µg/kg was used in this study.

Propofol dose required for induction of anaesthesia and isoflurane concentration required for maintenance of anaesthesia was lower in dogs administered medetomidine compared to those administered acepromazine. This is most likely a result of the greater sedative effect of medetomidine compared to acepromazine. There was also a difference between the two sedatives in intraoperative HR and blood pressure parameters. Heart rate was lower and blood pressure (SAP, DAP and MAP) higher in medetomidine groups compared to acepromazine groups. Medetomidine acts on peripheral α-2 receptors, resulting in vasoconstriction followed by an increase in blood pressure and a reflex decrease in heart rate (Rankin 2015). Conversely, acepromazine's action on peripheral α-1 receptors results in vasodilation and a subsequent decrease in blood pressure (Coulter et al. 1981), which explains the cardiovascular differences between the two sedatives. In addition, the dose of propofol and concentration of isoflurane required in acepromazine groups were greater than medetomidine groups, both of which cause a reduction in arterial blood pressure (Berry 2015; Steffey et al. 2015). Overall cardiovascular and respiratory values remained clinically acceptable throughout anaesthesia.

The dose of methadone used in the present study (0.3 mg/kg) was lower than the 0.5-1 mg/kg dose stated in the datasheet (Comfortan 10 mg/mL, NOAH Compendium). This lower dose is widely recommended since it anecdotally provides good analgesia and eliminates some of the dose-dependent adverse reactions such as respiratory depression and bradycardia seen at higher doses (Maiante et al. 2009; Credie et al. 2010). The results of the present study demonstrate that the lower dose of 0.3 mg/kg of methadone provides good analgesia and is well tolerated. The incidence of each adverse effect reported was similar between groups. A significant difference was shown in hypersalivation on recovery between acepromazine and medetomidine groups. However, this was most probably caused by the administration of atipamazole to antagonise medetomidine on recovery, since hypersalivation is a possible adverse effect of atipamazole (Atipam 5mg/mL SPC, 2013. Noah compendium).

The aim of this study was to provide clinical evidence to help clinicians in their decision-making regarding opioid use. Using methadone at a lower dose may dispel worries about analgesic efficacy, dose unfamiliarity and drug safety, factors which were identified by a recent survey as influencing clinicians when choosing an opioid for perioperative analgesia (Hunt et al. 2015).

In conclusion, our results support the hypothesis that methadone provides superior perioperative analgesia compared to buprenorphine in dogs undergoing ovariohysterectomy.

5. Cat Ovariohysterectomy Study

A comparison between methadone and buprenorphine within the QUAD protocol for perioperative analgesia in cats undergoing ovariohysterectomy

5.1. Abstract

Objective

The aim of this study was to investigate analgesic efficacy of methadone compared to buprenorphine within the QUAD protocol for anaesthesia in cats undergoing ovariohysterectomy.

Method

One hundred and twenty cats were recruited to an assessor-blinded, randomised, clinical trial. Cats received either methadone ($5mg/m^2$) or buprenorphine ($180\mu g/m^2$) combined with ketamine, midazolam and medetomidine intramuscularly. Anaesthesia was maintained with isoflurane in oxygen. Pain was assessed using the feline Composite Measure Pain Scale (CMPS-F) and a dynamic interactive visual analogue scale (DIVASpain). Sedation, pain, heart rate, and respiratory rate were measured prior to QUAD administration, before intubation, and 2, 4, 6 and 8 hours post QUAD administration. If indicated by the CMPS-F, rescue analgesia was provided with 0.5mg/kg of methadone administered intramuscularly. Meloxicam was administered after the last assessment. Differences in pain scores between groups were compared using a repeated measures ANOVA and requirement for rescue analgesia was compared using a Chi-squared test. Data are presented as mean ± SD.

Results

Cats administered methadone had lower CMPS-F scores over time ($p = 0.04$) and required less rescue analgesia ($p = 0.028$) compared with those administered buprenorphine.

Clinical Significance

Overall, methadone produced clinically superior post-operative analgesia compared to buprenorphine when used within the QUAD protocol in cats undergoing ovariohysterectomy.

5.2. Introduction

Neutering is essential for population control and is carried out on a large scale in animal shelters. There is often limited anaesthetic monitoring equipment in these situations and a safe but effective anaesthetic and analgesic regime is important. The QUAD protocol, comprises of medetomidine, ketamine, midazolam and buprenorphine. It was developed at the RSPCA Greater Manchester Animal Hospital (GMAH) to provide safe anaesthesia and analgesia for neutering in cats, particularly young cats, by providing a multimodal anaesthesia and analgesia technique and dosing on the basis of body surface area (Joyce & Yates 2011).

Both methadone and buprenorphine have been shown to provide adequate analgesia for feline ovariohysterectomy (Stanway et al. 2002; Taylor et al. 2010; Polson et al. 2012; Bortolami et al. 2013; Slingsby et al. 2014). However, there is some controversy over opioid efficacy in cats. No overall difference in postoperative analgesia following neutering (OVH and castration was found between methadone, buprenorphine and butorphanol, when combined with acepromazine (Bortolami et al. 2013). When combined with medetomidine (Slingsby et al. 2014) buprenorphine produced lower DIVAS pain scores at 3, 4, and 5 hours post premedication but showed no difference in rescue analgesia between opioids. In contrast, buprenorphine (Taylor et al. 2010) and methadone (Warne et al. 2013) have been shown to produce superior analgesia to butorphanol in cats undergoing OVH and buprenorphine produced inadequate analgesia post OVH when combined with alfaxalone (Warne et al. 2016). There are a limited number of studies which have directly compared methadone and buprenorphine, and to the authors' knowledge, none have compared the two drugs in the context of the QUAD or triple combination (medetomidine, buprenorphine/butorphanol, ketamine) protocols. We hypothesised that methadone would provide superior analgesia compared to buprenorphine in cats undergoing ovariohysterectomy when incorporated in the QUAD protocol.

5.3. Material and Methods

5.3.a. Study Design

An assessor blinded, randomised, prospective clinical trial was conducted at the Greater Manchester RSPCA Animal Hospital. The study protocol was approved by the University of Bristol Ethical Review Group (VIN/15/023) and was carried out under an Animal Test Certificate-S issued by the Veterinary Medicine Directorate.

5.3.b. Sample size

In the study by Polson and colleagues (2012), which compared analgesia post OVH provided by buprenorphine or butorphanol in the QUAD protocol with or without a non-steroidal anti-inflammatory drug, pain scores were not significantly different between groups but when all cats were grouped together pain scores were greatest at the 4-hour time point after surgery. At this point the mean (SD) DIVAS score in the buprenorphine group was 12 (19) mm (Polson et al. 2012). A reduction in DIVAS pain score of 10 mm was considered clinically relevant and this was also assumed to be the case for methadone groups. Therefore, considering an α value of 0.05 and power of 0.8, the number of animals in each group needed to detect a difference between opioids was 58.

5.3.c. Enrolment and inclusion

One hundred and twenty cats undergoing routine midline ovariohysterectomy were recruited. Written, informed consent for inclusion in the study was obtained prior to surgery. All cats underwent a pre-anaesthetic examination and only those classified as American Society of Anaesthesiologists (ASA) category 1 or 2 were included. Exclusion criteria comprised cats under 4 months of age, cats which had received analgesia, anaesthesia, or sedation within the previous 7 days, and cats that were not amenable to handling.

5.3.d. Randomisation

Cats were randomly allocated in the order of presentation to receive either buprenorphine or methadone (random number generator; www.random.org) as the opioid component within the QUAD protocol. There were 60 individuals in the following treatment groups: MET – methadone, medetomidine, ketamine, midazolam; BUP - buprenorphine, medetomidine, ketamine, midazolam.

5.3.e. Assessments

Assessments throughout the study period were conducted by the same assessor who was blinded to the treatment group. Methods used to measure sedation, pain and physiological parameters were similar in both studies and have been described in Chapter 3.

5.3.f. Pre-operative Assessments

Cats were fasted for a minimum of eight hours prior to anaesthesia but were provided with water until time of QUAD administration. Baseline HR, f_R, temperature (T), sedation and the MNT were recorded prior to QUAD administration. Parameters were also measured ten minutes after QUAD administration prior to intubation.

5.3.g. Administration of test drugs

The anaesthetic drugs were drawn up in the same syringe by the veterinary surgeon carrying out surgery and administered intramuscularly into the quadriceps muscles. The assessor was blinded to the treatment and not present when premedication was administered. Premedication comprised of 600 μg/m^2 medetomidine (Sedator 10mg/mL; Dechra Pharmaceuticals), 60 mg/m^2 ketamine (100mg/mL Anesketin; Dechra Pharmaceuticals), 3mg/m^2 Midazolam (5mg/mL Hypnovel; Roche) and either 5mg/m^2 methadone (Comfortan 10mg/mL; Dechra Pharmaceuticals) or 180μg/m^2 buprenorphine (Buprenodale 0.3mg/mL; Dechra Pharmaceuticals).

5.3.h. Anaesthesia

The QUAD combination includes ketamine which provides adequate anaesthesia for approximately 20 minutes. However, cats undergoing OVH were maintained on 0.5% ISO in oxygen initially and ISO concentration adjusted as necessary. The larynx was sprayed with lidocaine hydrochloride (Intubeaze 20mg/mL, Dechra Veterinary Products) to prevent laryngeal spasm and an appropriately sized non-cuffed endotracheal tube was placed. Maintenance and monitoring of anaesthesia have been described in Chapter 3.

5.3.i. Reversal and Recovery

Medetomidine sedation was antagonised with atipamazole at a dose of half the volume of administered medetomidine (Atipam 5mg/mL; Dechra Pharmaceuticals) thirty minutes after premedication or at the point of extubation if thirty minutes had already passed to prevent excitatory effect of ketamine postoperatively. The endotracheal tube was removed when it was clear the cat was making effective ventilatory movements and was maintaining an oxygen saturation of >95% when inhaling room air. Time from extubation to head lift, sternal

recumbency, and standing were recorded. Quality of recovery was evaluated using a SDS scale (Table 2). Rectal temperature was measured until it was >37°C.

5.3.j. Post-operative assessments

Heart rate, RR, sedation, pain and MNT were assessed 2, 4, 6 and 8 hours after QUAD administration in the same order. A post-operative CMPS-F score of 4 or greater out of total of 16 was considered to indicate the requirement for additional rescue analgesia. Methadone at a dose of 0.5 mg/kg was administered intramuscularly as rescue analgesia. The assessor was not blinded to rescue analgesia. Pain was assessed 30 minutes later and if required another dose of 0.5 mg/kg methadone given IM. All cats were administered meloxicam at 0.3 mg kg^{-1} subcutaneously (Metacam; Boehringer-Ingelheim) after assessments were completed at 8 hours post-surgery.

5.3.k. Statistical methods

Data were assessed for normality by visual inspection of histograms and the Shapiro-Wilk test (SPSS Statistics Version 23; IBM Corporation). Means of normally distributed data were compared between the treatment groups using a t-test. The means of nonparametric data were compared using a Mann-Whitney test. A mixed between-within group (two-way) ANOVA was used to compare repeated measures over time and between groups. MNT was analysed as a percentage change from baseline, with baseline values given a score of 100%, to account for the variation in pain threshold between individuals. Pain scores were corrected for rescue analgesia using the last observation carried forward method, where scores awarded before rescue analgesia administration were carried forward for the remaining time points. The proportion of cats in each group requiring rescue analgesia or experiencing adverse events was compared using a Chi-squared test. P values of ≤ 0.05 were considered statistically significant unless multiple comparisons were performed, when a Bonferroni correction was applied. Parametric data are reported as mean ± standard deviation and nonparametric data are reported as median (range).

5.4. Results

5.4.a. Demographical data

There was no difference with respect to age (p = 0.13), weight (p = 0.99) or surgery time (p = 0.94) between groups (Table 12).

Table 10. Age, weight and surgery time (mean ± SD) in cats administered preoperative methadone (n = 60) and buprenorphine (n = 60) as part of the QUAD protocol. There was no difference between groups in age, weight or surgery time

Group	Age (months)	Weight (Kg)	Surgery time (mins)
MET	13.3 ± 9.46	2.53 ± 0.57	14.9 ± 2.41
BUP	16.2 ± 14.4	2.53 ± 0.51	± 2.53

MET: methadone; BUP: buprenorphine

5.4.b. Preoperative assessments

5.4.b.i. Physiological parameters

Baseline HR and RR were within normal limits and did not differ between groups (p = 0.13 and 0.26 respectively). Ten minutes post QUAD administration, both HR and RR were significantly lower compared to baseline in both groups (p < 0.001) (Table 13). There was no significant difference in HR or RR between cats that received methadone and those that received buprenorphine post QUAD administration.

Table 11. Heart rate (HR) and respiration rate (f_R) before (baseline) and after QUAD administration (sedated) (mean ± SD) in cats administered preoperative methadone (n = 60) and buprenorphine (n = 60) as part of the QUAD protocol. There was no difference in HR or f_R between groups. However, all cats showed significantly lower HR and f_R after QUAD administration.

Group	Baseline HR (beats / minute)	Sedated HR (beats / minute)	Baseline RR (breaths / minute)	Sedated RR (breaths / minute)
MET	181 ± 35	135 ± 23	49 ± 9	28 ± 12
BUP	190 ± 29	133 ± 28	47 ± 11	29 ± 11

MET: methadone; BUP: buprenorphine

5.4.b.ii. Sedation

Sedation increased in all groups post QUAD (p < 0.0001) (MET: SDS 3(3-3), DIVAS 9.98 (9 – 10) cm; BUP: SDS 3 (3-3), DIVAS 10 (10 – 10) cm) compared to baseline (SDS 0, DIVAS 0). There was no significant difference in SDS or DIVAS sedation scores post QUAD between the two opioids.

5.4.b.iii. Pain

Pain scores, measured using DIVAS and CMPS-F were 0 in all cats at baseline and 10 minutes following administration of the QUAD protocol, with no significant differences between groups.

5.4.b.iv. MNT

Mechanical nociceptive threshold values were not significantly different between groups at baseline but significantly increased post QUAD administration in both groups ($p < 0.001$). There was no significant difference in the increase in MNT score post QUAD compared to baseline values with respect to opioid (Table 14).

Table 12. Mechanical nociceptive threshold before (baseline) and after (sedated) QUAD administration (mean ± SD) in cats administered preoperative methadone (n = 60) and buprenorphine (n = 60) as part of the QUAD protocol. There was no in MNT between groups post QUAD administration. There was a significant difference ($p < 0.001$) between baseline and sedated MNT.

Group	Baseline MNT (Newton)	Sedated MNT (Newton)	Sedated MNT (% change)
MET	2.66 ± 0.89	14.7 ± 1.57	618 ± 253
BUP	2.59 ± 0.78	15.0 ± 0.74	634 ± 207

MET: methadone; BUP: buprenorphine

5.4.c. Intraoperative assessments

Intraoperative variables were measured at six important time-points during surgery (skin incision, ligation of right and left ovarian pedicle, ligation of cervix, placement of the final closing sutures). There was no significant difference in ISO concentration, measured as the dialled vaporiser setting, between methadone and buprenorphine groups (Table 13). Physiological parameters (HR, RR, SBP, DBP, and MBP) were analysed as percentage change from baseline values measured prior to the point of incision and showed no significant difference between groups overtime or at any timepoint. There was no significant difference between groups with respect to $ETCO_2$ or SpO_2 (Table 15).

Table 13. Intraoperative data from 6 defined points during surgery (baseline, skin incision, ligation of right and left ovarian pedicle, ligation of cervix and placing of last suture) in cats administered preoperative methadone (n = 60) and buprenorphine (n = 60) as part of the QUAD protocol.

		Baseline		Incision		Ligation R ovarian pedicle		Ligation L ovarian pedicle		Ligation cervix		Last suture	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ISO vapouriser setting %	MET	1	0	1	0	1	1	1	0	1	0	1	0
	BUP	1	0	1	0	1	0	1	0	1	0	1	0
Heart rate (beats/minute)	MET	126	21	126	22	135	21	134	21	131	19	119	18
	BUP	126	19	124	28	131	25	133	21	132	22	120	18
Respiratory rate (breaths / minute)	MET	17	6	17	6	18	7	18	6	18	6	19	12
	BUP	19	8	19	7	19	7	21	7	20	7	19	7
Systolic blood pressure (mmHg)	MET	133	22	125	19	131	21	128	28	126	21	110	15
	BUP	135	21	127	18	136	19	139	23	135	24	118	18
Diastolic blood pressure (mmHg)	MET	92	19	83	17	90	17	88	19	84	23	70	17
	BUP	95	19	86	17	95	18	95	24	91	22	75	19
Mean blood pressure (mmHg)	MET	110	21	102	20	109	19	106	21	102	21	87	14
	BUP	111	20	104	17	111	23	115	23	109	24	94	17
End tidal CO2 (mmHg)	MET	34	11	33	10	32	10	32	9	31	9	30	8
	BUP	35	13	35	12	33	9	34	9	33	10	31	9
SPO2 (%)	MET	95	9	96	4	96	2	96	2	96	3	97	2
	BUP	95	7	95	7	95	3	95	7	95	7	96	7

MET: methadone, BUP: buprenorphine

5.4.d. Recovery

Recovery quality was generally good in all cats and there was no significant difference in recovery quality at extubation, head lift, sternal recumbency or standing unaided (Table 16). There was also no significant difference between groups in the time taken from removal of endotracheal tube to head lift/ sternal recumbency/ standing.

Table 14. Median recovery quality and recovery times (minutes) to head lift, sternal recumbency and unaided standing after removal of endotracheal tube in cats administered preoperative methadone (n = 60) and buprenorphine (n = 60) as part of the QUAD protocol.

	Recovery quality				Recovery time (minutes)		
	Extubation	Head lift	Sternal	Standing	Head lift	Sternal	Standing
MET	3 (3-3)	3 (2-3)	3 (2-3)	3 (2-3)	10 (2-30)	19 (5-40)	50 (5-55)
BUP	3 (2-3)	3 (2-3)	3 (2-3)	3 (3-3)	10 (4-40)	18 (4-56)	30 (9-79)

MET: methadone, BUP: buprenorphine

5.4.e. Postoperative measurements

5.4.e.i. Sedation

Post-operatively, SDS and DIVAS scores showed no significant difference in sedation scores at any time-point with respect to opioid. At the two-hour time point sedation scores were as SDS 1(0-2), DIVAS 0.7(0-4.5) for the methadone groups and SDS 1(0-2), DIVAS 1(0-6.7) for the buprenorphine group. All cats were fully awake at the 4-hour post QUAD timepoint and all subsequent sedation scores were 0 for both SDS and DIVAS.

5.4.e.ii. Pain

The methadone group had significantly lower CMPS-F pain scores compared to the buprenorphine group overtime (p = 0.04), but this was not statistically significant at any one particular timepoint (Figure 10a). There was no significant difference in DIVAS pain scores between groups overtime or at any one timepoint (p = 0.06) (Figure 10b).
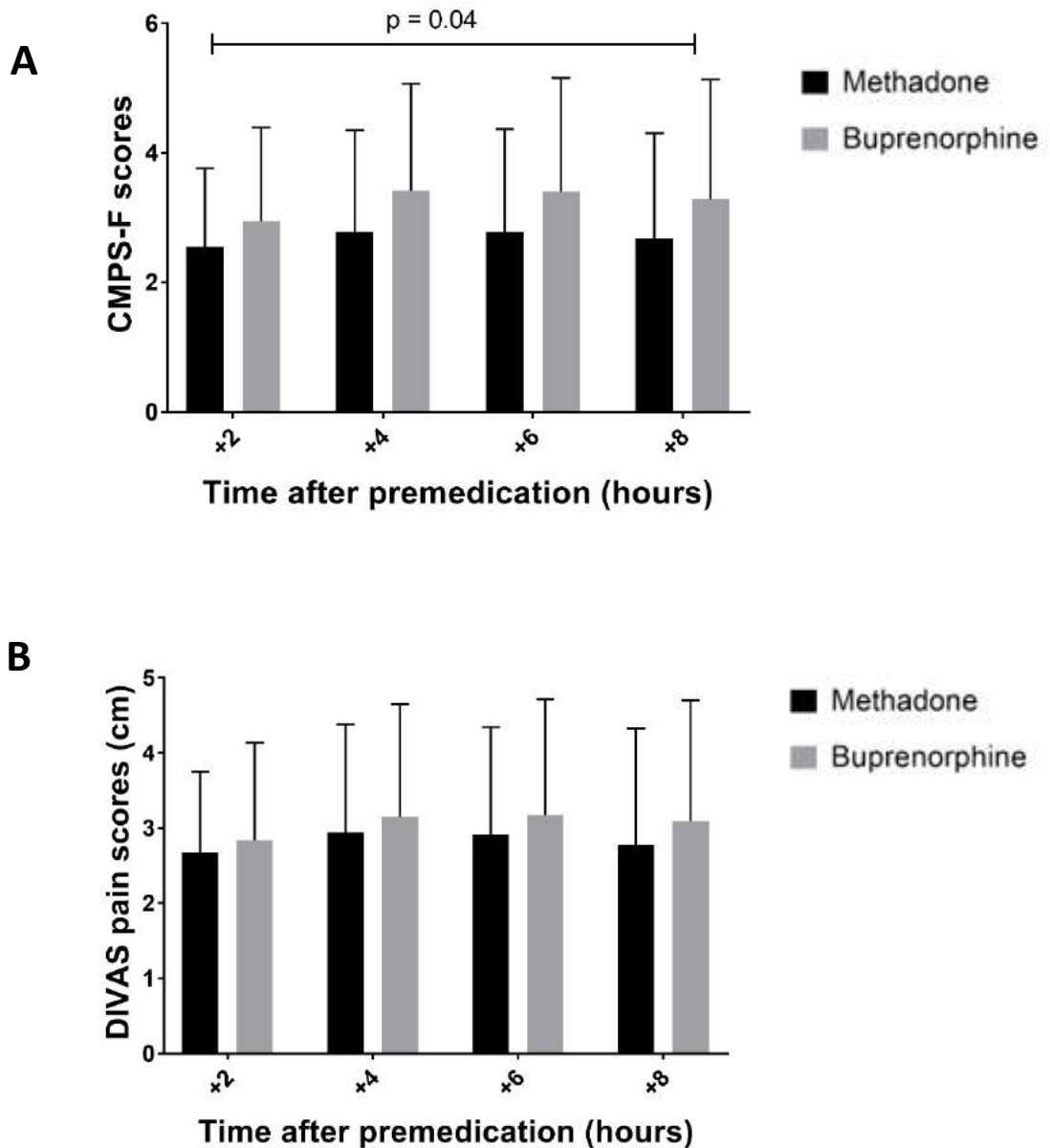
Figure 10. **(A)** Mean postoperative feline Composite Measure Pain Scale (CMPS-F) scores and **(B)** mean postoperative dynamic interactive visual scale (DIVAS) pain scores in cats administered preoperative methadone (n = 60) and buprenorphine (n = 60) intramuscularly as part of the QUAD protocol. Methadone groups had significantly lower CMPS-F pain scores (p = 0.04) compared to the buprenorphine group. DIVAS scores showed no difference between groups. Error bars indicate SD.

5.4.e.iii. Rescue analgesia

Eighteen of 60 cats (30%) in the methadone groups required rescue analgesia compared to 29 of 60 (48%) in the buprenorphine group (p = 0.04). All cats that received rescue analgesia required it within 6 hours post QUAD administration. CMPS-F scores one hour after rescue analgesia were significantly lower than scores before rescue analgesia (p < 0.0001). A Kaplan Meier survival graph was plotted using the number of cats requiring rescue analgesia at each time point. The two curves differed significantly (p = 0.028) (Figure 11).
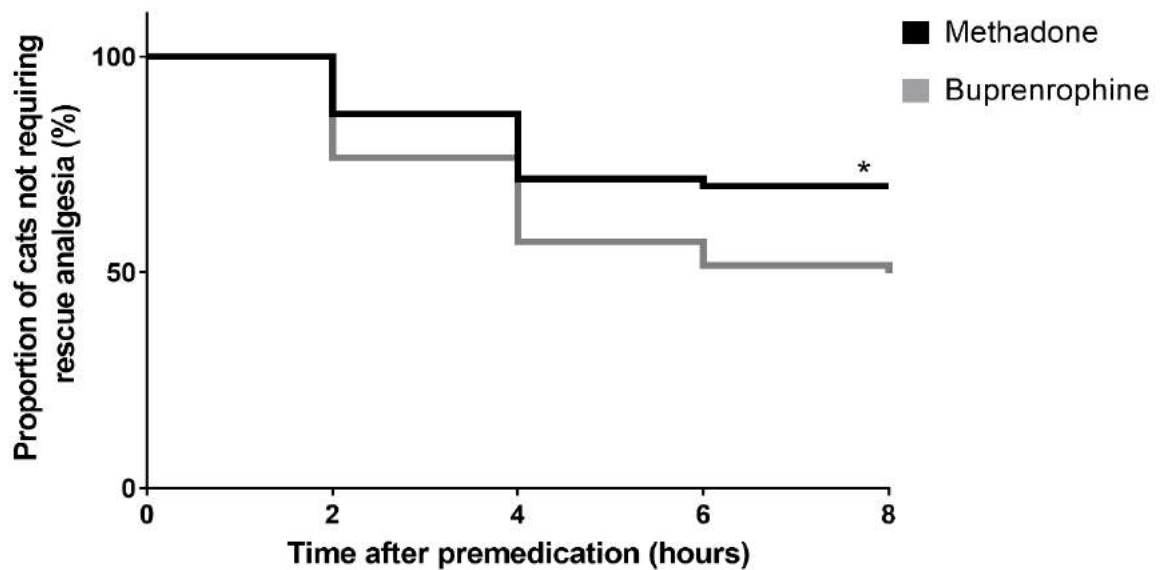
Figure 11. A Kaplan-Meier survival graph showing survival as the proportion of cats administered preoperative methadone (n = 60) and buprenorphine (n = 60) not requiring rescue analgesia at each time point postoperatively. * denotes a significant difference between the two curves (p = 0.028).

5.4.e.iv. MNT

There was no significant difference in MNT data between groups over time (p = 0.47), or at any specific time point (Figure 12).
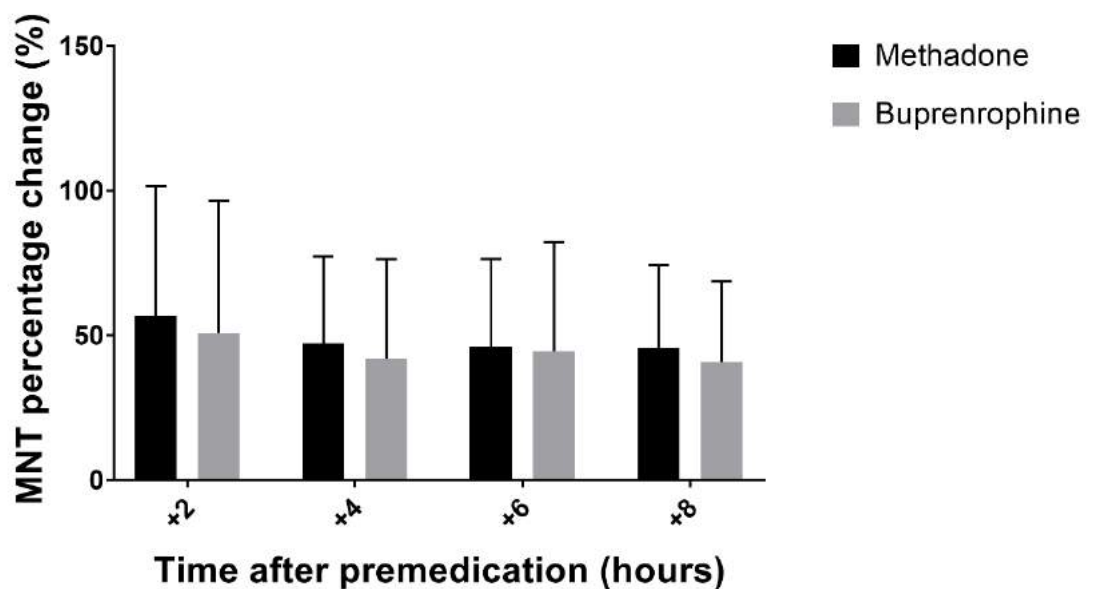
Figure 12. Mean postoperative mechanical nociceptive threshold (MNT) scores in cats that were administered preoperative methadone (n = 60) and buprenorphine (n = 60) intramuscularly as part of the QUAD protocol. Values are presented as percentage change from baseline (baseline scores were given a score of 100%). There was no difference in MNT scores between groups (p = 0.47) Error bars indicate SD.

The frequency of adverse effects was low. There was no significant difference between groups for any of the adverse effects observed during the study (Table 17).

Table 15. Adverse effects observed during the study and the number of cats in each group.

Adverse reaction	MET	BUP
Vomiting post QUAD administration	3	4
Licking lips post QUAD administration	2	0

MET: methadone, BUP: buprenorphine

5.5. Discussion

The aim of the study was to compare the characteristics of anaesthesia and analgesia between methadone and buprenorphine within the QUAD protocol in cats undergoing ovariohysterectomy. Our findings show that methadone results in lower overall CMPS-F pain scores and a lower requirement for rescue analgesia compared to buprenorphine. However, overall pain scores in both opioid groups were low and DIVAS pain scores showed no differences between opioid groups.

Recently, Bortolami et al. (2013) and Slingsby et al. (2015) compared methadone, buprenorphine and butorphanol combined with acepromazine and medetomidine respectively. Bortolami et al. found no difference between the opioids, however, data were not corrected for rescue analgesia. Slingsby et al. also showed no difference between the opioids when pain scores were not corrected for rescue analgesia but found buprenorphine to be superior to methadone and butorphanol at 3, 4 and 6 hours post opioid administration when corrected for rescue analgesia, possibly due to the longer duration of action. However, both studies included cats undergoing OVH and castration. Castration is less painful compared to ovariohysterectomy in cats (Väisänen et al. 2007) and the low pain scores in male cats may decreased the power of the studies to detect differences between groups. In addition, both studies used DIVAS as their pain measure. A recent study by Warne et al. (2016) used the UNESP-Botucatu composite pain scale and reported that premedication with buprenorphine and alfaxalone in cats undergoing OVH resulted in inadequate analgesia postoperatively and all cats required rescue analgesia (Warne et al. 2016). It is possible the DIVAS pain scale is less sensitive than the composite scales because it does not define and identify specific pain behaviours as the composite scales do. This is likely to be significant in cats as pain behaviours are subtle and may not drastically increase

with severity in pain. The disparity between CMPS-F and DIVAS scores in this study may be a result of this discrepancy and suggests that CMPS-F is more sensitive in detecting pain related behaviours in cats compared to DIVASpain.

Methadone was hypothesised to limit secondary hyperalgesia at the site adjacent to the surgical wound due to its ability to antagonise NMDA receptors. However, secondary hyperalgesia occurred in all cats with no significant difference in MNT scores between the two groups over time or at any time point in either raw or corrected data. These results are similar to many previous studies (Taylor et al. 2010; Polson et al. 2012; Slingsby et al. 2014). In contrast, Bortolami et al. (2013) reported that cats administered methadone in combination with acepromazine prior to ovariohysterectomy showed no significant variation in MNT scores over time compared to baseline scores, which supports the potentially anti-hyperalgesia action of methadone. However, OVH was carried out using a flank approach whereas the midline approach was used in the present study. The flank technique has been shown to cause greater wound tenderness because it damages muscle tissue, which has a greater number of nociceptors connecting to Aδ and C fibres compared to the connective tissue linea alba which is incised in the midline technique (Grint et al. 2006). In addition, ketamine is also a non-competitive NMDA receptor antagonist and may have reduced the ability to detect differences in MNT between methadone and buprenorphine. It is possible that a more invasive surgery may have resulted in more apparent differences in MNT between groups.

It could also be argued that ketamine has antinociceptive properties (Slingsby & Waterman-Pearson 2000) and may have influenced post-operative pain scores. However, there are limited data on the analgesic properties of single dose ketamine in cats and a study of the effect of a ketamine bolus in dogs undergoing ovariohysterectomy showed that the analgesic effects of pre-operative ketamine did not provide long lasting analgesia from a single dose (Slingsby & Waterman-Pearson 2000). In addition, a recent study investigating low-dose continuous rate infusion of ketamine in cats showed no increase in mechanical or thermal nociceptive thresholds (Ambros & Duke 2013).

No difference in adverse effects between the two opioids was detected. Vomiting post QUAD administration was the most common adverse effect, although this occurred in both buprenorphine and methadone groups and is possibly due to medetomidine, since vomiting is an occasional side effect of medetomidine in cats (Granholm et al. 2005). Both methadone and buprenorphine have been shown to cause limited adverse effects at clinical doses (Bortolami and Love 2015).

Overall our study suggests methadone produces greater analgesia in cats in the context of OVH and is well tolerated with minimal side effects at the doses used as part of the QUAD protocol. A dose of 0.5 mg/kg was used as rescue analgesia and this dose was also well tolerated. Methadone should be considered in cats undergoing OVH and procedures likely to produce moderate to severe pain and can be used as rescue analgesia in painful cats despite preoperative opioid choice.

6. General Discussion

6.1. Introduction

Analgesia is essential in preventing pain and suffering and is arguably one of the most important aspects of veterinary medicine. Most animals undergo a neuter surgery - an elective procedure, in which pain is inflicted on an otherwise pain-free animal. Ovariohysterectomy is one such procedure and is encouraged in dogs and cats to prevent reproductive behaviours and diseases. It is an example of a procedure with the potential to produce moderate -severe pain (Hardie et al. 1997) and is familiar to most veterinary surgeons. Choosing the right level of perioperative analgesia for any given procedure is fundamental to ensuring patient welfare and preventing longer lasting chronic pain states by reducing the level of peripheral and central sensitisation (Woolf 2011). A full range of analgesics must be available to veterinary surgeons to successfully achieve this.

Opioids are the cornerstone of peri-operative analgesia, of which methadone and buprenorphine are the two most commonly used in general practice in the U.K. However, the use of buprenorphine is far greater than the use of methadone in the UK (Hunt et al. 2015). Reasons for this disparity may stem from the fact that methadone has been licensed for use in dogs and cats relatively recently (2011) compared to buprenorphine. Therefore, clinicians may be less familiar with dosing, safety and adverse reactions. The purpose of this Master's research was to compare the analgesic properties of methadone and buprenorphine in the context of ovariohysterectomy in dogs and cats with a view to guide first opinion clinicians in their decision making when considering opioids and their analgesic efficacy.

6.2. Previous research

There is some discrepancy between previous studies that have compared the analgesic properties of different opioids. Some studies have reported buprenorphine to produce adequate analgesia post OVH in dogs and cats (Taylor et al. 2010; Hunt et al. 2013b), while others have shown it to provide inferior analgesia when compared to other analgesics such as NSAIDs and tramadol in the context of OVH and other moderate to severely painful procedures (Hunt et al. 2013a; Morgaz et al. 2013; Warne et al. 2014; Warne et al. 2016). Methadone has been shown to provide superior analgesia when compared to tramadol (Cardozo et al. 2014) and buprenorphine (Hunt et al. 2013a) in dogs undergoing orthopaedic surgery. These differences may be attributable to the difference in study protocols (dose of opioid, type of surgery, surgery technique, and experience of surgeon) as well as the sensitivity of pain scoring systems used.

To the author's knowledge only three studies have compared methadone and buprenorphine. Two studies in cats undergoing neuter surgeries have shown both buprenorphine and methadone to provide equal analgesia (Bortolami et al. 2013; Slingsby et al. 2015). However, both these studies included male cats undergoing castration and included only a small population of females. Castration is less painful compared to ovariohysterectomy in cats (Väisänen et al. 2007) and the low pain scores in male cats may have reduced the power of the studies to detect differences between groups. Small study samples increase the chance of a Type II error, and this may be especially important in pain studies where there is high variability in scores between individuals. Another study compared methadone to buprenorphine in dogs undergoing orthopaedic surgery and showed methadone to produce superior analgesia (Hunt et al. 2013a) . Few studies have compared methadone and buprenorphine in a large population of dogs or cats undergoing potentially moderate to severely painful surgery such as OVH. In addition, few studies have had the benefit of having the same surgeon and investigator for all animals.

6.3. Handling of data

There is some controversy over the optimum way to handle pain score data in clinical studies. Pain scores can be analysed as raw recorded data or corrected for rescue analgesia by carrying forward the score given at the time of rescue analgesia for all remaining time points. This maintains the assumed expected pain scores if additional analgesia had not been given and is termed the last observation carried forward (LOCF) method. The lower pain scores after rescue analgesia administration may influence the ability to detect differences between the two interventions by artificially decreasing the difference in pain scores between the opioid groups overtime. Therefore, LOCF increases the sensitivity of data analysis to discriminate between two interventions and allows analysis of trends over time. However, the disadvantage of LOCF is that it introduces a bias and should only be used if the assumption applied is justifiable. We predicted that pain scores without any further analgesia in painful animals would either remain the same or increase based on pain physiology. However, this is only an assumption and although unlikely it is possible the scores could have decreased over time even if rescue analgesia had not been given. We chose not to analyse data within the dog OVH study using the last observation carried forward (LOCF) method since a significant difference in raw pain scores was shown with uncorrected data. The difference in postoperative pain scores after OVH in cats was hypothesised to be smaller and the LOCF method was applied to data from this study. This is because the ovaries in cats are more mobile and easily exteriorised. Therefore, the suspensory ligaments do not need to be stretched/ torn and tissue handling is reduced compared to

ovariohysterectomy in the dog. This reduces tissue inflammation and resultant pain. In addition, ketamine and medetomidine were included in the QUAD protocol and their antinociceptive properties may have reduced the difference between groups.

6.4. Blinding to test opioid

Both studies were carried out as blinded clinical trials and the assessor was not aware of the test opioid being administered. This is important to eliminate any bias that results from predetermined opinion of the efficacy of a particular test drug. However, rescue analgesia could not be blinded as this decision was made by the assessor. Bias may have been introduced at this point. It could be argued therefore that subsequent pain scores should be treated as a separate group and either left out of analysis or analysed separately. The LOCF method used in the cat study aims to reduce this bias. However, this then introduces the assumption that pain scores remain the same or increase in animals that do not receive rescue analgesia. Although this assumption is logical and can be justified, it has not been proven because it is unethical to introduce a control group in which pain relief is withheld. In the dog study we did not use the LOCF method. However, we chose to include unblinded scores because animals required rescue analgesia at a variety of time points in the postoperative period. Removing all pain scores post rescue analgesia would result in many missing data points and limit analysis. Many analgesic efficacy studies have adopted the same approach (Taylor et al. 2010; Bortolami et al. 2013; Warne et al. 2013; Slingsby et al. 2014).

6.5. Pain assessments

The experience of pain incorporates physiological, emotional and cognitive components making it subjective to each individual and difficult to evaluate, especially in animals. In animals, pain assessment relies on behavioural and to an extent physiological observation. However, external environmental or situational factors can also affect a patient's response to pain and confound pain behaviours. The primary measure for pain in both studies was the GCPS since this pain scale has been validated for acute pain in dogs and cats in a clinical setting. Requirement of rescue analgesia was based on the validated intervention level of the GCPS (Reid et al. 2007; Calvo et al. 2014). DIVAS was used as an additional tool since pain is difficult to measure. More than one assessment tool is commonly used to help measure pain more accurately. DIVAS has been used in many veterinary pain studies (Lascelles et al. 1998; Stanway et al. 2002; Leece et al. 2005; Slingsby et al. 2011; Bortolami et al. 2013; Slingsby et al. 2015) and using it here may also allow better comparisons with other studies. Both GCPS and DIVAS are interactive scales and assess

the patient at rest and during movement to ensure relevant behavioural aspects of pain are assessed.

Both the canine and feline GCPS has been shown to reduce intra and inter-assessor variability by using defined behaviours weighted on severity (Reid et al. 2007; Calvo et al. 2014). The psychometric design helps to reduce subjectivity and provides a validated set intervention criterion. Therefore, the requirement for rescue analgesia was based on the GCPS score. A potential criticism of the GCPS is the confounding influence of sedation on the pain score. Medetomidine is known to produce profound sedation in both dogs and cats compared to other sedatives such as acepromazine (Taylor et al. 2010). Medetomidine was used in one population of dogs and all cats and was antagonised with atipamazole to eliminate the effect of sedation. Anxiety may also influence pain scoring since anxiety and pain can manifest in similar behaviours, especially in cats who are adapted to mask signs of pain. To reduce anxiety cats were kept in a separate cat ward and experienced similar environmental conditions to minimise situational and environmental confounding factors. Unfortunately, the environment for dogs postoperatively was more variable due to space limitations.

DIVAS was used as an additional measure of pain scoring. Although more sensitive than other forms of pain assessment such as the simple descriptive scale and numeric rating scale, DIVAS is subjective and influenced by factors such as experience and perception of 'worst possible pain' (Barletta et al. 2016). It was designed as a self-reporting tool and has been shown to be effective in human patients as it allows small variations in pain to be detected. However, the subjective nature of the pain experience and the inability of an assessor to fully assess the affective and emotive effect in an animal is likely to lead to inter and intra-assessor variability when using a non-linear scale with no specific anchor points. Variability is also more likely to occur when assessing changes in pain in the same individual over a period of time such as in analgesic efficacy studies (Holton et al. 1998). In human studies, repeated VAS assessments have been shown to vary by ±20 mm in the immediate postoperative period even when self-reporting (DeLoach et al. 1998). It is possible that this variability in a situation where the difference in pain scores was small between the two opioid groups, is the reason for the different outcomes of DIVAS and CMPS-F scores in the cat OVH study.

Experience has been shown to have an impact on pain assessment (Holton et al. 1998). In the present study, the assessor received training in using the SF-GCPS, CMPS-F and DIVAS prior to beginning data collection. It is possible that increased experience throughout the study resulted in increased sensitivity to pain. However, the effect of this was minimised by training in use of

the DIVASpain, GCPSs and MNT assessment techniques and the short duration of the studies. Data collection was completed over three months for each study. In addition, any shift in scoring based on experience would have applied equally across pain scales.

6.6. Rescue analgesia

The primary outcome measure in both studies was requirement of postoperative rescue analgesia since this was seen to be the most clinically relevant measure. In both cats and dogs rescue analgesia was required by significantly more animals in buprenorphine groups compared to methadone groups. In the population of cats and dogs that required rescue analgesia, the number of additional methadone administrations per rescue did not differ despite initial premedication opioid. This suggests that previous administration of buprenorphine did not antagonise the clinical analgesic effect of methadone. Previous studies have found contradictory results when investigating the interaction between buprenorphine and pure μ agonists. One study found that pre-treating dogs undergoing ovariohysterectomy with buprenorphine reduced the analgesic efficacy of the full μ opioid sufentanil (Goyenechea Jaramillo et al. 2006), whereas other studies, similar to the present study, found no antagonistic effects of buprenorphine on subsequent administration of postoperative methadone (Hunt et al. 2013a) or intra-operative fentanyl (Taylor & Walsh 2002). Recent findings in human medicine have also found that buprenorphine does not antagonise the analgesic efficacy of morphine (Oifa et al. 2009). Therefore, irrespective of opioid choice for premedication, methadone can still be given to painful patients postoperatively if necessary.

Rescue analgesic was required at a range of time points in the post-operative period in both cats and dogs illustrating individual variation in response to opioids and highlighting the need for regular postoperative pain assessments to ensure adequate pain management in all individuals

6.7. Overall pain scores

Overall pain scores were relatively low and overall means of SF-GCPS, CMPS-F and DIVAS pain scores were lower than the intervention level for rescue analgesia in both studies suggesting analgesia was adequate across all animals. Nevertheless, methadone resulted in lower pain scores compared to buprenorphine for both composite scales and DIVAS in dogs suggesting it provided greater analgesia. DIVAS scores did not differ in cats. A possible explanation might be the smaller overall difference in pain scores between opioids in cats, likely due to less tissue handling during the procedure, reduced noxious stimuli input and lower resultant pain intensity postoperatively. The antinociceptive propertied of ketamine and medetomidine may also have reduced the difference in pain scores between the two opioids. In addition, DIVAS may be less

sensitive to changes is pain compared to CMPS-F (Holton et al. 1998), especially in cats where pain behaviours are subtle.

A secondary analgesic such as a non-steroidal anti-inflammatory was not included as part of the anaesthetic protocol in order to gain a clearer understanding of the analgesic effects of methadone and buprenorphine without the confounding factor of additional analgesia. However, multi-modal analgesia and the addition of an NSAID is good clinical practice and is likely to have reduced the pain scores further (Shih et al. 2008). This may have decreased the difference in scores between methadone and buprenorphine. Despite this, a greater proportion of animals showed high enough pain scores to warrant additional analgesia in buprenorphine groups compared to methadone. Therefore, methadone is recommended for moderate to severely painful procedures such as ovariohysterectomy.

6.8. Mechanical nociceptive threshold

It was hypothesised that dogs and cats administered methadone would exhibit less hyperalgesia postoperatively than those treated with buprenorphine because methadone has antagonistic activity at the NMDA receptor (Inturrisi 2002). Secondary hyperplasia was measured using a hand held algometer because it does not require a Home Office licence and compared to other methods (e.g. Von Frey filaments and fixed actuators) is simple and quick to use. Overall mean MNT decreased in the different treatment groups at all time points in both dogs and cats and demonstrated that OVH caused postoperative secondary hyperalgesia. However, no difference was detected in MNT values between groups and variability in MNT data post-operatively was large. This may be because the level of hyperalgesia was not sufficient to distinguish between the two drugs. The painful aspect of OVH is usually the pulling and tearing of the suspensory ligament and there is little trauma around the incision site. Incisions are made through the linea alba and the muscle layer remains intact. The linea alba has few nociceptors and blood vessels (Grint et al. 2006). In addition, it was noted that sedation may have confounded MNT scores at the early time points after surgery and that some animals became conditioned to the uncomfortable stimulus from the PRoD, also found to the case in a study by Coleman et. al (2015). Age, weight, environment and position of animals have all also been shown to affect MNT scores and may have contributed to the high variability of scores found in our studies (Taylor & Dixon 2012; Briley et al. 2014; Harris et al. 2015). In cats it was difficult to manoeuvre the device underneath the abdomen and the pressure applied moved lighter animals possibly resulting in lower MNT scores.

6.9. Adverse effects

The incidence of adverse effects was low overall and similar between methadone and buprenorphine groups in both dogs and cat studies. The adverse effects of methadone are reported as respiratory depression, mild excitatory reactions such as lip licking, vocalisation, panting and salivation. Buprenorphine is generally considered to produce fewer side effects than methadone. However, the adverse effects of buprenorphine also include salivation, bradycardia, hypothermia and agitation (KuKanich & Wiese 2015). The adverse effects seen in the dog OVH study included hypersalivation, vomiting, sedation and intraoperative apnoea. It was difficult to attribute panting or vocalisation to opioid administration specifically in a busy and possibly stressful environment. Notable bradycardia was not seen. Adverse effects seen in the cat OVH study included hypersalivation and lip licking. No significant euphoria was seen. Kneading and mydriasis were occasionally seen but were not deemed to be an adverse effect. The incidence of adverse effects was low overall, and no difference was found between methadone and buprenorphine groups in both cats and dog populations at the doses used.

The aim of this study was to provide clinical evidence to help veterinarians in their decision-making regarding opioid use. The results of our studies show methadone is as well tolerated as buprenorphine when used at moderate doses. Using methadone at a lower dose may dispel worries about analgesic efficacy, dose unfamiliarity and drug safety, factors which were identified by a recent survey as influencing veterinarians when choosing an opioid for perioperative analgesia (Hunt et al. 2015).

6.10. Limitations

The studies were carried out at the RSPCA Greater Manchester Animal Hospital (GMAH) to allow recruitment of a large number of individuals since the RSPCA actively promotes neutering and ensures all animals entering rehoming centres are neutered. The aim of both studies was to investigate methadone and buprenorphine in a clinical setting and while GMAH provided this, the dog kennels were busy and often noisy. Kennel space was also limited. Disturbance during the 30 minutes after premedication given to allow for the onset of sedative effects may have inhibited full sedation, especially when animals were sedated with acepromazine since its sedative ability is moderate when compared to medetomidine. We found that animals were less reliably sedated with sedation scores lower than medetomidine groups, which is in contrast to previous studies comparing acepromazine and dexmedetomidine (Hunt et al. 2013b). The noisy environment may have increased anxiety levels and impacted postoperative pain scoring.

Another limitation to the study was the difficulty in measuring the MNT, especially in cats. A hand held algometer was used for both cats and dogs. The algometer was large and it was difficult to manoeuvre underneath a standing cat. MNT was measured in the standing position because it was thought that an escape response would be easier to detect giving more accurate results. However, cats required some restraint to allow the algometer to be placed and it was difficult to assess whether an escape response was due to the applied pressure or unwillingness to be handled. Lateral recumbency could be considered for MNT measurement but animals may feel vulnerable in this position and escape behaviours may be harder to recognise. Another solution would be to use a finger mounted algometer such as that described by Slingsby and colleagues (2011). This would have made it easier to access the abdomen and minimal restraint required.

Pain scoring was carried out by the same assessor in all animals. This ensured inter-assessor variability did not affect analysis of pain scores. However, a possible limitation is that it is difficult to measure an individual's pain scoring against the general population since pain assessment is subjective. If possible, pain scoring by another assessor and using an average of scores or comparing scores may help avoid possible bias.

7. Conclusions and Further work

Our studies have shown that methadone resulted in lower pain scores and less required rescue analgesia compared to buprenorphine in both cats and dogs undergoing ovariohysterectomy, supporting our hypothesis that the analgesic efficacy of methadone would be greater compared buprenorphine in the context of ovariohysterectomy. However, our studies have also highlighted the variability in response to opioid analgesic by individuals despite the initial choice of opioid emphasising the importance of regular pain assessments in the postoperative period. It is also important to note that although our studies did not administer non-steroidal analgesics during the study period, a multi-modal approach to analgesia is recommended.

Our overall aim was to help guide clinicians in their decision making regarding opioid choice for different procedures. Based on our results we believe that methadone is the better choice for ovariohysterectomy and this is likely to be true for other moderate to severely painful surgeries. However, there are a limited number of studies comparing methadone and buprenorphine and further studies comparing the two opioids in more painful soft tissue and orthopaedic surgeries would provide added information for their use in clinical practice. In addition, we were not able to illustrate methadone's ability to prevent secondary hyperplasia. This may be a consequence of low overall pain scores and studies investigating more painful procedures may be better

suited to research the role of methadone in the prevention of central sensitisation and secondary hyperplasia. The role of buprenorphine in prevention of central sensitisation has also not been elucidated. Finally, the dose of methadone (0.3 mg/kg) used in the dog ovariohysterectomy study was lower than that stated in the datasheet (0.5 – 1mg/kg) because it is thought to provide adequate analgesia with minimal side effects and is widely used by anaesthesiologists. However, no formal investigations into a clinically suitable dose range and corresponding adverse effects have been carried out for either methadone or buprenorphine in cats and dogs. Analgesia efficacy studies have used different doses with different routes of administration and this can be confusing for clinicians. Therefore, methadone and buprenorphine dose-response curves for analgesic efficacy may be beneficial.

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Appendices

Appendix 1: Glasgow composite measure pain scale for acute pain in dogs: SF-GCPS

A. Look at dog in Kennel
Is the dog
(i)

		(ii)	
Quiet	0	Ignoring any wound or painful area	0
Crying or whimpering	1	Looking at wound or painful area	1
Groaning	2	Licking wound or painful area	2
Screaming	3	Rubbing wound or painful area	3
		Chewing wound or painful area.	4

In the case of spinal, pelvic or multiple limb fractures, or where assistance is required to aid locomotion do not carry out section **B** and proceed to **C**

B. Put lead on dog and lead out of the kennel
When the dog rises/walks is it?
(iii)

C. If it has a wound or painful area including abdomen, apply gentle pressure 2 inches round the site. *Does it?*
(iv)

Normal	0	Do nothing	0
Lame	1	Look round	1
Slow or reluctant	2	Flinch	2
Stiff	3	Growl or guard area	3
It refuses to move	4	Snap	4
		Cry	5

D. Overall
Is the dog?
(v)

		Is the dog? *(vi)*	
Happy and content or happy and bouncy	0	Comfortable	0
Quiet	1	Unsettled	1
Indifferent or non-responsive to surroundings	2	Restless	2
Nervous or anxious or fearful	3	Hunched or tense	3
Depressed or non-responsive to stimulation	4	Rigid	4

Total Score (i+ii+iii+iv+v+vi) =

Appendix 2: Glasgow composite measure pain scale for acute pain in cats: CMPS – Feline

Choose the most appropriate expression from each section and total the scores to calculate the pain score for the cat, if more than one expression applies choose the higher score

LOOK AT THE CAT IN ITS CAGE
Question 1
Is it?
   Silent / purring / meowing   0
   Crying/growling / groaning   1
Question 2
   Relaxed   0
   Licking lips   1
   Restless/cowering at back of cage   2
   Tense/crouched   3
   Rigid/hunched   4
Question 3
   Ignoring any wound or painful area   0
   Attention to wound   1

APPROACH THE CAGE, CALL THE CAT BY NAME & STROKE ALONG ITS BACK FROM HEAD TO TAIL

Question 4
Does it?
   Respond to stroking   0
Is it?
   Unresponsive   1
   Aggressive   2

IF IT HAS A WOUND OR PAINFUL AREA, APPLY GENTLE PRESSURE 5 CM AROUND THE SITE. IN THE ABSENCE OF ANY PAINFUL AREA APPLY SIMILAR PRESSURE AROUDN THE HIND LEG ABOVE THE WOUND

Question 5
Does it?
   Do nothing   0
   Swish tail/flatten ears   1
   Cry/hiss   2
   Growl   3
   Bite/lash out   4
Question 6
General impression
Is the cat?
   Happy and content   0
   Disinterested/quiet   1
   Anxious/fearful   2
   Dull   3
   Depressed/grumpy   4

Pain Score …/ 16

Appendix 3: Written consent form for enrolment into the study

A STUDY COMPARING METHADONE AND BUPRENORPHINE FOR PAIN RELIF DURING AND AFTER SOFT TISSUE SURGERY IN DOGS AND CATS

University of Bristol, Division of Companion Animals, Langford House, Bristol, BS405DU
In association with:
Manchester RSPCA Hospital, 411 Eccles New Road, Salford, M5 5NN
Dechra Pharmaceuticals PLC., West Pavilion/Sansei Business Park, Shrewsbury SY4 4AS

Why are we performing this study?

The aim of the study is to compare the quality of pain relief between two drugs - methadone and buprenorphine, in dogs and cats undergoing soft tissue surgery. Animals will be randomly assigned to receive either buprenorphine or methadone during the premedication prior to anaesthesia. Both these drugs are licenced to provide adequate pain relief for soft-tissue surgery in veterinary medicine, however they work in slightly different ways. It is thought that methadone might provide more effective pain relief as well as contributing to a better overall anaesthetic compared to buprenorphine in surgical situations. However, this is currently unknown and both drugs are used very widely in general practice. The aim of the study is to scientifically investigate whether one drug is better than another in clinical practice.

What will be involved for you and your dog?

You will be asked to sign a consent form for anaesthesia and surgery and a separate consent form to enter the study. Once your animal has been admitted she will be given a routine pre-anaesthetic health check by a vet. If all is well she will be given a premedication of a sedative and painkiller – either methadone or buprenorphine. The study is a blind controlled study and the assessor (Meera Shah) will not know which drug has been given to the animal. Sometime after the drugs are given, anaesthesia will be induced and surgery performed. Sedation, pain and physiological parameters (heart rate, breathing rate, and temperature) will be measured at regular time points up to 8 hours after drug administration. Sedation and pain will be assessed by observation and by applying gentle pressure to the surgical wound to evaluate the degree of pain. Physical parameters will be assessed using a stethoscope and thermometer. If at any point the assessor feels that your animal is uncomfortable more pain relief will be given. All of the assessments are non-invasive. The study has been analysed and approved by the University of Bristol ethical review group and there are no ethical or welfare concerns for your dog.

If you have any further questions do not hesitate to email Meera Shah on
ms9387@my.bristol.ac.uk

Consent*:*

Owners Name…………………………………………………………………………………..

Dogs Name……………………………… ………………………………………………….

I hereby certify that:-

1. I have given consent for the animal(s) in my care, as detailed above, to be involved in the trial.
2. I understand the objectives of the trial and agree to the trial design as described in the trial protocol and information sheet.

If I am unclear of any health or safety aspects of the product, or any other query relating to the trial, guidance and advice can be sought from Meera Shah – ms9387@my.bristol.ac.uk.

Signature…………………………………………..Date……………………………………