ENHANCING ANALGESIC TECHNIQUES FOR THE MANAGEMENT OF PERIOPERATIVE ANALGESIA IN DOGS AND CATS

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This thesis is dedicated to my beloved wife

Kathren Sawicki

Whose unwavering love, patience, and encouragement have been my constant source of strength, even when the path was unclear. This work is as much yours as it is mine.

ABSTRACT

Effective pain management is a cornerstone of veterinary care. However, challenges persist in optimising perioperative analgesia across diverse clinical situations and in different species.

In a series of studies that form the backbone of this thesis, the electroencephalogram (EEG) and postoperative pain scores were used to evaluate a variety of drugs administered in the perioperative period and their analgesic efficacy in both feline and canine patients undergoing a range of surgical procedures resulting in nociception and postoperative pain.

Premedication involves the administration of an appropriate analgesic or combination of drugs to blunt nociceptive inputs during the intraoperative period, ideally before nociception begins. One study used the EEG to assess the efficacy of three commonly used opioids—morphine, buprenorphine, and methadone–as premedication in healthy cats undergoing castration. The results demonstrated that while all three opioids were effective in mitigating nociceptive input, methadone was significantly more effective than either morphine or buprenorphine.

Beyond the efficacy of individual premedication agents, the interactions between multiple drugs administered in combination are also critical in shaping intraoperative and postoperative analgesia. One study investigated EEG indices of nociception in

cats undergoing castration, examining how vatinoxan, an α_2-adrenoceptor antagonist, influenced the analgesic efficacy of dexmedetomidine, an α_2-adrenoceptor agonist, when given alone or in combination, with or without morphine. The findings indicated no significant attenuation of dexmedetomidine's antinociceptive effects when vatinoxan or morphine were added. Interestingly, high-dose morphine alone provided superior antinociception compared to dexmedetomidine, either alone or in combination with morphine and/or vatinoxan.

In dogs, intraoperative nociception and postoperative pain are often managed using multimodal analgesia-combinations of drugs designed to enhance analgesic efficacy and reduce side effects. One study evaluated intraoperative nociceptive indices and postoperative pain scores in dogs undergoing castration, comparing the effects of systemically administered morphine, locally administered bupivacaine via incisional block, and their combination to effectively control nociception and postoperative pain. While both morphine and bupivacaine alone effectively reduced intraoperative nociception, bupivacaine was superior in managing postoperative pain. However, the combination of both systemically administered morphine and locally administered bupivacaine proved most effective, completely suppressing intraoperative nociception.

Postoperative analgesic efficacy is influenced not only by the choice and combination, of drugs but also by the route of administration. This is particularly relevant when methods are adopted from human applications. A study assessing transdermally administered buprenorphine in dogs undergoing tibial tuberosity advancement orthopaedic surgery for the correction of cranial cruciate ligament rupture showed that

dogs receiving buprenorphine patches had significantly lower postoperative pain scores than those in the control group. These results support transdermal buprenorphine as a useful and effective alternative for postoperative pain management in dogs undergoing orthopaedic procedures.

These studies demonstrate that perioperative analgesic efficacy can be significantly enhanced by carefully considering key parameters, including drug selection, appropriate dosing, multimodal combinations, and the most suitable route of administration for the specific procedure.

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This thesis is presented in the hope that it will further advance the wellbeing of dogs and cats and offer a starting point to begin the conversations around improving analgesia in clinical practice for the countless animals entrusting their care to veterinarians worldwide.

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To all of you, thank you for being a part of this journey and for making this accomplishment possible.

The true art of healing lies in recognising pain in those who cannot speak it and relieving suffering in those who have no voice.

LIST OF PUBLICATIONS

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ABBREVIATIONS

ANOVA	analysis of variance
ASA	American Society of Anesthesiologists
BW	body weight
CMPS	composite measure pain scale
CMPS-SF	composite measure pain scale – short form
CNS	central nervous system
EEG	electroencephalogram
ET	endotracheal
F_{50}	median frequency of the EEG
F_{95}	spectral edge frequency of the EEG
$F_{E'}$Hal	end-tidal halothane concentration (%)
$F_{E'}$Iso	end-tidal isoflurane concentration (%)
f_R	respiratory rate (breaths/minute)
FFT	fast fourier transformation
HR	heart rate (beats/minute)
IB	incisional block
IM	intramuscular
IV	intravenous
LSM	least square means
MAC	minimum alveolar concentration
MAP	mean arterial pressure (mmHg)

MPS	Melbourne pain scale
MUAEC	Massey University Animal Ethics Committee
NMDA	N-methyl-D-aspartate
NRS	numerical rating scale
NS	nociceptive-specific
NSAID	non-steroidal anti-inflammatory drug
NZ	New Zealand
$paCO_2$	partial pressure of carbon dioxide (mmHg)
$PE'CO_2$	end-tidal carbon dioxide tension (mmHg)
pO_2	partial pressure of oxygen (mmHg)
PAG	periaqueductal gray
P_{tot}	total EEG power
SAP	systolic arterial blood pressure (mmHg)
SC	subcutaneous
SD	standard deviation
SDS	simple descriptive scale
SE	standard error
SPCA	Society for the Prevention of Cruelty to Animals
SpO_2	haemoglobin oxygen saturation (%)
T_1	testicle 1
T_2	testicle 1
TDS	transdermal delivery system
TDDS	transdermal drug delivery system
TPLO	tibial plateau levelling osteotomy

TTA	tibial tuberosity advancement
UK	United Kingdom
USA	United States of America
VAS	visual analogue scale
WDR	wide dynamic range

INTRODUCTION TO THESIS

THESIS STRUCTURE

The thesis is structured with each study presented as a chapter in this body of work. The studies are written as publication manuscripts for publication in peer-reviewed journals and for presentation at major veterinary conferences worldwide. All studies have either been accepted for or submitted to peer-reviewed veterinary journals and are presented in this thesis in the format required for each journal. Consequently, background information and methodology may be repeated in some of the chapters. Formatting and units may also differ between chapters as they mimic journal requirements for the specific publication. References are included at the end of each chapter and have been standardised to a single referencing style for the purpose of this thesis. Figures and tables are also labelled in sequential order as they appear in each chapter/manuscript and in the way they were submitted for publication. There will therefore be multiple Figure 1s, tables 1s, etc throughout this thesis. They will, however, be clearly identified where required by including the chapter number, such as Chapter 3, Figure 1, or Table 3.1, etc.

THESIS OUTLINE

Chapter 1 presents a comprehensive literature review that provides the reader with an overview of pain, including its definition, classification, and the methodologies used to assess pain across various species. This chapter also explores the different types of surgical pain, global trends in pain management, and the diverse methods of analgesia delivery. It highlights the implications of analgesia on central sensitisation and the importance of a multimodal approach to pain management. In addition, it reviews a range of analgesic drugs used in veterinary medicine and their applications across different species. The literature reveals that optimal analgesic protocols remain an area of active debate, with numerous unanswered questions and varying opinions among clinicians regarding the most effective approaches for pain management in different veterinary species.

Chapter 2 investigates the efficacy of three commonly used opioids—methadone, buprenorphine and morphine—administered intramuscularly as preoperative analgesia in cats undergoing castration. The study evaluates their ability to mitigate nociceptive input using electroencephalographic indices.

Chapter 3 examines the effect of vatinoxan, a peripherally acting α_2-adrenoceptor antagonist, on the electroencephalographic nociceptive indices in cats undergoing castration. The study assesses how vatinoxan influences the antinociceptive effects of dexmedetomidine, either alone or in combination with morphine.

Chapter 4 evaluates the effects of locally administered bupivacaine and systemically administered morphine, used separately and in combination, on electroencephalographic responses and postoperative pain in dogs undergoing castration. This study aims to assess the value of multimodal analgesia in managing surgical pain.

Chapter 5 explored the analgesic efficacy of transdermal buprenorphine patches compared to a negative control in dogs undergoing tibial tuberosity advancement (TTA) surgery for cranial cruciate ligament rupture. The findings contribute to the growing body of evidence investigating alternative routes of analgesia delivery in veterinary patients.

Chapter 6 provides a general discussion that synthesises the findings from previous chapters. It offers overall conclusions, reflects on the limitations of the experimental methodologies, and identifies future research directions that emerged from the studies.

A list of references is provided at the end of each chapter.

Ethical Statement

As detailed in each chapter, the protocol and conduct of all studies were approved by the Massey University Animal Ethics Committee (MUAEC), Palmerston North, New Zealand. Every effort was made to minimise the number of animals subjected to potentially painful procedures, and to reduce discomfort wherever possible. The studies involved animals that were already scheduled to undergo the respective surgical procedures as part of their routine medical care. The ethical justification for the

research lies in its potential to improve analgesic protocols in clinical veterinary practice, ultimately advancing animal health and welfare.

1 CHAPTER 1 – LITERATURE REVIEW

1.1 DEFINITION OF PAIN

Pain, in humans, is classically defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (International Association for the Study of Pain, 2018). In animals, pain is further defined as a sensory and emotional experience that is aversive and represents "an awareness by the animal of damage or threat to the integrity of its tissues and changes the animal's physiology and behaviour to reduce or avoid damage" and therefore to reduce recurrence of pain (Molony & Kent, 1997). It is a primary cause of stress, and may threaten an animal's well-being when severe (Moberg, 1999). Pain status is so important, that it is often considered the fifth clinical sign, after temperature, pulse rate, blood pressure, and respiratory rate (Muir, 2015).

The concept of pain is the result of two inter-connected processes: nociception, which is the result of suprathreshold stimulation of peripheral nociceptors, or the sensory component of pain that involves both sensory and motor systems working together to detect and respond to damaging or potentially damaging (noxious) stimuli (Dubin &

Patapoutian, 2010), and the emotional component which arises only when the nociceptive signals are transmitted to the areas of the brain associated with emotions and learning (Bateson, 1991). Noxious stimuli, whether mechanical or thermal, when present in sufficient intensity to result in tissue injury, lead to a cascade of responses which include withdrawal of the affected body part, alterations in autonomic outflow (manifesting clinically as an increased heart rate and blood pressure), as well as activation of the hypothalamopituitary axis, which results in an increase of stress hormones in circulation (Clark et al., 1997; Maier & Watkins, 1998; Moberg, 1999). Pain perception is also a very subjective experience that is affected by various individual differences (emotional, cognitive, sensory, behavioural) and therefore varies between individuals (Coghill, 2010).

1.2 NEUROPHYSIOLOGY OF PAIN

The connection between a noxious stimulus and resultant central processing resulting in pain is modulated by a neural process of encoding and processing the noxious stimulus (Loeser & Treede, 2008). This process has five components and utilises peripheral nerves, which act as an extension of the central nervous system (CNS) and consist of autonomic, sensory, and motor nerve fibres. A noxious stimulus is initially transduced (transduction) into electrical signals in the terminal ends of peripheral sensory neurones and transmitted (transmission) to the dorsal horn of the spinal cord. Here, they are modulated (modulation) and finally relayed (projection) to the brain (mainly along the spinothalamic tract) for final processing through the brainstem

onwards and eventual awareness (perception) of pain (Dubin & Patapoutian, 2010; Fields & Basbaum, 1999; Willis & Westlund, 1997) as shown in Figure 1.1.

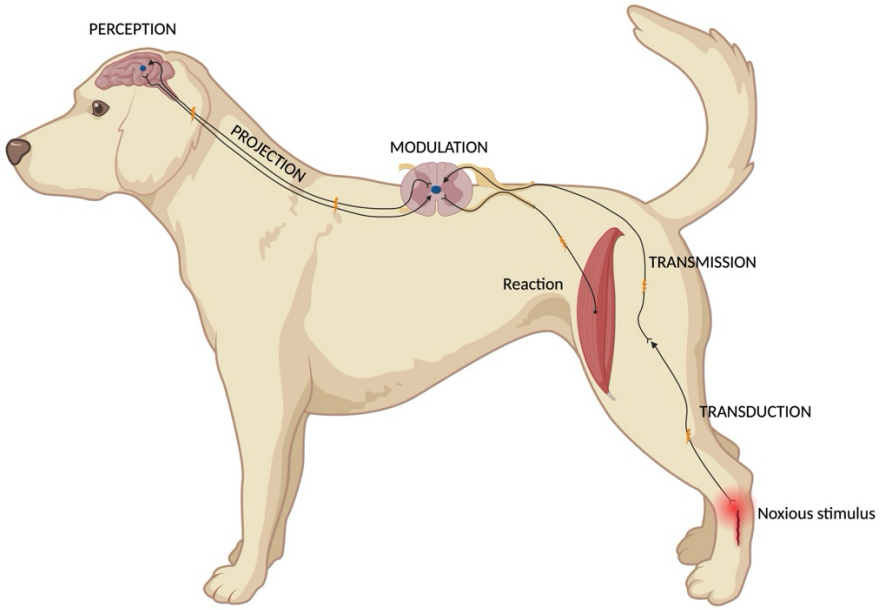

Figure 1.1. Pain pathways.(Created in BioRender. Sawicki, R. (2025) https://BioRender.com/v19l798)

1.2.1 Mechanisms of transduction and transmission

Noxious stimuli are detected by nociceptors (Figure 1.2), or primary sensory neurones providing critical information about the interface of the environment and the organism. They detect potentially damaging or threatening environmental inputs of high enough intensity to give rise to the sensation of pain. Nociceptors include a peripherally located terminal which innervates target tissue capable of transducing noxious stimuli, an axon for conducting the ensuing action potential generated to the

central nervous system, a cell body in the dorsal root ganglion, and finally a central terminal, at which information is transferred to second order neurones at central synapses (Woolf & Ma, 2007). Various nociceptors capable of responding to noxious chemical, thermal, or mechanical stimuli have been identified (Ramsey et al., 2006), and are classified according to the type of nerve fibre involved.

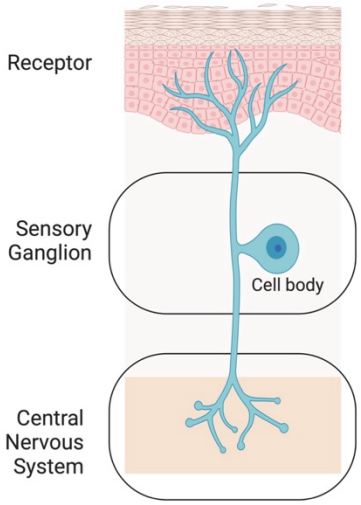

Figure 1.2. Nociceptors include a peripherally located terminal which innervates target tissue capable of transducing noxious stimuli, an axon for conducting the action potential generated to the central nervous system, a cell body in the sensory dorsal root ganglion, and finally a central terminal, at which information is transferred to second order neurones at synapses in the central nervous system. (Created in BioRender. Sawicki, R. (2025) https://BioRender.com/z43a384)

Nociceptors vary in their diameter and myelination, both of which have a direct effect on the relative speed of conduction of an action potential. They can be divided into two general types: medium to large diameter myelinated A-fibres, capable of fast

conduction and smaller diameter unmyelinated C-fibres, exhibiting slower conduction (Stucky et al., 2001).

Sensory A-fibres of the peripheral nervous system can be further subdivided into Aβ-fibres and Aδ-fibres. They are responsible for the pricking quality of pain (Stucky et al., 2001). Aβ-fibres are large axonal diameter (10 μm), highly myelinated fibres capable of a rapid conduction velocity (80 m/s) (McGlone & Reilly, 2010). These mechanoreceptors have a low threshold of activation (Brown & Iggo, 1967) and transduce tactile stimuli (Ikeda et al., 2014). Aδ-fibres are smaller in diameter (2.5 μm), thinly myelinated fibres capable of a slower conduction velocity (12 m/s) (McGlone & Reilly, 2010). They also possess higher activation thresholds and respond to both thermal and mechanical stimuli (D'Mello & Dickenson, 2008).

Sensory C-fibres comprise 70% of all nociceptors and are responsible for the slow quality of pain (Stucky et al., 2001). They are mostly responsible for sensing noxious thermal stimuli (Campero et al., 2001). Histochemical analysis has identified two subtypes of C-fibres, though their distinct functional roles remain unclear (Stucky et al., 2001). The first subtype contains neuropeptides, including substance P, while the second subclass expresses a surface carbohydrate that can be selectively labelled using the plant lectin isolectin B4 (IB4) (Julius & Basbaum, 2001; Snider & McMahon, 1998). These fibres are unmyelinated, possess the smallest axonal diameter (~ 1 μm), and have a slow conduction velocity (<1 m/s). Additionally, they also have the highest activation thresholds among sensory nerve fibres (McGlone & Reilly 2010).

1.2.2 Modulation of nociceptive signals

The dorsal horn of the spinal cord serves as the primary relay centre for sensory information from the peripheral nervous system, where incoming signals are processed before being transmitted to the brain. The central terminals of afferent fibres synapse with the spinal cord, facilitating this communication (D'Mello & Dickenson, 2008). Structurally, the dorsal horn is organised into distinct parallel laminae, arranged from the superficial to deep layers (Brown, 1981). Nociceptive Aδ- and C-fibres predominantly terminate in the superficial laminae (I-II), with a smaller proportion reaching deeper layers. In contrast, Aβ-fibres, which primarily convey nonnociceptive sensory input such as pressure and texture, predominantly innervate laminae III-VI (Takazawa & MacDermott, 2010; Todd, 2002).

Neuronal cell types within the spinal cord process peripheral sensory input and generate appropriate responses. Among these, nociceptive-specific (NS) neurons, located in the superficial dorsal horn, exclusively synapse with Aδ- and C-fibres, transmitting noxious stimuli from the periphery. In contrast, low-threshold (LT) neurones receive input from Aβ-fibres and respond solely to touch. A third group, wide dynamic range (WDR) neurones, integrate input from all three fibre types (Aβ, Aδ- and C-fibres), responding to a broad spectrum of stimuli, ranging from light touch to intense pain, heat, and chemical irritation (D'Mello & Dickenson, 2008). Repetitive activation of the neurones leads to 'wind-up', a phenomenon in which each successive stimulus increases evoked neuronal activity, amplifying pain perception (Dickenson & Sullivan, 1987). Additionally, the dorsal horn contains glutamatergic excitatory neurones and Gamma-amino butyric acid (GABAergic) inhibitory neurones, which

regulate NS and WDR neurone activity by either enhancing or dampening pain signals. Beyond neuronal mechanisms, non-neuronal cells, including astrocytes and microglia, also contribute to signal processing within the spinal cord, influencing pain transmission and neuroinflammation (Coyle, 1998).

Further modulation of nociceptive transmission occurs through descending axons from the brainstem, which synapse in the dorsal horn to regulate pain signalling (Bridgestock & Rae, 2013). Additionally, various neurotransmitters – released by sensory fibres of neighbouring dorsal horn neurones and afferent neurones – play a crucial role in shaping pain perception. These include ATP, substance P, glutamate, and GABA, which activate specific receptors on spinal cord neurones dependent on the pain state and the neurochemical changes present (Honore et al., 2000).

For instance, pain conditions exhibit distinct 'neurochemical signatures':

- Inflammation leads to significant upregulation of substance P and calcitonin-gene-related-peptide (CGRP)
- Neuropathic pain, in contrast, shows a downregulation of these same neurotransmitters
- Cancer pain, however, exhibits no significant change (Calzà et al., 1997; Zhang et al., 1995).

This variability explains why different pain states – such as cancer pain, neuropathic pain, and chronic inflammatory pain – respond best to different classes of analgesics. Given the vast number of interacting variables, combined with the subjective and

emotional aspects of pain perception, understanding how a specific nociceptive stimulus is processed into a painful experience remains a complex and challenging task.

1.2.3 Projection and final processing

Projection fibres connect the brain and spinal cord, consisting of both efferent and afferent nerve fibres. These fibres transmit sensory information to key brain regions, including the thalamus, periaqueductal grey (PAG), and the parabrachial area (PB) (D'Mello & Dickenson, 2008). Ascending pathways from the dorsal horn relay spinal output to higher brain centres, with somatic sensory information travelling to the reticular formation of the brain stem via multiple parallel circuits – spinothalamic, spinoreticular, spinomesencephalic, and postsynaptic dorsal column tracts – before converging in the thalamus (Fields et al., 1991).

Pain perception is further shaped by a complex interplay of excitatory and inhibitory mechanisms. Excitatory activity in the spinal cord is modulated by pre- and post-synaptic opioid (μ, κ, δ, OQF), noradrenergic (α-1, α-2), and muscarinic receptors (Baba et al., 1998, 2000b, 2000a). Additionally, neurones from laminae I project to the rostral ventromedial medulla (RVM) in the brainstem, a critical area involved in both ascending pain processing and descending modulation. The RVM, influenced by limbic structures, plays a pivotal role in the emotional and affective aspects of pain (D'Mello & Dickenson, 2008). In addition to relaying sensory inputs to higher brain centres, this region regulates descending inhibitory and excitatory influences that shape nociceptive processing at the spinal level. It contains both excitatory and inhibitory

neurones, which either facilitate or suppress nociceptive reflexes and behavioural response to pain (Fields & Basbaum, 1999).

The deeper neurones of the dorsal horn (lamina III-VI) form a major component of the spinothalamic tract, projecting to the thalamus and contributing significantly to pain transmission. In contrast, only 5-15% of spinothalamic tract neurones originating from lamina I (Klop et al., 2004a, 2004b, 2005). This ascending pathway relays sensory information to the thalamus, which plays a central role in shaping the sensory experience of pain. From the thalamus, pain-related signals are transmitted to various cortical regions, collectively known as the "pain matrix." This network includes the primary and secondary somatosensory cortices, insular cortex, anterior cingulate cortex, and prefrontal cortex (Tracey & Mantyh, 2007). Sensory information processed in the somatosensory cortex is further relayed to neighbouring structures, including the limbic system, which plays a critical role in the emotional and behavioural responses to pain.

The limbic system governs:

- Emotion and behaviour (cingulate gyrus)
- Conditioned fear and anxiety (amygdala)
- Memory formation (hippocampus)
- Sympathetic and autonomic regulation (hypothalamus)
- Arousal, vigilance, and behaviour (locus coeruleus)
- The fight-or-flight response and stress-induced analgesia (periaqueductal gray, PAG) (Charney et al., 1998a, 1998b; LeDoux et al., 1988).

1.2.4 Descending modulation of pain

In addition to ascending pathways, descending pathways play a crucial role in modulating pain perception. Two key neurotransmitters involved in descending inhibition are noradrenaline and 5-hydroxytryptamine (5-HT, serotonin) (Steeds, 2016). PAG neurones, activated in response to noxious stimuli, regulate antinociceptive and autonomic responses, effectively inhibiting pain transmission at the dorsal horn of the spinal cord (Charney et al., 1998b; Steeds, 2016). This descending control mechanism is essential for pain suppression, allowing the body to regulate pain intensity based on context and physiological state.

1.3 CLASSIFICATION OF PAIN

Pain can be classified by various parameters. One parameter used for clinical purposes is the duration of the presence of pain (acute vs. chronic). Acute pain has a distinct period of onset, is short in duration, and serves a protective role by enabling healing and tissue repair (Muir & Woolf, 2001). It is often associated with autonomic signs, including tachycardia and elevation in blood pressure (Max & Payne, 1999). Chronic pain is defined as pain that persists for longer than the time expected for healing, or as pain associated with a progressive, non-resolving disease. In non-progressive pain, 3 months is considered the temporal threshold dividing chronic pain from acute pain (Merskey & Bogduk, 1994). Chronic pain has little to no protective value and induces phenotypic and biochemical changes in the nervous system. These changes escalate

and alter sensory inputs, resulting in physiological and metabolic changes which further contribute to illness, and possibly death (Maier & Watkins, 1998).

Pain can also be classified according to the type of tissue affected, the location of the pain, and the body's response to tissue injury. This includes nociceptive pain, inflammatory pain, visceral pain, and neuropathic pain.

Nociceptive pain arises from an identifiable lesion that causes tissue damage and is initiated by the activation of nociceptors in somatic or visceral structures (Loeser & Treede, 2008). A subset of nociceptive pain, Inflammatory pain, is associated with body's response to the nociceptive stimulus through active inflammation. It is important to recognise pain classifications often overlap, incorporating multiple parameters. For instance, persistent inflammation, can lead to adaptive changes that further modify the nociceptive system over time (Woolf, 2004). Visceral nociceptive pain is yet another type of pain arising from the stretching, distention, or inflammation of internal organs. It is often described as a deep, cramping, aching, or gnawing pain that is poorly localised (Larauche et al., 2012). The poor localisation is due to a lower density of nociceptors on viscera and the fact that afferent fibres are not as well represented in cortical mapping (Anand et al., 2007).

Neuropathic pain results from direct injury to the somatosensory system and can be classified as peripheral or central, depending on the part of the nervous system affected. It is often severe and difficult to manage (Fornasari, 2017). Common causes include peripheral neuropathies, postherpetic neuralgia, nerve injury, stroke, spinal cord injury, and multiple sclerosis (Colloca et al., 2017). While opioids can be effective,

they are not first-line treatments due to concerns over adverse reactions and the potential for abuse and addiction by clients (Dowell et al., 2016). Instead tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors (SNRIs) are preferred for first-line management (Finnerup et al., 2015).

A comprehensive understanding of the neurophysiological mechanisms of pain and their clinical manifestations is essential for developing effectively pain management strategies. By identifying specific pain pathways and receptors, treatments can be precisely tailored using targeted pharmacological interventions. A major challenge in pain assessment is its inherently subjective nature – in humans, pain is best evaluated through self-reported experiences. In veterinary medicine, this is not possible, requiring clinicians to recognise signs of pain, minimise nociceptive inputs, and attempt to quantify both the intensity and nature of pain in nonverbal animals. Accurately assessing pain in these species is therefore critical to ensuring appropriate treatment and improving overall welfare.

1.4 RESPONSES TO PAIN

Pain responses in animals can be classified based on various parameters, including behavioural and physical. The most basic responses include excessive activity or lethargy (Hall et al., 2001). Pain responses can also be classified into distinct behavioural adaptations:

- Avoidance behaviours to prevent recurrence of pain

- Automatic protective responses, such as the withdrawal reflex
- Healing-driven behaviours that minimise pain and promote recovery
- Social responses designed to deter further pain or to elicit help (Mathews et al., 2014a; Molony & Kent, 1997).

Understanding normal behaviour is crucial, as deviations from it are often the clearest indicators of pain. Common signs include changes in behaviour, posture, gait, response to handling, and weight (Mathews et al., 2014a).

Physical indicators of pain may include ocular changes (tearing, pupil dilation), lameness, reduced activity, self-mutilation, anxiety, restlessness, personality shifts, and vocalisation (Short, 1998). Nociceptive inputs capable of causing pain can also concurrently activate physiological responses such as excessive salivation, tachypnoea, mydriasis, tachycardia, and biochemical changes, including elevated blood glucose, ACTH, cortisol, and catecholamines (Muir & Birchard, 1997).

1.4.1 Challenges in pain assessment

No single sign – or combination of signs – can definitively confirm or rule out pain. Anthropomorphism plays a role in pain assessment, as procedures known to cause pain in humans are assumed to have similar effects in animals. However, determining the intensity and subjective experience of pain in a species or individual remains challenging. It is important to recognise that physiological, behavioural, and biochemical responses are merely external manifestations of the complex and subjective perception of pain.

1.5 PAIN ASSESSMENT TECHNIQUES

While understanding pain is crucial, accurately recognising and quantifying it is essential for developing effective pain management strategies. Several factors make pain assessment in animals particularly challenging, including the inability to verbally communicate, interspecies and individual behavioural variability, the instinct to conceal pain, and the inherent subjectivity of pain evaluation. Importantly, a lot of animals exhibit pain-related behaviours that are different to our expectations and not readily perceptible to or intended for humans, meaning that the absence of visible distress does not equate to the absence of pain. However, given that animals share the same fundamental neuronal architecture as humans, it is reasonable to assume that their experiences of nociceptive stimuli are comparable.

One approach to quantifying pain is to adopt methodologies used in human medicine, classifying pain according to four primary strategies (Livingston, 2010):

1. Physical Response to Noxious Stimuli – Observing an animal's heightened reaction to pain exacerbation, such as palpation or manipulation of a painful area.
2. Behavioural Deviations – Assessing changes in behaviour relative to what is considered normal behaviour for that species or individual.
3. Cardinal Physiological Parameters – Monitoring fundamental parameters, such as pulse, temperature, respiration rate, and feeding/drinking patterns can provide indirect indicators of pain.

4. Neurophysiological Parameters – A key of this approach, particularly relevant to this review, is the use of neurophysiological parameters, such as electroencephalography (EEG), to quantify nociceptive stimuli capable of eliciting a painful response.
5. Effectiveness of Analgesia – Measuring pain reduction using a combination of the above criteria after implementing analgesic interventions.

By integrating these approaches, clinicians and researchers can more effectively evaluate pain in animals and refine strategies to alleviate suffering.

1.5.1 Observation based pain scales in animals

Most observation-based pain scales used in veterinary medicine are adaptations from those originally designed for human patients. The primary difference is that, in animals, the observer, rather than the patient, interprets and quantifies pain intensity. These scales are modelled after instruments used to assess pain in preverbal children (Elliott et al., 1987; McGrath, 1987; Tarbell et al., 1992). Three observation-based pain scales used in animals are the:

- Visual Analogue Scale (VAS)
- Numerical Rating Scale (NRS)
- Simple Descriptive Scale (SDS).

1.5.1.1 Pain scale descriptions

1. Visual Analogue Scales (VAS) – The observer places a mark on a 100mm horizontal line with anchors at each end (one end represents "no pain" and the other "unbearable or excruciating pain"). The intensity is then quantified by measuring the distance to the mark from zero (Scott & Huskisson, 1976). It is statistically different to the other two scales below as it is a continuous scale compared to the two categorical scales below.
2. Numerical Rating Scale (NRS) – A linear scale with fixed numbers (typically from 0 to 10 or 0 to 100), where 0 indicates no pain and the highest number indicates severe pain.
3. Simple Descriptive Scale (SDS) – Also a linear scale but with predefined categorical descriptions (e.g. no pain, mild pain, moderate pain, severe pain, etc.) instead of numbers (Anil et al., 2002).

1.5.1.2 Advantages of observation-based pain scales

- Ease of use – Simple and quick to implement.
- Minimal training required – Can be used by various observers with little prior instruction.
- Fast assessment – Requires minimal interaction with the animal.
- Reliable correlation between VAS and NRS – While there is less ability to detect subtle changes with the NRS, good agreement between these two scales has been shown in studies (Conzemius et al., 1997; Welsh et al., 1993).
- SDS has reasonable interobserver variability – Particularly useful in postoperative pain assessment (Holton et al., 1998).

- VAS is considered more sensitive – Due to its continuous scale, it may provide a more nuanced pain evaluation then pre-defined categories in SDS and NRS (Firth & Haldane, 1999).
- NRS has been used effectively in chronic pain assessment in clinical settings (de Haan et al., 1994).

1.5.1.3 Limitations of observation-based pain scales

- Subjectivity – Different observers may assign varying pain scores, leading to interobserver variability (Holton et al., 1998). They may also erroneously imply that equal distances between two scores on the scales denote equal distances in the dimension being measured (Pesudovs & Noble, 2005).
- Expectation of pain behaviours – These scales assume that animals will display observable pain behaviours, which may not always occur or occur in a way that is not perceivable, particularly in unnatural clinical settings or in prey animals where behaviours are targeted to be observed only by their own species.
- Impact of illness or trauma – Severely ill or traumatised animals may be too debilitated to exhibit typical pain-related behavioural, increasing the risk of undertreatment (Hansen & Hardie, 1993).

While observation-based pain scales have historically been valuable tools in veterinary medicine, their limitations highlight the need for complementary assessment methods to improve accuracy and reliability in pain evaluation.

1.5.2 Multifactorial pain scales in veterinary medicine

To enhance the accuracy of observation-based pain scales, researchers have developed multifactorial pain scales (MFPS), which assign weighted numerical values to specific behavioural observations. These categorised numerical rating scales typically assess three to seven behavioural and physiological categories such as vocalisation, movement, respiratory patterns, and posture. By evaluating each component individually, the observer can compile a comprehensive pain score (Holton et al., 2001; Reid et al., 2007).

One widely used MFPS is the Melbourne Pain Scale (MPS), which consists of six behavioural and physiological categories, each subdivided into three weighted scores, producing a total score out of 27 (Firth & Haldane, 1999). Similarly, the Colorado State University Pain Scale evaluates eight behaviour and physiological parameters indicative of pain (Hellyer & Gaynor, 1998). However, these early scales lacked detailed explanations regarding category selection and validation against actual animal subjects.

To address this, the Glasgow Composite Measure Pain Scale (CMPS) and its short-form (CMPS-SF) were developed. These scales were refined through input from 69 veterinary surgeons, using statistical validation to establish a more precise and standardised pain assessment tool (Holton et al., 2001; Reid et al., 2007).

1.5.2.1 Advantages of multifactorial pain scales

- Ease of use – These scales provide structured behavioural guidelines, improving observer consistency.
- Objective criteria – They offer statistically validated parameters with clear, repeatable assessment procedures.
- Improved reliability – Compared to simpler scales, MFPS provide a more comprehensive evaluation of pain (Holton et al., 2001).

1.5.2.2 Limitations and challenges

Despite their advantages, MFPS have notable drawbacks, particularly in severe pain. For example, a dog recovering from limb amputation just two days prior without analgesia appears motionless, depressed, and unwilling to eat or move for fear of causing more pain through action. While this dog is clearly experiencing severe pain, it would score only 4 out of 27 on the MPS, insufficient to warrant analgesia based on the scale's criteria (Hansen, 2003). Additionally, certain types of pain can suppress alertness and activity (Morton & Griffiths, 1985; Sanford, 1992). Species variability further limits their applicability, as these scales are often validated for only one species (e.g. dogs) and for pain type (e.g. acute pain) (Sanford, 1992). Another challenge in developing MFPS is the difficulty of appropriately weighting different categories that indicate pain. For instance, the CMPS-SF rates vocalisation on a scale from 0 to 3, while responses to wound pressure are scored from 0 to 5 (Reid et al., 2007). An increase of one step in vocalisation is not directly comparable to a one-step increase in wound pressure response; however, both contribute equally to the overall pain score.

This creates an inherent methodological limitation, as different behavioural categories may not proportionally reflect pain severity, leading to challenges in accurately quantifying pain and addressing these discrepancies.

1.5.2.3 Use of behavioural-based pain scales in veterinary research

Observation–based and behaviour–based pain scales have been widely applied in veterinary medicine, particularly in canine–based pain assessment. They have been utilised in:

- Castration and ovariohysterectomy studies (Aengwanich et al., 2019; Bendinelli et al., 2019; Bustamante et al., 2018; Firth & Haldane, 1999; Lascelles et al., 1997; Lemke et al., 2002; Slingsby et al., 2001; Slingsby & Waterman-Pearson, 2000).

- Numerous orthopaedic surgery studies (Adami et al., 2012; Barnes et al., 2019; Bartel et al., 2016; Brodbelt et al., 1997; Budsberg et al., 2002; Caniglia et al., 2012; Cerasoli et al., 2017; Conzemius et al., 1997; Davila et al., 2013; Day et al., 1995; Grisneaux et al., 1999; Heffernan et al., 2018; Hendrix et al., 1996; Lascelles et al., 1994; Mbugua et al., 1989; Nolan & Reid, 1993; Palomba et al., 2020; Pibarot et al., 1997; Sammarco et al., 1996; Taylor & Houlton, 1984; Vesal et al., 1996)

- Various other soft tissue and neurological surgeries (Aghighi et al., 2012; Bellei et al., 2011; Bienhoff et al., 2012; Chiavaccini et al., 2017; Dancker et al., 2020; Tayari et al., 2017).

However, these scales remain highly dependent on subjective interpretation, leading to significant variability in pain scores between observers and the animals themselves (Holton et al., 1998). While they represent a step forward in standardised pain assessment, further refinement is needed to enhance objectivity and improve pain evaluation across different species and conditions.

1.5.3 Biochemical and physiological methods

While physiological parameters such as heart rate, temperature, and respiratory rate are often used as indicators of pain, they can be unreliable and highly variable (Conzemius et al., 1997). These measures are frequently influenced by external factors, including environmental conditions, noises, physical activity, exercise, restraint, and even eating. For instance, even measuring heart rate in a flock animal like a sheep can trigger an autonomic stress response, leading to an increased heart rate independent of pain.

Even when subjective pain assessments are valid, they do not always correlate well with an animal's actual pain severity. In one study, postoperative pain severity in dogs, as measured by the Visual Analogue Scale (VAS) and Numerical Rating Scale (NRS), showed poor to no correlation with heart rate, respiratory rate, or blood pressure (Conzemius et al., 1997). It is also important to consider that the observational pain scores used in that study may not be entirely reliable indicators of pain in themselves.

Similarly, biochemical markers such as cortisol, adrenaline, and noradrenaline have also been explored as objective pain measures, but they are unreliable due to their susceptibility to non-pain-related influences. Factors such as species, age, sex, individual variation, previous experience, and the stress induced by sampling methods can significantly alter these biochemical readings, making it difficult to distinguish pain-related changes from other physiological responses (Dobromylskyj et al., 2000; Ladewig, 1987; Mellor et al., 2000; Pedersen, 1996).

A crucial challenge for clinicians is determining how much change in physiological or biochemical parameters truly reflects pain and suffering. Despite these limitations, physiological and biochemical markers can still serve as useful components in a broader, integrated pain assessment system rather than being relied upon as sole indicators of pain (Dobromylskyj et al.; 2000).

1.5.4 Neurophysiological techniques

Neurophysiological techniques have been widely utilised in both human and veterinary studies to assess pain perception and depth of anaesthesia (Dutton et al., 1996; Ekström et al., 1993; Kulka et al., 2012; Otto, 2008). One of the most commonly used tools is the electroencephalogram (EEG), which records the summated, spontaneous electrical activity of neurons in the cerebral cortex (Gugino et al., 2004; Tooley et al., 1984). The cerebral cortex plays a critical role in processing afferent nociceptive input, ultimately leading to the conscious perception of pain. Numerous studies have demonstrated a correlation between changes in EEG frequency spectra and noxious stimulation, with these alterations attenuated by preemptively administered analgesics

(Haga & Dolvik, 2005; Kongara et al., 2010, 2013a, 2013b, 2014; Murrell et al., 2003; Murrell & Johnson, 2006; Ong et al., 1997; Otto et al., 1996, 2000; Otto, 2007;).

To extract meaningful data from raw EEG recordings, the signal is transformed from a time-based to a frequency-base domain (Johnson et al., 2007). Several key variables of interest are derived from this transformation:

- Median Frequency (F_{50}): The frequency below which 50% of the total EEG power is located, offering a generalised measure of EEG activity.
- Spectral Edge Frequency (F_{95}): The frequency below which 95% of the total EEG power is located, reflecting high-frequency activity changes.
- Total EEG Power (P_{tot}): The area under the power spectrum curve, primarily influenced by lower-frequency activity (Murrell & Johnson, 2006).

Pain related nociceptive stimuli have been associated with increases in F_{50} and F_{95} and a decrease in P_{tot}, reflecting a shallower depth of anaesthesia and heightened nociceptive processing (Murrell et al., 2003). These findings highlight the potential of EEG-based monitoring in refining pain assessment and optimising anaesthetic and analgesic strategies.

1.5.5 Other pain assessment methods

Beyond commonly used traditional methods, several other techniques have been developed to objectively assess pain and nociception. These include:

- Nociceptive Threshold Testing (Analgesiometry): This method measures the minimum stimulus intensity required to elicit a nociceptive response, providing a quantitative assessment of pain sensitivity (Arendt-Nielsen & Yarnitsky, 2009).
- Behavioural Analysis vis Computerised Motion Tracking: Automated systems are used to analyse pain-associated behaviours, such as the amount of movement and associated patterns, using accelerometry in dogs (Hansen et al., 2007) and cats (Lascelles et al., 2007).
- Biochemical Pain Markers: This method measures levels of biomarkers of pain and inflammation (Zhang & An, 2007). One example of such a biomarker is adenosine deaminase, which has been implicated as an indicator of pain-related inflammation (Demir et al., 2014; Hu et al., 2016).
- Computerised Gait Analysis: Uses carefully applied joint markers, analysed motions, or the level of force applied as an objective assessment of gait abnormalities associated with pain and musculoskeletal dysfunction (Foss et al., 2018; Romans et al., 2005; Souza et al., 2019; Waxman et al., 2008; Wyatt et al., 2019).

These techniques have the potential to offer more precise and quantifiable insights into pain perception and locomotor changes. However, their practical application in daily clinical practice may be limited. Despite this, their use in research settings can significantly enhance pain management and treatment strategies across species.

1.6 SURGICAL PAIN

Advancements in pain research and measurement have also deepened our understanding of how pain manifests across different species following specific surgical procedures. This knowledge is essential for accurately quantifying pain and developing more effective treatment approaches. Gonadectomy is one such procedure, and the most common elective surgery in both cats and dogs.

1.6.1 Feline castration as a model for analgesia research

Most cats presenting for elective surgical neutering are young, healthy, and free of any pre-existing pain at the time of presentation (Dyson, 2008). This makes them ideal models for analgesic research, as pain responses can be assessed without the confounding effects of pre-existing conditions. The efficacy of administered treatments is commonly evaluated using ovariohysterectomy (Al-Gizawiy & Rudé 2004; Cagnardi et al., 2011; Giordano et al., 2010a) or orchiectomy (Kongara et al., 2013b; Slingsby et al., 2001; Väisänen et al., 2007) as standardised surgical pain models. Feline castration is known to cause moderate and sustained pain, significantly affecting behaviour for several days postoperatively.

In one study comparing long-acting carprofen to the short-acting pethidine, cats receiving carprofen experienced significantly less pain than those given pethidine, confirming that castration is a painful procedure with prolonged behavioural effects (Balmer et al., 1998). Various behavioural changes, indicative of pain, have been

observed after castration (Väisänen et al., 2007). Post-castration behavioural changes, such as hiding and withdrawal, are well recognised indicators of pain in cats (Robertson, 2005; Taylor & Robertson, 2004). A study by Väisänen et al., 2007 reported that even three to four days post-surgery, castrated cats exhibited a lowered body posture, altered responses to touch, reduced activity levels, decreased playfulness, increased sleeping duration, and heightened fearfulness.

Despite this evidence, analgesic use for feline castration remains alarmingly low. A study found that only 16% of veterinarians in Britain administered analgesics for routine cat castrations (Lascelles et al., 1999). Even more concerning, only 3.8% of veterinarians in Australia, New Zealand, and Britain provided post-discharge analgesia for castrated cats (Farnworth et al., 2014). These statistics highlight a significant gap in feline pain management, underscoring the need for increased awareness and improved analgesic protocols in veterinary practice.

1.6.2 Pain management in canine castration

Canine castration is one of the most common surgical procedures in small animal practice. It is associated with measurable levels of acute postoperative pain and is classified as a moderately painful procedure (Kongara et al., 2013a; Mathews, 2000). Perioperative analgesia plays a crucial role in alleviating this pain, which, if left untreated, can lead to numerous detrimental effects, including increased protein catabolism, decreased food intake, ventilation/perfusion mismatch, immunosuppression, and delayed wound healing. Beyond its physiological impact,

postoperative pain is also a significant animal welfare concern (Carroll, 1996; Gaynor, 1999; Hansen, 2005).

1.6.3 Local anaesthetic techniques in canine castration

As part of a balanced analgesic, intratesticular or incisional blocks are recommended in canine castration (Mathews et al., 2014b). Intratesticular injection of lidocaine has been shown to rapidly diffuse into the spermatic cord, providing effective analgesia in piglets (Ranheim et al., 2005). In dogs, intratesticular bupivacaine has been reported to reduce unwanted haemodynamic responses to castration (Huuskonen et al., 2013), and enhance perioperative analgesic efficacy when combined with hydromorphone and carprofen (Perez et al., 2013).

Evidence of local anaesthetic efficacy, however, remains inconsistent. In one study, for example, an intratesticular lidocaine/bupivacaine mixture failed to significantly reduce pain scores compared to saline injection (Stevens et al., 2013). Literature on the effects of local anaesthetic incisional blocks in canine castration and ovariohysterectomy is also inconsistent. One study found that bupivacaine infiltration at the incision site in ovariohysterectomised dogs provided no additional benefit in reducing postoperative pain scores (Fitzpatrick et al., 2010). Notably, there were no studies found evaluating the efficacy of incisional blocks alone in canine castration, highlighting a gap in research that warrants further investigation.

1.6.4 Canine orthopaedic surgery

Induced stifle joint inflammation has been widely used as a model of postoperative pain in dogs for decades (Cross et al., 1997; Guillot et al., 2013; van Arman et al.; 1970). Surgical repairs of cruciate ligament injuries are among the most frequently performed orthopaedic procedures in veterinary medicine. In a study by Tomas et al. (2015), stifle arthrotomy resulted in a significant reduction in limb usage for at least 72 hours postoperatively. This impairment was markedly improved by the administration of buprenorphine, confirming that the disuse was directly linked to acute pain. Orthopaedic procedures such as a tibial tuberosity advancement (TTA) and tibial plateau levelling osteotomy (TPLO) involve both an osteotomy and arthrotomy, make them highly invasive procedures causing significant postoperative pain in dogs. These surgeries are known to cause moderate to severe pain in animals (Mathews, 2000), providing a reliable framework for evaluating analgesic efficacy and refining pain management strategies.

1.7 GLOBAL TRENDS IN VETERINARY ANALGESIA

1.7.1 Analgesic use in New Zealand

In New Zealand, morphine and buprenorphine in dogs, and medetomidine and butorphanol in cats are the most commonly used preemptive analgesics, often administered with acepromazine in dogs and ketamine in cats as part of premedication or induction (Gates et al., 2020; Sano et al., 2018). Local anaesthetics are also widely

used due to their minimal systemic adverse effects compared to systemic analgesics (Sano et al., 2018). For postoperative pain management, morphine and buprenorphine remain popular choices, and non-steroidal anti-inflammatory drugs (NSAIDs) are typically administered immediately after anaesthesia in over 50% of cases (Sano et al., 2018). An earlier study reported that morphine and carprofen were the most commonly used analgesics, with the frequency with which analgesic drugs were prescribed preoperatively or intraoperatively ranging from 50% for feline castration to 91% for canine fracture repair. Although preemptive and multimodal analgesia were commonly used, the study also found disparities in veterinarians' perceptions of procedural pain severity and the consequence of analgesic administration (Williams et al., 2005). In a more recent study, canine ovariohysterectomies only received three days of postoperative analgesia in the form of orally administered NSAIDs, whereas feline ovariohysterectomies were only given a single injection of NSAID on the day of surgery, with no further postoperative analgesia (Gates et al., 2020).

1.7.2 Factors influencing analgesic choices

Several factors influence analgesic selection and administration, including: drug availability and accessibility; veterinary demographics, such as clinic size and location (Dohoo & Dohoo, 1996); accessibility of educational material; as well as client expectations (Demetriou et al., 2009).

For example:

- In Cameroon, glucocorticoids (dexamethasone and prednisolone) and NSAIDs (phenylbutazone, tolfenamic acid and meloxicam) are commonly used perioperatively (Kouamo & Kana, 2018).
- In Nigeria, xylazine and lidocaine are the frequently used analgesics, with selection based on behaviour, attitude, medical history, available information, and appetite. Many veterinarians source information from the internet, literature, and medication leaflets (Oguntoye & Eyarefe, 2017).
- In Italy, injectable opioids, NSAIDs, and tramadol are preferred for visceral pain; yet 74% of veterinarians do not use pain scoring systems, and 97% believe they need further education in pain management (Catanzaro et al., 2016).
- In Brazil, opioids (with tramadol more commonly used than morphine) and NSAIDs (meloxicam and ketoprofen) are most frequently administered – differing from New Zealand, where carprofen is the preferred NSAID (Lorena et al., 2014).

A comparative study examining analgesic use across New Zealand, Australia, and the United Kingdom (UK) revealed notable differences in prescribing habits. UK veterinarians were more likely to administer preoperative NSAIDs than those in New Zealand and Australia. By comparison, New Zealand and Australian clinicians were more likely to prescribe postoperative and post-discharge analgesia for cats undergoing gonadectomy than their UK counterparts (Farnworth et al., 2014).

Provision of analgesia is also influenced by the gender of the veterinarian and time since graduation (Lascelles et al., 1999) as well as practice size, with larger clinics more likely to offer comprehensive pain management (Raekallio et al., 2003). There are also species-specific trends in analgesic administration. Cats receive significantly less analgesia post-gonadectomy than dogs (Williams et al., 2005). Specific procedures also influence analgesia administered, with patients undergoing castration being less likely to receive analgesic coverage than patients undergoing an ovariohysterectomy, likely due to the perceived difference in pain severity between the two procedures (Dohoo & Dohoo, 1996; Lascelles et al., 1999; Raekallio et al., 2003; Williams et al., 2005).

These findings highlight more gaps in veterinary pain management and the need for further education. Research-based improvements in pain monitoring would allow for provision of analgesia tailored to the individual and the results of the pain experience, rather than standardised protocols that vastly differ between clinicians of differing demographics.

1.8 DELIVERY OF ANALGESIC DRUGS IN COMPANION ANIMALS

Pain is multidimensional, requiring a comprehensive approach to ensure effective management. It involves sensory (intensity, quality, location), postural (gait, stance), physiological (nociception, autonomic responses, endocrine effects), and behavioural (mood, appetite, activity) components. Thus, optimal pain relief must be tailored to each patient, selecting the most appropriate drug, dose, route, and modality to

maximise efficacy, minimise side effects, and enhance patient comfort. In this thesis, the route of administration plays a central role in evaluating analgesic efficacy across multiple studies. Previous research found that buprenorphine was ineffective when administered subcutaneously (Askar et al., 2020; Steagall et al., 2014, 2020;), which led to the decision to investigate its efficacy via the intramuscular route in **Chapter 2**. In **Chapter 4**, while the study compares two different drugs, they are administered through distinct routes, both systemically and locally, highlighting the impact of delivery methods on analgesic outcomes. **Chapter 5** examines whether a commonly used and well-researched analgesic, typically given via parenteral routes, maintains its efficacy when administered transdermally. Given the importance of these variations to the thesis, understanding the influence of different administration routes is a key aspect of this thesis.

1.8.1 Routes of drug Administration

Analgesic drugs can be administered via systemic or localised routes, depending on the desired effects and target tissue (Golan et al., 2016; Zempsky, 1998). Systemic routes include:

- Oral and rectal (enteral)
- Parenteral (subcutaneous, intramuscular, intravenous)
- Transmucosal (intranasal, buccal, sublingual)
- Pulmonary (inhalation)
- Transdermal

- Intraosseous (Jain, 2008).

Local delivery includes regional anaesthesia and can take the form of ring blocks, local nerve blocks, regional nerve blocks, epidurals, incisional blocks, and intratesticular blocks as specific examples. Regardless of the route, maintaining therapeutic drug levels is essential for effective analgesia.

1.8.2 Transdermal drug delivery

Transdermal drug delivery systems (TDDS) (Figure 1.3) are one example of a drug delivery method. They are composed of:

1. A protective backing to shield the entire patch from external factors.
2. A drug reservoir containing the analgesic formulation.
3. A porous membrane that regulates rate-controlled drug release.
4. An adhesive layer ensuring secure skin contact.
5. A liner that serves as a protective layer for both the patch and the adhesive (Zempsky 1998).

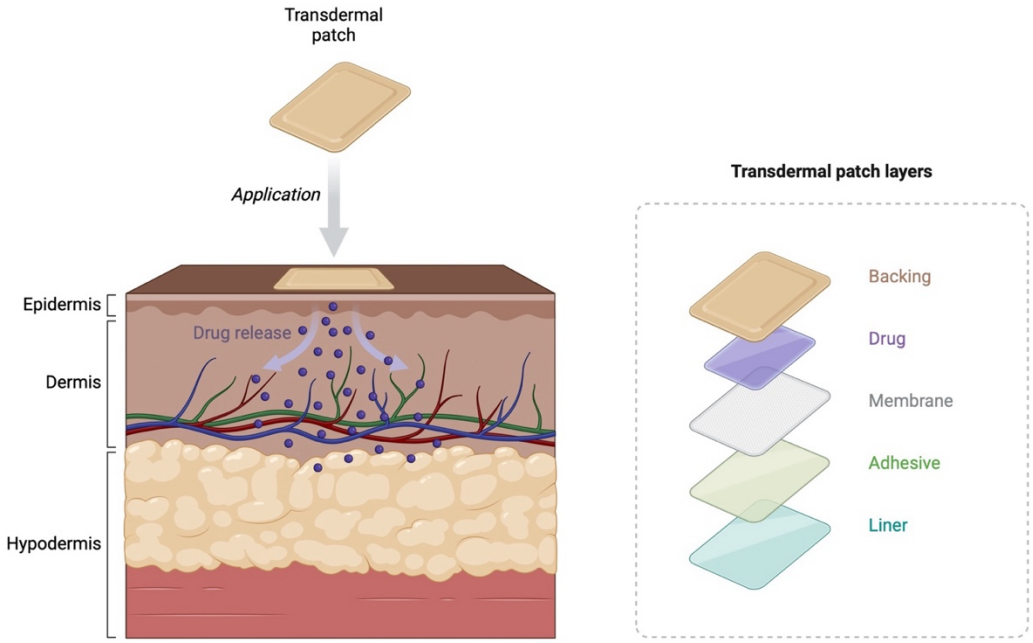

Figure 1.3 Transdermal Drug Delivery Systems (TDDS) Key components of Transdermal Drug Delivery Systems include a backing, drug, membrane, adhesive, and liner. (Created in BioRender. Sawicki, R. (2025) https://BioRender.com/h50o847)

While TDDS offer non-invasive, continuous drug delivery several factors influence their pharmacokinetics and efficacy, including: drug properties (pH, molecular size, lipid solubility, membrane permeability); skin factors (hydration, thickness, metabolism by skin flora, blood flow); and external conditions (temperature, site of application) (Balant et al., 1990; Berner & John, 1994; Clissold & Heel, 1985; de Boer et al., 1990; Moore & Chien, 1988; Ridout et al., 1988; Roy & Flynn, 1989, 1990; J. Singh & Roberts, 1988; S. Singh & Singh, 1993; Stevenson et al., 1993; Wada et al., 1993).

As a result, transdermal drug delivery systems have various advantages and disadvantages, which must be considered when prescribing TDDS to animals (Coelho et al., 2010):

Advantages:

- Prolonged drug activity and bioavailability
- Minimised systemic side effects
- Reduced dosing frequency
- Steady plasma drug levels
- Improved owner compliance

Disadvantages:

- Potential for toxicity
- Variable absorption and inconsistent drug delivery
- Risk of subtherapeutic dosing
- Higher cost

Currently, the fentanyl transdermal patch is the only long-acting analgesic formulation readily available, offering over 24 hours of pain relief. Research on fentanyl patch efficacy in dogs remains limited (Kyles et al., 1998). One approved transdermal fentanyl solution for dogs reaches therapeutic serum levels after 24 hours, providing sustained analgesia for an additional 48 hours (Kukanich & Clark, 2012). Due to slow onset and drug accumulation within the skin, concurrent opioid administration for immediate pain relief may increase the risk of respiratory depression (Bernstein, 1994; Lehmann & Zech 1992; Ridout et al., 1988). While fentanyl patches are convenient for acute pain management, they were originally approved in humans only for human cancer and chronic pain, and present a potential risk for misuse if sent home with

clients (Biddle & Gilliland, 1992; Calis et al., 1992; Payne, 1992; Portenoy et al., 1993; Skaer, 1993).

1.8.3 Parenteral drug delivery

Parenteral administration is the most common invasive route for drug delivery in veterinary medicine. Advantages of parenteral drug delivery include a rapid onset of action, predictable bioavailability, avoidance of gastrointestinal tract degradation, and a reliable route for critical patients. Disadvantages of parenteral drug delivery include fluctuating plasma drug levels, a limited ability to deliver protein-based drugs, and a potential for injection site complications (Jain, 2008).

1.8.4 Subcutaneous (SC) injections

SC injections deposit the drug into the subcutaneous fat layer, offering slow and sustained absorption. While relatively easy to administer, they have several limitations: an unpredictable absorption rate, local irritation and pain at the injection site, and a potential for drug accumulation with repeated injections (Jain, 2008), as seen specifically with buprenorphine administered SC.

1.8.5 Incisional infiltration (line blocks)

A subset of subcutaneous injections, incisional infiltration involves injecting local anaesthetic into the subcutaneous and fascial layers surrounding an area of actual or subsequent trauma, which may include surgical incisions. This technique effectively

reduces intraoperative and postoperative pain and is commonly used as part of multimodal analgesia (Campagnol et al., 2012; Carpenter et al., 2004; Fitzpatrick et al., 2010; McKune et al., 2014; Savvas et al., 2008).

1.8.6 Intramuscular (IM) injections

Intramuscular (IM) injections are given deep into skeletal muscles, leading to faster absorption than SC injection due to the increased vascularity of tissue, but slower than if administered intravenously (Jain, 2008). Absorption rates vary, depending on drug properties and muscle circulation and activity. Disadvantages of IM injections include: pain at the injection site; limited injection volume per site; inadvertent risk of intravenous injection; and the potential for nerve damage, haematoma or abscess formation (Jain, 2008).

1.8.7 Other drug delivery mechanisms

Several additional analgesic delivery methods are used in veterinary medicine, though they fall outside the scope of this review. These include: wound irrigation (Winkler et al., 1997); splash blocks; sublingual administration; analgesic implants (slow-release subcutaneous modules); and intravenous, intraarticular, intraperitoneal, intrathoracic, intrathecal, and epidural injections.

In addition to varying the route of administration, the timing of drug delivery is equally crucial. This is not only to ensure adequate analgesia for surgical pain but also to pre-empt central nervous system sensitisation.

1.9 Central Sensitisation and the Role of Preemptive Analgesia

1.9.1 Noxious stimuli and the development of postoperative pain

Noxious stimuli, including surgical incisions and perioperative nociceptive events, induce prolonged changes in central neuronal function contributing to postoperative pain (Woolf & Chong, 1993). Peripheral nociceptors are initially stimulated by noxious stimuli from surgical incisions, which trigger inflammatory pain in the dorsal horn of the spinal cord. This response is mediated by the release of C-fibre-dependent neuropeptides, excitatory amino acids, and other chemical mediators (Chiu et al., 2012; Middleton et al., 2021; Schmelz, 2015). These substances act as irritants to nociceptors, leading to central nervous system modulation via activity-dependent plasticity (Hoegh, 2023).

As a result, peripheral sensitisation develops, progressing to pathologic pain, which:

- Persists beyond the expected healing period
- Can outlast the half-life of the released inflammatory mediators
- Is excessive in intensity and spread
- Can be triggered by low-intensity stimuli (allodynia, hyperalgesia)
- Is hyperpathic in nature (Kelly et al., 2001).

Once peripheral neuronal excitability increases, the pain threshold decreases, allowing even mild nociceptive stimuli to activate and amplify central pain pathways (Kress et al., 1992). This sensitisation results in increased spontaneous activity, a lowered activation threshold, exaggerated responses to afferent inputs, enhanced responses to repeated inputs (wind-up phenomenon), an expansion of dorsal horn neuron receptive fields, and an altered population of sodium ion channels (Hoegh, 2023; Ingram et al., 2024; Jensen & Finnerup, 2014; Levinson et al., 2012).

1.9.2 Maladaptive plasticity and the development of chronic pain

These neuroplastic changes, referred to as maladaptive plasticity, amplify the intensity and duration of nociceptive signals (Coderre & Melzack, 1992; Kissin, 1994). This central sensitisation not only lowers future pain thresholds but also increases the risk of central hyperexcitability and chronic pain (Kelly et al., 2001). The long-term consequences of inadequate postoperative pain management have been well documented in human studies following:

- Thoracotomy (Kalso et al., 2001; Katz et al., 1996; Perttunen et al., 1999; Pluijms et al., 2006)
- Mastectomy (Poleshuck et al., 2006)
- Orthopaedic surgery (Nikolajsen et al., 2006)
- Inguinal herniorrhaphy (Aasvang & Kehlet, 2005; Manangi et al., 2014)
- Laparoscopic procedures (Blichfeldt-Eckhardt et al., 2014).

1.9.3 The role of preemptive analgesia

Preemptive analgesia, especially for the purposes of this review, involves administering analgesic treatment before the onset of surgery to prevent the establishment of central sensitisation caused by surgical and inflammatory injuries (Kissin, 2000). This approach aims to inhibit nociceptive transmission before pain pathways become hyperactive, thereby reducing postoperative pain intensity and duration. By providing analgesia prior to the surgical incision, it ultimately affects the way nociception develops intraoperatively and how pain is experienced in the early postoperative phase (22 to 48 hours post-surgery).

Pain can be very broadly classified into physiological, or protective and adaptive pain and pathological, or nonprotective and maladaptive pain. Treatment of physiological pain usually addresses the pain once it develops, whereas preemptive analgesia seeks to prevent the transition from acute physiological pain to pathologic pain, ultimately improving patient outcomes and reducing the risk of chronic postoperative pain syndromes (Mayoral Rojals et al., 2022).

1.10 MULTIMODAL ANALGESIA: A COMPREHENSIVE APPROACH TO PAIN MANAGEMENT

Multimodal analgesia refers to the use of multiple analgesic drugs with differing mechanisms of action, targeting various pathways and receptors to prevent or minimise nociceptive stimulation (Raffa, 2001; Rosenthal et al., 2004). This approach provides enhanced pain relief; reduces the need for high-dose single-drug

administration, which while increasing the range of potential side effects reduces their severity; and mitigates adverse effects while overcoming individual drug limitations (Raffa, 2001; Rosenthal et al., 2004). In human medicine, multimodal analgesia has been shown to reduce postoperative morbidity and mortality, improve patient satisfaction and quality of life, and lower inpatient costs (Skinner, 2004). As a result, it is widely recognised as a 'best practice' approach (Lascelles & Main, 2002). Veterinary studies similarly support the efficacy and benefits of multimodal analgesia.

A multimodal regimen typically involves:

1. Strong central (neuraxial) opioid analgesia
2. Non-opioid analgesics
3. Peripheral regional or neuraxial local anaesthetics (e.g. intercostal blocks, plexus blocks, and local anaesthetic infiltration of incisions) that act at varying sites of the pain pathway (Grape & Tramèr, 2007).

1.10.1 Complexity of pain and the need for multimodal analgesia

Despite its widespread clinical use, no controlled multimodal analgesic studies had been conducted in cats until recently (Lascelles et al., 2007). It has, however, been anecdotally reported as an effective and commonly used approach in feline pain management (Robertson, 2005). Multimodal analgesia is particularly crucial for managing chronic pain, as long-term pain states involve extensive central nervous system changes (Freedman, 2002; Manek & Lane, 2000; Mullican & Lacy, 2001). One

example of this is canine osteoarthritis, a progressive and chronic disease where multimodal analgesia is considered essential for effective pain control (Diehm & Tünsmeyer, 2018; Millward, 2017).

Given the numerous central and peripheral sensitising mechanisms involved in pain modulation amplification, single-drug analgesic regimens are rarely as effective as multimodal approaches. By targeting multiple pain pathways simultaneously, multimodal analgesia maximises pain relief while minimising side effects, making it an effective strategy for pain management in veterinary medicine (Lamont, 2008).

1.11 ANALGESIC DRUGS

1.11.1 Opioids in veterinary medicine

Opioids are widely regarded as some of the most effective, versatile, and potent analgesics with a relatively good safety margin. They form the backbone of acute pain management in veterinary species (Robertson, 2005).

1.11.1.1 Opioid receptor neurophysiology

Opioid receptors are distributed throughout the body, both centrally and peripherally, and are classified into four main subtypes:

- Mu (μ) – Primary receptor for analgesia, euphoria, respiratory depression, and dependence.

- Kappa (κ) – Modulates analgesia, sedation, and miosis.
- delta (δ) – Plays a supportive role in modulating μ-receptor activity
- Nociception/Orphanin FQ (NOP-R) – Involved in pain processing and modulation (Waldhoer et al., 2004).

Activation of μ receptor inhibits presynaptic release of glutamate, thereby blocking nociception from C and Aδ nerve fibres in the dorsal horn of the spinal cord. Additionally, postsynaptic inhibition in the dorsal root ganglion prevents the releases of excitatory neurotransmitters, suppressing nociceptive signalling (Barkin et al., 2005).

Activation of κ receptors increases phosphodiesterase activity, leading to an inhibitory neurotransmitter effect. This inhibitory effect hyperpolarises interneurons and raises their firing threshold, further dampening pain transmission. κ-opioid receptor-mediated analgesia, however, is generally short-lived and less effective than μ-mediated analgesia, except in birds, where κ-receptors predominate (Machelska & Celik, 2018).

The therapeutic goal of opioid therapy is to maximise analgesia while minimising side effects. Opioid analgesia is primarily mediated within the dorsal horn of the spinal cord (laminae I-V), whereas side effects such as sedation, dysphoria, nausea, and respiratory depression arise from μ-receptor activation in the brain (Wang, 2019).

1.11.1.2 Opioid analgesics in veterinary practice

(a) Morphine

Morphine is a full μ-opioid agonist that is widely used, has a slow onset of action, and does not induce excitement (Lascelles & Waterman, 1997; Robertson et al., 2003a). Its efficacy, however, is lower in cats than in dogs, likely due to limited production of its active metabolite, morphine-6-glucuronide (Bloomfield et al., 2001; Steagall et al., 2006). Morphine has a potent analgesic effect with reliable pharmacokinetics. It does, however, have a short half-life, requiring frequent administration, along with side effects of vomiting, respiratory depression, and bradycardia (Murphy et al., 2025).

(b) Methadone

Methadone a synthetic μ-opioid agonist with additional mechanisms of action, including N-methyl-D-aspartate (NMDA) receptor antagonism and monoamine re-uptake inhibition (Codd et al., 1995; Inturrisi, 2005). Unlike morphine, methadone does not undergo hepatic glucuronidation (Court & Greenblatt, 1997a, 1997b), making it more suitable for cats, which have deficient glucuronidation mechanisms (Ferreira et al., 2011). Compared to morphine, methadone has a longer duration of action, induces less vomiting and fewer cardiovascular side effects (Murrell, 2011). There are also a lot less studies looking at the effect of methadone in cats compared to morphine.

(c) Buprenorphine

Buprenorphine is a partial μ-opioid agonist with a unique pharmacological profile (Steagall et al., 2014) . It is widely used in veterinary medicine, particularly in mainland Europe, Australia, South Africa, and the UK (Joubert, 2001; Lascelles et al., 1999; Watson et al.; 1996). Buprenorphine administered subcutaneously has a slower onset

of action and a reduced analgesic efficacy compared to intramuscular and intravenous routes (Giordano et al., 2010b; Steagall et al., 2006). It has a duration of action ranging from 4 to 12 hours (Robertson et al., 2003a). Compared to morphine, while buprenorphine is less likely to cause vomiting and dysphoria and has no association with hyperthermia, it does have a more variable analgesic duration (Hall et al., 2001).

While opioids remain indispensable in veterinary analgesia, ongoing research aims to optimise their use, minimise side effects, and develop alternative pain management strategies to ensure safe and effective pain relief in companion animals.

1.11.2 Local anaesthetics

1.11.2.1 Mechanism of action

Local anaesthetics function by blocking nerve conduction, thereby preventing nociceptive signal transmission. Neurones maintain a resting membrane potential of -60 to -70 mV. When exposed to chemical, electrical, or mechanical stimuli, sodium (Na^+) channels open, allowing Na^+ influx, leading to membrane depolarisations. This triggers adjacent channels to open, creating a wave of depolarisation known as an action potential (Cohen, 1973).

Local anaesthetics bind to inactivated sodium channels, blocking sodium influx and potential propagation. By inhibiting this threshold depolarisation, they effectively halt nociceptive signal transmission, rendering the affected area insensitive to pain (Campoy & Read, 2015).

1.11.2.2 Efficacy and clinical use

Local anaesthetics are widely used in both human and veterinary medicine for pain prevention and management (Carpenter et al., 2004; Skarda & Tranquilli, 2007). They are primarily metabolised in the liver and can be administered in various ways, some of which are:

- Splash blocks – infiltration into surgical wounds, traumatic injuries, or fractures (Duke, 2000; Lamont, 2002).
- Regional blocks – Epidural analgesia and spinal anaesthesia
- Nerve blocks – targeting peripheral nerves.

Local anaesthetics are cost-effective, require minimal legal control and record keeping, and provide effective pain relief with fewer systemic effects (Savvas et al., 2008).

Common local anaesthetics include lidocaine, mepivacaine, bupivacaine, levobupivacaine, and ropivacaine. These agents vary in their speed of onset, duration of action, and potential for cardiovascular and CNS toxicity (Cuvillon et al., 2009). In companion animal practice, bupivacaine and ropivacaine are commonly used local anaesthetics in clinical practice, due to their longer duration of action.

1.11.2.3 Efficacy in veterinary literature

The effects of local anaesthetics have been evaluated in several veterinary studies. Significantly lower postoperative pain scores have been reported in dogs following

ovariohysterectomy and celiotomy when local anaesthetics were used (Campagnol et al., 2012; Carpenter et al., 2004; Cicirelli et al., 2022; Savvas et al., 2008; Song et al., 2024). However, in canine castration, the use of intratesticular local anaesthetic blocks has yielded inconsistent results. While these blocks are recommended as part of a balanced analgesic approach (Mathews et al., 2014a), studies have reported contradictory findings regarding their ability to effectively reduce nociception and pain (Huuskonen et al., 2013; McMillan et al., 2012; Perez et al., 2013; Stevens et al., 2013). Additionally, several studies in dogs and cats have found no significant analgesic benefit from postoperative local anaesthetic infiltration (Fitzpatrick et al., 2010; McKune et al., 2014; Winkler et al., 1997). These findings suggest that while local anaesthetics play a crucial role in multimodal analgesia, their efficacy varies depending on the type of procedure, administration technique, and species-specific responses.

1.11.3 α_2-adrenoceptor agonists

1.11.3.1 Mechanism of action

α_2-adrenoceptor agonists are sedative-analgesic drugs that act on α_2-adrenergic receptors in the central nervous system (CNS). While they are not considered first-line analgesics like NSAIDs and opioids, their use as analgesic adjuvants is increasing due to their ability to provide muscle relaxation, sedation, and reduced anaesthetic requirements. Their clinical use, however, is limited by significant cardiovascular side effects.

There are three main types of α_2-receptors subtypes:

- α_{2A} receptors – Located in the brainstem and spinal cord, they are responsible for sedation, analgesia, and anaesthetic sparing effects.
- α_{2B} receptors – Located in the brainstem and spinal cord, they are primarily involved in peripheral vasoconstriction
- α_{2C} receptors – Found in the spinal cord, these receptors are involved in regulating hypothermia (Lakhlani et al., 1997; Maze & Fujinaga, 2000; Schwartz et al., 1999).

These receptors exist on noradrenergic neurons (supraspinal sites) and non-noradrenergic (dorsal horn of spinal cord) neurons (Budai et al., 1998; Millan et al., 1994). Analgesia is mediated via two mechanisms:

1. Presynaptic inhibition – α_2-agonists bind to receptors on afferent C-fibres, reducing calcium influx, which decreases the release of nociceptive neurotransmitters (e.g. glutamate, substance P).
2. Postsynaptic hyperpolarisation – Activation of α_2-receptors on spinal neurons inhibits ascending nociceptive transmission (Buerkle & Yaksh, 1998).

The supraspinal α_2-adrenoceptors are primarily responsible for the sedative-hypnotic effects observed with α_2-agonists (Giovannitti et al., 2015).

1.11.3.2 Adverse effects

Cardiovascular effects

Immediately after administration, α_2-agonists induce vasoconstriction via vascular α_2-adrenoceptors, resulting in increased systemic vascular resistance. This triggers a baroreceptor-mediated reflex bradycardia, which can reduce cardiac output by up to 70% and may lead to arrhythmias (Pypendop & Verstegen, 1998).

Respiratory effects

α_2-agonists decrease the respiratory rate, central respiratory drive, and minute ventilation. $paCO_2$, pO_2, and pH generally remain stable (Lerche & Muir III, 2004; Pypendop & Verstegen, 1999).

Gastrointestinal effects

Up to 90% of cats and 20% of dogs vomit following administration of medetomidine or dexmedetomidine (Vainio, 1989).

Other systemic effects

Renal effects of α_2-agonists include significantly increased urine output (Saleh et al., 2005), endocrine effects which include hyperglycaemia due to insulin suppression (Ambrisko & Hikasa, 2002), and blunting of the perioperative stress response in dogs (Benson et al., 2000).

1.11.3.3 α_2-agonist drugs and their uses

The most commonly used α_2-agonists in veterinary medicine include detomidine, romifidine, medetomidine, dexmedetomidine, and xylazine (Pypendop, 2015), of which medetomidine, dexmedetomidine, and xylazine are registered for use in cats and dogs in New Zealand. These drugs are rapidly absorbed, undergo hepatic metabolism, and are primary excreted via the kidneys.

(a) Medetomidine and dexmedetomidine

Medetomidine and dexmedetomidine are widely used in veterinary medicine for their sedative and analgesic properties, primarily through central and spinal α_2-receptor interactions. Despite their sedative, analgesic and minimum alveolar concentration (MAC) sparing effects, their cardiovascular side effects limit their use in certain patients.

Medetomidine and dexmedetomidine exert their cardiovascular effects by acting on both peripheral and central α_2-receptors (Lascelles & Waterman, 1997; Robertson et al., 2003). They are often combined with opioids for a dose-sparing effect, enhancing sedation and providing superior analgesia. Both drugs exhibit dose-dependent sedation with a ceiling effect, where increasing the dose prolongs sedation rather than intensifying it (Bloor et al., 1989; Kuusela et al., 2000).

1.11.4 α_2-adrenoceptor antagonists

α_2-antagonists are used to reverse the effects of α_2-agonists, particularly in cases of overdose or severe cardiovascular depression.

1.11.4.1 Atipamezole

Atipamezole is the most commonly used α_2-antagonist, administered intramuscularly (IM) to reverse sedation and cardiovascular effects (Sinclair, 2003). Rapid IV administration can induce hypotension, especially in dogs, so reversal should be performed IM or titrated IV over 2-3 minutes (Vainio, 1990).

1.11.4.2 Vatinoxan (previously known as MK-467)

Vatinoxan, formerly known as MK-467, is a peripheral α_2-adrenoceptor antagonist that effectively reduces the vasoconstrictive and cardiovascular side effects of medetomidine and dexmedetomidine, without significantly affecting their sedative properties (Honkavaara et al., 2008, 2011). By selectively targeting peripheral α_2-receptors, vatinoxan mitigates the haemodynamic disturbances induced by α_2-agonists while preserving their desired central sedative and analgesic effects (Doze et al., 1989; Restitutti et al., 2011; Vainionpää et al., 2013).

Vatinoxan's ability to prevent peripheral vasoconstriction without interfering with sedation appears to be due to its limited penetration into the CNS. While its effects have been demonstrated originally in rats and marmosets (Clineschmidt et al., 1988),

further research is needed to confirm its effect on the analgesic properties of α_2-agonists and safety in clinical veterinary applications.

1.11.5 Additional analgesic options and modalities

A comprehensive, multimodal approach to pain management may incorporate various drugs with analgesic properties, including:

- NSAIDs
- Corticosteroids
- N-methyl-D-aspartate (NMDA) receptor antagonists
- Antidepressants
- Anticonvulsants (e.g. gabapentinoids)
- Monoamine reuptake inhibitors
- Neurokinin type-1 (NK1) receptor antagonists.

While these drugs play a crucial role in a clinician's pain management arsenal, they fall outside the scope of this review.

1.12 RESEARCH AIMS AND OBJECTIVES

The studies described in this thesis aim to investigate deficiencies in perioperative analgesia and address knowledge gaps to enhance analgesic efficacy by addressing specific questions spanning the entire perioperative period, including the preoperative,

intraoperative, and postoperative phases. The different aspects addressed by this thesis include the improvement of pre-medicant opioid efficacy in cats undergoing surgery, the effect of non-analgesic drugs on nociception in the preoperative period, the effect of multimodal analgesia intraoperatively and postoperatively using local anaesthetics as local area blocks in dogs undergoing routine surgical procedures, and finally assessing the efficacy of sustained transdermal analgesia on postoperative pain in dogs undergoing orthopaedic surgical procedures.

In **chapter 2**, the focus is on the preoperative period and optimising opioid drug selection amongst three commonly used opioids in cats undergoing routine surgical castration. Though analgesic use has been increased in veterinary patients, pain in cats is still undertreated due to a lack of reliable methods to recognise it, limited knowledge on analgesic mechanisms, species-specific pharmacokinetics of analgesics, and a unique temperament which makes pain assessment in the cat difficult (Wright, 2002). Currently, a limited number of approved and licensed medications are available for the treatment and management of pain in cats, compared to those approved for use in species such as dogs (Steagall et al., 2022). This is largely due to the lack of reliable evidence on the efficacy and adverse effects of commonly used analgesics in cats. Subjective pain assessment in cats can also prove to be difficult due to their unique temperament and subtle expression of pain behaviours (Wright, 2002), and often leads to an underestimation of the pain that cats experience. The objective nature of the EEG enhances its usefulness in studying nociception in cats. This chapter aims to determine the efficacy of methadone, buprenorphine, and morphine in ameliorating the EEG indices of nociception in cats undergoing castration.

Chapter 3 investigates the effects of adjunct drugs given alongside pre-medicants in cats undergoing routine castration. They are used to ameliorate undesirable side effects of commonly administered pre-medication drugs. Their effect on the analgesic efficacy of the co-administered drug is explored as part of this study. Dexmedetomidine, a commonly used drug in the pre-medication period, is a potent, selective α_2-agonist with significant sedative, anaesthetic dose sparing and stress relieving properties (Murrell & Hellebrekers, 2005). Dexmedetomidine also possesses analgesic properties; however, its strong sedative effect and numerous unwanted physiological side effects makes it challenging to assess analgesic efficacy using behavioural or physiological pain evaluation methods (Slingsby et al., 2009, 2010). The undesirable effects of α_2-agonist agents can be mitigated with vatinoxan, a peripheral α_2–adrenoceptor antagonist. When administered alongside dexmedetomidine, vatinoxan attenuates its harmful cardiovascular effects while having little or no impact on sedation (Bryant et al., 1998; Honkavaara et al., 2008; Pypendop & Jones, 2015; Restitutti et al., 2011). This effect is possible as vatinoxan does not markedly cross the blood-brain–barrier (Clineschmidt et al., 1988). This chapter aims to investigate the effects of dexmedetomidine when used separately and in combination with vatinoxan, with and without the addition of morphine in ameliorating the EEG indices of nociception in healthy cats undergoing routine surgical castration.

Chapter 4 compares a multi-modal analgesic technique, using both an opioid and locally administered analgesic, to using each drug individually in providing intraoperative antinociception and postoperative pain relief in dogs undergoing routine surgical castration. While opioids have been commonly used for pain control in

veterinary practice, they can cause clinically significant side effects (Hall et al., 2001). When administered alone, they may not provide sufficient analgesia (Fitzpatrick et al., 2010), and increasing the dosage to achieve adequate pain relief increases the incidence and severity of side effects (Hall et al., 2001). The addition of local anaesthetics in a multi-modal approach can provide additional analgesia without overly relying on one specific drug. Local anaesthetics have been used in a variety of ways in human and veterinary practice to prevent or treat pain (Carpenter et al., 2004). In the veterinary literature, Carpenter et al. (2004) demonstrated the benefit of intraperitoneal and incisional infiltration of local anaesthetic demonstrating significant decreases in postoperative pain scores in dogs undergoing ovariohysterectomy. This was supported by Savvas et al. (2008), who showed that a preoperative incisional block with bupivacaine was a useful adjunct for controlling pain after celiotomy in dogs. They also offer additional advantages, such as potent analgesia by regional blockade, lower cost, reduced legal control and no need for record keeping (Savvas et al., 2008). The objective of this study was to investigate the effects of preoperatively administered bupivacaine and morphine separately and in combination, on EEG responses to nociception and postoperative pain perception in dogs undergoing castration.

Chapter 5 investigates the efficacy of a novel administration method for a commonly used opioid in the postoperative period. Specifically, it examines whether a long-acting transdermal formulation of buprenorphine can provide effective pain relief in dogs following orthopaedic surgery. Transdermal delivery systems (TDS) have numerous advantages in that they: provide sustained and relatively consistent plasma concentrations over an extended period of time; offer a non-invasive analgesic

approach to the patient (i.e. reducing anxiety in patients from handling and avoiding repeated injections); reduce the nursing workload when compared to intravenous (IV) infusions or repeated injections; and minimise side effects seen with intermittent delivery (Arndt et al., 1984; Kyles et al., 1996; Riviere & Papich, 2001). Transdermal buprenorphine patches have been developed for the treatment of human chronic pain. They have previously been studied and compared to injectable buprenorphine in the control of postoperative pain resulting from soft tissue surgery (Loeser & Treede, 2008). The transdermal formulation lasts for seven days, reduces the amount of interaction necessary with the patient in an already stressful environment, and may also be more cost effective than injectable buprenorphine and morphine. However, they have not been evaluated for the control of orthopaedic surgical pain in dogs. Orthopaedic surgical pain involving a surgical osteotomy is a surgical procedure causing moderate to severe pain (Mathews, 2000). The objective of this study was to compare the efficacy of transdermal buprenorphine patches as an analgesic protocol to currently used intramuscular (IM) administration of morphine and buprenorphine to control postoperative pain following orthopaedic surgery in dogs.

The four clinical trials that comprise the four studies were conducted in client owned healthy dogs and cats.

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2 CHAPTER 2 - COMPARATIVE EFFECTS OF METHADONE, BUPRENORPHINE, AND MORPHINE ON THE ELECTROENCEPHALOGRAM OF CATS UNDERGOING CASTRATION

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2.1 ABSTRACT

AIMS: To compare the efficacy of methadone, buprenorphine and morphine administered intramuscularly as preoperative analgesia in ameliorating the encephalographic indices of nociception in healthy cats undergoing castration.

METHODS: Cats (n = eight per group) randomly received either methadone (0.3 mg/kg), buprenorphine (0.03 mg/kg), or morphine (0.3 mg/kg) IM at the time of anaesthetic premedication. Anaesthesia was induced with intravenous propofol to effect and maintained with halothane in oxygen. Electroencephalogram electrodes were placed SC and the electroencephalogram continuously recorded. All cats underwent castration via a scrotal approach. The median frequency (F_{50}), 95% spectral edge frequency (F_{95}) and total power (P_{tot}) were compared between treatment groups and between surgical time-points using mixed model analysis.

RESULTS: There were no significant differences in the F_{50}, F_{95} and P_{tot} of the EEG between the three treatment groups. Methadone significantly decreased the F_{50} and F_{95} and significantly increased P_{tot} of the EEG.

CONCLUSIONS: Significant increases in F_{50} and F_{95} or decreases in P_{tot} in the EEG are indicative of nociception in animals. Intramuscularly administered methadone, buprenorphine, and morphine were all able to effectively prevent EEG changes indicative of nociception in cats undergoing castration.

CLINICAL RELEVANCE: While all three opioids were effective at ameliorating EEG indices indicative of nociception during feline castration, methadone was more effective than either morphine or buprenorphine in cats undergoing castration.

KEYWORDS: methadone, buprenorphine, morphine, feline castration, electroencephalogram, analgesia, nociception

2.2 INTRODUCTION

Effective pain management is essential to achieve positive health outcomes, while ensuring animal well-being. Inadequate pain relief in the perioperative period results in prolonged recovery, increased patient morbidity and complications such as decreased food intake, immunosuppression, and delayed wound healing (Holte and Kehlet 2002; Hansen 2005; Mathews et al. 2014). Various drug combinations are used as pre-anaesthetic medications in small animals, with an opioid often being a common choice (Hall et al. 2001). The choice of the opioid used depends on the breed, procedure, availability, side effects, and personal preference (Pascoe 2000a). Buprenorphine, morphine, and methadone are among the most commonly used analgesics in balanced anaesthetic protocols. A study conducted in New Zealand found that one of these medications is administered as a pre-anaesthetic in 93% of dogs and 55% of cats undergoing anaesthesia (Sano et al. 2018).

Opioids bind to opioid receptors, inhibiting the release of excitatory neurotransmitters in the brain and spinal cord, thereby reducing transmission of nociceptive signals and perception of pain in response to nociceptive stimuli without affecting motor function

(Pascoe 2000b; Wright 2002). Opioids are frequently included in anaesthetic pre-medication combinations to provide perioperative analgesia and sedation, while also reducing the required doses of drugs used for anaesthetic induction and maintenance. (Golder et al. 1998; Hall et al. 1999; Bortolami et al. 2013). Morphine (morphine sulphate) is regarded as the prototypical, 'gold-standard' μ opioid receptor agonist (Clarke and Trim 2013) and one against which analgesic efficacy is often measured. Although historically thought to produce excitement in cats (Fertziger et al. 1974), when used at appropriate doses and dosing intervals, it is effective in treating acute pain (Robertson and Taylor 2013) with minimal side effects such as mydriasis and transient hypothermia (Wallenstein and Wang 1979; Posner et al. 2010). Methadone is a synthetic opioid that is structurally unrelated to other opium-derived analgesics. It exists as a racemic mixture: the d-isomer which acts as a noncompetitive antagonist at the NMDA receptor, inhibiting the reuptake of norepinephrine, and the l-isomer, which serves as an agonist for the μ opioid receptor (Inturrisi 2005). Methadone's antagonism of the NMDA receptor inhibits central sensitisation, prevents hyperalgesia, and may prevent remodelling of pain pathways, all of which can help manage chronic pain (Latremoliere and Woolf 2009; Sotgiu et al. 2009). A third analgesic effect of methadone is its capacity to block the reuptake of serotonin and norepinephrine, which contribute to modulating and inhibiting pain within the central nervous system (KuKanich and Papich 2018). Buprenorphine is a highly lipophilic semi-synthetic partial μ opioid receptor agonist (Rang et al. 1999) which has been used for many years in dogs and cats for analgesia, sedation, and pre-anaesthetic medication. It has been shown to be the most commonly used analgesic in small animal practice in the United States, United Kingdon, and New Zealand (Capner et al. 1999; Lascelles et

al. 1999; Williams et al. 2005; Kogan et al. 2019). However, a wide variation in the duration of antinociceptive and analgesic effects has been reported with buprenorphine (Bortolami and Love 2015). It has a delayed onset of action in cats, provides a moderate level of analgesia, and has a duration of action that is shorter in cats than in other species (Steagall et al. 2014).

The cerebral cortex plays a central role in processing afferent nociceptive inputs, leading to the conscious perception of pain (Schnitzler and Ploner 2000). Neurophysiological techniques, such as the electroencephalogram (EEG), are able to record cortical activity and have been widely used in human and animal studies to measure anaesthetic depth and pain perception (Ekström et al. 1993; Kulka et al. 2012). The EEG provides a record of summated spontaneous electrical activity of neurons, displayed as waveforms of various frequencies over time (Gugino et al. 2004). Exposure to noxious stimuli induces predictable changes in the EEG pattern, marked by a shift towards high-frequency and low-amplitude activity (Murrell et al. 2003; Haga and Ranheim 2005; Johnson 2005). Under controlled conditions, such as those provided by the minimal anaesthesia model, the EEG can be used to capture and quantify these changes in cortical activity (Murrell and Johnson 2006). The cerebral cortex remains responsive to noxious stimuli during minimal halothane anaesthesia (Murrell et al. 2003), making this technique particularly valuable. By minimizing the confounding effects of deeper anaesthesia, this approach ensures data more accurately reflect nociceptive processing in the cerebral cortex. This approach allows for the ethical and conscientious investigation of potentially painful procedures and facilitates the comparison of different analgesic drugs. Such analyses provide critical insights into

pain perception, offering a closer representation of how nociception may be experienced in awake animals.

Johnson et al (2007) described the transformation of EEG data from a time-based domain to a frequency-based domain using fast Fourier transformation. Variables of interest were derived from this transformation including the median frequency (F_{50}), the 95% spectral edge frequency (F_{95}), and total EEG power (P_{tot}). Increases in F_{50} and F_{95} have been linked to nociception, and a decrease in P_{tot} has been related to a decrease in depth of anaesthesia during a nociceptive stimulus (Gibson 2007). Studies have associated changes in the EEG frequency spectrum with noxious stimulation, and subsequent amelioration of these changes with pre-emptively administered analgesics (Ong et al. 1997; Murrell et al. 2003; Murrell and Johnson 2006; Kongara et al. 2010, 2013a, 2014).

The objective of this study was to characterise the effect of three commonly used opioids in feline anaesthesia on the EEG indices of nociception in cats undergoing castration. We hypothesise that opioid administration at the time of premedication will reduce EEG changes indicative of noxious stimulation in cats undergoing castration.

2.3 MATERIALS AND METHODS

2.3.1 Study Design

All aspects of the current study design were reviewed and approved by the Massey University Animal Ethics Committee (Approved protocol number - MUAEC 14/43). Previous EEG studies (Kongara *et al.* 2013a, 2014) have demonstrated that a sample size of 8 animals per group is sufficient to demonstrate a measurable and statistically significant difference in similar circumstances.

2.3.2 Animals

A total of 24 male cats were studied. The cats were client-owned animals recruited through the Palmerston North SPCA and presented for routine castration. Cats were considered healthy based on history and physical examination; they were admitted to the study on the day of surgery with written owner consent obtained prior to enrolment into the study. Food was withheld for 12 hours prior to surgery with unlimited access to water until the morning of the procedure.

2.3.3 Treatment Groups

Each cat was randomly assigned to one of three treatment groups (n = eight/group). Randomization was performed by the online software QuickCalcs (GraphPad Software, CA, USA). Cats were considered healthy based on history and physical examination. Cats in the treatment groups received one of the following treatments intramuscularly: 0.3 mg/kg methadone (Phebra Pty Ltd., NSW, Australia), 0.03 mg/kg buprenorphine (Temgesic, Reckitt Benckiser, Berkshire, England) or 0.3 mg/kg morphine (Mayne Pharma Private Ltd., VIC, Australia). The analgesic treatments were

combined with 0.05mg/kg acepromazine (Acezine-2, 2%; Ethical Agents, New Zealand) and administered IM via the lumbar epaxial muscles using a 1ml syringe, 30-40 minutes prior to the induction of anaesthesia.

2.3.4 Anaesthesia

A 22-gauge catheter (Optiva IV Catheter Radiopaque; Smiths Medical International Ltd, Luton, UK) was then percutaneously placed in a cephalic vein. The catheter was connected to a PRN-adapter (Interlink; Baxter, Deerfield, IL, USA) and taped to the limb with surgical tape (Durapore; 3M, St. Paul, MN, USA). Anaesthesia was induced using 4 mg/kg propofol (Repose; Norbrook NZ Ltd, New Zealand) given IV to effect. Following endotracheal intubation using a cuffed endotracheal tube, anaesthesia was maintained with halothane (Halothane-Vet; Merial NZ Limited, New Zealand) delivered in oxygen via a Mapleson F non-rebreathing circuit, with a fresh gas flow rate of 1.5 L/min. Halothane delivery was adjusted to maintain an end tidal halothane concentration required to keep the cats suitably anaesthetised for surgical castration. Systolic arterial blood pressure was recorded every 5 minutes using a doppler ultrasound pressure transducer (Parks Medical Electronics Inc., OR, USA) positioned over the palmar aspect of the metacarpus, with an occluding cuff positioned dorsal to the carpus and operated via a sphygmomanometer. The width of the cuff was approximately 30-40% of the measured circumference. Respiratory rate, pulse rate (measured using a pulse oximeter probe placed over the tongue), end-tidal partial pressure of carbon dioxide ($PE'CO_2$), and end-tidal halothane (FE´Hal) were recorded every 5 minutes and continuously measured using a monitor (Hewlett Packard

M1025B; Hewlett Packard, Germany). Anaesthetic induction, maintenance of anaesthesia, and anaesthetic monitoring was carried out by a single person, who was not aware of the animals' group allocations of administered analgesics.

2.3.5 Surgery

Surgery was performed via two separate skin incisions, one over each caudal pole of the scrotal sac. Removal of each testicle was completed using a figure-of-eight knot instrument tie in a closed castration technique with the cat in dorsal recumbency. The same surgeon performed the removal of the first testicle (T_1) in each cat for study purposes, while the second testicle (T_2) castration was completed by a final year veterinary student under the supervision of the veterinary surgeon.

2.3.6 EEG recording

The EEG was recorded using three subcutaneous, 27-gauge stainless-steel needle electrodes (Viasys Healthcare, Surrey, UK). The non-inverting electrode was placed over the zygomatic process of the frontal bone, the inverting electrode over the mastoid process of the temporal bone, and the ground electrode caudal to the occipital process. (Kongara *et al.* 2010, 2012, 2013a). Once the animal was stabilized under anaesthesia, the EEG was recorded continuously with a sample rate of 1 kHz and a band pass of 0.1–100 Hz, using an amplifier (Iso-DAM8A; World Precision Instruments, Sarasota, FL, USA) and analogue to digital converter (Powerlab 4/20 data acquisition system; AD Instruments Ltd, Sydney, Australia). A baseline consisting of a

period of 2 minutes was recorded prior to any manipulations, including skin incision. Further EEG data were divided into sections, starting with T_1, which commenced with the skin incision over the first testicle, the exteriorisation of the testicle, clamping, the instrument tie, surgical removal of the testicle and tightening of the auto ligation knot. After a period of two minutes without any further stimulation, section T_2 was commenced and included removal of the second testicle using the same procedure as above. Data for 45 seconds following the beginning of each of the events were sampled to include all surgical manipulations (for the removal of each testicle), averaged over each time period and presented as baseline (prior to any surgical manipulations), testicle 1 (T_1), and testicle 2 (T_2) for statistical comparison.

Raw EEG data were analysed offline after the completion of each experiment. Median frequency (F_{50}), spectral edge frequency (F_{95}) and total EEG power (P_{tot}) were calculated for consecutive non-overlapping 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson-Massey University, Palmerston North, New Zealand).

2.3.7 Postoperative pain

After surgery, cats were moved to a recovery cage and given 0.2 mg/kg meloxicam (Metacam 0.5mg/ml; Boehringer-Ingelheim NZ Ltd., Auckland, NZ) SC. The endotracheal tube was removed after the restoration of laryngeal reflexes.

2.3.8 Statistical Analysis

Summary statistics (mean and standard deviation) were calculated for each group to assess the central tendency and variability of the data. The ANOVA test was performed to determine whether there were any significant differences in the means of the groups. EEG variables (F_{50}, F_{95}, and P_{tot}), recorded during a 20 second period prior to surgery (baseline) and during the removal of each testicle (T_1 and T_2), were compared between the treatment groups as well as between surgical time-points within groups, using generalized linear mixed model analysis (Littell *et al.* 1998) in SAS 9.4 (SAS Institute Inc. Cary, NC, USA). The linear mixed model included the fixed effects of treatments, time and their interaction, and random effects of animals. Baseline values were treated as a covariate because there were significant between-group differences in the baseline values for the three EEG variables. The covariance error structure for repeated measures over time points, within animals within group was determined using Akaike's information criterion. A first-order autoregressive model was found to be the most appropriate error structure. The distribution of the EEG data were tested for normality (Shapiro-Wilk, Kolmogorov-Smirnov, Anderson-Darling and Crame´r-von Mises tests). Since the residuals of data were not normally distributed, log-transformed variables were used in the analysis.

2.4 RESULTS

There were no significant differences between groups for age, bodyweight, and duration of surgery. Median (range) age was 5.5 (min 3.0, max 24) months, bodyweight was 2.9 (min 1.9, max 4.2) kg, and duration of the surgical procedure was 15 minutes (min 10, max 25) minutes. Heart rate (beats/minute), respiratory rate (breaths/minute)

and blood pressure (mmHg) were recorded for clinical purposes and remained within normal values throughout the study. Overall means for PE´CO_2 (mmHg) were 34.4 ± 6.6, 33.6 ± 2.7, and 31.7 ± 2.2 in the methadone, buprenorphine, and morphine groups, respectively, with no significant difference between the groups. Overall means for FE´Hal were 1.22 ± 0.25, 1.36 ± 0.24 and 1.38 ±0.24 in the methadone, buprenorphine, and morphine groups, respectively, with no significant difference between the groups. All anaesthesia recoveries were uneventful; no intraoperative or postoperative adverse effects were observed, and no intraoperative interventions were necessary.

Values for Least Square Means - LSM (SEM) for F_{50}, F_{95}, and P_{tot} of the encephalogram during surgery are presented with baseline in each group (Tables 2.1-2.3). The median frequency (F_{50}) was not significantly increased from baseline for all three groups during T_1 and T_2. However, in the methadone group, F_{50} values were significantly decreased from baseline during T_1 ($p < 0.001$) and T_2 ($p = 0.009$). In the buprenorphine group, F_{50} values were also significantly ($p = 0.045$) lower compared to baseline during T_2. During T_1, F_{50} values in the methadone group were significantly ($p = 0.004$) lower when compared to those in the morphine and buprenorphine groups (Table 2.1).

Table 2.1. Least square means (SEM) for the median frequency (F_{50}) of the electroencephalogram prior and during surgery in anaesthetised cats after treatment with IM morphine 0.3 mg/kg (Morphine), IM methadone 0.3 mg/kg (Methadone) and IM buprenorphine 0.03 mg/kg (Buprenorphine). (n = 8 per group).

Group	Baseline (Hz)	Testis 1 (Hz)	Testis 2 (Hz)
Morphine	9.56 (SEM 0.32)	9.61 (SEM 0.32)	9.44 (SEM 0.32)
Methadone	9.60 (SEM 0.22)	8.45 (SEM 0.22)*,†	9.03 (SEM 0.22)*
Buprenorphine	9.56 (SEM 0.25)	9.23 (SEM 0.25)	8.95 (SEM 0.25)*

*Statistically significant ($p < 0.05$) from baseline within group

†Statistically significant ($p < 0.05$) versus other groups within given time-point

The spectral edge frequency (F_{95}) was not significantly increased from baseline for all three groups during T_1 and T_2. However, in the methadone group, F_{95} was significantly decreased from baseline during T_2 (Table 2.2).

Table 2.2. Least square means (SEM) for the spectral edge frequency (F_{95}) of the electroencephalogram prior and during surgery in anaesthetised cats after treatment with IM morphine 0.3 mg/kg (Morphine), IM methadone 0.3 mg/kg (Methadone) and IM buprenorphine 0.03 mg/kg (Buprenorphine). (n = 8 per group).

Group	Baseline (Hz)	Testis 1 (Hz)	Testis 2 (Hz)
Morphine	25.64 (SEM 0.20)	25.67 (SEM 0.20)	25.45 (SEM 0.20)
Methadone	25.65 (SEM 0.16)	25.33 (SEM 0.16)	25.13 (SEM 0.16)*
Buprenorphine	25.67 (SEM 0.22)	25.52 (SEM 0.22)	25.31 (SEM 0.22)

*Statistically significant ($p < 0.05$) from baseline within group

The total power (P_{tot}) was not significantly decreased from baseline for all three groups during T_1 and T_2. During T_2 values for the methadone group were significantly ($p = 0.043$) higher when compared to those in the buprenorphine group (Table 2.3).

Table 2.3. Least square means (SEM) for the total power (P_{tot}) of the electroencephalogram prior and during surgery in anaesthetised cats after treatment with IM morphine 0.3 mg/kg (Morphine), IM methadone 0.3 mg/kg (Methadone) and IM buprenorphine 0.03 mg/kg (Buprenorphine). (n = 8 per group).

Group	Baseline (μV^2)	Testis 1 (μV^2)	Testis 2 (μV^2)
Morphine	29.02 (SEM 1.08)	30.31 (SEM 1.08)	31.27 (SEM 1.08)
Methadone	28.89 (SEM 2.54)	31.49 (SEM 2.54)	33.79 (SEM 2.54)*
Buprenorphine	29.27 (SEM 1.28)	28.09 (SEM 1.28)	28.27 (SEM 1.28)

* Statistically significant ($p < 0.05$) from buprenorphine within given time-point.

Overall, no significant increases in F_{50} and F_{95} or decreases in P_{tot} from baseline were found in any of the three opioid groups. In the methadone group, however, there were significant decreases from baseline in both F_{50} and F_{95} and a significant increase in P_{tot} compared to buprenorphine.

2.5 DISCUSSION

This study compared the antinociceptive effects of three opioids used as pre-anaesthetic medication in cats undergoing surgical castration – methadone, morphine, and buprenorphine. The effects were measured with the electroencephalogram using the Minimal Anaesthesia Model. The Minimal Anaesthesia Model allows for the

investigation of antinociceptive effects of various analgesic agents using a protocol that has been validated in numerous species (Murrell *et al.* 2003; Johnson 2005; Johnson *et al.* 2005; Kongara *et al.* 2010, 2012, 2013b; Kells *et al.* 2017). Noxious stimulation results in specific changes in electroencephalographic indices. These include an increase in the median frequency (F_{50}), an increase in the spectral edge frequency (F_{95}), and a decrease in the total power (P_{tot}) of the EEG (Johnson *et al.* 2007). Antinociceptive agents are able to obtund this response. In this study, all three opioids were able to prevent an increase in the median frequency and spectral edge frequency as well as a decrease in the total power of EEG signals. The data in this study, using EEG measurements, are in agreement with numerous other studies assessing IM efficacy of various opioids (Petty and Murrell 2019; Lawrence 2024; Moretti *et al.* 2024).

In this study, buprenorphine administered IM at a clinically accepted therapeutic dose (KuKanich and Papich 2018) was able to attenuate all EEG signals indicative of nociception due to castration. There was no increase observed in the F_{50} or F_{95} nor decrease in the P_{tot}. The only observed change was a significant decrease from baseline in the F_{50} during castration of testicle two. This is in contrast to a previous study, where buprenorphine did not abolish the changes in EEG frequencies in responses to castration when administered subcutaneously (Kongara *et al.* 2013b). This is correlated by other studies, where a standard formulation of buprenorphine administered SC provided significantly less analgesia when compared to the IM or IV routes of administration (Giordano *et al.* 2010; Steagall *et al.* 2013; Askar *et al.* 2020). In one study, SC absorption of buprenorphine was so erratic, that pharmacokinetic modelling could not be performed, with negligible plasma concentrations seen after SC administration

(Steagall *et al.* 2013). In another clinical study, SC administered buprenorphine had a significantly higher incidence of treatment failure (52%) when compared to IM administration (16%) (Giordano *et al.* 2010). Based on the present study, the IM route of buprenorphine administration appears to be more dependable than the SC route of administration.

In one study looking at a clinical setting, drugs were often preferentially administered SC in companion animals as it was perceived to be less painful, require less restraint, and thought to be less stressful compared to the IM route (Gurney *et al.* 2009). Furthermore, clinicians often relate patient behavioural changes after drug administration with evidence that clinical analgesic efficacy has been achieved. However, this may not always be the case, as in the case of buprenorphine. Numerous studies have shown that while buprenorphine does not provide adequate analgesia after SC administration, the behavioural effects are the same as seen during IM administration (Steagall *et al.* 2006, 2013).

Methadone and morphine are both widely used opioids for managing pain in small animals. In this study, both drugs effectively reduced nociceptive responses to castration in cats, but methadone proved to be more effective than morphine. This difference in efficacy may be attributed to the distinct metabolic pathways of these drugs in cats. Unlike in dogs, the primary morphine metabolites in the cat are sulphate conjugates due to a deficiency of glucuronidation in the cat and limited production of the active metabolite of morphine, morphine-6-glucoronide (Yeh *et al.* 1971). As a result, pharmacokinetic studies of morphine in the cat indicate a smaller volume of distribution and slower clearance in comparison to dogs (Taylor *et al.* 2001; KuKanich

et al. 2005; Bruno H. Pypendop *et al.* 2014). Conversely, methadone does not undergo hepatic glucuronidation. This may explain why methadone showed better suppression of EEG signals compared to morphine. Ultimately, the choice between the two full μ opioid receptor agonists should be guided by clinical judgment, the specific needs of the patient, and the context of their treatment.

One limitation of this study involves the fact that two different surgeons removed each testicle on the cats, and each cat had a different surgeon for one of the testicles. This study was carried out in a veterinary teaching hospital, where veterinary students perform procedures as part of their education. The second testicle (T_2) was removed by final year veterinary students as part of their veterinary training, under the supervision of a veterinary surgeon. In comparing analgesics using both testicles as sequential nociceptive events, this study assumed that all cats received the same degree of surgical stimulation and nociceptive inputs during the removal of both testicles. It is possible, however, that less skilled students introduced variability between groups and between T_1 and T_2 in terms of tissue handling and the degree of noxious stimulation produced.

In summary, morphine, methadone, and buprenorphine administered IM were able to completely obtund the changes in EEG indices of nociception in cats during surgical castration. While all three analgesics appears to provide significant antinociception for this surgical procedure, use of these opioids in clinical practice should be guided on the side effects of each drug, patient presentation, as well as prescriber comfort with

each medication. Further studies looking at different surgical procedures in cats and administration of analgesics at different dosages is recommended.

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3 CHAPTER 3 - INVESTIGATION OF THE EFFECTS OF VATINOXAN ON THE ANTINOCICEPTION PRODUCED BY DEXMEDETOMIDINE AND MORPHINE IN CATS UNDERGOING CASTRATION

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3.1 ABSTRACT

Objective To investigate the effect of vatinoxan, a peripherally acting α2-adrenoceptor antagonist, on the electroencephalographic (EEG) indices of nociception produced by dexmedetomidine or dexmedetomidine-morphine in cats undergoing castration.

Study design Randomised blinded clinical study.

Animals 50 healthy mixed-breed male cats.

Methods Cats (n = 10 per group) were randomly assigned to receive either dexmedetomidine (25 μg kg-1), dexmedetomidine and vatinoxan (25 μg kg-1 and 600 μg kg-1, respectively), dexmedetomidine and morphine (12.5 μg kg-1 and 0.2 mg kg-1, respectively), dexmedetomidine and vatinoxan and morphine (12.5 μg kg-1, 300 μg kg-1, and 0.2 mg kg-1, respectively), or morphine (0.4 mg kg-1) IM 30 minutes before induction of anaesthesia. Anaesthesia was induced with intravenous (IV) propofol to effect and maintained with halothane in oxygen. Electroencephalogram electrodes were placed subcutaneously and the EEG continuously recorded. The median frequency (F_{50}), 95% spectral edge frequency (F_{95}) and total power (P_{tot}) were compared between treatment groups and between time-points using mixed model analysis. Statistical significance was accepted with $p < 0.05$.

Results There were no significant differences in the F_{50}, F_{95} and P_{tot} between groups receiving dexmedetomidine. The four groups had significantly higher F_{50}, F_{95}, and lower P_{tot} EEG variables when compared to baseline. No significant differences were

observed in EEG variables between the baseline period and surgical castration in the high-dose morphine group.

Conclusion and clinical relevance High-dose morphine appeared to provide significantly better antinociception compared to dexmedetomidine alone and dexmedetomidine with morphine. While vatinoxan attenuates the unwanted effects of dexmedetomidine, it did not significantly impair its antinociceptive properties in this study.

Keywords dexmedetomidine; electroencephalogram; feline castration; MK-467; morphine; vatinoxan.

3.2 INTRODUCTION

Dexmedetomidine is an α2-adrenoceptor agonist used to produce sedation, analgesia, and to decrease inhalational anaesthetic requirements in cats (Granholm et al., 2006). The analgesic effects of α2-adrenoceptor agonists are mediated by receptors in the superficial laminae of the dorsal spinal cord and in the brain stem (Ossipov et al., 1989). They are used for antinociceptive and opioid-sparing effects in the treatment of postoperative pain (Lemke, 2004), acute inflammatory pain (Malmberg et al., 2001) and chronic neuropathic pain unresponsive to opioids (Weinbroum and Ben-Abraham, 2001). However, dexmedetomidine can cause significant cardiovascular depression due to an initial increase in vascular resistance, leading to baroreflex-

mediated bradycardia and decreased cardiac output (Pypendop et al., 2011), which limits its use in sick and older cats.

Vatinoxan (previously known as L-659,066 and MK-467) is an α_2-adrenoceptor antagonist with minimal ability to cross the blood-brain barrier (Honkavaara et al., 2020). The beneficial effects of dexmedetomidine, including analgesia, sedation, and anaesthetic sparing ability are mainly centrally mediated, while dexmedetomidine's deleterious cardiovascular effects are mostly peripherally mediated. When administered in combination with dexmedetomidine, vatinoxan attenuates bradycardia and hypertension without significantly affecting sedative properties (Honkavaara et al., 2017a, 2017b). Vatinoxan influences the disposition of dexmedetomidine, but dexmedetomidine only minimally influences the disposition of vatinoxan (Pypendop et al., 2016).

Opioids bind to receptors in the nervous system, inhibiting excitatory neurotransmitter release and reducing pain without affecting motor function (Pascoe, 2000). Morphine (sulphate), regarded as the 'gold-standard' μ opioid receptor agonist, (Clarke and Trim, 2013) is used as a benchmark for analgesic efficacy. Although historically thought to produce excitement in cats (Fertziger et al., 1974), when used at appropriate doses and dosing intervals, it is effective in treating acute pain (Robertson and Taylor, 2013).

The cerebral cortex participates in the processing of afferent nociceptive inputs that result in the conscious perception of pain (Schnitzler and Ploner, 2000). Neurophysiological techniques such as the electroencephalogram (EEG) record cortical activity have been widely used in humans and animals as important tools in

measuring the depth of anaesthesia and assessing pain (Ekström et al., 1993; Kulka et al., 2012). The EEG is a record of the summated, spontaneous electrical activity of cortical neurons displayed as waveforms of various frequencies over time (Gugino et al. 2004). Raw EEG data is transformed from a time based to a frequency-based domain (Johnson et al., 2007). Variables derived from this transformation include the median frequency (F_{50}), the 95% spectral edge frequency (F_{95}), and total EEG power (P_{tot}). Increases from baseline in F_{50} and F_{95} have been linked to nociception and decreases in P_{tot} have been related to a decrease in depth of anaesthesia during a nociceptive stimulus (Murrell et al., 2003). Studies link EEG changes to noxious stimuli and their subsequent attenuation with pre-emptively administered analgesics (Murrell et al., 2003; Kongara et al., 2013, 2014).

The objective of this study was to characterise the effect that vatinoxan has on the antinociceptive effects of dexmedetomidine separately or in combination with morphine in cats using the EEG. We hypothesise that vatinoxan will have no significant effect on the antinociceptive properties of dexmedetomidine or morphine.

3.3 MATERIALS AND METHODS

This study was conducted and reported in accordance with the ARRIVE 2.0 guidelines for animal research (Percie du Sert et al., 2020).

3.3.1 Cohort selection

The study was approved by the Massey University Animal Ethics Committee, Palmerston North. Sample size calculation was undertaken using previous EEG studies in cats (Kongara et al., 2013, 2014). At a power of 80%, a sample size of ten cats per group (total 50 cats over the five groups) was deemed adequate considering the standard deviation of 0.2 for the F_{50} variable (Kongara et al., 2013) and an assumed F_{50} mean difference of 0.21 between groups. A total of 50 male cats were studied. The cats were client-owned animals recruited through the Palmerston North SPCA and presented for routine castration. Cats were admitted to the study on the day of surgery after obtaining written owner consent. Each cat was randomly assigned into one of five treatment groups of ten animals in each group. Randomization was performed by the online software QuickCalcs (GraphPad Software, CA, USA). Cats were considered healthy based on history and physical examination. Food was withheld for 12 hours prior to surgery with unlimited access to water until the morning of the procedure.

3.3.2 Analgesia and Anaesthesia

Cats in the treatment group M received morphine 0.4 mg kg^{-1} (Mayne Pharma Private Ltd., VIC, Australia) intramuscularly (IM). Cats in the treatment group D received dexmedetomidine 25 μg kg^{-1} (Dexdomitor, Zoetis, Auckland, New Zealand) IM. Cats in the treatment group DV received 25 μg kg^{-1} dexmedetomidine + 600μg kg^{-1} vatinoxan (Recipharm, Sweden) IM. Cats in the treatment group DM received 12.5 μg kg^{-1} dexmedetomidine + 0.2 mg kg^{-1} morphine IM and cats in the treatment group DMV received 12.5 μg kg^{-1} dexmedetomidine + 0.2 mg kg^{-1} morphine + 300 μg kg^{-1} vatinoxan IM. The vatinoxan solution was prepared immediately before use in a sterile

vial containing 25mg vatinoxan powder by adding 5mL sterile isotonic saline to produce a final vatinoxan concentration of 5 mg mL^{-1}.

Cats received the treatment into the lumbar epaxial muscles 30 minutes prior to the induction of anaesthesia. After 20 minutes, the degree of sedation was assessed for each cat using a simple descriptive scale (SDS) of: 0 (no sedation), 1 (can stand but is wobbly), 2 (in sternal recumbency), 3 (can lift its head) and 4 (fast asleep/no response to hand clap) (Table 3.1). The sedation scores were based on a subjective evaluation of the cat's behaviour or attitude and its response to a hand clap. A 22-gauge catheter (Optiva IV Catheter Radiopaque; Smiths Medical International Ltd, Luton, UK) was then percutaneously placed in a cephalic vein. The catheter was connected to a PRN-adapter (Interlink; Baxter, Deerfield, IL, USA) and taped to the limb with surgical tape (Durapore; 3M, St. Paul, MN, USA).

Table 3.1. Simple Descriptive scale of scores used to assess a cat's level of sedation.

Score	Sedation
0	No sedation
1	Can stand but is wobbly
2	In sternal recumbency
3	Can lift its head
4	Fast asleep/no response to hand clap

Anaesthesia was induced using propofol (Repose; Norbrook NZ Ltd, New Zealand), given to effect. Following endotracheal intubation using a cuffed endotracheal tube, anaesthesia was maintained with halothane (Halothane-Vet; Merial NZ Limited, New Zealand) delivered in oxygen via a Mapleson F non-rebreathing circuit, with a fresh gas flow of 1.5 L minute^{-1}. Halothane concentration was adjusted to the lowest halothane end-tidal concentration required to achieve a suitable plane of surgical anaesthesia, which was between 0.9 and 1.1%; this is in line with previous studies using the Minimal Anaesthesia Model which keeps animals at a suitable surgical anaesthesia while not affecting EEG signal response to nociception. All study subjects breathed spontaneously without need for mechanical ventilation; intermittent positive pressure ventilation would have been provided as necessary to maintain an end-tidal partial pressure of carbon dioxide ($PE´CO_2$) at 35-40 mmHg (4.7-5.3 kPa). Systolic arterial blood pressure was recorded every 5 minutes using a Doppler ultrasound pressure transducer (Parks Medical Electronics Inc., OR, USA) positioned over the palmar aspect of the metacarpus, with an occluding cuff positioned dorsal to the carpus and operated via a sphygmomanometer. The width of the cuff was approximately 30-40% of the measured circumference. Respiratory rate, pulse rate (measured using a pulse oximeter probe placed over the tongue), end-tidal partial pressure of carbon dioxide ($PE´CO_2$), and end-tidal halothane concentration (FE´Hal) (measured using sampling tubing connected to an outlet in the endotracheal tube adapter) were connected to and continuously measured using a monitor calibrated daily (Hewlett Packard M1025B; Hewlett Packard, Germany) and recorded every 5 minutes. Anaesthetic induction, maintenance of anaesthesia, and anaesthetic monitoring was carried out by a single

person, who was unaware of the animals' group allocations and administered medication.

After surgery, cats were moved to a recovery cage and given 0.2 mg kg^{-1} meloxicam (Metacam 0.5mg mL^{-1}; Boehringer-Ingelheim NZ Ltd., Auckland, NZ) subcutaneously. Post-operative monitoring was maintained in all cats until protective reflexes returned, and they were able to maintain sternal recumbency.

3.3.3 Surgery

Surgery was performed via two separate skin incisions, one over each caudal pole of the scrotal sac. Removal of each testicle was completed using a figure-of-eight knot instrument tie in a closed castration technique with the cat in dorsal recumbency. The same surgeon performed the removal of the first testicle (T_1) in each cat for study purposes, while the second testicle castration was completed by a final year veterinary student under the supervision of the veterinary surgeon for clinical completion of the surgery.

3.3.4 EEG recording

The EEG was recorded using three subcutaneous, 27-gauge stainless-steel needle electrodes (Viasys Healthcare, Surrey, UK). The non-inverting electrode was placed over the zygomatic process of the frontal bone, the inverting electrode over the

mastoid process of the temporal bone, and the ground electrode caudal to the occipital process (Kongara et al., 2013). Once the cat was stabilized under anaesthesia, the EEG was recorded continuously with a sample rate of 1 kHz and a band pass of 0.1–100 Hz, using an amplifier and analogue to digital converter (Powerlab 4/20 data acquisition system; AD Instruments Ltd, Sydney, Australia). A baseline consisting of a period of 2 minutes was recorded prior to any manipulations, including skin incision. EEG data were also recorded during removal of testicle 1 (T_1), which included the skin incision over the first testicle, the exteriorisation of the testicle, clamping, the instrument tie, surgical removal of the testicle and tightening of the auto ligation knot. Data from the baseline as well as from the beginning of the skin incision to 45 seconds following the surgical removal of the testicle were sampled, averaged over each time-period and presented as baseline and testicle 1 (T_1) for statistical comparison. No data from the period of the removal of the second testicle were analysed for inclusion in the study.

Raw EEG data were analysed after the completion of each experiment. The median frequency (F_{50}), spectral edge frequency (F_{95}) and total EEG power (P_{tot}) were calculated for consecutive non-overlapping 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, New Zealand).

3.3.5 Statistical analysis

EEG variables recorded during the baseline period (before surgery began) and during the removal of testicle 1 (T_1), were compared between the treatment groups as well as between surgical time-points within a group, using generalized linear mixed model analysis in SAS 9.4 (SAS Institute Inc. Cary, NC, USA). The linear mixed model included the fixed effects of treatments, time and their interaction, and random effects of animals. Baseline values were treated as a covariate because there were significant between group differences in the baseline values for the three EEG variables. The covariance error structure for repeated measures over time points, within animals within group was determined using Akaike's information criterion. A first-order autoregressive model was found to be the most appropriate error structure. The distribution of the EEG data was tested for normality (Shapiro-Wilk, Kolmogorov-Smirnov, Anderson-Darling and Crame´r-von Mises tests).

Differences in body weight, age and surgery time among groups, were tested by the Kruskal-Wallis test in SAS 9.4.

Heart rate, respiratory rate, PE´CO_2, and FE´Hal of the dogs recorded at 5-minute intervals during surgery were analysed as repeated measures employing linear mixed model analysis. The model includes the fixed effects of treatment, time and their interaction, and random effects of the animal. Results were considered significant if $p < 0.05$.

3.4 RESULTS

There were no significant differences in age, bodyweight, or duration of surgery between groups. Median (range) age was 10 (4.5-60) months, bodyweight was 3.9 (2.0-5.4) kg, and duration of the surgical procedure (from initial cut on T_1 to complete removal of T_2) was 15 (5-30) minutes. Heart rate, respiratory rate, and systolic blood pressure were recorded for clinical purposes and remained within normal limits (100 – 180 beats minute^{-1}, 10 – 20 breathes minute^{-1}, and 95 – 130 mmHg, respectively) throughout the study. Summated means for PE´CO_2 were 39.2 ± 4.3, 35.1 ± 4.2, 32.5 ± 9.4, 35.8 ± 5.3 and 34.4 ± 3.7 mmHg (5.2 ± 0.6, 4.7 ± 0.6, 4.3 ± 1.3, 4.8 ± 0.7 and 4.6 ± 0.5 kPa) in the D, DV, DM, DMV, and M groups, respectively, with a significant difference (p = 0.017) between the D and M groups. Summated means for FE´Hal were 0.78 ± 0.26, 0.85 ± 0.20, 0.75 ± 0.20, 0.71 ± 0.13 and 0.95 ± 0.21 in the D, DV, DM, DMV, and M groups, respectively, with significant difference (p = 0.009) between the DMV and M groups. Summated means for sedation scores were 3.7 ± 0.3, 4 ± 0.5, 3.1 ± 1.4, 4 ± 0, and 0.6 ± 0.9 in the D, DV, DM, DMV, and M groups, respectively, with a significant difference between all groups containing dexmedetomidine (D, DV, DM, DMV) and morphine ($p = 1 \times 10^{-6}$, $p = 7 \times 10^{-6}$, p = 0.003, and p = 0.001, respectively). All anaesthesia recoveries were uneventful; no postoperative adverse effects were observed.

Values for Least Square Means (LSM) ± SE for F_{50}, F_{95}, and P_{tot} of the encephalogram during surgery are presented with baseline as well as a proportion of the baseline value in each group. The median frequency (F_{50}) was not significantly different from baseline for all four dexmedetomidine groups during T_1; however, high dose morphine alone showed a significant decrease from baseline during T_1 (Table 3.2). When compared as

a proportion of baseline (Table 3.3), there were no significant differences between dexmedetomidine alone (D), or in combination with vatinoxan (DV), morphine (DM), or both vatinoxan and morphine (DMV) during T_1. High dose morphine (M), however, had a significantly lower median frequency compared to the remaining four groups during T_1.

Table 3.2. Least square means ± standard error for the median frequency (F_{50}) of the electroencephalogram prior to and during surgery in anaesthetised cats after administration of intramuscular (IM) dexmedetomidine 25 μg kg^{-1} (D), IM dexmedetomidine 25 μg kg^{-1} and vatinoxan 600μg kg^{-1} (DV), IM dexmedetomidine 12.5 μg kg^{-1} and morphine 0.2mg kg^{-1} (DM), IM dexmedetomidine 12.5 μg kg^{-1}, morphine 0.2 mg kg^{-1} and vatinoxan 300μg kg^{-1} (DMV), or IM morphine 0.4 mg kg^{-1} (M) (n = 10 per group).

	D	DV	DM	DMV	M
Baseline (μV^2)	6.18 ± 0.33	6.42 ± 0.22	5.61 ± 0.43	6.62 ± 0.54	6.43 ± 0.29
Testis 1 (μV^2)	6.45 ± 0.33	6.42 ± 0.22	6.31 ± 0.43	7.17 ± 0.54	6.00 ± 0.29*

*Statistically significant ($p < 0.05$) difference from baseline within group

Table 3.3. Least square means ± standard error for the median frequency (F_{50}) of the electroencephalogram (as a proportion of baseline) prior to and during surgery in anaesthetised cats after administration of intramuscular (IM) dexmedetomidine 25 μg kg^{-1} (D), IM dexmedetomidine 25 μg kg^{-1} and vatinoxan 600μg kg^{-1} (DV), IM dexmedetomidine 12.5 μg kg^{-1} and morphine 0.2mg kg^{-1} (DM), IM dexmedetomidine

12.5 μg kg^{-1}, morphine 0.2 mg kg^{-1} and vatinoxan 300μg kg^{-1} (DMV), or IM morphine 0.4 mg kg^{-1} (M), transformed for normality (n = 10 per group).

	D	DV	DM	DMV	M
Testis 1 (μV^2)	104.32±3.21	100.75±3.50	113.47±8.11	108.75±8.56	93.00±2.65*

*Statistically significant ($p < 0.05$) difference of group M from D, DM, and DMV

The spectral edge frequency (F_{95}) increased significantly in all groups containing dexmedetomidine with the exception of dexmedetomidine in combination with vatinoxan (DV) in T_1. High dose morphine (M) was not significantly different to baseline during T_1 (Table 3.4). There was no significant difference between the dexmedetomidine alone (D), with vatinoxan (DV), or in combination with vatinoxan and morphine (DMV) groups during T_1, while dexmedetomidine and morphine alone (DM) was significantly increased as a proportion of baseline compared to dexmedetomidine and vatinoxan (DV) during T_1. High dose morphine (M) was significantly lower as a proportion of baseline than all 4 other groups in the study at the spectral edge frequency (Table 3.5).

Table 3.4. Least square means ± standard error for the spectral edge frequency (F_{95}) of the electroencephalogram prior to and during surgery in anaesthetised cats after administration of intramuscular (IM) dexmedetomidine 25 μg kg^{-1} (D), IM dexmedetomidine 25 μg kg^{-1} and vatinoxan 600μg kg^{-1} (DV), IM dexmedetomidine 12.5 μg kg^{-1} and morphine 0.2mg kg^{-1} (DM), IM dexmedetomidine 12.5 μg kg^{-1}, morphine 0.2 mg kg^{-1} and vatinoxan 300μg kg^{-1} (DMV), or IM morphine 0.4 mg kg^{-1} (M) (n = 10 per group).

	D	DV	DM	DMV	M
Baseline (μV^2)	24.31 ± 0.19	24.53 ± 0.26	23.99 ± 0.33	24.78 ± 0.28	23.89 ± 0.37
Testis 1 (μV^2)	24.66 ± 0.19*	24.71 ± 0.26	24.92 ± 0.33*	25.29 ± 0.28*	23.74 ± 0.37

*Statistically significant ($p < 0.05$) difference from baseline within group

Table 3.5. Least square means ± standard error for the spectral edge frequency (F_{95}) of the electroencephalogram (as a proportion of baseline) prior to and during surgery in anaesthetised cats after administration of intramuscular (IM) dexmedetomidine 25 μg kg^{-1} (D), IM dexmedetomidine 25 μg kg^{-1} and vatinoxan 600μg kg^{-1} (DV), IM dexmedetomidine 12.5 μg kg^{-1} and morphine 0.2mg kg^{-1} (DM), IM dexmedetomidine 12.5 μg kg^{-1}, morphine 0.2 mg kg^{-1} and vatinoxan 300μg kg^{-1} (DMV), or IM morphine 0.4 mg kg^{-1} (M), transformed for normality ($n = 10$ per group).

	D	DV	DM	DMV	M
Testis 1 (μV^2)	101.44 ± 0.53	100.71 ± 0.66†	103.89 ± 1.40	102.11 ± 1.00	99.31 ± 0.74*†

*Statistically significant ($p < 0.05$) difference from D and DMV

†Statistically significant ($p < 0.05$) difference from DM

The total power (P_{tot}) decreased significantly in the dexmedetomidine and morphine with (DMV) and without (DM) vatinoxan during T_1 (Table 3.6). In between group comparison, there was no significant difference in P_{tot} in any of the five groups during T_1 (Table 3.7).

Table 3.6. Least square means ± standard error for the total power (P_{tot}) of the electroencephalogram prior to and during surgery in anaesthetised cats after administration of intramuscular (IM) dexmedetomidine 25 μg kg^{-1} (D), IM dexmedetomidine 25 μg kg^{-1} and vatinoxan 600μg kg^{-1} (DV), IM dexmedetomidine 12.5 μg kg^{-1} and morphine 0.2mg kg^{-1} (DM), IM dexmedetomidine 12.5 μg kg^{-1}, morphine 0.2 mg kg^{-1} and vatinoxan 300μg kg^{-1} (DMV), or IM morphine 0.4 mg kg^{-1} (M) (n = 10 per group).

	D	DV	DM	DMV	M
Baseline (μV^2)	27.82 ± 2.08	22.59 ± 2.21	29.62 ± 4.02	30.45 ± 4.39	33.98 ± 3.48
Testis 1 (μV^2)	26.91 ± 2.08	21.14 ± 2.21	27.13 ± 4.02*	28.22 ± 4.39*	32.64 ± 3.48

*Statistically significant ($p < 0.05$) difference from baseline within group

Table 3.7. Least square means ± standard error for the total power (P_{tot}) of the electroencephalogram (as a proportion of baseline) prior to and during surgery in anaesthetised cats after administration of intramuscular (IM) dexmedetomidine 25 μg kg^{-1} (D), IM dexmedetomidine 25 μg kg^{-1} and vatinoxan 600μg kg^{-1} (DV), IM dexmedetomidine 12.5 μg kg^{-1} and morphine 0.2mg kg^{-1} (DM), IM dexmedetomidine

12.5 μg kg^{-1}, morphine 0.2 mg kg^{-1} and vatinoxan 300μg kg^{-1} (DMV), or IM morphine 0.4 mg kg^{-1} (M), transformed for normality (n = 10 per group).

	D	DV	DM	DMV	M
Testis 1 (μV^2)	97.79 ± 3.57	95.74 ± 4.02	90.4 ± 4.29	93.53 ± 3.31	97.09 ± 4.86

No statistically significant differences found between groups.

3.5 DISCUSSION

This study compared EEG-derived indices of antinociceptive effects of dexmedetomidine and morphine with and without the addition of vatinoxan. The study findings suggest that the addition of vatinoxan (600ug kg^{-1} or 300ug kg^{-1}) to dexmedetomidine (25ug kg^{-1} or 12.5ug kg^{-1}, respectively), with and without low dose morphine (0.2mg kg^{-1}) did not significantly affect the antinociceptive properties of dexmedetomidine during castration in halothane-anesthetized cats. There was also no significant difference in the antinociceptive properties of dexmedetomidine alone compared to dexmedetomidine with low dose morphine.

All treatments except morphine given alone induced deep clinical sedation in all cats when assessed on a SDS of 0 to 4 (Steagall et al., 2009). This sedative effect was mostly mediated by dexmedetomidine, which is commonly used in the clinical setting as a sedative. α_2-agonists provide sedation through noradrenaline outflow reduction within the central nervous system, which also decreases central sympathetic tone (Sinclair, 2003). Vatinoxan, when added to dexmedetomidine, with or without morphine, did

not appear to have any effect on the depth of sedation experienced by cats prior to anaesthetic induction. This is consistent with other studies which also found that the time to onset of sedation is increased (Restitutti et al., 2017) and the duration of sedation is decreased (Honkavaara et al., 2017b) when vatinoxan is combined with dexmedetomidine.

In this study, morphine (0.4mg kg^{-1}) alone was added as a positive control as a way to contextualise the antinociceptive effect of all the other groups' drug combinations. High dose morphine was the only treatment that was associated with no significant difference between baseline and T_1 and so provided superior antinociception than a combination of morphine and dexmedetomidine or dexmedetomidine alone. Combining an opioid with α_2-agonists has been shown to have numerous beneficial effects when compared to administering each drug alone. In pharmacokinetic studies of morphine administered with dexmedetomidine, it was found that the elimination half-life (T1/2) and clearance rate of morphine was significantly lower when combined with dexmedetomidine, which may lead to an increased dosing interval of morphine (Karna et al., 2020). Dexmedetomidine also exhibited enhanced antinociception when administered in combination with morphine or methadone intramuscularly to dogs when evaluated using the pedal withdrawal reflex (Cardoso et al., 2014). One reason this finding was not observed in the current study could be that, in the combination doses, dexmedetomidine and morphine were administered at half the dose (12.5 μg kg^{-1} and 0.2 mg kg^{-1}, respectively), compared to the higher doses used in the dexmedetomidine (25 μg kg^{-1}) and morphine (0.4 mg kg^{-1}) alone groups, which contrasts with the studies in dogs mentioned earlier.

The addition of vatinoxan to dexmedetomidine, morphine, and their combination did not significantly affect the antinociceptive properties of either drug during surgical castration of cats in this study. The analgesic effects of dexmedetomidine administered IM were previously found to be dose-dependent (Slingsby et al., 2010). Previous studies of antinociceptive effects of dexmedetomidine or medetomidine combined with vatinoxan have been carried out in dogs (Bennett et al., 2016; Huuskonen et al., 2020). In one study, administration of vatinoxan with medetomidine did not substantially decrease visceral antinociceptive effects of medetomidine, with non-inferiority also detected at certain time points in somatic antinociception (Huuskonen et al., 2020). In another study, however, the combination of dexmedetomidine and vatinoxan led to a significant reduction in somatic antinociception of dexmedetomidine (measured by pedal withdrawal due to squeezing the nail bed with haemostats) (Bennett et al., 2016). In that study, only 10μg kg^{-1} of dexmedetomidine was administered with 250μg kg^{-1} vatinoxan IV; this is notably less dexmedetomidine than used in this study in cats. Dose rates and resulting plasma concentrations of dexmedetomidine associated with analgesia are reported to be three times higher than those associated with sedation (van Oostrom et al., 2011). When equal plasma concentrations are reached, sedative effects are also longer lasting in the dog compared to analgesic effects at the same dose (Kuusela et al., 2000). These observations may explain why dexmedetomidine given at the dose in this study supported antinociceptive effects in cats that were not abolished by vatinoxan. Further study is warranted to quantify the required concentrations and doses for sedation and analgesia, and how the amount of drug given affects the sedative and analgesic onset and length in cats.

The primary antinociceptive effects of α_2-agonists are believed to be mediated at the level of the spinal cord by activation of α_2-adrenoceptors (Sabbe et al., 1994) and any decrease in dexmedetomidine may affect its ability to provide analgesia. Previously, observations of reduced antinociception in dogs suggest that decreased sedative and antinociceptive effects of dexmedetomidine are more likely a result of lower concentrations of dexmedetomidine due to vatinoxan's effect on dexmedetomidine's disposition rather than vatinoxan's central effects due to its ability to permeate the blood-brain barrier to any significant extent (Honkavaara et al., 2012; Bennett et al., 2016). This is supported by studies in rats and dogs, where injection of vatinoxan resulted in minimal brain concentration of the drug (Clineschmidt et al., 1988; Honkavaara et al., 2020). Minimal spinal cord penetration, however, cannot be fully ruled out and may have caused the lack of antinociception seen in other studies. In studies in cats as well as in dogs, vatinoxan increased the minimum alveolar concentration (MAC) of inhalational anaesthetics and inhibited dexmedetomidine's MAC reducing effect of isoflurane (Hector et al., 2017; Pypendop et al., 2019). This is interesting, as MAC is primarily mediated at the level of the spinal cord (Eger et al., 2002). One possible explanation is in the difference between the penetration of vatinoxan into the brain compared to the penetration into the spinal cord. Atipamezole, for example, has the ability to reverse central as well as peripheral α_2-agonist effects and has not shown any effect on the MAC of isoflurane in rats and dogs. The same mechanism of vatinoxan influencing the MAC of inhalational anaesthetics may have a minimal effect on the antinociception of α_2-agonists through

the same mechanism. Further studies are warranted to explore this possible central effect and the extent to which it influences the antinociceptive effects of α_2-agonists.

The main limitation of this study was the lack of a group with no analgesic premedication to act as a negative control. For the purpose of pain studies, a negative control represents the absence of any pain attenuation methods in the face of the applied surgical stimulation; this can include the administration of a placebo or no treatment at all (Charlesworth et al., 2017). One advantage of the Minimal Anaesthesia Model is that it compares EEG indices of nociception during painful procedures to the animal's baseline EEG, recorded prior to the procedure. This baseline EEG accounts for factors such as anaesthetic drugs and other variables, so that the only difference between the baseline and the surgical procedure is the nociceptive input. In this study, however, including a negative control group would more effectively determine the unattenuated nociceptive input induced by surgical castration on the EEG, without the influence of analgesics. This would also allow for a clearer quantification of the antinociceptive effects of dexmedetomidine, both with and without the addition of morphine and/or vatinoxan.

In summary, vatinoxan did not significantly affect the antinociceptive properties of dexmedetomidine at the doses administered in this study. It is important to note that higher dexmedetomidine concentrations are required for analgesia than for sedation, meaning that the doses used in this study were higher than would be typically administered in clinical practice (Robertson et al., 2018). Additional studies looking at the effects of vatinoxan on the temporal nature of dexmedetomidine's antinociceptive properties as well as its effects on lower doses of dexmedetomidine are warranted.

3.6 CONCLUSIONS

This study found no statistically significant difference in EEG-derived indicators of antinociception between dexmedetomidine and dexmedetomidine with morphine during feline castration. The addition of vatinoxan had no significant effect on the antinociceptive properties of dexmedetomidine either with or without morphine. Higher dose morphine alone, was able to ameliorate all EEG changes indicative of nociception and appeared to provide superior antinociception than either drug alone, or in combination.

3.7 ACKNOWLEDGEMENTS

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4 CHAPTER 4 - EFFECTS OF BUPIVACAINE AND MORPHINE ON THE ELECTROENCEPHALOGRAM AND POSTOPERATIVE PAIN IN CASTRATED DOGS

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4.1 ABSTRACT

Objective To compare the effects of locally administered bupivacaine and systemic morphine, separately or in combination, on the electroencephalogram (EEG) and postoperative pain in castrated dogs.

Study design Randomised blinded clinical study.

Animals A group of 21 healthy mixed-breed dogs.

Methods Dogs were randomly assigned to one of three groups (n = 7 per group): morphine (0.5 mg kg^{-1}); bupivacaine (2 mg kg^{-1}); or a combination of both. Premedication consisted of acepromazine (0.05 mg kg^{-1}) (all groups), and morphine (morphine and combination groups only) administered subcutaneously. Anaesthesia was induced with intravenous propofol to effect and maintained with halothane in oxygen. Bupivacaine was infiltrated at the incision site 30 minutes prior to start of surgery in bupivacaine and combination groups. Systolic arterial blood pressure (SAP), heart rate (HR) and haemoglobin oxygen saturation (SpO_2) were recorded. All dogs underwent an open castration via a routine pre-scrotal approach. EEG was recorded throughout anaesthesia. Median frequency (F_{50}), 95% spectral edge frequency (F_{95}) and total power (P_{tot}) were derived from raw EEG traces. Postoperative pain was assessed at 1, 3, 6 and 9-hours using a short form of the Glasgow composite measure pain scale. EEG and pain scores were compared among groups using a generalized linear mixed model and Wilcoxon-Mann-Whitney odds macro, respectively. Data were presented as mean $\pm$ standard deviation ($p < 0.05$).

Results The morphine and bupivacaine groups had a significantly higher F_{50}, F_{95} and a lower P_{tot} than the combination group during castration. Postoperative pain scores did not significantly differ between bupivacaine and combination groups, but both groups had significantly lower pain scores than the morphine group. SAP, HR and SpO_2 did not differ significantly among groups.

Conclusion and clinical relevance Presurgical incisional infiltration of bupivacaine alone or with a systemic opioid reduces intraoperative nociception and postoperative pain in dogs undergoing castration.

Keywords bupivacaine; canine castration; electroencephalogram; local analgesia; morphine

4.2 INTRODUCTION

Effective perioperative analgesia is essential in small animal surgery. Canine castration, a moderately painful procedure, causes measurable intraoperative encephalographic changes and postoperative pain (Kongara et al. 2013). Perioperative analgesia mitigates nociception, which can otherwise result in detrimental effects such as increased protein catabolism, reduced food intake, ventilation/perfusion mismatch, immunosuppression and delayed wound healing (Hansen 2005). Postoperative pain is also a welfare concern in animals.

Opioids, despite well-documented adverse effects, remain widely used for pain control in veterinary practice (Clarke & Trim 2013). Morphine, the prototypical μ-opioid is

commonly administered perioperatively in dogs (Clarke & Trim 2013). Its short duration of action (4-6 hours), necessitates repeated doses, increasing the risk of clinically significant side effects such as urinary retention, ileus, and constipation (Epstein 2015).

Local anaesthetics are widely used in human and veterinary practice for pain management (Carpenter et al. 2004). However, the evidence for their postoperative analgesic efficacy in both human and veterinary studies is inconclusive. In veterinary literature, local anaesthetics significantly lower pain scores in dogs after ovariohysterectomy and coeliotomy (Campagnol et al. 2012; Carpenter et al. 2004). In canine castration, intratesticular blocks with local anaesthetics, have yielded differing effects on nociception and pain (Huuskonen et al. 2013; Stevens et al. 2013).

Neurophysiological techniques, such as electroencephalography, have been extensively used to assess pain and anaesthetic depth/plane in both human and veterinary studies (Ekström et al. 1993; Kulka et al. 2012). The electroencephalogram (EEG) is a record of the summated, spontaneous electrical activity of neurons in the cerebral cortex (Gugino et al. 2004). The cerebral cortex participates in the processing of afferent nociceptive inputs that results in the conscious perception of pain. Several studies have correlated changes in the EEG frequency spectrum with noxious stimulation, and subsequent amelioration of these changes with pre-emptively administered analgesics (Ong et al. 1997; Murrell et al. 2003; Murrell and Johnson 2006; Kongara et al. 2010, 2013, 2014). The raw EEG data are transformed from a time-based domain to a frequency-based domain (Johnson et al. 2007). Variables derived from this

transformation include the median frequency (F_{50}), the 95% spectral edge frequency (F_{95}), and total EEG power (P_{tot}). The median frequency is the frequency below which 50% of the total power of the EEG is located, the spectral edge frequency is the frequency below which 95% of the total power of the EEG is locate, and the total EEG power represents the total area under the amplitude *versus* frequency graph. An increase in F_{50} and F_{95} have been linked to nociception, and a decrease in P_{tot} has been related to a decrease in depth of anaesthesia during a nociceptive stimulus (Murrell et al. 2003).

This study investigated the effects of pre-incisional bupivacaine infiltration and subcutaneous morphine, separately and in combination, on EEG responses and postoperative pain in dogs undergoing castration. We hypothesized that using bupivacaine with systemically administered morphine would reduce pain scores and EEG markers of nociception more effectively than either drug alone.

4.3 MATERIALS AND METHODS

This study was conducted and reported in accordance with the ARRIVE 2.0 guidelines for animal research (Percie du Sert et al., 2020).

4.3.1 Cohort selection

The study was approved by the Massey University Animal Ethics Committee, Palmerston North. Since there were no reports describing the effect of a bupivacaine

block on the EEG during canine castration, the sample size calculation was undertaken using EEG F_{50} data in dogs administered morphine from our previous study (Kongara et al. 2013). At a power of 80%, a sample size of seven dogs per group (total 21 dogs over the three groups) was deemed adequate considering the standard deviation of 0.2 for the F_{50} variable (Kongara et al. 2013) and an assumed F_{50} mean difference of 0.4 between morphine and bupivacaine groups. A total of 21 healthy mixed breed dogs weighing more than five kg and aged less than two years old were used from dogs presenting to the Massey University Veterinary Teaching Hospital for routine castration. Dogs were admitted to the study on the day prior to surgery with consent obtained prior to enrolment into the study. Dogs were completed in groups of seven at a time. Within each group of seven dogs, groups were randomly allocated to one of three groups as they were presented for castration. Randomization was performed by the online software QuickCalcs (GraphPad Software, CA, USA). All dogs were considered healthy based on physical examination. Each dog was scored using the short form Glasgow Composite Measure Pain Scale (CMPS-SF) to assess the presence of pain prior to the surgical procedure. Dogs were fasted for 12 hours prior to anaesthesia with free access to water until anaesthetic induction.

4.3.2 Analgesia and Anaesthesia

Dogs in the morphine group (n = 7) received morphine (0.5 mg kg^{-1}; Morphine Sulphate Inj., 30 mg mL^{-1}, Mayne Pharma Private Ltd., VIC, Australia) subcutaneously (SC). Dogs in the bupivacaine group (n = 7) were administered bupivacaine hydrochloride (2 mg kg^{-1}; Marcaine 0.5% Bupivacaine Injection, AstraZeneca, NSW,

Australia) as an incisional block (IB) - infiltrated in the pre-scrotal area at the site of the incision using a 25-gauge needle in multiple, equidistant blebs 0.5–1.0 cm apart in an ellipse, 15 minutes after anaesthetic induction and 30 minutes before the start of surgery. Dogs in the combination group (n = 7) were administered 0.5 mg kg^{-1} morphine SC and 2 mg kg^{-1} of 0.5% bupivacaine hydrochloride IB. Dogs in all three groups were premedicated with acepromazine (0.05 mg kg^{-1}; Acezine-2, 2%; Ethical Agents, Auckland, New Zealand) SC. Acepromazine was administered concurrently with morphine, when applicable. Group randomisation and drug administration was completed by one researcher who was not involved in monitoring and maintenance of anaesthesia, surgery, or pain scoring.

In all groups, 45 to 60 minutes after pre-anaesthetic medication, anaesthesia was induced with an intravenous (IV) injection of propofol (Repose; Norbrook, Auckland, New Zealand) to effect. Following endotracheal intubation, anaesthesia was maintained with halothane (Halothane-Vet; Merial NZ Limited, New Zealand) in oxygen administered via a rebreathing circuit (VentiFlow, Flexicare, United Kingdom). The precision vaporiser (MSS-2, MSS Ltd, United Kingdom) was adjusted to obtain the minimal amount of end tidal halothane required to maintain the dogs at a suitable plane of surgical anaesthesia. All study subjects breathed spontaneously without need for mechanical ventilation. Systolic arterial blood pressure was recorded every 5 minutes using a doppler ultrasound pressure transducer with cuff (Parks Medical Electronics Inc., OR, USA). Throughout the anaesthetic period, Hartmann's solution (Baxter Healthcare Ltd., NSW, Australia) was administered IV at a rate of 10 mL kg^{-1} $hour^{-1}$. Systolic arterial blood pressure was maintained above 100 mmHg. Respiratory

rate (f_R), heart rate (HR), end-tidal CO_2 ($PE'CO_2$), and end-tidal halothane ($FE'Hal$) were monitored every 5 minutes using an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Germany). Anaesthetic induction, maintenance of anaesthesia, and anaesthetic monitoring were performed by the hospital anaesthetists and final year students on their anaesthesia rotation; they were blinded to the animals' group allocations and administered analgesics.

4.3.3 Surgery

Final year veterinary students under the supervision of a single veterinary surgeon, who was blinded to the animals' group allocation, performed open castrations via a routine pre-scrotal approach with the dog in dorsal recumbency.

4.3.4 EEG Recording

The EEG was recorded using three subcutaneous, 27-gauge stainless-steel needle electrodes (Viasys Healthcare, Surrey, UK). The non-inverting electrode was placed over the zygomatic process of the frontal bone, the inverting electrode over the mastoid process of the temporal bone, and the ground electrode caudal to the occipital process (Kongara et al., 2010, 2012, 2013). Once anaesthesia was stable, the EEG was recorded with a sample rate of 1 kHz and a band pass of 0.1–100 Hz, using an amplifier and analogue to digital converter (Powerlab 4/20 data acquisition system; AD Instruments Ltd, NSW, Australia). EEG data were recorded continuously throughout anaesthesia. The baseline period consisted of data recorded over a period of two

minutes prior to draping and the skin incision. Further EEG data were recorded during exteriorisation of and placement of traction on the spermatic cord, clamping of the spermatic cord, and incision into tissue to remove the testicle after application of the suture. Data for 30 seconds following each of the three events were sampled, averaged over each period and presented as testicle 1 (T_1) and testicle 2 (T_2) for statistical comparison.

The raw EEG was analysed after the completion of each experiment. The median frequency (F_{50}), spectral edge frequency (F_{95}) and total EEG power (P_{tot}) were calculated for consecutive non-overlapping 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, New Zealand).

4.3.5 Postoperative pain assessment

A single observer (blinded to the animal's group allocation and analgesic drugs administered) was responsible for evaluating pain. Each dog was assessed using the CMPS-SF at 1, 3, 6 and 9 hours postoperatively. Rescue analgesia (0.3 mg kg^{-1} morphine intramuscularly-IM) was administered if CMPS-SF scores were ≥ 6/24 or 5/20 (for dogs that were not yet fully recovered from anaesthesia and able to walk). Dogs that received rescue analgesia continued to be scored at subsequent time points to ensure rescue analgesia was effective. This methodology allowed animals to be assigned into specific categories based on their scores at each time point and the resulting categories compared based on the intervention administered to each group.

4.4 STATISTICAL ANALYSIS

4.4.1 EEG data

EEG variables recorded during the baseline period (before surgery began) and during the removal of each testicle (T_1 and T_2), were compared among the groups as well as between surgical time-points within a group, using generalized linear mixed model analysis in SAS 9.4 (SAS Institute Inc. Cary, NC, USA). The linear mixed model included the fixed effects of treatments, time and their interaction, and random effects of animals. Baseline values were treated as a covariate because there were significant differences among groups in the baseline values for the three EEG variables. The covariance error structure for repeated measures over time points, within animals within group was determined using Akaike's information criterion. A first-order autoregressive model was found to be the most appropriate error structure. The distribution of the EEG data was tested for normality (Shapiro-Wilk, Kolmogorov-Smirnov, Anderson-Darling and Crame´r-von Mises tests) and residuals of data were found to be normally distributed.

4.4.2 CMPS-SF scores

Pre-surgical pain scores were all zero and hence were not considered further for statistical analysis. Postsurgical pain scores at each of the recorded times were categorised into three classes (Kongara et al. 2012): class 1 (pain score 0–2; no pain), class 2 (pain score 3–5; mild pain) and class 3 (pain score ≥ 6; moderate pain). The

pooled number of dogs within each pain class at different time points were summarised for each group. To test differences in post-surgical pain scores between-groups as well as between surgical time-points, within a group, the raw pain scores were rank-transformed using 'proc rank' procedure in SAS 9.4 (SAS Institute, NC, USA) and a mixed model analysis of the transformed pain scores was undertaken using 'proc mixed' procedure. The employed mixed model included the fixed effects of treatment (group), time and their interaction, and random effects of dogs. Based on Akaike's information criterion, a compound symmetry error structure was deemed appropriate to model the covariance associated with repeated measures. Pain scores were presented as least square mean plus / minus standard error (LSM ± SE), in original scale.

Differences in body weight, age and surgery time among groups, were tested by the Kruskal-Wallis test in SAS 9.4 (SAS Institute, NC, USA).

HR, f_R, $PE'CO_2$, and FE´Hal of the dogs recorded at 5-minute intervals during surgery were analysed as repeated measures employing linear mixed model analysis. The model included the fixed effects of group, time and their interaction, and random effects of the animal. Results were considered significant if $p < 0.05$.

4.5 RESULTS

There were no significant differences among the groups for age, bodyweight, and duration of surgery. Median (range) age was 7.3 (4–24) months; bodyweight was 30.6 (5.7–41) kg; and duration of surgical procedure was 46.7 (30–55) minutes. There were

also no significant differences ($p > 0.05$) in HR, f_R, systolic arterial pressure (SAP), $P_{E'}CO_2$, and $F_{E'}$Hal. Overall least-square mean ± SE for HR (beats minute^{-1}) in morphine, bupivacaine and combination groups were 82 ± 9, 96 ± 7 and 83 ± 7, while those for f_R were 16 ± 3, 19 ± 3 and 22 ± 3, respectively. Similarly, overall least-square mean ± SE for SAP (mmHg) in morphine, bupivacaine and combination groups were 106 ± 6, 103 ± 3 and 97 ± 4, and $P_{E'}CO_2$ (mmHg) in the respective groups were 43 ± 2, 39 ± 2 and 43 ± 2, respectively. Overall least-square mean ± SE for $F_{E'}$Hal were 0.85 ± 0.1, 1.05 ± 0.1, and 0.90 ± 0.1 in the morphine, bupivacaine, and combination groups, respectively. All anaesthesia recoveries were uneventful; no intraoperative or postoperative adverse effects were observed, and no intraoperative interventions were necessary.

In the combination group, there was no significant difference in the F_{50}, F_{95} and P_{tot} between baseline and T_1 ($p = 0.41$) and T_2 ($p = 0.92$). In the morphine group, F_{50} showed no significant increase at T_1 ($p = 0.052$) but increased at T_2 ($p = 0.029$) compared to baseline (Table 4.1). F_{50} was significantly higher compared to the combination group at both T_1 ($p = 0.027$) and T_2 ($p = 0.013$). In the bupivacaine group, F_{50} increased significantly from its baseline value at T_1 ($p = 0.016$), but did not increase significantly ($p = 0.057$) when compared to combination group during castration of testicle 1. Dogs in the bupivacaine group had a lower P_{tot} mean compared to that of the combination group during T_1 ($p = 0.018$), while dogs in both morphine ($p = 0.043$) and bupivacaine ($p = 0.017$) groups had significantly lower P_{tot} values than those in combination group during T_2 (Table 4.2). Dogs in both the morphine and bupivacaine groups had an increase ($p = 0.051$ and $p = 0.042$, respectively) in the F_{95} compared to

that of the combination group during T_1. No significant differences were found in the F_{95} of dogs in the three treatment groups during T_2 (Table 4.3).

Table 4.1. Least square means ± standard error for the median frequency (F_{50}) of the electroencephalogram prior to and during surgery in anaesthetised dogs after administration of subcutaneous (SC) morphine 0.5 mg kg^{-1} (Morphine), incisional (IB) bupivacaine 2 mg kg^{-1} (Bupivacaine) or a combination of SC morphine 0.5 mg kg^{-1} and IB bupivacaine 2 mg kg^{-1} (Combination). (n = 7 per group).

Group	Baseline (Hz)	Testis 1 (Hz)	Testis 2 (Hz)
Morphine	7.74 ± 0.88	9.53 ± 0.88	10.21 ± 0.88*
Bupivacaine	7.20 ± 0.47	8.57 ± 0.47*	7.96 ± 0.47†
Combination	7.53 ± 0.57	7.11 ± 0.57†	7.47 ± 0.56†

*Statistically significant ($p < 0.05$) from baseline within group

†Statistically significant ($p < 0.05$) versus morphine at given time-point

Table 4.2. Least square means ± standard error for the total power (P_{tot}) of the electroencephalogram prior to and during surgery in anaesthetised dogs after administration of subcutaneous (SC) morphine 0.5 mg kg^{-1} (Morphine), incisional (IB) bupivacaine 2 mg kg^{-1} (Bupivacaine) or a combination of SC morphine 0.5 mg kg^{-1} and IB bupivacaine 2 mg kg^{-1} (Combination). (n = 7 per group).

Group	Baseline (μV^2)	Testis 1 (μV^2)	Testis 2 (μV^2)
Morphine	75.48 ± 11.97	51.54 ± 11.97	31.2 ± 11.87*
Bupivacaine	62.43 ± 5.29	46.45 ± 5.13*	43.91 ± 5.29*
Combination	58.84 ± 2.2	60.04 ± 2.19†	57.68 ± 2.16‡

*Statistically significant ($p < 0.05$) from baseline within group

†Statistically significant ($p < 0.05$) versus bupivacaine at given time-point

‡Statistically significant ($p < 0.05$) versus both morphine and bupivacaine groups at given time-point

Table 4.3. Least square means ± standard error for the spectral edge frequency (F_{95}) of the electroencephalogram prior to and during surgery in anaesthetised dogs after administration of subcutaneous (SC) morphine 0.5 mg kg^{-1} (Morphine), incisional (IB) bupivacaine 2 mg kg^{-1} (Bupivacaine) or a combination of SC morphine 0.5 mg kg^{-1} and IB bupivacaine 2 mg kg^{-1} (Combination). (n = 7 per group).

Group	Baseline (Hz)	Testis 1 (Hz)	Testis 2 (Hz)
Morphine	25.53 ± 0.53	26.28 ± 0.53	26.85 ± 0.53*
Bupivacaine	25.14 ± 0.32	25.96 ± 0.32*	25.79 ± 0.32
Combination	25.46 ± 0.20	25.15 ± 0.20‡	25.84 ± 0.19†

*Statistically significant ($p < 0.05$) from baseline within group

†Statistically significant ($p < 0.05$) from T1 within group

‡Statistically significant ($p < 0.05$) versus bupivacaine at given time-point

4.5.1 CMPS-SF scores

Postoperatively, no dogs in the combination or bupivacaine groups required rescue analgesia, while one dog in the morphine group needed it at 1-hour post-surgery (class 3; pain score ≥ 6) (Table 4.4). This dog was then removed from the study for the purpose of further pain scoring, and the value of pain class 3 was held over to all subsequent time-points. This approach is recommended by Max and Laska (1991), and previously utilised in veterinary pain score studies (Slingsby & Waterman-Pearson 1998, 2000). In the combination group, all dogs were in the pain score class 1 (pain

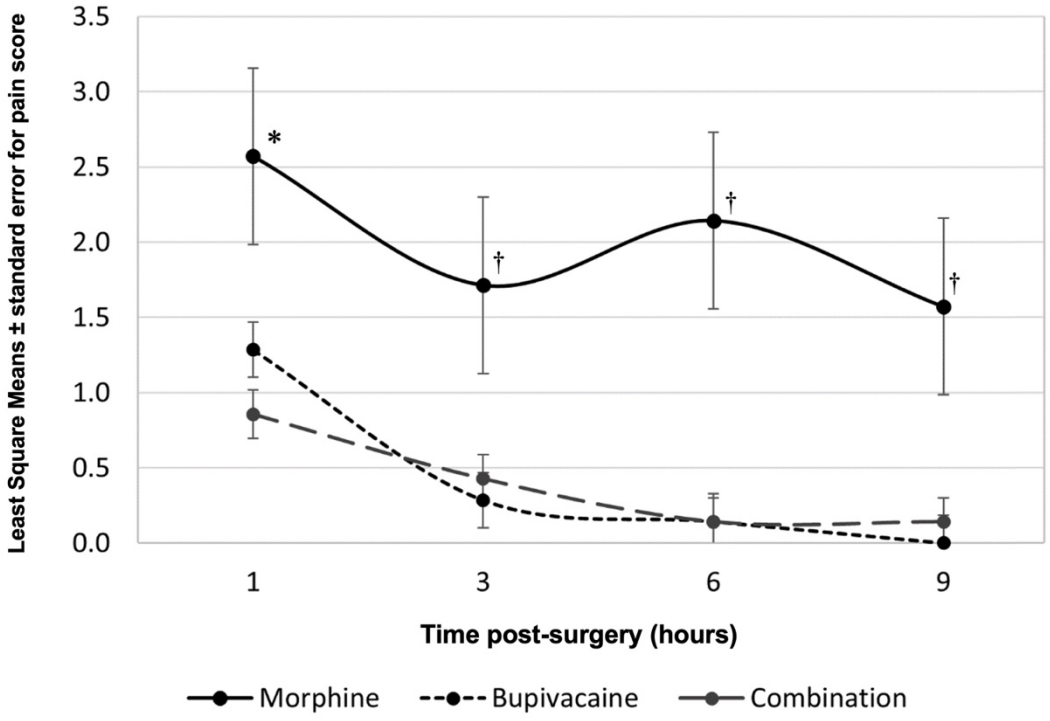
score 0–2; no pain) at all postoperative time-points. The mean pain scores at different post-surgical time-points in the three groups are presented in Figure 4.1. The overall pain score of the dogs (across all time-points) in either the combination group - morphine and bupivacaine ($p = 0.0002$) or group bupivacaine alone ($p = 0.0004$) was significantly lower than that of dogs in group morphine alone. Pain scores in the combination and bupivacaine groups were significantly lower than in the morphine group at all post-surgical time points, except at 1 hour in the bupivacaine group ($p = 0.1852$). No difference was found between the overall pain score of dogs in the bupivacaine group and combination group ($p = 0.8056$). Similarly, there were no significant differences in pain scores at any of the post-surgical time-points, between these two groups.

Table 4.4. Distribution of dogs scored with the Glasgow Composite Measure Pain Scale into pain class 1 (pain scores between 0 and 2), pain class 2 (pain scores between 3 and 5), and pain class 3 (pain scores 6 and above), at different time-points post castration after treatment with subcutaneous (SC) morphine 0.5 mg kg^{-1} (Morphine), incisional (IB) bupivacaine 2 mg kg^{-1} (Bupivacaine) or a combination of SC morphine 0.5 mg kg^{-1} and IB bupivacaine 2 mg kg^{-1} (Combination). (n = 7 per group).

Group	Class	Number of dogs at postoperative time points (hours)			
		1	3	6	9
Morphine	1	5	4	4	4
	2	1	2	2	2
	3	1	1	1	1
Bupivacaine*	1	7	7	7	7
	2	0	0	0	0
	3	0	0	0	0
Combination*	1	7	7	7	7
	2	0	0	0	0
	3	0	0	0	0

*Statistically significant ($p < 0.05$) from Morphine group on overall pain scores

Figure 4.1. Least square means ± standard error of post-surgical Glasgow Composite Measure Pain Scale pain scores at various post-surgical time points (in hours) after treatment with subcutaneous (SC) morphine 0.5 mg kg^{-1} (Morphine), incisional (IB) bupivacaine 2 mg kg^{-1} (Bupivacaine) or a combination of SC morphine 0.5 mg kg^{-1} and IB bupivacaine 2 mg kg^{-1} (Combination). *Statistically significant ($p < 0.05$) when compared to combination group at a given time point. †Statistically significant when compared to both bupivacaine and combination groups at given time points.



4.6 DISCUSSION

In this study, the use of a pre-incisional bupivacaine injection in combination with a systemically administered opioid appeared more effective in reducing EEG indices of nociception during canine castration than either drug administered alone. In previous studies, significant increases in F_{50} and F_{95} and decrease in P_{tot} were associated with nociception during surgical stimulation (Murrell et al. 2003; Johnson 2005; Kongara et al. 2013, 2014). Lidocaine injection into the testes or spermatic cord of piglets, or IV infusion prior to castration of horses, prevented these changes (Murrell et al. 2003; Ranheim et al. 2005). Similar EEG changes indicating nociception were observed following velvet antler removal in red deer and surgical dehorning in calves, but pre-emptive lidocaine ring blocks around antlers or horn buds abolished these changes (Johnson et al. 2005; Gibson 2007). In the current study, bupivacaine infiltration alone at the incision site could not prevent the changes in EEG indices of nociception in response to castration.

In canine castration, intratesticular or incisional blocks are recommended as part of a balanced analgesic approach (Mathews et al. 2014). Intratesticular injection of lidocaine has been shown to rapidly diffuse into the spermatic cord and produce analgesia in piglets (Ranheim et al. 2005). In dogs, intratesticular injection of lidocaine reduced the unwanted haemodynamic responses to castration (Huuskonen et al. 2013). In another study by Stevens et al. (2013), however, a lidocaine/bupivacaine mixture administered intratesticularly was not effective at reducing pain scores compared to that of saline injection. Infiltration of bupivacaine at the incision site of dogs undergoing ovariohysterectomy provided no additional benefit in terms of a reduction of post-

surgical pain scores (Fitzpatrick et al. 2010; Kalchofner Guerrero et al. 2016). There were no studies found that evaluated the efficacy of incisional blocks alone in canine castration.

There is limited literature on the extent of distribution of local anaesthetics in tissue of conscious dogs. In the bupivacaine group, it is possible that the deeper surgical tissues were unable to attain a high enough concentration of bupivacaine within the time-period needed for effective blockade of nerve conduction. This may have allowed afferent transmission during surgery, which was reflected as changes in the EEG indices of nociception. During castration, tension on the spermatic cord and cremaster muscle may cause visceral nociception (Gaynor and Muir 2015). The dense nature of connective tissue between the dermal area and the spermatic cord probably limits the diffusion of bupivacaine infiltrated at the pre-scrotal area into the spermatic cord and cremaster muscle for effective blockade of afferent transmission. Therefore, it did not prevent nociception in response to traction on the tunic or traction and clamping of the spermatic cord in this study.

In our study, the combination of SC morphine and IB bupivacaine blocked the changes in nociceptive markers of the EEG, indicating effective antinociception. Although their separate administration allowed afferent noxious transmission, morphine and bupivacaine might have been additive in blocking the nociception effectively when administered together prior to castration. Pre-emptive, multi-modal analgesia provides superior pain relief with reduced analgesic-related side effects (Joshi 2005).

Attempts to quantify clinical pain in dogs using physiological variables is inconsistent and unreliable (Holton et al. 1998). Instead, behavioural responses are often used to quantify postoperative pain in animals of various species (Morton et al. 2005). The Glasgow pain scale, which is based on various behavioural changes, has been validated in dogs for the assessment of acute pain (Morton et al. 2005). The short form has been further simplified for use in clinical practice (Reid et al. 2007).

The overall pain scores of dogs in the morphine group were significantly higher than those of the combination and bupivacaine groups. In analgesia studies based on visual appraisal of pain, the effect of morphine was expected to last for 2–4 hours (Guedes et al. 2007). In the current study, the time between preoperative administration of morphine and the first assessment of postoperative pain was approximately 2.5 to 3 hours. This amount of time increases with subsequent postoperative pain assessment time-points (3, 6 and 9 hours). Because of the short duration of effect (2–4 hours), it is possible that morphine, administered at the time of pre-anaesthetic medication, had worn off by 1, 3, 6 and 9 hours after castration. There was no significant difference in the pain scores of dogs between the bupivacaine and combination groups. All dogs in these two groups were in the pain score class 1 (pain score 0–2; no pain) at all time-points. Although bupivacaine did not restrict the changes in F_{50} and P_{tot} to the same extent as the combination group, the magnitude of changes in these EEG indices of nociception were less than that of the morphine. While it appears that bupivacaine might allow some afferent transmission during surgery due to its slow absorption, it could produce effective analgesia in the postoperative period due to its long duration of action.

A limitation of this study was a possible inconsistency of surgical stimuli and the degree of tissue manipulation. Since these were client owned dogs and comprised the teaching hospital's clinical caseload, castrations and accompanying anaesthesia were performed by final year veterinary students. Duration of surgery and degree of tissue manipulation would have been more consistent if one surgeon carried out each surgery. All students, however, were supervised by the same surgeon and an identical protocol was followed throughout. Wagner et al. (2000) showed that the amount of surgeon experience and the duration of surgery may not influence the frequency or severity of pain-related behaviour postoperatively. It may, however, alter biochemical markers that are indicative of differences in the amount of surgical trauma (Michelsen et al. 2012).

There are limitations in assessing pain using behavioural indicators in the clinical setting despite using a validated pain scale. Pain scoring may have been affected by the behaviour and demeanour of certain overly anxious dogs housed in an unfamiliar environment (Anil et al. 2002). Differences in behavioural and physiological variables indicative of pain expression may also have been influenced by individual temperament, breed and external stressors (Mathews et al., 2014).

4.7 CONCLUSION

In this study, we found that combining a bupivacaine incisional block with systemic morphine reduced intraoperative nociception during canine castration compared to either drug alone. Bupivacaine as an incisional block alone results in smaller EEG changes and lower postoperative pain scores than systemic morphine. Postoperatively,

both the combination and bupivacaine alone were superior to morphine alone, but the combination offered no additional benefit over bupivacaine alone.

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5 CHAPTER 5 - EVALUATION OF TRANSDERMAL BUPRENORPHINE PATCHES FOR POSTOPERATIVE ORTHOPAEDIC PAIN CONTROL FOLLOWING TIBIAL TUBEROSITY ADVANCEMENT

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5.1 ABSTRACT

Objective To investigate the analgesic efficacy of transdermal buprenorphine patches compared to a negative control in dogs undergoing a tibial tuberosity advancement surgery.

Study design Randomised blinded clinical study.

Animals A group of 38 healthy adult mixed breed dogs presented for tibial tuberosity advancement surgery

Methods Dogs were randomly divided into two groups (n = 19) to receive either two buprenorphine patches or two placebo patches (control). Patches were placed 18 hours prior to surgery. Premedication consisted of acepromazine (0.02 mg kg^{-1}) and buprenorphine (test group) or morphine (control group) administered intramuscularly. Anaesthesia was induced with intravenous (IV) propofol to effect and maintained with isoflurane in oxygen. All dogs underwent a surgical tibial tuberosity advancement (TTA). Postoperative pain was assessed using the short form Glasgow Composite Measure Pain Scale (CMPS-SF) at t = 0.25, 1, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 48 hours post extubation. Dogs with a CMPS-SF pain score >4/20 or >5/24 at any time point received rescue analgesia. If rescue analgesia was required, 0.02 mg kg^{-1} buprenorphine intramuscularly (IM) was provided and a treatment failure recorded from that point onwards for the patient. The CMPS-SF pain scores and rescue analgesia injections were analysed over 48 hours. The last observation carried forward method was applied in case of treatment failure. Data were analysed using a Mann-

Whitney U test and presented as median (minimum-maximum range), with significance set at $p < 0.05$.

Results Four of 19 dogs in the test group and 11/19 dogs in the control group received rescue analgesia ($p = 0.04$). The test group had significantly lower mean CMPS-SF pain scores across the postoperative period (t = 4 to t = 48 hours post extubation).

Conclusion and clinical relevance Transdermal buprenorphine was effective in managing the postoperative pain associated with a tibial tuberosity advancement procedure in dogs. Transdermal buprenorphine patches are a useful alternative for managing orthopaedic pain in dogs undergoing surgery.

Keywords analgesia, buprenorphine, postoperative, transdermal

5.2 INTRODUCTION

Initially developed for human use, transdermal delivery systems (TDS) have numerous advantages in that they: provide sustained and relatively consistent plasma concentrations over an extended period of time; offer a non-invasive analgesic approach to the patient (i.e. reducing anxiety in patients from handling and avoiding repeated injections); reduce the nursing workload when compared to intravenous (IV) infusions or repeated injections; and minimise side effects seen with intermittent delivery (Arndt et al., 1984; Kyles et al., 1996; Riviere and Papich, 2001). Fentanyl patches are the most commonly used and studied TDS in veterinary medicine,

primarily used for managing acute postoperative and chronic cancer-related pain (Kyles et al., 1996, 1998; Egger et al., 2007). However, using fentanyl patches in animals presents several challenges, including: lower than expected plasma fentanyl concentrations when human-designed patches are applied to animal skin; high inter-animal variability; and a prolonged or inconsistent time to reach steady-state plasma concentrations (Kyles et al., 1996, 1998; Andaluz et al., 2009). Due to fentanyl's narrow safety margin, accidental overdose – such as a patch inadvertently adhering to a family member or ingestion of a used patch – can result in severe complications, including death (Zanon et al., 2020).

Buprenorphine is a highly lipophilic, potent partial μ-opioid receptor agonist and κ-opioid receptor antagonist, exhibiting strong receptor affinity and a pronounced antihyperalgesic effect. (Pergolizzi et al., 2010). Compared to other opioids, buprenorphine offers several advantages. In dogs, buprenorphine has a relatively long duration of action, negligible cardiovascular effects, and a tendency not to induce vomiting (Martinez et al., 1997; Pascoe, 2000; Budd, 2002). At recommended doses, buprenorphine's analgesic efficacy in the dog is similar to that of morphine administered at 0.3 – 0.8 mg kg^{-1} (Kerr, 2007). Buprenorphine has several properties that make it optimal for transdermal formulation, including its low molecular weight, high lipophilicity, and high potency (Aiyer et al., 2018). Unlike fentanyl, buprenorphine also has a significantly wider safety margin, making it a safer option for transdermal applications with a much lower risk of abuse (Yassen et al., 2008).

Previous studies have evaluated the use of transdermal buprenorphine patches for managing pain following soft tissue surgical procedures in dogs (Moll et al., 2011; Galosi et al., 2022). To the authors' knowledge, their analgesic efficacy for postsurgical orthopaedic pain has not yet been investigated. Research has shown significant variability in buprenorphine plasma concentrations in dogs, ranging from 0.1 ng mL^{-1} to 2 ng mL^{-1} over 100 hours with a 70 μg $hour^{-1}$ buprenorphine patch (Andaluz et al., 2009; Moll et al., 2011). Similar levels (0.8 - 2.3 ng mL^{-1}) were also reported following the placement of a smaller 52.5 μg $hour^{-1}$ patch (Pieper, 2011). In humans, the minimum effective analgesic plasma concentration is 0.1 ng mL^{-1} (Sittl et al., 2003). It remains unclear how plasma levels of 0.1 to 2 ng mL^{-1} can adequately manage the moderate to severe pain associated with orthopaedic surgeries in dogs.

The objective of this study was to characterise the effect of transdermal buprenorphine patches in controlling postoperative pain in dogs undergoing TTA surgery for the correction of cranial cruciate ligament rupture. We hypothesised that transdermal buprenorphine would provide analgesia equivalent to the current clinical practice of IM administered morphine and buprenorphine for controlling postsurgical orthopaedic pain.

5.3 MATERIALS AND METHODS

This study was conducted and reported in accordance with the CONSORT guidelines and checklist when writing this report (Schulz et al., 2010).

5.3.1 Cohort selection

The study was approved by the Massey University Animal Ethics Committee, Palmerston North. Thirty-eight client-owned dogs aged 19 months and older were selected from those presented to the Massey University Veterinary Teaching Hospital for elective surgical correction of cranial cruciate ligament rupture using a tibial tuberosity advancement. Written informed owner consent was obtained for all dogs. Dogs were admitted to the study on the day before surgery. Each dog was randomly assigned into one of two treatment groups of nineteen dogs (n = 19) in each group. Randomization was performed by using a "draw from the hat" technique. Group randomisation and drug administration was completed by one researcher who was not involved in directly monitoring and maintenance of anaesthesia, surgery, or patient pain scoring. Dogs were considered healthy based on history and physical examination and were assessed as American Society of Anesthesiologists (ASA) 1 or 2 on pre-anaesthetic examination. Food was withheld for 12 hours prior to surgery with unlimited access to water until the morning of the procedure.

5.3.2 Anaesthesia and analgesia

Dogs in both groups had a square area of skin shaved on either side of the lateral thorax for the application of patches. The clipped skin was cleaned with warm water and dried. Skin was prepared and patches were applied 18 hours prior to surgery. Dogs in the buprenorphine group had a total of two 20 μg hour^{-1} buprenorphine patches (Norspan; Mundipharma, Cambridge, United Kingdom) placed directly on their skin,

with one patch applied on either side of the thorax. The patches were covered with a flesh-coloured self-adherent cohesive bandage (Coban; 3M, MN, USA), and the entire area was covered with a light dressing of an adherent elasticated bandage (Elastoplast; Beiersdorf, Hamburg, Germany). Dogs in the control group had the same area of skin clipped, cleaned, and covered with the cohesive bandage and elasticated bandage only to blind the scorer.

Dogs were administered acepromazine (0.02 mg kg^{-1}; Acezine-2, 2%; Ethical Agents, Auckland, New Zealand) with buprenorphine hydrochloride (0.02 mg kg^{-1}; Temgesic, 0.3 μg mL^{-1}, Reckitt Benckiser, Slough, UK) (test group) or morphine (0.5 mg kg^{-1}; Morphine Sulphate Inj., 30 mg mL^{-1}, Mayne Pharma Private Ltd., VIC, Australia) (control group) intramuscularly (IM) 45 minutes prior to anaesthetic induction.

In all groups, 45 minutes after pre-anaesthetic medication, anaesthesia was induced with an intravenous (IV) injection of propofol (Repose; Norbrook, Auckland, New Zealand) until jaw relaxation and suppression of pharyngeal reflex was adequate to allow orotracheal intubation. Following intubation, anaesthesia was maintained with isoflurane (MedSource; Ashburton, New Zealand) in oxygen administered via an appropriate rebreathing circuit. Dogs in both groups were given bupivacaine hydrochloride (1 mg kg^{-1}; Marcaine 0.5% Bupivacaine Injection, AstraZeneca, NSW, Australia) administered epidurally in the lumbosacral space prior to surgery. All study subjects breathed spontaneously without need for mechanical ventilation. Systolic arterial blood pressure was recorded every five minutes using a doppler ultrasound pressure transducer with cuff (Parks Medical Electronics Inc., OR, USA). Throughout the anaesthetic period, Hartmann's solution (Baxter Healthcare Ltd., NSW, Australia)

was administered IV at a rate of 10 mL kg^{-1} $hour^{-1}$. Respiratory rate (f_R), heart rate (HR), end-tidal CO_2 ($P_{E'}CO_2$), and end-tidal isoflurane ($F_{E'}Iso$) were monitored every five minutes using an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Germany). In the event a physiological response to nociception was suspected based on an increase in HR and/or mean arterial pressure (MAP) greater than 15% in comparison with the previous value (when normotensive), an IV bolus of fentanyl at 2 μg kg^{-1} (Fentanyl Citrate, 50 μg mL^{-1}, Mercury Pharma, Auckland, NZ) was administered. If dogs experienced hypotension (MAP < 70 mmHg), dopamine (Dopamine Concentrate, 40 mg mL^{-1}; Max Health Ltd., Auckland, NZ) was administered at a starting dose of 5 μg kg^{-1} $minute^{-1}$, increasing to a maximum of 9 μg kg^{-1} $minute^{-1}$ as needed to become normotensive. Anaesthetic induction, maintenance of anaesthesia, and anaesthetic monitoring were performed by the hospital anaesthetists and final year students on their anaesthesia rotation; they were also blinded to the animals' group allocations and administered analgesics.

5.3.3 Procedures

A routine tibial tuberosity advancement through a medial skin and arthrotomy approach was performed by one of two board-certified surgeons experienced in the technique. All dogs received cefazolin (Cefazolin-AFT; AFT Pharmaceuticals, Auckland, NZ) administered IV at 22 mg kg^{-1} following induction of anaesthesia and during the perioperative period. Dogs in the control group were given morphine (0.3 mg kg^{-1}; Morphine Sulphate Inj., 30 mg mL^{-1}, Mayne Pharma Private Ltd., VIC, Australia) intramuscularly (IM) at extubation. All dogs were moved to a recovery cage

and given 4 mg kg^{-1} carprofen (Rimadyl 50 mg mL^{-1}; Zoetis NZ Ltd., Auckland, NZ) IM. Intervals between injections and first pain assessment were kept consistent for all patients. All dogs continued to receive 4 mg kg^{-1} carprofen once daily given orally for a further 7 days.

5.3.4 Pain scoring

Pain scoring was completed at extubation (time = 0), and 0.25, 1, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 48 hours post-extubation. Pain was assessed by observing the dog in its cage, approaching the cage and opening the door, encouraging the dog to stand and walk if capable, and by applying gentle palpation using the fingers of one hand approximately 3cm from the wound. Pain scores were assigned using the short form Glasgow Composite Pain Score (CMPS-SF). If a dog scored greater than 5/20 (if non-ambulatory) or 6/24 (if ambulatory), additional analgesia was provided with 0.03 mg kg^{-1} buprenorphine IM and pain reassessed 30 minutes later. Dogs that received rescue analgesia were removed from the study, although pain scoring and monitoring was continued, to assess whether additional pain medication was needed and to monitor for adverse clinical signs.

5.3.5 Statistical analysis

Statistical power analysis for sample size calculation was carried out using online software (http://hedwig.mgh.harvard.edu/sample_size/size.html). Pain scores typically have a high standard deviation (SD) that varies depending on the type of pain.

It is not possible to provide an accurate measure of the expected variability with this study design. A parallel study design was selected with a quantitative two-sided measurement, a *p* value of 0.05 and a power of 0.8, where a sample size of 38 patients could detect a difference between treatments of 0.934 times the SD, deemed sufficient for this study.

Parametric data were tested for normality using the Shapiro-Wilk test. Non-normally distributed data were analysed using a Wilcoxon Rank-Sum test, whereas normally distributed data were analysed using an independent samples t-test. The median and range were reported for data that were not normally distributed, and the mean ± SD were reported for data that were normally distributed. Differences in body weight, age, anaesthesia time, and surgery time among groups were tested using the Kruskal-Wallis test.

Dogs that received rescue analgesia were removed from the study and the Last Observation Carried Forward (LOCF) method was applied to subsequent data points (Dancker et al., 2020). Pain scores were analysed as the total sum over t = 4 to t = 48 hours.

Non-parametric within group pain score differences were compared using a Friedman repeated measures analysis of variance on ranks. The Wilcoxon Rank-Sum test was used to compare time to rescue analgesia between the groups. A fisher's exact test was used for the incidence of postoperative rescue analgesia.

All statistical analyses were performed using R statistical software version 4.4.2 (R Development Core Team 2024, R Foundation for Statistical Computing, Vienna, Austria). Significance was set at $p < 0.05$.

5.4 RESULTS

The dogs were between 1.6 and 12.8 years of age with a median age of four years. The body weight of dogs was between 19.5 and 52 kg with a median of 30.2 kg. There were no significant differences between the groups for age or bodyweight (Table 5.1). There was no significant difference between the groups in the number of intraoperative fentanyl administration events or the total amount of fentanyl administered. No adverse surgical events or complications were encountered during any of the procedures performed. Only three dogs (one in the buprenorphine group and two in the control group) were reported to have hypotension during the surgery period, which was treated successfully with dopamine in each case.

Mean ± SD HR, f_R, SAP, PE´CO2, and FE´Iso were not different among groups. Duration of anaesthesia and duration of surgery were not different among groups or between surgeons. Duration of anaesthesia among groups ranged from 200 ± 38.9 minutes to 207 ± 32.4 minutes. Duration of surgery among groups ranged from 91.4 ± 15.4 minutes to 99.3 ± 18.3 minutes (Table 5.1). All anaesthesia recoveries were uneventful; no postoperative adverse effects were observed.

Table 5.1. Demographics for 38 client-owned dogs that underwent unilateral TTA surgery for treatment of cranial cruciate ligament rupture that received either two 20 μg hr^{-1} transdermal buprenorphine patches for the buprenorphine group (B) or two transdermal placebo patches for the control group (C). (n = 19 per group).

	Group		
Variable	Buprenorphine	Control	p - value
Age (months)	50.7 ± 25.5	65.5 ± 27.3	0.06
Weight (kg)	32.7 ± 8.64	31.7 ± 4.57	0.8
Duration of surgery	91.4 ± 15.4	99.3 ± 18.3	0.2
Duration of anaesthesia	200 ± 38.9	207 ± 32.4	0.6

Figure 5.1. Kaplan-Meier curve for time to rescue analgesia (i.e. fraction of patients not requiring rescue analgesia) for 48 hours following unilateral TTA surgery for treatment of cranial cruciate ligament rupture at various post-surgical time points (in hours) after receiving either two 20 μg hr^{-1} transdermal buprenorphine patches for the buprenorphine group (B) or two transdermal placebo patches for the control group (C).

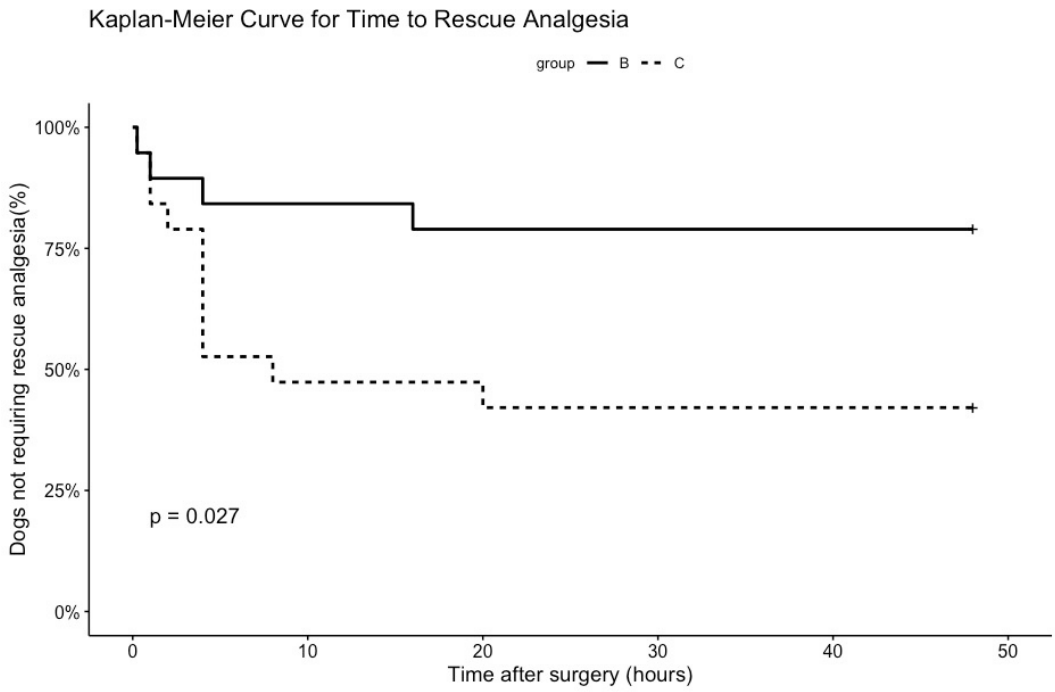

Figure 1 illustrates the percentage of dogs by time-point in each group that did not require rescue analgesia and so remained in the study in the form of a Kaplan-Meier graph. There was a significant difference between the buprenorphine group and control group (p = 0.027). The number of dogs requiring rescue analgesia was significantly different in each group (p = 0.04), with 11 dogs requiring rescue analgesia in the control group (57.9%) compared to four dogs requiring rescue analgesia in the buprenorphine group (21.1%). Time to rescue analgesia administration was not different among groups (Table 5.2).

Table 5.2. Summary of rescue analgesia data for 38 client-owned dogs that underwent a unilateral TTA surgery for treatment of cranial cruciate ligament rupture that received either two 20 μg hr^{-1} transdermal buprenorphine patches for the buprenorphine group (B) or two transdermal placebo patches for the control group (C). (n = 19 per group).

Variable	Buprenorphine (B)	Control (C)
No. of dogs requiring rescue analgesia	4	11*
Median time to rescue analgesia administration (h)	2.5 hours (1 – 16)	4 hours (0.25 – 20)
Total no. of rescue analgesia doses given per group	5	18
Time of earliest rescue analgesia administration (h)	1	0.25

*Statistically significant ($p < 0.05$) from buprenorphine (B) group

The median (range) hours to administration of the first rescue analgesia was 2.5 (0.25 – 16) for the buprenorphine group and 4 (0.25 – 20) for the control group. Time to rescue analgesia did not differ significantly ($p = 0.7$). Mean pain scores from t = 4 to the end of the study (t = 48) hours were significantly higher in the control group (5.03) than in the buprenorphine group (3.58) ($p = 0.04$). Pain scores within each group were significantly different from each other. Pain scores at individual time points (Table 5.3) were significantly different between groups, with pain scores in the control group being significantly higher at t = 6 ($p = 0.0014$), t = 8 ($p = 0.0396$), t = 20 ($p = 0.0475$), and t = 24 ($p = 0.0098$). Although not statistically significant, differences approached

significance at t = 12 (p = 0.064). There was no difference in pain scores between surgeons at all time points except t = 40 (p = 0.018).

Table 5.3. Median + range modified GCMPS scores of 38 client-owned dogs that underwent unilateral TTA surgery for treatment of cranial cruciate ligament rupture that received either two 20 μg hr^{-1} transdermal buprenorphine patches for the buprenorphine group (B) or two transdermal placebo patches for the control group (C). (n = 19 per group).

	No. of dogs remaining in study		Median (range) pain score		
Time Point	Buprenorphine (B)	Control (C)	Buprenorphine (B)	Control (C)	*P* value
Pre-surgery	19	19	1 (0 – 1)	1 (0 – 1.2)	0.934
15 min	19	19	1.2 (1 – 12)	2.4 (1.2 – 8.4)	0.501
1 h	18	18	2.4 (1.2 – 7.2)	1.2 (1 – 6)	0.542
2 h	17	16	2.4 (1.2 – 12)	1.2 (0 – 7.2)	0.984
4 h	17	15	2.4 (1 – 8.4)	3 (0 – 8.2)	0.0988
6 h	16	10	2 (1 – 8.4)	4 (2 – 8)*	0.0014
8 h	16	9	2.4 (1 – 6)	3 (1.2 – 7)*	0.0396
12 h	16	9	3 (1 – 6)	4 (1 – 7)	0.0635
16 h	16	9	3 (1 – 8)	3 (1 – 11)	0.178
20 h	15	9	3 (1 – 6)	3 (1 – 8)*	0.0475
24 h	15	8	2 (1 – 6)	3 (1 – 8)*	0.0098
32 h	15	8	3 (0 – 7)	2 (1 – 6)	0.368
40 h	15	8	3 (1 – 6)	2 (1 – 6)	0.311
48 h	15	8	2 (1 – 5)	2 (1 – 6)	0.194

*Statistically significant (p < 0.05) from buprenorphine (B) group

5.5 DISCUSSION

This study compared the quality and duration of analgesia produced by long-acting transdermal buprenorphine patches in dogs undergoing an orthopaedic procedure compared to a negative control group only receiving an injection of morphine at anaesthetic recovery. The results of this clinical study provide evidence that transdermal buprenorphine at a dose of two 20 μg hour^{-1} patches applied 18 hours prior to the surgical stimulus was superior to the negative control group as assessed by CMPS-SF pain scores and the number of dogs requiring rescue analgesia postoperatively in dogs undergoing orthopaedic surgery. The need for postoperative rescue analgesia was significantly decreased in the group with buprenorphine patches.

Due to the limited availability of transdermal buprenorphine patch sizes in New Zealand, two 20 μg hour^{-1} patches were applied to each dog in our study, regardless of weight. With a median patient weight of 30.2 kg, this delivered approximately 1.3 μg kg^{-1} hour^{-1} of buprenorphine. This dosage is considerably lower than in previous studies that demonstrated analgesic efficacy using 70 μg hour^{-1} patches on smaller dogs (mean weights of 13.75 kg and 12.67 kg), delivering 5.1 μg kg^{-1} hour^{-1} and 5.5 μg kg^{-1} hour^{-1}, respectively (Andaluz et al., 2009; Moll et al., 2011). The higher doses used in those studies resulted in steady state buprenorphine plasma concentrations of 0.7 – 1 ng mL^{-1} (Andaluz et al., 2009). In humans, the minimum effective buprenorphine plasma concentration for analgesia is assumed to be 0.1 ng mL^{-1} (Terlinden and Stadler, 2000; Sittl et al., 2005). Notably, two of the four buprenorphine-treated dogs requiring rescue analgesia in our study were significantly

heavier, with a mean weight of 47.5 kg. It is possible that buprenorphine plasma concentrations did not achieve analgesic threshold in these animals.

The median time to rescue analgesia in the buprenorphine group (2.5 hours) was earlier than in the control group (4 hours). Although not statistically significant, this may be due to plasma buprenorphine concentrations in some dogs not reaching steady-state levels, leaving them at sub-analgesic concentrations. Pharmacokinetic studies on transdermal buprenorphine patches in dogs demonstrated that plasma levels continued to rise over the first 36 hours (Andaluz et al., 2009) and may not even reach measurable concentrations 36 hours after application (Pieper, 2011). Similar plateau times of 36–60 hours have been reported in human studies as well (Terlinden and Stadler, 2000). In our study, patches were applied 18 hours prior to the scheduled surgery anaesthetic induction time. Patches were therefore only in contact with the skin for 20 – 24 hours before extubation when anaesthesia and surgery time is taken into account. This may not have been enough time for plasma buprenorphine levels to reach analgesic levels in some animals. Inter-individual variability in effective plasma concentration, however, cannot be ruled out as a cause of these results.

The mean pain scores for individual patients were analysed between groups from t = 4 hours post-extubation to the end of the pain scoring period (t = 48 hours). The first 4 hours were excluded from the analysis of the overall mean pain scores because the control group received IM morphine upon extubation, which provides analgesic effects for at least 4 hours (Lucas et al., 2001), thereby suppressing pain in the control group during that period. This aligns with the median time to rescue analgesia of t = 4 hours, with 5 out of 11 dogs (45.5%) in the control group requiring their first dose of

rescue analgesia at that time. Consequently, no statistically significant differences in pain scores were observed between the two groups from t = 0 to t = 4 hours, supporting the conclusion that when the control group had morphine analgesia, pain scores were comparable between the two groups.

One limitation of this study was the sample size that was able to be recruited in the specified time. Ethics approval originally expected 40 dogs in each group to maximise the ability to show a significant difference between the groups. Due to the intensity of the study and ability to recruit patients, only 19 dogs were able to be enrolled in each group. Despite this, we were able to show that buprenorphine patches are an effective analgesic for orthopaedic pain in dogs and show statistically significant effects at most time points compared to the control group. There were also time points where the difference approached significance but was not statistically significant. These results may have been more definitive with the originally planned group sizes.

Another limitation in our study was using more than one individual to pain score dogs. Inter-individual variability in using pain scales can cause differences in pain scoring that are not due to pain but due to differing individual scoring techniques (Marco-Martorell et al., 2024). While the majority of pain scores were scored by one individual, certain pain scoring that was due to be completed outside of normal working hours was completed by individual nursing staff or students on rotation at the time. All pain scorers were unaware of patient group assignments.

In our study, dogs in the buprenorphine group that required rescue analgesia were scored as painful 3 hours after receiving buprenorphine, while dogs in the control

group were scored as painful at 15 minutes and 1 hour following IM morphine administration, despite the opioid still being effective and expected to provide analgesia during this period. This may have been influenced by increased anxiety or dysphoric recovery, which could have affected the behavioural parameters assessed by the CMPS-SF. Although the CMPS-SF pain scale is validated for evaluating pain in dogs, it remains a psychometric tool reliant on the interpretation of patient behaviour (Reid et al., 2007).

Validated pain scales can be influenced by age, breed, and temperament of dogs (Mathews et al., 2014) as well as by the effects of anaesthetic drugs (Guillot et al., 2011; Rialland et al., 2012). Our study included a variety of dog breeds, each of which may exhibit unique behavioural responses to stress, analgesics, sedatives, or pain. Even if breed variation were controlled, individual patient experiences prior to study enrolment could also impact behaviour. For instance, a dog with prior hospital visits, anaesthesia, or surgery might behave differently compared to a dog with no such experiences, despite similar signalment. In this study, animals were not screened or assessed for this variability in temperament or anxiety and group variance in resulting pain scores could have differed due to different effects present in each group.

Several factors, including postoperative anxiety, can influence an observer's perception of pain severity. Conversely, pain itself may exacerbate anxiety, as demonstrated by Ellwood and Murison (2022) in dogs following orthopaedic (stifle) surgery. Disentangling behavioural changes caused by the hospital environment or dysphoria from those due to pain can be challenging. This overlap may lead to elevated pain scores, triggering rescue analgesia and contributing to perceived treatment failure.

5.6 CONCLUSION

Transdermal buprenorphine patches effectively managed postoperative pain following tibial tuberosity advancement surgery in dogs for cranial cruciate ligament rupture. The patches were well tolerated, with no observed adverse effects, suggesting they may serve as a viable alternative for pain management in dogs undergoing orthopaedic surgery. Further research is needed to establish the optimal weight-based dosage and evaluate their efficacy for other common surgical procedures.

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6 CHAPTER 6 - GENERAL DISCUSSION AND CONCLUSIONS

6.1 INTRODUCTION

Pain management in veterinary medicine has undergone significant advancements in recent years, with an increasing focus on multimodal analgesia and innovative drug delivery systems. Despite these developments, there remains considerable variability in analgesia techniques used for common surgical procedures in veterinary practice. A study surveying veterinarians in New Zealand highlighted this diversity, reporting 74 different drug combinations for premedication and induction, as well as 41 unique postoperative analgesic protocols for a single routine neutering procedure in dogs (Gates et al., 2020). More research is needed to provide veterinarians with evidence-based guidance on preoperative drug selection across different species and procedures, the interactions between different analgesics, and the most effective postoperative pain management strategies in terms of both duration and efficacy.

This thesis was undertaken not only to assess the safety and efficacy of various analgesic drugs but, more importantly, to evaluate the research methodologies used to measure analgesic effectiveness. Establishing reliable techniques to assess pain management across different perioperative periods is crucial for improving patient care and welfare in companion animals.

This chapter synthesises the key findings from experiments presented in this thesis, discusses research limitations, provides an overall conclusion, and outlines future directions for analgesia research using the methodology employed in these studies.

The four experiments presented in this dissertation include:

- **Evaluation of opioid efficacy in feline castration:** A study investigating the effectiveness of three intramuscularly administered opioids in reducing electroencephalographic indices of nociception in cats undergoing castration.

- **Analgesic interactions between dexmedetomidine and vatinoxan:** A study assessing the impact of vatinoxan, a peripherally acting α_2-adrenoceptor antagonist that does not cross the blood-brain barrier, on the antinociceptive properties of dexmedetomidine alone and in combination with morphine in cats undergoing castration.

- **Comparison of systemic morphine and locally administered bupivacaine for canine castration:** A clinical study evaluating the analgesic efficacy of systemically administered morphine, locally administered

bupivacaine as an incisional block, and their combination in preventing nociception and postoperative pain in dogs undergoing castration.

- **Transdermal buprenorphine for postoperative pain in orthopaedic surgery:** A clinical study using the Glasgow Composite Measure Pain Scale (CMPS-SF) to assess the effectiveness of transdermally delivered buprenorphine in managing postoperative pain in dogs undergoing orthopaedic surgery for cranial cruciate ligament repair.

By integrating findings from these studies, this thesis aims to contribute to the ongoing refinement of analgesic protocols in veterinary medicine, ultimately enhancing pain management strategies and improving patient welfare.

6.2 THE PREMEDICATION PERIOD FOR ANALGESIA

The study in **Chapter 2** highlights the critical role of the premedication period in veterinary anaesthesia, particularly in the selection of analgesic agents. For the purpose of this thesis, the premedication period is defined as the time before the start of the surgical incision, during which drugs are administered to mildly sedate the patient, reduce anxiety, enhance anaesthetic efficiency, and provide analgesia prior to the start of surgery. In some cases, premedication is also used to achieve additional benefits, such as reducing salivation, minimising the risk of gastric content aspiration during intubation, decreasing the likelihood of emesis, and even preventing central sensitisation (wind-up pain) (Schwenk & Mariano, 2018).

Premedication protocols can be complex, often involving multiple drugs administered at different time points. It is essential to ensure that these drugs achieve the intended effect in the species they are being used on while minimising the number of agents required. When designing a premedication protocol, clinicians must consider factors such as species and breed differences, drug availability, potential side effects, the surgical procedure being performed, and personal familiarity with specific drugs (Pascoe, 2000).

Beyond these considerations, the premedication period directly influences the level of analgesia available to the patient during surgery – the time of greatest noxious stimulation. One of the primary challenges in researching the intraoperative efficacy of analgesics is the difficulty in assessing immediate antinociceptive effects using conventional pain assessment techniques while the animal is anaesthetised. The most commonly used methods, such as observation-based pain scales, multifactorial pain scales, and behaviour-based pain assessments rely on patient responses, behaviours, and movements, all of which are absent during general anaesthesia. This limitation makes the EEG particularly valuable, as it allows researchers to detect encephalographic changes in response to noxious stimuli that would otherwise be perceived as pain in an awake animal. This is especially relevant when studying multiple, often subtle differences between drugs.

Even when evaluating the efficacy of analgesic drugs administered in isolation, numerous variables can influence their level of antinociception, many of which are not always considered when designing premedication protocols. In the comparison of the

three opioids used in **Chapter 2**, several key factors affected their intraoperative efficacy:

- **Opioid receptor activity:** Methadone and morphine are full μ-opioid agonists, while buprenorphine is a partial μ-opioid agonist. The greater receptor efficacy of methadone and morphine may have provided them with an advantage in blocking nociceptive signals and managing pain.

- **NMDA receptor activity:** While both methadone and morphine act as full μ-opioid agonists, methadone also exerts analgesic effects at NMDA receptors (Carpenter et al., 2000), which may offer additional benefits in nociceptive modulation and pain management.

- **Route of administration:** The method of drug administration significantly affects analgesic efficacy. For example, buprenorphine has been shown to have poor efficacy when administered subcutaneously (Rodríguez-Muñoz et al., 2012). When administered intramuscularly, as in this study, it resulted in effective antinociception.

- **Species-specific responses:** Different species exhibit unique physiological reactions to analgesics, influencing their overall effectiveness. For example, in cats, opioids frequently cause marked mydriasis, which occurs before the drug's analgesic effects take hold and persist even after its pain-relieving properties subside (Dixon et al., 2002). This phenomenon is not as

pronounced in dogs. Many analgesic studies have previously historically been conducted in dogs and cannot be directly extrapolated to feline patients.

These variables can significantly alter the expected efficacy of analgesics, sometimes producing results that contradict findings in other species. This study demonstrated that all three opioids, including buprenorphine, effectively eliminated EEG indices of nociception in cats undergoing castration. While subcutaneous buprenorphine has shown inconsistent analgesic effects in a previous study, intramuscular administration proved highly effective. This is likely due to the erratic absorption of buprenorphine when given subcutaneously (Steagall et al., 2014).

One unexpected difficulty and side effect in this study included the amount of sedation offered by each drug. While this was not part of the research in this study, cats receiving buprenorphine were less sedated than those receiving morphine or methadone. This made it more challenging in handling the cats, especially when putting in intravenous catheters. Additional clinical effect of drugs other than analgesic ones, like the sedative effects will undoubtedly be taken into account when choosing the optimal drug for a patient in clinical practice.

These findings highlight the importance of considering species-specific responses, administration routes, and receptor activity when designing analgesic protocols. Future research should continue refining multimodal analgesic strategies, especially focusing on the premedication period, to maximise efficacy while minimising side effects.

6.3 Impact of Drug Interactions on Analgesic Efficacy

When selecting drugs for the premedication period, it is essential not only to ensure that the chosen combinations achieve their intended effects but also to confirm that they do not interfere with each other or produce unwanted, unintended, or unexpected consequences. Drug interactions can be synergistic, additive, or antagonistic (Chabot-Doré et al., 2015), and understanding these relationships is crucial in determining compatibility and optimising dosing when multiple drugs are administered together.

6.3.1 Key findings

In **Chapter 3**, we investigated the interaction between vatinoxan, a novel peripherally acting α2-adrenoceptor antagonist, and dexmedetomidine, an α2-adrenoceptor agonist. Dexmedetomidine provides centrally mediated sedation and analgesia, but also induces peripheral side effects, particularly cardiovascular changes with increased cardiac work secondary to profound vasoconstriction (Jaeger et al., 2019; Pypendop et al., 2017a). Vatinoxan, when administered concurrently with dexmedetomidine, counteracts these peripheral cardiovascular effects without crossing the blood-brain barrier (Clineschmidt et al., 1988; J. M. Honkavaara et al., 2020), thereby preserving dexmedetomidine's sedative properties. Notably, this study confirmed that vatinoxan does not significantly impair dexmedetomidine's antinociceptive properties.

This study also explored combinations of low-dose morphine (0.2 mg/kg) with and without dexmedetomidine, with and without vatinoxan, as well as a higher dose

morphine group (0.4 mg/kg). The higher dose morphine provided significantly better antinociception than dexmedetomidine or its combination with morphine. However, there was no difference in nociceptive responses between dexmedetomidine alone and dexmedetomidine combined with morphine, nor did the addition of vatinoxan alter these effects.

6.3.2 Study limitations and considerations

One limitation of this study was its focus on a single, specific time point of noxious stimulation (testicle removal during castration) which occurred within a relatively short timeframe after anaesthetic induction. Previous research has shown that while vatinoxan does not reduce the initial level of sedation produced by dexmedetomidine, it does shorten its overall duration (Pypendop et al., 2019). This could be due to either minimal CNS penetration of vatinoxan over time, or more likely, an increased rate of dexmedetomidine absorption and metabolism. Supporting this hypothesis, vatinoxan has been shown to accelerate drug absorption when co-administered intramuscularly (Kallio-Kujala et al., 2018), likely by reducing vasoconstriction and increasing cardiac output, leading to a faster volume of distribution and elimination of dexmedetomidine (J. Honkavaara et al., 2012; Pypendop et al., 2017b). It is therefore hypothesised, but beyond the scope of this study's design, that vatinoxan may also reduce the duration of dexmedetomidine's analgesic effect.

A recent study investigating vatinoxan's effects on dexmedetomidine-ketamine anaesthesia found that while these combinations were effective for sedation, they did not consistently induce complete anaesthesia, as tested by applying a noxious stimulus

to the animal (Law et al., 2024). The study also observed that adding vatinoxan significantly shortened the time to first movement in response to noxious stimuli. Interestingly, none of the tested drug combinations, with or without vatinoxan, reliably prevented movement in response to noxious stimuli. The authors hypothesised that this could be due to suboptimal dosing or inappropriate drug combinations for complete antinociception or that the noxious stimulus used was stronger than typical surgical pain.

This finding is relevant to our study, as dexmedetomidine, whether alone or combined with morphine or vatinoxan did significantly alter EEG indices of nociception between groups, though they were significantly different to the high-dose morphine group. This suggests that dexmedetomidine and dexmedetomidine with lower dose morphine are not as potent of antinociceptive agents as morphine alone at a higher dose. Our study does not quantify how much antinociception dexmedetomidine provides above and beyond groups with no analgesia at all. We know that it is less than higher dose morphine, but we don't know how much more than no analgesia at all.

6.3.3 Future research directions

In this study, median frequency (F_{50}) EEG measurements were compared to each cat's baseline, allowing each animal to serve as its own control. However, even with this approach, increases in EEG frequencies were observed, and while these changes can be measured as a percentage of baseline, they do not provide a direct comparison to cats receiving no analgesia during the EEG monitoring phase. Including a no analgesia control group would be ethically feasible, as the cats are fully anaesthetised and do not

experience pain during the noxious stimulus. Appropriate analgesia could then be administered immediately after data collection, and before anaesthesia is discontinued and the cat regains consciousness.

The introduction of a novel drug inevitably raises many unanswered questions, even after it becomes available on the market. Ongoing research is essential to better understand the efficacy, interactions, and long-term effects of new drugs. Studies like this one play a crucial role in defining how new drugs function in combination with others and in refining clinical protocols. The EEG has proven to be a powerful research tool in evaluating the efficacy of analgesia. Further research using this method will be valuable in answering not only the remaining questions about vatinoxan and dexmedetomidine, but also to clarify new questions which arise as a result of completed research, such as in the above discussion points.

6.4 MULTIMODAL VS. SINGLE AGENT ANALGESIA

The study presented in **Chapter 4** demonstrated that locally administered bupivacaine, given as an incisional line block, provided intraoperative antinociception comparable to and postoperative pain control better than systemically administered morphine. When administered as single agents, however, both bupivacaine and morphine alone were unable to completely ameliorate EEG indices of nociception during canine castration. When bupivacaine was combined with systemically administered morphine, all EEG indices of nociception were abolished, showing significantly greater analgesic efficacy than either drug alone. In the postoperative period, both bupivacaine alone

and the combination of bupivacaine and morphine effectively controlled pain, with no dogs requiring rescue analgesia. While the combination of a local anaesthetic and a systemic opioid at the given dose proved superior, bupivacaine alone provided more effective perioperative analgesia than morphine, which has always been considered the prototypical analgesic (Morgan et al., 2017).

At the time this study was conducted, there were few published studies evaluating local analgesia for canine castration outside of intratesticular blocks, and no studies assessing the efficacy of incisional blocks alone for this procedure. Systemic opioids were the cornerstone of perioperative analgesia, while local anaesthetic blocks were rarely used in the veterinary teaching hospitals and were almost unheard of in clinical practice. Since the completion of this study, the veterinary teaching hospital has widely adopted multimodal analgesia protocols which incorporate local anaesthetics into perioperative pain management. The success of this approach has also led to the expansion of local analgesia use in more complex orthopaedic procedures and the implementation of advanced regional nerve blocks.

The study also emphasised the importance of assessing analgesic efficacy across the entire perioperative period rather than just taking a single snapshot of analgesic efficacy at a single time point. Morphine's relatively short duration of action (4-6 hours) raised concerns about its ability to produce sufficient postoperative analgesia. Conversely, while bupivacaine's prolonged duration of action made it advantageous for postoperative pain control, its localised effect was initially thought to limit its efficacy beyond the immediate surgical incision site during the intraoperative period. The results demonstrated that both drugs were equally effective at reducing nociceptive

input intraoperatively, but bupivacaine was superior for controlling postoperative pain. This highlights the need for a balanced, multimodal analgesic protocol that considers the full perioperative period rather than focussing solely on intraoperative pain management. The findings from **Chapter 4** underscore that bupivacaine, when administered as an incisional block, is far more effective than systemically administered morphine. Additionally, it offers multiple advantages as it is inexpensive, has fewer adverse side effects, and eliminates the logistical challenges associated with handling controlled substances.

A well-designed multimodal analgesic protocol should take the form of a checklist, rather than a recipe. While standardising key categories of analgesic agents, it must also allow for flexibility in drug selection and dosage to accommodate patient-specific factors, including the type of procedure, prior analgesic history, comorbidities, and individual pain thresholds. Based on the results of this study, non-opioid analgesics should serve as the foundation of multimodal analgesic regimens. These agents not only avoid the numerous side effects associated with opioids but also offer superior targeting of postoperative pain while minimising overall drug-related complications.

This study played a pivotal role in terms of changes around local anaesthesia instituted into the Massey Veterinary Teaching Hospital. The finding that a simple local incisional block, comprising 3-4 cm of injected bupivacaine, could effectively control both intraoperative nociception and postoperative pain was unexpected. Even more striking was its superior efficacy to systemic morphine. One possible explanation for this effectiveness is that local anaesthetic transforms the subcutaneous tissues into a slightly gelatinous layer, helping to retain the bupivacaine in place before the incision. Once

the tissue is incised, the bupivacaine "bathes" the underlying layers, likely extending its analgesic effect. However, it is unlikely that a significant amount of the local anaesthetic permeated the testicular parenchyma or spermatic cord. Given this, combining an incisional block with an intratesticular block could provide even more comprehensive analgesia. By ensuring local anaesthetic coverage across the entire surgical field, this approach may have the potential to block all nociceptive signals, making its effectiveness comparable to that observed in the combination group.

6.5 Innovative Approaches to Enhancing Analgesic Efficacy

Finding more effective and efficient ways to accomplish a task is always a welcome advancement. When these improvements not only enhance efficiency but also maintain or improve patient outcomes, they become even more exciting. Many such advancements in veterinary medicine originate from protocols used in human medicine. One promising example is transdermal drug delivery, though it comes with certain challenges.

Most transdermal systems are designed for human use, providing medication at precise doses that do not always correlate to those used in veterinary medicine. Additionally, these systems are tailored to human skin, which has very relatively uniform thickness, composition, and temperatures – factors that vary significantly across animal species and individual animals. Despite these limitations, the study in **Chapter 5** demonstrated

that transdermal buprenorphine patches could serve as a viable analgesic option for dogs undergoing orthopaedic surgery.

6.5.1 Challenges in the transdermal study

Conducting this study presented numerous challenges. It required recruiting a large cohort of patients referred to the teaching hospital from various veterinary clinics across New Zealand, each with different expectations of standards of care, and integrating them into a single specialist referral setting. The prolonged and continuous data collection period for each patient made standardisation with minimal researcher involvement difficult and further complicated the process, as pain scores needed to be collected throughout a continuous 48-hour period. It was therefore impossible to have a single blinded observer score each dog across all time points over a continuous 48 hour period.

Additionally, the study involved large breed dogs which required multiple large patches applied to shaved skin. Blinding was essential, necessitating careful concealment of whether a patch was present. A brown elasticated bandaged, layered under elasticated tape, effectively masked the treatment and maintained blinding, even if the tape underneath became slightly displaced.

One significant improvement for future studies would be adjusting drug dosage, and therefore patch size, based on body weight rather than using a standardised amount and size. Some dogs weighed nearly twice as much as others and still had the same dose of patches applied, which likely influenced drug efficacy and response.

6.5.2 Buprenorphine as a versatile analgesic

Buprenorphine is an opioid increasingly incorporated into routine analgesic protocols and is available in various formulations, including extended release, transmucosal, and intranasal formulations (Hiroko Enomoto et al., 2022; Michele Barletta et al., 2018; Porters et al., 2014). Its broad safety margin compared to other opioids makes it a safer option, reducing the risk of abuse and overdose (Yassen et al., 2008).

Using a transdermal buprenorphine patch offers several advantages. It allows animals to receive sustained opioid analgesia beyond their hospital stay, reducing stress and minimising handling, which can improve overall patient comfort and welfare. This was not seen in the cohort studied, as each dog needed to be pain scored and therefore interacted with on a regular basis. In a clinical setting, however, having a TDS for medication reduces the number of interactions between clinic staff and animals, allowing for more distance assessment, thereby decreasing stress in already anxious animals. Transdermal buprenorphine's effectiveness in managing pain following orthopaedic surgery suggests its potential use beyond less painful soft tissue procedures. Further research could explore its application in providing analgesia for animals experiencing cancer-related pain.

6.5.3 Assessing analgesic efficacy with objective vs. subjective measures

Determining the effectiveness of analgesia also depends on the assessment method, each with its strengths and limitations. The EEG provides an objective measure of

nociception by detecting neural responses to noxious stimuli. This facilitates the analysis of a measure of nociception before its conscious interpretation by the individual, making the EEG an excellent tool for intraoperative assessment of nociception. While this study did not use the EEG in assessing intraoperative nociception, the requirement for intraoperative fentanyl analgesia did not differ between the two groups. The emotional and sensory interpretation of pain is what makes it invariably more complex to assess than nociception.

Pain scales, on the other hand, offer valuable clinical insight by including this interpretation and evaluating how an animal consciously experiences pain. Yet, their accuracy depends on observer interpretation and can vary between individuals. While the EEG (as used in **Chapter 2 and 3**) provided an objective dataset, pain scales (as used in **Chapter 5**) allowed for prolonged pain assessment over time, albeit with some additional subjective variability.

Chapter 4 combined both the EEG and pain scale assessments, creating a more comprehensive approach to evaluating analgesic efficacy. This dual-method strategy revealed insights that would have been missed if either method were used in isolation. While each technique serves distinct purposes, integrating both can provide a more complete clinical picture, improving pain management strategies for veterinary patients.

6.6 CONCLUSION

The results of the research presented in this thesis provide evidence that:

6.6.1 Chapter 2

In cats undergoing castration, it was demonstrated that all three intramuscularly administered analgesics, including morphine, methadone, and buprenorphine, effectively prevented EEG changes associated with nociception. This finding enables clinicians to select the opioid that best suits their specific scenario without concern that it will compromise antinociceptive efficacy for their patient.

6.6.2 Chapter 3

In this study, vatinoxan successfully reduced the unwanted effects of dexmedetomidine without significantly compromising its antinociceptive properties. Additionally, high dose morphine provided significantly superior antinociception compared to dexmedetomidine alone or the combination of dexmedetomidine and morphine.

6.6.3 Chapter 4

In dogs undergoing routine castration, pre-surgical infiltration of bupivacaine as an incisional block, either alone or in combination with systemically administered morphine, significantly reduced intraoperative nociceptive input and postoperative pain compared to morphine alone. While systemically administered morphine was able to reduce intraoperative nociceptive inputs, it was unable to effectively control postoperative pain. Furthermore, the combination of morphine and bupivacaine was

not only able to reduce nociceptive inputs but completely obliterated all signals reaching the CNS.

6.6.4 Chapter 5

In the final study, transdermally administered buprenorphine effectively managed postoperative pain following tibial tuberosity advancement in dogs undergoing cranial cruciate ligament repair. This suggests that innovative ways of delivering pain relief, such as transdermal buprenorphine patches, may be valuable alternatives for pain management in dogs undergoing orthopaedic surgery.

These findings can be incorporated into veterinary analgesia clinical protocols in order to improve current analgesic techniques and reduce morbidity in surgical patients. They also serve as the basis for further research into veterinary analgesia aimed at improving and simplifying analgesia protocols for clinical general practice veterinarians using research techniques utilised in these studies.

6.7 FUTURE DIRECTIONS

The studies conducted in this PhD showcase a range of innovative techniques that are highly relevant to the field of pain management. By addressing multiple aspects of the perioperative period, these studies offer practical and accessible strategies for enhancing analgesia in surgical patients, making them applicable to a broad spectrum of veterinary practitioners. The research primarily focused on improving pain relief

through straightforward, cost-effective methods that utilise readily available drugs, explore drug interactions, and minimise both overall drug use and the reliance on controlled substances.

This work encompasses key areas of analgesia, including preoperative pain management, multimodal approaches, and pain control in both soft tissue and orthopaedic surgeries. With an emphasis on effective postoperative pain relief, the potential for future research applications is vast and virtually limitless. Rather than limiting the discussion to specific drugs, surgical procedures, or species, this research serves as a foundation for targeted investigations aimed at refining analgesic protocols. By identifying key areas where further study can optimise pain management, these findings contribute to the development of simple, yet effective, solutions that enhance patient welfare and advance veterinary analgesia.

The premedication period, for example, is a crucial stage in pain management. Providing adequate analgesia before nociceptive stimuli occur is essential for ensuring effective intraoperative nociceptive control and for preventing the development of chronic pain and central sensitisation (wind-up pain) following surgery. Further research should focus on utilising the EEG to gain deeper insights on how different drugs and drug combinations affect various species and their ability to block nociceptive inputs, ultimately improving analgesic strategies in veterinary medicine.

Another example is multimodal analgesia, which is a fundamental component of pain management protocols in small animal medicine. With the widespread integration of local anaesthetics into balanced multimodal analgesic plans, coupled with the increased

availability of advanced equipment such as ultrasound machines and nerve stimulators, the use of various local anaesthesia techniques is rapidly expanding. This growth has prompted numerous questions regarding the intraoperative analgesic efficacy of different nerve blocks, ranging from simpler techniques such as splash and intratesticular blocks to more advanced methods used in complex procedures. For example, quadratus lumborum (QL) and transverse abdominis plane (TAP) blocks are gaining interest for analgesia during ovariohysterectomy, while sciatic and femoral nerve blocks are being used more commonly in orthopaedic surgeries in the hind limb (Read et al., 2024). Investigating the effectiveness of these local anaesthetic techniques in small animal surgical procedures, as well as their interactions with current best practices, remains a critical and highly relevant area of research.

The bupivacaine and morphine study presented in **Chapter 4** demonstrated that an optimal multimodal analgesic plan can completely block all nociceptive inputs to the CNS that would otherwise cause pain in an awake dog. This was achieved using only two commonly available analgesics. The study also found that the local anaesthetic alone was more effective in controlling postoperative pain management than a systemically administered opioid. By enabling more targeted pain management, this approach reduces costs and minimises the reliance on controlled substances. Further research should focus on evaluating commonly used analgesic protocols and developing novel strategies that achieve complete nociceptive blockade and optimal analgesia while using the minimal necessary number and dose of drugs. This would help reduce side effects and improve patient outcomes. Furthermore, comparing other commonly used drug combinations to individual targeted agents might reveal that

increasing the complexity of analgesia protocols does not always enhance pain control, and in some cases, could result in more severe adverse effects.

While these areas of future research focus on the specifics of perioperative analgesia, they do account for other influential factors in decision-making around pain management. Before optimising drug protocols for surgical patients, it is first crucial to establish consensus among all clinicians regarding the necessity of postoperative analgesia. A significant concern is that some veterinarians fail to provide any form of postoperative pain relief for inherently painful procedures such as canine and feline ovariohysterectomies (Gates et al., 2020). Research into the motivational drivers behind drug selection, as well as the effectiveness of continuing education programmes on analgesia and pain prevention, would be highly valuable. Understanding these factors could inform the development of targeted educational initiatives aimed at promoting behavioural change among clinicians and improving pain management practices.

From a clinician's perspective pain management often ends at patient discharge. However, the patient's comfort and pain experience at home in a familiar environment must also be considered. Behavioural changes indicating postoperative discomfort can persist for several days after surgery and should be factored into assessments of analgesic efficacy and the design of postoperative pain control protocols (Väisänen et al., 2007). In human medicine, the caregiver is considered an important stakeholder when implementing analgesia plans for patients (Bhatia & Buvanendran, 2019). Additionally, despite extensive research, debates persist regarding the administration of post-incisional local analgesics (Hewson et al., 2006; Kalchofner Guerrero et al.,

2016), and even the necessity of postoperative analgesia itself (Hugonnard et al., 2004; Lascelles et al., 1999; Williams et al., 2005), though hopefully this has been resolved in recent years. This highlights the need for further investigation into the social and behavioural aspects of analgesia research, as these factors play a critical role in shaping clinical practice and should be integrated into conventional pain management studies.

6.8 REFERENCES

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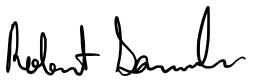
Student's signature:		Main supervisor's signature:	

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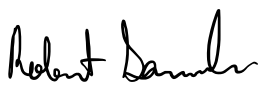
Student's signature:		Main supervisor's signature:	

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