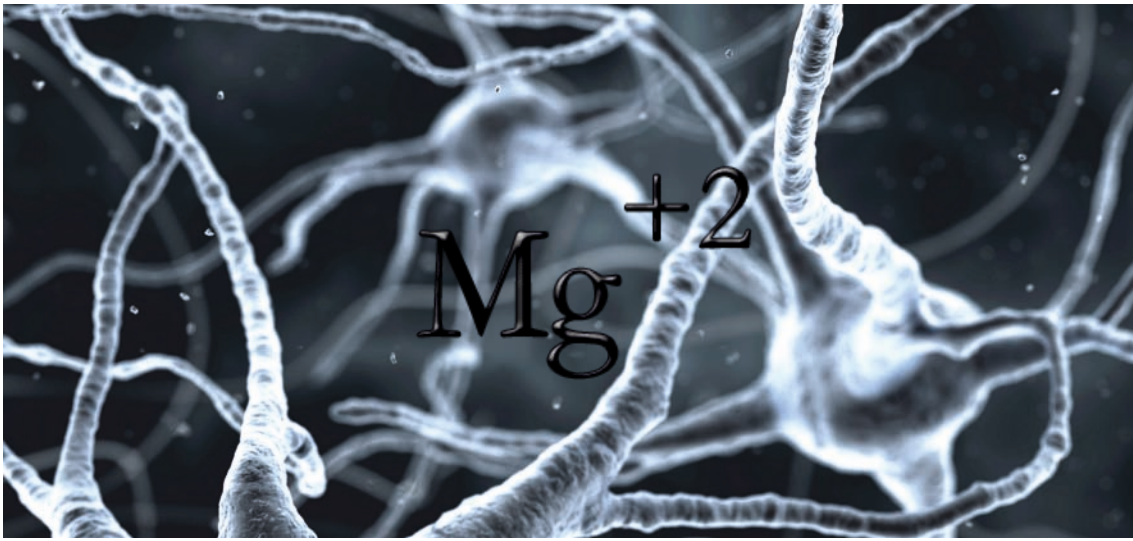
ANNE BAHRENBERG

Antinociceptive effects of epidural magnesium sulphate alone or in combination with morphine in dogs



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Antinociceptive effects of epidural magnesium sulphate alone or in combination with morphine in dogs

INAUGURAL DISSERTATION

for the acquisition of the doctoral degree

at the Faculty of Veterinary Medicine

Justus-Liebig-University Giessen, Germany

submitted by

Anne Bahrenberg

Veterinarian from Dortmund

Giessen 2014

With permission of the Faculty of Veterinary Medicine of
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Day of Disputation: 4th November 2014

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Declaration

I declare that I have completed this dissertation without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and referenced all text passages that are derived literally from or are based on content of published or unpublished work of others, and all information that relates to verbal communications. I have abided by the principle of good scientific conduct laid down in the charter of the Justus-Liebig-University of Giessen in carrying out the investigations described in the dissertation.

Giessen, Anne Bahrenberg

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My parents for their immeasurable support.

In Erinnerung an meine Groβeltern

Abbreviations

%	Percent
AB	Anne Bahrenberg
ACTH	Adrenocorticotrophic hormone
ACVA	American College of Veterinary Anesthesiologists
AMPA	Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BD	Brighton Dzikiti
Cm	Centimetre
CNS	Central nervous system
Co	Control treatment
CO_2	Carbon dioxide
Cp	Carpal pad
CRF	Corticotrophin releasing factor
CRI	Constant rate infusion
CSF	Cerebrospinal fluid
ER	Eva Rioja-Garcia
G	Gauge
gr	Gram
GABA	Gamma-aminobutyric acid
IQR	Interquartile range
IV	Intravenous
JCAHO	Joint Commission on Accreditation of Healthcare Organisations
kg	Kilograms
Mt	Metatarsus
mg	Milligrams
Mg	Magnesium treatment
Mg^{2+}	Ionized magnesium
$MgSO_4$	Magnesium sulphate
Mm	Magnesium and morphine treatment
Mo	Morphine treatment
mL	Millilitre

Na^+	Sodium
NK1R	Neurokinin 1 receptor
NMDA	N-methyl-D-aspartate
NSAID's	Non-steroidal anti-inflammatory drugs
PAG	Periaqueductal grey matter
PKC	Protein kinase C
PTH	Parathyroid hormone
sc	Subcutaneous
SD	Standard deviation
Th	Thorax
VSCC	Voltage sensitive calcium channel

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1 Introduction

Pain is defined as “an unpleasant emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (International Association for the Study of Pain, 1979).

The physiological consequences of pain involve endocrine and sympathetic nervous system activation (Gaynor and Muir, 2009). Non-adequately treated or untreated pain in animals in clinical settings is known to cause suffering, a decrease in immunity, and an increase in morbidity and mortality (Silverstein, 2009). Furthermore, constant pain can alter the pain transmission, modulation and perception, which can result in peripheral and central sensitization (Woolf, 2011). The main receptor responsible for central sensitisation at the level of the spinal cord is the N-methyl-D-aspartate (NMDA) receptor. This receptor is activated by the neurotransmitter glutamate and glycine. Magnesium is a natural antagonist on the NMDA receptor as the magnesium ion (Mg^{2+}) blocks the central canal of the ionic receptor inhibiting calcium influx and preventing neuronal depolarisation (Mayer *et al.*, 1984; Petrenko *et al.*, 2003).

Based on the Mg^{2+} interaction on the NMDA-receptor several investigations have focused on a possible analgesic effect mediated by systemic administration of magnesium.

The majority of studies in humans showed a decrease in inter- and postoperative opioid requirements in patients undergoing soft tissue and orthopaedic surgeries (Koinig *et al.*, 1998; Kara *et al.*, 2002; Unlügenç *et al.*, 2003; Hwang *et al.* 2009; Kogler, 2009; Gupta *et al*., 2011; Kiran *et al.*, 2011). However, other studies found no beneficial effect of magnesium when administered systemically to human patients undergoing soft tissue surgery (Wilder-Smith *et al.*, 1998; Zarauza *et al.*, 2000; Ko *et al.*, 2001; Tramèr and Glynn 2007; Sullivan *et al.*, 2012). In a systematic review of 14 human randomized clinical trials, it was concluded that there was no effect of systemic administration of magnesium on post-operative pain intensity and analgesic requirements (Lysakowski *et al.*, 2007).

Only a few studies have investigated the effect of systemic magnesium administration in animals. Systemic administration of magnesium reversed mechanical hyperalgesia induced by magnesium deficiency (Begon *et al.*, 2001) and reduced allodynia in rats (Xiao and Bennett, 1994). However, intravenous administration of magnesium failed to show a clear antinociceptive effect in dogs undergoing ovariohysterectomy (Rioja *et al.*, 2012).

The main site of magnesium action is at the level of the spinal cord, but the ability of serum magnesium to cross the blood-brain barrier remains unclear (McCarthy *et al.*, 1998; Ko *et al.*, 2001). Therefore, the neuraxial administration of magnesium has been investigated in human studies and animal trials.

Epidural and intrathecal administration of analgesic drugs, such as local anaesthetics and opioids, in humans and in animals are commonly used methods to achieve multimodal analgesia and anaesthesia (Grass, 2000; Valverde, 2008). The benefits of neuraxial administration include less systemic absorption, using lower doses, longer duration of the effects, which leads to fewer side effects and a superior analgesic effect compared to systemic administration of analgesic drugs (Bonath, 1986; Valverde, 2008). However, when local anaesthetics are administered neuraxially, this results in motor paralysis (Tranquilli, *et al.*, 2007) which may be undesirable. The administration of opioids in combination with other drugs that do not cause motor paralysis, such as magnesium, has been investigated.

In rats, magnesium administered intrathecally enhanced spinal anaesthesia induced by opioids (Kroin *et al.*, 2000) and delayed the development of opioid tolerance (McCarthy *et al.*, 1998). Furthermore, intrathecal magnesium in rats induced sedation and sensory block (Bahar *et al.*, 1996) and motor block (Karasawa *et al.*, 1998). The addition of magnesium to epidural local anaesthetics or ketamine induced a prolonged analgesic effect in goats (Bigham *et al.*, 2009), horses (Bigham and Shafiei, 2008), cattle (Dehghani and Bigham, 2009b) and sheep (DeRossi *et al.*, 2012).

In human clinical trials, magnesium administered epidurally or intrathecally in combination with opioids and/or local anaesthetics provides a longer duration of

analgesia (Buvanendran *et al.*, 2002; Ozalevli *et al.*, 2005; Yousef and Amr, 2010; Shukla *et al.*, 2011; Nath *et al.*, 2012), a post-operative opioid sparing effect (Arcioni *et al.*, 2007; Ouerghi *et al.*, 2011; Khezri *et al.*, 2012) and a decrease in post-operative pain scores (Sun *et al.*, 2012) in patients undergoing soft tissue and orthopaedic surgeries. However, epidural administration of magnesium showed no effect on postoperative pain and analgesia requirement in paediatric patients undergoing surgery (Birbicer *et al*., 2007)

The purpose of this study was to determine whether lumbosacral epidural administration of magnesium would have an antinociceptive effect on its own and whether it would enhance morphine antinociception in dogs. It was hypothesised that magnesium would produce an antinociceptive effect when administered alone and that it would enhance morphine antinociception when administered in combination.

2 Literature review

2.1 Pain

2.1.1 The definition of pain

The International Association for the Study of Pain defines pain as “an unpleasant emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (International Association of the study of Pain, 1979).

Pain occurs as a conscious awareness of discomfort resulting from injury, disease or emotional stress. A series of complex neurophysiologic processes are involved in creating the experience of pain. These neurophysiologic processes can be divided into four distinct components: transduction, transmission, modulation and perception. The biological function of pain is to warn the individual of a harmful situation and to avoid tissue damage by leading to motor action and a change in behaviour, which results in avoiding, escaping or destroying the factors responsible for the nociceptive stimulus (Gaynor and Muir, 2009).

Pain of high intensity or longer duration can alter the neurophysiologic processes and induce peripheral and central sensitisation which could result in pathological pain. Pathological pain has no biological advantage for the animal and can be seen as a disease on itself (Woolf and Ma, 2007).

Animals are unable to verbally communicate the pain experience; therefore, the assessment of pain in animals is challenging. However, there is scientific evidence that all vertebrates and some invertebrates can experience pain but the way pain is experienced and expressed depends on the degree of development of the central and peripheral nervous systems (Gaynor and Muir, 2009). Nevertheless, the uncertainty about the presence, quality and intensity of the pain experience by animals does not preclude the administration of adequate pain treatment (Hellyer, 2004).

Over the last decades, the understanding of pain and its appropriate treatment have been improved and pain management is becoming more and more an important component of good medical practice in human and veterinary medicine (Dohoo and Dohoo, 1996; Hellyer, 2002).

In human medicine, the Joint Commission on Accreditation of Healthcare Organisations (JCAHO) elevated pain to the fifth vital sign (together with temperature, respiration, pulse and blood force) in 2000. They state that "appropriate pain management is good medicine because it results in quicker clinical recovery; shorter hospital stays, fewer readmissions, and improved quality of life, leading to increased productivity" (Phillips, 2000).

In 2003, the American Animal Association followed the JCAHO's guideline and elevated pain to the fourth vital sign (along with temperature, pulse and respiration). The American College of Veterinary Anaesthesiologist (ACVA) acknowledged in their position paper on the treatment of animal pain (1998) that "animal pain is a clinically important condition that adversely affects an animal's quality of life". Furthermore, they state that "the prevention and alleviation of animal pain is an important and tenable therapeutic goal in veterinary medicine". Therefore, veterinarians are morally and medically obligated to address pain in animals and to avoid, assess and treat pain in their patients (Hellyer, 2002).

2.1.2 Peripheral nociception

The experience of pain involves a series of complex neurophysiologic processes, which can be divided into four distinct components: transduction, transmission, modulation and perception (Gaynor and Muir, 2009). In this chapter the process of transduction will be discussed.

Transduction is the transformation of a noxious stimulus into an electrical signal in a sensory nerve ending. Free nerve endings can respond to both low-intensity (non-painful) and high-intensity (painful) stimuli. Only the nerve endings that respond to high-

threshold stimuli are called nociceptors. Nociceptors can be found in skin, muscle, joints, periosteum and viscera. The type of noxious stimuli that the nociceptors can detect are thermal, chemical or mechanical stimuli or a combination of the three (Stoelting and Hillier, 2005).

In normal tissue, nociceptors are inactive until a noxious stimulus, exceeding the threshold of excitation, activates them and as a consequence an electrical excitatory signal (action potential) is generated and transmitted to the dorsal horn of the spinal cord through the nerve fibre (Stoelting and Hillier, 2005). The greater the intensity of the stimulus, the greater the number of electrical signals that are generated by the free nerve ending. Also, a stimulus of long duration produces a prolonged electrical signal (Gaynor and Muir, 2009).

The nerve fibres are divided on the basis of their conduction velocity into Aα, Aβ, Aδ and C-fibres, in order of greatest to lowest conduction velocity. Aα and Aβ-fibres are low-threshold fibres and respond to mechanical stimuli. These fibres are regarded as the ones responsible for transducing innocuous sensory information. Aδ-fibres can be nociceptors or not depending on their threshold of excitation. Approximately 25% of the Aδ-fibres are nociceptors (Gaynor and Muir, 2009) and aproximately 85% of the C-fibres are nociceptors (Gaynor and Muir, 2009).

The Aδ-fibre nociceptors can be sub-divided into three groups depending on the type of activating stimulus: high-threshold mechano nociceptors, mechano-heat nociceptors and mechano-cold nociceptors. The mechano-heat nociceptors are further divided into Type I and Type II (Djouhri and Lawson, 2004). Type I mechano-heat nociceptors have a higher heat threshold and a lower mechanical threshold than Type II. Type I mechano-heat nociceptors can also respond to chemical stimuli. Therefore, these Aδ-fibres can be referred to as polymodal nociceptors (Djouhri and Lawson, 2004.

The excitatory signals in Aδ-fibres are transmitted with a high discharge and a rapid conduction velocity (12 to 30 m/s) due to their myelinated axon. The activation of these fibres is responsible for the pricking sharp sensation associated with the initiation of pain (Tranquilli *et al*, 2007).

The C-fibre nociceptors are mostly high-threshold fibres that respond to more than one type of stimuli and they can also be referred to as polymodal. They are unmyelinated and respond with slow conduction velocities of 0.5 m/s to 2 m/s. The activation of these nociceptors is associated with a slow and burning type of pain, which is poorly localised and less specifically related to the stimulus (Stoelting and Hillier, 2005).

Silent nociceptors are nociceptors with a high threshold of excitation that are normally not activated (Woolf and Ma, 2007). C and Aδ-fibres can be silent nociceptors. However, this threshold can be reduced by tissue-inflammatory mediators such as prostaglandins and leukotrienes, which will lead to activation of these silent nociceptors in the presence of massive tissue inflammation. It is presumed that their activation is one mechanism for primary hyperalgesia, also called peripheral sensitisation (Woolf and Ma, 2007).

2.1.3 Central nociception

Central nociception consists of the neurophysiologic processes of transmission, modulation and perception in the central nervous system (CNS) (Stoelting and Hillier, 2005).

Central nociception commences when the primary afferent nerve fibres enter the spinal cord. The spinal cord is divided into white matter formed by the axons from projection neurons and grey matter formed by the cell bodies (Gaynor and Muir, 2009). The grey matter contains interneurons also known as gate cells and cell bodies from ascending neurons. The grey matter is divided into three anatomic regions: the dorsal horn, the intermediate zone and the ventral horn. Sensory information is received, transmitted and modulated in the dorsal horn (Gaynor and Muir, 2009).

The grey matter is further subdivided into ten Laminae based on similar function of the neuronal cells. Laminae I to VI are located in the dorsal horn and participate in pain transmission and modulation (Gaynor and Muir, 2009).

Lamina I plays an important role in pain sensation. It receives sensory input mostly from Aδ-fibres in the skin, musculoskeletal system and viscera and contains specific nociceptive neurons, wide-dynamic range neurons as well as interneurons. Lamina II is also known as *substantia gelatinosa* and is composed of mostly C-fibres and a large number of interneurons. Lamina II integrates sensory information together with Lamina I. Due to the large number of interneurons in Lamina II, it is believed that this lamina plays a key role in the transmission and modulation of pain. Laminae III to VI, also known as *nucleus proprius*, receive tactile, thermal and mechanical sensory information from the periphery and furthermore they receive descending information from the brain. Lamina X is located around the central canal of the spinal cord and receives and transmits sensory information to the brain. Finally, Laminae VII to IX are located in the intermediate and ventral zones of the spinal cord and are not involved in pain transmission (Gaynor and Muir, 2009).

The primary sensory neurons enter the spinal cord through the dorsal root, where they synapse with secondary afferent neurons. Two different types of secondary afferent neurons are described: nociceptive specific neurons and second-order wide dynamic range neurons (Tranquillie *et al*. 2007). Nociceptive specific neurons are dedicated purely to nociceptive stimuli and in consequence the ascending stimulus results in a more discriminative nociception. In contrast, wide dynamic range neurons are stimulated by noxious and non-noxious sensory stimuli and as a result the conveyed nociception is less discriminative. The wide dynamic range neurons are also characterised by reacting to afferent noxious stimuli from the skin and the viscera and this results in the phenomenon of “referred pain”. Referred pain occurs when a noxious stimulus received from the viscera is perceived as having originated in the skin (Tranquillie *et al*. 2007).

Interneurons play an important role in modifying and regulating sensory information. Melzack and Wall in 1965 developed a concept of pain modulation in the spinal cord that they called “the gate control theory” (Melzack and Wall, 1965), although this theory seems to be somewhat inaccurate, it is still used to understand the modulation of pain. This theory implies that afferent sensory impulses from nerve fibres entering the spinal cord underline a modulating feedback in the *substantia gelatinosa* mediated by interneurons. Nociceptive C- and Aδ-fibres and non-nociceptive A-fibres connect with

wide dynamic range neurons. The non-nociceptive neurons additionally synapse with interneurons and the interneurons have inhibitory properties on the wide dynamic range neurons. This results in less activation of the wide dynamic range neurons and subsequently of the projection neurons. Summarising, non-nociceptive nerve impulses "close the gate" for nociceptive stimuli (Melzack and Wall, 1965).

The transmission and modulation of pain perception in the spinal cord is regulated by a multitude of neurotransmitters. They can be divided into excitatory, inhibitory and facilitating neurotransmitters. The most important neurotransmitters are amino acids. The dicarboxylic amino acids glutamate and aspartate are the most important excitatory neurotransmitters while the monocarboxylic amino acids like gamma-aminobutyric acid (GABA), glycine and alanine act as inhibitory neurotransmitters (Gaynor and Muir, 2009).

Glutamate and aspartate have excitatory effects and they act on multiple receptor subtypes. The receptors are subdivided into ionotropic receptors (i.e. ligand-gated ion channels) and metabotropic receptors (i.e. G-protein coupled receptors). The ionotropic receptors are named according to their specific agonists in vitro. Subsequently ionotropic receptors can be divided into: NMDA-receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-receptors (AMPA) and kainate-receptors (Zimmermann, 2004).

The NMDA-receptor is the main receptor in terms of transmission of nociceptive stimuli by afferent neurons in the central nervous system and is widely spread at the level of the spinal cord and the brain (Petrenko *et al.*, 2003). The excitatory transmitter glutamate binds to the NMDA-receptor site and the co-transmitter glycine binds to the modulatory site of the receptor. Both binding sites must be occupied for the channel to open. The activation of the NMDA-receptor results in an influx of calcium ion causing post-synaptic depolarisation and triggering a cascade of events including activation of the protein kinases (Petrenko *et al.*, 2003). Mayer et al. discovered in 1984 that the NMDA receptor is normally occupied by Mg^{2+} at physiological extracellular concentrations, which causes blockade of the ion channel, and that this Mg^{2+} block is voltage dependant (Mayer *et al.*, 1984). Mayer et al. also showed that a decrease in the extracellular Mg^{2+} concentration results in a reduction of the voltage-sensitivity of the receptor (decreased voltage-threshold of activation). This activation of the NMDA-receptor caused by Mg^{2+} deficiency induces a state of hyperalgesia (Begon *et al.*, 2001). Several exogenous

antagonists of this receptor are known. The anaesthetic and analgesic action of ketamine is attributable to its antagonistic effect on this receptor (Petrenko *et al.*, 2003).

The NMDA-receptor consists of three subunits: NR1, NR2 (types A, B, C and D) and NR3 (types A and B). At least the subunit NR1 and one of the NR2 subunits are required to form the receptor. Different types of subunits NR2 have been shown to influence the pharmacological properties of the receptor. The affinity for agonist and antagonist drugs is determined by the type of subunits forming the receptor. The sensitivity for blockade by Mg^{2+} is also influenced by the subunits type and it is exaggerated by type NR2A and NR2B (Petrenko *et al.*, 2003). The NR3 subunit can be co-expressed and influences the receptor activity. When this subunit is present, the receptor turns into an excitatory glycine receptor, unaffected by glutamate, impermeable to calcium and resistant to Mg^{2+} block. The role of the NR3 subunit on the pain mechanism has not been investigated yet (Petrenko *et al.*, 2003).

The NMDA-receptor does not participate in normal pain transmission as it is normally blocked by Mg^{2+} (Figure 1). Constant afferent input alters the NMDA-receptor properties mediated by the protein kinase C (PKC) and the tyrosin kinase, resulting in removal of the Mg^{2+} block; therefore, calcium influx occurs (Figure 2). The increase in postsynaptic calcium concentration leads to a PKC activation and thus exponentiation of the NMDA-receptor response due to phosphorylation (Figure 3) (Petrenko *et al.*, 2003).

Figure 1: Normal synaptic transmission: Presynaptic calcium influx through voltage sensitive calcium channel (VSCC) results in glutamate release. Glutamate activates AMPA-receptors leading to sodium influx into the postsynaptic dorsal horn neuron extrapolated from, Petrenko *et al.*, 2003.

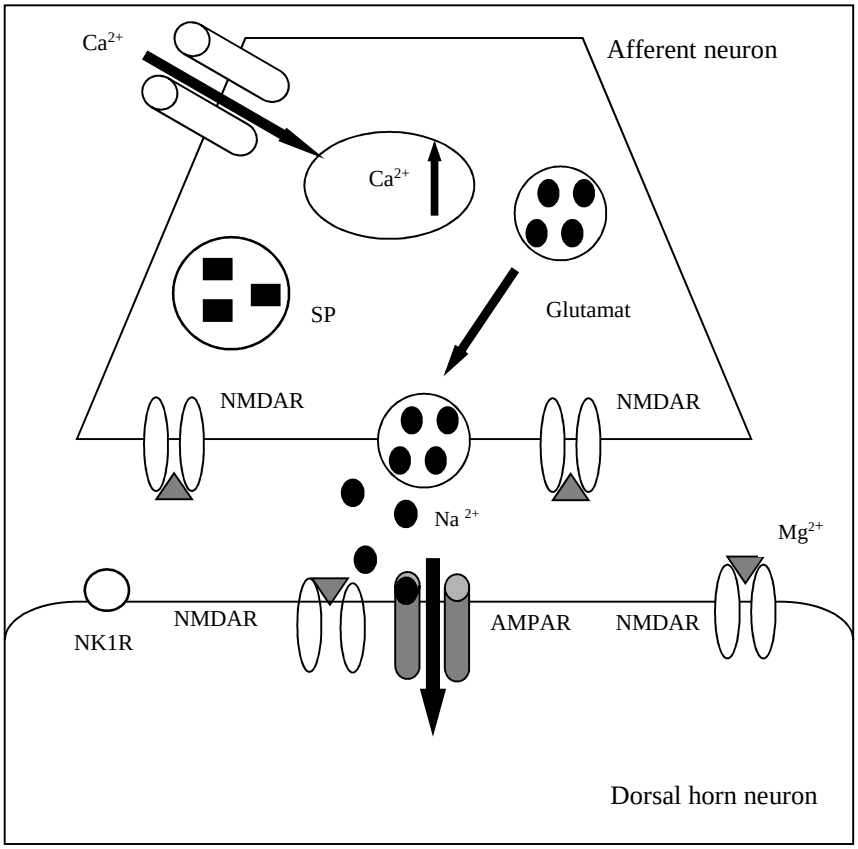


Figure 2: Constant afferent input leads to constant presynaptic Calcium influx. glutamate and substance P (SP) release increase. SP leads to neurokinin 1 receptor activation (NK1R), whereas glutamate activates AMPA-receptors leading to Sodium influx into the postsynaptic dorsal horn neuron. The depolarisation of the postsynaptic membrane leads to removal of the Mg^{2+} block of the NMDA-receptors and calcium influx occurs. NMDA-receptor becomes activated and facilitate the response extrapolated from, Petrenko *et al.*, 2003.

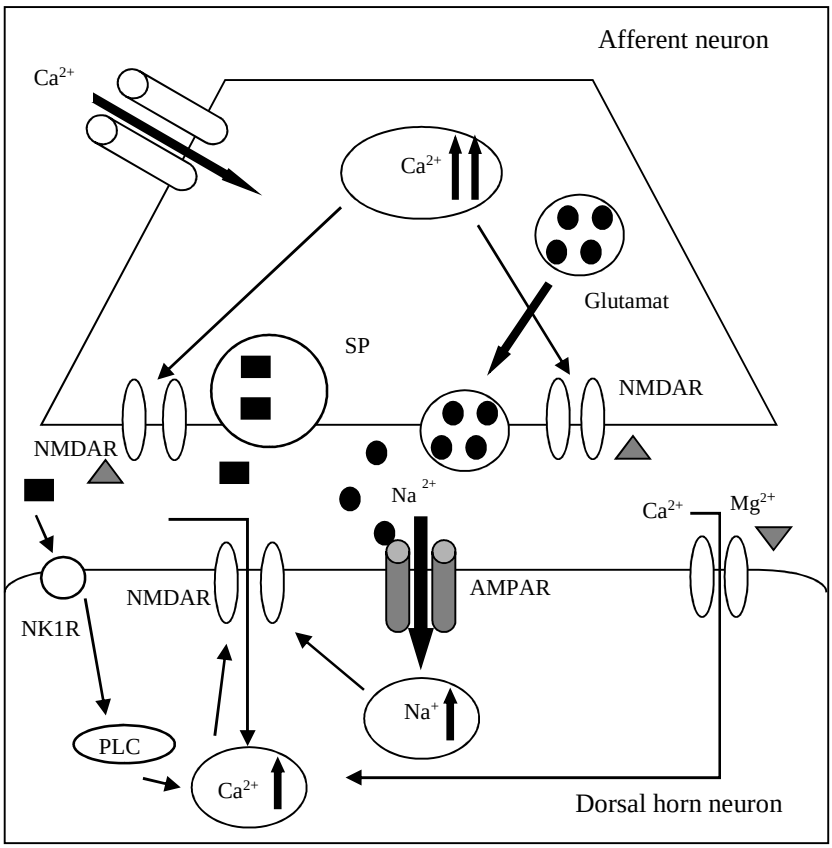
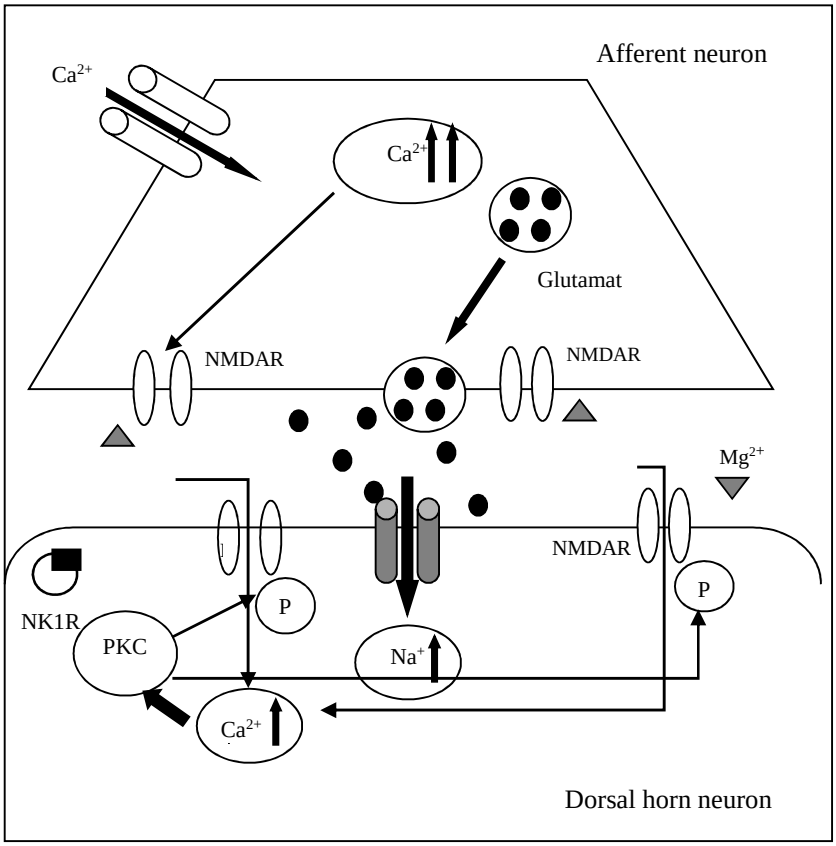


Figure 3: Calcium influx leads to posttranslational modification of the NMDA-receptor, where PKC phosphorylates the NMDA-receptor leading to a prolonged channel opening time and decrease in voltage dependent Mg^{2+} block extrapolated from, Petrenko *et al.*, 2003.



Pain projection at the level of the spinal cord includes multiple nociceptive pathways and their functions are overlapping and complex (Tranquilli *et al*. 2007). The spinal cord can be divided into the following conveying ascending tracts: spinothalamic tract, spinoreticular tract, spinohypothalamic tract and spinomesencephalic tract.

The final perception of pain occurs at the level of the brain, which results from the integration, processing and recognition of the ascending information. Different areas of the brain are involved in these processes and information is transmitted through multiple pathways to ensure an adequate input into the CNS. These multiple pathways are called “parallel processing” (Gaynor and Muir, 2009).

The ascending information from the spinoreticular tract terminates in the reticular formation. This area is composed of cores (most important is the *raphe nucleus*) extending from the medulla oblongata to the diencephalon and is involved in consciousness as well as mediation of sensory, autonomic and motor functions (Gaynor and Muir, 2009). The reticular formation sends collaterals to other nuclei which are located in the brainstem, hypothalamus, thalamus and cerebral cortex (Lamont *et al.*, 2000).

The hypothalamus is responsible for processing sensory and hormonal information (Desborough, 2000). It plays a key role in emotional reactions and vegetative responses. Activation of the hypothalamus leads to sympathetic nervous system and pituitary responses causing the release of catecholamines and glucocorticoids.

The limbic system contains cores in the cortical and subcortical regions. Some autonomic functions such as thermoregulation and respiration are controlled in this area in addition to emotional responses composed of physiological, cognitive and behavioural changes (Tranquilli *et al*. 2007). Deregulation or over activity of the limbic system can lead to aggression, fear, anxiety or depression (Silverstein, 2009).

The cerebral cortex performs the higher neurological functions and nociception in this area is described as cognitive-evaluative, which is affected by experience, learning, attention and memory. Complex behaviour patterns are attributable to this structure (Tranquilli *et al*. 2007).

The perception of pain is also dependant on the activation of the descending pain pathway. The periaqueductal grey matter (PAG) is a core of grey matter located in the midbrain and is a key structure in ascending and descending control of sensory information (Lamont *et al.*, 2000). The PAG receives input from higher brain centres such as the cerebral cortex, the limbic system and the hypothalamus. The PAG is known to have a high density of opioid receptors and its stimulation results in release of endogenous opioids and enkephalins (Tranquilli *et al*. 2007).

The PAG synapses with the *nucleus raphe magnus* located in the reticular formation, from which adrenergic and serotonergic nerves descend to the spinal cord and transmit inhibitory signals mediated by the PAG. The endogenous release of opioids can induce inhibitory and analgesic effects in the brain and at the level of the spinal cord (Lamont, 2008).

2.1.4 Classification of pain

Pain can be categorized based on different aspects, such as according to underlying disease (e.g. arthritis, cancer), anatomy (e.g. back, orthopaedic), general region (e.g. superficial, deep), duration (e.g. acute, chronic) and intensity (mild, moderate, severe) (Gaynor and Muir, 2009). However, these categories are purely descriptive and they do not explain the underlying mechanism responsible for the pain. Additionally, these categories do not provide any therapeutic advice.

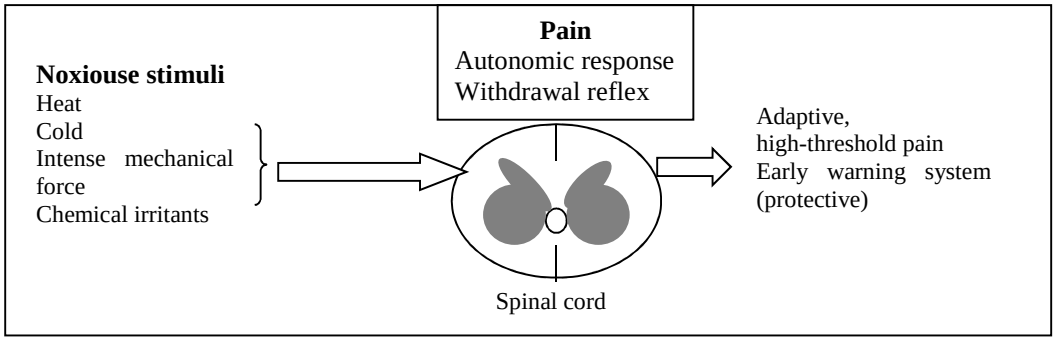
Amongst experts, pain is categorised most often according to the mechanism responsible for its production.

One common classification of pain is into physiological pain, caused by noxious stimuli, pathophysiological pain, caused by a change in organ function (e.g. due to inflammation) and neuropathic pain, caused by damage of the nervous system (Pfannkuche, 2008).

Another classification of pain is into nociceptive pain and neuropathic pain. Nociceptive pain is further subdivided into visceral pain and somatic pain. Visceral pain is described as diffuse, poorly localized and often causing autonomic nervous system activation. Somatic pain originates from the skin and musculoskeletal system and is characterized as a sharp, pricking and well-localized pain. Neuropathic pain involves damage of the peripheral or central neural pathways and it is described as a burning type of pain (Stoelting and Hillier, 2005).

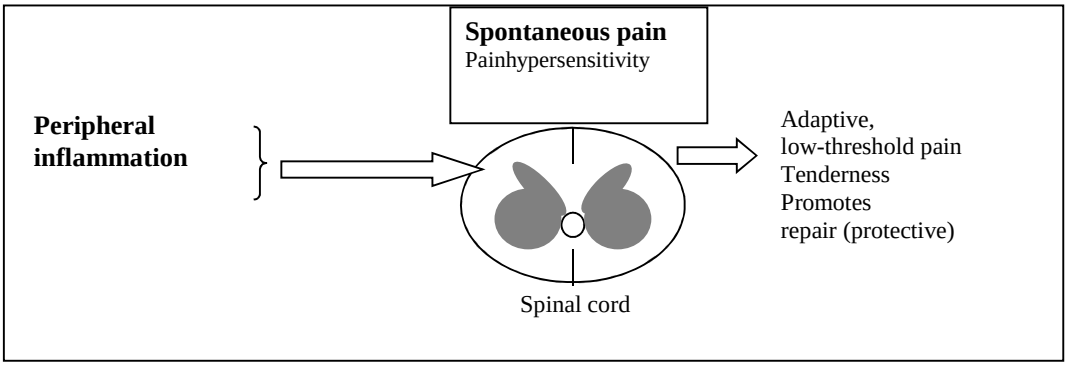
Woolf classified pain into adaptive and maladaptive pain based on its biological function (Woolf, 2010). Adaptive pain includes nociceptive pain and inflammatory pain. Nociceptive pain functions as a warning system and evokes an immediate response such as a withdrawal reflex, allowing the animal to avoid the potential damaging stimuli (Figure 4).

Figure 4: Nociceptive pain extrapolated from, Woolf, 2010.



Inflammatory pain is also adaptive and protective and appears after tissue damage. It leads to an increase in sensitivity and results in decreased movement and avoidance of further damage of the tissue, thereby promoting and assisting the healing process (Figure 5).

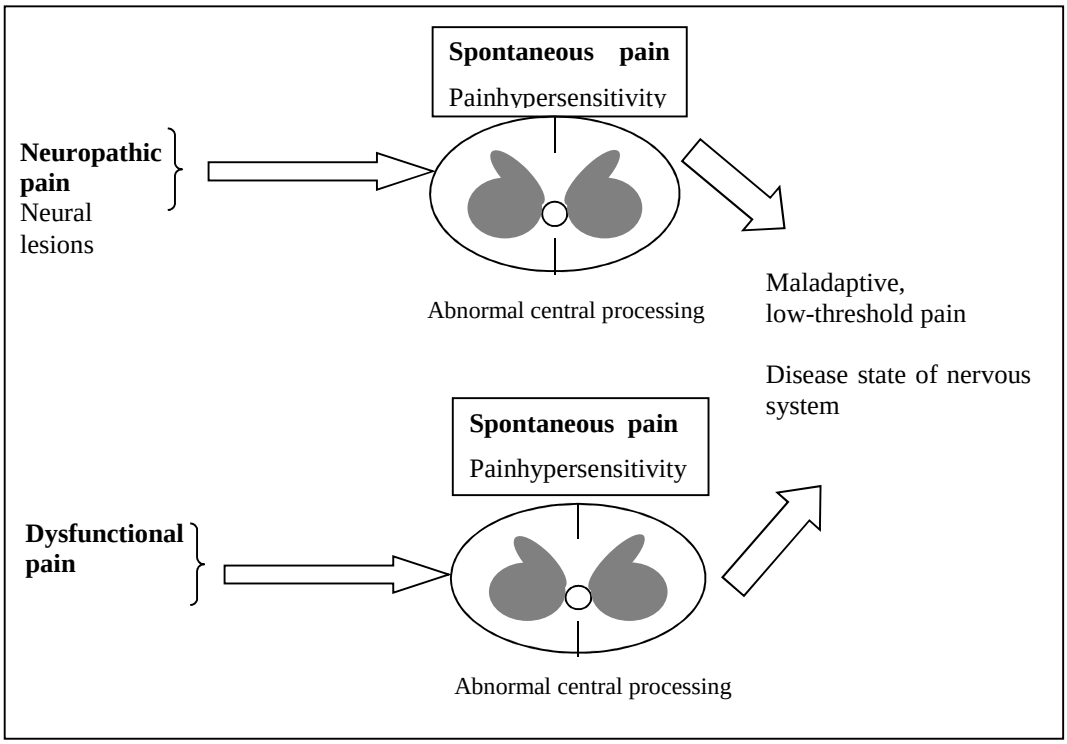
Figure 5: Inflammatory pain extrapolated from, Woolf, 2010.



In contrast, maladaptive pain results from an abnormal function of the central nervous system and it is called pathological pain. Woolf subdivided pathological pain into neuropathic and dysfunctional pain. Neuropathic pain originates from damage to the nervous system itself, whereas dysfunctional pain is largely evoked by non-noxious

stimuli (e.g. touch), which induces an exaggerated and excessive response in the nervous system (Figure 6).

Figure 6: Pathological pain extrapolated from, Woolf, 2010.



From a therapeutic point of view, Woolf's classification of pain seems to be the most useful.

Neuropathic pain originates from injury of the nervous system and neuroplastic changes in it (Woolf, 2000). Neuropathic pain has no biological advantage. The underlying pathophysiological mechanism is not clearly understood, but a lack of modifiability and plasticity of the nervous system is considered to be responsible (Woolf, 2010; Tranquilli *et al.*, 2007).

Peripheral sensitisation occurs due to a change in the chemical milieu resulting from the disruption of cells, secretions of inflammatory cells, mast cells degranulation and induction of enzymes (Woolf and Ma, 2007). A variety of substances have been identified but new substances are still being identified. Well-studied substances are

kinins, amines, prostaglandins, cytokines, chemokines and growth factors. This so-called "inflammatory soup" causes a decrease of the nociceptor threshold of activation and leads to an exaggerated response to noxious stimuli (Woolf and Ma, 2007). These changes result in a condition called "primary hyperalgesia" (Tranquilli *et al.*, 2007). Furthermore, silent nociceptors, with are normally inactive, are also recruited and innocuous stimuli start being sensed as noxious (Woolf and Ma, 2007). The hyperexcitability of the nociceptors induces spontaneous depolarisations originating in the axon or in the cell body in the absence of a sensory stimulus. This change in sensitivity can lead to spontaneous pain in the absence of any noxious stimulus (Woolf and Ma, 2007). This condition is termed "allodynia" (International Association for the Study of Pain, 1979).

Central sensitisation is triggered by a high discharge rate and long duration of excitatory input in the spinal cord (Woolf, 2011). It is characterized by an increased synaptic efficacy that lasts longer than the duration of the conditioning stimulus. This excitatory input leads to synaptic plasticity characterised by changes in the microglia, gap junctions, membrane excitability and gene transcription. The threshold, kinetics and activation of the receptors and nerve terminals in the spinal cord change, resulting in an increase in pain transmission and perception (Woolf, 2011). The NMDA-receptor has been shown to play a key role in the central sensitization process (Petrenko *et al.*, 2003; Zimmermann, 2004). In normal synaptic transmission, the post-synaptic NMDA-receptor is voltage-dependently blocked by extracellular Mg^{2+}. The increased excitatory input leads to post-synaptic depolarisation mediated by glutamate, which results in sodium influx through the AMPA-receptor. A strong and prolonged post-synaptic depolarisation reduces the voltage-dependent Mg^{2+} block of the NMDA-receptor. Calcium influx through the NMDA-receptor into the postsynaptic cells occurs. Additionally, intracellular calcium leads to activation of the PKC and this mediates an enhanced opening of the NMDA-receptor. Pre-synaptic NMDA-receptors have also been identified. Their activation results in substance-P release, enhancing the excitatory transmission at the level of the post-synaptic membrane (Petrenko *et al.*, 2003). These changes result in a condition called "secondary hyperalgesia" (Woolf, 2011).

Peripheral and central sensitisation, which lead to primary and secondary hyperalgesia and may also result in allodynia, are likely to play an important role in the development of neuropathic pain (Woolf, 2011).

The term “wind up” is used to describe the central plasticity stimulated by a constant and rapid activation of C-fibres, which leads to an increase in action potential firing over the course of stimulus (Tranquilli *et al.,* 2007). Wind up has been associated with activation of the NMDA-receptor (Gaynor and Muir, 2009).

Central sensitisation and “wind up” result in pain perception causing continuing and severe pain (Gaynor and Muir, 2009).

2.1.5 Physiological consequences of pain

The physiological effects of pain on the body are multiple and they aim to protect and prepare the organism against the insult by mobilising energy sources. Pain results in behavioural modulation, activation of the sympathetic and neuroendocrine systems (Gaynor and Muir, 2009) as well as immunological and haematological changes. The physiological changes caused by injury and trauma are referred to as stress response (Desborough, 2000).

The behavioural modulation depends on the species and comprises both a learned and a memory component. Behavioural changes often seen in dogs during pain and fear are avoidance, immobility and aggressive behaviour. The goal is to avoid and escape tissue damage and to maintain homeostasis (Gaynor and Muir, 2009). The learned and memory components are processed in the cerebral cortex (Tranquilli *et al.,* 2007), while the limbic system and the hypothalamus are responsible for the fear and anxiety, as well as the behavioural response (Tranquilli *et al.,* 2007).

The main change in endocrine function is caused by the neuroendocrine axis and the activation of the sympathetic nervous system. Afferent impulses stimulate the secretion of corticotrophin releasing factor (CRF) and vasoactive intestinal peptide by the

hypothalamus, which leads to an increase in pituitary secretion of adrenocorticotropin hormone (ACTH), propriomelanocortin, growth hormone, vasopressin and prolactin. Propriomelanocortin is the link substrate between the pituitary-adrenal axis and the endogenous opioid system. Propriomelanocortin is metabolised to ACTH and β-endorphine. Additionally, CRF stimulates catecholamine and endogenous opioid release from the adrenal medulla (Tsigos and Chrousos, 2002).

Adrenocorticotropic hormone increases the release of glucocorticoids, in particular cortisol, from the adrenal cortex. Cortisol is known as the key mediator of the stress response. It creates a catabolic state by stimulating gluconeogenesis, increasing protein breakdown, enhancing the sensitivity of fat tissue towards the action of lipolytic hormones and causing insulin resistance. The consequence of these effects is to ensure glucose delivery to the brain and to provide energy sources. The ability of cortisol to stimulate the adrenomedullary secretion of catecholamines enhances the stress response and aids in maintaining cardiovascular stability. A negative feedback is mediated from the glucocorticoids on ACTH production but it seems to be ineffective in trauma due to surgery (Desborough, 2000). Another beneficial effect of cortisol is to prevent an overreaction of the immune system by inhibiting the migration of macrophages and neutrophils into inflamed tissue and by decreasing the amount of inflammatory mediators such as prostaglandins (Tsigos and Chrousos, 2002). There is a direct relationship between the amount of ACTH and cortisol release and the degree of trauma (Weissmann, 1990).

The additional release from the pituitary gland of growth hormone, vasopressin and prolactin contribute in different ways to the hormonal changes that occur due to the stress response (Desborough, 2000).

Growth hormone also known as somatostatin is secreted from the anterior pituitary. Most of the effects are manifested through increased transcription of the insulin-like growth factors in a variety of tissues. Insulin-like growth factor creates an anabolic effect by enhancing protein synthesis, inhibiting protein breakdown, promoting lipolysis and it also has an anti-insulin effect. As a result, the plasma becomes hyperglycaemic and the glucose dependent tissues (e.g. brain) can be adequately supplied (Desborough, 2000). Different studies have shown that there is a decrease in insulin and an increase in

glucagon related to surgeries. The major effect is an increase in gluconeogenesis (Weissman, 1990).

Vasopressin is also known as antidiuretic hormone. Its secretion is stimulated by changes in plasma osmolality and also influenced by changes in blood force and blood volume during stress and fear (Weissman, 1990). Vasopressin activates the vasopressin-2 receptors in the renal tubules causing an increase in number of aquaporin water channels. Renin is secreted from the juxtaglomerular cells and angiotensin II production increases, which leads to a release of aldosterone. Aldosterone leads to increased water reabsorption due to Na^+ reabsorption in the kidney. The main effect of vasopressin and aldosterone is an increase in water absorption and thereby a stabilisation of the body fluid volume (Desborough, 2000).

The activation of the sympathetic nervous system results in release of adrenaline by the adrenal medulla. Additionally, noradrenaline is released from the sympathetic nerve terminals and spills over into the plasma. The major catecholamine effects are related to the cardiovascular system causing tachycardia, hypertension, an increase in cardiac output, with a consequent increase in myocardial oxygen consumption, to provide adequate perfusion to the body tissues and organs (Tsigos and Chrousos, 2002). Additionally, adrenaline increases gluconeogenesis, glycogenolysis and lipolysis, decreases insulin release and causes peripheral insulin resistance (Weissman, 1990). The increase in ventilation and heart rate due to the sympathetic response can cause major problems in patient with compromised cardiovascular function, when they are not able to compensate (Weissman, 1990).

Damaged tissue due to injury or infection leads to activation of cytokines. This group of proteins include the interleukins and the tumour necrosis factors. They are produced from leucocytes, fibroblasts, macrophages, monocytes and endothelial cells and play a major role in the acute inflammatory response. Their local effects include chemotaxis, which stimulates migration of lymphocytes, monocytes and neutrophils to inflamed tissue, while their systemic effects include fever, activation of the acute phase response and an increase of ACTH release from the pituitary gland. After surgery the major cytokines are interleukin-1, tumour necrosis factor-α and, in a secondary phase of

cytokine release, interleukin-6. The stimulation of ACTH leads to an increase in cortisol, which inhibits cytokine expression. The cortisol plasma concentration during major surgery in sufficient to depress the cytokine concentration by the negative feedback mechanism (Sheeran and Hall, 1997; Desborough, 2000).

To summarise, the stress response results in a catabolic state causing an increase in blood glucose to mobilize energy in order to supply the damaged tissue (Tranquilli *et al.*, 2007). This stress response and the accompanied physiological changes are meant to be acute and of limited duration. However, in clinical settings the evoked stress response due to pain and trauma is argued to be unnecessary (Desborough, 2000) as it can lead to weight loss and muscle wastage as well as decreased immunity due to high plasma cortisol levels (Tranquilli *et al.*, 2007). This stress response has been shown to increase mortality and morbidity in the clinical setting (Morrison *et al.*, 2003).

Hyperglycaemia produced by multiple hormonal interactions has been related to a higher mortality rate in critical ill patients after major surgeries (Egi *et al.*, 2009). It is controversial if the hyperglycaemia is only a reflection of the severity of the illness or if it may cause harm on its own. The potential underlying mechanisms of hyperglycaemia-induced mortality include promotion of sepsis, delayed wound healing and neuromyopathy (Kavanagh and McCowen, 2010).

Moreover, excessive trauma and stress can cause widespread release of endogenous mediators such as cytokines that can subsequently result in the systemic inflammatory response syndrome, multiple organ failure and death (Silverstein, 2009).

With analgesic and anaesthetic agents the stress response related to surgery or medical conditions can be controlled (Weissmann, 1990; Desborough, 2000).

2.2 Pain management

Analgesic drugs can be divided into non-steroidal anti-inflammatory drugs (NSAIDs), α_2-adrenoreceptor agonists, opioids, local anaesthetics and others (Gaynor and Muir, 2009). These drugs act on different steps along the pain pathway.

Monotherapy with only one agent is often not sufficient to achieve adequate analgesia in clinical settings; therefore, multimodal pain therapy has become the standard practice in human and veterinary medicine. Multimodal analgesia consists of the administration of more than one analgesic drug that acts at different levels on the pain pathway (Hellyer, 2004). An advantage of this multimodal therapy is that pain is better controlled because of additive or synergistic analgesic effects of the drugs. Another advantage is that lower doses of each drug are required, thereby reducing or even eliminating the potential adverse effects (Tranquilli *et al*., 2007; Lamont, 2008). The choice of drugs should be based on the mechanisms responsible for the pain pathogenesis (Woolf, 2000). Systemically administered drugs and regional anaesthetic techniques with local anaesthetics are often combined.

2.2.1 Epidural analgesia

A commonly used regional analgesic technique in veterinary medicine is the epidural administration of drugs (Bonath *et al.*, 1984; Valverde, 2008). Epidural analgesia using local anaesthetics has been used in veterinary medicine since the 1950s. After the development of safer general anaesthetic agents epidural techniques were displaced, but with the discovery of the opioid action on the spinal cord in the 1980s (Yaksh and Noueihed, 1985), there was a re-emergence of epidural techniques as analgesic effects could be achieved using opioids without the side effects of motor paralysis due to administration of local anaesthetics (Valverde, 2008). Epidural techniques are now widely used for intra- and postoperative pain control and new drugs are being investigated (Hansen, 2001).

Epidural administration of drugs reduces the need for systemic analgesic drugs (Torske and Dyson, 2000; Jones, 2008) and thereby reduces the development of systemic

adverse effects. The analgesia achieved by an epidural injection has been proven to have a faster onset of action, a higher potency and a longer duration compared to the systemic administration of the same drug (Bonath, 1986). By inhibiting the pain-pathway at the level of the spinal cord, central sensitisation can be avoided and the stress response is markedly decreased (Woolf, 2011). Furthermore, epidural analgesia leads to a better postoperative outcome (Grass, 2000; Rodgers *et al.*, 2000; Jones, 2008).

Before performing an epidural injection the patient should be carefully selected. Contraindications for epidural injections include septicaemia, coagulation disorders, trauma or infection in the area of injection and deformity of the anatomy (Hansen, 2001; Valverde, 2008)

Epidural injections are commonly performed in the lumbosacral intervertebral space in small animals as it provides the largest access to the spinal canal (Jones, 2001). The dorsal intervertebral space in medium size dogs is approximately 2-4 mm. To insert a spinal needle in the epidural space the following anatomic structures need to be pierced: the skin, the subcutaneous fascia, the interspinous ligament and the prominent interarcuate ligament or *ligamentum flavum*. The meninges of the spinal cord from outermost to innermost are the dura mater, the arachnoidea and the pia mater. The dura mater is divided into an external and internal laminae. The external lamina is represented by the periosteum of the vertebral canal and only the internal lamina surrounds the spinal cord. The epidural injection is performed between the two laminae, which actually represents the intradural space. The epidural space is filled with fat to prevent the spinal cord from injury. The next meningx surrounding the spinal cord is the arachnoidea, which contains the cerebrospinal fluid (CSF). The arachnoid mater is named after its spider web appearance provided by trabeculae and fibrous tissue, which are in close contact with the pia mater. Injection of drugs into the fluid filled subarachnoid space is known as subarachnoid, spinal or intrathecal injection. The meningx closest to the spinal cord is the pia mater, which contains blood vessels to supply the spinal cord with nutrition and oxygen (Valverde, 2008).

In foetuses, the spinal cord extends as far as the sacrum. During growth, it shrinks within the vertebral canal as the growth of the vertebrae is faster than the spinal cord

growth. In large breed dogs the spinal cord terminates as the *filium terminale* at the fifth lumbar vertebra and in small breeds it ends at the level of the lumbosacral point. This anatomic feature makes it more likely to accidentally perform an intrathecal injection in smaller dog breeds and in paediatrics (Valverde, 2008). The subarachnoid space and the sac of the dura mater extend around 2 cm beyond the *filium terminale*. The sacral and caudal spinal roots form the *cauda equina*.

The needle size should be chosen depending on the size of the dog. A 2.5 cm, 22 Gauge (G) needle is recommended for small dogs, a 3.8 cm, 20 G needle for medium dogs and a 7.5 cm, 18 G needle for large dogs (Valverde, 2008).

The epidural injection should be performed in sedated or anaesthetised animals to ensure correct needle placement by avoiding movement of the patient (Torske and Dyson, 2000).

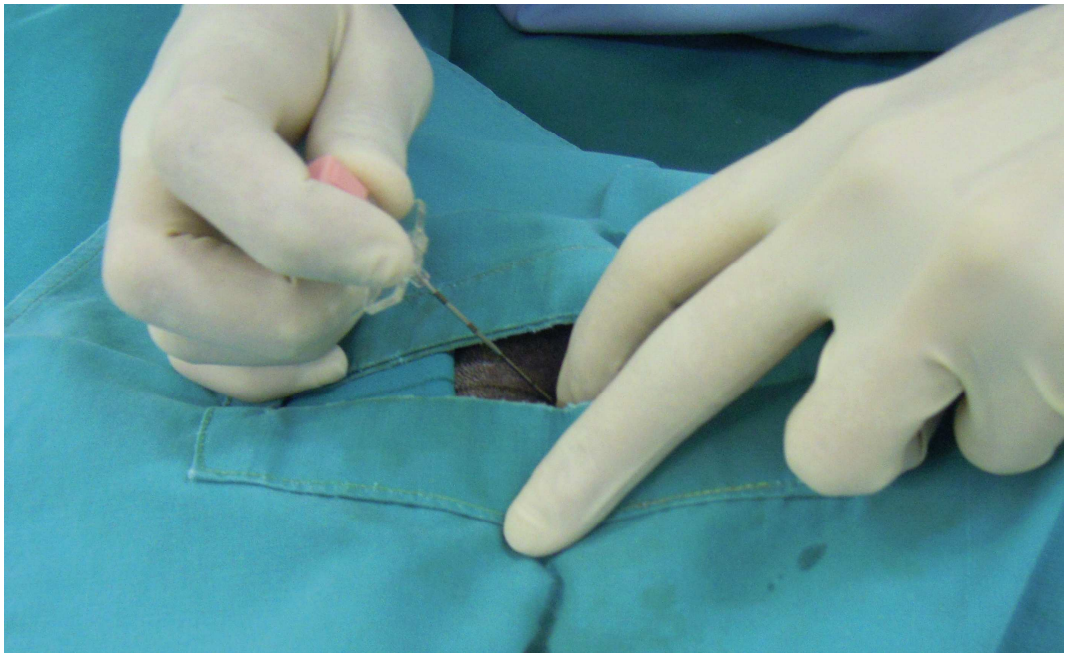
Positioning of the animal in sternal recumbency is recommended as it is easier to insert the needle in the midline compared to a dog placed in lateral recumbency (Jones, 2008). The hind limbs can be pulled forward to maximise the intervertebral space. Rotation of the patient in right or left lateral recumbency or in dorsal recumbency can be performed to allow increased spread of the drug over the desired vertebral bodies. For lumbosacral epidural injection, the anatomical landmarks include the external angles of the iliac crests (*tuber coxae*), the dorsal spinous process of the 7th lumbar vertebra and the sacrum. The area is prepared using a sterile technique and the needle is inserted in a straight angle through the skin (Jones, 2008). When the *ligamentum flavum* is pierced an increased resistance can be felt and is described as a “pop”. Correct placement in the epidural space is tested by injecting a small amount of air, sterile water or saline solution with lack of resistance. Other methods to ensure correct placement include the “hanging drop” technique, the measurement of force waves from the epidural space, the use of electrical stimulation (Valverde, 2008) and the injection of a small amount of radiological contrast (epidurogram) (Bartynski *et al.*, 2005). In the study by Troncy et al. in 2002, epidural injection failure occurred in 7% of dogs undergoing surgery. The epidural

injections were performed in 242 anaesthetised dogs and a failure was considered as an inability to decrease the requirement of inhalant agent, which occurred in 17 dogs (Troncy *et al.*, 2002). If cerebrospinal fluid is obtained the needle was inserted too far into the subarachnoid space. The spinal needle should be withdrawn to position it in the epidural space or, alternatively, the drug may be injected intrathecally but the injected dose should be reduced (Valverde, 2008). Torske et al. recommended a general drug reduction of 40% to 60% when drugs are injected intrathecally (Torske and Dyson, 2000).

Another technique performed in humans is an epidural in combination with an intrathecal injection. An epidural catheter is placed to ensure a prolonged block and intrathecal injection is performed with a low dose to obtain a rapid onset of action. This technique has been successful performed in a dog by Bonath et al. in 1984 (Bonath *et al.*, 1984) and Novello and Corletto in 2006 (Novello and Corletto, 2006).

For a repeated or constant delivery of analgesic drugs, an epidural catheter may be placed (Hansen, 2001). A commercial kit is used containing a catheter and a Tuohy needle. The Tuohy needle has a round tip with a bevel to direct the catheter into the epidural space. The Tuohy needle is placed in the same manner as the spinal needle, but the “pop” is more pronounced as the needle is blunter. The stylet is removed and the epidural catheter threaded through the needle into the epidural space. If the needle is in the correct position the catheter can be inserted without any resistance. To secure the catheter it should be threaded far enough cranially so that movement of the skin will not retract the catheter. After this procedure the needle can be removed. To ensure adequate catheter placement radiographs may be obtained. The entry of the catheter through the skin should be protected with aseptic solutions and a bacterial filter is placed on the catheter (Hansen, 2001).

Figure 7: Lumbosacral epidural Tuohy needle placement in a dog.



The cranial spread of the drug is largely dependent on the administered volume (Lee *et al.*, 2004). Recommended volumes are 1 mL per 5 kg of body weight to extend up to the first lumbar vertebra, with a maximum volume of 6 mL as the epidural space is a fixed volume space and cannot contain excessive volumes (Torske and Dyson, 2000). Other authors recommend 0.3–0.5 mL per 10 cm from the occiput to the seventh lumbar vertebrae (Westhues and Fritsch, 1960).

Local anaesthetic drugs are widely used in veterinary medicine (Tranquilli, *et al*., 2007). They block the sodium-selective voltage-dependent ion channel in nerve fibres. As result the sodium influx in the afferent nerve fibres is decreased and depolarisation of the cell membrane becomes less likely (Mazoit, 2012). After the epidural injection, the local anaesthetic will diffuse into the intervertebral area and act on the distal part of the dorsal nerve roots. The drug will also spread from the intradural space through the arachnoidea into the subarachnoid space where it acts on the nerve roots. A direct action on the spinal cord is also suspected. The action of local anaesthetics extends to all nerves entering and leaving the spinal cord resulting in motor, sensory and autonomic blockade of nerve transmission (Torske and Dyson, 2000; Kokki, 2012). The onset of action depends on the diameter of the nerve fibre, with sympathetic blockade first, followed by sensory and

finally motor nerves. Therefore, local anaesthetics are likely to lead to motor paralysis, which results in ataxia and pelvic-limb weakness. Excessive cranial spread of the local anaesthetic into the thoracic vertebrae will result in sympathetic block with hypotension and decreased cardiac output (Valverde, 2008).

Another group of drugs used for epidural analgesia are the opioids. Opioids gained attention and popularity as epidural or intrathecally administered drugs after the landmark study in 1976 by Yaksh et al. (Yaksh and Rudy, 1976).

Opioids can be classified into opioid agonists, opioid agonist-antagonists and opioid antagonists. Opioid receptors are classified as μ, κ and δ receptors. The μ receptors are further subdivided into μ_1, μ_2 and μ_3 receptors (Stoelting and Hillier, 2005). The μ_1 receptors mediate analgesia and euphoria and can lead to urinary retention, whereas the μ_2 receptors mediate analgesia and cause respiratory depression, bradycardia and physical dependence (Stoelting and Hillier, 2005). The μ_3 receptors are located in peripheral nerves and lead to hyperpolarisation due to inflammation (Gaynor and Muir, 2009). The κ receptors are known to cause sedation, analgesia and dysphoria, and cause less physical dependence (Stoelting and Hillier, 2005). Lastly, the δ receptors modulate μ receptor activity (Gaynor and Muir, 2009).

Opioid receptors are found in the periphery and in the CNS. They are widely distributed in pre- and postsynaptic neurons in the spinal cord, thalamus and cortex and are also part of the descending pain-pathways like the periaqueductal grey matter, nucleus raphe magnus and medulla (Inturrisi, 2002). All classes of opioid receptors are G-protein coupled and mediate inhibition of the adenylate-cyclase. They decrease presynaptic excitatory neurotransmitter release and inhibit postsynaptic conduction by hyperpolarising the cells. On presynaptic nerve terminals, they decrease calcium influx, which results in less substance-P release (Stoelting and Hillier, 2005). This effect is mainly seen on C-fibres and to a lesser extent and depending on the dose, on Aδ-fibres (Valverde, 2008). In postsynaptic neurons, they increase potassium efflux resulting in hyperpolarisation of the cells. In addition, they inhibit GABAergic inhibition action on inhibitory pain neurons in the central nervous system (Inturrisi, 2002). Another suspected site of action of opioids is at the NMDA-receptor in the dorsal horn of the spinal cord, enhancing the effects of NMDA-antagonists (Inturrisi, 2002). As a result, opioids lead to

a reduced neuronal action, which results in analgesia and/or sedation (Stoelting and Hillier, 2005).

The main site of action of epidurally administered opioids is the dorsal horn of the spinal cord. After epidural injection, the drug binds to fat in the epidural space and penetrates the meninges. The arachnoid mater is the primary barrier for the administered drug. The possibility to penetrate this barrier is influenced by the lipid solubility. Opioids with a high lipid solubility such as fentanyl cross the arachnoid mater rapidly, which results in a fast onset of action and a short duration due to a high systemic absorption. Less lipid soluble drugs like morphine have been shown to have a slow onset and a long duration of effect, which makes this opioid the preferred one for epidural techniques in animals. Another pharmacokinetic factor affecting opioid effect is the epidural blood flow. An increased blood flow leads to an increase clearance from the epidural space and a higher systemic absorption rate. Sympathetic and motor blocks do not occur due to opioid administration (Valverde, 2008).

When morphine is administered at a dose of 0.1 mg kg^{-1} epidurally to animals the onset time is 20–60 min (Jones, 2008; Valverde, 2008) and the analgesic duration of action varies from 10 to 24 hours (Torske and Dyson, 2000; Troncy *et al.*, 2002; Valverde, 2008; Jones, 2008). This contrast with the systemic administration of morphine, which results in an analgesic effect lasting between 4 and 6 hours. Therefore, the epidural administration provides a much longer analgesic duration (Inturrisi, 2002). The rostral spread of morphine is extensive as there is a slow clearance from the CSF (Valverde, 2008).

Side effects due to epidural opioid administration are dose dependent; therefore, side effects are more likely to occur when lipohilic opioids are administered as their systemic absorption and plasma levels are higher (Valverde, 2008). Complication rate due to epidural morphine administration is described at 0.75% in dogs and cats (Torske and Dyson, 2000). With regards to the cardiovascular system, epidural fentanyl and oxymorphone showed a dose dependent decrease in heart rate, a decrease in blood force and an increase in arterial carbon dioxide tension, whereby dogs treated with epidural morphine had better blood forces and cardiac output compared with the control group (Troncy *et al.*, 2002). Respiratory depression is potentially the most serious adverse side

effect of epidural opioids. It manifests as a decrease in respiration rate and an increase in arterial carbon dioxide tension in dogs receiving morphine (Troncy *et al.*, 2002). Usually the respiratory depression is related to a wide rostral spread and is delayed in relation to the time of administration. Additionally, urinary retention is described as an adverse effect, which occurs more commonly in humans (Valverde, 2008). Other adverse effects reported in humans are nausea and vomiting due to action on the medullary chemoreceptor trigger zone in the brain (Inturrisi, 2002).

Adverse effects due to epidural injections are rare and epidural techniques are relatively safe. In humans, post-dural puncture headaches are reported as well as neurological symptoms (Kokki, 2012). Mechanical injury, abscesses and spinal cord infection have been described (Remedios *et al.*, 1996; Swalander *et al.*, 2000). Accidental intravascular injection can lead to systemic toxicity (Mulroy *et al.*, 1997). However, the side effects are reduced and the analgesic effect is improved with epidural administration compared with systemic drug administration (Valverde, 2008).

2.3 Magnesium

2.3.1 Magnesium physiology

Magnesium is the fourth most common mineral salt present in the human body after phosphorus, calcium and potassium. Mg^{2+} is the second most common intracellular cation after potassium (Dubé and Granary, 2003).

Approximately 99% of the body magnesium is stored inside the cells. Of this amount, 67% of magnesium is in the bones together with calcium and phosphorus, 20% is stored in muscle tissue and 11% is found in other soft tissues. Only 1% of the total body magnesium is located outside the cells in the extracellular space. The exchange between the extracellular and intracellular magnesium stores is difficult to study but it appears that there is only a very slow exchange between bone and muscle and the extracellular space. Soft tissue seems to be much more able to liberate magnesium to the extracellular pool. The 1% extracellular magnesium is presented in three forms. The ionized form (active form, 55%) (Mg^{2+}), the protein-bound form (20–30%) and the anion complexed form

(bound to phosphates and citrate, 15–25%). It is suspected that there is a shift between the free ionized form and the complex form (DiBartola, 2006).

The maintenance of an adequate magnesium balance is complex and mainly controlled by intestinal absorption and renal excretion. It is closely linked to other electrolytes like sodium, potassium and calcium (Reinhart, 1990).

The absorption of magnesium takes place between the ileum and the colon. Two pathways for intestinal magnesium absorption are well known. One pathway is the passive paracellular route, through the tight junctions between epithelial cells. The forces for this movement are the transepithelial magnesium concentration gradient, the transepithelial voltage gradient formed by water and salt absorption and the permeability of the tight junction to magnesium. The transepithelial magnesium gradient is influenced by the gut intraluminal Mg^{2+} concentration and the total dietary intake of magnesium as well as the amount of magnesium that is chelated. A small positive intraluminal voltage created by net movement of salt and water results in transepithelial cation movement. Additionally, cation movement results in solvent drag created by sodium and water absorbtion. The permeability of the tight junction is created by numerous ion channels. A specific magnesium ion channel has not been conclusively identified (DiBartola, 2006). The second existing pathway in the gut is the active transcellular route. At the moment there are a lot of investigations in this field of study, which focus on the hypothesis that several magnesium transport proteins exist (Quamme and Rouffignac, 2000). Parathyroid hormone (PTH) has been identified to have a positive influence on the magnesium absorption in the gut (Hardwick *et al.*, 1991). The primary factor of the percentage of magnesium absorbed by transcellular and paracellular mechanisms is the dietary concentration of magnesium. A high magnesium intake creates a large concentration gradient and most absorption occurs through the paracellular route. Conversely, a poor magnesium intake results in a less efficient paracellular absorption and active transcellular magnesium transport becomes more important for adequate magnesium balance (DiBartola, 2006).

Magnesium transport in the kidney is influenced by calcium and several hormones. It is likely that similar control mechanisms influence magnesium absorption in the gut. The kidney provides the most sensitive control for magnesium balance (Quamme

and Rouffignac, 2000). In the glomerulus, 80% of total serum magnesium is filtered. Approximately only a small fraction of 15% is reabsorbed within the proximal tubuli. The reabsorbtion is mainly via passive and unsaturable mechanisms through paracellular transport. A large amount of magnesium (60%) is reabsorbed in the cortical thick ascending loop of Henle. Paracellular pathways through tight junctions seem to be the most important mechanism. The principal force allowing magnesium transport is the electropositive luminal environment created by the movement of sodium and chloride from the lumen to the interstitial space. In addition, magnesium movement in the interstitial space occurs as a result of solvent drag through the tight junctions. This mechanism implies that a change in transepithelial voltage influence the permeability for magnesium and additionally increase the absorption of magnesium. An increase in salt movement from the lumen will elevate the transepithelial electrical potential and facilitate magnesium absorption. Calcitonin, PTH, glucagon, antidiuretic hormone, aldosterone and insulin are known to increase magnesium absorption. On the other hand, prostaglandins, hypocalcaemia, hyphosphataemia and acidosis can decrease magnesium absorption. The distal convoluted tubuli do not act as mass transporter of magnesium but constitute the site that determine the final amount of magnesium excretion. Reabsorbtion of magnesium in this area appears to be mainly through active transcellular routes (Quamme and Rouffignac, 2000).

Magnesium has a fundamental role in many cellular functions. It is involved as a co-factor in more than 300 enzymatic reactions related to energy metabolism and nucleic acid synthesis (Fawcett *et al.*, 1999). Magnesium has modulatory effects on sodium and potassium currents by regulating the Na^{2+}-K^{+}-ATPase, thus mediating a membrane stabilising effect (Herroeder *et al*., 2011). Magnesium acts by regulating and controlling different ion channels and its calcium antagonistic effects are well studied. Magnesium regulates calcium channels in cell membranes and sarcoplasmic reticulum. These results in a direct competitive antagonistic action directed against calcium influx into cells and outflow of calcium from the sarcoplasmic reticulum (Dubé and Granary, 2003).

In muscles, magnesium and calcium and have opposite effects. Hypomagnesaemia results in contraction and hypocalcaemia induces relaxation. The mechanism behind this effect is that hypomagnesaemia causes a rapid passive release of calcium by the sarcoplasmic reticulum, which leads to contraction. Magnesium influences

the neuromuscular transmission by blocking the entry of calcium into presynaptic endings which leads to a decrease in acetylcholine release. The decrease in acetylcholine has been shown to increase the threshold for axonal excitation. In conclusion, hypermagnesaemia causes neuromuscular weakness while hypomagnesaemia induces neuromuscular hyperexcitability (Dubé and Granary, 2003).

Magnesium is known to inhibit catecholamine release by blocking calcium channels thus preventing calcium influx into symphathetic nerve endings. This results in modulation of the sympathetic reaction to nociceptive stimuli and stress response (Shimosawa, 2004).

In the spinal cord, Mg^{2+} is a natural non-competitive NMDA-receptor antagonist and leads to an increased activation threshold (Mayer *et al*., 1984). It has been shown to induce analgesia (Mayer *et al.*, 1984; Woolf, 2000; McCartney *et al.*, 2004; Soave *et al.*, 2009) and has neuroprotective effects (Simpson *et al.*, 1994).

The measurement of magnesium to diagnose magnesium deficits is difficult and controversial. At present there is no simple, rapid and accurate laboratory test available to assess the amount of total body magnesium (Swaminathan, 2003). The fact that only 1% of the body magnesium is extracellular and only 55% of this is in the ionized form presents a diagnostic challenge to detect deficits. There are two different methods to assess magnesium clinically: either Mg^{2+} or the total magnesium in various tissues, most commonly blood.

Total serum magnesium is the most commonly used method of assessing magnesium as it is easy to obtain serum samples from patients and the assay is easy to perform and widely available (DiBartola, 2003). Other tissues (red blood cells, white blood cells, muscle tissue) have been used to measure magnesium concentration. However, because of the complexity of the assays, these methods are not routinely used in clinical practice.

Another method to assess magnesium deficit is to assess the renal magnesium handling by testing the renal retention of magnesium. This assay is based on the idea that renal retention of magnesium occurs during magnesium deficit. Consequently, this assay can not be used in patients with inadequate renal function. However, these assays are not widely used in veterinary practice (DiBartola, 2003).

Normal total serum magnesium concentration for humans ranges between 0.75–0.95 mmol/l (Musso, 2009), 0.76–0.96 mmol/l (Fawcett *et al.*, 1999), 0.7–1 mmol/l (Herroeder *et al*., 2011), and 0.7–1.1 mmol/l (Swaminathan, 2003). In dogs, the normal range is 0.6–1.2 mmol/l (Clinical Pathology Laboratory reference range, Department of Companion Animal Clinical Studies, University of Pretoria).

Magnesium disorders such as hypomagnesaemia can be found in hospitalised patients and it is common in critically ill patients. Hypomagnesaemia is often associated with other metabolic disorders, as for example, hypokalaemia and hypophosphatemia. There are several causes of hypomagnesaemia. Common causes include disorders of the two regulating organs: kidney and gut. This results in a lack of input, less absorption or excessive elimination. Some of these conditions are for example: malnutrition, malabsorption, inflammatory bowel disease, diarrhoea, pancreatitis, hypercalcaemia, hyperaldosteronism, diabetes mellitus and hypoparathyroidism (Dubé and Granary, 2003). Hypomagnesaemia manifests typically as cardiac and/or neuromuscular disorders. Clinical symptoms of hypomagnesaemia include anorexia, nausea, vomiting, generalized weakness, convulsion, tetani and changes in the electrocardiogram (Dubé and Granary, 2003; Herroeder *et al*., 2011).

Hypermagnesaemia is less frequent and occurs in patients with chronic renal failure and due to rhabdomyolysis, or iatrogenically after excessive use of antacids or laxatives containing magnesium-salts or treatments for hypomagnesaemia. Clinical symptoms can range from nausea, vomiting and somnolence to deep coma (Dubé and Granary, 2003).

2.3.2 Magnesium as a systemic analgesic

Magnesium as a physiological NMDA-receptor antagonist is thought to have analgesic properties by inhibiting central pain transmission at the level of the spinal cord as well as inhibiting and preventing central sensitisation caused by peripheral nociceptive stimulation of long duration (Mebazaa *et al.*, 2011).

Different studies in humans and animals have been performed on the analgesic effect of intravenous (IV) magnesium administration. Most studies administered magnesium sulphate ($MgSO_4$) whilst others used magnesium as laevulinate (Wilder-Smith *et al.*, 1997), gluconate (Steinlechner, 2006) or chloride (Felsby *et al.*, 1996). Most commonly, a single magnesium IV bolus was administered followed by a constant rate infusion (CRI) during surgery or for a certain period. But also single doses have been tested (Schulz-Stübner *et al*., 2001; Tramèr and Glynn 2007). The majority of these studies focused on the effect of magnesium on the total analgesic consumption in the intraoperative and postoperative periods. Some studies evaluated the effect of magnesium on neuropathic pain (Brill *et al.*, 2002) and one study compared magnesium with the NMDA-receptor antagonist ketamine (Felsby *et al.*, 1996).

The magnesium doses used in different studies are very wide. Described IV bolus doses range from 5 mg kg^{-1} to 50 mg kg^{-1} (Apan *et al.*, 2004, Ryu *et al.*, 2008), and doses for CRI range from 8 mg kg^{-1} h to 500 mg h in humans (Kara *et al.*, 2002; Apan *et al.*, 2004). In animals, different doses of magnesium have been used, from a single injection of 600 mg kg^{-1} subcutaneously in rats (Xiao and Bennett, 1994), to a 50 mg kg^{-1} bolus followed by a 15 mg kg^{-1} h CRI (Rioja *et al.*, 2012) and a 50 mg kg^{-1} bolus followed by a 12 mg kg^{1} h CRI (Anagnostou *et al.*, 2008).

When magnesium was administered in humans during soft tissue surgery, such as hysterectomy or cardiac surgery, the opioid requirements decreased in the majority of studies (Tramer *et al*., 1996; Schulz-Stübner *et al*., 2001; Kara *et al.*, 2002; Unlügenç *et al.*, 2003; Apan *et al.*, 2004; Seyhan, 2006; Steinlechner, 2006; Mentes *et al*., 2008; Ryu *et al.*, 2008; Gupta *et al.*, 2011; Kiran *et al.*, 2011; Lee *et al.*, 2011; Olgun *et al.*, 2012), while it did not have any effect in other studies (Wilder-Smith *et al.*, 1997; Zarauza *et al*., 2000; Ko *et al.*, 2001; Bhatia *et al.*, 2004; Paech *et al.*, 2006; Tramèr and Glynn, 2007;

Sullivan *et al.*, 2012). When magnesium was administered in humans undergoing orthopaedic surgery, the opioid consumption decreased (Koinig *et al.*, 1998; Telci *et al.*, 2002; Levaux *et al.*, 2003; Hwang *et al.* 2009; Kogler, 2009). Furthermore, the administration of magnesium resulted in less postoperative discomfort and better quality of sleep (Tramer *et al*., 1996; Bhatia *et al.*, 2004) and a lower incidence of postoperative shivering (Ryu *et al.*, 2008).

A study compared ketamine with magnesium chloride in humans suffering from peripheral neuropathic pain (Felsby *et al.*, 1996). Ketamine or magnesium was administered by a bolus infusion followed by a CRI and pain was assessed using pain scales as well as mechanical and thermal threshold testing. Ketamine infusion, but not magnesium, reduced spontaneous pain and allodynia significantly. Mechanical and thermal thresholds were unchanged by both administered drugs.

In a systematic review of 14 human randomized clinical trials, it was concluded that there was no effect of systemic administration of magensium on post-operative pain intensity and analgesic requirements (Lysakowski *et al.*, 2007).

In studies in rats, magnesium reduced allodynia (Xiao and Bennett, 1994). When magnesium was administered to dogs undergoing ovariohysterectomy it did not affect the analgesic consumption (Rioja *et al.*, 2012).

Possible reasons for the different results found in the previously mentioned studies include different dosages (Lysakowski *et al.*, 2007), single bolus administration (Tramèr and Glynn, 2007), small number of patients (Rioja *et al.*, 2012) and limited ability of magnesium to cross the blood brain barrier (Ko *et al.*, 2001).

2.3.3 Magnesium as a neuraxial analgesic

Intravenous administration of magnesium is known to reduce intra- and postoperative analgesic requirements by acting as a physiological antagonist on the NMDA-receptor in

the dorsal spinal cord. Whether or not systemically administered magnesium is able to penetrate the blood brain barrier remains unclear and an increase in serum magnesium concentration does not seem to increase the CSF concentration of magnesium (McCarthy *et al*., 1998; Ko *et al*., 2001; Sun *et al*., 2012). Therefore, the neuraxial administration of magnesium has been investigated in animal and human models of pain.

The administration of high doses of magnesium at the level of the spinal cord is proven to have no toxicity in rat models (Chanimov *et al*., 1997; Takano *et al*., 2000) as well as in dogs (Simpson *et al*., 1994) and cats (Tsai *et al*., 1994). However, dose dependant neurological dysfunction, neurotoxicity and no protective effect against ischaemic spinal cord injuries has been reported after intrathecal magnesium administration in rabbits (Saeki et al., 2004).

In rat models of pain, intrathecal magnesium enhanced spinal analgesia induced by opioids (Kroin *et al*., 2000) and delayed the development of opioid tolerance (Mc Carthy *et al*., 1998). Furthermore, intrathecal Magnesium induced motor block (Karasawa *et al*., 1998), sedation and sensory block (Bahar *et al*., 1996). Magnesium administered intrathecally reversed hyperalgesia induced by a magnesium deficiency (Begon *et al*., 2001). High doses of magnesium injected intrathecally showed analgesic effect using the formalin test in rats (Takano *et al*., 2000). Lower magnesium doses and antinociception tested with other nociceptive tests failed to show any antinociceptive properties (Takano *et al*., 2000). The authors argued that this outcome could be due to the acute type of pain evoked by the different antinociceptive tests.

The antinociceptive effect of epidurally administered magnesium in different species has been investigated. Cross-over studies in goats (Bigham *et al*., 2009), horses (Bigham and Shafiei 2008) and cattle (Dehghani and Bigham, 2009b) have been performed using 1 ml of 10% magnesium (100 mg) combined with 2% lidocaine in all studies. Time to onset of analgesia, duration of analgesia, standing time, cranial spread and vital parameters were assessed. The analgesic effect was measured recording the response to superficial and deep muscular pinpricks (Bigham *et al*., 2009) and pinpricks and force from haemostatic clamps (Bigham and Shafiei 2008; Dehghani and Bigham, 2009b). In all species, the onset of analgesia was significantly prolonged in the lidocaine

combined with magnesium treatment compared with the lidocaine treatement, but also the duration of analgesia was prolonged. Mild ataxia was observed in cattle and horses when only lidocaine was administered (Bigham and Shafiei 2008; Dehghani and Bigham, 2009b). No difference in standing time was observed in goats (Bigham *et al*., 2009). The vital parameters did not differ significantly from baseline in both treatment groups (Bigham and Shafiei 2008; Bigham *et al*., 2009; Dehghani and Bigham, 2009b). In sheep, epidural administration of 50 mg of magnesium produced analgesia for approximately 29 min and prolonged analgesia achieved by epidural ketamine (DeRossi *et al.*, 2012).

Different studies have been performed using intrathecal magnesium in clinical human trials. Most studies focused on the effect of magnesium on the onset, degree and duration of analgesia when administered in combination with opioids and/or local anaesthetics (Buvanendran *et al.*, 2002; Ozalevli *et al.*, 2005; Arcioni *et al.*, 2007; El-Kerdawy, 2008; Yousef and Amr 2010; Shukla *et al.*, 2011; Nath *et al.*, 2012) and the effect on postoperative opioid consumption (Bilir *et al*., 2007; Birbicer, et al., 2007; El-Kerdawy, 2008; Yousef and Amr 2010; Ouerghi *et al.*, 2011).

The first trial dealing with magnesium intrathecally in humans included 26 patients requesting analgesia for labour. Patients received fentanyl and magnesium (50 mg) intrathecally. The duration of analgesia was defined by the time the patient requested additional drugs for pain management. Duration of analgesia was significantly prolonged in the fentanyl plus magnesium treatment (75 min) compared with the fentanyl alone treatment (60 min). The authors argued that the prolonged duration might have limited clinical relevance, but that it might be due to the single bolus and the low dose of magnesium administered (Buvanendran *et al.*, 2002).

Similar results were observed in Ozalevli et al's study with patients undergoing lower extremity surgery (Ozalevli *et al.*, 2005). Spinal anaesthesia was achieved by intrathecal administration of bupivacaine (10 mg), fentanyl (25 µg) and additional 50 mg magnesium. The main findings were that the onset of motor and sensory block were delayed and the duration of spinal anaesthesia was prolonged (median 173 min vs. 155 min) in the group receiving additional magnesium.

In another study in patients undergoing lower extremity orthopaedic surgery, a 50 mg magnesium bolus followed by a CRI of 100 mg h magnesium was added to bupivacaine (10 mg) and fentanyl (25 µg) intrathecally (El-Kerdawy, 2008). The onset of spinal anaesthesia was significantly delayed in the group receiving magnesium, but the duration of analgesia was significantly longer. The overall postoperative fentanyl consumption was significantly lower in the magnesium group.

In patients undergoing lower abdominal or lower limb procedure, 50 mg magnesium intrathecally delayed the onset of analgesia and prolonged analgesia achieved by bupivacaine (15 mg), but the analgesic duration was shorter compared to the group receiving additional dexmedetomidine (10 µg) (Shukla *et al.*, 2011).

Intrathecal magnesium (50 mg) in addition to fentanyl (25 µg) and morphine (3 mg) in humans undergoing thoracotomy revealed a reduced postoperative analgesic requirement (Ouerghi *et al.*, 2011).

Magnesium (100 mg) administered intrathecally in combination with fentanyl (25 µg) and bupivacaine (12.5 mg) in patients undergoing hysterectomy produced a delayed onset of sensory and motor blocks and prolonged the duration of analgesia (Nath *et al.*, 2012) .

The postoperative fentanyl consumption was also significantly lower in a study including patients undergoing hip surgery (Bilir *et al.*, 2007). One group (25 patients) received a continuous epidural infusion of fentanyl, whereas the other group received fentanyl combined with a 50 mg kg^{-1} magnesium bolus followed by a 100 mg kg^{-1} per day continuous infusion. Visual analogue scale scores were lower in the group receiving additional magnesium and the overall fentanyl consumption was significantly less.

In another study, epidural magnesium was added (500 mg) to bupivacaine (25 mg epidural and 10 mg intrathecal) and fentanyl (100 µg) in women undergoing caesarean section (Yousef and Amr, 2010). The magnesium group showed significantly

better muscle relaxation and delayed onset of postoperative pain, whilst postoperative analgesic requirements were significantly reduced.

A study compared the different routes of neuraxial administration of magnesium (intrathecal, epidural, combined epidural and intrathecal) (Arcioni *et al.*, 2007). Postoperative morphine consumption was assessed using patient controlled analgesia. Morphine consumption during 36 hours post-surgery was 38% lower in patients receiving magnesium epidurally, 49% lower in patients receiving magnesium intrathecally and 69% lower in patients administered a combined intrathecal and epidural injection of magnesium.

However, epidural administration of 50 mg magnesium in combination with ropivacaine showed no effect on postoperative pain and analgesia requirement in paediatric patients undergoing lower abdominal or penoscrotal surgery (Birbicer, et al., 2007).

No difference in incidence of undesirable effects like bradycardia, hypotension or sedation in comparison to the control treatment was reported (Ozalevli *et al.*, 2005; Ouerghi *et al.*, 2011)

To the author's knowledge no studies of neuraxial magnesium administration have been performed in dogs to date.

2.4 Types of threshold testing

Assessing pain in animals in an objective way presents a challenge in clinical, as well as in research settings. Different types of pain scales based on behavioural changes have been developed. Also the use of physiological parameters to assess pain has been investigated (Hansen 2003). Another method to assess pain is by using algometry, also called nociceptive threshold testing (Love *et al.*, 2011). However, at present there is no established "gold standard" to assess pain as all the methods have limitations (Gaynor and Muir, 2009).

To investigate analgesic effects of drugs and to evaluate hyperalgesia, different nociceptive testing techniques have been used. The principle behind nociceptive testing is to apply a quantified nociceptive stimulus to a body part until a behavioural or reflex response is noticed, which indicates the pain threshold. The behavioural or reflex response is defined as the "end-point" and terminates the application of the nociceptive stimulus. The ideal analgesiometer was described by Beecher and is characterized by repeatability and a good reliability. In addition, it should be easy to apply and the end-point should be easy to detect. The applied stimulus should be quantifiable, reproducible and non-invasive, causing no tissue damage and should be associated with perceived pain; furthermore, it should show a dose-response relationship (Beecher, 1957).

There are several limitations to nociceptice algometry. The determination of the end-point can be challenging as it is dependent on the species and individual variability (Love, 2001). Furthermore, an experimenter bias is possible as the experimenter has to judge whether there was a response to the applied stimulus or not (Bove, 2006). Also the significance of the chosen end-point is important as a reflex response indicates a less complex conscious perception of pain than a more complex response (Bove, 2006).

Nociceptive stimuli can be evoked by applying thermal, electrical, chemical and mechanical stimulation (Love, 2001).

Thermal threshold testing, is commonly used in laboratory animals (Le Bars *et al.*, 2001; Zhang *et al.*, 2013) and domestic animals (Love, 2001; Pypendop *et al.*, 2008).

By using thermal nociceptive stimuli Aδ-fibres mechano-heat nociceptors and polymodal C-fibers are activated (Zhu and Lu, 2010). Other studies have demonstrated that the activation of the nociceptive fibres is dependent on the speed at which the temperature is increased. A rapid rate of heating activates Aδ-fibres whereas slow rate of heating activates polymodal C-fibres (Yeomans and Proudfit, 1996).

Commonly used thermal tests in laboratory animals are the “tail-flick” reflex response test, the “hot-plate” test (Beecher, 1957), the “tail-immersion” test (Luttinger, 1985) and the “hind-paw thermal withdrawal” test (Dirig *et al.*, 1997). These tests usually record the latency to a response following application of a constant temperature (Love, 2001). In horses, a source of radiant heat (lamp) has been used for thermal testing with the end-point being latency period to hoof withdrawal or skin twitches (Carregaro *et al.*, 2007). An increase in time until a response occurs has been interpreted as an antinociceptive effect of tested drugs. One limitation of these methods is the inaccuracy associated with timing. If the time between exposure to the heat source and the response is short, this factor becomes more significant (Love, 2001). Animals may learn to avoid the nociceptive stimulus. Up to 50% of the horses showed avoidance towards the heat-lamp before the stimulus was applied (Kamerling *et al.*, 1985). Introducing a “sham test” in the study protocol by exposing the horses regularly to a non-heat producing lamp is suitable to eliminate the learning effect (Kamerling *et al.*, 1985). An advantage of using radiant heat is that there is no contact between the skin and the heat source; therefore, there is no sensation of touch or force, which could evoke a reflex response (Beecher, 1957).

Another method of thermal threshold testing is using the thermode based system. A probe containing a heating element and a temperature sensor is held against a clipped area of skin. The contact of the probe with the skin can be regulated and modified by using a blood force cuff. The heating element heats at a constant rate until a change in behaviour is noticed. The behavioural change varies according to the species being tested. It can be a skin twitch or turn of the head in horses (Love, 2001) or jumping, turning the head, flicking the tail or licking and biting the probe in cats (Pypendop *et al.*, 2008). The temperature at which the animal responds is referred to as the thermal threshold. In order to avoid tissue damage, should an animal not respond, a cut-out temperature has been set. In horses, different heating rates have been investigated. The slower the rate the more clear the end-point and the more consistent the threshold temperature (Love, 2001). These thermal threshold testing systems are criticised as they are in contact with the skin and can thereby evoke mechanical stimulation (Le Bars *et al.*, 2001).

Electrical stimuli are superior to other type of stimuli as they are quantifiable, non-invasive and reproducible, but this kind of stimulus also has disadvantages as it is not

a natural type of stimulus and activates all kinds of afferent fibres, from selective nociceptor Aδ and C-fibres up to large diameter non-nociceptive fibres. Furthermore, the impedance of the stimulated tissue can vary and makes it necessary to monitor the given current as well as the voltage required to generate the current (Le Bars *et al.*, 2001).

Tests using chemical stimuli differ from tests using other stimuli as chemical stimuli change progressively over time, are of longer duration and inescapable. They are known to be the closest experimental models to clinical pain. Usually a chemical agent is injected intradermally or intraperitoneally. Injections at other regions are less common. The injection of formalin is the most widely performed, but hypertonic saline, capsaicin and other substances have also been used. Pain response is dependent on the dose and concentration of the injected agent. The behavioural response (licking, biting, rest and protection of the injected limb) can be assessed and scored. Formalin causes a biphasic behavioural response in rats and mice. The first phase results from a direct activation of the nociceptors and the second response is due to inflammatory pain. Therefore, chemical stimuli can be used to evaluate analgesic effects of both opioids and NSAIDs. Opioids have been shown to suppress both phases of the pain response while NSAIDs only block the second phase (Le Bars *et al.*, 2001).

2.4.1 Mechanical threshold testing

Mechanical threshold testing is mostly performed applying gradually increasing force over a given area of the skin until a behavioural end-point is reached. The responses can vary from a simple spinal reflex-response to vocalisation and complex changes in behaviour (Love, 2001).

The main criticism of mechanical threshold testing is that the applied force may be difficult to measure with precision and that a repetition of the stimulus can lead to an increase in threshold of the assessed body part (Le Bars *et al.*, 2001). Activated receptors include low-threshold mechanoreceptors as well as nociceptors; therefore, this type of stimulus has been criticised for being not specific. To avoid the activation of low-threshold mechanoreceptors it is necessary to apply a relatively high force, which leads to a weak sensitivity for nociceptive detection and can cause tissue damage and inflammation (Le Bars *et al.*, 2001).

Different devices for different species have been developed (Le Bars *et al.*, 2001). The main group of devices are based on von Frey's investigations in 1922, when he developed the first monofilament by attaching a mammalian hair to a handle. Semmes and Weinstein further developed a set of nylon monofilaments of different diameters fixed on a handle. The theory behind monofilaments is to apply the monofilamet of a given thickness to a surface until it bends. The force generated remains constant throughout the bending excursion and the force is dependent on the thickness of the monofilament. When no response is evoked the next thicker filament is chosen and applied until a response is evoked and the threshold is determined (Bove, 2006).

Different configurations of the monofilaments have been investigated. The probe has been fixed to a controlled mechanical advancer, producing a controlled force as with the Dynamic Plantar Aesthesiometer (Modell 37450; Ugo Basile Srl, Italy). On the other hand, the electronic von Frey device contains a rigid tip, which is manually applied, and the maximum force in grams that elicits an end-point is electronically recorded.

Figure 8: Electronic Von Frey device, monitor and handle with rigid tip.

The main limitations of the mechanical threshold methods have been discussed by Bove et al. (Bove, 2006). Bending of the filament results in a change of the tip surface

and results in an edge being applied to the skin. The sharpness and area of the filament tip is dependent on the bending degree and increases with strength of the surface to which it is applied. This leads to an unpredictable force generation, dependent on tip geometry and strength of tissue to which it is applied (Bove, 2006). Some mechanical threshold devices, like the electrical von Frey device (Electronic von Frey, Model 23931; IITC Life Science, California, USA) are using rigid tips. Therefore, limitations discussed for flexible tips do not apply for the rigid tips. Other studies report that filaments meant to produce the same force do not, depending on the manufacturer (Booth and Young, 2000), and that interfilament variation from the same manufacturer also occurs (McGill *et al.*, 1998). Further discussed is an experimenter bias, as the experimenter decides which filament is used and which behavioural change is considered as the end-point (Bove, 2006). Another point of criticism is the different ways of reporting monofilament threshold testing. The application of the monofilaments varies from not reporting duration of application (KuKanich *et al.*, 2005), applying the tip for only one second (Duque *et al.*, 2004; Lindegaard *et al.*, 2009) or for up to more than 10 sec (Bove, 2006). The recording of the force applied varies from grams (Redua *et al.*, 2002; Lindegaard *et al.*, 2009) to newtons (Lascelles *et al.*, 1997) to pascals (Bove, 2006). The reported changes are expressed in percentage changes from baseline (Lascelles *et al.*, 1997; KuKanich *et al.*, 2005) and changes in grams from baseline converted to logarithm scales (Redua *et al.*, 2002; Duque *et al.*, 2004). Therefore, a comparison between different studies and findings is very difficult.

Other limitation are that a learning behaviour may occur, as the animal may learn to avoid the nociceptive stimulus (Love, 2001). However, in a study using von Frey filaments in dogs the threshold values in the control group did not change significantly over time compared to baseline (KuKanich *et al.*, 2005). Another study comparing mechanical thresholds in horses on non-incision and incision site found no changes in mechanical thresholds at the non-incision site, while changes were found at the incision site, which proved absence of a learning process (Redua *et al.*, 2002).

Furthermore, mechanical threshold testing may be affected by environmental or external factors (e.g. time of the day, visual stimuli, noise, which may cause distraction and increase the thresholds) and internal factors like behaviour (e.g. frightened animals or very active animals would respond earlier than calm and friendly animals) (Bove, 2006).

In summary, all types of nociceptive stimuli have their limitations, advantages and disadvantages as well as different patterns of fibre activation. Overall, there is no “Gold Standard” in threshold testing.

3 Objectives and Hypotheses

3.1 Objectives

3.1.1 Primary objectives

- To study whether magnesium administered in the lumbosacral epidural space in dogs produces antinociceptive effects determined using von Frey mechanical thresholds.
- To study the possible antinociceptive interaction between magnesium and morphine when administered in combination in the lumbosacral epidural space in dogs determined using von Frey mechanical thresholds.

3.1.2 Secondary objectives

- To study the onset and duration of the antinociceptive effects produced by magnesium alone or in combination with morphine when administered in the lumbosacral epidural space in dogs.
- To study whether magnesium administered alone or in combination with morphine in the lumbosacral epidural space in dogs produces motor deficits.

3.2 Hypotheses

3.2.1 Primary hypotheses

- Magnesium administered in the lumbosacral epidural space in dogs will produce an antinociceptive effect detectable using von Frey mechanical thresholds.
- The combined administration of magnesium and morphine in the lumbosacral epidural space in dogs will produce a greater antinociceptive effect compared with the administration of each drug alone.

3.2.2 Secondary hypotheses

- The onset of action of the antinociceptive effect of magnesium administered in the lumbosacral epidural space in dogs will be longer and the duration shorter than morphine alone or the combination of magnesium and morphine.
- Administration of magnesium alone or in combination with morphine in the lumbosacral epidural space in dogs will not produce any motor deficits.

4 Material and Methods

4.1 Dogs

A total of six healthy, adult, neutered research Beagle dogs (3 male, 3 female) were enrolled in the study. The weight and age of the dogs were 15.2 ± 1.5 kg and 4 ± 1 years, respectively. Dogs were determined to be healthy prior to enrolment based on a clinical examination and blood work including a complete blood count, total serum protein and creatinine concentrations. Also, the dogs' total serum magnesium concentration were determined to exclude states of hypomagnesaemia, as so exclude interfere with the study aim. Additionally, a clinical examination was performed every morning before the dogs were anaesthetized. Dogs were housed in cages and had daily regular access to a free run, they were fed with commercial dog food and had access to fresh water *ad libitum*. The University of Pretoria Animal Use and Care Committee approved the study (V074-11).

4.2 Study design

This was an experimental, randomized, blinded, crossover study. Dogs received four treatments in a random order with a one week washout period between treatments. Epidural injections consisted of:

- Treatment control (Co): sterile water (0.115 mL kg^{-1}) (Sabax water for injection 10 mL Adcock Ingram Critical Care, South Africa).
- Treatment magnesium (Mg): $MgSO_4$ 50% alone (0.005 mL kg^{-1}, 2.5 mg kg^{-1}) (Sabax Magnesium sulphate 50%; Adcock Ingram, South Africa).
- Treatment morphine (Mo): morphine alone (0.1 mg kg^{-1}) (Morphine Sulphate-Fresenius PF 10 mg mL^{-1}; Fresenius Kabi for Bodene, South Africa).
- Treatment magnesium and morphine (Mm): a combination of $MgSO_4$ (0.005 mL kg^{-1}) and morphine (0.1 mg kg^{-1}).

Sterile water was added to treatments Mg, Mo and Mm to obtain a total volume of 0.115 mL kg^{-1}. The above-described solutions were always prepared by the same anaesthetist (BD), who was not involved in the antinociceptive evaluations.

4.2.1 Anaesthesia

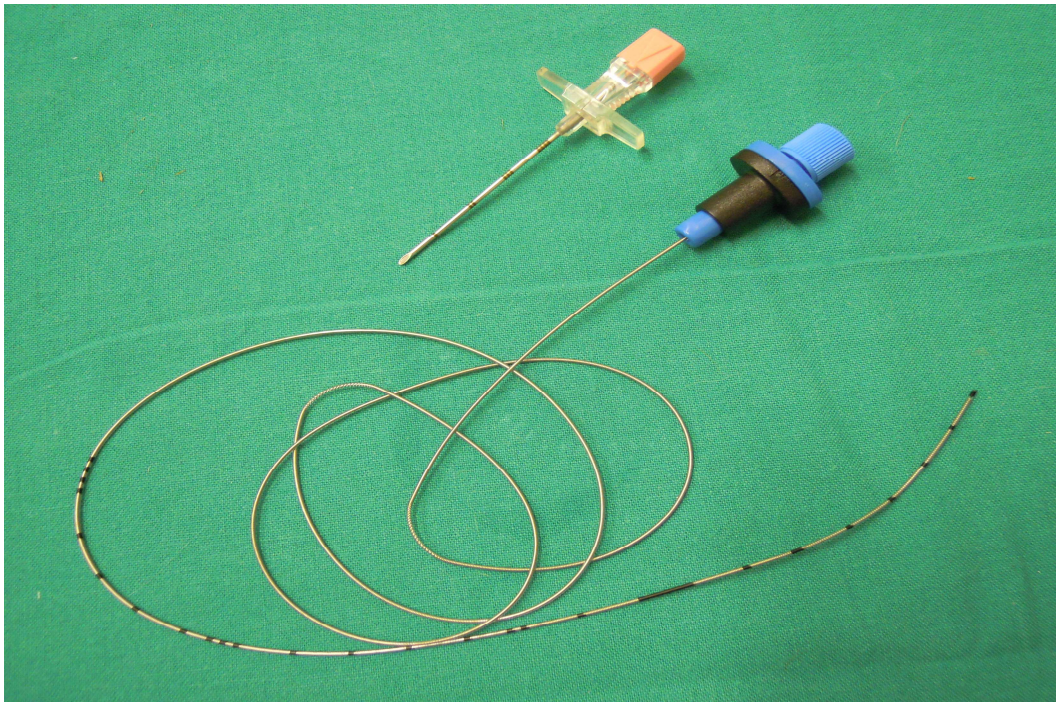
A clinical examination was performed on the dogs every week in the morning before each treatment. Food was withdrawn for a minimum of six hours prior to anaesthesia, which was performed in the morning of the day of the treatment. A 20 G catheter (Jelco® I.V. Catheter Radiopaque purple 20 G x 1.75"; Smiths Medical International, UK) was aseptically placed in the cephalic vein. Anaesthesia was induced in study dogs using propofol (Propofol 1% Fresenius; Fresenius Kabi South Africa, South Africa), administered intravenously to effect (ranging from 6–8 mg kg^{-1}) until loss of consciousness, and the trachea was intubated with a cuffed polyvinyl chloride endotrachel tube (size 8). Anaesthesia was maintained with isoflurane (Isofor Inhalation Anaesthetic; Saffeline Pharmaceuticals, South Africa) in 100% oxygen via a circle circuit rebreathing system, with a fresh gas flow rate of 1 L min^{-1}. Vital parameters were continuously monitored during anaesthesia using a multiparameter monitor (SurgiVet Tm; Smiths Medical PM, Wisconsins, USA) including: respiration rate, haemoglobin oxygen saturation, expired CO_2, electrocardiogram and arterial blood forces, measured noninvasively with an oscillometer. Dogs received 4 mL kg^{-1} h^{-1} of Lactated Ringer's solution (Sabax Ringer-Lactate [Hartmann's Solution]; Adcock Ingram, South Africa) intravenously during anaesthesia. Rectal temperature was also measured before anaesthesia and in recovery. Dogs recovered from anaesthesia in sternal recumbency and under continuous observation from the primary researcher.

4.2.2 Epidural catheter placement and drug administration

Anaesthetised dogs were placed in sternal recumbency. The lumbosacral area was clipped and aseptically prepared using chlorhexidine and 90% alcohol. An 18 G x 4.45 cm Tuohy needle was inserted in the lumbosacral epidural space with the bevel pointing cranially. A volume of 0.5 to 2.5 mL of sterile water was injected to verify placement by lack of resistance to injection of a small volume and corroborate correct epidural needle placement. A 20 G epidural catheter was then introduced through the needle into the epidural canal (Epidural Catheterization Set with Flex-Tip Plus® Catheter for Pedriatric Lumbar Placement; Arrow International Special Order Products, South Africa) (Figure 9). The epidural catheter was advanced 2 to 4 cm into the epidural space. Corresponding drugs were immediately administered through the epidural catheter with the dog still under anaesthesia. The order of administration of the drugs was done in a consistent

manner and time of injection was recorded. After the end of the epidural injection the epidural catheter was removed and the dogs were allowed to recover from anaesthesia. Epidural catheter placement and injection were always performed by a single experienced anaesthetist (ER), who was blinded to the treatments.

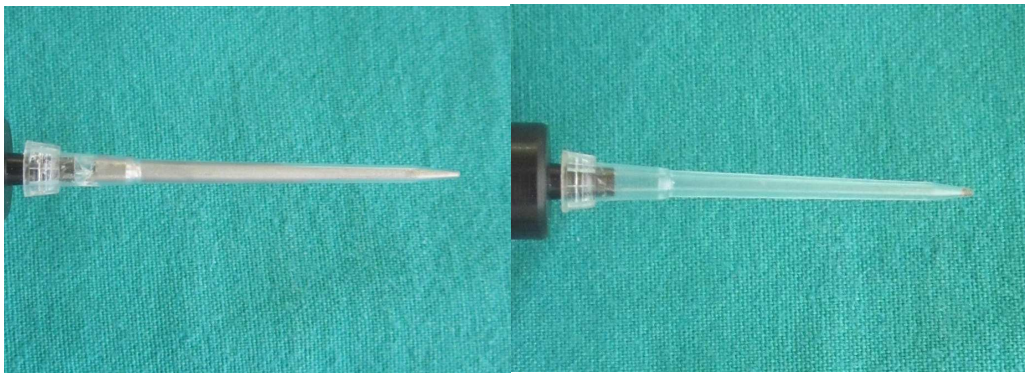
Figure 9: Epidural catheter set, top-left 18 G x 4.45 cm Tuohy needle, bottom-right 20 G catheter with injection port attached (Epidural Catheterization Set with Flex Tip Plus® Catheter for Pedriatric Lumbar Placement; Arrow International Special Order Products, South Africa).



4.2.3 Antinociceptive threshold testing using the von Frey device

Antinociceptive threshold testing was performed using a von Frey device (Electronic von Frey, Model 23931 [modified]; IITC Life Science, California, USA). The device consisted of a load cell, a handle, a monitor and a rigid tip. The plastic tip (4.5 cm in length, 0.5 cm diameter) was custom built and modified by filling it completely with an epoxy putty (Repair Metal power Epoxy; Pattex, Germany), to increase the rigidity (Figure 10).

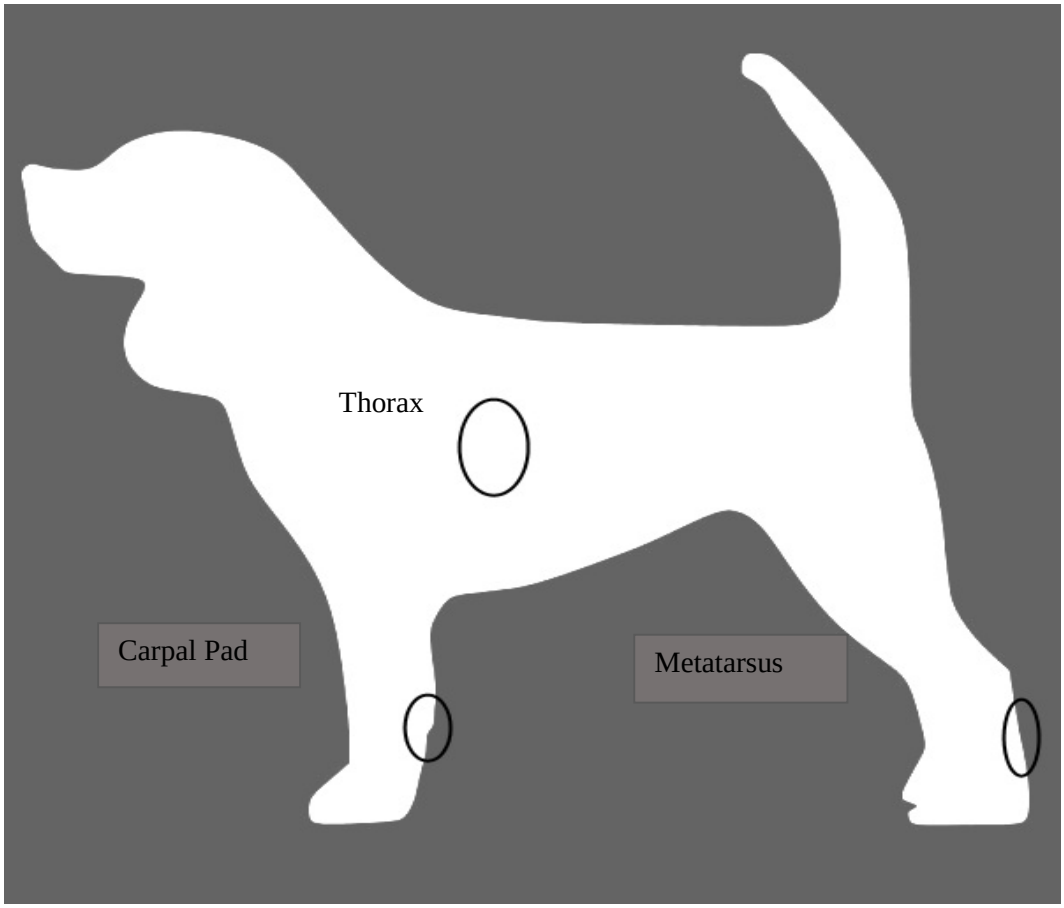
Figure 10: Rigid von Frey device custom built plastic tip (right) and tip filled completely with an epoxy putty (left).



The load cell is designed to measure an applied force of 1 g to 1000 g. Calibration was performed each day of the study prior to data collection. The monitor retrains the maximum force applied in grams before withdrawal occurred. The operator, always the same person (AB), was trained to increase the applied force in a slow constant manner until a nociceptive response occurred.

The repeatability of von Frey measurements was assessed prior to commencement of the experimental evaluation of treatments (phase 1 of the study). Three investigators (AB, ER and BD) performed two sets of measurements on two separate days, with three measurements on each region on the six dogs. Tested areas included the carpal pad, lateral surface of the epicondylus lateralis of the humerus, thoracic wall at the intercostal spaces 6 or 7, lateral surface of the thigh and plantar metatarsus, on both sides. These areas were clipped bilaterally for consistency. The most consistent results were obtained at the carpal pad (Cp), thorax (Th) and metatarsus (Mt) and, therefore, these areas were selected for the study (Figure 11).

Figure 11: Regions evaluated with the Von Frey device: carpal pads, both sides of thorax and metatarsi.



Threshold testing was performed in a temperature-controlled room with minimal restraint and minimal distraction of the dogs. Dogs were allowed to stand or lie in lateral recumbency during the measurements. During the experimental (phase 2 of the study) evaluation, measurements were performed by a single investigator (AB) who was blinded to the administered treatment.

During the experimental evaluation, threshold testing was always performed in the morning prior to anaesthesia and epidural injection, which was considered the baseline and at 30 minutes, 1, 2, 4, 6, 12, 18 and 24 hours after the epidural injection. Three consecutive measurements were obtained at each region and on the left and right side at each time point. The maximum force at which a response was noted (the von Frey threshold) was recorded by a second observer also blinded to treatment group. The

measured von Frey threshold were expressed in grams and the three measurements averaged for statistical analysis.

Briefly, the tip was applied on each region perpendicular to the body surface on clipped skin and force was applied in a consistently increasing manner until a nociceptive response was obtained. A nociceptive response was considered withdrawal of the limb (Cp and Mt), a skin twitch or turning of the head (Th). A withdrawal reflex obtained in response to touching with the tip was not recorded as a nociceptive response. A maximum cut off force of 600 g was set. The investigator was notified to stop if this force was reached and it was recorded as the von Frey threshold.

The assessments of the antinociceptive thresholds on the Cp were always performed with the dogs standing. The antinociceptive thresholds measured at the Th and the Mt were obtained whilst the dog was standing or in lateral recumbency, depending on the dog's preference (Figure 12). The measurements were obtained first at the Cp, followed by the Th and lastly at the Mt. The left and right sides were assessed in random orders.

Figure 12: Electrical von Frey device applied to the metatarsus of a Beagle in lateral recumbency.

Tested regions were visually inspected weekly for possible signs of tissue damage caused by the applied force.

4.2.4 Additional measurements

Dogs' behaviour during the threshold testing was assessed using a numerical descriptive scale, with 0 being frightened, shy and quiet; 1 being calm and cooperative and easy to work with; 2 being anxious, unsettled and restless, but still possible to work with; and 3 being excited, non-cooperative and difficult to work with.

The level of sedation after recovery from anaesthesia was also scored using a numerical descriptive scale, with 0 being not sedated, 1 being mildly sedated, 2 being moderately sedated, and 3 being severely sedated

Tail tone was assessed to evaluated motor effects of the treatments. The degree of tail tone was scored using a numerical descriptive scale, with 0 having a normal tail tone, 1 having a mild decrease in tail tone, 2 having a moderate decrease in tail tone, and 3 having no tail tone.

Additionally, ataxia of the pelvic limb was assessed to evaluated motor effects of the treatments. Ataxia of the pelvic limbs was scored with the dog walking three meters in a straight line using a numeric descriptive scale, with 0 being no ataxia, 1 mild ataxia, 2 moderate ataxia, and 3 severe ataxia.

Additional measurements were always obtained by the same person (AB), who was unaware of the treatments. Sedation, tail tone and ataxia were evaluated immediately prior to the von Frey thresholds measurement and behaviour was assessed after threshold testing at each time point.

Room temperature and humidity were recorded at each time point during data collection with a combined thermometer/hygrometer (HOBBO Data Logger-U14-001; Onset, Massachusetts, USA).

4.3 Statistical analysis

Data were assessed for normality through the plotting of histograms, calculation of descriptive statistics and the Anderson-Darling test for normality. Outcome variables violating the normality assumption were transformed using natural logarithms or ranks prior to statistical analysis. Repeatability was assessed by calculating the coefficient of variation (standard deviation divided by the mean) of the three repeated measurements and by performing a variance components analysis. A linear mixed model was used to

analyse the effect of treatment and time on the von Frey thresholds. Dog was included as a random effect in the model and behaviour, side, region and week were included as fixed effects. Week of the study was evaluated as a potential confounder or effect modifier in the evaluation of treatment effects. Multiple pairwise comparisons were adjusted using Bonferroni correction. A non-parametric Freidman test was used to compare the distance of the epidural catheter within the canal among treatment. Data were analyzed using commercially available software (SPSS version 17.0; SPSS Inc; Chicago, Ill. USA) and results interpreted at the 5% level of significance.

5 Results

5.1 Dogs

Data are expressed as mean ± SD unless otherwise specified. Haematology and clinical examination prior to the study revealed no abnormalities in any dog. The blood serum total magnesium concentration was 0.8 ± 0.1 mmol/L, which was within the normal range of the University of Pretoria clinical pathology laboratory (0.6–1.2 mmol/L).

5.2 Anaesthesia, epidural catheter placement and drug administration

Anaesthesia was induced using 6.6 ± 1.3 mg kg^{-1} of propofol and total anaesthesia time was 13.0 ± 4.3 min. All vital parameters monitored during anaesthesia were within normal limits and no complications occurred.

Lumbosacral epidural catheter placement could be performed in all dogs. The epidural catheter was advanced 2.68 ± 1.06 cm into the epidural canal. The epidural injection could be completed in all dogs without any complications. The volume of injected drugs was 1.73 ± 0.17 mL. Additionally, a total of 1.42 ± 0.51 mL of sterile water was added to the injected drugs. Therefore, the total epidural volume was 3.17 ± 0.68 mL.

Induction, maintenance and recovery from anaesthesia were uneventful in all dogs.

No signs of inflammation or tissue damage at the insertion site of the epidural catheter were noticed during the study.

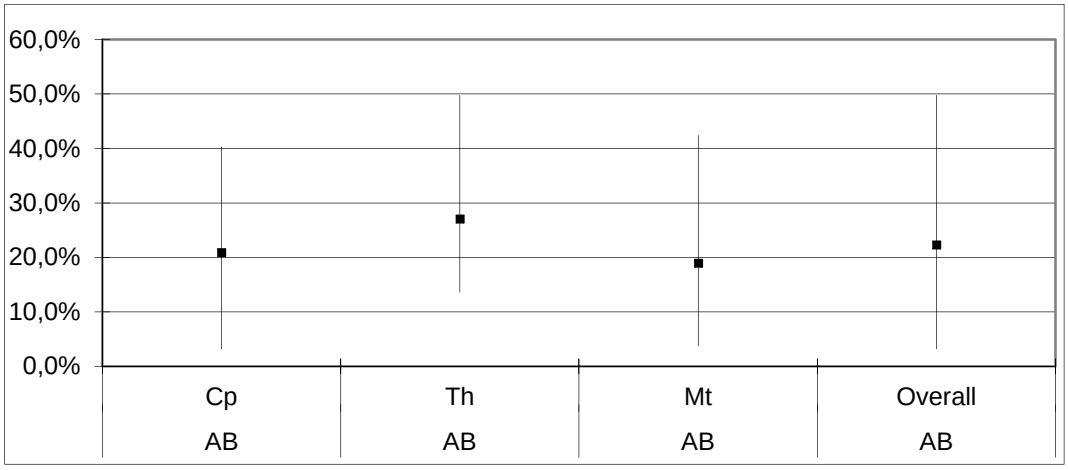
5.3 Antinocicpetive threshold testing using the von Frey device

Testing with the von Frey device was well tolerated by all dogs. There was no evidence of tissue damage, injury or lameness at any time point due to the applied force of the von Frey mechanical threshold testing.

5.3.1 Repeatability of the von Frey threshold

During phase 1 of the study, data collected from the Cp, Th and Mt had the highest repeatability (data not shown) and were selected for the evaluation of treatment effects. The mean coefficients of variation (range) of the von Frey thresholds for the three regions for the investigator AB was 20.8% (3.2%-40.3%); 27% (13.6%–49.8%) and 18.9% (3.7%–42.4%) at the Cp, Th and Mt sides, respectively (Figure 13). The majority (74%) of variability in the von Frey mechanical thresholds was unexplained, but 18.4% was attributed to the operator, 3.4% to the dog, 3.3% to the region and 0.7% to the day.

Figure 13: Coefficients of variation (%) (mean [minimum, maximum]) of the von Frey threshold for the investigator (AB), at the CP, Th, Mt and all regions combined.



5.3.2 Antinocicpetive effects of the treatments

Mechanical von Frey threshold values are presented as the median (interquartile range) grams.

During phase 2 of the study, there was a significant effect of treatment and time in all regions. Threshold values varied significantly by region ($p<0.001$). Threshold values for Th were the highest, followed by Mt and Cp (Table 1). Baseline thresholds at each region were similar throughout the study and did not significantly vary by week.

Table 1: Overall mechanical threshold values in gram (median [interquartile range]) obtained with the von Frey device at the Cp, Th and Mt.

Regions	Median
Cp	138 (118-165)
Th	172 (140-214)
Mt	162 (136-192)

Treatment had a significant effect on the von Frey threshold values when combined over all regions ($p<0.001$). Overall threshold values for treatment Co were significantly lower compared with the three other treatments ($p<0.001$). Overall threshold values for treatment Mm were significantly lower compared with Mg ($p=0.022$). No differences in overall threshold values when combined over all regions were found between Mo and Mg treatments.

Treatment also had a significant effect on the von Frey threshold values at the three independent regions, Cp ($p<0.001$), Mt ($p<0.001$) and Th ($p<0.001$).

At the Cp, treatment Co had the lowest obtained threshold readings. Threshold values at the Cp obtained with treatment Co were significantly lower than with treatment

Mo ($p=0.019$) and treatment Mg ($p<0.001$), but they were not significantly different from treatment Mm ($p=0.099$) (Table 2).

At the Th, treatment Co had the lowest obtained threshold readings. Threshold values at the Th obtained with treatment Co were significantly lower than with treatment Mo ($p=0.014$), treatment Mg ($p<0.001$) and treatment Mm ($p=0.012$) (Table 2).

At the Mt, treatment Co had the lowest obtained threshold readings. Threshold values at the Mt obtained with treatment Co were significantly lower than with treatment Mo ($p<0.001$), treatment Mg ($p<0.001$) and treatment Mm ($p=0.003$) (Table 2).

There were no significant differences in threshold values comparing Mo, Mg and Mm analysed for each region (Table 2).

Table 2: Overall mechanical threshold values in gram (median [interquartile range]). Individual epidural treatments consisted of Co, Mg, Mo and Mm. Results are shown for the three region Cp, Th and Mt, combined and on independent regions. * # ǂ significant differences with respect to treatment Co. τ difference with respect to treatment Mg.

	Treatments				
Region	Co	Mg	Mo	Mm	p values
Combined	145 (120-179)	164 (135-200)*	156 (129-195)*	158 (131-192)* τ	*<0.001, τ 0.022
Cp	130 (111-155)	144 (124-174)*	137 (118-168)#	140 (119-166)	*<0.001, #0.019
Th	160 (125-199)	186 (152-224)*	171 (142-216)#	174 (139-213)ǂ	*<0.001, #0.014, ǂ0.012
Mt	153 (128-182)	170 (136-199)*	162 (136-197)#	166 (141-192)ǂ	*<0.001, #<0.001, ǂ0.003

5.3.3 Changes in the antinociceptive threshold over time for regions

Time had a significant effect on the von Frey threshold values. Overall von Frey threshold values (when treatments and regions were combined) were significantly increased at 30 min, 2, 4, 6 and 12 hours after injection of the treatments compared with baseline values ($p<0.001$, $p=0.002$, $p=0.001$, $p=0.01$, $p<0.001$, respectively). The measurement obtained at 1 hour after treatment administration was increased compared to baseline, but it did not reach statistical significance ($p=0.073$) (Table 3).

At the Cp, time significantly influenced the mechanical threshold values at the time point 30 min ($p<0.001$) after injection of the treatments (Table 3).

At the Th, time did significantly influence the obtained mechanical thresholds values. The threshold values were significantly elevated compared to baseline at 30 min, 1, 2, 4, 6 and 12 hours after injection of the treatments ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.002$, respectively) (Table 3).

At the Mt, time did significantly influence the obtained mechanical threshold values. The threshold values were significantly elevated compared to baseline at 30 min and 2 hours after injection of the treatments ($p=0.007$, $p=0.003$, respectively) (Table 3)

Table 3: Mechanical threshold values (pooled for treatments) in gram (median [interquartile range]) overtime obtained at different time points in hours from epidural injection of the treatments, measured on the three regions combined and on independent regions * Value significantly different compared to baseline ($p<0.05$).

	Time (hours)								
Regions	Baseline	1/2	1	2	4	6	12	18	24
Combined	146 (124-178)	166 (138-208)*	155 (128-190)	159 (130-198)*	161 (132-199)*	157 (129-195)*	162 (131-198)*	146 (122-173)	153 (123-190)
Cp	134 (118-155)	155 (126-199)*	132 (113-159)	137 (117-164)	142 (120-176)	138 (118-160)	143 (121-174)	130 (114-162)	133 (114-162)
Th	152 (122-195)	175 (148-220)*	174 (139-217)*	180 (141-220)*	178 (149-230)*	188 (148-228)*	177 (141-216)*	162 (128-189)	167 (135-207)
Mt	158 (135-185)	168 (141-208)*	160 (134-188)	178 (140-204)*	166 (132-200)	163 (131-193)	165 (137-193)	150 (132-168)	165 (133-197)

5.3.4 Changes in the antinociceptive threshold over time for treatments

Von Frey threshold values when regions were combined showed a significant effect of time for the different treatments (Table 4).

The Co treatment had a significantly elevated threshold compared to baseline at 30min, 2, 4 and 12 hours after injection of the treatments (Table 4).

The Mg treatment had a significantly elevated threshold compared to baseline at 30min after injection of the treatments (Table 4).

The Mo treatment had a significantly elevated threshold compared to baseline at 4 hours after injection of the treatments (Table 4).

The Mm treatment had a significantly elevated threshold compared to baseline at 2 hours after injection of the treatments (Table 4).

Table 4: Mechanical threshold values (pooled for region) in gram (median [interquartile range]) overtime. Individual epidural treatments Co, Mo, Mg and Mm. Results are shown at different time points after epidural injection. * Value significantly different from baseline within a region ($p < 0.05$).

	Time (hours)								
Treatment	Baseline	0.5	1	2	4	6	12	18	24
Co	126 (110-159)	153 (123-184)*	139 (118-177)	148 (122-197)*	151 (126-180)*	148 (125-178)	153 (125-185)*	141 (117-163)	150 (116-177)
Mg	158 (134-192)	179 (149-227)*	158 (127-191)	159 (137-191)	171 (133-211)	171 (135-211)	170 (142-205)	155 (135-187)	153 (131-199)
Mo	152 (127-181)	175 (147-227)*	158 (136-185)	153 (128-196)	165 (134-215)*	161 (133-211)	154 (131-193)	141 (117-167)	160 (125-192)
Mm	148 (132-175)	161 (135-197)	160 (127-204)	174 (143-208)*	164 (134-190)	156 (133-189)	165 (138-203)	148 (123-177)	149 (119-188)

Figure 14 illustrates the actual mechanical threshold values over time obtained at the Cp for all four treatments separately. No statistical significance could be detected at any time point.

Figure 14: Mean (SD) threshold values obtained at the Cp over time with the four treatments.

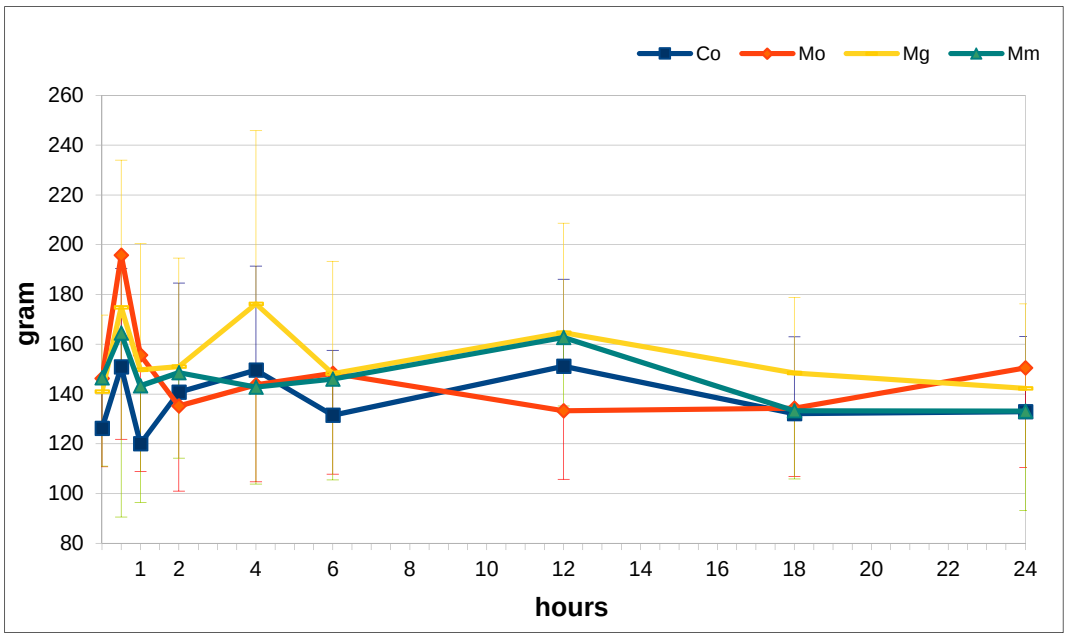


Figure 15 illustrates the predicted mechanical threshold values over time obtained at the Cp for all four treatments separately.

Figure 15: Mean (SD) predicted threshold values obtained at the Cp over time with the Mm eatments.

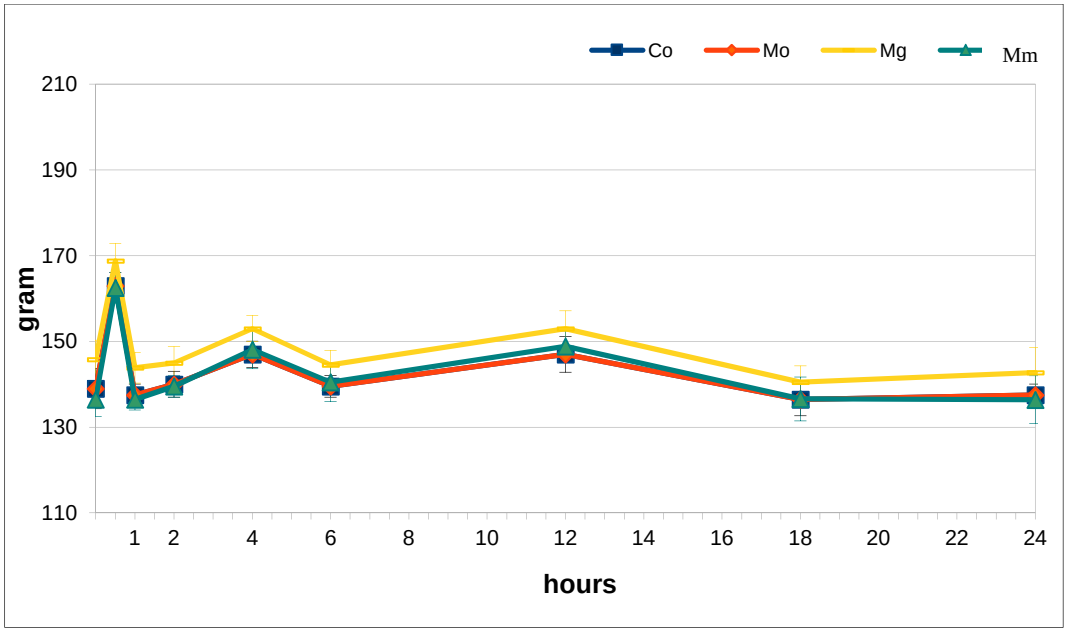


Figure 16 illustrates the actual mechanical threshold values over time obtained at the Th for all four treatments separately. No statistical significance could be detected at any time point.

Figure 16: Mean (SD) actual threshold values obtained at the Th over time with the four treatments.

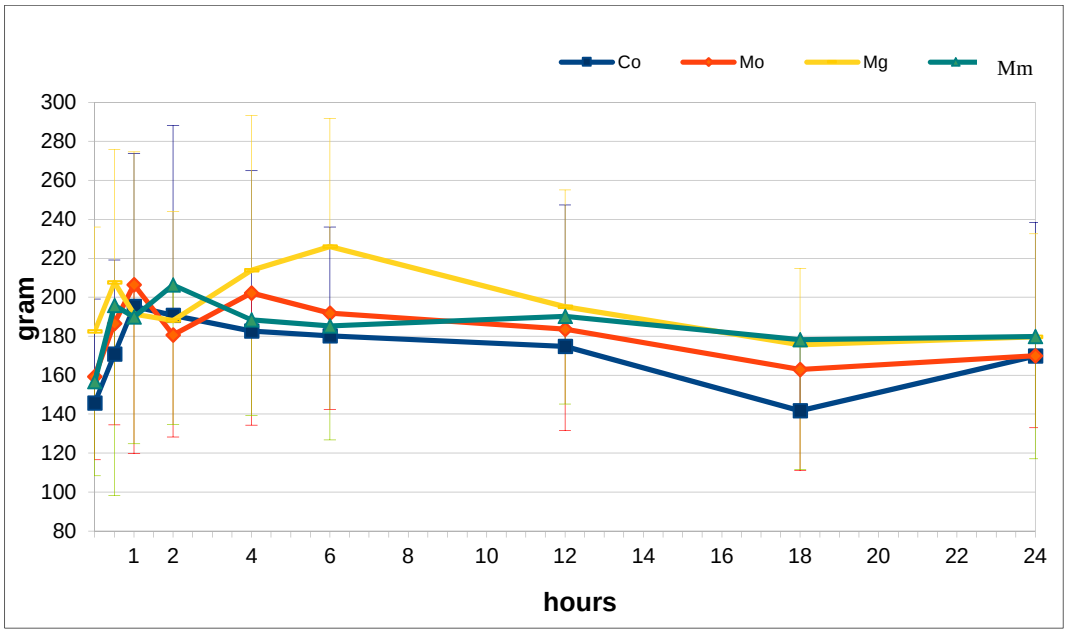


Figure 17 illustrates the predicted mechanical threshold values over time obtained at the Th for all four treatments separately.

Figure 17: Mean (SD) predicted threshold values obtained at the Th over time with the four treatments.

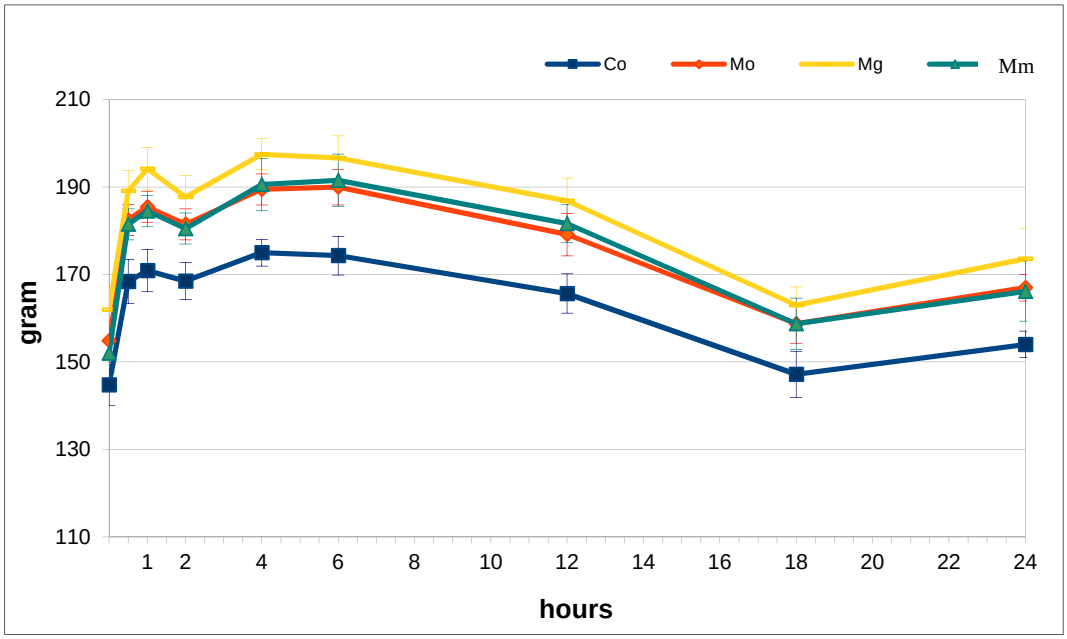


Figure 18 illustrates the actual mechanical threshold values over time obtained at the Mt for all four treatments separately. No statistical significance could be detected at any time point.

Figure 18: Mean (SD) actual threshold values obtained at the Mt over time with the four treatments.

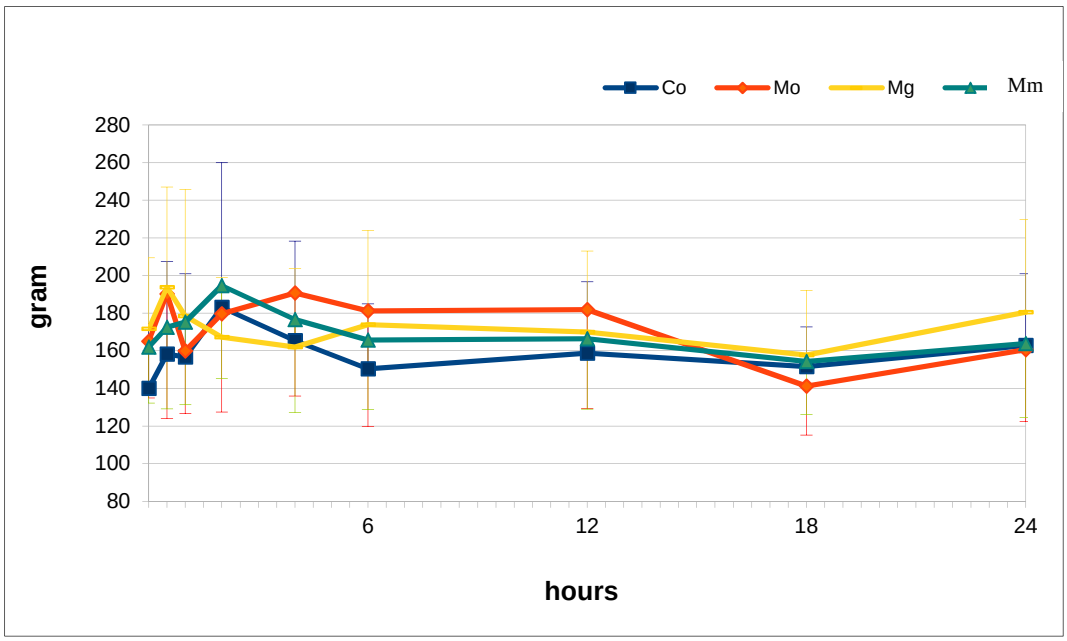
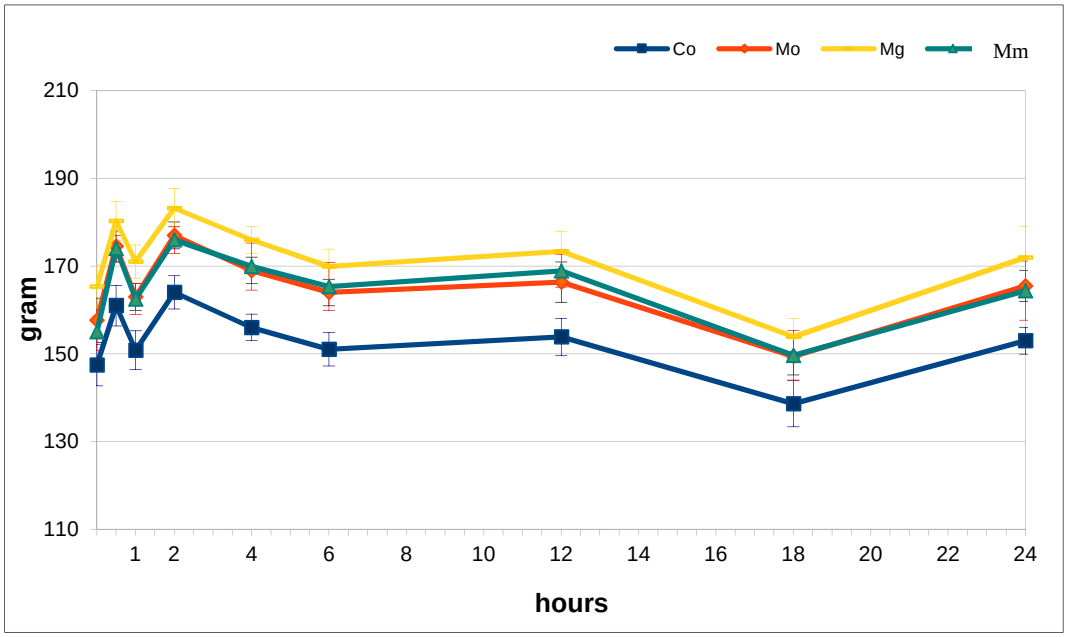


Figure 19 illustrates the predicted mechanical threshold values over time obtained at the Mt for all four treatments separately.

Figure 19: Mean (SD) predicted threshold values obtained at the Mt over time with the four treatments.



In summary, a trend for onset and duration of the antinociceptive effects of the different treatments could be illustrated, although no significant differences were found between time points for individual treatments, only when all treatments were combined. There was an increase in the threshold values 30 min after administration of all treatments in all regions. Threshold values seemed to increase again between 4-6 hours after drug administration. Return to baseline values seemed to occur 18 hours post-injection. Finally, a slight increase in threshold values seemed to take place 24 hours post-injection.

5.3.5 Changes in the antinociceptive thresholds over time in individual dogs for each treatment

5.3.5.1 Changes in the antinociceptive threshold over time for treatment Co

Figure 20: Threshold values obtained at the Cp over time for treatment Co.

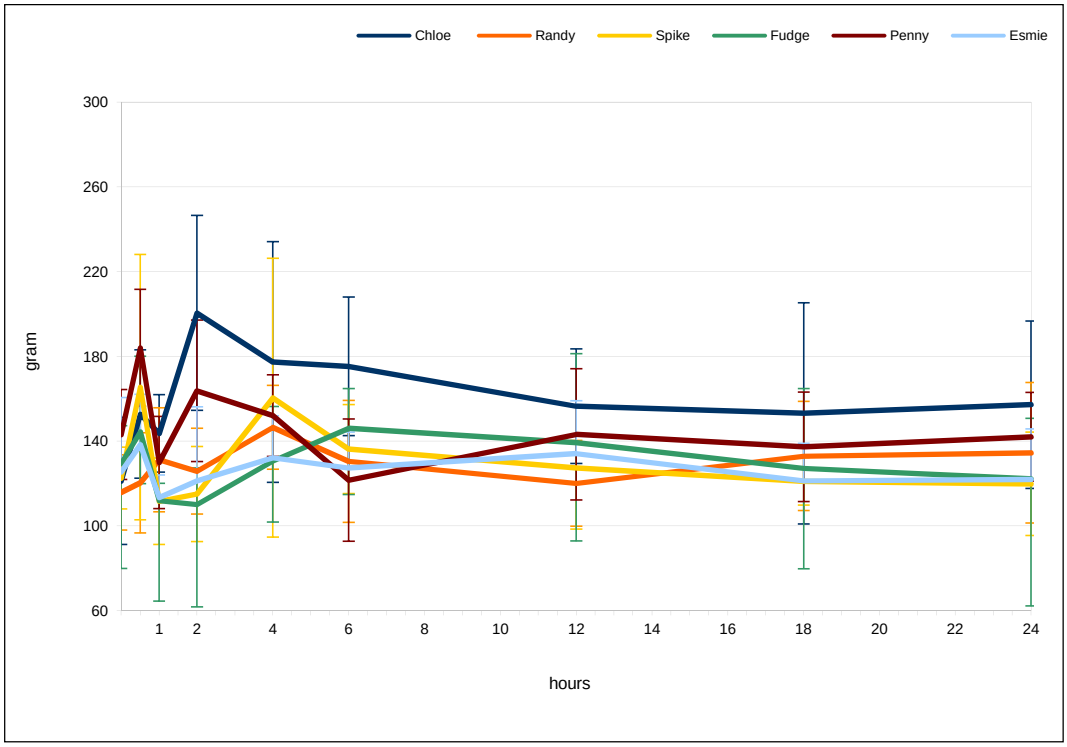


Figure 21: Threshold values obtained at the Th over time for treatment Co.

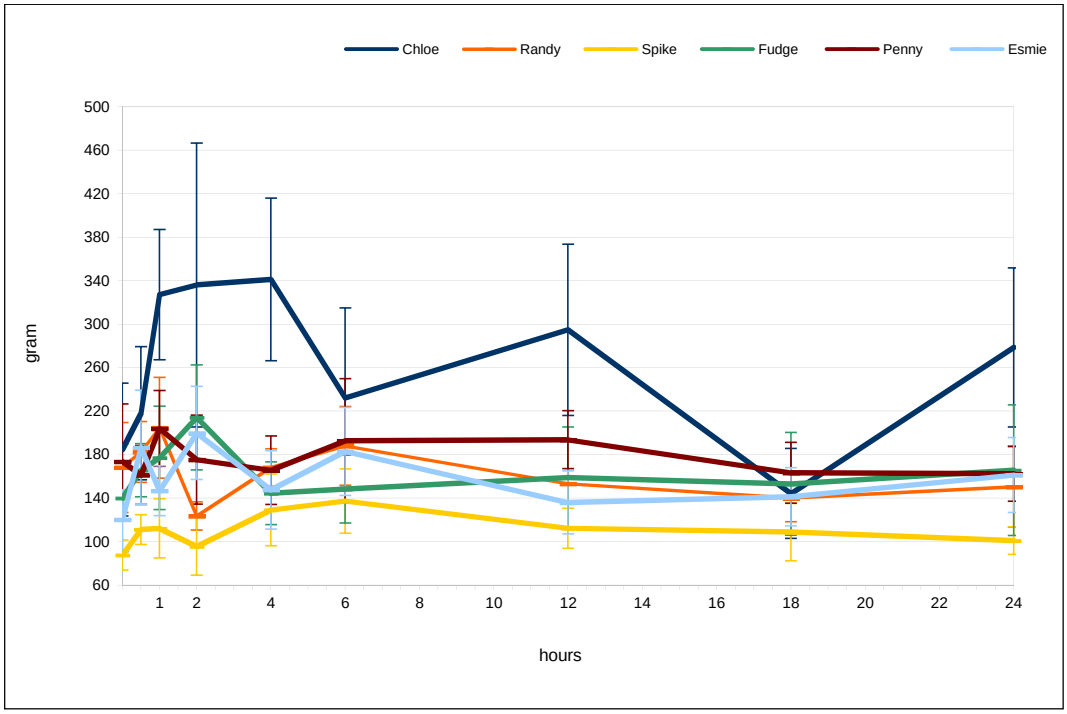
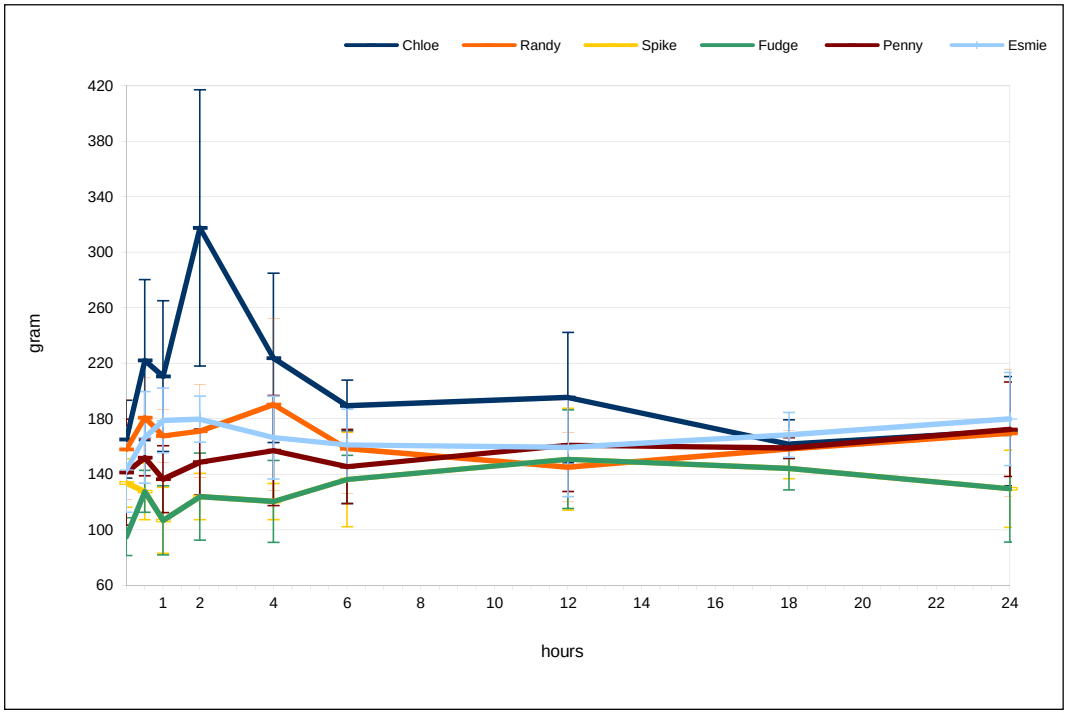


Figure 22: Threshold values obtained at the Mt over time for treatment Co.



5.3.5.2 Changes in the antinociceptive threshold over time for treatment Mo

Figure 23: Threshold values obtained at the Cp over time for treatment Mo.

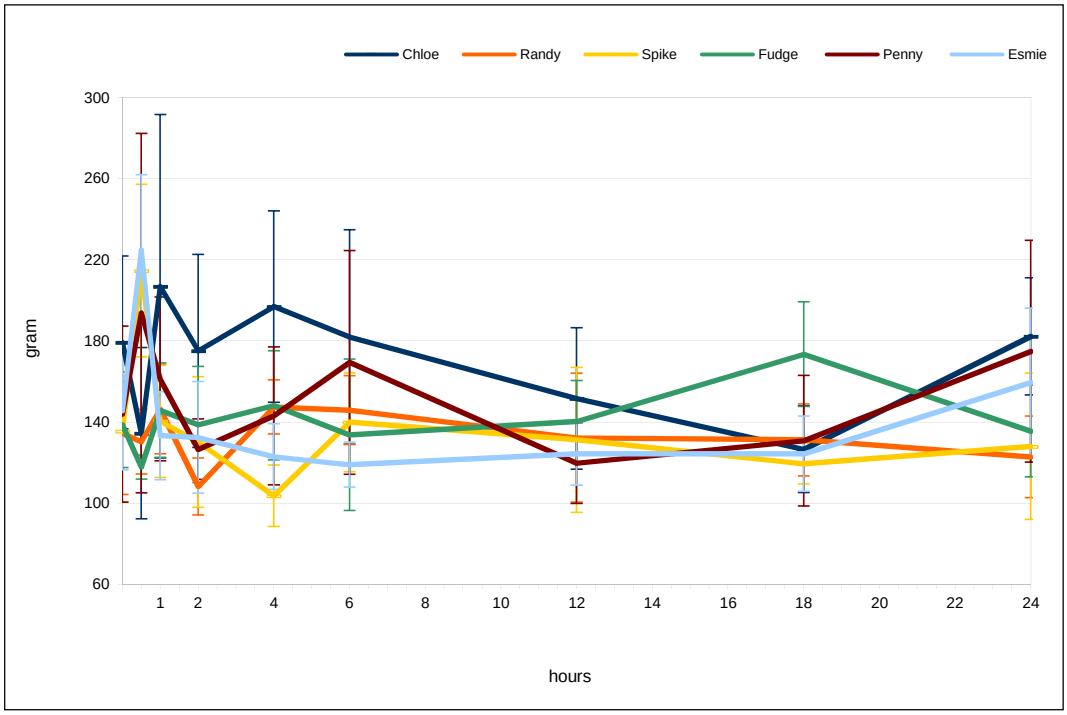


Figure 24: Threshold values obtained at the Th over time for treatment Mo.

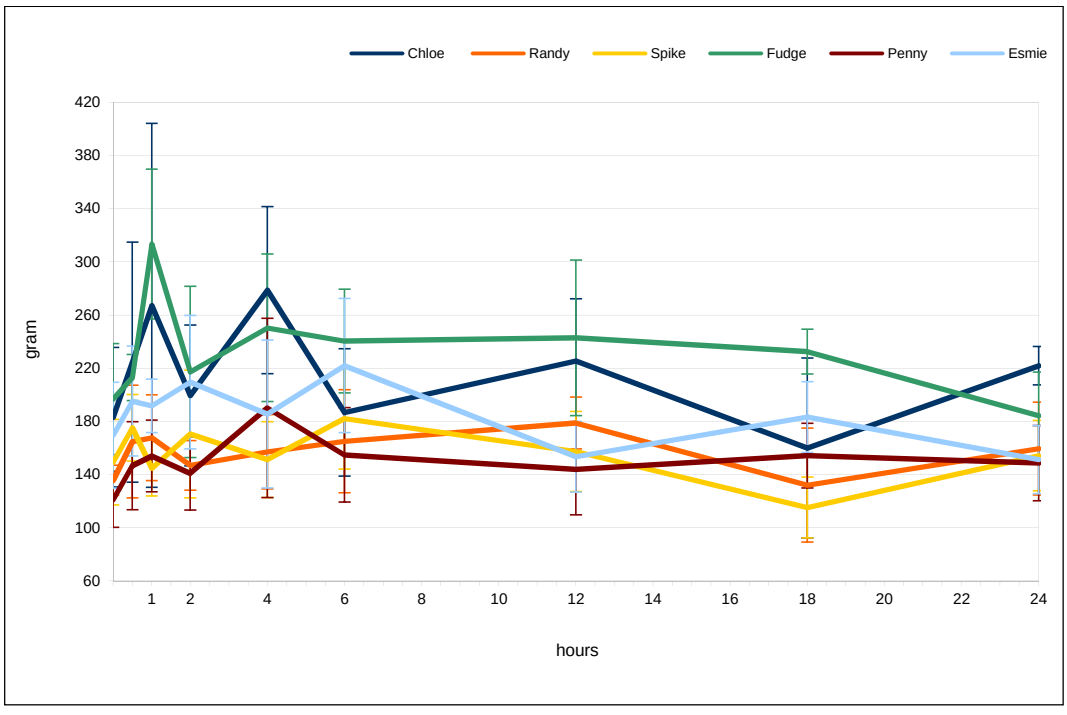
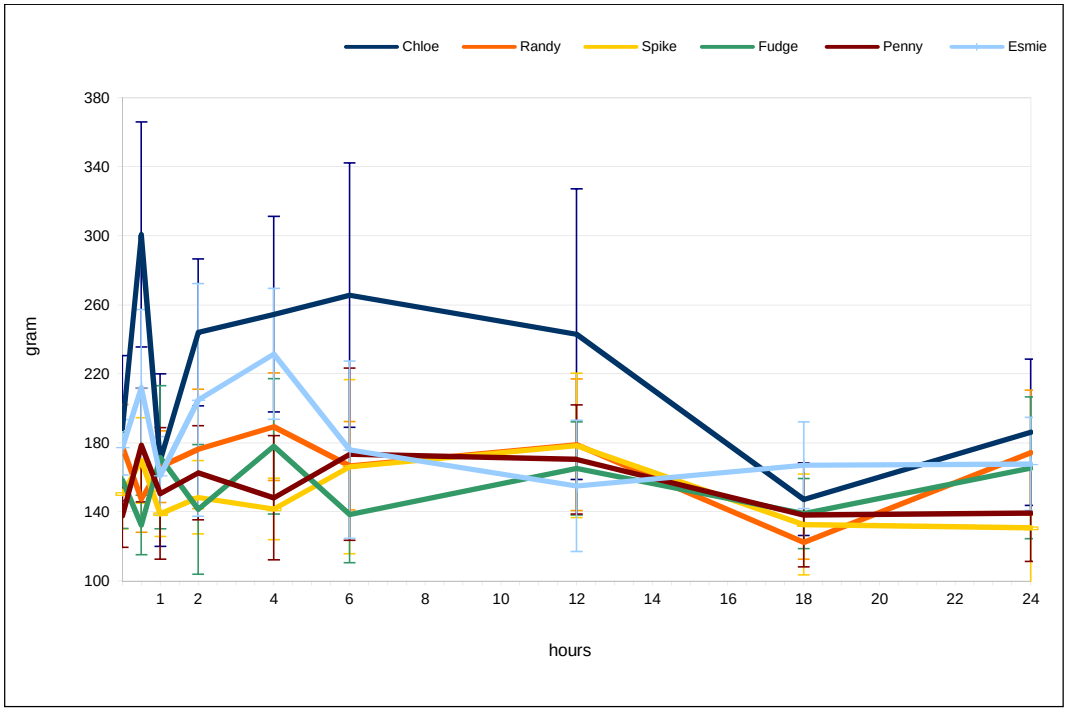


Figure 25: Threshold values obtained at the Mt over time for treatment Mo.



5.3.5.3 Changes in the antinociceptive threshold over time for treatment Mg

Figure 26: Threshold values obtained at the Cp over time for treatment Mg.

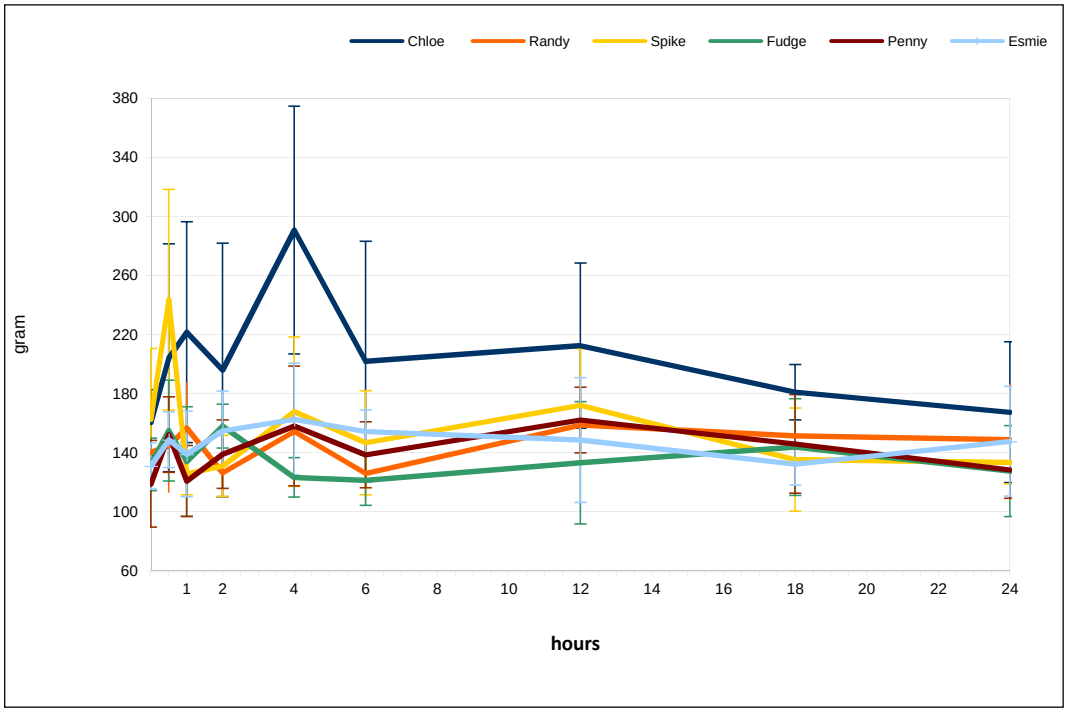


Figure 27: Threshold values obtained at the Th over time for treatment Mg.

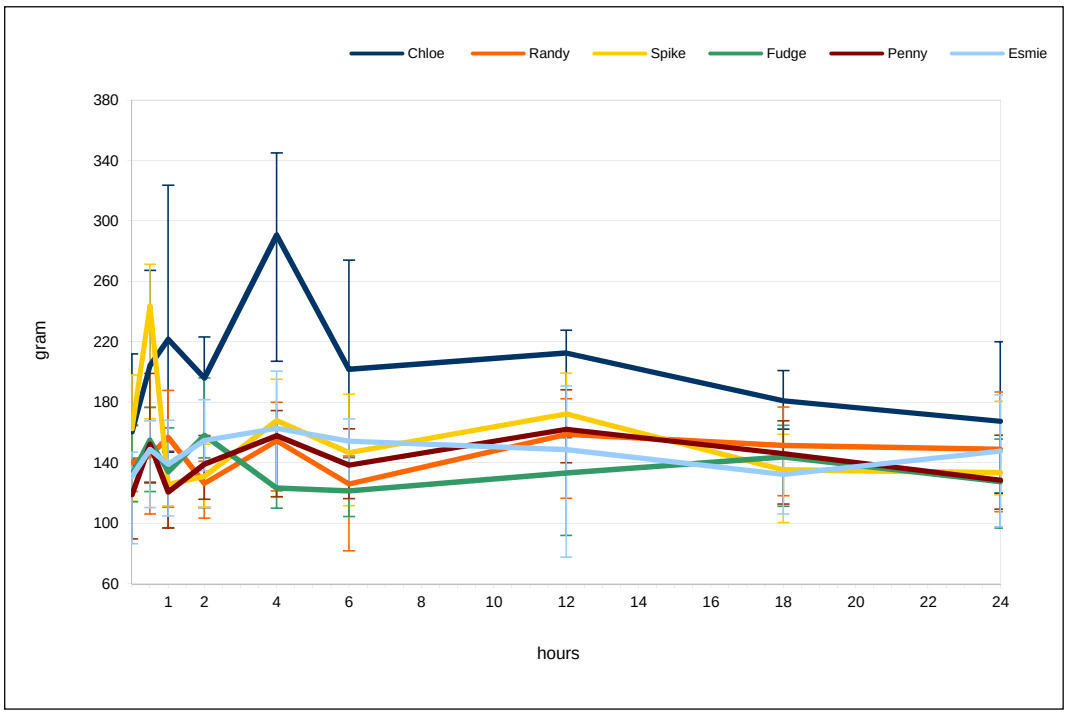
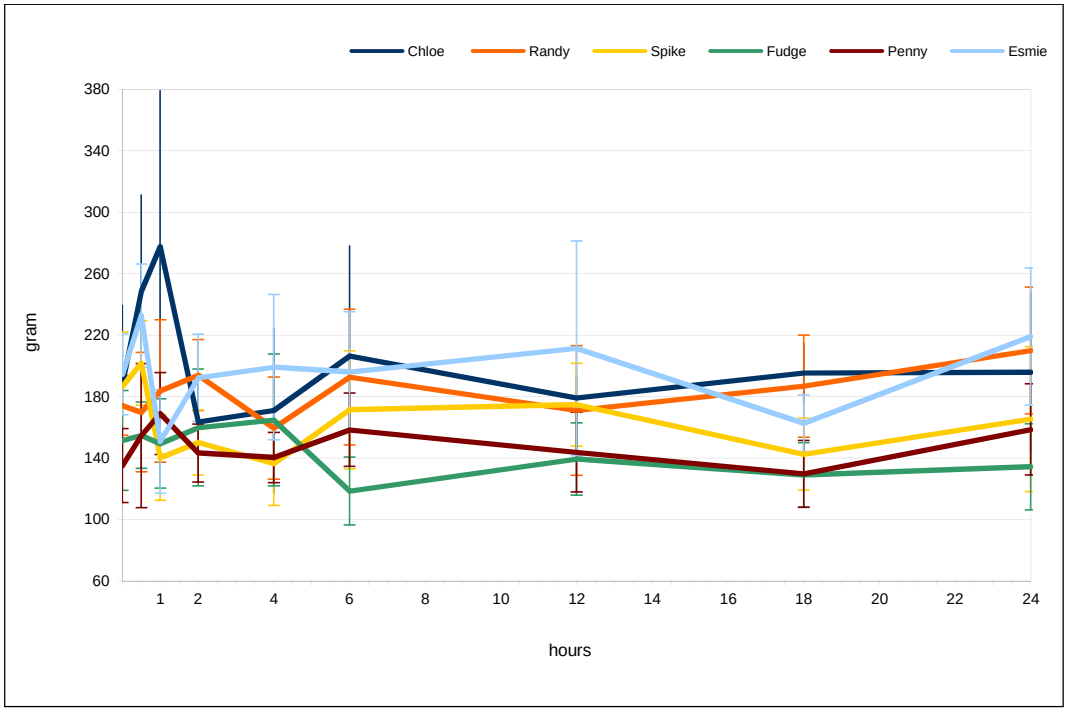


Figure 28: Threshold values obtained at the Mt over time for treatment Mg.



5.3.5.4 Changes in the antinociceptive threshold over time for treatment Mm

Figure 29: Threshold values obtained at the Cp over time for treatment Mm.

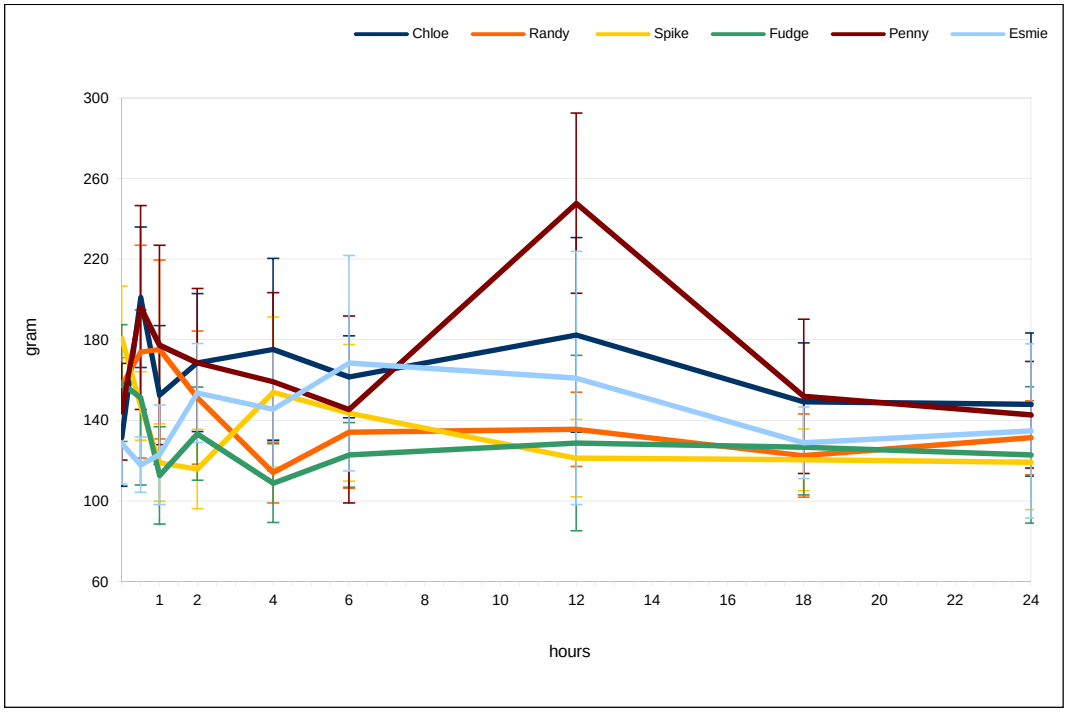


Figure 30: Threshold values obtained at the Th over time for treatment Mm.

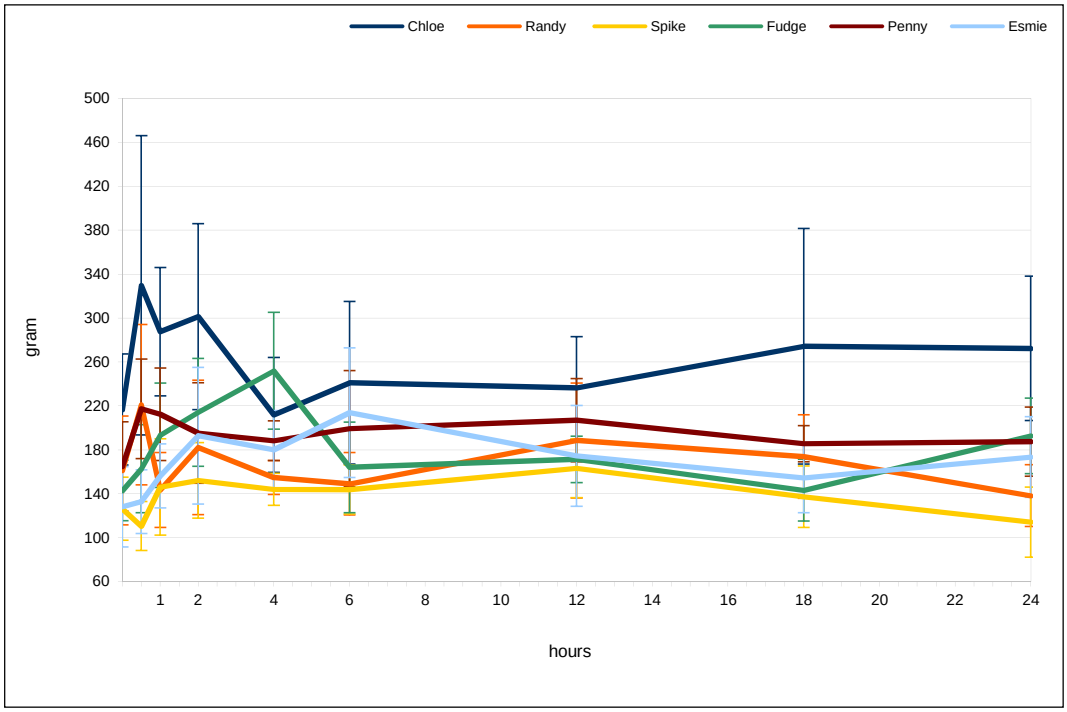
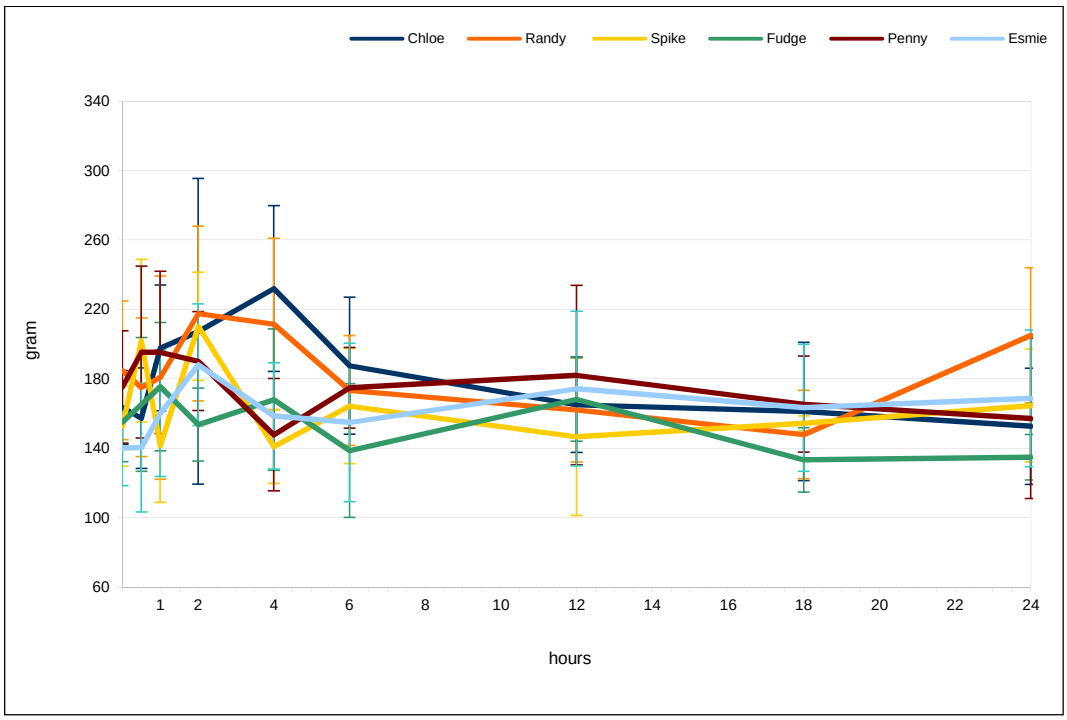


Figure 31: Threshold values obtained at the Mt over time for treatment Mm.



5.3.6 Effect of side on the thresholds

There was a significant effect of side on the mechanical threshold values ($p<0.001$) on all three regions (Table 5).

Table 5: Median (range) mechanical threshold values obtained on the right and left sides in grams. * Significant difference compared to the right side ($p<0.05$).

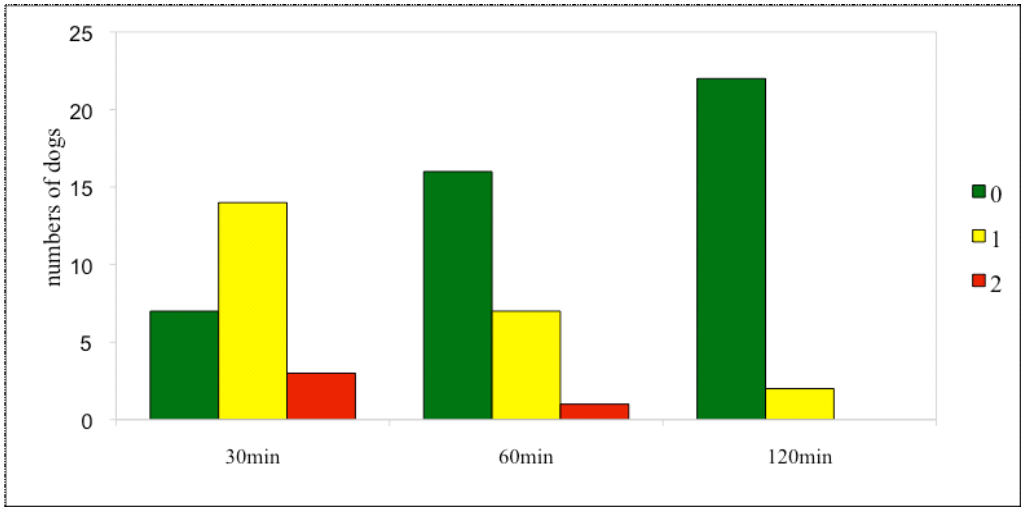
	Left	Right
Regions combined	157 (130, 193) *	154 (126, 189)
Cp	141 (120, 171) *	135 (115, 159)
Th	171 (140, 213) *	174 (139, 216)
Mt	165 (136, 196) *	160 (133, 188)

5.4 Additional measurements

5.4.1 Sedation

Thirty minutes after epidural injection, the majority of dogs were mildly (58%) to moderately (13%) (Figure 30) sedated. Sixty minutes after epidural injection, most dogs were non-sedated (67%) and 120 minutes after epidural injection, only two dogs (4%) still showed mild signs of sedation (both receiving Mo treatment).

Figure 32: Sedation score obtained 30, 60 and 120 minutes after epidural injection of the treatments; no sedation (0), mild sedation (1) and moderate sedation (2)



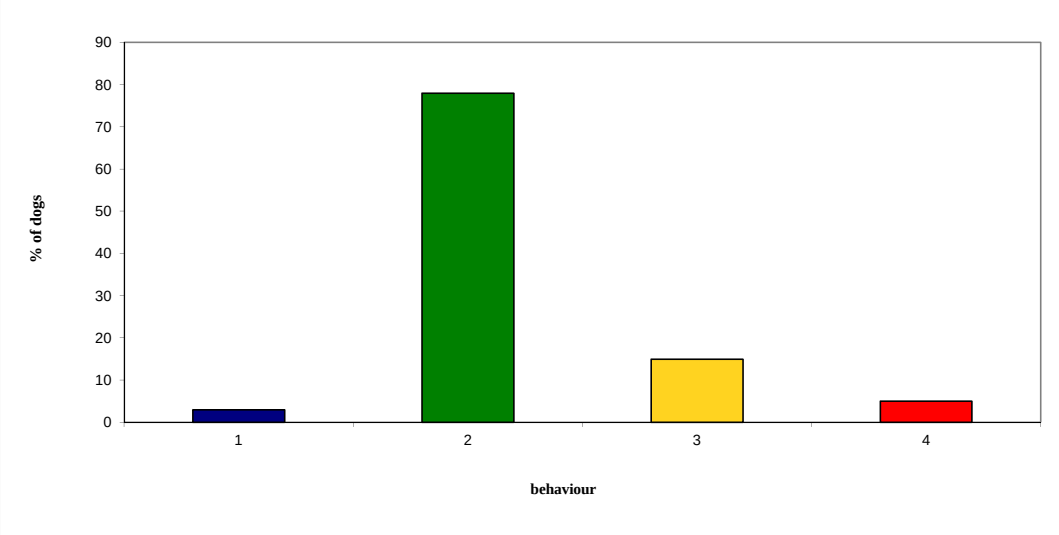
5.4.2 Behaviour

Behaviour score 1 was most commonly given (78%), followed by score 2 (15%) and score 3 (5%). Behaviour score 0 was given 5 times in only two dogs (3%) (Figure 31).

Dogs with behaviour score 0 had significantly higher overall threshold values (166 [137,189]) compared to dogs with a behaviour score 2 (164 [133, 197]) ($p = 0.045$).

At the Mt, dogs with a behaviour score 3 had significantly higher threshold values (185 [156, 215]) than dogs with a behaviour score 2 (172 [145, 197]) ($p = 0.029$). At the other regions, no significant differences in mechanical thresholds could be detected between the behaviour scores.

Figure 33: Illustration of behavior scores observed in dogs in %.



5.4.3 Motor effects

The tail tone scores were always 0 on all dogs at all time points with all treatments.

The scores for ataxia were always 0 on all dogs at all time points with all treatments.

5.4.4 Room temperature and humidity

The median (range) room temperature was 24.9°C (26.8°C–21.4°C) and humidity was 65% (79%–49%) throughout the study.

6 Discussion

Lumbosacral epidural injections in dogs can be challenging and failures of epidural injection are reported to range from 7% (Troncy *et al.*, 2002) to 12% (Heath, 1992). To avoid epidural injection failures and therefore, false negative results an epidural catheter was placed in all dogs before each treatment. Correct epidural catheter placement was assessed by lack of resistance to advancement of the catheter cranially and lack of resistance to injection of sterile water. A more accurate method to ensure correct epidural needle placement is the performance of a fluoroscopy and epidurogram (El-Khoury *et al.*, 1988; Bartynski *et al.*, 2005). However, due to ethical consideration regarding the X-ray exposure these methods could not be performed in the dogs enrolled in the study.

The used morphine dose of 0.1 mg kg^{-1} is the recommended dose for epidural administration in dogs by several authors (Tranquilli *et al*., 2007; Valverde, 2008). Side effects due to epidural morphine administration are rare including respiratory depression, urinary retention and pruritus at the injection site (Torske and Dyson, 2000). In this study no side effects could be observed.

There are no previous studies investigating the antinociceptive effects of epidural magnesium administration in dogs in the literature. The doses of neuraxial magnesium described in the literature are very variable. In humans, a single dose of 50 mg of magnesium has been most commonly used intrathecally with no apparent adverse effects (Buvanendran *et al.*, 2002; Ozalevli *et al.*, 2005; Shukla *et al.*, 2011). Epidurally, 500 mg bolus (Yousef and Amr, 2010) and 50 mg bolus with intrathecal CRI of 100 mg hour has been investigated (Bilir *et al*., 2007; El-Kerdawy, 2008). Epidural doses of 0.18 mg kg^{-1} have been administered in horses (Bigham & Shafiei 2008), 0.21 mg kg^{-1} in cattle (Dehghani and Bigham, 2009a), and 2 mg kg^{-1} in goats (Bigham *et al.*, 2009) in combination with local anaesthetics. A dose of 3 mg kg^{-1} of magnesium administered intrathecally to dogs did not cause any adverse effects and it seemed to possess neuroprotective effects (Simpson et al. 1994). However, neurotoxicity was observed after 3 mg kg^{-1} intrathecal magnesium administration in rabbits (Saeki et al., 2004). Based on the lack of clinically recommended doses and the apparent safety of epidural magnesium, a relatively high dose of 2.5 mg kg^{-1} was used in the present study.

The antinociceptive effect of magnesium extended up to the thoracic limbs. The dispersion of a drug in the epidural space is dependent on the injected volume, the force within the epidural space (Torske and Dyson, 2000) and the lipid solubility of the drug, as these factors facilitate the absorption across the dura membrane and into the cerebrospinal fluid (Valverde, 2008). The total volume administered was approximately equivalent to a volume of 0.2 mL kg^{-1}, which has been described to migrate up to the thoracolumbar area (Torske and Dyson, 2000; Valverde, 2008). The observed effect on the thoracic limbs has been previously described with lumbosacral epidural morphine (Valverde, 2008) and subsequently explained the absorption of the drug into the cerebrospinal fluid, which promoted its cranial migration (Valverde, 2008).

The von Frey device was used to determine increase in antinociceptive mechanical thresholds following morphine and magnesium epidural administration. The von Frey device is validated for antinociceptive threshold testing in dogs and various other species (Jensen and Yaksh, 1986; Redua *et al.*, 2002; Vivancos *et al.*, 2004; KuKanich *et al.*, 2005). In this study, the rigidity of the tip was increased using an epoxy putty to avoid bending of the tip with application of high force as described in KuKanich et al.'s study in 2005 (KuKanich *et al.*, 2005). Bending of the tip would lead to a change in the surface area being applied to the body, thereby producing an unpredictable change in the force applied (Bove, 2006). With this modification no bending occurred in any of the measurements, regardless of the force applied. The same tip was used throughout the whole study to avoid a source of possible variability (Booth and Young, 2000).

The end-point of threshold testing was defined in a pilot study before commencement of the study and the principal investigator (AB) was trained to apply the force in a constant manner. Furthermore, to avoid possible observer bias (Bove, 2006), the principal investigator was blinded to the maximum reading obtained, which was recorded by a second person.

The negative control, treatment Co, had overall the lowest thresholds at all regions and therefore, as expected, no detectable analgesic effects. There was no evidence of change in thresholds obtained for treatment Co overtime that could be attributed to tolerance, hyperaesthesia or learning behaviour.

The Mo treatment, used as a positive control, showed significantly higher thresholds compared to the negative control treatment Co. This effect could be observed at all three measured regions separately as well as in the overall analysis. The significant increase in threshold at the carpal pads indicates that the analgesic effect of morphine reached up to the front limbs as described in another study (Valverde *et al.*, 1989). The analgesic effect of morphine is mainly due to its action on C-fibres in the dorsal horn of the spinal cord, on central pain perception as well as on descending pain pathways (Stoelting and Hillier, 2005) and only at high doses it also affects Aδ fibres (Djouhri and Lawson, 2004). The nociceptive stimulus elicited by the von Frey device is mechanical and causes activation of Aδ (high-threshold mechano-heat and mechano-cold nociceptors) and polymodal C-fibres (Djouhri and Lawson, 2004). The pain elicited by the von Frey device can be described as similar to naturally occurring pain (Le Bars *et al.*, 2001). The results of the present study show that the administered morphine did have an analgesic effect and that the von Frey device was sensitive enough to show this effect.

As hypothesised, the magnesium treatment showed significantly higher thresholds compared with treatment Co. This analgesic effect was identified on all measured regions. This implies that epidural magnesium mediated an analgesic effect that spread up to the front limbs. Interestingly, the thresholds obtained in magnesium treatment were numerically the highest of all groups, although this was not statistically significant. Similar results were found in the study by Bahr et al. in 1996 (Bahar *et al.*, 1996), where intrathecal magnesium also induced sensory block, although in this study they also showed motor block and sedation. The analgesic effect of magnesium is thought to be due to its antagonistic action at the NMDA-receptor (Dubé and Granary, 2003). The NMDA-receptor is mainly activated by continuous nociceptive input from C-fibres and contributes to the development of central sensitisation (Bhatia *et al.*, 2004). Other possible mechanisms for the antinociceptive effect of magnesium include inhibition of catecholamine release causing a decrease in neuronal activity (Shimosawa, 2004), inhibition of acetylcholine release or membrane stabilization (Herroeder *et al.*, 2011). All these mechanisms are due to the calcium-antagonistic effects of magnesium (Fawcett *et al.*, 1999). The obtained serum magnesium levels were all within normal limits (0.6-1.2 mmol/L) and all dogs were healthy; hence a state of hypomagnesaemia in these dogs was

unlikely. Therefore, the antinociceptive effect of magnesium was most likely due to its direct effect on the spinal cord and not due to the correction of a magnesium deficiency.

Neuraxial administration of magnesium (epidural and intrathecal) in combination with opioids and/or local anaesthetics provides a longer duration of analgesia (Buvanendran *et al.*, 2002; Ozalevli *et al.*, 2005; Yousef and Amr, 2010; Shukla *et al.*, 2011; Nath *et al.*, 2012), a post-operative opioid sparing effect (Arcioni *et al.*, 2007; Ouerghi *et al.*, 2011; Khezri *et al.*, 2012). A synergistic effect between magnesium and opioids has been previously postulated (Tramer *et al.*, 1996). This possible interaction was investigated in this study by administering both drugs in combination. Treatment Mm had significantly higher thresholds compared with treatment Co when all regions were analysed together and at the thorax and metatarsi. At the carpal pads, thresholds obtained were numerically higher than in treatment Co, but it did not reach statistical significance. It is possible that with a greater sample size an effect on the front limbs could have been also observed. However, no enhancement of antinociception was observed when magnesium was combined with morphine since the thresholds were not significantly different from those obtained with morphine or magnesium administered alone. Therefore, no synergistic or additive effect could be demonstrated in this study. A possible explanation could be the limited sample size of six dogs. Another possibility is that the nociception elicited by the von Frey device is of different quality and intensity than pain elicited by surgeries (Ozalevli *et al.*, 2005; El-Kerdawy, 2008; Yousef and Amr, 2010), and therefore, the synergistic effect of opioids in combination with magnesium seen in patients undergoing surgeries might be due to the difference in pain experience. Both opioids and magnesium have an antagonistic action on calcium channels, preventing calcium influx into pre-synaptic cells leading to a decrease in excitatory transmitter release (Tranquilli *et al*., 2007; Fawcett *et al.*, 1999). Therefore, a ceiling effect on the inhibition of the pre-synaptic calcium channels could have been reached when both drugs were administered together, which may have prevented an enhancement of the antinociceptive effect. Another possibility is that the dose of magnesium was either too high or too low to observe a synergistic interaction with morphine. A dose-finding study would be necessary to establish this.

An increase in mechanical thresholds was observed 30 min after drug administration with all treatments and decreased 1 hour after injection compared to

baseline. The measurement obtained 1 hour after injection showed no significant increase compared with baseline. During the first von Frey threshold testing 30 min post-injection most of the dogs were mildly to moderately sedated, whereas 1 hour post-injection less than half of the dogs showed signs of sedation. Therefore, the increase in threshold at 30 min could have been due to sedation. A sedated dog is more likely to respond only to nociceptive stimuli of greater intensity and the reaction time might also be prolonged compared with a non-sedated dog (Beecher, 1957). However, in a study performed by KuKanich et al. in 2005 (KuKanich *et al.*, 2005), a high dose of intravenous morphine was administered to dogs and the reported sedation lasted for 7-12 hours whereas antinociception, evaluated with the von Frey device, only lasted for 4 hours. Based on these results the authors suggested that von Frey mechanical thresholds were able to discriminate between antinociceptive effects and sedation. In our study, the observed sedation of the dogs was most likely due to residual anaesthesia from isoflurane (Lopez *et al.*, 2009), which might be different from the sedation mediated by IV morphine. Another possible explanation for the increase in thresholds 30 min post-injection is the rapid initial systemic absorption of the injected drugs causing systemic rather than neuraxial analgesia, as has been shown following epidural morphine in dogs (Valverde *et al.*, 1992).

An increase in thresholds was observed from 30 min to 12 hours, excluding 1 hour, compared with baseline following administration of all treatments when all regions were pooled together. An onset and duration of the individual treatments could not be established.

Interestingly the control group showed a significant increase in threshold over time compared to baseline when treatments were analysed separately. These observation remains unexplained. An increase of power might be necessary to detect differences in the pairwise comparisons for the individual treatments. A larger sample size would be necessary to detect onset and duration of each individual treatment. An onset of action for epidural morphine is reported to be 20-60 min (Jones, 2001; Valverde, 2008). In the literature magnesium is described to delay the onset of analgesia achieved with opioids and local anaesthetics (Ozalevli *et al.*, 2005; El-Kerdawy, 2008). The duration of the analgesic action of epidural morphine in dogs reported in the literature varies from 10 to 23 hours (Torske and Dyson, 2000), 12 to 24 hours (Valverde, 2008), 16 hours (Troncy *et*

al., 2002) and 16 to 24 hours (Jones, 2001). It was expected that magnesium would prolong the analgesic effect of morphine as previously described (Ozalevli *et al.*, 2005; Shukla *et al.*, 2011; Nath *et al.*, 2012); however, this could not be determined with the methods used in this study.

Little is known about onset of action of epidural magnesium. In a study performed in humans the onset of systemically administered magnesium is approximately 30 min (Brill *et al.*, 2002). A delayed onset of analgesia is described in humans when magnesium is added to spinal anaesthesia (Ozalevli *et al.*, 2005; El-Kerdawy, 2008) Epidural injection of magnesium in combination with lidocaine in goats (Bigham *et al.*, 2009), horses (Yousef and Amr, 2010) and cattle (Dehghani and Bigham, 2009b) results in a rapid onset of analgesic action of a few minutes, which could be due to the rapid onset of action of lidocaine (Valverde, 2008). Interestingly, these studies report a delayed onset of a few minutes when magnesium is added compared to the administration of lidocaine alone.

Little is known about the duration of antinociception of epidural magnesium administered alone. In sheep epidural magnesium produced an analgesic effect lasting approximately 29 minutes (DeRossi *et al.*, 2012). When magnesium is administered in combination with lidocaine, the duration of analgesia is reported to be approximately 3 hours (Bigham *et al.*, 2009; Dehghani and Bigham, 2009b; Yousef and Amr, 2010). In humans, intrathecal magnesium in combination with a local anaesthetic and an opioid resulted in an analgesic effect of approximately 2 hours (Ozalevli *et al.*, 2005).

A potentiation of the analgesic effect of morphine with magnesium may be demonstrated by obtaining higher thresholds or by observing a prolonged duration of effect compared to each drug administered individually (Ozalevli *et al.*, 2005; Shukla *et al.*, 2011; Nath *et al.*, 2012). An increase in the duration of the antinociceptive effect could not be demonstrated in this study. Possible explanations could be the limited sample size, the lack of clinical pain and/or the lack of sensitivity of the methods used.

Interestingly, the thresholds obtained on the left side were significantly higher than on the right side in all regions. The position of the patient after an epidural injection

is known to influence the contact of the agent with the target tissue and therefore, influence the spread of the drug (Valverde, 2008). In the present study, the epidural injection was performed with the dogs in sternal recumbency and during recovery the dogs remained in sternal recumbency until they were able to stand and walk on their own. Also, during the von Frey testing, dogs remained in standing or sitting position. During threshold measurements at the thorax, some dogs were positioned in lateral recumbency; however, this was approximately 35 min after injection and the spread of epidurally administered drugs is believed to be complete after 5 minutes post-injection (Tranquilli *et al*., 2007). Therefore, it is unlikely that the change in position at this point in time might have influenced the spread of the drugs. Another possible explanation is the lateralization of the epidural catheter during its introduction into the epidural canal. The same person (ER) performed all the catheter placements and used the same technique. This investigator is right handed and this could have influenced the lateralization of the catheter towards the left side.

It has been previously hypothesized that mechanical threshold testing may be affected by environmental or external factors (e.g. time of the day, visual stimuli, noise, which may cause distraction and increase the thresholds) and internal factors like behaviour (e.g. frightened animals or very active animals would respond earlier than calm and friendly animals) (Bove, 2006). In the present study behaviour significantly affected the mechanical thresholds obtained, with more active dogs obtaining greater threshold values than calm dogs. In calm dogs the device could be applied more accurately, with slower increasing force and it was easier to see a clear end-point than in very active dogs. All the evaluations were performed in a familiar room, separated from the wards, with minimal restraint and minimal distraction of the dogs to try to exclude the influence of external factors. However, some environmental factors could not be totally controlled as for example the time of the day when dogs were fed, which was in the morning and probably influenced the results. Nonetheless, behaviour was included in the model to account for its possible effects on the mechanical thresholds.

As hypothesized, epidural magnesium injection did not cause a decrease in tail tone or ataxia. By acting mainly on the NMDA-receptor, magnesium causes analgesia, but it is only an exaggerated antagonistic action on the NMDA-receptor that may cause ataxia and motor incoordination as observed previously in rats (Bahar *et al*., 1996;

Karasawa *et al.*, 1998). At the doses used in the present study, it seems that magnesium may be safely added to epidural morphine without causing any motor deficits.

Room temperature may affect nociception due to its effect on skin vasoconstriction and vasodilatation (Love, 2011). The humidity may affect the bending filaments, but it is unlikely to affect the rigid tips. In this study, room temperature and humidity were controlled and maintained relatively constant; therefore, it is very unlikely that they influenced the threshold readings obtained in these dogs.

One limitation of this study is the fact that it was performed in dogs; therefore, the end-point for antinociception (i.e. withdrawal of limb or turning the head at the probe) is subjective and might be influenced by other factors as discussed above. Unfortunately, there is currently no "Gold Standard" method for antinociceptive testing in animal studies of pain. In this study using von Frey mechanical thresholds the antinociceptive effect of morphine, our positive control treatment, could be detected; therefore, it seems that the methodology was appropriate.

Another limitation of the study is the sample size, which was limited to six dogs. However, by performing a cross-over study the variability is decreased; therefore, the statistical power is increased. The sample size was large enough to detect overall differences between treatments in the 3 studied regions, but it was insufficient to detect an onset and duration of effect of the individual treatments as previously discussed.

A possible source of variability is the observer performing the testing. The intra- and inter-observer coefficients of variation were calculated before commencement of the study and they were within the range of 20-30%, which is considered acceptable for the validation of serological tests (Jacobson, 1998). Inter-observer variability could be excluded as only one person performed the measurements. The principal investigator performing the measurements practiced using the von Frey device before the actual study and learned to increase the force gradually. Therefore, this source of variability should have had a minimal impact on the results of this study.

7 Conclusion

In conclusion, the present study showed that a lumbosacral epidural injection of 2.5 mg kg^{-1} $MgSO_4$ produces an antinociceptive effect in dogs without causing any motor deficits when administered alone or in combination with morphine. The antinociceptive effect of magnesium could be observed in the Cp, Th and Mt indicating that antinociception reached up to the thoracic limbs. The onset and duration of the antinociceptive effect could not be determined, although a tendency could be observed.

No potentiation of the antinociceptive effect could be demonstrated between morphine and magnesium.

The von Frey aesthesiometer was able to detect the antinociceptive effects mediated by morphine and magnesium.

To what extend these results can be extrapolated to clinical cases needs further investigation. As this was not a clinical trial and no clinical pain was present in these dogs, results obtained are not directly applicable to clinical cases with acute pain, chronic pain or central sensitization. Clinical studies are necessary to determine whether epidural administration of magnesium would be beneficial.

In addition, the fact that a potentiation of the antinociceptive effect was not observed between morphine and magnesium contradicts the findings from the reviewed literature. Further studies with other type of stimuli, different dosages or performed in clinical cases with naturally occurring pain are warranted to demonstrate a possible positive interaction effect between these drugs.

8 Summary

Antinociceptive effects of epidural magnesium sulphate alone or in combination with morphine in dogs

The analgesic properties of magnesium meditated by its physiological antagonistic action on the NMDA-receptor is of great interest in human and veterinary medicine. The primary objectives of this study were to investigate whether the lumbosacral epidural injection of magnesium could produce an antinociceptive effect and to determine whether there was possible potentiation of the antinociceptive effect between magnesium and morphine when administered epidurally in combination in dogs. A secondary goal was to study the onset and duration of the antinociceptive effect of epidural magnesium alone or in combination with morphine in dogs. Furthermore, the possible motor deficits induced by epidural magnesium were investigated.

Six healthy, adult, neutered research Beagle dogs (3 male and 3 female) were used in a randomized blinded crossover study with a one-week wash-out period between treatments. Treatments consisted of an epidural injection of: 0.115 mL kg^{-1} of sterile water (treatment Co); 0.1 mg kg^{-1} of morphine (treatment Mo); 2.5 mg kg^{-1} of $MgSO_4$ 50% (treatment Mg); and 2.5 mg kg^{-1} of $MgSO_4$ together with 0.1 mg kg^{-1} of morphine (treatment Mm). Sterile water was added to treatments Mo, Mg and Mm to receive a total volume of 0.115 mL kg^{-1}. Dogs were anaesthetized with propofol and isoflurane to place a lumbosacral epidural catheter for the administration of the treatments. Antinociceptive effects were evaluated at different time points for 24 hours post-injection using von Frey mechanical thresholds. Three threshold measurements on both sides were obtained at the carpal pads, thorax and metacarpi at each time point and then averaged for statistical analysis. Maximum applied force eliciting a nociceptive response was recorded and compared between treatments. Within each treatment, measurements obtained at different time points were compared with baseline values. Tail tone, level of ataxia, level of sedation and behaviour were scored at each time point. Data were analysed using a linear mixed model with significance set as $p<0.05$.

The treatment groups Mg, Mo and Mm had significantly higher thresholds at all three measured regions compared with treatment Co (except for the carpal pads with treatment Mm). There was a significant increase in threshold values over time obtained at the thorax and at all three regions pooled together. No motor deficits were observed with any of the treatments at any time point. Behaviour influenced the mechanical thresholds and was included in the statistical model as a fixed effect.

In conclusion, 2.5 mg kg^{-1} $MgSO_4$ administered in the lumbosacral epidural space in dogs produces antinociception without causing motor effects. The antinociceptive effect of magnesium reached up to the thoracic limbs. No potentiation of the antinociceptive effect could be detected between magnesium and morphine. Onset and duration of analgesia could not be determined although there was a significant effect of time on the threshold values.

The present study suggests that a lumbosacral epidural injection of magnesium in dogs might be useful to provide analgesia to the thoracic and pelvic limbs, as well as the thorax. However, to what extend magnesium causes analgesia in clinical cases and in states of central sensitisation requires further investigation.

9 Zusammenfassung

Anti-nozizeptive Effekte von epidural verabreichtem Magnesiumsulfat allein und in Kombination mit Morphin beim Hund

Die analgetische Wirkung von Magnesium als physiologischer Antagonist am NMDA Rezeptor ist von großem Interesse in der Human- und Tiermedizin. Ziel dieser Studie war zu untersuchen, ob die epidurale lumbosacrale Administration von $MgSO_4$ beim Hund eine analgetische Wirkung besitzt und ob eine Potenzierung durch gemeinsame Verabreichung mit Morphin erzielt werden kann. Des Weiteren wurde der Eintritt und die Dauer der analgetischen Wirkung von $MgSO_4$ allein und bei gemeinsamer Administration mit Morphin untersucht. Das mögliche auftretenden motorische Funktionsausfälle wurde ebenfalls studiert.

Sechs gesunde, ausgewachsene, kastrierte Beagle (3 männlich und 3 weiblich) wurden in einer randomisierten, blinden, „cross-over“ Studie verwendet. Die Auswaschzeit zwischen den verschiedenen Behandlungen betrug eine Woche. Die Behandlungen bestanden aus epiduralen Injektionen von: 0,115 ml kg^{-1} steriles Wasser (Gruppe Co); 0,1 mg kg^{-1} Morphin (Gruppe Mo); 0,005 ml kg^{-1} Magnesium (Gruppe Mg); 0,005 ml kg^{-1} Magnesium und 0,1 mg kg^{-1} Morphin (Gruppe Mm). Steriles Wasser wurde zu den Gruppen Mo, Mg und Mm hinzugefügt, um ein absolutes Volumen von 0,115 ml kg^{-1} zu erhalten. Die Hunde wurden anästhesiert und ein Katheter, für die Verabreichung der Behandlungen wurde in den lumbosakral in den Epiduralraum eingeführt. Die anti-nozizeptive Wirkung wurde zu verschiedenen Zeitpunkten über einen Zeitraum von 24 Stunden evaluiert. Zur Evaluierung des anti-nozizeptive Effekts wurde der mechanische Schwellenwertes unter zur Hilfenahmen des Von Frey Gerätes bestimmt. Jeweils drei Schwellenwert-Messungen an der linken und rechten Körperseite am Carpus, Thorax und Metatarsus wurden durchgeführt. Der maximale zugefügte Druck, der zu einer Schmerzreaktion führte, wurde aufgezeichnet. Die Werte wurden gruppenweise verglichen. Des Weiteren wurden die Werte in den Gruppen mit den Ausgangswerten verglichen. Zusätzlich wurde die Spannung der Rute, Grad der Sedation und das Verhalten der Hunde zu jedem Messzeitpunkt bewertet.

Die Gruppen mit der Behandlung Mg, Mo und Mm hatten signifikant höhere Schmerzschwellenwerte an jedem der untersuchten Körperareale verglichen mit Gruppe Co. Ausgenommen davon waren die Werte von MM, die kein signifikant höheren Schwellenwert am Carpus aufwiesen. Über den Zeitverlauf war ein signifikanter Anstieg des Schmerzschwellenwerts am Thorax und an allen drei gemessenen Körperareale zu bemerken, vorausgesetzt diese wurden gemeinsam analysiert (ausgenommen 1 Stunde nach der Injektion). Das Vergleichen von Schwellenwerten innerhalb jeder Gruppe zum jeweiligen Ausgangsschwellenwert zeigte keinen signifikanten Anstieg des Schwellenwertes über den Zeitverlauf. Das Verhalten der Hunde beeinflusste den Schwellenwert und wurde demzufolge in der statistischen Auswertung mit berücksichtigt. Eine Änderung der Rutenspannung trat zu keinem Zeitpunkt auf. Die Hunde waren nach der Anästhesie während der ersten Messungen gering bis mittelgradig sediert.

Diese Studie schlussfolgert, dass $MgSO_4$ epidural verabreicht, bei Hunden zu einem analgetischen Effekt führt, ohne paralytisch zu wirken. Eine synergistische Wirkung zwischen Magnesium in der verwendeten Dosis und Morphin konnte nicht festgestellt werden. Der Beginn und die Dauer der Analgesie konnte nicht bestimmt werden, wenn auch der Faktor Zeit eine Rolle zu spielen scheint.

Die vorliegende Studie lässt vermuten, dass die lumbosakrale epidurale Injektion von Magnesium zur analgetischen Wirkung an der Vorder-, Hintergliedmaße und am Thorax führt. In welchem Umfang Magnesium im klinischen Einsatz und bei zentraler Sensibilisierung analgetische Wirkungen vermittelt, bedarf weiteren Untersuchungen.

10 References

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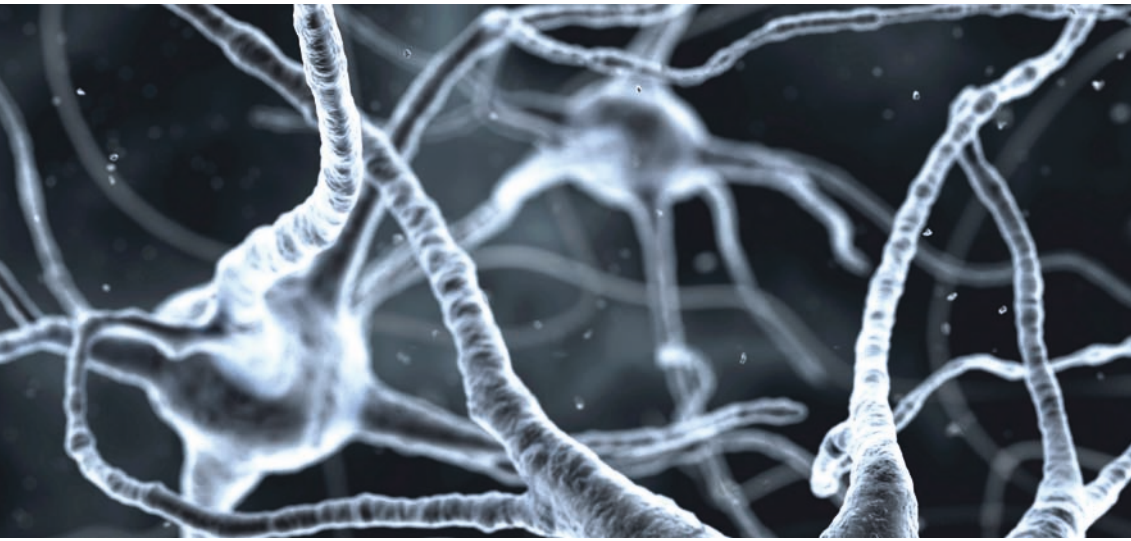

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