UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

EFFECTIVENESS OF ROPIVACAINE BLOCKS IN ELECTIVE OVARIOHYSTERECTOMY IN DOGS FOR CONTROL OF POST-OPERATIVE PAIN

VALDO ALEXANDRE SILVA MONTEIRO

CONSTITUIÇÃO DO JÚRI

Doutora Luísa Maria Leal Mateus

Doutor José Manuel Chéu Limão

Doutora Berta Maria Fernandes Ferreira São Braz

ORIENTADORA

Doutora Paula Larenza Menzies

CO-ORIENTADORA

Doutora Berta Maria Fernandes Ferreira São Braz

2015

LISBOA

UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

EFFECTIVENESS OF ROPIVACAINE BLOCKS IN ELECTIVE OVARIOHYSTERECTOMY IN DOGS FOR CONTROL OF POST-OPERATIVE PAIN

VALDO ALEXANDRE SILVA MONTEIRO

DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

CONSTITUIÇÃO DO JÚRI

Doutora Luísa Maria Leal Mateus

Doutor José Manuel Chéu Limão

Doutora Berta Maria Fernandes Ferreira São Braz

ORIENTADORA

Doutora Paula Larenza Menzies

CO-ORIENTADORA

Doutora Berta Maria Fernandes Ferreira São Braz

2015

LISBOA

Dedicatory

This work is dedicated to a very special person, who stood beside me in all the good and bad moments of these last seven years and has taught me several skills that will be very useful through all my life. She has also taught me to be the better person and has come to settle herself in a very cozy and comfortable chamber in my heart.

For this and much more, I am grateful to be your friend.

My *mashiara*, Ba.

"*Primum non nocere*"

"Sedare dolorem opus divinum est"

– Hippocrates

Acknowledgements

I'd like to thank my Co-Supervisor Doctor Berta São Braz for taking me in as her trainee and putting up with my despair and lack of patience, and also for encouraging me to not give up and to keep on going even when things looked grim and the opposite of what we expected.

I'd also like to thank my Supervisor Doctor Paula Larenza for accepting me as her trainee and all the help she gave me in putting up the study, for all the knowledge she has passed on to me and for the warm greeting in a very cold country.

Also to Dr. Ana Murta for accepting to participate in my study, for all the knowledge, good humour, the late hours in surgery and the sushi. Because without sushi, there would be no surgery.

To the director of the Hospital Escolar da Faculdade de Medicina Veterinária da Universidade de Lisboa, Dr. António Ferreira, a deep thanks for authorizing the realization of the study.

I would like to thank Dr. Carlos Cabral of BBRAUN for their contribution, which made this study a lot easier to accomplish.

Thank you to Dr. Alexandra Dugdale for her comments and help on the thesis research and all her good disposition, quick response to emails and availability to discussion, even with her busy schedule.

A big 'Thank You' to the staff teams of both hospitals, Veterinary Teaching Hospital of the University of Helsinki and Hospital Escolar da Faculdade de Medicina Veterinária, because without your help and your patience this study couldn't possibly be finished, especially to those who helped me gather patients for the study.

A special thanks to the Erasmus Departments of both Faculties of Veterinary Medicine of the Universities of Lisbon and Helsinki and their respective coordinators (Leonilde Ribeiro and Janna Koivisto) for their help in taking care of all business related to the programme and for allowing a very pleasant and profitable experience outside my country. Also, huge thanks to Johanna Haapalainen and all the Erasmus students (especially my tutors) for the warm welcome in the cold land of Finland and their unending sympathy.

Last, but absolutely not least, a very deep thanks to Dr. Telmo Nunes for everything related to statistical analysis in this work and his review of my discussion. Your help has been invaluable and you've helped me in one more battle against the statistic demons!

EFFECTIVENESS OF ROPIVACAINE BLOCKS IN ELECTIVE OVARIOHYSTERECTOMY IN DOGS FOR CONTROL OF POST-OPERATIVE PAIN

Abstract

Nowadays, pain is an ever-present theme in veterinary practice. The number of available tools to fight painful stimuli has increased and practicians are more accurate in recognizing pain in their patients. However, much is still to be discovered and full drug potential hasn't yet been achieved.

The present study was meant to test the efficacy of the use of ropivacaine as a mean to control post-operative pain, by subcutaneous administration over the incision line, in elective ovariohysterectomies in female dogs. Glasgow's Composite Measure Pain Scale was used as pain assessment tool. Pain assessment began thirty minutes post-extubation and was repeated hourly, up until 6 hours post-local block (limit of ropivacaine's action).

In this study, 14 dogs were received at the Veterinary Teaching Hospital of the Veterinary Medicine Faculty of the University of Lisbon (HEFMV-ULisbon) for elective ovariohysterectomy. They were randomly distributed into two groups – Ropivacaine Group (RG) and Saline Group (SG). The observer was blinded to the distribution during the study period. RG was submitted to a subcutaneous infiltration of 1 mg/kg of ropivacaine, while SG patients were subject to a saline infiltration – volume calculation as per ropivacaine's dose and concentration. During the procedure, heart and respiratory rate were monitored, with special attention to 5 crucial time-points. Post-operatively, rescue analgesia (carprofen 4 mg/kg + buprenorphine 0,015 mg/kg) were given to either patients whose score was 5 or greater in pain assessment or after 6 hours had passed, whichever came first. After the administration of rescue analgesia, the study was ended for that patient. Statistical analysis was done with R v2.1.2.

There were no statistical differences between groups in age, weight, duration of procedure and duration of anesthesia ($p = 0.743$, $p = 0.318$, $p = 0.796$ and $p = 0.337$, respectively). Comparison between groups wasn't statistically significant in heart and respiratory rate in the 5 chosen time-points ($p = 0.990$ e $p = 0.529$, respectively) nor in pain scores ($p = 0.638$).

In conclusion, anesthesia with methadone, acepromazine, propofol and isoflurane provided reliable analgesia during and after the procedure, since no supplemental analgesics were needed in the control group. The pre-incisional administration of 1 mg/kg SC ropivacaine did not prove effective in lowering post-op pain scores.

Keywords: Analgesia, local anesthetic, pain evaluation, ropivacaine, dog, ovariohysterectomy

EFICÁCIA DE BLOQUEIOS ANESTÉSICOS COM ROPIVACAÍNA EM OVARIOHISTERECTOMIAS ELECTIVAS EM CADELAS PARA CONTROLO DA DOR PÓS-OPERATÓRIA

Resumo

A temática da dor está, hoje em dia, sempre presente na prática veterinária. As ferramentas de combate aos estímulos álgicos têm aumentado e os veterinários estão mais eficazes a reconhecer estados de dor nos seus pacientes. No entanto, ainda muito está por descobrir e ainda é possível aumentar a potencialidade de alguns fármacos disponíveis para o efeito.

O presente estudo teve como objectivo averiguar a eficácia da utilização de ropivacaína como meio de controlo de dor pós-cirúrgica, através da sua infiltração subcutânea na linha de incisão em ovariohisterectomias electivas de cadelas. Para efeitos de avaliação de dor pós-cirúrgica, foi usada a Escala Composta Multidimensional de Avaliação de Dor da Universidade de Glasgow, com início da avaliação trinta minutos após a extubação e novamente de hora a hora, sendo a última avaliação quando se completaram as seis horas pós-infiltração (tempo de acção da ropivacaína).

Neste estudo participaram 14 animais que compareceram no Hospital Escolar da Faculdade de Medicina Veterinária da Universidade de Lisboa (HEFMV-ULisboa), que foram posteriormente aleatóriamente colocados em dois grupos – Grupo Ropivacaína (GR) e grupo Salino (GS). O observador não teve conhecimento do grupo a que cada paciente pertencia durante o decorrer do estudo. O GR foi sujeito a uma infiltração subcutânea de 1 mg/kg de ropivacaína, enquanto que o GS foi sujeito ao mesmo procedimento com soro salino, sendo o volume calculado tendo em conta a dose e a concentração da ropivacaína. Durante o procedimento foram monitorizados a frequência cardíaca e a frequência respiratória, tendo destaque 5 momentos temporais cruciais do procedimento. No pós-cirúrgico, a analgesia de resgate (carprofeno 4 mg/kg + buprenorfina 0,015 mg/kg) foi administrada a pacientes que apresentassem um *score* de 5 ou mais durante as monitorizações ou após finalizadas as 6 horas pós-infiltração. Após esta administração, o estudo deu-se por terminado para o paciente em questão. A análise estatística foi realizada com base no *software* R v3.1.2.

Neste estudo, não houve diferenças significativas entre grupos para idade, peso, duração do procedimento e duração da anestesia ($p = 0.743$, $p = 0.318$, $p = 0.796$ e $p = 0.337$, respectivamente). A comparação entre grupos em termos de frequência cardíaca e respiratória dos pacientes nos 5 momentos escolhidos ($p = 0.990$ e $p = 0.529$, respectivamente) e de *scores* de dor ($p = 0.638$) não foi estatisticamente significante.

Conclui-se então que o protocolo anestésico foi apropriado para o procedimento, uma vez que não foi necessária analgesia suplementar para nenhum paciente do grupo de controlo. Conclui-se também que a administração SC pré-incisional de 1 mg/kg de ropivacaína não contribui para uma redução dos *scores* de dor dos pacientes após OVH.

Palavras-chave: Analgesia, anestésico local, avaliação de dor, cão, ovariohisterectomia, ropivacaína

Index

List of Tables

List of figures

Abreviations

A2A – α-2 agonists

AMPA – δ-amino-3-hidroxi-5-metil-4-isoxazol propionate acid

Ca^{2+} - calcium ion

cm - centimeter

CTZ – chemoreceptor trigger zone

GIT – gastrointestinal tract

H^{+} - hydrogen ion

HEFMV-ULisboa - Hospital Escolar da Faculdade de Medicina Veterinária da Universidade de Lisboa

IM - intramuscular

IV - intravenous

K^{+} - potassium ion

Kg - kilogram

LA – local anaesthetic

m/s – meters per second

mg - milligram

mcg - microgram

Mg^{2+} - magnesium ion

mm - millimetre

Na^{+} - sodium ion

NDS – numeric descriptive scale

NGF – nerve growth factor

NMDA – N-metil-D-aspartate

NSAID's – non-steroidal anti-inflammatory drugs

O^{2} – oxygen

OVH - ovariohysterectomy

PG - prostaglandin

pH – hydrogenionic potential

pKa – acid dissociation constant

SC – subcutaneous

tCO_2 – tidal carbon dioxide

TX - tromboxane

VAS – visual assessment scale

Vs - versus

α - alfa

δ - delta

κ - kappa

μ - mu

Part I - Bibliographic review

1. Pain and its definitions

Pain is the single, hardest emotion to describe. It is usually classified as an unpleasant feeling, often related to tissue lesion. There have been numerous attempts of defining it, though many of them aren't explicit enough to fully describe what pain is. The official definition of pain by the International Association for the Study of Pain (IASP) is "an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, or described in terms of such damage".

Molony has also defined pain in a most interesting way, taking into account animal pain: "An aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of tissues. It changes the animal's physiology and behavior to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery." (Molony & Kent, 1997)

Both definitions equally state that pain isn't just a physical phenomena (the lesion *per se*), but it also has psychological, behavioral and cognitive elements as well (the latter only in the species with important cortical development – i.e. primates). As an example of the interaction between those elements, the limbic system (a system responsible for some emotions, like fear and anxiety) gives feedback to the cortex, which means that fear and anxiety (psychological emotions) can increase pain perception. These two feelings are, thus, unwanted in the painful patient (Dugdale, 2010).

In order to fully understand the subject, some light must be shed upon the concepts used to explain what pain is and its effects on any subject. As it is, the word "pain" is very vague and its definition is merely related to sensation. Biologically, the word that relates to pain is nociception. Nociception is the whole physiological process through which there is a neuronal response to a noxious stimuli, which in the end leads to the perception of pain (from the Greek *Nocere* - Noxious). If this process becomes interrupted, the stimulus doesn't elicit a neuronal response. Thus, a state of analgesia is established. Analgesia is the total absence of pain or of the ability to perceive it. This state is very difficult to achieve as, despite being able to inhibit the perception or the transmission of noxious stimuli, they still exist and still trigger biological processes. What we can usually achieve is hypoalgesia, or a state of reduced perception of pain or reduced intensity of the painful stimuli. This can be achieved through

the use of drugs or in certain stressful situations, such as a predator attack, due to endogenous mechanisms that will be further explained later. This is an important biological function, which allows the subject to walk away from the painful event into safety. The opposite of hypoalgesia is also possible to occur, especially due to inflammatory reaction. Hyperalgesia is an exaggerated pain response to a stimulus due to decreased receptor threshold and increased electric impulse production. This phenomenon can occur in the periphery, at the lesion site and adjacent structures (peripheral sensitization) or it can occur centrally, perpetuating the perceived pain even after the stimulus is removed. Hyperalgesia can become an extreme condition, when even non-noxious stimuli elicit a pain response from the subject. This level of sensitization is referred to as allodynia. (Viñuela-Fernández et al, 2007; Traquilli, Thurmon, & Grimm, 2007; Dugdale, 2010)

2. Classification of pain

Different painful stimuli have different origins. Most of the pain we feel originates from inflammatory response to some kind of lesion, since the inflammatory mediators activate pain receptors at the lesion site. However, nerve damage or excessive dilation of viscera can also produce painfully stimuli. Intensity and duration are the most important pain features to its classification and allow distinction between physiologic pain and pathologic pain (Dugdale, 2010). Nevertheless, there are several ways of classifying pain, as seen below.

2.1. Acute pain

Pain which results from sudden tissue lesion caused by thermal, mechanical or chemical stimuli, which begins abruptly and has a brief duration. This kind of pain can be easily abolished by analgesic drugs (Dugdale, 2010).

2.2. Chronic pain

Pain that persists over time and that cannot be reduced by adopting a protective behavior. It is difficult to treat fully with analgesic drugs, frequently needing a multi-modal approach to its treatment (analgesic drugs along with physiotherapy and environment manipulation, etc) (Traquilli, Thurmon, & Grimm, 2007).

2.3. Somatic pain

Somatic pain occurs on the skin or in musculoskeletal areas of the body. It can be easily localized and differs from visceral pain because these areas possess a higher density of receptors with small sensitive fields (Lemke, 2004).

2.4. Visceral pain

In opposition to somatic pain, visceral pain is ill-localized, diffuse and prompted by mechanical changes in internal organs (distension, ischemia, dilation). These organs have a low density of receptors and each receptor has a larger sensitive field than the somatic ones, thus being less specific (Lemke, 2004).

2.5. Neuropathic pain

Neuropathic pain can be recognized when the patient's pain cannot be diminished by normal pain medication (medication used to reduce inflammatory pain). It is originated by nerve lesions, which result in ectopic activity due to the accumulation of sodium channels in the lesion site. This accumulation can spread to the neuronal bodies in the dorsal ganglia and to other neurons, including effector neurons. It all might result in spontaneous pain and in increased sensitivity in the peripheral nociceptors (D'Mello & Dickenson, 2008).

2.6. Psychological pain

Some patients report pain perception even when no lesion can be seen or detected. These cases can be related to psychological pain – a phenomenon usually occurring after extreme episodes of pain which, through severe neuromodulation of the pain pathways, keep occurring despite the treatment and disappearance of the initial lesion (Dugdale, 2010).

2.7. Adaptative (physiologic) pain

Physiologic pain is the most frequent type of pain felt and, as the name implies, has a physiologic function. Its purpose is to potentiate the subject's survival by enacting protective behavior. This will prevent further damage to the affected area and allow the subject to recover into full health. This type of pain requires the activation of high-threshold receptors (nociceptors) and it is an adaptive pain, well localized in time and space, and its electrical impulses are conducted through A-δ fibers. (Bishop, 1980; Lemke, 2004; Viñuela-Fernández et al, 2007; Hellyer et al, 2007; Dugdale, 2010)

2.8. Maladaptative (pathological) pain

Maladaptive or pathological pain, as the name suggests, is a disease. It is triggered by severe trauma or other highly painful events (like a car crash or surgery) and its presence is highly debilitating to the subject. It generates morbidity and is often difficult to abolish. Besides the normal nociceptors, silent receptors (normally associated with non-painful stimuli, like tactile sensation) with low excitement thresholds are also activated, increasing the number of electrical signals produced. This creates a diffuse pain sensation that is difficult to localize, isn't specific to any stimuli (as it may continue even after the initial stimuli has ended) and is

slowly adapting. The electrical impulses that are responsible for pathological pain are conducted by C fibers. (Bishop, 1980; Lemke, 2004; Viñuela-Fernández et al, 2007; Hellyer, et al., 2007; Dugdale, 2010)

The veterinarian surgeon must act in every instance where a patient feels pain, but it is in pathological pain cases that his action is life-saving, as it is a condition that will not go away on its own. Hence, the veterinarian must ease and, preferably, prevent pathological pain from occurring to the best of his abilities. (Bishop, 1980; Lemke, 2004; Viñuela-Fernández et al, 2007; Dugdale, 2010)

3. The physiology of pain

3.1. Pain pathways

Nociception is a sequential process beginning at the level of nociception receptors through the transduction of physical or chemical stimuli to electrical impulses and continuing with their conduction by an afferent neuronal pathway up to the dorsal horn of the medulla. From here, the impulses are transmitted to the spinal and supraspinal centers, where modulation and perception of the stimuli occur. Nociceptive pathways are a very complex system. However, its structure can be explained in a rather simplistic manner, to allow for a better understanding of the path an electrical impulse must go through. There are three levels of neurons by which the nociceptive stimulus is transmitted: the primary neurons, the projector neurons and the supraspinal neurons (D'Mello & Dickenson, 2008; Dugdale, 2010).

The primary neurons (also called afferent neurons) have their cellular body located in the ganglia of the dorsal root of the medulla and its axons extend peripherically to their target-organs and centrally to the dorsal horn of the medulla. These neurons connect with specific sites on the dorsal horn, which is divided in six lamellae whose function is to process the incoming information. The neurons with specific (nociceptive) information link with lamellae number one and two (the most superficial), while the more unspecific neurons link with the deepest lamellae (Figure 1) (D'Mello & Dickenson, 2008).

The projector neurons receive the electrical sign from the medulla and establish the connection with the spinal and supra-spinal centers (thalamus, pons, midbrain, hypothalamus, etc). Then, the supra-spinal neurons take the nociceptive impulse even further into the brain, more specifically to the cortical and sub-cortical centers, where perception occurs. (Lemke, 2004; Viñuela-Fernández et al, 2007; D'Mello & Dickenson, 2008; Dugdale, 2010)

Figure 1 - Pain pathways from periphery to brain. (D'Mello & Dickenson, 2008)

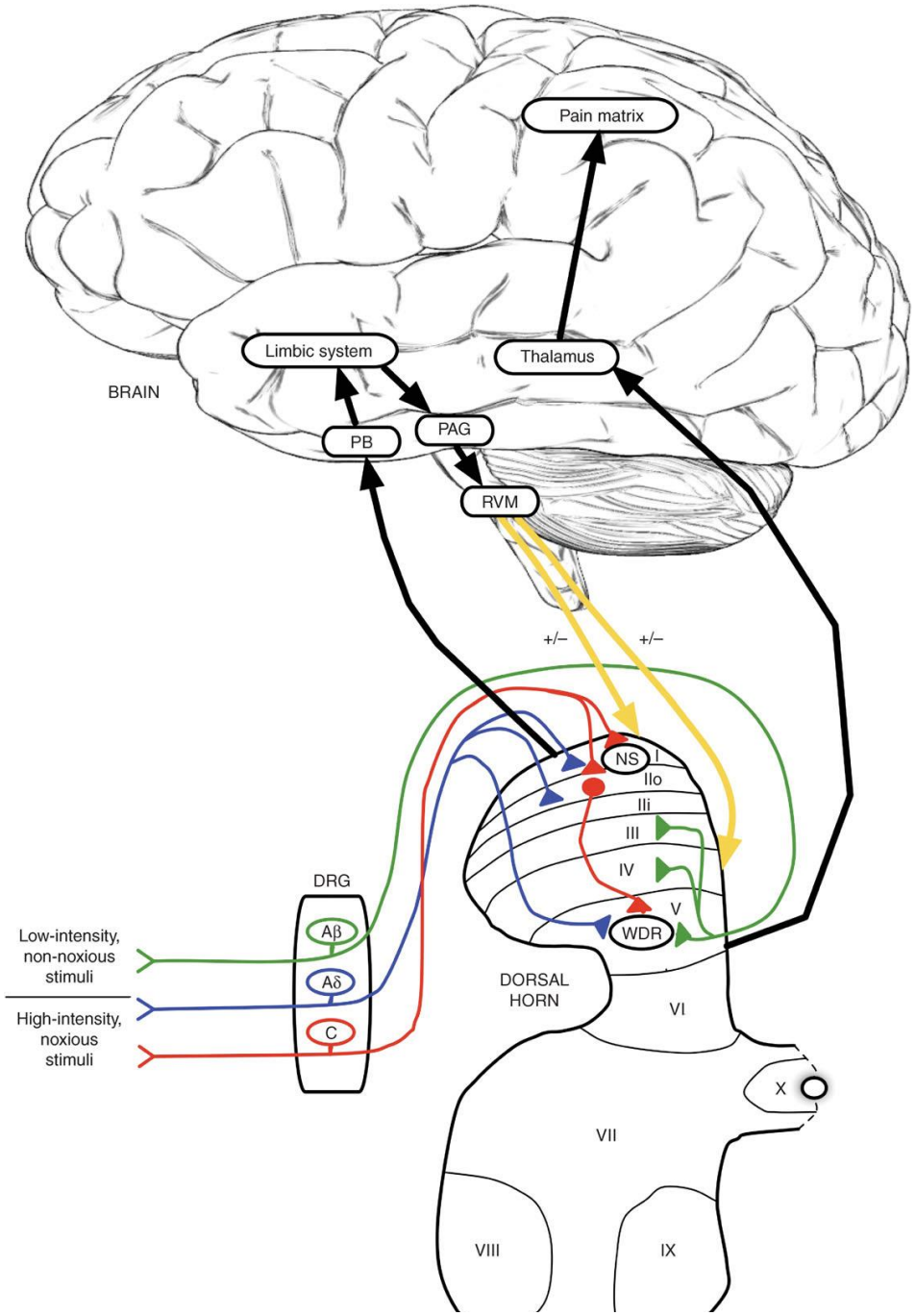


Image description: The primary afferent fibers lead impulses towards the dorsal root ganglion (DRG) and into the dorsal horn of the spinal cord. The superficial dorsal horn is divided into several laminae. The most superficial laminae (I and II) contain the nociceptive specific cells (NS), while the deeper laminae have the wide dynamic ranges cells (WDRs; lamella V). The parabrachial area (PB) and the periaqueductal grey (PAG) are innervated by projection neurons from lamina I and are also affected by limbic areas. From these areas and some brainstem nuclei (i.e. rostral ventromedial medulla - RVM), the descending pathways have their origin (yellow arrows) and are activated to modulate spinal processing. Lamina V neurones mainly project to the thalamus through the spinothalamic tract and from here the various cortical regions forming the 'pain matrix' are activated.

3.2. Pain processing

Upon establishing contact with a noxious stimuli, a series of events occur almost simultaneously, which transform the stimuli into an electrical signal and conduct it towards the highest neuronal centers, where the signal is read, perceived and an appropriate answer is activated (Lemke, 2004; Traquilli, Thurmon, & Grimm, 2007; D'Mello & Dickenson, 2008; Dugdale, 2010). These events can be divided in four stages as follows:

3.2.1. Transduction

If the intensity of a stimulus is sufficient to overcome the nociceptor's activation threshold, it triggers a response and the physical energy is transformed into an electrical signal. The transduction is thus the initial step that leads into pain perception.

3.2.2. Transmission

This stage comprises the conduction of the electrical signal throughout the neural pathways to the highest neuronal centers. It is done by pain fibers (A-delta fibers for acute pain and C-fibers for chronic pain) primarily towards the dorsal horn of the medulla, then to the thalamus and reticular system and finally to the cortex and higher centers. However, before reaching the higher centers, the signal goes through a stage of modulation.

3.3.3. Modulation

At the dorsal horn of the medulla and supraspinal levels, the pain signals are subject to some modifications derived from the endogenous descending pathways. This occurs at several levels and the descending pathway system is responsible for increasing, decreasing or somehow altering the nature of the stimulus. This step is responsible for the complexity of the relation between formation of stimuli and their perception, as some of them might get nullified by the descending pathways' intervention. The result of signal modulation is dependent on the careful balance between excitatory and inhibitory impulses.

3.3.4. Perception

When the noxious signals reach the highest neuronal centers, perception of the stimulus occurs. The conscious, subjective and emotional experience of pain is felt at this level only. Hence, perception of a painful stimulus is totally dependent on the successful transduction, transmission and modulation of the signal.

3.4. Ascending pathways

3.4.1. Afferent fibres and their nociceptors

Conduction of the electric signals is made through a number of nerve axons grouped together to form fibers. Three different types of fibers exist, which differ in composition and in the type of electrical signal that travels within them. Not all of these fibers participate directly in pain transmission, but all of them are needed to perceive the exact nature of the stimulus.

A-β fibers are related to non-painful stimuli, like tactile sensation. Due to their large diameter and myelinated membrane, these are the fastest transmission fibers. Despite not being used in normal transmission of pain, they help in determining its nature. Still myelinated, but with a thinner diameter, A-δ fibers carry pain impulses generated by mechanothermal stimuli. These fibers respond to extreme changes by these types of stimuli, transporting the electric signals at high-speed (3 – 30 m/s) to the medulla. Pain sensation induced from these fibers is called epicritic pain and is usually acute (first pain), well localized and adaptative (Lemke, 2004; D'Mello & Dickenson, 2008). Finally, the thinnest fibers, C fibers, are the slowest in signal conduction and are not coated with myelin. Their receptors are polymodal, meaning that they respond to any kind of stimuli, as long as it is sufficient to overcome their high threshold level. Conducting speed is less than 3 m/s and the pain transmitted by them is slowly adaptative (prolonged in time), diffuse and unspecific. Pain transmitted through these fibers is also called protopathic pain. Along with A-δ and A-β fibers, the three fibers are called 'pain fibers'. There is also a less known type of fibers that usually doesn't take part in any process, until an inflammatory response is formed. The 'silent fibers' do not respond to stimuli until the moment of their activation by inflammatory mediators. From then on, their excitement thresholds are sufficiently lowered to be activated by mechanothermal stimuli. These fibers are similar to C-fibers, since the pain transmitted by them has the same characteristics as the latter. Despite the name, they have an important role in peripheral sensitization, increasing the pain sensation at the target site (Lemke, 2004; Viñuela-Fernández et al, 2007; D'Mello & Dickenson, 2008; Dugdale, 2010).

3.5. Descending pathways

Descending pathways carry impulses generated in the brain. These impulses may have excitatory or inhibitory nature, thus increasing or decreasing stimuli transmission. Inhibitory descending stimuli are strong enough to prevent pain signals from reaching the brain, thus its interest to medicine. Pavlov's experiments demonstrate a perfect example of the descending inhibitory pathway at work: the dogs, before presentation of food, were hurt with electrical

shocks or cut in the nose or paws. Despite the noxious stimuli, after a period of habituation, they either showed no signal of perception of pain or stopped interpreting these stimuli as painful, interpreting them instead as a pre-feeding signal (Melzack & Wall, 1965; Lemke, 2004).

Descending inhibitory pathways work at four different levels: cortex and thalamus, midbrain's periaqueductal gray matter, raphe magnus nuclei in the pons and medulla oblongata plus spinal medulla. The most important and better understood of them all is the periaqueductal gray matter (Dugdale, 2010).

3.5.1. Periaqueductal gray matter

This descending pathway is very important due to the high concentration of opioid receptors. Its effect is most potent against pain impulses at spinal level. However, they must be activated by GABA, serotonin, glutamate, noradrenaline, acetylcholine, adenosine or endorphins to work. By identifying the molecules that activate this pathway, doors to the discovery of new tools to fight against pain will be opened (Dugdale, 2010).

3.6. Pain modulation

Neuromodulation is one of the SNC's responses to long-lasting pain. Pain perception mechanisms can be changed by the presence of painful stimuli. The two biggest phenomena that can be observed due to this biologic behavior are sensitization and dessensitization (Dugdale, 2010).

3.6.1. Dessensitization

It is a physiological way of the organism to deal with frequently felt low-level pain. As referred earlier, this process increases the threshold to a certain type of stimuli and prevents it from triggering the nociceptors. It can completely negate a painful stimulus or merely attenuate its intensity. This property is easily seen when comparing pain thresholds of a senior to a junior animal. As the subject grows older, it becomes desensitized to a number of stimuli which improve the subject's survivability. However, this phenomenon may not always occur and the reasons or necessary conditions for its occurrence are still unknown (Dugdale, 2010).

3.6.2. Sensitization

As an opposite of desensitization, this process' outcome is the increased perception of a painful stimulus. The pain threshold is lowered for a certain stimuli, increasing its intensity. It can be divided in peripheral and central sensitization (Dugdale, 2010).

3.6.2.1. Peripheral sensitization

As the name implies, this process occurs peripherally, at the wound site. The presence of inflammatory mediators (prostaglandins, histamine, serotonin, substance P) and other algogenic agents (H^+ and K^+ ions) lowers the thresholds of the silent fibers and nociceptors, resulting in an increase of electric signal emission. This prolonged and more intensive activation of receptors ultimately results in the increased perception of pain at the wound site, which spreads to the lesion's adjacent areas (Gogny, 2006).

3.6.2.2. Central sensitization

This occurs at a central level, in the SNC. Any pain stimulus can cause central sensitization, but long-lasting changes are mostly caused by prolonged, high-intensity and/or high-frequency stimuli. These stimuli cause a reaction of the raquidian nociceptive neurons, which start reacting in an excessive way to the received signals (wind-up). The wind-up effect can be so dramatic that pain continues even after removal of the initial stimulus (Gogny, 2006).

Several molecules are involved in central sensitization, particularly glutamate, which is a neurotransmissor that can link to NMDA (N-Metil D-Aspartate) and AMPA (α–amino–3–hidroxi–5–metil–4–isoxazol-proprionate acid) receptors. These receptors are associated and emit long and short duration stimuli, respectively. Central sensitization can be reversed naturally after the removal of painful stimulus or with medication, but despite the advancements in pain management medicine it remains a very difficult condition to treat. The only way of effectively treating central sensitization is by preventing pain (pre-emptive analgesia). (Gogny, 2006; Dugdale, 2010)

3.6.3. NMDA and AMPA receptors

AMPA receptors are responsible for the establishment of a base response from the medulla to painful and non-painful stimuli. They control ion flux and channels of rapid activation by glutamate. If the received stimuli arrive from C fibers and possess high intensity and frequency, then NMDA receptors are also activated (Viñuela-Fernández et al, 2007).

NMDA receptors control non-specific cation flux channels in nervous cells (Na^+ and Ca^{2+} influx and K^+ efflux) and indirectly control gene expression, cellular signaling and receptor synthesis. These receptors may be found in great concentration in the dorsal horn of the medulla (Viñuela-Fernández et al 2007). They are unique receptors, due to their double-activation mechanism: in order to become active, NMDA receptors need to be subject to a persistent membrane depolarization, which will alter their conformation and release Mg^{2+} ions from the active site in order to allow the linkage of glutamate and glycine (co-agonist). Influx

of sodium and calcium ions through the opened ion channels will perpetrate membrane depolarization and initiate cellular signaling cascades that are responsible for long-term neuromodulation. These channels remain open for a longer period than normal ion channels, thus being perfect for the neuromodulation role. (Viñuela-Fernández et al, 2007; D'Mello & Dickenson, 2008; Dugdale, 2010)

Antagonist molecules to NMDA receptors (ketamine, petidine and some methadone isomers) were discovered by modern medicine and their success has been proven (Dugdale, 2010). Dextrometorphane and memantine may also antagonize them (D'Mello & Dickenson, 2008). All of them are used in an attempt to revert central sensitization. Other possible effective molecules include nitric oxide, xenon and benzodiazepines (Dugdale, 2010). Despite their properties, the therapeutical window is extremely small and their adverse effects make their use a very difficult matter (D'Mello & Dickenson, 2008).

3.7. New approaches to central pain management

Hefti *et al* tested the theory that the nerve growth factor (NGF) is essential in pain signaling after lesion and inflammation. NGF antagonist molecules were used in some animal models with good results, showing high effectiveness with no adverse effects (Hefti, Rosenthal, & Walicke, 2006).

Glutamate receptors such as AMPA and NMDA are seductive targets for pain management, but their wide distribution along the CNS prevents the wide and common use of their antagonists, due to the generated adverse effects, as can be seen with the use of ketamine, so further clinical studies are needed (Viñuela-Fernández et al, 2007).

4. Pain assessment

4.1. Pain assessment and evaluation in small animal practice

Pain assessment in small animals can be challenging, depending on the skill of the observer, his relation with the patient, the patient's normal behavior and the patient's species. While animals like dogs or monkeys express pain in a clear way, other species prefer to hide the pain from possible threats. Cats and sheep are perfect examples of such behavior. Also, the exotic pet species (i.e. reptiles) are a different challenge, as their primitive neural development hasn't evolved to allow open expression of pain. Finally, owners perceive pain differently, according to the species being observed: while lumbar pain is easily recognized by most dog-owners, the same isn't true for cat-owners. Diseases like osteoarthritis, intervertebral disc

disease and spondylosis are under-diagnosed due to the common thought that the changes in behavior occur due to old age rather than pain. Due to all of these reasons, pain assessment must be individualized for each patient. The same surgical procedure can cause different levels of pain to an adult in comparison to a senior animal, since the older one might feel musculoskeletal pain due to surgery positioning, in addition to the normal, predicted pain from the procedure itself (Hellyer et al., 2007).

Pain evaluation is the second challenge in veterinary pain management. Despite the fact that the existence of a painful stimulus alters the normal behaviour of an animal (Holton et al, 2001; Morton et al, 2005; Dugdale, 2010), some of those changes might not be pain-specific. In fact, those changes in behaviour are also typical of fear-provoking events or in the presence of a predator, in complete absence of a painful condition (Barr, 1998; Chamberset al, 1999; Anil, S.S., Anil, L. & Deen, J., 2002; Morton et al, 2005). Furthermore, most behaviour changes may not describe the full extent of the pain the animal is feeling; fearful animals may be over-expressing their pain or it can be confused with attention-seeking behaviour. With such a high chance for error, the medical community has tried over and over again to overcome these obstacles and come up with some sort of mechanism to successfully assess animal pain. The answer came in the form of pain scales.

4.2. Pain measurement through scales

4.2.1. Visual Assessment Scale

The Visual Assessment Scale (VAS) consists on a 100mm line with limits labeled as “no pain” and “worst pain possible” and where the human patient must place a cross at the level where his pain is. Very simple to use in any human patient that can hold a pen, this method alone provides some help understanding the need for analgesia, but is easily biased by the patient (and also easily abused). However, it can be used in conjunction with other scales to make the general pain assessment method more sensitive. In animals, it is still useful, though the person marking the scale is the observer. If the observations are repeatedly done by the same trained observer, the usefulness of this method is significant. (Holton et al, 1998; Anil, Anil, & Deen, 2002; Morton et al, 2005)

4.2.2. Numeric Descriptive Scale

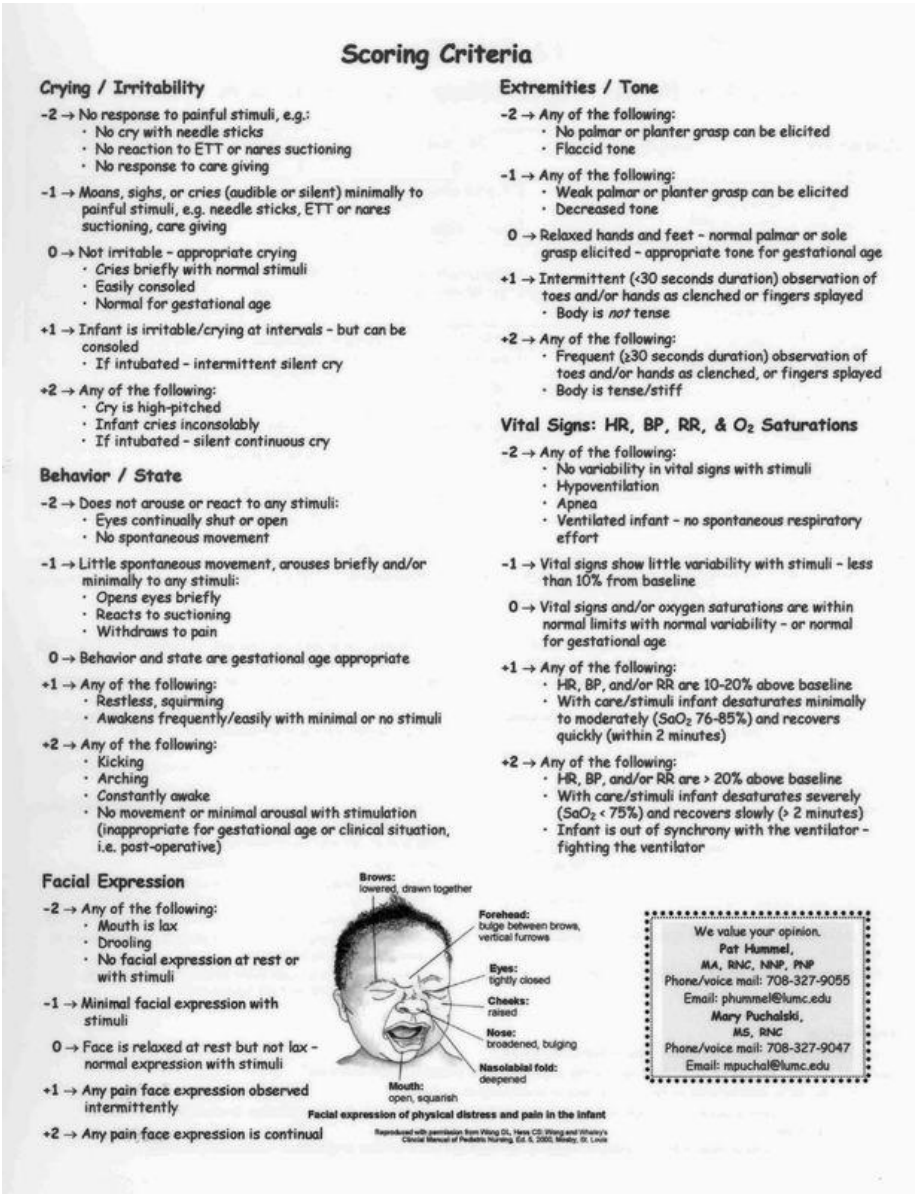
The Numeric Descriptive Scale (NDS) can also be used by the observer and is composed of several classes with a descriptor and the observed pain should fit into one of those. These descriptors can be something as simple as “soft pain, mild pain and strong pain” or have more or less descriptors. Usually the classes are numbered and, if several classes are present, the

score is added up. Patients scoring over a certain value are considered to be eligible to receive analgesia or to have their analgesia protocol reviewed. (Holton, et al., 1998; Anil, Anil, & Deen, 2002; Morton et al, 2005)

4.3.3. Composite pain scales

The above methods, however, didn't prove sufficient to identify all painful patients, as they aren't specific enough or the inter-observer variability can be too high (Holton et al., 1998). Furthermore, the task of attributing a specific class to a patient might be a difficult one, when the pain perceived by the observer might be simultaneously on the high-side of one class or in the lower-side of the next class. Sometimes the observer attributes an inconsistent and variable gap between classes, which makes the NDS a non-interval scale (Morton et al, 2005).

Figure 2 – Detail of neonatal pain scale. (Hummel P, 2008)



Scoring Criteria

Crying / Irritability

-2 → No response to painful stimuli, e.g.:
- No cry with needle sticks
- No reaction to ETT or nares suctioning
- No response to care giving

-1 → Moans, sighs, or cries (audible or silent) minimally to painful stimuli, e.g. needle sticks, ETT or nares suctioning, care giving

0 → Not irritable – appropriate crying
- Cries briefly with normal stimuli
- Easily consoled
- Normal for gestational age

+1 → Infant is irritable/crying at intervals – but can be consoled
- If intubated – intermittent silent cry

+2 → Any of the following:
- Cry is high-pitched
- Infant cries inconsolably
- If intubated – silent continuous cry

Behavior / State

-2 → Does not arouse or react to any stimuli:
- Eyes continually shut or open
- No spontaneous movement

-1 → Little spontaneous movement, arouses briefly and/or minimally to any stimuli:
- Opens eyes briefly
- Reacts to suctioning
- Withdraws to pain

0 → Behavior and state are gestational age appropriate

+1 → Any of the following:
- Restless, squirming
- Awakens frequently/easily with minimal or no stimuli

+2 → Any of the following:
- Kicking
- Arching
- Constantly awake
- No movement or minimal arousal with stimulation (inappropriate for gestational age or clinical situation, i.e. post-operative)

Facial Expression

-2 → Any of the following:
- Mouth is lax
- Drooling
- No facial expression at rest or with stimuli

-1 → Minimal facial expression with stimuli

0 → Face is relaxed at rest but not lax – normal expression with stimuli

+1 → Any pain face expression observed intermittently

+2 → Any pain face expression is continual

Extremities / Tone

-2 → Any of the following:
- No palmar or planter grasp can be elicited
- Flaccid tone

-1 → Any of the following:
- Weak palmar or planter grasp can be elicited
- Decreased tone

0 → Relaxed hands and feet – normal palmar or sole grasp elicited – appropriate tone for gestational age

+1 → Intermittent (<30 seconds duration) observation of toes and/or hands as clenched or fingers splayed
- Body is *not* tense

+2 → Any of the following:
- Frequent (≥30 seconds duration) observation of toes and/or hands as clenched, or fingers splayed
- Body is tense/stiff

Vital Signs: HR, BP, RR, & O_2 Saturations

-2 → Any of the following:
- No variability in vital signs with stimuli
- Hypoventilation
- Apnea
- Ventilated infant – no spontaneous respiratory effort

-1 → Vital signs show little variability with stimuli – less than 10% from baseline

0 → Vital signs and/or oxygen saturations are within normal limits with normal variability – or normal for gestational age

+1 → Any of the following:
- HR, BP, and/or RR are 10-20% above baseline
- With care/stimuli infant desaturates minimally to moderately (SaO_2 76-85%) and recovers quickly (within 2 minutes)

+2 → Any of the following:
- HR, BP, and/or RR are > 20% above baseline
- With care/stimuli infant desaturates severely (SaO_2 < 75%) and recovers slowly (> 2 minutes)
- Infant is out of synchrony with the ventilator – fighting the ventilator

Facial expression of physical distress and pain in the infant

We value your opinion.
Pat Hummel,
MA, RNC, NNP, PNP
Phone/voice mail: 708-327-9055
Email: phummel@lumc.edu
Mary Puchalski,
MS, RNC
Phone/voice mail: 708-327-9047
Email: mpuchal@lumc.edu

A different approach should be used and the first steps were taken in human pediatric care (Figure 2). Human doctors have similar difficulties to identify pain in newborns as veterinarians have with animals, since they also are unable to speak. For these pediatric patients, several scales were built and tested, which relied on facial expressions and describing the way they cried to try and perceive when a neonate would be in pain (Peters et al, 2003). Though far from perfect, this method of pain assessment proved very fruitful and able to identify most instances when analgesia was required. The same type of observation was used for animals in several instances, originating several composite pain scales.

4.3.3.1. University of Melbourne Pain Scale (UMPS)

The main objective of this scale is assessing post-operative pain in dogs. The authors used ovariohysterectomy (OVH) as the surgical procedure in the test groups (3 test groups, with different post-operative analgesia protocols: carprofen, butorphanol or no analgesia), with two witness groups that only underwent anesthesia and were medicated either with only acepromazine maleate or with acepromazine plus butorphanol. The scale includes physiological measurements (heart rate, respiratory rate) and several behavioural responses (response to palpation, activity, mental status, posture, and vocalization) (Figure 3). The authors proved that the scale was accurate to detect pain and to differentiate pain levels between groups and it was also reproducible by different observers. This scale, though, is only fit to assess acute pain post-operatively and it was created and experimented in animals undergoing elective surgery, meaning that it might not give reliable results when evaluating a patient that has undergone different procedures or whose pain is from different origin (i.e. somatic pain) (Firth & Haldane, 1999).

Figure 3 - University of Melbourne's Pain Scale

Category	Descriptor	Score
Physiological data		
a)	Physiological data within reference range	0
b)	Dilated pupils	2
c) *Choose only one:*	Percentage increase in heart rate relative to baseline	
	>20%	1
	>50%	2
	>100%	3
d) *Choose only one:*	Percentage increase in respiratory rate relative to baseline	
	>20%	1
	>50%	2
	>100%	3
e) Rectal temperature exceeds reference range		1
d) Salivation		2
Response to palpation		
a) *Choose only one:*	No change from preprocedural behavior	0
	Guards/reacts[a] when touched	2
	Guards/reacts[a] before touched	3
Activity		
a) *Choose only one:*	At rest- sleeping or semiconscious	0
	At rest- awake	1
	Eating	0
	Restless (pacing/getting up and down)	2
	Rolling, thrashing	3
Posture		
a)	Guarding or protecting affected area (includes fetal position)	2
b) *Choose only one:*	Lateral recumbency	0
	Sternal recumbency	1
	Sitting/standing, head up	1
	Standing, head hanging down	2
	Moving	0
	Abnormal posture (prayer position, hunched)	2
Vocalization[b]		
a) *Choose only one:*	Not vocalizing	0
	Vocalizing when touched	2
	Intermittent vocalization	2
	Continuous vocalization	3
Mental status		
a) *Choose only one:*	Submissive	0
	Overtly friendly	1
	Wary	2
	Aggressive	3
	Melbourne score	**4**

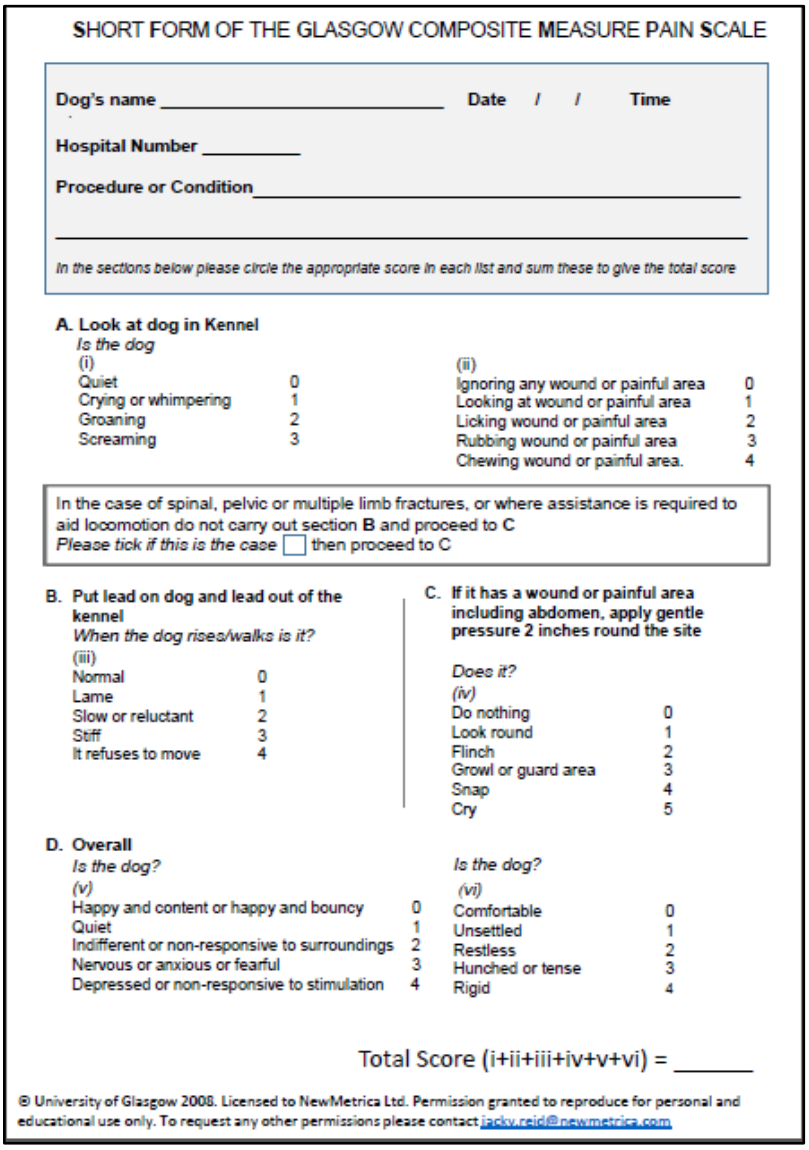
[a]Turning head toward affected area, biting, licking, scratching at the wound; snapping at handler; or tense muscles and a protective (guarding) posture.
[b]Does not include alert barking.

4.3.3.2. Glasgow's Composite Measure Pain Scale (GCMPS)

This scale was built on a general use basis. It can be used by trained and untrained people alike, with any kind of acute pain, originated either from orthopedic or soft tissue surgery, trauma or a simple medical intervention. The authors' objective was to create a valid and objective way of assessing pain in the clinical setting using an interval system, so that it is valid in any kind of intervention. Based on the McGill's pain questionnaire and on pre-existing veterinary pain scales, their defects and strong points were analyzed in order to make one cohesive scale, easy to use, with strictly determined wording that reduces inter-observer variability as much as possible. The initial study resulted in 39 behavioral expressions and 8 physiological parameters, spread through 10 categories, with each of the expressions being given an intensity value. The sum of all the values for a single patient translates into its pain score and that score is then used as the base for a therapeutic decision (Holton et al, 2001). This scale was tested in patients subjected to different procedures (orthopedic and soft tissue

surgeries, medical management in the hospital and control group) and its objective was to be a valid pain scale to use in any dog or cat in any situation where acute pain might present itself and also to allow reliable statistical data to be taken from case studies that involved pain assessment. It proved effective at doing so, though it still lacks some sensibility to detect slight changes in the pain sensation by an in-dwelling patient (Morton, Reid, Scott, LL, & Nolan, 2005). The GCMPS short form can be seen in figure 4.

Figure 4 - Short form of the Glasgow Composite Measure Pain Scale.



SHORT FORM OF THE GLASGOW COMPOSITE MEASURE PAIN SCALE

Dog's name _____ **Date** / / **Time**

Hospital Number _____

Procedure or Condition _____

_____

In the sections below please circle the appropriate score in each list and sum these to give the total score

A. Look at dog in Kennel

Is the dog

(i)

Quiet	0
Crying or whimpering	1
Groaning	2
Screaming	3

(ii)

Ignoring any wound or painful area	0
Looking at wound or painful area	1
Licking wound or painful area	2
Rubbing wound or painful area	3
Chewing wound or painful area.	4

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

B. Put lead on dog and lead out of the kennel

When the dog rises/walks is it?

(iii)

Normal	0
Lame	1
Slow or reluctant	2
Stiff	3
It refuses to move	4

C. If it has a wound or painful area including abdomen, apply gentle pressure 2 inches round the site

Does it?

(iv)

Do nothing	0
Look round	1
Flinch	2
Growl or guard area	3
Snap	4
Cry	5

D. Overall

Is the dog?

(v)

Happy and content or happy and bouncy	0
Quiet	1
Indifferent or non-responsive to surroundings	2
Nervous or anxious or fearful	3
Depressed or non-responsive to stimulation	4

Is the dog?

(vi)

Comfortable	0
Unsettled	1
Restless	2
Hunched or tense	3
Rigid	4

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

Further studies revealed that physiological parameters are not specific to pain, thus are not reliable indicators to include in pain evaluation, so the scale was reduced to 7 classes (Morton

et al, 2005). This resulted in an easy-to-use scale that has significant sensitivity to assess patient's pain and that has become extremely useful in clinical practice.

5. Pain pharmacology

5.1. Physio-pharmacology

Pharmacological treatment of pain is a much desired path ever since the discovery of several target areas where certain molecules are able to interfere with the conduction of the signals produced by noxious stimuli. Hence, it is possible to modulate pain signals at four different stages of transmission (Dugdale, 2010).

5.1.1. Noxious signal transduction sites

If the transformation of the noxious stimulus into an electrical signal is prevented, there is no pain. Local anesthetics are used to prevent the activation of nociceptors in the periphery and the formation of an electric signal (more information in the Local Anesthetics chapter). NSAID's can also be used to obtain the same result since they reduce the concentration of pro-inflammatory molecules on the lesion site, reducing secondary sensitization. Opioids and α-2 agonists might have some peripheral effects because receptors for both molecule types are evident in the inflamed tissues (Traquilli, Thurmon, & Grimm, 2007; Dugdale, 2010).

5.1.2. Signal transmission sites

Once again, the use of local anesthetics is advantageous. These drugs can block specific nerves (nerve block), block signals coming from a specific area (line blocks, ring blocks) or prevent the arrival of the signal to the central neurons in the medulla (regional blocks). This type of action blocks the signal transmission to higher centers (Traquilli, Thurmon, & Grimm, 2007; Dugdale, 2010).

5.1.3. Signal modulation sites

Modulation occurs at the medulla and thalamus, where signals can be modified and travel further to the cortex. Opioids, α-2 agonists and NMDA antagonists can be used to alter these sites' function. Drugs can be administered systemically or injected directly into the site of choice (i.e. epidurals) (Traquilli, Thurmon, & Grimm, 2007; Dugdale, 2010).

5.1.4. Signal perception sites

These sites include the cortex, reticular formation and limbic system and are the only point where the perception of the pain signals occurs. Medical intervention at these sites is possible

through the use of anxiolytics/sedatives (like benzodiazepines and phenothiazines), opioids (those with more sedative effects) and even injectable or inhalable general anesthetics (Dugdale, 2010).

5.2. Common analgesic agents used in the perioperative period

5.2.1. Opiates and opioids

5.2.1.1. Introduction

Opiates are derivates from opium, which is extracted from the poppy flower (*Papaver somniferum*), and have important analgesic properties. Morphine was the first substance to be identified and is currently the representative of this drug class. All other opioids are compared to morphine, in order to assess their relative potency. All drugs from this class are analgesics but are also considered narcotics, because narcosis (a feeling of sedation, sleepiness) is one of their adverse effects. *Opiates* are the natural molecules taken from opium; *opioids* are synthetic molecules with a similar mechanism of action to that of opiates. However, o*pioid* is the most commonly used expression to refer to this group and will be the one adopted throughout this text (Dugdale, 2010).

5.2.1.2. Mechanism of action

Opioids take action in the opioid receptors, which are wide spread through the body. These receptors were first known to exist in the CNS but more recently were found to exist also in peripheral tissues, like the gastrointestinal tract or joint tissue, especially during inflammation. Their action differs with the target species, due to different distribution and abundance in the tissues. Thus, the same dose of morphine can cause sedation in dogs but may cause euphoria (a typical overdose effect) in cats or horses, which have a higher density of opioids receptors in the CNS. There is also a variety of different opioid receptors and each molecule has different affinity to them (i.e. birds and reptiles have more κ receptors than μ), which means the same molecule may provide good analgesia on one species and sub-optimal analgesia on another (Epstein, 2012).

Discovered opioid receptors are: δ receptors, the receptor site for endogenous encephalins; μ receptors, the main receptors for opioid molecules, β-endorphins and some encephalins too; κ receptors, similar to μ receptors, but with a distribution mainly in the CNS; σ receptors, once considered opioid receptors but now considered to be related to NMDA receptors; ε receptors, thought to be endorphin receptors (Dugdale, 2010).

The most important effect of opioid drugs occurs against diffuse pain, due to the density of receptors being highest in the periaqueductal gray matter, where the inhibitory descending pathways are stimulated. Effectiveness against sharp acute pain isn't very noticeable because that type of pain is modulated by the reticular formation, which has low density of opioid receptors (Dugdale, 2010; Epstein, 2012).

5.2.1.3. Classification

Classification of opioids is based on the interactions with the receptors. Drugs can be agonists (full or partial), antagonists or agonist-antagonists to one or more receptors. Opioid agonists connect to the receptors and elicit a biological response (with partial agonists being weaker than full agonists), while the antagonists trigger no response from the receptors. The latter ones are, usually, used as antidotes for opioid overdose or simply to stop their action when needed. Agonist-antagonists have a mixed effect, depending on the receptors they connect to, and in certain situations they can be used to antagonize a full-agonist (Epstein, 2012).

Each molecule has different strengths, depending on affinity to receptors, type of activity and the density of receptors in the target species. As explained above, all opioids are compared to morphine to assess their relative potency. This means that the comparison between them is to assess which dose of a certain opioid is able to block a standardized pain signal and then compare it to the morphine's action efficacy (i.e. fentanyl's dose to block a painful stimulus is a hundred times smaller than morphine's dose to achieve the same effect, thus, fentanyl is a hundred times more potent than morphine). This classification is very useful, since it is based on clinical efficacy and allows the practitioner to successfully plan his analgesic protocol according to the level of pain expected in each procedure. However, other factors must be observed, namely the preparations' physical characteristics (pKa and liposolubility), the route of administration and the target species (Traquilli, Thurmon, & Grimm, 2007; Dugdale, 2010; Epstein, 2012).

5.2.1.4. Pharmacokinetics

Some active metabolites may prolong the effect beyond the original molecule's expected time of action. Opioid metabolism occurs in the liver tissue and excretion is biliary and urinary. Thus, practitioners should be cautious of using these molecules in animals with any kind of hepatic or renal impairment (Plumb, 2011).

5.2.1.5. Expected and adverse effects

Opioid effects in the CNS result in supraspinal, spinal and peripheral analgesia, along with light to moderate sedation. However, as opioid receptors are wide spread along the organism, some adverse effects also occur. These are usually dose and molecule-dependent (Dugdale, 2010).

Opioid drugs affect the sensitivity of respiratory centers to changes in blood tCO^2, causing respiratory rate depression (bradypnea). This might pose a problem in non-tachypneic animals, whose only complaint is pain, though it has been proven that when pain is present the respiratory effects are almost absent (McMillan, 2012).

According to the liposolubility of each substance, opioids can either stimulate or inhibit emesis. It depends on whether the molecule used reaches the chemoreceptor trigger zone (CTZ) or the emesis centre first, respectively. They might also increase smooth muscle motility. However, this effect doesn't mean faster food transit in the gastrointestinal tract (GIT) food transit because the increased motility isn't coordinated between muscles. Exception to this rule is petidine with its anti-spasmolytic effect, along with other anticholinergic effects (Dugdale, 2010).

There are also molecule and species-dependent effects in the cardiovascular system. Rapid IV administration of some opioids may induce histamine release with consequent hypotension. Hypotension can also be centrally induced by morphine, along with bradycardia, through a vago-mimetic effect. However, etorphine and carfentanyl have hypertensive effects (Trim, 2008; Dugdale, 2010; Epstein, 2012).

5.2.1.6. Example 1 - Methadone

This opioid has been known in human medicine for a long time and was mostly used to treat opioid addiction, since its withdrawal effects were much less prominent than those of heroin or morphine. Similar in effect and classification to morphine (full μ agonist), methadone has a longer half-life than her counterpart. Its effects extend beyond the normal opioid effects. It is reported to have NMDA antagonist activity, making it useful in some central sensitization and chronic pain cases, as well as causing inhibition of the reuptake of serotonin and norepinephrine, improving its analgesic activity. Its activity in the NMDA receptors also prevents development of tolerance towards opioid effects, a useful fact to keep in mind in prolonged opioid administration. Its activity is synergic with the administration of other opioids (Riviere & Papich, 2009).

Administration of methadone can be done through several routes. It can be given SC, IM or IV with increasing availability respectively, and it can even be administrated orally through the transmucosal absorption effect in cats (Ferreira et al, 2011). Methadone's half-life is longer than morphine's due to slower elimination, thus it should be administered in longer time intervals than morphine (every six to eight hours against every four hours for morphine) (Riviere & Papich, 2009).

Besides the normal opioid adverse effects, it has been reported increased systemic vascular resistance and coronary vascular resistance following administration. Thus, it should be carefully dosed and administered in patients prone to congestive heart failure. It also causes less vomiting and less excitation than other μ agonists (Plumb, 2011).

Methadone is commercially available as a 50:50 racemic mixture, but only the L-methadone has analgesic properties. However, both L and D enantiomers have affinity to NMDA receptors. Levomethadone is composed exclusively of L-methadone and thus, regarding analgesia, is more potent than normal methadone. When using levomethadone, doses should be halved (Riviere & Papich, 2009).

5.2.1.7. Example 2 - Buprenorphine

Buprenorphine is a mixed agonist-antagonist opioid, having agonist properties when linking to μ receptors and antagonist properties when linking to κ receptors. In terms of analgesia, sedation and side-effects, it exhibits the same effects as all other opioids but it has a plateau effect, after which additional doses do not increase intensity of analgesia. It is a safe molecule to use, with toxic doses being several dozen times higher than therapeutic ones. Its use along with other opioids should be avoided, as buprenorphine has a higher affinity to the μ receptor than full agonists, and thus can result in some reversal-like activity, displacing the full agonists from the receptors. The positive side of its high affinity is the long duration of action (four to six hours). It is approximately thirty times as potent as morphine, but studies on its analgesic efficacy differ, some saying that it is lower than morphine, others saying that it is higher in the cat. Administration routes include SC, IM, IV and oral transmucosal in cats (Riviere & Papich, 2009; Plumb, 2011).

5.2.1.8. Special case - Tramadol

Tramadol is a molecule with some resemblance to opioid drugs, but its mechanism of action is hybrid, generating both opioid and non-opioid effects. Its potency is about 1/10 of

morphine, but it has less adverse effects. Despite its reduced potency, some metabolites are more potent than the parent drug (Dugdale, 2010).

Tramadol's mechanism of action comprises several mechanisms which include a weak agonism to μ receptors as well as weak alfa-2 receptor agonism. It also inhibits synaptic reabsorption of noradrenalin and serotonin, while stimulating the release of the latter, which contributes to the activation of inhibitory descendent noradrenergic and serotoninergic pathways. Last but not least, it has a possible effect in reducing substance P release (Dugdale, 2010; Plumb, 2011). This range of the effects can be attributed to the different enantiomers present in the marketed racemic solution. The positive enantiomer is responsible for the opioid-like activity, while the negative enantiomer is responsible for the other mechanisms. Its numerous metabolites also play an important role in its efficacy (Riviere & Papich, 2009).

Adverse effects are limited to nausea, emesis and drowsiness/sedation (Riviere & Papich, 2009; Dugdale, 2010). Care should be taken in calculating dosages for cats and dogs, as cats metabolize the drug more slowly and thus it has a longer duration of action than dogs (12 hours against 6-8 hours, respectively) (Riviere & Papich, 2009).

5.2.2. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

5.2.2.1. Introduction

This group is vast and is comprised of drugs which have in common their anti-inflammatory, anti-pyretic and analgesic properties. NSAIDs are weak organic acids that can be divided into two categories, based on their molecular composition: enolic acids (pyrazolones, pyrazolidines and oxicams) and carboxylic acids (salicylic, acetic, proprionic, fenamic and nicotinic acids). The only exception to this is paracetamol, which isn't an acid and, unlike the other molecules, has a much better antipyretic action than both analgesic and anti-inflammatory actions (Dugdale, 2010).

5.2.2.2. Mechanism of action and main effects

The mechanism of action of NSAIDs is based on inhibition of ciclo-oxygenase (COX) activity, the most important enzyme in the arachidonic acid cascade, preventing the formation of several pro-inflammatory molecules, like thromboxanes (TX) and prostaglandins (PGs). This inhibitory effect is responsible for the analgesic, antipyretic, anti-inflammatory, antithrombotic and antiendotoxemic effects of NSAIDs. Their variety of effects makes for a wide use in several different clinical situations.

5.2.2.3. Classification

According to their specificity to either COX-1 or COX-2 (two isoforms of the COX enzyme), these molecules can be classified as non-selective, partially COX-2 selective or COX-2 selective. Theoretically, as selectiveness increases, adverse effects decrease in number and severity. However, several studies have proven that this isn't exactly true and thus the interaction between NSAIDs and COX molecules needs to be better understood in order to improve the effectiveness of these drugs (Riviere & Papich, 2009; Dugdale, 2010; Plumb, 2011).

This theory arises from the fact that COX-1 is associated with physiological functions in the organism (by producing PGs that are used in the maintenance of several protecting mechanisms of different organs: production of the gastric mucous layer, auto-regulation of renal blood flow, etc) and COX-2 participates in the production of pro-inflammatory molecules through its action over arachidonic acid. However, it was discovered that COX-2 also has some physiological functions, participating in angiogenesis of wound healing, for example, which indicates that full COX-2 inhibition might not be the way to go. (Riviere & Papich, 2009; Dugdale, 2010)

5.2.2.4. Adverse effects

Due to their inhibition of both COX isoforms, the effects referred above aren't the only ones that occur. The most known adverse effects of NSAIDs are gastrointestinal hemorrhages or ulceration due to the gastric protective mucous layer decreased production. They also have renal adverse effects caused by inhibiting the production of maintenance PGs that keep optimal renal blood flow and then leading to kidney ischemia. Since they are metabolized through hepatic biotransformation, hepatotoxicity might also occur. Salicylic acids and ketoprofen's anti-platelet aggregation properties might be harmful to the patient if hemorrhage is present (such as that induced by gastric ulceration). Other adverse effects include birth delay and teratogenicity in the fetus of pregnant females. For asthmatic patients NSAIDs should be used with care. Most molecules inhibit COX enzymes while not having an effect in lipo-oxygenase (LOX) activity, thus favoring tromboxane (TX) production. Increased TX production may lead to episodes of severe bronchic constriction (Riviere & Papich, 2009; Dugdale, 2010; Plumb, 2011).

5.2.3. Local anesthetics

5.2.3.1. Introduction and classification

The use of local anesthetics (LAs) for analgesia during surgery under general anesthesia is a relatively recent event. Up until now they were used to perform small surgeries in non-sleeping patients or during a lameness clinical exam in horses, but never considered for use along with other analgesia drug classes for procedures such as surgery (Jones, 2002). Their use, however, recently extended to epidural anesthesia, intra-operative analgesia and even post-operative analgesia, by using a local infiltration technique resorting to a subcutaneous or intramuscular-placed catheter (Dugdale, 2010).

Local anesthetics are weak bases that effectively block sodium (and other) ion channels in the peripheral nerves' membranes, preventing membrane depolarization and, thus, stopping electrical signal conduction. Their molecular structure is composed by an aromatic group (with lipophilic properties), an intermediate link composed by carbon and hydrogen atoms and an amide group (with hydrophilic properties). The nature of the intermediary link allows the division of the drugs into an ester-linked group (composed by cocaine, procaine, tetracaine, etc.) or an amide-linked group (lidocaine, bupivacaine, ropivacaine, etc.). Both groups provide good local analgesia though they differ in several properties (see Table 1). Local anesthetics are supplied as slightly acidic solutions which contain a higher percentage of ionized molecules than non-ionized molecules. Upon administration, the local pH (around 7.4) quickly normalizes that percentage, depending on the substance's pKa. Both forms of the substance are needed to achieve the nerve-blocking effect (Casati & Putzu, 2005; Riviere & Papich, 2009; Dugdale, 2010).

Table 1 - Comparison of properties between local anaesthetic agent groups (adapted from Dugdale, 2010).

Properties of amino-esters	Properties of amino-amides
Poor tissue penetration	Good tissue penetration
Short duration of action (rapid metabolism)	Long duration of action (slower metabolism)
Fast elimination = decreased chance of toxicity	Slow elimination = increased risk of toxicity
Increased chance of allergic reactions due to para-amino benzoic acid (pABA) as metabolite.	Possibility of allergic reactions due to methylparaben as preservative (can break down into pABA)

5.2.3.2. Mechanism of action

LAs' mechanism of action is not completely understood. These drugs are highly liposoluble with an alkaline pKa and are rapidly absorbed by the tissues. This allows a quick linkage to the sodium channels of the peripheral nerve fibers, blocking their signal conduction properties. This block is performed by ionized and non-ionized molecules. Due to their pKa (see Table 2), LAs are dissociated into these two forms, which perform the same action through different mechanisms. Ionized molecules block the sodium channels from the exterior side of the fibers, while the non-ionized molecules are absorbed into the fibers and perform the block from the inside. It is important to keep this in mind when using LAs to anesthetize an inflamed tissue, because in these conditions the local pH is altered. This may lead to a delayed onset of action which needs to be taken into consideration (Dugdale, 2010).

The three most important characteristics of a local anesthetic are pKa, which controls the amount of ionized and non-ionized molecules available after administration and thus regulates speed of onset of action; lipid solubility, which relates to a substance's potency; tissue protein binding, which is the property that relates to duration of action. A combination of these three properties and also the tissue's condition regulates the drug's tissue penetration. (Casati & Putzu, 2005; Dugdale, 2010)

5.2.3.3. Effects and pharmacokinetics

The complete blockage of impulses in the affected nerves causes a lack of sensation in the innervated area and, thus, an absence of pain when certain procedures are done to an anesthetized area.

It must be taken into account, though, that all tissues have sodium channels, with some variations in type and density depending on the tissue in question. If a sufficient dose of an LA is systemically absorbed, those tissues' channels will be inhibited, causing depressant effects, especially in both the CNS (sedation, seizures, depression, coma, respiratory arrest) and the cardiovascular system (cardiac arrest). This fact explains the risk of using high doses of LAs. After absorption, the local anesthetic may inhibit the ion channels of a primary organ (brain or heart, for instance) and cause severe problems. These effects are more pronounced with more lipophilic substances. Vasoconstrictor drugs can be used to delay the systemic absorption (lidocaine, i.e.), though the more recent molecules already have a certain vasoconstricting effect (bupivacaine, ropivacaine and levobupivacaine) (Casati & Putzu, 2005). Other adverse effects can arise from nerve toxicity (detergent-like activity of high

doses of LAs, preservatives of commercial solutions, excessive vasoconstrictor effect) or allergic reactions (especially with ester-linked group molecules). (Riviere & Papich, 2009; Dugdale, 2010)

Table 2 - Physicochemical properties of some local anaesthetic agents. (adapted from Dugdale, 2010)

	pKa	Onset	Relative lipid solubility	Toxicity	Relative potency	Protein binding	Duration of action
Lidocaine	7.9	Fast	150	Medium	2	65%	Intermediate
Bupivacaine	8.16	Moderate	1000	High	8	95%	Long
Ropivacaine	8.1	Moderate	400	Medium	6	95%	Long

Care should be taken when using LAs in patients with impaired liver function, as all molecules are liver-metabolized (Plumb, 2011).

5.2.3.4 Example 1 - Ropivacaine

Ropivacaine is an amide-linked local anesthetic with many similarities to the well-known bupivacaine. It is very similar to bupivacaine, differing from it for being a pure left-isomer and having one less carbon in the side-chain. Left-isomers are proven to be less toxic than racemic mixtures or right-isomers. Also, the reduced number of carbons in the side chain makes it less lipophilic than bupivacaine. These two characteristics explain the advantages of ropivacaine compared to its predecessor: less inherent CNS and cardiovascular toxicity (which makes it a safer drug to use, in case large volumes are needed), less systemic absorption (due to its lower liposolubility, it is harder to cross the blood-brain-barrier and leads to the next advantage) and increased duration of action (because it remains in the target area for a longer period of time) (Casati & Putzu, 2005; Dugdale, 2010).

In terms of potency, it is classified as being 50% less potent than bupivacaine, in controlled trials. However, in clinical studies, this difference isn't relevant. In fact, it is noticed that ropivacaine blocks produce the same analgesia (although the doses are 50% higher than bupivacaine) but less motor blockage, which allows for an earlier recovery of the patients. This remains true only as long as 0,5% or 0,75% concentrations of ropivacaine are used (Casati & Putzu, 2005).

5.2.4. α-2 receptor agonists

5.2.4.1. Introduction

Composed by xylazine, detomidine, medetomidine and dexmedetomidine, among others, this drug class is very well known and used around the globe by physicians and veterinarians alike ever since they came around in the 20th century. However, after the discovery of medetomidine and even dexmedetomidine, along with the increasing worries about managing pain during procedures, practitioners began to use them with more frequency due to their excellent combination of sedative and analgesic properties (Kraus, 2012). They are seldom used alone for any anesthetic protocol. Usually, α-2 agonists (A2As) are used in conjunction with opioids, benzodiazepines or other tranquilizers/sedatives for a greater sedation effect, longer duration of action and reduced dosages of all substances. A2As and opioids have synergistic analgesic effects that should be taken advantage of. Finally, the use of A2As with general anesthetics highly reduces the dosage needed to achieve the target plane of anesthesia, both with fixed and inhalable drugs (propofol, thiopental, isoflurane, sevoflurane, etc…). With high doses of dexmedetomidine, inhalable anesthetic needs can be reduced as much as 89% (isoflurane; in dogs, with a 20 mcg/kg/IV dose) (Teixeira-Neto, 2009).

5.2.4.2. Mechanism of action and main effects

A2A's connect to the adrenaline receptors across the central and peripheral nervous system and displace norepinephrine from there, creating a negative-feedback that prevents adrenaline release and the propagation of stimulus. However, because these receptors are not limited to the nervous system, when they connect to the cardiovascular receptors they also cause peripheral vasoconstriction that increases systemic blood pressure, while reflexively lowering heart rate. Vomiting might also be seen in some patients (Plumb, 2011; Shaffran, 2012).

Alfa-2 antagonists can be used to control adverse effects and the duration of sedation. The most common drug used is atipamezole, which is safe and guarantees an effective reversal effect in a short period of time (usually 5-15 minutes). Yohimbine and tolazoline might also be used, but they often come with undesired side effects, since besides displacing the agonists' molecules from the receptors, they also stimulate the CNS. Atipamezole has no effect on CNS, thus making it the safer choice, as well as having a longer duration than dexmedetomidine which prevents the re-sedation effect (animals becoming sedated again after the antagonists' effect has run off) (Shaffran, 2012).

Their action at the adrenergic receptors in the locus coeruleus and spinal cord is responsible for their sedative and analgesic properties. Most A2A's also act at the imidazoline receptors, this site being responsible for their anti - arrhythmogenetic effect (Shaffran, 2012).

5.2.4.3. Adverse effects and pharmacokinetics

Adverse effects are related to the agonistic action on the α_1-receptors, which translate especially into an excessive bradycardia or excessive hypertension, usually present when a high dose of one of the drugs is administered. Respiratory depression is usually not seen in healthy patients but may be an issue in ill patients. Nonetheless, oxygen supply should be available because some patients have an important reduction in O_2 blood saturation, after being given a high dose of A2A (Traquilli, Thurmon, & Grimm, 2007; Teixeira-Neto, 2009; Dugdale, 2010; Plumb, 2011).

A2As are mostly excreted in urine, thus the veterinarian needs to be careful when using these in animals with impaired renal function. Patients with known cardiac disease or with liver function impairment are not eligible for an anesthesia protocol involving A2As.

5.2.5. NMDA antagonist - Ketamine

5.2.5.1. Introduction

Ketamine is the shortest acting phencyclidine derivative, with tiletamine being a longer lasting version of it. It is widely used in veterinary medicine due to the dissociation effect seen between the thalamocortical and limbic systems. Plus, ketamine has a potent analgesic effect, which labels it as a very useful tool to use in analgesia protocols. It is also one of the few molecules able to lessen or even reverse the central sensitization phenomenon (Riviere & Papich, 2009).

5.2.5.2. Mechanism of action and pharmacokinetics

Ketamine acts as a non-competitive antagonist of the NMDA receptors, preventing their activation by connecting to a specific site inside the receptor (phencyclidine binding site) and physically blocking Ca^{2+} entrance. Besides this, it also has antagonist effect in μ opioid receptors, agonist effect in κ and δ opioid receptors, granting it some opioid-like analgesic effects, and even on monoaminergic and muscarine receptors and voltage-sensitive calcium channels, which explains the effects in the cardiovascular system, respiratory system and muscular tone (Riviere & Papich, 2009; Dugdale, 2010; Plumb, 2011). Anesthesia with these agents causes a state of altered consciousness, also known as catalepsy. This means that the

patients are aware of their surroundings but they stop caring about it and cease responding to the stimuli. Dissociative agents are also analgesic and prevent/revert central sensitization effects (Dugdale, 2010).

Ketamine is mainly metabolized in the liver, but in cats it is excreted almost in nature through the urine. Thus, it should be used cautiously in dogs with hepatic or renal disease and in cats with renal impairment (Plumb, 2011).

5.2.5.3. Adverse effects

Ketamine's adverse effects are related to its use in patients who have diseases of the organs in which it acts, with exception to the wake-up delirium that patients go through. It can be dangerous for the patient and for the people handling it, so drugs that counter this state of mind should be administered in conjunction with ketamine (i.e. benzodiazepines). This drug has some contra-indications like: it should not be used in head trauma patients or any patient with a disease that involves increased intra-cranial pressure, as the administration of ketamine increases it; patients with diagnosed hypertension are not eligible for anesthesia with this drug due to the increased sympathetic tone granted by ketamine; finally, patients with renal failure might experience a longer duration of a ketamine administration (Riviere & Papich, 2009; Plumb, 2011).

5.3 Multimodal and pre-emptive analgesia

Several years ago, during the advent of analgesia as a discipline, the analgesic protocols were very simplistic. They were often composed of single drugs (the number of available drugs was also much lower than nowadays) and their dose was titrated to effect. In some patients, the dose would be so high that the adverse effects of the drug used would become serious considerations before or during the procedure. As new drugs were discovered and new studies were made, a better understanding of the mechanisms of pain and analgesic drugs was obtained and the use of a mixture of drugs to fight pain was attempted. The results were great and it slowly became the norm. Multimodal analgesia (the use of several analgesic drugs in a single analgesic protocol) is an almost mandatory step in our days, as the benefits highly surpass the risks of mixing several drugs in the patient. Since the pain pathway is blocked or intercepted in several different points, the pain signals find a series of impassable hurdles, thus successfully preventing the signals from reaching the highest brain centers. Furthermore, the use of several analgesic drugs allows a major reduction of each drug's dose, greatly

reducing the occurrence of adverse effects and allowing the fine tuning of analgesia depth to our desired purpose. (Gogny, 2006; Hellyer et al, 2007)

The concept of prevention of pain has evolved even more, though. In modern days, we also recur to Pre-emptive Analgesia, or the administration of analgesic drugs prior to a painful procedure. In theory, if the analgesic agents are already present in the organism when a painful stimulus occurs, the painful signal will be almost immediately suppressed by their action, since the steps of administration and distribution of the drug in body tissues are skipped. Furthermore, since the only way to prevent peripheral and central sensitization is to successfully prevent any nociceptive signal to reach the highest centers, this procedure seems to be the only one that can avoid them. However, many controversial studies are published and a general consensus about this pain management strategy hasn't been reached. (Victory et al, 1995; Vallejo et al, 2006; Viñuela-Fernández et al, 2007; Rica et al, 2007; Bamigboye & Justus, 2008)

Part II – Experimental study

1. Introduction

General anesthesia and opioid plus non-steroidal anti-inflammatory drug combinations are often used in surgical procedures, as tools of guaranteeing patient immobility and preventing pain. The principles of multimodal analgesia state that different drugs should be used in order to interrupt the pain pathways at several levels and thus prevent central sensitization successfully. Secondly, if under the effect of a drug the transformation of a painful stimulus into an electrical signal is prevented, then pain sensation is blocked at the beginning of the pain process and all of its effects are prevented. This is the key concept behind the use of local anesthetics, drugs that have been known to veterinary medicine since the 1890's (Riviere & Papich, 2009). However, their use in combination with general anesthesia wasn't described until 2000 (Nolan, 2000). After this description, there has been a growing interest in the utility of this kind of approach to peri-operative pain and a multitude of drugs and infiltration techniques have been tested in order to assess which of them might be the most useful in a certain procedure.

The inclusion of local anesthetics like lidocaine and bupivacaine in analgesic protocols for a variety of procedures has been tested in several studies and although the theoretical reason for their success is clear, results have been surprisingly controversial (Victory et al, 1995; Carpenter et al, 2004; Casati & Putzu, 2005; Vallejo et al, 2006; Savvas et al, 2008; Bamigboye & Justus, 2008; Campagnol et al, 2012;). The use of these drugs brings great benefits regarding pain management, but their side-effects must always be taken into account, especially when considering patients with underlying heart or CNS diseases, since the possibility of interfering with the normal electrical function of the heart due to an over-dosage of local anesthetic is real and often irreversible. Through extensive retrospective analysis of several studies regarding the differences between bupivacaine, levobupivacaine and ropivacaine, it was concluded that despite the differences in their relative potency at a molecular level, there doesn't seem to be a significant difference between their effects, determined by the degree of success of the procedure, following administration of one of these drugs (Casati & Putzu, 2005). Furthermore, ropivacaine has a safer profile compared to bupivacaine (Casati & Putzu, 2005; Riviere & Papich, 2009). It becomes obvious that there is interest in generalizing the use of this drug, in order to obtain safer protocols.

Several studies of ropivacaine's effectiveness exist in human medicine (Rica et al, 2007; Bamigboye & Justus, 2008; Gutton et al, 2013;), which might indicate that its analgesic properties and safer profile can be used safely in animals as well. Since its use has been so restricted, its usefulness hasn't been tested in the full range of existing surgical procedures, with most of its use being concentrated in laboratory trials (Feldman et al, 1997; Takahashi, et al., 2004; Charlet, Rodeau, & Poisbeau, 2011; Loon et al, 2012) and clinical studies (Klein & Benveniste, 1999; Marzo et al, 2012), in procedures ranging from epidurals, intravenous regional anesthesia, cervical blocks or braquial plexus blocks.

Furthermore, in the Veterinary Teaching Hospital of the University of Helsinki, the surgical team has great care in assuring that their patients are given the best and most appropriate anesthetic protocols to their surgeries. Lately, they've been trying to develop a better analgesic protocol for ovariohysterectomies, since they felt their protocol was lacking and that they could give a better post-operative comfort level to their patients. Thus, several ideas have come up, from instilling local anesthetic before the ligature of the ovarian pedicles to the use of a local anesthetic to alleviate abdominal pain during the post-operative period. The author then decided to contribute to this research by picking up one of the suggestions (the local anesthetic administration) and studying its efficacy. As for drug choice, the lack of available information on ropivacaine for its use in more routine procedures led the author into designing a study with which a contribution to this gap in medical knowledge could be given.

2. Objective

This was a blind, randomized controlled clinical trial. Its objective was to assess the effectiveness of a subcutaneous infiltration of ropivacaine over the incision line in preventing post-operative pain, in healthy female dogs after elective ovariohysterectomy (OVH). More specifically, the objective was to determine if the group treated with ropivacaine had lower pain scores than the group that only received a saline administration.

The experimental protocol was submitted to and approved by the Animal Ethical and Welfare Committee of FMV-ULisboa.

3. Material and Methods

3.1 Patients and inclusion criteria

The study was performed at the HEFMV-ULisboa. A total of 15 female dogs that were scheduled for elective ovariohysterectomy were selected for this study, based on the data gathered at the pre-surgery consultation. Patient info can be seen in table 3. Inclusion criteria included age between 6 months and 9 years old, weight between 2.5 and 50 kg and to be healthy and non-pregnant as determined by anamnesis, physical exam and basic blood analysis (haematocrit, total proteins, glucose, ALT and urea). Any alterations in any of the parameters meant exclusion from the study. One of the patients was excluded from the study, as explained below.

Patients were kept in a separate recovery room from the other surgery patients, where the observer had easy access to them and could easily monitor them. All patients were walked outside at least once during the study.

Table 3 - Patient list and respective study group.

Group	Patient nº	Age	Breed	Weight (kg)
NaCl	**1**	1 year	French Bulldog	11,4
	4	8 mo.	X Husky	19,6
	5	2 years	Jack russel	4,5
	7	1 years	SRD	23,6
	8	5 years	SRD	7,2
	11	1 year	SRD	19,3
	13	10 mo.	SRD	11,8
Ropi	**2**	9 mo.	SRD	7
	3	3 years	SRD	10,4
	6	9 mo.	SRD	15
	9	6 years	Boxer	31,3
	10	1 year	Yorkshire	2,7
	12	1 year	Yorkshire	3,1
	14	2 years	French Bulldog	10,5

3.2 Study groups

After approval into the study, dogs were divided randomly into two groups: Group Ropivacaine (GR) and Group Saline (GS). The observer was blinded to patient distribution during the study. Both groups were subjected to an SC administration over the incision line, during surgery preparation, approximately 15-20 minutes prior to surgery start. GR got 1

mg/kg ropivacaine and GS got the same volume of saline. Aside from this, both groups received the same anesthetic protocol.

3.3 Therapeutic protocol

Patients were pre-medicated with 0.2 mg/kg of methadone (Semfortam®, Eurovet animal health B.V., Bladel, Netherlands) and 0.01-0.02 mg/kg of acepromazine (Calmivet®, Vétoquinol SA, Lure, France) 15 to 30 minutes before being taken to preparation. Patients under 10 kg of weight were not given acepromazine. Once in preparation and after catheter placement, induction was attained with 2 – 4 mg/kg of propofol (Lipuro®, BBRAUN Vet Care SA, Rubí, Spain) and intubation was performed. Maintenance of anesthesia was achieved with isoflurane (IsoVet®, BBRAUN Vet Care SA., Rubí, Spain). After induction, all patients received 12.5 mg/kg amoxycilin plus clavulanic acid subcutaneously (Synulox®, Pfizer Italia, S.r.I., Latina, Italy). The local anesthetic block was performed during the site washing and disinfection. GR received 1 mg/kg of ropivacaine (Naropeine 0,5%®, AstraZeneca Produtos Farmacêuticos Lda., Barcarena, Portugal) and GS received a volume of saline (Soro fisiológico BBRAUN Vet Care SA, Rubí, Spain) calculated using ropivacaine's dose and concentration. Rescue analgesia was given to all dogs in this study, either at the end of the monitoring period (6 hours post-local block) or if they scored over 5 during pain assessment, whichever came first. Rescue analgesia consisted of 4 mg/kg of carprofen (Rimadyl®, Pfizer laboratories Lda – Animal Health Division, Guarulhos – São Paulo, Brazil) and 0,015 mg/kg of buprenorphine (Bupaq®, Richter Pharma AG, Wels, Austria). Fluidotherapy was done using sodium chloride 0,9% (Soro fisiológico BBRAUN Vet Care SA., Rubí, Spain) at a rate of approximately 5 mL/kg/h. The therapeutic protocol is summarized on table 4.

Table 4 -Pharmacotherapeutical protocol.

	Drug name	Dose	Route	Obs.
Pre-med	Acepromazine + Methadone	0,01-0,02 mg/kg + 0,2 mg/kg	IM	Ace not given to patients <10kg. 15-30 min. prior to patient preparation.
Induction	Propofol	2 – 4 mg/kg	IV	To effect (allow intubation).
Maintenance	Isoflurane	1 – 4 %		-
Rescue	Carprofen + Buprenorphine	4 mg/kg + 0,015 mg/kg	SC	If pain score ≥ 5 or 6h post-block.
Antibiotherapy	Amoxicilin + Clavulanic acid	12,5 mg/kg	SC	-

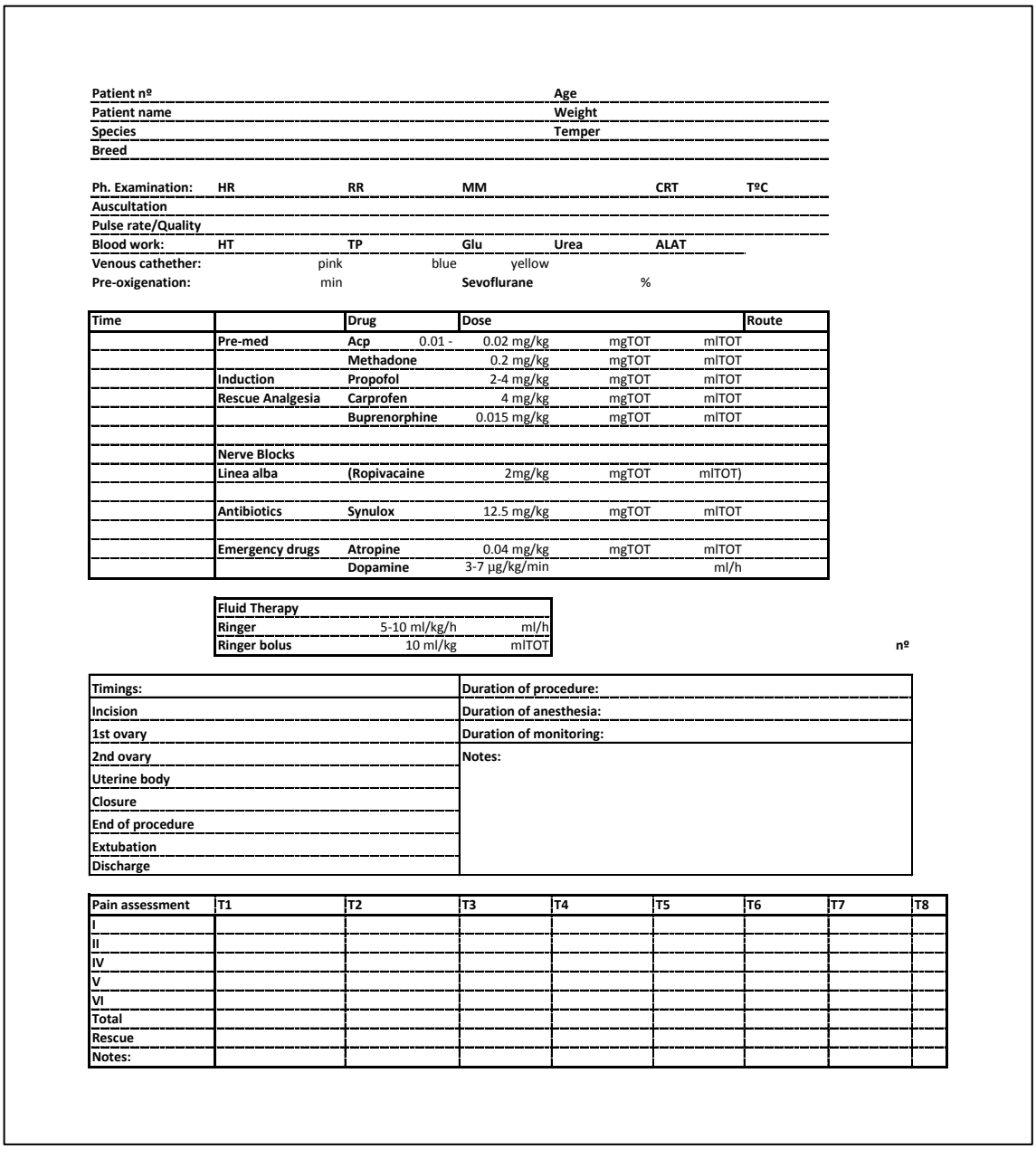
3.4 Surgical technique

An incision 2 to 4 cm long was done immediately caudal to the umbilical scar. Subcutaneous fat was debrided until the muscular layer was reached. Then, the linea alba was incised with a scalpel blade and the incision extended with the use of a Mayo scissors, while tenting the abdominal wall with the help of an Adson-Brawn forceps. Once the opening was satisfactory, the surgeon manually searched for the uterus, usually trying to find the right uterine horn first. Once found, the right ovary was lifted from its position and an haemostatic clamp was set between the ovary and the uterus horn, to secure the structure for ligature placement. A small hole in the broad ligament was done, to allow the passage of a suture in order to do the ligatures. Two ligatures were performed on the ovarian pedicle. Once tightly secured, two clamps were set between the ovary and the ligatures and the pedicle was incised in between them. The ligated pedicle was checked for hemorrhage and if it wasn't present, slowly replaced in its place. Finally, the broad and round ligaments were ligated as well, before being incised. Then, following that uterine horn, the left uterine horn was found and the left ovary pulled up. The same procedure was repeated for this ovary. Once the left broad and round ligaments were incised, both horns were pulled caudally to expose the uterine body and cervix. The uterine body was ligated with a simple ligature first, followed by a transfixation ligature. Once that was accomplished, the uterine horn was clamped with two haemostatic clamps just cranial to the ligatures and the uterus incised in the middle of them. The uterine stump was checked for hemorrhage and if no hemorrhage was seen, the stump was replaced in its proper place and the pedicles' ligatures re-checked for hemorrhages. If none were seen, the surgeon initiated the closure of the abdominal cavity. The peritoneum, the muscle layers and the muscle fascia were sutured together using a simple continuous pattern, reinforced with a simple interrupted pattern. Then an approximation suture was performed in the subcutaneous tissue and finally skin closure was achieved with an intradermic continuous pattern.

3.5 Monitoring parameters

Each patient had its own monitoring sheet, which comprised of all the data related to that patient, from physiological parameters to its behaviour. The individual doses for the pharmacotherapeutical protocol were registered, along with pre-medication, induction and other surgical procedure timings. Finally, it includes a table for pain assessment. This patient sheet can be seen in figure 5.

Figure 5 - Monitoring sheet. (original photo)



Patient nº			Age	
Patient name			Weight	
Species			Temper	
Breed				

Ph. Examination:	HR	RR	MM	CRT	TºC
Auscultation					
Pulse rate/Quality					
Blood work:	HT	TP	Glu	Urea	ALAT
Venous cathether:	pink	blue	yellow		
Pre-oxigenation:	min		Sevoflurane	%	

Time		Drug	Dose			Route
	Pre-med	Acp	0.01 - 0.02 mg/kg	mgTOT	mlTOT	
		Methadone	0.2 mg/kg	mgTOT	mlTOT	
	Induction	Propofol	2-4 mg/kg	mgTOT	mlTOT	
	Rescue Analgesia	Carprofen	4 mg/kg	mgTOT	mlTOT	
		Buprenorphine	0.015 mg/kg	mgTOT	mlTOT	
	Nerve Blocks					
	Linea alba	(Ropivacaine	2mg/kg	mgTOT	mlTOT)	
	Antibiotics	Synulox	12.5 mg/kg	mgTOT	mlTOT	
	Emergency drugs	Atropine	0.04 mg/kg	mgTOT	mlTOT	
		Dopamine	3-7 µg/kg/min		ml/h	

Fluid Therapy		
Ringer	5-10 ml/kg/h	ml/h
Ringer bolus	10 ml/kg	mlTOT

nº

Timings:	Duration of procedure:
Incision	Duration of anesthesia:
1st ovary	Duration of monitoring:
2nd ovary	Notes:
Uterine body	
Closure	
End of procedure	
Extubation	
Discharge	

Pain assessment	T1	T2	T3	T4	T5	T6	T7	T8
I								
II								
IV								
V								
VI								
Total								
Rescue								
Notes:								

3.5.1 Physiological parameters

From induction to the end of surgery, heart rate and respiratory rate were measured every 5 minutes. It was not possible to measure blood pressure during the intervention due to the unreliability of the monitoring equipment and the lack of proper sized cuffs. Five time-points were set during surgery, corresponding to the most painful moments of surgical handling: skin incision (S1), clamping of first ovary (S2), clamping of second ovary (S3), ligature of the uterine body (S4) and beginning of closing sutures (S5). These were set in order to allow statistical analysis of these parameters.

3.5.2 Pain scoring

Pain assessment was initiated 30 minutes after extubation (T1) and continued every hour, up until 6 hours post-local block (T2 – T5). Only one observer scored the animals (the author) and the short form of the Glasgow's Composite Measure Pain Scale (GCMPS) was used for this purpose, due to its ease of use for untrained people and because of its sensibility. The observer began the assessment by approaching the cage silently and watching the animal's behaviour from afar for approximately one minute. Then, the observer opened the cage and greeted the patient, petting him on the head and over its back, observing the response (i.e. if the animal is awake, sedated, actively responding or just ignoring the stimuli). Then, with careful movements, the surgical site was palpated with two fingers gently pressing around and finally on the surgical incision, while recording carefully the patient's reaction. Finally, the patient was left alone and watched from a distance to see if he stood awake or went to sleep again. Between T3 and T5, all patients were walked outside, after palpation of the wound site.

3.6 Results analysis

Differences between group age, weight, duration of procedure and of anesthesia were analyzed with a two-sample Wilcoxon test. Statistical differences in heart rate (HR), respiratory rate (RR) and pain scores (PS) between groups were calculated using a repeated measures ANOVA analysis. Minutes to HR and RR time-points were analyzed with descriptive statistical methods only. Statistical analysis was done with R v2.1.2.

4. Results

4.1 Study group

From the 15 dogs that fulfilled the criteria, 14 were included in the study (see table 4). The one rejection was due to pregnancy, discovered during surgery (not included in table 4). Half of the patients didn't belong to a specific breed. Breeds represented in the study are French Bulldog (x2), Yorkshire Terrier (x2), Boxer (x1), Husky (x1), and Jack Russel (x1). Breed influence was not analyzed.

4.2 Age, weight, duration of procedure and of anesthesia

Ages ranged from 8 months to 6 years and weights ranged from 2.7 kg to 31.3 kg. Average values for SG were: age = 1.63 ± 1.55 years, weight = 13.9 ± 7.0 kg, duration of surgery = 38.7 ± 6 minutes and duration of anesthesia = 80.7 ± 13 minutes; and for RG were: age = 2.05 ± 1.93 years, weight = 11.4 ± 9.7 kg, duration of surgery = 37.7 ± 8 minutes and duration of

anesthesia = 71.1 ± 12 minutes. No signs of local anesthetic toxicity were seen during the study. Statistically, patients' age, weight, duration of procedure and duration of anesthesia did not differ between groups (p = 0.743, p = 0.318, p = 0.796 and p = 0.337, respectively) (see Table 5).

Table 5 - Descriptive statistics and statistical significance of age, weight, duration of procedure and of anestesia.

Parameter	SG	RG	P value
Age (y)	1.63 ± 1.55	2.05 ± 1.93	0.743
Weight (kg)	13.9 ± 7.0	11.4 ± 9.7	0.318
Duration of procedure (min)	38.7 ± 6	37.7 ± 8	0.796
Duration of anestesia (min)	80.7 ± 13	71.1 ± 12	0.337

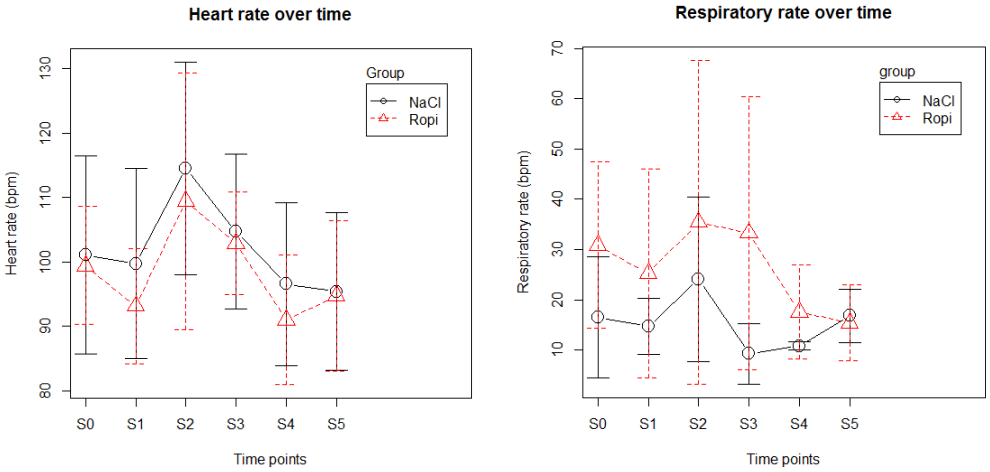
4.3 Heart and respiratory rate

During surgery, the heart rate and breathing rate data was collected in the 5 time-points described in 3.5.1. Visually analyzing table 6, it is clear that those time points were very similar between groups, which mean the painful stimuli were inflicted roughly after a similar period of time had passed since administration of ropivacaine. Neither HR nor RR have statistically significant differences between groups (p = 0.990 and p = 0.529, respectively). There was a peak in both the monitorized parameters at S2 (see figure 6), coinciding with the pull on the first ovary pedicle, which might indicate that this is the most painful moment of the surgery and thus the anesthetist should be paying close attention to the patient at this time and act in order to prevent it as much as possible. Because HR and RR are extremely unreliable parameters for pain assessment when used individually, evaluation of anesthesia depth through the palpebral reflex was done simultaneously. Whenever possible, additional patient's biological parameters should also be measured (i.e. blood pressure and pulse).

Table 6 - Average time to time points. Average differences between groups were under 5 minutes.

Time points	Time to crucial time-points (min.)	
	NaCl	Ropi
S1	21±5	17±7
S2	24±5	21±6
S3	31±4	28±5
S4	37±5	33±6
S5	44±6	39±6

Figure 6 - HR and RR over time. S1-S5 are the crucial time points. S0 is an average of the values between induction and incision. Bars represent a 95% confidence level.



4.4 Rescue analgesia

During the monitoring period, rescue analgesia was given to three dogs before ropivacaine's effect had ended. However, none was due to a high pain score. Dog number 2 became very defensive during monitoring and wouldn't allow the approach of the observer but was resting comfortably, ignoring the wound area and was happy and bouncy when he came out walking, dog number 4 was picked up by the owner sooner than expected and dog number 7 was mistakenly given the rescue analgesia before time. No dog exceeded the limit score that set the need for rescue analgesia (PS $\geq$ 5).

4.5 Pain scores

This study showed no differences in pain scores between groups ($p = 0.638$). It is interesting to see that by looking at the scores alone (Table 7), it might seem that patients that received ropivacaine infiltration have higher scores than the control patients. The reason for this trend is difficult to pinpoint, but some reasons might be: an unexpected reaction to ropivacaine (Does it sting or somehow bring some discomfort? Is the numbing sensation of a local anesthetic bothersome to some patients?), the result of a sub-optimal dose of local anesthetic (1 mg/kg was used instead of the usual 2 mg/kg from literature) or some other unknown reason. Since the sample size is small, the question remains if the same trend would remain if more patients were added to the study or if the recommended dose of ropivacaine was administered.

Table 7 - Pain scores of all the patients and respective groups.

Group	Patient nº	T1	T2	T3	T4	T5
NaCl	**1**	2	0	0	0	0
	4	2	3	1	NA	NA
	5	1	0	0	0	0
	7	3	2	4	NA	NA
	8	1	0	0	0	0
	11	0	1	1	1	1
	13	3	4	3	2	2
Ropi	**2**	0	4	1	NA	NA
	3	0	2	0	0	0
	6	2	4	1	1	1
	9	1	2	2	2	0
	10	2	1	3	3	3
	12	4	4	3	3	2
	14	0	0	0	0	0

5. Discussion

There are no studies on the use of ropivacaine in abdominal surgery in animals. However, in humans, it is proven to lower the need for post-operative analgesics, including the need for opioids, in women undergoing cesarean delivery (Bamigboye & Justus, 2008). Twenty-four women out of fifty of the ropivacaine group compared to forty-seven out of fifty of the control group, required pethidine or experienced severe pain during the first hour post-op. The use of other analgesics was also reduced in the ropivacaine group, compared to the control group (Bamigboye & Justus, 2008). The authors of that study administered ropivacaine directly into the surgical wound and sprayed in the peritoneum. In the present study, ropivacaine was administrated before surgical incision in a linear injection, subcutaneously over the area to be incised. The amount of ropivacaine also differed between studies: in this study, 1 mg/kg of ropivacaine was used, which is lower than the recommended dosage for local blocks, but was chosen due to volume issues with the 2 mg/kg dose (ropivacaine's presentation was 0,5% or 5 mg/ml), since the surgical incision was very small (2 -4 cm); in the human study, 30ml of 0.75% ropivacaine (equivalent to 7,5 mg/ml) were used in every patient (Bamigboye & Justus, 2008).

There are two studies that followed a similar approach, using lidocaine and bupivacaine instead and administering it in the wound incision and sprayed in the peritoneum (Carpenter, Wilson, & Evans, 2004; Campagnol, Teixeira-Neto, Monteiro, Restitutti, & Minto, 2012).

Their results were in favor of the use of intraperitoneal bupivacaine as a valid method of analgesia post-op in this surgery, with lower pain scores obtained in these groups, compared to control groups. Due to short supply of ropivacaine, it wasn't possible to use it via intra-peritoneum in the present study. Yet another study had a similar design to this one and the two mentioned above, but used only bupivacaine in a SC+IM administration technique and patients included weren't limited to OVH procedures, but rather any celiotomy (Savvas, Papazoglou, Anagnostou, Tsioli, & Raptopoulos, 2008).

This study failed to prove any advantage in the use of ropivacaine in the analgesic protocol of OVH in dogs. Due to the small differences between groups, the author concludes that in order to have statistical differences, the sample size should have been larger. Another similar study reached the same conclusion (Campagnol, Teixeira-Neto, Monteiro, Restitutti, & Minto, 2012). Nonetheless, there is evidence that the SC use of a local anesthetic can be of benefit in these cases (Carpenter, Wilson, & Evans, 2004; Savvas, Papazoglou, Anagnostou, Tsioli, & Raptopoulos, 2008). In one of the studies the administration of local anesthetic was done SC before wound closure, along with IP spray (Carpenter, Wilson, & Evans, 2004) and in the other study the local anesthetic was given SC+IM (Savvas, Papazoglou, Anagnostou, Tsioli, & Raptopoulos, 2008). According to Savvas et al, there was evidence of effectiveness of this approach, if the administration was done pre-incision rather than pre-wound closure.

Due to the context of this study, there were some obstacles that might have contributed to the lack of observable differences. Since this study was set-up during the period of curricular training and in a public veterinary health establishment, its design had to be inserted in the routine of the hospital's staff without causing much disturbance and it depended on the available resources for its realization. Thus, the author did not manage to gather the amount of patients originally pretended and the pre-surgical blood analysis couldn't be too extensive, to avoid excessively charging the owners. Furthermore, the absence of a fully-fledged veterinary anesthetist limited the creation of a better adapted anesthetic protocol to the study's objectives by restraining the drug selection to those the in-house surgeon felt comfortable using. Finally, ropivacaine's availability in Portugal is quite limited, both in quantity and in presentation, fact that ended with the author using a presentation that had a sub-optimal concentration, which led to the change in the ropivacaine's dose in the protocol.

Despite the lack of results, the author still believes that they could've been obtained if the study was designed with slight adjustments, noticed and learned throughout the study period.

Patient selection should be tighter, taking into account animal behaviour, in order to prevent too fearful or too aggressive animals to be included in the study. These attitudes highly hinder pain assessment and might induce the observer into scoring a patient wrongly. It should also be useful to not include pediatric and geriatric patients in the same study, as both groups of patients have slight differences in pain perception (interference of dessensitization phenomenon or presence of chronic pain originated from geriatric health problems). Blood pressure should be monitored during surgery, as it grants one more parameter for nociception assessment during the procedure. The surgery protocol could be revised, giving preference to short-duration analgesia for the intra-operative period and a higher dose of local anesthetic, normalized to a 0.75% concentration, to assure a good spread of the anesthetic. This would assure that in the post-op period analgesia would be provided solely by the local anesthetic and that it was administered in a proper dose. Finally, the administration technique should be changed into SC+IM administration, as it has proven to be a reliable technique (Savvas, Papazoglou, Anagnostou, Tsioli, & Raptopoulos, 2008).

In conclusion, anesthesia with methadone, acepromazine, propofol and isoflurane provided reliable analgesia during and after the procedure, since no supplemental analgesics were needed in the control group. The pre-incisional administration of 1 mg/kg SC of ropivacaine did not show any effectiveness in lowering post-operative pain scores.

Bibliography

Anil, S., Anil, L., & Deen, J. (2002, February 1). Challenges of pain assessment in domestic animals. *Journal of the American Veterinary Medical Association, 220*(3), 313-319.

Bamigboye, A. A., & Justus, H. G. (2008). Ropivacaine abdominal wound infiltration and peritoneal spraying at cesarean delivery for preemptive analgesia. *International Journal of Ginecology and Obstetrics*, 160-164.

Barr, R. (1998). Reflections on measuring pain in infants: dissociation in responsive systems and "honest signalling". *Archives Of Disease In Childhood, 79*(2), 152-156.

Bishop, B. (1980, Janeiro). Pain: Its physiology and rationale for management. *unknown*, pp. 24-37.

Campagnol, D., Teixeira-Neto, F., Monteiro, E., Restitutti, F., & Minto, B. (2012). Effect of intraperitoneal or incisional bupivacaine on pain and the analgesic requirement after ovariohysterectomy in dogs. *Veterinary Anesthesia and Analgesia, 39*, 426-430.

Carpenter, R., Wilson, D., & Evans, A. (2004). Evaluation of intraperitoneal and incisional lidocaine or bupivacaine for analgesia following ovariohysterectomy in the dog. *Journal of Veterinary Anesthesia and Analgesia*, 46-52.

Casati, A., & Putzu, M. (2005). Bupivacaine, levobupivacaine and ropivacaine: Are they clinically different? *Best Practice & Research Clinical Anaesthesiology* , 247–268.

Chambers, C., Giesbrecht, K., Craig, K., Bennet, S., & Huntsman, E. (1999). A comparison of faces scales for the measurement of pediatric pain: children's and parents' ratings. *PAIN, 83*(1), 25-35.

Charlet, A., Rodeau, J., & Poisbeau, P. (2011). Radiotelemetric and symptomatic evaluation of pain in the rat after laparotomy: long-term benefits of perioperative ropivacaine care. *The Journal of Pain, 12*(2), 246-256.

D'Mello, R., & Dickenson, A. (2008). Spinal cord mechanisms of pain. *British Journal of Anesthesia*, 8-16.

Dugdale, A. (2010). *Veterinary anaesthesia: principles to practice.* Oxford: Wiley-Blackwell.

Durrance, D. (2007, February). Overcoming compassion fatigue. *DVM Newsmagazine*, pp. 44-46.

Epstein, M. E. (2012, February 19-23). *Opioids: A Practical Guide and New Developments*. Retrieved March 8, 2014, from www.vin.com: http://www.vin.com/doc/?id=5603996

Feldman, H., Dvoskin, S., Halldin, M., Ask, A., & Doucette, A. (1997). Comparative local anesthetic efficacy and pharmacokinetics of epidurally administered ropivacaine and bupivacaine in the sheep. *Regional Anesthesia and Pain Medicine, 22*(5), 451-460.

Ferreira, T. H., Rezende, M. L., Mama, K. R., Hudachek, S. F., & Aguiar, A. J. (2011). Plasma concentrations and behavioral, antinociceptive, and physiologic effects of methadone after intravenous and oral transmucosal administration in cats. *American Journal of Veterinary Research*, 764-771.

Firth, A., & Haldane, S. (1999). Development of a scale to evaluate postoperative pain in dogs. *Journal Of The American Veterinary Medical Association, 214*(5), 651-659.

Gogny, M. (2006). Maneio da dor nos doentes críticos. *Waltham Focus*, 2-8.

Gutton, C., Bellefleur, J., Puppo, S., Brunet, J., Antonini, F., Leone, M., & Bretelle, F. (2013). Lidocaine versus ropivacaine for perineal infiltration post-episiotomy. *International Journal of Gynecology and Obstetrics, 122*, 33-36.

Hefti, F., Rosenthal, A., & Walicke, P. (2006). Novel class of pain drugs based on antagonism of NGF.(nerve growth factor). *Pain Digest, 16*(4), 183.

Hellyer, P., Rodan, I., Brunt, J., Downing, R., Hagedorn, J. E., & Robertson, S. A. (2007). AAHA/AAFP Pain management guidelines for dogs & cats. *Journal of the American Animal Hospital Association*, 235-248.

Holton, L., Reid, L., Scott, E., Pawson, P., & Nolan, A. (2001, April 28). Development of a behaviour-based scale to measure acute pain in dogs. *The Veterinary Record*(148), 525-531.

Holton, L., Scott, E., Nolan, A., Reid, J., Welsh, E., & Flaherty, D. (1998). Comparison of three methods used for assessment of pain in dogs. *Journal of the American Veterinary Medical Association, 212*(1), 61.

Hummel P, P. M. (2008). Clinical reliability and validity of the N-PASS: neonatal pain, agitation and sedation scale with prolonged pain. *Journal of Perinatology*, 55-60.

Jones, R. S. (2002). A history of veterinary anaesthesia. *Anales Veterinaria de Murcia, 18*, 7-15.

Klein, S., & Benveniste, H. (1999, March-April). Anxiety, vocalization, and agitation following peripheral nerve block with ropivacaine. *Regional Anesthesia and Pain Medicine, 24*(2), 175-178.

Ko, J. (2009, October 12-15). *Dexmedetomidine and its Injectable Anesthetic-Pain Management Combinations*. Retrieved March 08, 2014, from www.vin.com: http://www.vin.com/doc/?id=4108562

Kraus, B. L. (2012, February 19-23). *Getting Friendly with Dexmedetomidine*. Retrieved March 8, 2014, from www.vin.com: http://www.vin.com/doc/?id=5603794

Lemke, K. (2004, May). Understanding the pathophysiology of perioperative pain. *Canadian Veterinary Journal*, pp. 405-413.

Loon, J., Menke, E., Doornenbal, A., Back, W., & Hellebrekers, L. (2012). Antinociceptive effects of low dose lumbosacral epidural ropivacaine in healthy ponies. *The Veterinary Journal, 193*, 240-245.

Marzo, C., Crovace, A., Monte, V., Grimaldi, D., Iarussi, F., & Staffieri, F. (2012, December). Comparison of intra-operative analgesia provided by intravenous regional anesthesia or brachial plexus block for pancarpal arthrodesis in dogs. *Research in Veterinary Science, 93*(3), 1493-1497.

Mathews, K. (2000). Pain Assessment. *Veterinary Clinics of North America: Small Animal Practice*, 729-755.

Mathews, K., & Dyson, D. (2003). *Animal Pain: Figuring Out What Is Going On*. Retrieved March 23, 2014, from Veterinary Information Network: http://www.vin.com/doc/?id=3849379

McMillan, S. (2012, April 11-15). *Safe Emergency Sedation*. Retrieved March 8, 2014, from www.vin.com: http://www.vin.com/doc/?id=5328226

Melzack, R., & Wall, P. (1965). Pain mechanisms: a new theory. *Science*, 971-979.

Molony, V., & Kent, J. (1997). Assessment of acute pain in farm animals using behavioral and physiological measurements. *Journal of Animal Science*, 266-272.

Morton, C., Reid, J., Scott, E., LL, H., & Nolan, A. (2005). Application of a scaling model to establish and validate an interval level pain scale for assessment of acute pain in dogs. *American Journal of Veterinary Research, 66*(12), 2154-2166.

Nolan, A. (2000). In P. Flecknell, & A. Waterman-Pearson, *Pain Management in Animals* (pp. 32-33). London: W.B. Saunders.

Peters, J., Koot, H., Grunau, R., Boer, J., Druenen, M., Tibboel, D., & Duivenvoorden, H. (2003). Neonatal Facial Coding System for Assessing Postoperative Pain in Infants: Item Reduction is Valid and Feasible. *The Clinical Journal of Pain*(19), 353-363.

Plumb, D. C. (2011). *Plumb's Veterinary Drug Handbook.* Stockholm, Winsconsin: PharmaVet Inc.

Rica, M., Norlia, A., Rohaizak, M., & Naqiyah, I. (2007, January). Preemptive ropivacaine local anaesthetic infiltration versus postoperative ropivacaine wound infiltration in mastectomy: postoperative pain and drain outputs. *Asian Journal of Surgery, 30*(1), 34-39.

Riviere, J. E., & Papich, M. G. (2009). *Veterinary Pharmacology and Therapeutics.* Iowa, USA: Willey-Blackwell.

Savvas, I., Papazoglou, L., Anagnostou, T., Tsioli, V., & Raptopoulos, D. (2008, March/April). Incisional block with bupivacaine for analgesia after celiotomy in dogs. *Journal of the American Animal Hospital Association, 44*, 60-66.

Shaffran, N. (2012, February 19-23). *Alpha-2 Agonists: Much More than Sedation*. Retrieved March 08, 2014, from www.vin.com: http://www.vin.com/doc/?id=5603704

Takahashi, Y., Yamaguchi, S., Tezuka, M., Kimura, Y., Nagao, M., Yamazaki, H., & Hamaguchi, S. (2004). Comparison of 0.2% ropivacaine, 0.125% bupivacaine, and 0.25% bupivacaine for duration and magnitude of action in peripheral arterial blood flow induced by sympathetic block in dogs. *Regional Anesthesia and Pain Medicine, 29*, 441-445.

Taylor, P., Pascoe, P., & Mama, K. (2002). Diagnosing and treating pain in the horse. Where are we today? *The Veterinary Clinics Equine Practice*(18), 1-19.

Teixeira-Neto, F. (2009, July 21-24). *Dexmedetomidine: A New Alpha-2 Agonist for Small Animal Practice*. Retrieved March 8, 2014, from www.vin.com: http://www.vin.com/doc/?id=4252628

Traquilli, W. J., Thurmon, J. C., & Grimm, K. A. (2007). *Lumb & Jones' Veterinary Anesthesia and Analgesia.* Iowa, USA: Blackwell Publishing.

Trim, C. M. (2008, February 17-21). *Opioid Analgesia*. Retrieved March 8, 2014, from www.vin.com: http://www.vin.com/doc/?id=3862553

Vallejo, M., Phelps, A., Sah, N., Romeo, R., Falk, J., Johnson, R., . . . Edington, H. (2006, May/June). Preemptive analgesia with bupivacaine for segmental mastectomy. *Regional Anesthesia and Pain Medicine, 31*(3), 227-232.

Victory, R., Gajraj, N., Van Elstraete, A., Pace, N., Johnson, E., & White, P. (1995). Effect of preincision versus postincision infiltration with bupivacaine on postoperative pain. *Journal of Clinical Anesthesia, 7*, 192-196.

Viñuela-Fernández, I., Jones, E., Welsh, E., & Fleetwood-Walker, S. (2007). Pain mechanisms and their implication for management of pain in farm and companion animals. *The Veterinary Journal 174*, 227-239.

Wetmore, L. A. (2012, September 8-12). *Opioids: The Good Drugs - Why They Work, Why They Don't*. Retrieved March 8, 2014, from www.vin.com: http://www.vin.com/doc/?id=5478499