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EVALUATION AND EFFICACY OF GABAPENTIN FOR INTRAOPERATIVE AND POSTOPERATIVE PAIN CONTROL IN DOGS UNDERGOING LAPAROSCOPIC OVARIECTOMY

Valutazione ed efficacia del gabapentin nel controllo del dolore
intraoperatorio e postoperatorio nelle ovariectomie in laparoscopia del cane

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Abstract

Laparoscopic ovariectomy is an elective surgical procedure commonly performed in veterinary clinical practice, requiring anaesthetic protocols that ensure safety and hemodynamic stability, while providing an adequate depth of anaesthesia and effective analgesia.

This pilot study evaluated the intraoperative and postoperative analgesic effects of preoperative gabapentin administration in dogs undergoing laparoscopic ovariectomy, with particular focus on opioid rescue analgesia requirement during the intraoperative and postoperative periods and a potential isoflurane-sparing effect.

Fourteen client-owned female dogs were enrolled and randomly allocated to gabapentin or control treatment groups. All dogs received the same anaesthetic protocol, including methadone and dexmedetomidine premedication, propofol induction, and isoflurane maintenance. Intraoperative nociceptive responses were managed with intravenous fentanyl boluses according to predefined criteria. The intraoperative analysis assessed hemodynamic and respiratory parameters, peripheral oxygen saturation, end-tidal carbon dioxide and end-tidal isoflurane concentrations. Postoperative pain was also assessed hourly using the Glasgow Composite Measure Pain Scale-Short Form and physiological parameters assessment. The requirement for rescue analgesia, physiological parameters, and end-tidal isoflurane concentrations was compared between groups.

Preoperative gabapentin appeared to be a safe component of a multimodal anaesthetic and analgesic protocol and was associated with improved postoperative pain scores and a lower requirement for rescue analgesia. Although a numerical reduction in intraoperative fentanyl administration was observed, no statistically significant opioid-sparing effect was demonstrated. Moreover, no statistically significant isoflurane-sparing effect was demonstrated. Future studies with larger sample sizes will be necessary to consolidate these findings.

Riassunto

L'ovariectomia laparoscopica è una procedura chirurgica elettiva comunemente eseguita nella pratica clinica veterinaria, che richiede l'impiego di protocolli anestesiológicos in grado di garantire la sicurezza e la stabilità emodinamica, fornendo al contempo un'adeguata profondità anestetica e un'efficace analgesia.

Il presente studio ha valutato gli effetti analgesici intraoperatori e postoperatori della somministrazione preoperatoria di gabapentin in cani sottoposti a ovariectomia laparoscopica, con particolare attenzione alla eventuale aggiunta di oppioidi durante la procedura chirurgica e nel postoperatorio e a una potenziale diminuzione del consumo di isoflurano.

Sono stati inclusi 14 cani femmina di proprietà, assegnate casualmente al gruppo trattato con gabapentin o al gruppo di controllo. Tutti i soggetti sono stati sottoposti a premedicazione con metadone e dexmedetomidina, induzione con propofol e mantenimento con isoflurano. Le risposte nocicettive a stimoli dolorifici intraoperatori sono state gestite mediante la somministrazione di boli endovenosi di fentanyl, somministrati secondo i criteri prestabiliti. L'analisi statistica dei dati ha preso in considerazione i parametri emodinamici e respiratori, la saturazione periferica di ossigeno, la concentrazione di anidride carbonica di fine espirazione e la concentrazione di isoflurano di fine espirazione. Il dolore postoperatorio è stato inoltre valutato con cadenza oraria regolare mediante la Scala Glasgow e attraverso la valutazione di parametri fisiologici. Sono stati confrontati tra i due gruppi il ricorso ad una analgesia aggiuntiva mediante oppioidi, i parametri fisiologici e le concentrazioni di isoflurano di fine espirazione.

La somministrazione preoperatoria di gabapentin si è dimostrata una componente sicura all'interno di un protocollo anestesiológico e analgesico multimodale ed è risultata associata ad una migliore valutazione del dolore postoperatorio e ad una minore necessità di analgesia aggiuntiva. Sebbene sia stata osservata una riduzione numerica della quantità di fentanyl somministrata durante l'intervento, non è stato evidenziato un effetto analgesico statisticamente significativo sulla somministrazione intraoperatoria di oppioidi. Analogamente, non è stato dimostrato alcun effetto statisticamente significativo sull'utilizzo di una minore quantità di isoflurano durante la procedura chirurgica. Saranno necessari studi futuri con campioni numericamente più ampi per consolidare queste evidenze.

Introduction

Gabapentin is a member of the gabapentinoids class of drugs, and it is a synthetic analogue of γ -aminobutyric acid (GABA), sharing structural similarities with this neurotransmitter (Bockbrader et al., 2010). Gabapentin was originally approved for human use as an adjunctive therapy in the treatment of partial seizures (Russo et al., 2023). Subsequently, it has been used for pain indications (Goodman & Brett, 2019), including for the management of postoperative pain, with the aim of reducing opioid consumption and pain intensity (Verret et al., 2020). Similar to its use in human medicine, gabapentin was initially introduced extra-label into veterinary medicine as an antiepileptic drug; however, to date is more commonly used for neuropathic and chronic pain management (Di Cesare et al., 2023). Despite its widespread use in veterinary clinical practice, the evidence supporting its analgesic efficacy remains limited. This is particularly evident in the postoperative setting, where studies have reported variable results. Nevertheless, when used as part of a multimodal analgesic protocol, gabapentin appears to reduce the need for opioids and rescue analgesia after surgery (Di Cesare et al., 2023).

Multimodal analgesia consists of the concomitant use of different analgesic drugs or analgesic techniques, with the aim of improving pain control while reducing the dose and the potential adverse effects associated with individual drugs. The expected outcome of multimodal analgesia is therefore to improve pain control, enhanced functional recovery and reduced opioid-related adverse effects. Effective pain management is a fundamental aspect of veterinary clinical practice, as achieving adequate postoperative analgesia is essential for ensuring patient welfare (O'Neill & Lirk, 2022).

Therefore, the aim of the present study was to evaluate the effects of preoperative gabapentin administration in dogs undergoing laparoscopic ovariectomy, with particular focus on its intraoperative and postoperative analgesic effects. Within a multimodal analgesic approach, the study investigated whether the addition of gabapentin to the pre-anaesthetic protocol could reduce the requirement for additional analgesic drugs and inhalational anaesthetic agents.

General Topics

Chapter 1: Physiology of Pain

1.1 Definition and Classification of Pain

In 1978 the International Association for the Study of Pain (IASP) recommended definitions of “Pain Terms” to the IASP Council. In this occasion Pain was defined as *“An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage”*. The IASP definition of pain has become accepted globally by health care professionals and researchers in the pain field all around the world (Raja et al., 2020).

This definition is expanded upon with the addition of six key notes:

- *“Pain is always a subjective experience that is influenced to varying degrees by biological, psychological, and social factors”.*
- *“Pain and nociception are different phenomena: the experience of pain cannot be reduced to activity in sensory pathways”.*
- *“Through their life experiences, individuals learn the concept of pain and its applications”.*
- *“A person’s report of an experience as pain should be accepted as such and respected”.*
- *“Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being”.*
- *“Verbal description is only one of several behaviours to express pain; inability to communicate does not negate the possibility that a human or a non-human animal experiences pain”* (Raja et al., 2020)

In animals the nature of pain is equally complex as in humans, although the experience and expression of it are not likely to be identical. The physiology of pain is similar across mammalian species, and the capacity of animals to suffer is well established in law in many countries (Lamont, 2024).

Pain represents a vitality physiological sensation, so much that the lack of this function, in subjects with congenital absence of pain sensibility, reduces life expectations. Functioning by organism’s alarm signal, pain acts as a protective system against potentially harmful external events, to prevent or minimize tissue damages. In this case pain is defined as *physiological* due to the activation of the nociceptive system by a noxious stimulus (Bufalari et al., 2012).

About the classification of pain, it can be defined based on three macro categories: transitory, nociceptive and neuropathic.

- Transitory Pain

Transitory pain is the most common pain sensation, and it develops when the potentially noxious stimulus does not cause a tissue damage due to his short-lived. Pain evokes a response in the subject and functions as an alarm signal that triggers the withdrawal reflex. In this context, pain can be considered protective and physiological and resolves rapidly following stimulus cessation (Gamba, 2020).

- Nociceptive Pain

“Pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors” (<https://www.iasp-pain.org/Resources/Terminology/>, n.d.).

Nociceptive pain can be classified into acute and chronic forms and arises from damage to superficial tissue, such as skin and mucosa, or to deeper somatic and visceral structures. Regardless of the underlying cause, nociceptive pain has inflammatory origin (Gamba, 2020). Following tissue injury, inflammatory mediators are released, activating nociceptors and generating the nociceptive input. At the same time, peripheral sensitization develops, leading to an increased response to stimuli. As tissue healing progresses and inflammatory resolves, nociceptive activity and pain perception gradually decrease (Gamba, 2020).

In the presence of a tissue lesion, pain is an integral component of the inflammatory process and contributes to the protective response of the organism. Changes in the nervous system during inflammation increase the sensitivity of the nociceptive system, leading to phenomena such as hyperalgesia, defined such as an *“increased pain from a stimulus that normally provokes pain”* (<https://www.iasp-pain.org/Resources/Terminology/>, n.d.) and allodynia, defined as *“pain due to a stimulus that does not normally provoke pain”* (<https://www.iasp-pain.org/Resources/Terminology/>, n.d.). These phenomena reflect a temporary alteration in pain processing mechanism and tend to resolve as the tissue heals (Bufalari et al., 2012).

Acute pain is generally defined as short-lasting and cause-correlated pain. It is typically well localized and disappears when the nociceptive stimulus or the inflammatory process ceases. Example of acute pain include postoperative pain, trauma-related pain or pain associated with acute inflammatory processes (Bufalari et al., 2012).

On the other hand, chronic pain does not present a well-defined causal origin and due to this is characterized by its persistence beyond the resolution of the initial noxious stimulus. It can therefore be defined as a *disnociceptive* or *no-nociceptive* pain. The chronification of pain is caused by damage or dysfunction of the nervous system and/or the development of alterations (Bufalari et al., 2012). Nociceptive pain can also be classified according to the site of injury; it can therefore be defined as superficial, deep somatic and deep visceral pain:

- superficial nociceptive pain concerns noxious stimuli acting on skin, teeth, eyes and mucosal tissue;
 - deep somatic nociceptive pain can be further subdivided into Musculoskeletal pain and Nerve trunk pain. The first one involves the activation of nociceptors located in muscles, bones, joints, blood vessels, parietal serosa and dura mater. The second one is a neurogenic non-neuropathic pain resulting from inflammation of the *nervi nervorum*, which constitute the intrinsic innervation of the nerve;
 - deep visceral pain concerns all the noxious stimuli acting on internal organs, such as the irritant action of chemical substances, distension of the wall and contraction of the muscular layer in hollow viscera, rapid distension of the capsule in parenchymal organs, transient ischemia or necrosis of the muscular layer, traction on visceral ligaments, and vascular disorders of the visceral vessels (Bufalari et al., 2012).
- Neuropathic Pain

Neuropathic pain has been defined in 1994 by the International Association for the Study of Pain (IASP) as “*pain initiated or caused by a primary lesion or dysfunction in the nervous system*”. In 2008 the IASP Special Interest Group on Neuropathic Pain (NeuPSIG) proposed a new definition that omitted the term “dysfunction”, defining neuropathic pain as “*pain arising as a direct consequence of a lesion or disease affecting the somatosensory*

system”, which has been further rewritten as *“pain caused by a lesion or disease of the somatosensory nervous system”* (Finnerup et al., 2016).

In contrast to nociceptive pain, neuropathic pain originates from altered nociceptors activation, which may occur even in the absence of a stimulus. For this reason, neuropathic pain can also be defined as *disnociceptive*; indeed, it is not attributable to another disease, as it is itself a pathological condition. It has no biological protective function, so it is therefore considered *maladaptive* (Gamba, 2020). In clinical medicine, the term *“neuropathic”* refers to functional changes and damages to a nerve as potential causes of pain. Primary sensory neurons (first-order neurons) encode specific sensory modalities: they respond to specific stimuli by generating electrical impulses and communicate with secondary sensory neurons through synapses in the spinal cord via the release of neurotransmitters. The maintenance of this mechanism requires complex biological processes, indeed, in the presence of nerve damage, the electrical properties, neurochemical profile and central connectivity of these neurons may be altered, with consequences for normal sensory perception and, in some cases, leading to neuropathic pain (Gamba, 2020). Based on pathogenesis and lesion site, neuropathic pain can be classified into five types:

- pain resulting from persistent nociceptor hyperexcitability. Despite cessation of the noxious stimulus, nociceptors do not return to their resting state;
- demyelination-related pain. Damage to nerve fibres involves only the myelin sheath, causing its hyperexcitability and the transmission of pathological nociceptive signals to the central nervous system;
- axonal neuropathy. The whole nerve is affected, involving both afferent and efferent fibres. The distal nerve stump degenerates, whereas the proximal stump attempts to reorganized itself;
- deafferentation pain. The damage is only pregangliar. In this case, the distal nerve stump undergoes degeneration, but no attempt by the proximal stump to reorganized itself;
- central neuropathic pain. The damage affects the tracts from the dorsal horn to higher centers up to the cortex (Gamba, 2020).

In addition to these three macro categories, pain can also be described as psychogenic, referred and reflex pain. Psychogenic pain includes conditions such as muscle tension pain, hallucinatory pain, pain of unknown or uncertain origin and phantom limb pain (Bufalari et al., 2012). Referred pain results from the convergence of nociceptive inputs from deep somatic or visceral structures onto the same central neurons that also receive the afferents from other deep or superficial structures; therefore, the central nervous system cannot precisely identify the origin of the stimulus (Bufalari et al., 2012). Reflex pain occurs as a consequence of nociceptor activation triggered by efferent activity induced by a primary nociceptive input, such as pain associated with muscle spasm or visceral reflex pain (Bufalari et al., 2012).

1.2 Nociception: transduction, transmission, modulation and perception

Nociception is defined as: *“The neural process of encoding noxious stimuli”* (<https://www.lasp-pain.org/resources/terminology/>, n.d.).

The nociceptive system can be activated by a wide range of stimuli, including mechanical, electromagnetic, electrical, thermal and chemical modalities. All these stimuli represent potential threats to the integrity of the organism and therefore possible causes of tissue damage (Gamba, 2020). Although nociceptive stimulation usually results in the sensation of pain, some pharmacological research and brain lesion studies have shown that it can occur without resulting in pain experience. Several human neuroimaging studies support this finding, demonstrating that nociception and pain are not always associated in terms of brain activity (Mischkowski et al., 2019). Nociceptive information is mediated via the dorsal root ganglion and propagated to brain via spinothalamic tracts or via the trigeminal ganglion. Each tract ascends to respective brain areas, such as the reticular formation, periaqueductal gray, limbic system, hypothalamus, basal ganglia, and the insular cingulate and somatosensory cortices. In addition, brain can mediate descending control over pain; in particular, activation of midbrain and medullary areas can reduce nociception. The periaqueductal gray receives inputs from other brain areas and can produce an analgesic effect; the rostroventromedial medulla can inhibit nociception and contributes to the regulation of descending pain processes (Sneddon, 2018).

Nociceptive pain is the result of four distinct physiological events. The first step is the transduction of a nociceptive stimulus into an electrical signal by nociceptors, which are located in the functional free nerve terminals of afferent fibres (Gamba, 2020). Afferent fibers consist of A δ myelinated nerve fibres and C unmyelinated nerve fibres, which are characterized by a high

activation threshold (Bufalari et al., 2012). The second step is the transmission of this signal from nociceptors to the spinal cord and subsequently to the brain stem, ascending reticular formation, thalamus and finally the somatosensory cortex. Thereafter the modulation of the signal within the spinal cord, thalamus and other medulla oblongata and brain regions. At the end there is the integration of all these processes at the cortical level (Bufalari et al., 2012).

Transduction represents the first step in the nociceptive process and consists of the conversion of physical energy into electrical signal at the level of peripheral nociceptors. This energy must exceed the nociceptors' activation threshold in order to initiate an action potential (Gamba, 2020). The resting potential of nociceptive afferents is approximately -40 mV; the action potential threshold ranges from -35 mV to -55 mV. For sensory information to be propagated to the central nervous system, an action potential has to be generated through membrane depolarisation induced by the transduction process. This event termed "generator potential" can be initiated in three ways: the opening of an ion channel, the closing of K⁺ channels or the activation of low-threshold voltage-gated ion channels that are in close association with ion channels capable of driving membrane depolarization (Lamont, 2024).

These processes occurred at the level of nociceptors, also called transducers, can be described by the type and origin of the stimulus, their tissue type association and if they are extrinsic or intrinsic to the primary afferent fibres (Lamont, 2024). A noxious stimulus can activate these nociceptors either directly or indirectly by causing tissue damage and initiating an inflammatory process, leading to the release of various inflammatory mediators. In the presence of tissue damage and inflammatory mediators, nociceptors can lower their activation threshold resulting in an enhanced response to stimuli. This phenomenon is called peripheral sensitization, whereby low-intensity signals evoke allodynia and primary hyperalgesia (Bufalari et al., 2012).

The process then continues with transmission, also referred to as conduction, and represents the second stage of the nociceptive process, involving the propagation of the nerve impulse from the peripheral nervous system via afferent A δ myelinated fibres and unmyelinated polymodal C nerve fibres to the spinal cord (Gamba, 2020). The transmission of depolarization waves, which carry the nociceptive signal to the spinal cord dorsal horns, is mediated by the voltage-gated sodium channels (Bufalari et al., 2012).

At the spinal level, signal transmission is modulated in the dorsal horns of the spinal cord before reaching higher centers. This modulation consists of either amplification or attenuation of the signal prior to its cortical processing, but it can also be modulated after leaving higher centres, upon returning to the periphery through descending pain pathways (Gamba, 2020).

Finally, perception constitutes the final stage of the nociceptive process. At this level, pain becomes consciously perceived in terms of intensity and localization. This occurs mainly in the somatosensory cortex after ascending pathways relay the modulated signal to the thalamus and subsequently to the cortical areas (Gamba, 2020).

1.3 Nociceptive receptors and nerve fibers (A δ e C)

Nociceptive receptors are defined by IASP as *“a high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli”* (<https://www.lasp-pain.org/resources/terminology/>, n.d.).

They basically consist of specialized neurons that detect and respond to potentially damaging forms of energy. Nociceptors mediate this function through the expression of molecules, which are responsible for detecting and signalling the presence of potential harm (W. D. Tracey, 2017). A plethora of studies, concerning mammalian animals, have identified a variety of somatosensory nociceptors, which have been classified according to conduction velocity, diameter, amount of myelination, and the types of stimuli to which they respond. In this context, nociceptors are generally categorized as polymodal, when activated by multiple classes of noxious stimuli, or as specialized, when they respond to a single modality (W. D. Tracey, 2017). Tissue damage may be caused by a variety of noxious stimuli, which can be provoked by high mechanical pressure, extremes of temperature (<10°C and >40°C in mammals), and chemicals. All these stimuli present specific receptors (Sneddon, 2018). Despite this, the understanding of somatosensory mechanisms, involved in the detection of pressure, touch and temperature, remains limited, largely due to the incomplete identification of the receptors mediating these physical stimuli (Caterina & Julius, 2026).

Acid-sensing ion channels (ASICs), gated by H⁺ and modulated by neuropeptides are a class of nociceptors present in the mammalian nervous system, both peripherally in sensory ganglia and in the brain. They are classified in four different subunit ASIC1, ASIC2, ASIC3 and ASIC4, although their functional roles are partially overlapping. Their involvement in mechanoreception and pain

has been supported by the observation that physiological or pathological disturbances are often associated with an acidic shift in acid-base homeostasis (Krishtal, 2003). Another important class of nociceptors is represented by the transient receptor potential channels (TRP). These ligand-gated ion channels mediate the detection of mechanical and chemical stimuli through nociceptor activation and regulate multiple sensory modalities, including temperature, mechanical changes and nociceptive inputs (Sneddon, 2018). The TRP channels family presents other mammalian subfamily, which share a thermotransduction role with two-pore potassium channels (K2P): transient receptor potential cation vanilloid (TRPV), ankyrin (TRPA) and melastatin (TRPM) (Leigh Lamont, 2024). Among them, TRPA1 is also involved in chemical nociception, and its activation occurs through nucleophilic attack by the damaging molecules, and covalent modification of specific intracellular cysteine residues mechanism. In mammals, additional ion channels implicated in the modulation of noxious heat detection include the chloride-permeable channel TMEM16A/anoctamin, as well as the previously mentioned two-pore potassium channels TREK1, TREK2 and TRAAK (W. Daniel Tracey, 2017). Opioid receptors and endogenous opioids, comprising three G-protein-coupled receptors subtypes (μ -, δ -, and κ -), are also closely linked to nociception and pain (Sneddon, 2018).

Following the previously described molecular basis of nociception, nociceptive information is conveyed through distinct anatomical pathways involving both peripheral and central components.

Nociceptive signals are generated at the level of free nerve endings, which consist of plexiform arborizations in cutaneous, muscular and joint tissues. These signals are transmitted by fibers organized within peripheral nerves toward the spinal cord. Peripheral receptors transduce nociceptive events for processing by first-order sensory neurons in the dorsal root ganglion, whose central projections terminate in the dorsal horn of the spinal cord (Gamba, 2020). According to this, the cell bodies of nociceptor neurons are located in the peripheral nervous system, in particular in the dorsal horn and trigeminal ganglia (W. D. Tracey, 2017). Dorsal horn is divided into parallel laminae by the density of the neurons. Lamina I neurons contribute to the spinothalamic and spinoreticular tracts, both of which are involved in the processing of pain and temperature information. Lamina II, named substantia gelatinosa, is the primary site for nociceptive inputs, from C and A δ fibers, arriving in the spinal cord. This lamina shows a well-defined modular organization of neurons that differentially modulate and transmit afferent

inputs from C and A δ fibers (Lamont, 2024). C and A δ fibers are both characterized by a high activation threshold and run in parallel within peripheral spinal nerves (Gamba, 2020). C fibers are unmyelinated, have a smaller diameter, and conduct signals more slowly than myelinated A δ fibers. Anatomical studies indicate that the majority of cutaneous fiber types in mammalian are predominantly nociceptive, about ~68%, including A δ fibers (12%), C polymodal fibers (30%), C mechanothermal or heat-sensitive fibers (20%), and silent C fibers (5%). The remaining ~32% are involved in the detection of touch and pressure, comprising A β fibers (22%), non-nociceptive A δ fibers (6%), and low-threshold touch C fibers (~5%). Among C fibres, approximately 30% are polymodal, responding to noxious chemical, mechanical, and thermal stimuli, in contrast to the predominantly mechanothermal properties of A δ fibers. A δ mechanoreceptors show slow adaptation and maintain their response for the duration of the stimulus and are believed to mediate “first pain” due to their relatively fast conduction velocity. In contrast, C fibers are associated with the prolonged sensation of pain, consistent with their slower conduction velocity. In humans, A δ fibre activation is typically perceived as “pricking” pain, C fibre-mediated sensations are described as “dull” or “pressing” pain (Sneddon, 2018).

Based on their phenotype and sensitivity to neurotrophins, C fibers can be divided into two groups, even if the functionally differentiation of them is not completely clear. One group is sensitive to nerve growth factor (NGF) and is characterized by the expression of substance P, calcitonin gene-related peptide (CGRP), and galanin (peptidergic fibers), whereas the second group is defined by binding sites for lectin isolectin B4 and by the expression of the purinergic receptors P2X3 and P2Y1 (non-peptidergic fibers). The central projections of these two groups are differently located: peptidergic C fibers, which innervate other tissues in addition to the deeper layers of the skin, extend into lamina I and the outer part of lamina II; non-peptidergic fibers extend to the central portion of lamina II and innervate the superficial layers of the skin (Lamont, 2024).

Overall, these properties of nociceptors constitute the basis for the transmission and processing of nociceptive information within the nervous system, playing a fundamental role in the perception of pain.

1.4 Ascending pain pathways

Ascending pain pathways consist of specialised neural tracts responsible for the transmission of noxious stimuli from the periphery to the spinal cord and finally to the cortex, where the higher centres process nociceptive information.

It was already clarified how, due to the nociceptive transmission process, the stimulus is propagated from the periphery, by the first-order neurons, to the spinal cord, from which second-order neurons project (Gamba, 2020). In particular, the signal reaches the dorsal horn of the spinal cord, which represents the central pathways for the beginning of processing nociceptive information and the interneural networks within it are responsible not only for the transmission of that information, but also for its modulation (Willis & Westlund, 1997). Concerning signal modulation, it may be either amplified or attenuated. Accordingly, two types of ascending pathways can be distinguished: excitatory pathways, which release excitatory neuropeptides to amplify the signal, and inhibitory pathways, which conversely release inhibitory neuropeptides to attenuate it (Gamba, 2020).

In addition to being classified as excitatory or inhibitory, spinal neurons can also be categorized as interneurons or projection neurons. Interneurons are essential for dorsal horns microcircuitry, whereas projection neurons determine whether a stimulus is perceived as painful (Motzkin et al., 2024). In terms of anatomical localization, nociceptive projection neurons in the spinal cord transmit information to various regions of the brainstem and diencephalon, such as thalamus, periaqueductal gray, parabrachial region, and bulbar reticular formation. They also terminate into limbic structures such as the hypothalamus, amygdaloid nucleus and septal nucleus (Willis & Westlund, 1997). Based on these regions, the ascending tracts are generally named according to where they terminate in the brain (Lamont, 2024). Starting from the anterolateral quadrant: the spinothalamic tract terminates in the thalamus (Lamont, 2024) and mediates the sensations of pain, cold, warmth and touch (Willis & Westlund, 1997); in particular this tract plays a key role in the localisation of these sensations (Lamont, 2024). The spinomesencephalic tract includes cells distributed in the spinal cord similar to spinothalamic tract cells and their projections terminate in different areas in the midbrain. Spinomesencephalic neurons are nociceptive, responding either to painful stimuli and innocuous ones and have complex receptive fields on separated areas of the body (Willis & Westlund, 1997). The spinoreticular tracts present cell located in the deep layers of the dorsal horn and anatomic studies have shown that some spinoreticular neurons are collateral branches of spinothalamic ones, considering that both tracts

ascend together in the ventrolateral spinal cord and brainstem (Willis & Westlund, 1997). The spinobulbar tract, which terminates in hindbrain and midbrain regions, is involved in pain processing by mediating the affective component and intensity of the stimulus. Projections from this pathway reach the amygdala and hypothalamus via the parabrachial nucleus and it can modulate descending pathways regulating spinal cord output.

In conclusion, all these ascending pathways consist of specialised neurons that convey nociceptive information to distinct brain regions, where the stimulus undergoes processing and from which descending pathways subsequently arise, ultimately resulting in the release of neurotransmitters.

1.5 Descending pain pathways and pain modulation

The descending pain modulatory system is an anatomical network involved in the regulation of nociceptive processing, which can either facilitate (pronociception) or inhibit (antinociception) this process. Over the last several decades, many studies have shown that a variety of brain regions contribute to descending pain modulation and that include the frontal lobe, anterior cingulate cortex, insula, amygdala, hypothalamus, periaqueductal gray (PAG), nucleus cuneiformis, and rostral ventromedial medulla (RVM) (I. Tracey & Mantyh, 2007). Although descending pathways are mainly implicated in the transmission of the pain stimulus from the brain to the periphery, it has also been demonstrated that there is a close association with the ascending pathways; in fact, a large population of projection neurons is present in lamina I of the dorsal horn, which project to the PAG, thalamus and also to the RVM that can consequently influence descending controls (Lamont, 2024). The periaqueductal gray, localized in the midbrain, represents one of the most important structures associated with the descending pain control system, receiving information from the thalamus, hypothalamus, amygdala, somatosensory and cingulate cortices and also from the ascending pathways (Lamont, 2024). It is an essential locus of top-down control with a direct influence on dorsal horn nociceptive processing, besides playing a role in coordinating survival responses to threat (Motzkin et al., 2024). Together with PAG, the RVM is capable of modulating nociceptive information, particularly through inhibitory mechanisms, thereby influencing pain sensation to the higher centres. This mechanism is mediated by inputs from cortical and subcortical areas, which may explain the brain's ability to modulate pain perception in order to give precedence to competing stimuli or maintain homeostasis and survival (Sneddon, 2018). The above-mentioned modulation

is mediated by excitatory and inhibitory interneurons within the dorsal horn, together with descending facilitatory and inhibitory pathways arising from the brain (Dubin & Patapoutian, 2010). Excitatory ascending pathways rely on the release of excitatory neurotransmitters, such as aspartate, neurokinin A, glutamate, which activates the AMPA and NMDA post-synaptic receptors and substance P, which activates NK receptors (Bufalari et al., 2012). All of these neurotransmitters increase postsynaptic sodium permeability and facilitate nociceptive transmission (Gamba, 2020). Excitatory descending pathways, whose function is typically to increase attentional processing of external stimuli in the animal, originate in the brainstem and enhance the propagation of spinal projections, thereby facilitating signal ascent (Gamba, 2020). In contrast, signal attenuation is mediated by descending inhibitory pathways that reduce the release of pronociceptive mediators through endogenous opioids, adrenaline and serotonin as well as inhibitory local circuits, operating according to the gate control theory (Gamba, 2020).

The gate control theory substantially explains how the sensation of pain can be reduced by closing a “gate” at the ascending neurons synaptic level in the spinal cord (Heitler, 2023). It also postulates how afferent nociceptive stimuli are modulated by the activation of non-nociceptive afferent fibres (Bufalari et al., 2012), considering that nociceptive sensory A δ and C fibres converge together with tactile and pressure A β afferent fibres in lamina II of the spinal cord (Gamba, 2020). According to this theory, the closure of the “gate” can also be elicited by stimulating tactile afferents with receptive fields corresponding to the anatomical area where the nociceptive input originates (Heitler, 2023). Before synapsing with spinal neurons, both nociceptive and tactile fibres give off collateral branches that interact with interneurons producing the endogenous opioid enkephalin (Gamba, 2020). Tactile A β fibres promote the release of enkephalin; therefore, when a non-nociceptive stimulus activates these fibres, inhibitory interneurons are recruited, suppressing the transmission of nociceptive signals to the brain and resulting in the closure of the “gate” (Gamba, 2020). By contrast, sensory C fibres inhibit the enkephalinergic interneuron and so the enkephalin production, allowing the painful stimulus to reach the brain and resulting in the opening of the “gate” (Gamba, 2020). Accordingly, when painful and tactile inputs occur simultaneously, pain perception is attenuated as a result of the excitatory effect of the A β fibres on enkephalinergic interneurons (Gamba, 2020).

1.6 Neurotransmitters and mediators involved

Neurotransmitters represent the chemical substances that carry information between neurons. Following release into the synaptic cleft, neurotransmitters activate postsynaptic receptors through diffusion across the synapse (Hyman, 2005). Synapses can be classified as electrical, which allow the direct flow of ions between cells through gap junctions, or chemical, which are the most common type and enable the transmission of excitatory, inhibitory and complex biochemical signals between cells (Hyman, 2005). The chemical synapse is mainly composed of a presynaptic nerve terminal and, on the opposite side a postsynaptic structure that contains neurotransmitter receptors and an intracellular signaling system. Between these two structures lies the synaptic cleft, the space through which signal transmission occurs. In the presynaptic terminal, more precisely into the active zones, there is a high density of voltage-gated Ca^{2+} channels and in these zones synaptic vesicles are clustered, which contain several thousand molecules of neurotransmitters (Hyman, 2005). The vesicles are released by presynaptic neurons and bind to receptors, localized on dendrites of the postsynaptic membranes; consequently, chemical information is converted into electrical information by the activation of ion channels (Hyman, 2005). Depending on which of these channel types are activated, either hyperpolarizing negative or depolarizing positive charges can enter the cells. These changes in charge trigger an action potential, which in turn activates voltage-gated Ca^{2+} channels, ultimately resulting in the release of presynaptic neurotransmitters into the synaptic cleft (Hyman, 2005). Neurotransmitters can be categorized as inhibitory or excitatory based on their postsynaptic effects. Inhibitory neurotransmitters such as GABA, noradrenaline, serotonin, dopamine, opioids, cannabinoids and adenosine (Gamba, 2020) activate anion channels (Cl^-) (Hyman, 2005); meanwhile, excitatory neurotransmitters such as acetylcholine, glutamate, P substance, neuronal growth factor (NGF), aspartate, neurokinin A and cholecystokinin (Gamba, 2020) depolarize cation channels (Na^+ or Ca^{2+}) (Hyman, 2005).

Inhibitory neurotransmitters noradrenaline (norepinephrine) and serotonin (5-hydroxytryptamine) represent the major transmitters in the descending control pathways and modulate the outputs of the dorsal horn neurons. Noradrenaline modulates nociception: in the LC-spinal descending pathway, the central noradrenergic system inhibits pain, but it is also demonstrated that an altered noradrenergic activity, like during acute inflammation and nerve injury, can lead to hypersensitivity. Noradrenaline is also involved in the regulation of vigilance,

attention and cognitive functions (Lamont, 2024). On the other hand, the main excitatory neurotransmitters include glutamate and substance P. In the dorsal horn of spinal cord, glutamate binds to several receptors on postsynaptic neurons, including AMP, NMDA and KA and several G-protein coupled glutamate receptor subtypes. These receptors are crucial for enhancing the excitability of nociceptive neurons. While they are not involved in basal nociception, they play a fundamental role in the development of central sensitization (Latremoliere & Woolf, 2009). Central sensitization is also promoted by substance P, binding to the neurokinin-1 (NK1) G-protein-coupled receptor, which is expressed by spinothalamic and PAG neurons, and its activation induces a prolonged membrane depolarization (Latremoliere & Woolf, 2009).

1.7 Peripheral and central sensitisation

The term sensitisation refers to the nervous process, defined by the IASP as an *“increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally subthreshold inputs”* (<https://www.iasp-pain.org/Resources/Terminology/>, n.d.).

Sensitisation can be classified as either central or peripheral.

Central sensitisation is essentially a phenomenon of synaptic plasticity involving central pain pathways, including pathways that do not normally transmit pain signals, following noxious insults (Ji et al., 2018). This term was first used and the phenomenon studied in animal models by CJ Woolf in 1983 to refer to a specific spinal disorder mechanism that was responsible for amplifying ongoing peripheral nociceptive inputs (Harte et al., 2018). These studies showed that neurons become hyperexcitable over time and the chemical, structural and functional changes in the central nervous system can be maintained with or without continued peripheral inputs (Volcheck et al., 2023). The pathophysiologic changes associated with central sensitisation include structural and functional changes in the thalamus, hypothalamus and amygdala, hyperexcitability of the cell membrane of central neurons, decreased action potential threshold, increased synaptic strength, decreased descending inhibitory transmission and enlarged receptive fields. All these changes contribute to the development of phenomena such as hyperalgesia, allodynia and global sensory hyperresponsiveness (Volcheck et al., 2023). The molecular mechanism inducing and maintaining this phenomenon was dependent on NMDA receptors and so correlated to the neurotransmitter glutamate. The mechanism can be divided in two temporal phases: the early phosphorylation-dependent and transcription-independent

phase is primarily mediated by rapid alterations in glutamate receptor and ion channel properties; in contrast, the later and more persistent transcription-dependent phase promotes the synthesis of new proteins that underlie the prolonged form of central sensitisation observed across several pathological conditions (Latremoliere & Woolf, 2009). NMDA receptors are both a trigger and effector of central sensitisation; in fact, under normal conditions, these channels are blocked in a voltage-dependent manner by Mg^{2+} ion and the removal of this block allows entry of Ca^{2+} ion in the neuron, which activate numerous synaptic intracellular pathways and beyond a certain threshold level produces the functional changes that manifest as central sensitisation (Latremoliere & Woolf, 2009). Central sensitisation also contributes to neuropathic and inflammatory pain. It occurs in response to inflammation as a protective mechanism, whereby enhanced nociceptor sensitivity promotes protection of the injured body region, thereby preventing further tissue damage (Smith & Lewin, 2009). The mechanism through which inflammatory states and central sensitisation are correlated can be explained considering the large number of endogenous factors released during inflammation in the vicinity of the free endings of the nociceptors, which moreover express specific patterns of receptors for inflammatory mediators (Lamont, 2024). It is also important to report that peripheral inflammation induces a phenotypic change in primary sensory neurons, involving alterations in their neurochemical characteristics and functional properties as a result of changes in transcription and translation processes (Latremoliere & Woolf, 2009); in fact, through signal transduction, the neurotransmitters, released in increased amounts as a consequence of the peripheral inflammation itself, produce a state of neuronal hyperactivity and hyperexcitability in the spinal cord and consequently in the brain (Ji et al., 2018).

Likewise, peripheral sensitisation represents an *“increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields”* (<https://www.lasp-pain.org/resources/terminology/>, n.d.); therefore, sensory neurons modify their firing properties following inflammation or injury, becoming more readily triggered by their usual ligands and activators, such as hot or cold temperature, low pH, noxious mechanical activation, damaging chemicals and endogenously produced proinflammatory molecules, like growth factors and cytokines (Li et al., 2025).

In conclusion, sensitisation consists in a neuroplastic process driven by persistent nociceptive stimuli, which may lead to the development of chronic pain even after the initial stimuli have

ceased. In this case, neuroplasticity has primed the nerves to become more sensitive to stimulation, and pain signalling is not merely a protective response (Volcheck et al., 2023).

Chapter 2: Acute Pain

Pain was defined by Williams and Craig in 2016 as *“an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”*; according to this definition, pain should be regarded as a genuine experience that necessarily requires brain activity to be perceived (Bell, 2018). It is also considered as a subjective and individual experience, and its classification is necessary prior to discussion. Many studies refer to pain as a spectrum ranging from adaptative pain, which serves a protective function, to maladaptive pain, which is no longer beneficial to the organism. Considering this, a correct management of acute pain may prevent permanent anatomic changes in the nervous system that promote the progression toward chronic maladaptive pain (Downing & Della Rocca, 2023). Nevertheless, the distinction between acute and chronic pain is not precisely established but based on an arbitrary temporal criterion, chronic pain is commonly defined as pain that persists for more than three months (Sander et al., 2025).

2.1 Alterations in physiological parameters and behaviour

Acute pain refers to a specific stimulus, and it may be described as a localized and transient sensation, that ceases upon the removal of the stimulus (Gamba, 2020). Untreated pain is associated with clinical, functional and behavioural consequences, such as reduced or absent ambulation, leading to muscle atrophy, a deterioration in general condition, an inability to rest or sleep adequately, together with significantly increased stress levels (Bufalari et al., 2012). It may arise as a result of trauma, inflammatory disorders, diagnostic procedures or surgical intervention (Gamba, 2020). Severe tissue injury, resulting in intense pain, triggers a variety of sensory signals that alert the organism and, in attempt to restore homeostasis, a complex cascade of physiological events is initiated, involving spinal reflex, endocrine, metabolic, neural and cortical responses (Bufalari et al., 2012). Severe acute pain triggers the release into the systemic circulation of pro-inflammatory cytokines, including interleukin-1, interleukin-6, interferon- γ , TNF α , as well as the activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Collectively, these processes result in cardiovascular and respiratory haemodynamic alterations, increased metabolic and caloric demands, disturbances

in fluid and electrolyte homeostasis, and in the most severe cases also in suppression of immune function (Bufalari et al., 2012). Many studies confirm that also under general anesthesia the autonomic nervous system, triggered by a noxious stimulation, activates multiple physiological mechanisms, resulting in alterations in physiological parameters such as blood pressure, skin conductance and heart rate (Ben-Israel et al., 2013). It should also be noted that since nociception cannot be accurately inferred from changes in a single physiological variable, owing to the influence of numerous physiological, psychological, and procedural factors, including measurement conditions and anesthesia. Therefore, pain assessment is likely to benefit from the combined analysis of multiple parameters (Jiang et al., 2019).

At the cardiovascular level, pain-induced activation of the sympathetic nervous system results in increased heart rate and cardiac output, peripheral vasoconstriction and a consequent rise in arterial blood pressure. Increased cardiac stimulation and the centralisation of the circulation results in greater myocardial oxygen consumption, predisposing to the development of arrhythmias, as well as increasing oxygen demands in other organs. At the peripheral level, however, vasoconstriction reduces oxygen delivery, potentially leading to tissue hypoxia and the development of acidosis (Bufalari et al., 2012). These alterations are particularly detrimental in patients with pre-existing cardiovascular disease. However, when pain persists, even otherwise healthy animals may progress to overt shock, characterised by circulatory collapse, including bradycardia and hypotension, disseminated intravascular coagulation (CID), increased vascular permeability and, in severe cases, neurogenic shock (Bufalari et al., 2012). On the other hand, respiratory changes may manifest as either hyperventilation or, when pain interferes with normal respiratory movements, hypoventilation. Pain-induced hypoventilation leads to a reduced oxygen supply, which contrasts with the increased oxygen demand associated with sympathetic cardiovascular activation. This imbalance rapidly results in moderate hypoxaemia, followed by disturbances in fluid and electrolyte homeostasis and the development of both respiratory and metabolic acidosis. Although initially compensated by blood and tissue buffer systems, the persistence of hypoventilation eventually exhausts these systems, resulting in progressive multiorgan dysfunction (Bufalari et al., 2012). In addition, progressively reduced tidal volumes and prolonged recumbency may lead to the development of pulmonary atelectasis, where impaired mucociliary clearance can predispose patients to secondary pneumonia (Bufalari et al., 2012).

Sympathetic activation and hypoxaemia also promote cortisol release from the adrenal cortex. Cortisol and catecholamines, together with increased sympathetic tone, lead to generalised immunosuppression through impaired lymphocyte function, reduced leukocyte proliferation, decreased cytokine production and impaired phagocytic activity. This results in suppression of both humoral and cellular immunity, reduced host defences, delayed wound healing, increased susceptibility to infection, and a higher risk of postoperative complications (Bufalari et al., 2012). In addition to inducing physiological alterations, acute pain also gives rise to a range of behavioural changes; including vocalizing, ambulatory activity, changes in appetite, urination and defecations (Gamba, 2020). For this reason, knowledge of the animal's baseline behaviour in the absence of pain is essential for the accurate recognition and interpretation of behavioural changes associated with pain.

2.2 Veterinary Pain Scale for assessment of acute pain

Pain recognition and assessment are essential prerequisites for ensuring the health and well-being of patients. For this reason, failure to recognize pain not only represents a welfare concern, but may also result in adverse physiological consequences, including activation of the sympathetic nervous system, impaired wound healing, immunosuppression, increased morbidity, potential effects on disease progression and altered metabolism (Menéndez et al., 2023). On the other hand, prompt recognition and treatment of acute pain may reduce the release of inflammatory cytokines and the associated inflammatory stress response, improve haemodynamic stability, and help prevent postoperative complications and chronic pain states (Marco-Martorell et al., 2024). Considering these factors, several guiding principles for pain recognition and management should be adopted: the physical examination should include observation of posture, gait and behaviour, including those reported by the owner (Gruen et al., 2022). Furthermore, it is essential to differentiate pain assessment approaches between cats and dogs, as signs of pain may not always be overt and can vary considerably between species (Menéndez et al., 2023). Regarding dogs, this species manifests signs of pain through inappetence and calm behaviour, while adjusting its demeanour, posture and movement in order to minimize pain (Gruen et al., 2022). Furthermore, facial expressions constitute an important form of non-verbal communication, in both humans and animals, serving a key role in social interactions and conveying information about emotional states (Chiavaccini et al., 2024).

In veterinary medicine, given the inability of the patient to verbally express pain and suffering, the development of composite scales has become essential for measuring these feelings (Morton et al., 2005). Pain scales are valuable diagnostic tools, providing veterinarians with a standardised and readily applicable method for pain assessment. By assigning a score to a painful condition, clinicians can objectively identify a therapeutic approach that is proportional to the degree of pain (Della Rocca et al., 2018). Both unidimensional and multidimensional pain scales are available. The former only measure a single parameter associated with pain, thereby indicating its intensity, and require the observer to make a subjective judgment of the animal's condition. This represents a challenge in pain assessment when these scales are used in busy clinical settings, as multiple observers may assess the same animal and assign different pain levels (Della Rocca et al., 2018). Moreover, several factors, including dog breeds and anxiety levels, which may increase in patients during clinical visits or in the post-operative period, can influence the perception of pain severity. Likewise, sensation of pain can increase anxiety itself (Marco-Martorell et al., 2024). Therefore, differentiating between behavioural changes resulting from hospital environment and pain may be difficult, especially using unidimensional scales (Marco-Martorell et al., 2024). For these reasons, multidimensional pain scales represent a better tool for pain recognition, as they reduce the influence of observer subjectivity (Della Rocca et al., 2018). The psychometric approach to scale development follows a rigorous and well-established methodology involving item generation and selection, questionnaire construction, and testing for validity, reliability, and responsiveness (Della Rocca et al., 2018). Regarding the validity contest, consideration should be given to criterion validity, which is the agreement of a new instrument with an existing "gold standard", which, however, does not exist in the case of animal pain; content validity concerns the appropriateness and completeness of the items constituting the instrument; and construct validity that is established when the instrument provides results that support predefined hypotheses concerning the construct being measured (Della Rocca et al., 2018).

In veterinary medicine, the three main unidimensional scales were originally developed for use in humans: the Simple Descriptive Scale (SDS), the Numeric Rating Scale (NRS) and the Visual Analog Scale (VAS) (Morton et al., 2005). As previously stated, no "gold standard" currently exists for the assessment of pain in animals. Nevertheless, the NRS is generally considered a more appropriate reference to use in dogs than the VAS, although the latter has been identified as the "gold standard" in human medicine (Morton et al., 2005). Moreover, these scales have been

shown to be unreliable in veterinary settings, considering that they do not provide the necessary discriminatory properties required for assessment of modern analgesia and do not fulfill the necessary criteria of a valid and reliable scale (Morton et al., 2005).

By contrast, several multidimensional scales have been developed, including the University of Melbourne Pain Scale, the 4AVet Scales, the UNESP-Botucatu Multidimensional Composite Pain Scale and the Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF) (Della Rocca et al., 2018). The latter is the most commonly used scale in veterinary medicine for the assessment of pain in dogs and was derived from the original Glasgow Composite Measure Pain Scale (CMPS) (Della Rocca et al., 2018). This scale is substantially a structured questionnaire, designed using psychometric principles and conducted by an observer following a standardised protocol, that includes the assessment of spontaneous and evoked behaviours, interactions with the patient and clinical observation (Della Rocca et al., 2018). The Short Form comprises seven behavioural categories: posture, activity, vocalisation, attention to wound or painful area, demeanour, mobility and response to touch. Each category includes a set of items from which the observer selects the term that best describes the behaviour of the patient and then assign a score to it (Reid et al., 2007). The total score ranges from 0 to 24 in ambulatory patients and from 0 to 20 in non-ambulatory patients (Marco-Martorell et al., 2024).

However, despite the availability of validated pain assessment scales, several studies evidence that their utilisation remains limited among veterinarians, due to a lack of established practice routine, time constraints and limited clinical personnel (Menéndez et al., 2023).

Chapter 3: Gabapentin

3.1 Gabapentin pharmacology

Gabapentin, 1-(aminomethyl) cyclohexane acetic acid, is a member of the gabapentinoids class of drugs, which also includes Pregabalin (Lamont, 2024). Gabapentin and pregabalin are synthetic analogues of γ -aminobutyric acid (GABA) and share structural similarities with this neurotransmitter (Bockbrader et al., 2010). In fact, the chemical structure of gabapentin includes a structure resembling GABA, with an additional cyclohexane ring incorporated into its structure. Gabapentin is a water soluble, white crystalline substance (Rose & Kam, 2002), with a molecular weight of 171.24 g/mol, an acid dissociation constant (pKa) of 3.7, and a base dissociation constant (pKb) of 10.7 (Bockbrader et al., 2010).

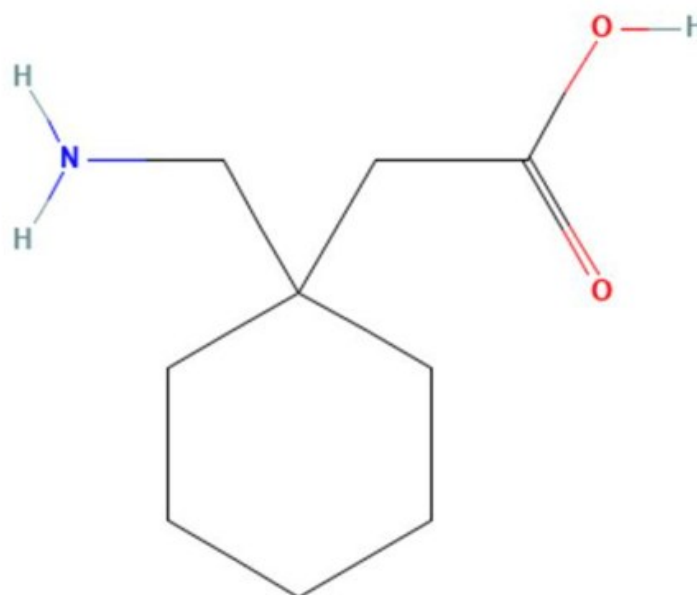


Figure 1: Gabapentin chemical structure (Di Cesare et al., 2023)

Gabapentin was originally approved for human use in the United States by the Food and Drug Administration (FDA) in December 1993 as an adjunctive therapy in the treatment of partial seizures in adults at an effective dose of 900-1800 mg/day (Radulovic et al., 1995).

Subsequently, in 2002, it was also approved for post-herpetic neuralgia (Russo et al., 2023), namely for pain indication (Goodman & Brett, 2019).

Over the last decade, gabapentinoids have been routinely used in several countries for the management of postoperative pain, with the aim of reducing opioid consumption and pain intensity. Moreover, their off-label use for the treatment of acute nociceptive and neuropathic pain has increased considerably (Verret et al., 2020). As mentioned above, Gabapentin was originally designed as an antiepileptic drug; nevertheless, in patients with central lesions or peripheral nerve injuries, it provided significant relief of ongoing spontaneous and paroxysmal pain (Gu & Huang, 2001).

3.2 Mechanism of action

Gabapentin's dual role as both an anticonvulsant and an analgesic reflects its effects on distinct therapeutic pathways underlying its principal clinical indications. The current understanding of gabapentin's mechanisms of action is based largely on evidence from rodent models; however, this makes it challenging to fully understand the complexities of functional and molecular effects on human sensory neurons (Demeter et al., 2025).

Although gabapentin was originally designed as a brain penetrant GABA analogue, it does not appear to exert its effects through central GABAergic mechanisms and exhibits little affinity for GABA receptors (Di Cesare et al., 2023). In particular, extensive evidence has demonstrated that gabapentin does not interact with the GABA_A receptors, instead exhibits minimally selective activity for GABA_B receptors (Cheng & Chiou, 2006), activating presynaptic GABA_B heteroreceptors on glutamatergic terminals, but not GABA_B autoreceptors on GABAergic terminals (Di Cesare et al., 2023). It has been demonstrated that gabapentin may cause an increase in GABA extracellular concentration in some brain regions due to the increased glutamic acid decarboxylase activity, which produces GABA. On the other hand, there is no evidence that gabapentin exerts any GABA-mimetic action or increases intraneuronal GABA concentrations (Rose & Kam, 2002).

Regarding its anticonvulsant effect, one of the main theories concerns the interaction with the L-amino acid transporter system (Rose & Kam, 2002), a protein responsible for the entry of α -amino acids into cells and their intestinal absorption (Cheng & Chiou, 2006). The chemical structure of gabapentin contains both amine and carboxylic acid functional groups, resembling that of an α -amino acid. Therefore, gabapentin may be recognized by L-amino acid transporters, facilitating its transport across the blood-brain barrier and its intestinal absorption. However, these transporters may not be directly involved in the cellular actions of gabapentin (Cheng & Chiou, 2006). Several additional molecular mechanisms have been proposed to explain anti-epileptic effect of gabapentin, but none demonstrated sufficient pharmacodynamic relevance. The proposed mechanisms include modest reductions in monoamine neurotransmitters release, such as dopamine, noradrenaline and serotonin, as well as attenuation of sodium-dependent action potentials following prolonged gabapentin exposure (Rose & Kam, 2002).

Regarding the analgesic effects of gabapentin, the principal mechanism is thought to involve its interaction with voltage gated Ca²⁺ channels (VGCCs). However, several other mechanisms have been proposed, including effects at the spinal and supraspinal levels mediated by enhanced inhibitory GABAergic input, reduced excitatory neurotransmission, and antagonism of NMDA receptors. Among these proposed mechanisms, the most widely accepted hypothesis is that gabapentin exerts its effects through the inhibition of VGCCs-mediated neurotransmission. This is supported by the evidence showing that GABA receptor antagonists do not reduce the anti-allodynic effect of gabapentin, thus it seems unlikely that potentiation of inhibitory GABA-ergic pathways could be responsible for gabapentin action (Rose & Kam, 2002).

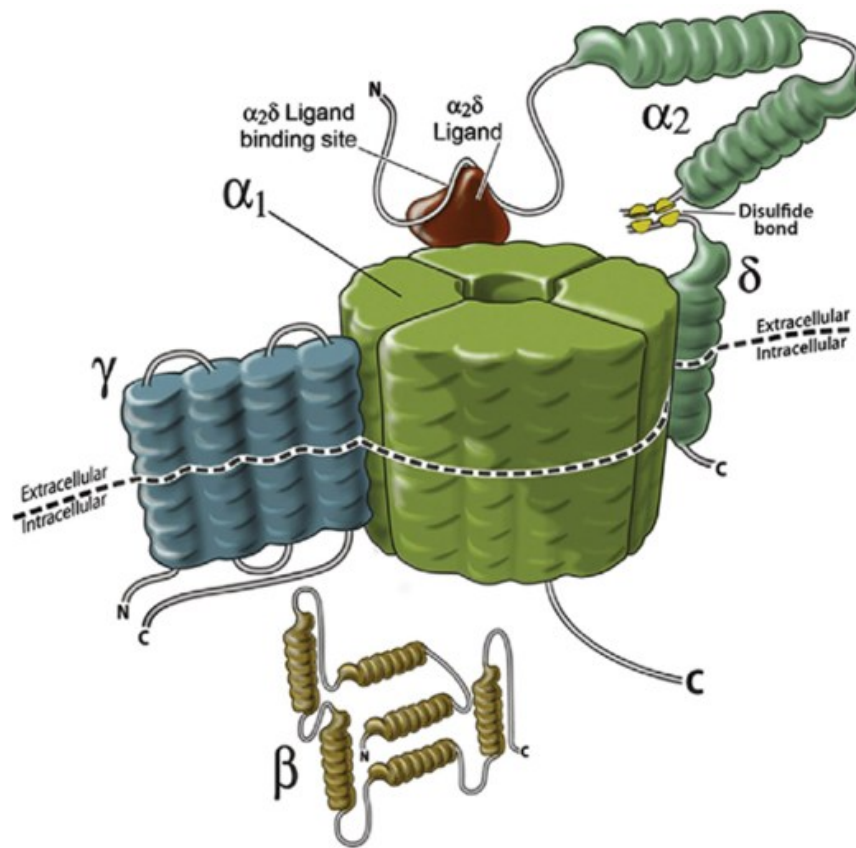


Figure 2: Voltage-gated Calcium Channel (Chincholkar, 2018)

VGCCs are classified into high-voltage activated channels (L-, P/Q-, N-, R-type) or low-voltage activated channels (T-type) (Chincholkar, 2018). Among these, evidence indicates that only the P/Q- and N-type channels are involved in nociception (Gu & Huang, 2001). VGCCs are formed by multiple subunits: the α_1 subunit, which comprises four homologous domains with six transmembrane helices each, allows the entry of calcium ions; the α_2 subunit, located extracellularly and attached to the δ subunit via a disulfide linkage, has four isoforms (1-4); and the β subunit, which is located entirely intracellularly (Chincholkar, 2018). The $\alpha_2\delta$ subunit plays a role in Ca^{2+} channel membrane incorporation and, associated to the β subunit, constitute the auxiliary subunits of all types of Ca^{2+} channels. The coexpression of these two subunits, together with the pore-forming α_1 subunit, normally promotes the Ca^{2+} current (Cheng & Chiou, 2006). The $\alpha_2\delta$ -1 isoform is principally expressed in dorsal root ganglion (DRG) and spinal dorsal horn (Russo et al., 2023), but it is also located in the brain, skeletal, cardiac and smooth muscle (Chincholkar, 2018).

The $\alpha_2\delta$ -1 subunit represents the principal site of binding of gabapentin to VGCCs, and its interaction with gabapentin results in the inhibition of channel function at both spinal and

supraspinal levels (Gordh et al., 2008). In turn, the direct inhibition of the channels and the interference with the function of $\alpha 2\delta$ -1-associated components, such as the $\alpha 1$ subunit of VGCCs, N-methyl-D-aspartate receptors (NMDARs), neurexin-1 α , and thrombospondin (Demeter et al., 2025), results in reduction of presynaptic Ca^{2+} influx, with the subsequently inhibited releasement of excitatory neurotransmitters from presynaptic nerve terminals (Chincholkar, 2018). Nevertheless, this assumption is not totally correct, considering that acute application of gabapentin does not reduce calcium currents (Chincholkar, 2018). Rather, gabapentin binding to $\alpha 2\delta$ -1 subunit of VGCCs, thereby reducing Ca^{2+} influx and, consequently, neurotransmitter release (Demeter et al., 2025). More recent genomic studies have provided further insights into the mechanism of action of gabapentin. In particular, chronic administration of this drug has demonstrated that the reduction in neurotransmitter release is not simply a consequence of its binding to the $\alpha 2\delta$ -1 subunit (Chincholkar, 2018); (Demeter et al., 2025). Rather, inhibition of the $\alpha 2\delta$ -1 subunit reduces the trafficking and membrane expression of the $\alpha 1$ subunit of VGCCs, which in turn decreases calcium influx and neurotransmitter release (Demeter et al., 2025). Furthermore, gabapentin interference with $\alpha 2\delta$ -1 subunit can also reduce neuronal hyperexcitability (Demeter et al., 2025). Gabapentin also binding to the $\alpha 2\delta$ -2 subunit, highly expressed in cerebellum and brainstem, leading to modest analgesic effect, corticospinal plasticity and regeneration after spinal cord injury in mice models (Russo et al., 2023).

In 2019, a dual mechanism of action at the locus coeruleus (CL) was proposed to explain the role of gabapentin in reducing chronic neuropathic pain. Gabapentin was suggested to act in two complementary ways: first, by interacting with the $\alpha 2\delta$ subunit in the LC to reduce presynaptic GABA release; and second, by activating glutamate transporter-1-dependent mechanisms, thereby promoting glutamate release from LC astrocytes (Di Cesare et al., 2023). This dual mechanism exerts complementary effects on LC neurons by inhibiting VGCC function while simultaneously increasing extracellular glutamate levels, ultimately enhancing LC neuronal activity and activating descending pain inhibition (Di Cesare et al., 2023). Another proposed mechanism by which gabapentin may alleviate neuropathic pain involves the disruption of the $\alpha 2\delta$ -1 – NMDAR complex. NMDARs are key mediators of synaptic plasticity and neuropathic pain. The interaction between them has been demonstrated to enhance synaptic transmission in the dorsal horn following nerve injury (Russo et al., 2023). However, the effect of gabapentin on NMDARs appears to be plastic, as the drug seems to exert its action only when the intracellular

expression of protein kinases C, an inflamed mediator, exceeds a critical threshold in cells affected by nerve injury (Gu & Huang, 2001).

Despite the numerous studies investigating this issue, the precise mechanism of action of gabapentin remains currently largely unclear. As discussed above, several hypotheses have been proposed, suggesting that gabapentin do not exert its effects through a single mechanism of action, but rather interact with multiple molecular targets (Gu & Huang, 2001).

3.3 Pharmacokinetic

The pharmacokinetic of gabapentinoids have been extensively studied, particularly in rodents and humans, providing insights into their absorption, distribution, metabolism and elimination properties.

Gabapentin is available exclusively as an oral formulation (Rose & Kam, 2002). Due to its high-water solubility, it dissolves rapidly following oral administration (Bockbrader et al., 2010). In the small intestine, its absorption occurs primarily through the system-L-transporter family, which mediates the uptake of large neutral amino acids and contributes to the intestinal absorption of gabapentin. In particular, in preclinical studies, LAT1 transporter has been established as the sole transporter mediating gabapentin uptake, resulting in a ready saturable transport process and thus dose-limited absorption. Depending on the presence of the transporters along the gastrointestinal tract, which are assigned to the passage of the molecules from the intestinal lumen to the systemic circulation, the rate and extent of absorption are modulate (Bockbrader et al., 2010). Moreover, in veterinary medicine, transporter expression levels vary among species (Di Cesare et al., 2023). The absorption of gabapentin is characterized by a relatively slow rate, resulting in peak plasma concentrations approximately 3 hours postdose (Bockbrader et al., 2010). With regard to the extent of drug absorption, the saturable nature of its transporter leads to a reduction in absorption with increasing administered doses. Regarding gabapentin bioavailability, this is inversely proportional to dose, with an approximately bioavailability of 80% at 100-mg, which is the lowest dose studied (Bockbrader et al., 2010), 60% at 300-mg dose and 40% at 600-mg (Rose & Kam, 2002). Although mean gabapentin steady-state minimum plasma concentrations ($C_{min,ss}$) increase with increasing doses, the relationship is not proportional. Likewise, maximum plasma concentrations (C_{max}) exhibit a similar trend, with the time to reach the C_{max} (t_{max}) dependent on the administered dose. In human models, studies have shown that, at low doses of 100 mg, gabapentin reaches the t_{max} approximately 1.7 hours after

administration, whereas higher single doses are associated with a longer $t_{\max}$ of 3-4 hours. Neither $C_{\max}$ nor $t_{\max}$ are affected by food intake; the only observed change is an approximately 10% increase in $C_{\max}$ following either a standard meal or a high-fat meal. However, this finding is not considered to be clinically relevant (Bockbrader et al., 2010). Regarding gabapentin distribution, in humans, gabapentin plasma concentration results similar to the whole-blood concentration, indicating that it can penetrate red blood cells. In contrast, no plasma protein binding has been demonstrated, thus suggesting that drug-drug interactions with highly plasma protein-bound drugs are unlikely. After single-dose administration, sparse sampling studies revealed that gabapentin concentrations in the cerebrospinal fluid (CSF) reached approximately 9-14% of the corresponding plasma concentration, with a CSF/plasma concentration ratio increased with time and following multiple-dose administration (Bockbrader et al., 2010). Studies in rodent models have demonstrated preferential distribution of gabapentin to pancreatic and renal tissues, with concentrations reaching approximately eight- and four-times higher levels than those measured in serum, respectively (Rose & Kam, 2002). The volume of distribution (V_d) of gabapentin, based on pharmacokinetic analyses and independent of drug bioavailability, corresponds of 0.8 L/kg, which is a relatively small volume, similar to that of total body water (Bockbrader et al., 2010). In humans, gabapentin is not metabolised by the liver, and it is eliminated unchanged in the urine (Rose & Kam, 2002), while in dogs it is metabolized to the corresponding N-methyl metabolite (Bockbrader et al., 2010). Regarding gabapentin excretion, the only pathway for systemic elimination of the drug is essentially the renal excretion, considering that gabapentin plasma clearance is highly correlated with renal function. The elimination half-life of gabapentin has been reported to range between 5.0 and 7.0 hours, suggesting that within 24-48 hours following administration of this drug, the steady state is achieved (Bockbrader et al., 2010).

Despite the extensive characterization of gabapentin pharmacokinetic in other species, its pharmacokinetic profile in dogs has been poorly investigated. The main studies on this topic are those by Louis L. Radulovic et al. in 1995, using Beagle dogs as animal models, and by Butch KuKanich and Rachel L. Cohen in 2009, using Greyhound dogs. Findings from these studies elucidated that, as noted above, dogs are the only species known to metabolize gabapentin prior to its renal excretion, at concentrations representing approximately 30-35% of the administered dose (Lamont, 2024), with an oral bioavailability of 80% at a dose of 50 mg/kg (Radulovic et al., 1995). In KuKanich and Cohen study, gabapentin was administered orally to 6 healthy Greyhound

dogs at doses of 10 mg/kg on day 1 and 20 mg/kg on day 2, with the following results of C_{max} values of 8.54 and 13.22 mcg/mL, respectively, t_{max} values of 1.31 and 1.51 hours, and terminal half-lives values of 3.3 and 3.4 hours (KuKanich & Cohen, 2011). In human studies, gabapentin efficacy is associated with 2 mcg/mL plasma concentration; consequently, the optimal dosing regimen and dosing interval required to maintain the same concentration in dogs were investigated by KuKanich and Cohen. The concentration of 2 mcg/mL was achieved in the 10 mg/kg dose group in 6/6 dogs at 6 hours, 4/6 dogs at 8 hours and 0/6 dogs at 12 hours after dosing; while it is achieved in the 20 mg/kg dose group in 6/6 dogs at 8 hours and 2/6 dogs at 12 hours. Based on these data, in order to maintain a plasma concentration equivalent to 2 mcg/mL, it would be necessary to administer gabapentin at doses of 10-20 mg/kg at least every 8 hours (KuKanich & Cohen, 2011).

3.4 Gabapentin clinical use in human medicine

Initially approved in 1993 as an adjunctive medication to control partial seizures and generalised tonic-clonic seizures in patients over 12 years of age (Rose & Kam, 2002), gabapentin gradually emerged as an effective analgesic agent. By the mid-1990s, antiseizure medications had gained increasing recognition as therapeutic options for neuropathic pain that was refractory to conventional treatments, including opioids (Peckham et al., 2018). Although gabapentin continued to be primarily prescribed as an antiepileptic drug, research conducted in the late 1990s increasingly investigated its potential therapeutic applications beyond seizure control. In particular, Dodrill et al. (1999) investigated the cognitive and psychosocial effects of gabapentin and, reported improvements in mood and psychological adjustment were observed among patients receiving lower doses of gabapentin. According to the authors, the perceived improvement in cognitive abilities was more likely attributable to improved mood than to genuine enhancement of cognitive performances. Nevertheless, patients also reported reduced anxiety and discouragement (Dodrill et al., 1999).

Subsequently, after 2001, considering its favourable safety profile compared with other antiseizure medications, gabapentin began to be increasingly prescribed also for nonseizure conditions, with 83% of its prescriptions being for off-label indications. Following this development, in 2002 the FDA approved gabapentin in the United States also for the treatment of a neuropathic pain condition (Peckham et al., 2018). However, at that time, the evidence supporting its analgesic effects were considered only sufficient enough to justify its approval for

postherpetic neuralgia (Peckham et al., 2018), a neuropathic pain condition that persists for at least three months after the crusting of the acute herpes zoster rash. Generally, herpes zoster-associated pain resolves spontaneously. However, in some patients, the sensation of pain becomes chronic and may persist for several years, leading to the development of hyperalgesia or allodynia (Rowbotham et al., 1998). Supporting this, gabapentin utility in postherpetic neuralgia treatment was examined by Rice et al. (2001), elucidating that patients, receiving gabapentin at a dosage of 1800 and 2400 mg/day, showed improvements in both pain reduction and sleep interference (Rice et al., 2001). Subsequently, the use of gabapentin for off-label indications gained traction, including other neuropathic pain conditions, such as diabetic neuropathy, complex regional pain syndrome, restless legs syndrome (RLS), trigeminal neuralgia (Peckham et al., 2018), and reflex sympathetic dystrophy (Cheng & Chiou, 2006); but also for other non-seizure-related indications, such as bipolar disorder, attention deficit disorder, periodic limb movement, premenstrual syndrome, migraine headache, drug and alcohol withdrawal seizures, and disorders of sleep (Peckham et al., 2018). Regarding the latter pathological condition, Liu et al. (2017) conducted a systematic review to evaluate the efficacy and tolerability of gabapentin in adults with sleep disturbance secondary to medical illness, including RLS, neuropathic pain, alcohol dependence, hot flashes in menopause, fibromyalgia, phantom limb pain, HIV-associated sensory neuropathies, and bipolar disorders. From this study emerged that, without consideration of the type of sleep outcomes, treatment with gabapentin was associated with shorter sleep latency, fewer awakenings, reduced fast-wave sleep, increased slow-wave sleep, longer total sleep time, improved sleep efficiency, and better quality of sleep. However, the authors noted that the mean daily dose of approximately 1,800 mg could be associated with a higher risk of treatment discontinuation and drug withdrawal, compared with placebo group (Liu et al., 2017). Other conditions, including low back pain and radiculopathy, represent some of the most common indications for the off-label use of gabapentin. Gabapentin has also been prescribed off-label for the treatment of fibromyalgia, possibly due to its lower cost, despite pregabalin being the only approved gabapentinoids for this indication (Goodman & Brett, 2019). Furthermore, gabapentin is used frequently, combined with opioids, to severe cancer pain treatment (Gu & Huang, 2001).

In recent years, the use of gabapentin has increasingly expanded to include the management of pain, owing to its well-documented analgesic properties. In particular, gabapentin has been widely used as an adjuvant therapy for postoperative pain, where it may provide synergistic

analgesic effects when combined with opioids and non-steroidal anti-inflammatory drugs (NSAIDs) (Chincholkar, 2018). Gabapentin has been increasingly considered as part of multimodal analgesic strategies due to its capacity to reduce the need for opioid analgesics, which may contribute to a reduction around 20% of opiate use in the first 24h after surgery, and also to a reduction in opioid related adverse effects and improving the quality and speed of postoperative recovery (Baos et al., 2025). Moreover, researchers have also investigated its potential role in the preoperative setting, with the aim of determining whether preoperative administration could improve postoperative pain control and related clinical outcomes. Gabapentinoids are effective antinociceptives agents in numerous animal models of inflammatory pain and have been shown to reduce inflammatory mediators, suppress dorsal horn activity, and attenuate the neuroinflammatory response, which is partly driven by increased levels of proinflammatory cytokines. Several studies, conducted in animal models, evaluated gabapentin antinociceptive properties following its administration (Chincholkar, 2018). Specifically, Feng et al. (2003) reported that intraperitoneal administration of gabapentin significantly reduced acetic acid-induced visceral nociception in rodents (Feng et al., 2003). Similarly, Whiteside et al. (2004) found that gabapentin administration attenuated mechanical hyperalgesia and tactile allodynia response, following a surgical incision of rat paw (Whiteside et al., 2004). However, acute gabapentin administration, unlike NSAIDs, did not improve reduced activity. Only chronic gabapentin treatment alleviated ambulatory-evoked pain (Chincholkar, 2018). Collectively, evidence from animal studies of surgical pain models and clinical studies of inflammatory pain demonstrates that both conditions induce allodynia and hyperalgesia, both of which are susceptible to treatment with gabapentin. Antihyperalgesic agents, such as gabapentin, represent promising options for postoperative analgesia because they may selectively inhibit pathological pain, while preserving physiological nociceptive responses. Therefore, of particular interest in the postoperative setting is the evidence that the analgesic effects of gabapentin are distinct from those of opioids. Indeed, gabapentin's antihyperalgesic activity does not appear to rely on opioid receptor activation and does not impair gastrointestinal motility in rats, which is a major side-effect of opioids in postoperative patients (Dahl et al., 2004). In addition to its use in postoperative pain management, gabapentin has also been evaluated in clinical trials as a preoperative medication, with the aim of reducing postoperative pain and improving analgesic outcomes. Indeed, the concept of pre-emptive analgesia was introduced to prevent the development of hyperalgesia and allodynia and to protect the central nervous system from the

deleterious effects of noxious stimulus. Syal et al. (2010) conducted a study to evaluate the effects of protective premedication on postoperative analgesia in patients undergoing open cholecystectomy, administering acetaminophen, gabapentin or a combination of both agents before surgery. From this study emerged that the combination of gabapentin and acetaminophen reduced the total analgesic requirement by 51%, while gabapentin alone achieved a 48% reduction compared with the placebo group. Moreover, gabapentin alone group had lower VAS scores, both at rest and during movement, at all postoperative time points compared with the placebo group (Syal et al., 2010).

Despite gabapentin has been proposed as a valid alternative to antiseizure drugs and a strategy to reduce opioid consumption, some dose dependent and reversible mild adverse effects have been reported, such as dizziness and somnolence, occurring in >20% of patients (Bockbrader et al., 2010). In addition to these principal side effects, others have been reported, including nervous system disorders, such as ataxia, amnesia, abnormal gait, incoordination, asthenia; psychiatric disorders, such as confusion, euphoria, abnormal thinking; and eye disorders, such as amblyopia and blurred vision (Meaadi et al., 2023). Moreover, has been reported that gabapentin may cause reversible acute renal allograft dysfunction (Rose & Kam, 2002), and Stevens-Johnson syndrome (Gonzalez-Sicilia Leandro et al., 1998). On the other hand, the risk of adverse effects appears to increase when gabapentin is administrated with other central nervous system depressants, such as opioids (Peckham et al., 2018).

In conclusion, although gabapentinoids are extensively used in pain management and other off-label conditions, the available scientific evidence supporting their clinical utility remains limited (Goodman & Brett, 2019).

3.5 Gabapentin clinical use in veterinary medicine

Similar to its use in human medicine, gabapentin was initially introduced extra-label into veterinary medicine as an antiepileptic drug, primarily for dogs and cats. The mechanism of action, as an anticonvulsant agent, has not been fully elucidated. Unlike several conventional antiepileptic drugs, gabapentin probably does not interact with voltage-dependent sodium channels, common targets of several anticonvulsant agents. Furthermore, it has not been shown to exhibit significant affinity for common neurotransmitters receptors, enzymes, or other ion channels. To date, despite the lack of evidence of efficacy and of correspondence of neuropathic

pain conditions in human and animals, gabapentin is more commonly used for neuropathic and chronic pain management. Regarding the main indications in dogs, gabapentin has been shown to be potentially beneficial for the treatment of epilepsy, chronic, neuropathic and post-operative pain, and anxiety; while in cats for post-ovariohysterectomy-related pain, and anxiety management (Di Cesare et al., 2023).

The first report describing the use of gabapentin as an anticonvulsant agent in dogs was presented in 2003 at the 16th Annual Symposium of the European College of Veterinary Neurology. Since then, gabapentin has been used in dogs as an adjunctive therapy to improve seizure control in patients for which conventional antiepileptic drugs are either ineffective or become toxic. To date, the available evidence supports the use of gabapentin as an add-on therapy for patients suffering from partial seizures, resistant to conventional therapies, with no studies demonstrating its efficacy as monotherapy (Di Cesare et al., 2023). Subsequent studies have explored the antiepileptic effects of gabapentin, as well as its potential therapeutic role in other psychological disorders, such as anxiety. Govendir et al. (2005) conducted a study on 17 dogs with refractory epilepsy to determine the potential benefit of adding gabapentin to the therapeutic regime, already composed by phenobarbitone and potassium bromide, for achieving better seizure control. Among these 17 dogs, after four months of supplementary therapy administration, 3 became seizure free shortly after the introduction of gabapentin, whereas 13 patients continued to experience generalised motor seizure despite receiving triple therapy. However, in eight of these thirteen patients, seizures appeared less severe, with reduced frequency and shorter duration compared with the period preceding gabapentin administration. Additionally, both postictal depression and recovery time were reduced (Govendir M et al., 2005).

Regarding the use of gabapentin as an anxiolytic agent, anxiolytic-like effects has been reported in several behavioural tests on rodents, including the rat conflict test, the mouse light/dark box test, and the rat elevated X-maze test. Similar behavioural responses, suggestive of anxiolysis activity, have also been observed in the marmoset human threat test. In companion animals, gabapentin has shown promising anxiolytic properties, with clinical benefits described for the management of stormia phobia, anxiety, and fear reduction, especially in relation to veterinary visits. These effects may be associated with a modulation of the physiological stress response, including a reduction in catecholamine release during the “flight and fight” response (Di Cesare et al., 2023). Bleuer-Elsner et al. (2021) conducted a study on 18 dogs suffering from storm

phobia. This study was based on two exposures to a natural thunderstorm event, in which each dog received gabapentin before one exposure at a single dosage of 25-30 mg/kg, and a placebo before the other one. The findings of this study suggest that gabapentin, administered orally 90 min before exposure, was effective in reducing fear responses in the studied dog population when exposed to thunderstorms (Bleuer-Elsner et al., 2021). On the other hand, the potential efficacy of gabapentin in alleviating anxiety associated with veterinary visits has been investigated more extensively in cats than in dogs; however, Stollar et al. (2022) conducted a study to evaluate the effects of a single dose of orally administered gabapentin, at 50 mg/kg dose 2 hours before the beginning of each visit, in 22 healthy client-owned dogs. No significant differences were observed between the gabapentin and placebo groups in terms of physiological parameters, including salivary cortisol concentrations and eye surface temperature. Conversely, significant differences were identified in behavioural parameters, including lip licking frequency during the physical examination phase and the total frequency in the pre- and post-physical examination phases. Lip licking was considered a behavioural indicator of stress and anxiety during the veterinary visit. Indeed, a substantial reduction in licking behaviour was observed in the gabapentin group throughout the entire examination period, supporting the hypothesis of the authors (Stollar et al., 2022).

Regarding other clinical applications in dogs, gabapentin has been evaluated in several studies for the management of postoperative pain as well as other pathological conditions. Nevertheless, evidence supporting its clinical efficacy remains limited. In most cases, the primary advantage of gabapentin was its association with a lower incidence of adverse effects compared with other therapeutic agents. Plessas et al. (2015) conducted a study investigating the effects of gabapentin versus topiramate, administered as adjunctive therapies to carprofen, on the quality of life of dogs with clinical signs of neuropathic pain secondary to Chiari-like malformation and syringomyelia. Quality of life was evaluated using a Visual Analogue Scale (VAS) at baseline (day 0), following 1 week of treatment with carprofen alone, after 2 weeks of treatment with carprofen in combination with gabapentin, and after 2 weeks of treatment with carprofen in combination with topiramate. At the end of the study, no substantial differences in quality of life were detected between the gabapentin and topiramate groups. Nevertheless, a more detailed analysis revealed a greater improvement in quality of life in the gabapentin group compared with the initial treatment phase with carprofen alone. Moreover, gabapentin was associated with a more favourable safety profile, with fewer adverse effects reported compared with topiramate

(Plessas et al., 2015). In the context of neurological disorders, Wagner et al. (2010) and Aghighi et al. (2012) conducted studies investigating the use of gabapentin as an adjunctive therapy to improve postoperative analgesia. The study by Wagner et al. (2010) is a clinical investigation evaluating the perioperative administration of gabapentin in dogs undergoing forelimb amputation. Thirty client-owned dogs were randomly assigned to one of two treatment groups prior to surgery. One group received gabapentin 10mg/kg PO on the afternoon before surgery, followed by 5 mg/kg PO every 12 hours for an additional 3 days, whereas the other received placebo, consisting of an orally administered gelatine capsule. However, the study found no significant differences between the gabapentin and placebo groups, as pain scores did not differ over time regardless of the pain assessment method used. Thus, gabapentin, at the dose and frequency used in this study, did not provide a significant benefit for the management of acute perioperative pain as an adjunct to other analgesics and anaesthetics. Despite these negative findings, the authors highlighted several limitations that may have influenced the study outcomes. They suggested that the gabapentin dosage may have been too low, the duration of treatment may have been insufficient, and the concomitant administration of other analgesic agents may not have provided adequate multimodal analgesia (Wagner Ann E. et al., 2010). Similarly, Aghighi et al. (2012) also found no evidence supporting the efficacy of gabapentin in the management of postoperative pain. In their clinical evaluation of postoperative pain following thoracolumbar intervertebral disc surgery in 63 client-owned dogs, authors reported that oral gabapentin (10 mg/kg twice daily), administered as an adjunct to standard opioid analgesia, did not significantly reduce pain-related behaviour compared with opioid analgesia alone. Nevertheless, a trend toward lower pain scores was observed in dogs receiving gabapentin (Aghighi et al., 2012). Additional studies have also investigated the use of gabapentin for postoperative pain management in dogs undergoing mastectomy. In the study by Crociolli et al. (2015), dogs received a single oral dose of gabapentin (10 mg/kg) 120 minutes prior to surgery, and then continuing for three days after surgery, beginning 12 hours after the preoperative dose. In addition to gabapentin or placebo administration, the premedication analgesic protocol included 0.03 mg/kg acepromazine and 0.3 mg/kg morphine intramuscularly, and 0.2 mg/kg meloxicam intravenously 5 minutes prior to surgery. The pain level was measured, using the modified Glasgow Composite Measure Pain Scale and the Visual Analogue Scale, prior to premedication up to 72 hours after extubation. Although median pain scores did not differ significantly between the two groups, this study demonstrated that dogs receiving gabapentin

had a significantly lower incidence of rescue analgesic administration compared with the placebo group. Consequently, this may suggest improved analgesic efficacy when gabapentin is administered perioperatively. Indeed, it was concluded in the study that its administration reduced the requirement for rescue morphine doses by 44% in dogs (Crocioni et al., 2015).

Finally, further investigations have examined the effect of orally administered gabapentin on the minimum alveolar concentration (MAC) of isoflurane in dogs. Because isoflurane commonly induces vasodilation and may consequently contribute to hypotension, premedication with agents such as tranquilizers, analgesics, and anxiolytics is generally recommended to reduce the required dose of induction agents and decrease the MAC of inhalational anaesthetics. For this reason, Johnson et al. (2019) investigated the effect of 20 mg/kg oral administration of gabapentin 2 hours before an anaesthetic event. Consequently, the study evaluated variations in isoflurane MAC in the same patient during two separate sedation sessions. The study results indicated that MAC of isoflurane was significantly lower after gabapentin administration ($0.71 \pm 0.12\%$) than after isoflurane alone ($0.91 \pm 0.26\%$), which resulted in a mean reduction in MAC of $20 \pm 14\%$. Other parameters were also investigated, including heart rate, SAP, DAP and MAP, which did not differ significantly between treatments; while the time to extubation was minor after gabapentin treatment (6 ± 4 minutes) than after isoflurane alone (23 ± 15 minutes) (Johnson Brittney A. et al., 2019). Similarly, Aiello et al. (2025) compared the effect of administering, 2 hours before anaesthesia, oral gabapentin (20 mg/kg) and trazodone (8 mg/kg) on the MAC of isoflurane in 6 adult dogs. Likewise, the findings of this study indicated that the administration of gabapentin in combination with trazodone as part of a premedication protocol reduced isoflurane requirements, resulting in a decrease in MAC of $0.33 \pm 0.04\%$, with no significant effect on hemodynamic variables (Aiello et al., 2025).

Concerning the clinical applications of gabapentin in cats, early reports were primarily based on anecdotal evidence, and data regarding the safety and efficacy of its long-term administration were lacking. In recent years, the use of gabapentin in feline internal medicine has been increasingly investigated, as mentioned above, for its analgesic and anxiolytic effects. Nevertheless, current knowledge regarding gabapentin anticonvulsant role in this species remains limited, with scarce evidence available to support its safety and efficacy (Di Cesare et al., 2023). A topic of particular interest is the potential role of gabapentin as an adjunctive postoperative analgesic in cats undergoing ovariohysterectomy. About this topic, the study by Steagall et al. (2017) aimed to evaluate, in 52 adult cats undergoing ovariohysterectomy, the

analgesic efficacy of a gabapentin (50 mg PO) - buprenorphine (0.02 mg/kg IM) combination, compared with meloxicam (0.2 mg/kg SC) - buprenorphine or buprenorphine alone with a placebo capsule. Gabapentin or placebo capsules were administered orally 12 and 1 hour before the surgical procedure, depending on group allocation. Postoperative pain was evaluated for up to 8 hours after surgery using a Multidimensional Composite Pain Scale (MCPS) and the Glasgow Composite Measure Pain Scale – Feline (rCMPS-F). However, the results differed according to the pain assessment scale used. Specifically, postoperative analgesia did not differ significantly among the treatment groups, using MCPS. In contrast, assessment using the rCMPS-F suggested that both gabapentin-buprenorphine and meloxicam-buprenorphine groups provided more effective analgesia than buprenorphine alone. Moreover, the authors reported that, in healthy cats undergoing ovariohysterectomy, treatment with gabapentin did not result in higher sedation scores or an increased incidence of clinically detectable adverse effects, compared with the other treatment protocols (Steagall et al., 2018). Fantinati et al. (2020) further demonstrated that, following ovariohysterectomy, food intake was significantly higher in cats treated with either gabapentin or mirtazapine than in those receiving placebo. Notably, 30% of cats treated with gabapentin covered their resting energy requirements, while none of the cats in the placebo group achieved this target (Fantinati et al., 2020).

In conclusion, the current literature indicates that gabapentin is widely used in veterinary medicine, particularly for the management of anxiety. While evidence regarding its postoperative analgesic efficacy is variable, its use as an adjunct to multimodal analgesia appears to reduce the requirement for rescue analgesia following surgery.

Chapter 4: Analgesia in veterinary

4.1 Multimodal analgesia

Multimodal treatment is defined by the IASP as: *“the concurrent use of separate therapeutic interventions with different mechanisms of action within one discipline aimed at different pain mechanisms”* (<https://www.lasp-pain.org/resources/terminology/>, n.d.). Essentially, multimodal analgesia is based on the concomitant use of two or more analgesic agents or analgesic techniques with the aim of reducing the dose and the associated adverse effects of each individual drug, while maintaining overall efficacy. The expected outcome of multimodal

analgesia is therefore improved pain control, enhanced functional recovery and reduced opioid-related adverse effects. Pain management is a fundamental aspect of both human and veterinary clinical practice, as achieving adequate postoperative analgesia is essential for ensuring patient welfare. However, effective postoperative pain control can sometimes be challenging, as the effectiveness of pain management depends on multiple factors related to the procedure, patient characteristics, and available treatment techniques (O'Neill & Lirk, 2022). A major challenge associated with combination therapy is that the onset and duration of action of different drugs do not always overlap, which may complicate the assessment of their individual and combined efficacy. Therefore, the selection of drugs should consider their sites and mechanisms of action to ensure a rational combination (Patel & Dickenson, 2022).

Opioids remain among the most widely used analgesic in both the preoperative and postoperative periods. However, several other drugs have emerged as valuable components of multimodal analgesic protocols to achieve optimal pain control, including acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), and selective cyclooxygenase-2 (COX-2) inhibitors. More recently, several additional drugs have been increasingly used in the postoperative period, including N-methyl-D-aspartate (NMDA) receptor antagonists as ketamine, $\alpha 2$ agonists such as dexmedetomidine, anticonvulsants such as gabapentin and pregabalin and corticosteroids (Joshi, 2005).

Moreover, of particular relevance are regional or local analgesia techniques, such as epidural analgesia, peripheral nerve blocks, wound infiltration and intra-articular or intracavity administration of local anaesthetics (Joshi, 2005). The administration of local anaesthetics as part of a multimodal analgesic protocol, in combination with systemic analgesics, has been shown to reduce both intraoperative nociceptive indicators and postoperative pain scores compared with systemic analgesics alone. Indeed, these drugs exert their analgesic effect by preventing nociceptive impulses from reaching the central nervous system (CNS), primarily by blocking voltage-gated sodium channels in peripheral nerves (Grubb & Lobprise, 2020).

In conclusion, the selection of analgesic combinations should consider both their efficacy and adverse effect profile, as superior pain relief may not necessarily translate into clinical benefit when associated with an increased incidence of adverse events. Although multimodal analgesia can improve postoperative pain control, the optimal combination for specific patients and surgical procedures remains unclear (Joshi, 2005).

4.2 Opioids

Opioids are a class of analgesic drugs widely prescribed for the treatment of acute and chronic pain. The use of opium, along with the recognition of its pharmacological effects, dates to approximately 5000 BCE, when the Sumerians cultivated the opium poppy (*Papaver somniferum*) and extracted it for ritualistic and medicinal purposes (Gamba, 2020).

The first documented administration of opium to domestic animals is attributed to Robert Boyle in 1659, when he intravenously injected an opium infusion into a superficial vein of the pelvic limb of an experimental dog, inducing deep stupor. Subsequently, in 1664, Robert Hooke introduced the concept of using opiate narcosis in surgical procedures. However, given the limited utility of opium as a surgical anaesthetic agent, James Moore described its postoperative analgesic properties in 1784 (Lamont, 2024). Despite its long history of use, the modern clinical use of opium began in 1817, with the first isolation of its primary active constituent, morphine (Gamba, 2020).

The term *opioid* refers to any endogenous or exogenous compound, whether natural, semisynthetic or synthetic, that produces morphine-like effects. In contrast, the term *opiate* specifically refers to the naturally occurring alkaloids derived from opium (Gamba, 2020). Both opioids and opiates mediate their effects through opioid receptors. The opioid receptors belong to the family of G-protein-coupled receptors and comprise four main receptor types: the μ (MOP), δ (DOP), and κ (KOP) opioid receptors, and the nociception/orphanin FQ (N/OFQ) or nociception opioid peptide (NOP) receptors. These receptors are extensively expressed throughout the body, including the central and peripheral nervous systems, as well as in several peripheral tissues. Consequently, their activation results in a wide range of physiological effects. Their localization in pain-related pathways mediates the analgesic effects of opioid agonists; however, receptor distribution varies among species, and increased spinal μ opioid receptor density has been reported in chronic pain states. Opioids can also be classified based on their receptor interactions. Full agonists produce a dose-dependent response with maximal receptor activation. Similarly, partial agonists also produce a dose-dependent response; however, they achieve a lower maximal effect compared with full agonists. Finally, antagonists bind opioid receptors with high affinity but without activating them, thereby blocking the effects of agonists (Lamont, 2024).

4.2.1 Main molecules used in dogs

Full opioid agonists commonly used in veterinary medicine include morphine, methadone, fentanyl and its structural analogues, remifentanyl and sufentanyl. Buprenorphine is the main representative of partial opioid agonists, whereas butorphanol exhibits mixed opioid agonist-antagonist activity. Finally, naloxone is an opioid antagonist primarily used as an antidote for opioid-induced effects and toxicity.

- Morphine is a natural alkaloid derived from the opium, and it is considered a full agonist at the μ -opioid receptor, with a higher binding affinity for the μ_1 subtype compared to the μ_2 subtype. In dogs, it is used for perioperative analgesia, and it has been shown to provide effective pain control for a wide range of surgical procedures. Morphine efficacy has been demonstrated across multiple routes of administration, including parenteral, epidural, intrathecal, and intra-articular. Following intravenous administration in dogs, morphine has a reported effective dose of 0.71 mg/kg, an onset of analgesia of approximately 10 minutes and an analgesic duration of 2-4 hours (Lamont, 2024).
- Methadone is a full agonist of μ -opioid receptors, with an analgesic potency approximately 1.0-1.5 times greater than morphine, while exhibiting similar analgesic properties (Pascoe, 2000). It binds also to monoamine, and it is an antagonist of NMDA receptors, for this reason it could be beneficial in the treatment of chronic pain, hyperalgesia and central sensitization (Lamont, 2024). In dogs, methadone oral bioavailability is lower than in humans with terminal half-lives range from 1.75-4 hours following IV administration and 2-12 hours following SC administration (Ingvast-Larsson et al., 2010). It is principally administered intravenously or intramuscularly, and its main application is in the perioperative setting (Lamont, 2024). It may be used as a sole agent or, more frequently, combined with sedatives and tranquilizers as part of a preanesthetic medication protocol (Bufalari et al., 2012) The recommended dose range of single shot methadone in dogs is 0.1-0.5 mg/kg IV and IM (Gamba, 2020).
- Fentanyl is a highly protein-bound, lipophilic derivate, with a rapid onset of action (Lamont, 2024), of about 2-3 minutes (Pascoe, 2000). Due to its rapid redistribution from the central to the peripheral compartment, it has a short duration of action and for this reason it is often administered as CRI. The recommended dose range of single shot fentanyl in dogs is 2-10 μ g/kg IV, followed by 3-20 μ g/kg/h IV CRI. In dogs, fentanyl is rapidly adsorbed following SC administration, exhibiting a pharmacokinetic profile not

markedly different from that observed after IV injection. It is an effective analgesic for the management of acute pain. However, transdermal fentanyl patches also exist and can provide prolonged postoperative analgesia, with effects lasting up to 72-96 hours and the same adverse effects observed following parenteral administration (Lamont, 2024).

- Buprenorphine is a partial agonist of μ -opioid receptor, with an antagonism for κ -opioid receptors. Its role as a partial agonist results in pharmacological effects similar to those of full agonist, but without eliciting the same degree of effect (Pascoe, 2000). In Europe, buprenorphine is available as a 0.3 mg/mL injectable formulation for intravenous (IV) and intramuscular (IM) administration, providing analgesia for 6-8 hours in dogs. It can also be administered via the subcutaneous (SC), intranasal (IN), and oral transmucosal (OTM) routes and is available in transdermal patches, similarly to fentanyl (Lamont, 2024). The recommended dose range of single shot buprenorphine in dogs is 5-20 μ g/kg IV, IM, SC (Gamba, 2020).
- Butorphanol exhibits mixed activity at opioid receptors, acting as an antagonist to partial agonist at μ -opioid receptors and as an agonist at κ -opioid receptors. This receptors profile allows to attenuate some of the effects mediated by μ -opioid receptors, while still producing analgesic effects (Lamont, 2024). Despite exhibiting a receptor binding potency approximately 7-10 times greater than that morphine, butorphanol appears to be less effective as an analgesic in patients experiencing severe pain (Pascoe, 2000). Butorphanol efficacy has been demonstrated across multiple routes of administration, including IV (bolus and CRI), IM or PO. It produces antitussive and mild sedative effects, but no analgesic effect following oral administration (Lamont, 2024). The recommended dose range of single shot butorphanol in dogs is 0.1-0.4 mg/kg IV, IM (Gamba, 2020), with a duration of activity of at least 2 hours (Pascoe, 2000).
- Naloxone, as a short-acting opioid receptor antagonist, is primarily used to reverse the sedative and the other μ -opioid receptor mediated effects, induced by full agonists. It has a rapid adsorption following IV and IM administration (Lamont, 2024), and the recommended dose range of single shot naloxone in dogs is 10-40 μ g/kg IV (Gamba, 2020). An alternative route of administration for naloxone is the intranasal route, with a required dose four times higher than the IV dose, and it is mainly employed in emergency settings for the management of accidental opioid exposure (Lamont, 2024).

4.2.2 Physiological and collateral effects

Opioids interact with multiple organ systems and can therefore produce both therapeutic and adverse effects. Among the principal effects, those on the nervous system are particularly prominent, especially their analgesic properties. Opioids provide potent, sustained, and dose-dependent analgesia in both acute and neuropathic pain (Gamba, 2020). In addition, activation of opioid receptors, expressed on the peripheral terminals of primary afferent neurons innervating inflamed tissues can produce potent antinociceptive effects.

The choice of opioid should be guided by the type of pain being treated, as well as by patient-related and drug-specific factors. In patients with acute pain, particularly acute surgical pain, full μ -opioid receptor agonists are generally preferred because they provide greater analgesic efficacy, whereas partial and mixed agonists may require greater receptor occupancy to achieve comparable analgesia (Lamont, 2024). However, opioids may also produce pronociceptive effects through mechanisms involving sensitization of the nervous system. Strategies to mitigate these effects include opioid rotation, reduction of the μ -agonists dose, and the use of a multimodal analgesic approach combining different analgesic agents (Gamba, 2020).

The most common adverse effects of opioid therapy on the nervous system include euphoria, dysphoria and dose-dependent sedation, although the degree of sedation does not always correlate with the level of analgesia. In addition to individual patient-related factors, differences of species also influence opioid induced sedation. For example, cats often exhibit euphoria and/or dysphoria instead of consistent sedation following opioid administration. Similarly, dogs sedated with inhalant anaesthetics and μ -agonists have been reported to display dysphoric behaviours (Lamont, 2024).

Regarding cardiovascular system, opioids may induce bradycardia and occasional hypotension through increased parasympathetic tone and reduced sympathetic activity. The reduction in heart rate is associated with the dose-dependent effect on cardiac index (CI). In fact, studies have reported that in conscious dogs, 120 minutes after intravenous administration of morphine (1 mg/kg) HR and CI resulted reduced by 26% and 19%, respectively; whereas methadone, at similar doses, produced even more marked cardiovascular depression (Lamont, 2024).

Respiratory effects of opioids primarily include respiratory depression, bronchoconstriction and reduced cough reflex activity. Opioids produce a dose-dependent reduction of the responsiveness of medullary respiratory centres to hypoxic and hypercapnic stimuli, and this

respiratory depressant effect is inseparable from their analgesic action, even at low doses. Bronchoconstriction is instead attributed to the direct action of opioids on airway smooth muscle, mediated by histamine release, induced by certain agents, such as morphine (Gamba, 2020).

The gastrointestinal effects of opioids represent some of the most clinically secondary adverse effects. Furthermore, a clear correlation exists between the opioid dose administered and the incidence of adverse effects. The mechanisms underlying opioid-induced nausea and vomiting are both peripheral and central. Peripheral mechanisms involve delayed gastric emptying, due to reduced activity of gastric longitudinal muscle fibres, whereas central mechanisms involve stimulation of the Chemoreceptor Trigger Zone. Delayed gastrointestinal transit and reduced secretions induced contribute to constipation, the most common adverse effect of long term opioid therapy (Gamba, 2020).

Opioids also exert effects on the renal system, promoting urinary retention through increased tone of the circular fibres of the bladder sphincter and reduced contractility of longitudinal bladder fibres (Gamba, 2020).

Chapter 5: Laparoscopic ovariectomy in dogs

Laparoscopic ovariectomy is one of the most commonly performed minimally invasive soft tissue procedure in veterinary medicine, along with laparoscopic ovariohysterectomy. Originally, these procedures were reserved for medium- to large-breed dogs because of the difficulties associated with the limited working space and the use of rigid instruments in small patients. However, they can currently be performed in dogs of any breeds and also in cats. Dogs and cats of different ages, sizes and breeds can be suitable candidates for laparoscopic sterilization, but it must be considered that small working space contributes to longer operative times and consequently to hypothermia and associated risks (Fransson & Mayhew, 2015).

The most common indication for ovariectomy is elective sterilization in patients with normal uterus. In contrast, in the presence of uterine pathology, ovariohysterectomy is generally preferred (Johnston & Tobias, 2018). Relative contraindications to laparoscopic ovariectomy are few and may include patients in active estrus, early pregnancy or those affected by pyometra. Pathological conditions such as cardiopulmonary compromise or known diaphragmatic hernia should also be considered contraindications because carbon dioxide insufflation may be dangerous in these cases (Fransson & Mayhew, 2015).

5.1 Relevant Anatomy

The anatomical structures of primary relevance to this procedure include: ovaries, ovarian pedicles, suspensory ligaments, uterine horns and associated connective tissues (Fransson & Mayhew, 2015).

In dogs and cats, the ovaries are located caudal to the kidneys and occasionally the left ovary is larger than the right one. They are characterized centrally by the medulla, which contains blood and lymphatic vessels, nerves, smooth muscle fibers, and connective tissue; peripherally by the cortex, a connective tissue stroma containing follicles; and a hilar region with the rete ovarii. On the surface, connective tissue forms a capsule called the tunica albuginea, which is covered by the peritoneum. The peritoneum forms a double fold around the ovary, creating the ovarian bursa (Johnston & Tobias, 2018). In dogs, the ovaries are usually completely concealed in the ovarian bursa with the associated fat obscuring, consequently, the view of the actual ovary. The ovaries are anchored to the dorsolateral abdominal wall through the mesovarium, a peritoneal fold containing the ovarian vasculature and connected with the suspensory ligament cranially and the mesometrium caudally (Fransson & Mayhew, 2015). Together, mesovarium and mesometrium constitute the broad ligaments. The ovarian vascularization is composed by the paired ovarian arteries and the ovarian veins, which anastomose with the uterine vein. The lumbar lymph nodes represent the main lymphatic drainage station for the ovarian lymphatic vessels. Ovarian innervation is constituted by the sympathetic division of the autonomic nervous system (Johnston & Tobias, 2018).

The uterus is composed by the cervix, the body, located in the pelvic and abdominal cavities, and the uterine horns. Histologically, it is divided into three tunics: the serosa, the muscularis layer, called the myometrium, and the mucosa, called the endometrium (Johnston & Tobias, 2018). It is attached to the dorsolateral body wall by the mesometrium, which contains the uterine arteries and veins (Fransson & Mayhew, 2015). The vascularization is composed by uterine arteries and by the anastomosis with the ovarian arteries. The hypogastric and lumbar lymph nodes represent the main lymphatic stations for the uterine lymphatic vessels. The uterine innervation is supplied by the pelvic plexus; the parasympathetic innervation is paired through pelvic nerves, while the sympathetic innervation through left and right hypogastric nerves (Johnston & Tobias, 2018).

5.2 Patient Preparation

Patient surgical preparation is similar to that required for any surgical procedures. It begins the night before with at least 8-12 hours of fasting; while water is allowed overnight but removed the morning of surgery. Following admission to the hospital, the patient should be subjected to a physical examination and appropriate preoperative blood testing. Depending on the timing of surgery, it may be particularly helpful to walk patients scheduled for laparoscopy to encourage urination before the procedure, as the working space during caudal abdominal procedures may be limited by a distended bladder, which may also increase the risk of bladder rupture. Subsequently, premedication is administered and an intravenous catheter is placed. Following anaesthetic procedures, including induction, endotracheal intubation and placement of monitoring equipment, the abdominal surgical field is prepared by hair clipping. In the absence of transabdominal ovarian suspension, surgical preparation of the ventral abdomen for the laparoscopic ovariectomy is performed in a similar manner to that used for open ovariohysterectomy. Hair clipping extends laterally beyond the mammary line bilaterally, cranially to the rib cage and caudally to the pubis, thereby allowing conversion to an open procedure if necessary. Conversely, when transabdominal ovarian suspension is planned, a more extensive area of hair clipping is required, extending to approximately 50% of the distance between the ventral and the dorsal midline.

In the operating room, after positioning the patient in dorsal recumbency with the legs secured at all four corners of the table, a surgical scrub should be performed. For this procedure, the use of a tilt table can be highly beneficial. In fact, during the laparoscopy, the patient is flipped almost in lateral recumbency to move the intraabdominal organs away from the underlying ovary, especially on the left side, where the spleen generally cover the ovary. Furthermore, appropriate tower alignment with the patient must also be taken into consideration. For laparoscopic ovariectomy, the tower can be set up also at the head or back of the operating table (Fransson & Mayhew, 2015).

5.3 Surgical technique and Complications

Laparoscopic ovariectomy requires specific equipment, including: a telescope, laparoscopic Kelly or Babcock forceps for manipulating the proper ligament or uterine horn, cannulae for the exportation of the tissues and some version of vessel sealing device (VSD). The surgical technique can be performed by the placement of three, two or one portals (Fransson & Mayhew, 2015).

- The three-port technique encompass both the approach involving the placement of one median portal at the umbilicus with two lateral portals and the approach in which all ports are placed along the ventral midline (Fransson & Mayhew, 2015).

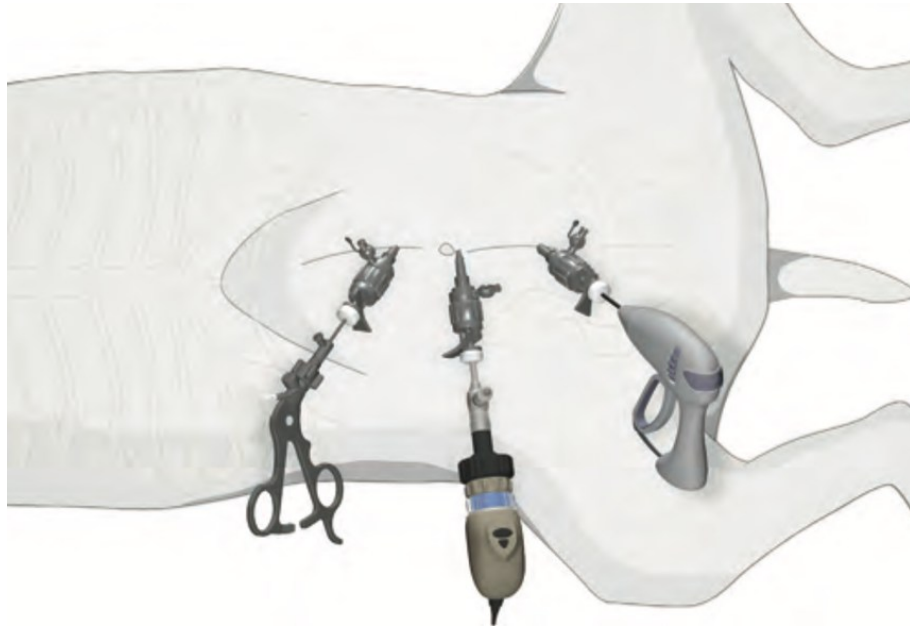


Figure 3: Three-port technique (Fransson & Mayhew, 2015)

- The two-port technique, which use the ovariectomy needle suspension method, is considered the most advantageous technique in terms of pain control and surgical times. It consists in placing one port 1 cm caudal to the umbilicus and one port 2 to 5 cm cranial to the pubis. After port placement, and the CO₂ insufflation, to distend the abdomen, the patient should be rotated approximately 15 to 30 degrees toward the surgeon to displace the viscera away from the targeted ovary, which is generally located directly caudal to the kidney. Once the uterine horn has been identified, the proper ligament is grasped using laparoscopic Kelly or Babcock forceps and elevated toward the body wall. Consequently, the ovary is suspended by using an OVE hook or a half-circle needle that is placed percutaneously through the body wall, incorporating the proper ligament or cranial uterine horn or mesometrium and is then brought back out through the body wall. The suture is then clamped with mosquito haemostats to hold the ovary suspended tight against the body wall. At this point, the Kelly or Babcock forceps can be removed from the abdomen, thereby providing space for the VSD. The VSD should be applied along a line extending from the suspensory ligament to the proximal uterine horn, maintaining

close proximity to the ovary and uterine body while ensuring avoidance of the ureters and large mesometrial vessels.

Once the ovary has been separated from the uterus, it should be left suspended to the body wall by the suture. The VSD is then removed and the patient repositioned to dorsal recumbency. At this stage of the procedure, it is recommended to complete both sides and then remove both ovaries at the end through the caudal port, in order to avoid enlarging the surgical port site and losing the pneumoperitoneum established by insufflation or losing the ovary in the abdomen. At the end, after removing both the ovaries, the cannulae are removed and the body wall is closed routinely (Fransson & Mayhew, 2015).

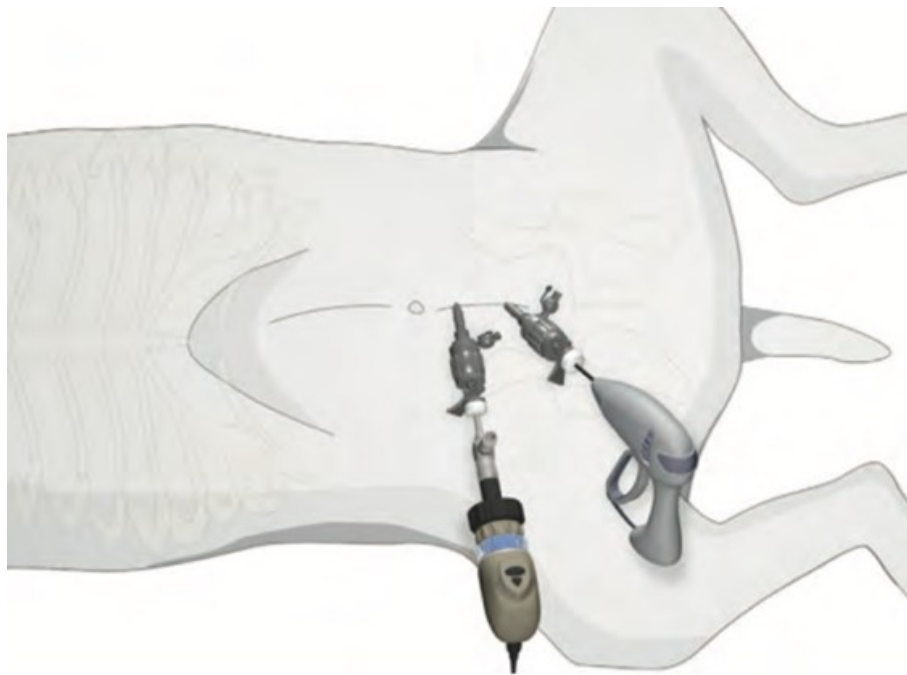


Figure 4: Two-port technique (Fransson & Mayhew, 2015)

- The one-port technique involves the placement of a 12 mm portal 1 to 2 cm caudal to the umbilicus, through which a 10 mm telescope with an operating channel is introduced (Fransson & Mayhew, 2015).

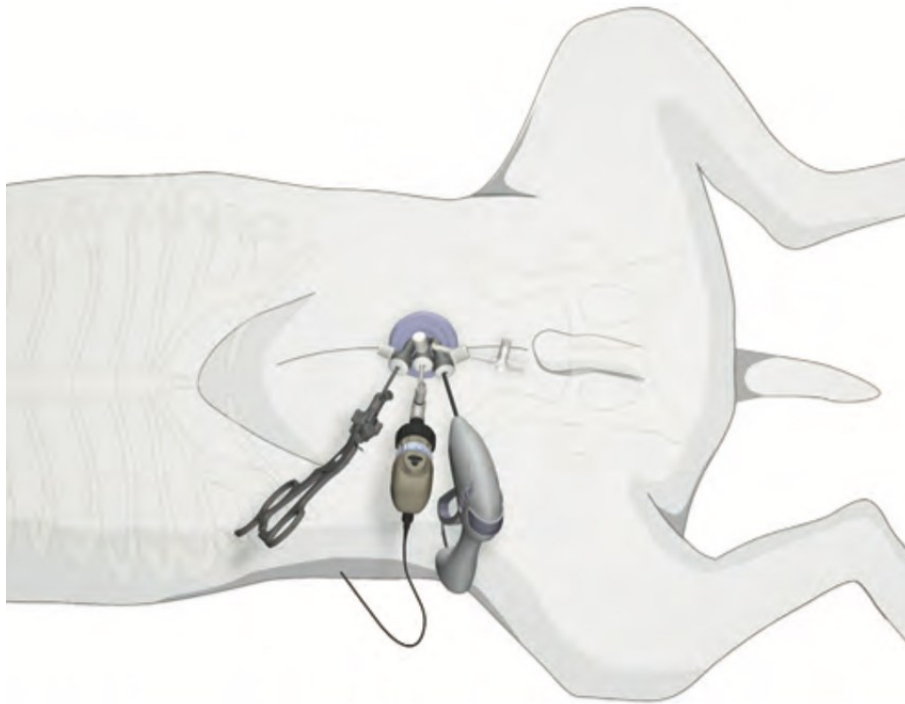


Figure 5: One-port technique (Fransson & Mayhew, 2015)

Complications may occur during any surgical procedure. In this case, complications are relatively rare but should nevertheless be taken into consideration. They can be divided into intraoperative and postoperative complications. Intraoperative complications include insertional trauma to intraabdominal organs, especially the spleen, which intra-abdominal haemorrhage reported in 5% to 18.7% of cases; urinary bladder puncture; bleeding from the pedicle; dropping the ovary; burning the peritoneum or other organs with the VSD; and leakage of CO₂ from cannulae into subcutaneous tissue.

Although conversion to laparotomy is occasionally required, it should not be regarded as a complication, but rather as a consequence of certain complications described above. The most frequent indication is uncontrolled haemorrhage from the spleen or vascular pedicle. On the other hand, reported postoperative complications rate of 3.9% to 31% and include incisional problems including seroma and hematoma at the suspension suture site; port site infection; retained ovarian tissue; intermittent vaginal haemorrhage; herniation of omentum through an incision site; and ongoing bleeding from a pedicle (Fransson & Mayhew, 2015).

Experimental Topics

Objective

The aim of the present study was to evaluate the effects of preoperative gabapentin administration in dogs undergoing laparoscopic ovariectomy, with particular focus on its intraoperative and postoperative analgesic effects. Within a multimodal analgesic approach, the study investigated whether the addition of gabapentin to the pre-anaesthetic protocol could reduce the requirement for additional analgesic drugs, particularly opioids, both during and after surgery.

To evaluate its intraoperative effects, physiological parameters were monitored throughout anaesthesia, with particular attention to changes occurring in response to the most nociceptive surgical stimuli. The same parameters were also assessed during the postoperative period, together with pain assessment using the Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF). In addition, end-tidal isoflurane concentrations were monitored throughout the intraoperative period to evaluate the potential contribution of gabapentin to a multimodal anaesthetic approach and its possible isoflurane-sparing effect.

The primary objective was therefore to determine whether preoperative gabapentin administration could reduce intraoperative and postoperative opioid requirements. The secondary objective was to evaluate its potential isoflurane-sparing effect, as an additional component of multimodal anaesthetic and analgesic management in dogs undergoing laparoscopic ovariectomy.

Materials and Methods

A total of 14 female dogs undergoing elective laparoscopic ovariectomy at the OVUD (Ospedale Veterinario Universitario Didattico) of the University of Parma were included in this study.

Client-owned dogs were enrolled, while dogs scheduled to undergo concomitant gastropexy at the surgeon's discretion were excluded. No specific restrictions were imposed regarding breed, body weight, or age. Inclusion criteria required dogs scheduled to undergo elective sterilization surgery and to be classified as anaesthesiologic risk categories ASA 1 or 2, therefore considered clinically healthy based on physical examination, complete blood count and serum biochemical analysis.

All dogs underwent a thorough pre-anaesthetic examination before the surgical procedure. During this assessment the general clinical condition of each patient was evaluated, and the owners were instructed to withhold food for at least 8 hours before anaesthesia. This fasting period was recommended to reduce the risk of regurgitation and aspiration during anaesthesia and to ensure the safety of the anaesthetic procedure.

Before anaesthesia was induced, owners were informed about the nature of the procedure and its potential risks and provided written informed consent.

The dogs were randomly assigned to two groups: group G received 10 mg/kg gabapentin (Gabapentin Teva Pharma®, Teva Pharma B.V., Netherlands) 30 minutes before the anaesthetic induction, by an external operator who was not involved in the perioperative and postoperative assessment of the patient, this approach was adopted to minimize the risk of observer bias during the subsequent evaluation of the dogs; whereas group C served as the control group and did not receive gabapentin.

All dogs included in the study received the same anaesthetic premedication, consisting of 3 mcg/kg of dexmedetomidine (Dexdomitor®, Vetoquinol Italia S.r.l., Italy) and 0,2 mg/kg of methadone (Semfortan®, Eurovet Animal Health B.V., Netherlands), both administered intramuscularly. Approximately 20 minutes after premedication, the degree of sedation was assessed. Following clipping and aseptic preparation of the skin, an intravenous catheter was placed in one of the cephalic veins. Prior to induction, the patients were preoxygenated with 100% oxygen using an appropriately sized face mask. Once an adequate level of sedation had been confirmed, anaesthetic induction was performed. General anaesthesia was induced by intravenous administration of propofol (Proposure®, Merial, Italy) to effect, until loss of the palpebral reflex and the achievement of an adequate depth of anaesthesia for orotracheal intubation. Following intubation, the patients were connected to an appropriate breathing system and monitoring system (GE HealthCare B125M), and intravenous fluid therapy with lactated Ringer (RL, SALF, Italy) solution was initiated at a rate of 2-3 mL/kg/h. In both groups, anaesthesia will be maintained with the lowest effective concentration of isoflurane (IsoFlo®, Zoetis Spain S.L., Spain) in 60% oxygen. All interventions were systematically recorded and documented in the anaesthetic record.

The patient was subsequently transferred to the operating room, where it was positioned in dorsal recumbency within a dedicated positioning cradle and mechanically ventilated (GE

HealthCare Carestation™ 620) to maintain an end-tidal carbon dioxide (ETCO₂) concentration between 35 and 45 mmHg.

Throughout the procedure, all patients were monitored at 5-minute intervals using a multiparameter monitor. The depth and adequacy of anaesthesia were assessed by evaluating the following clinical parameters: heart rate (HR), respiratory rate (RR), systolic arterial pressure (SAP), mean arterial pressure (MAP), and diastolic arterial pressure (DAP) (measured using either non-invasive or invasive method), peripheral oxygen saturation (SpO₂), end-tidal carbon dioxide concentration (ETCO₂), and end-tidal isoflurane concentration (ETISO%). All these parameters were also monitored at specific time points during surgery:

1. Positioning on the operating table (T0)
2. Placement of Backhaus clamps, following surgical field preparation (T1)
3. Surgical incision (T2)
4. Insertion of the first portal (T3)
5. CO₂ insufflation in patient abdomen (T4)
6. Clamping of the first ovary (T5)
7. Clamping of the second ovary (T6)
8. Closure of the portal insertion site (T7)
9. Completion of surgery (T8)

Intraoperative nociceptive responses were assessed by continuously monitoring the physiological parameters of each patient, with particular attention to heart rate. Changes in these parameters were evaluated in relation to the surgical phase and to the baseline values recorded before the occurrence of the suspected stimulus. In particular, an increase in heart rate of 20% or more compared with the baseline value was considered indicative of a possible nociceptive response and was therefore interpreted as a sign of intraoperative pain, provided that no other identifiable cause could explain the change. To manage the nociceptive stimulus, the anaesthetic protocol included the administration of a fentanyl (Fentadon®, Eurovet Animal Health BV, Netherlands) bolus at a dose of 1,5 mcg/kg. If, 5 minutes after administration of the bolus, heart rate value did not return to baseline, a second bolus was administered at the same dose. If the second bolus failed to achieve the desired effect, a Controlled Rate Infusion (CRI) of fentanyl was initiated, with the infusion rate adjusted according to the patient's requirements.

At the end of the procedure, the isoflurane concentration used for anaesthetic maintenance was gradually reduced until complete discontinuation. Additionally, all patients received 0,2 mg/kg of meloxicam (Meloxydil®, Ceva Santé Animale, France) intravenously. Once the palpebral reflex had returned, followed by the laryngeal reflex, the patients were extubated and transferred to the recovery room, where they were continuously monitored by an experienced operator until complete recovery.

During the surgical procedure, any episodes of bradycardia (HR <60pm) and hypotension (MAP <60mmHg) were documented, as they are considered relevant indicators for evaluating the hemodynamic stability of the patient. If such events occurred and persisted, the patient would have been excluded from the study and treated accordingly. In cases of hypotension, initial treatment consisted of a bolus of lactated Ringer (RL, SALF, Italy) at 3-5 mL/kg over 10 minutes. If blood pressure failed to normalize, norepinephrine (Noradrenalina Tartrato Galenica Senese 2mg/ml®, Industria Farmaceutica Galenica Senese S.r.l., Italy) was initiated at 0,05 mcg/kg/min and titrated by 0,05 mcg/kg/min every 5 minutes until MAP reached = 60 mmHg. In the presence of bradycardia associated with hypotension, atropine (Atropina Solfato Galenica Senese 1,0 mg/ml®, Industria Farmaceutica Galenica Senese S.r.l., Italy) was administered intravenously at a dose of 20 mcg/kg.

Every hour after the completion of the surgical procedure, an operator assessed each patient to evaluate postoperative pain. Postoperative monitoring was continued for a minimum of 5 hours after the end of the surgery. At each assessment, the following physiological parameters were recorded: temperature, heart rate (HR), respiratory rate (RR), systolic arterial pressure (SAP), mean arterial pressure (MAP), and diastolic arterial pressure (DAP) (measured using non invasive method). In addition to monitoring physiological parameters, a clinical assessment of each patient was performed every hour using the Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF) (Reid et al., 2007). The scale used in the study consists of six assessment criteria to determine the degree of pain experienced by the patient. The parameters considered include the behaviour of the dog in the cage, its interest in the surgical wound and the presence of pain upon palpation, its gait, and its emotional state. To each of these aspects is assigned an individual score. The individual scores are then summed to obtain a total score, which provides an overall assessment of the patient's condition. A total score greater than 5 indicates that the patient is

experiencing pain. If the total score exceeds 5, the patient receives analgesic medication at the discretion of the attending operator and is excluded from further monitoring for the study.

SHORT FORM OF THE GLASGOW COMPOSITE PAIN SCALE

Dog's name _____

Hospital Number _____ **Date** / / **Time** _____

Surgery Yes/No (delete as appropriate) _____

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

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

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

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

Figure 6: Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF) (Reid et al., 2007)

Statistical Analysis

For all dogs included in the study, body weight (Kg) and age (years) were recorded and analysed. Several continuous intraoperative parameters were also monitored and evaluated, including heart rate (HR), respiratory rate (RR), systolic (SAP), mean (MAP), and diastolic (DAP) arterial blood pressure, peripheral oxygen saturation (SpO₂), end-tidal carbon dioxide concentration (ETCO₂), and end-tidal isoflurane concentration (ETISO%), as well as the number of additional fentanyl boluses administered during the procedure. Quantitative variables were described using the mean and standard deviation; when appropriate, the median and data distribution were also reported. The Mann-Whitney U test was used to compare the two groups for summary variables and variables that were not normally distributed, whereas categorical variables were compared using the exact test of Fisher. Physiological variables measured from T0 to T8 were analysed using repeated-measures ANOVA, assessing the within-subject effect of TIME, the between-GROUP interaction, and the TIME×GROUP interaction, indicating whether changes over time differed between the two groups. In cases where the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied. As a complementary within-group analysis, the non-parametric Friedman test was performed separately for each group, followed by pairwise comparisons with Bonferroni correction. Postoperative parameters were also evaluated, including temperature (T), HR, RR, SAP, MAP, DAP, and the Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF) assessments. The availability of data progressively decreased at later postoperative time points. Therefore, between-group comparisons at individual time points were performed using all subjects available at each time point, employing the Mann-Whitney U test. Temporal trends within each group were assessed using the Friedman test, restricting the analysis to time points for which an adequate number of subjects with complete measurements were available. Post-hoc pairwise comparisons were adjusted using the Bonferroni correction. Results at later time points with extremely small sample sizes were considered descriptive only. The significance threshold was set at $p < 0,05$.

Results

All dogs enrolled in the study were successfully examined, and none were excluded from the analysis. The subjects differed in terms of breed, body weight, and age. In group G, the mean body weight was 16,5 kg (SD = 11,08), whereas in group C, the mean body weight was 10,4 kg

(SD = 8,35). The mean age of patients was 2,4 years (SD = 1,43) in group G and 1,3 years (SD = 0,76) in group C (Tables 1 and 2).

Group G		
Breed	Body weight (Kg)	Age (years)
Dachshund	3,5	2 aa
Dachshund	4,4	3 aa
Staffordshire Bull Terrier	13,7	1 aa
Rhodesian	32,6	3 aa
Toy Poodle	12,5	1,5 aa
Nova Scotia Duck Terrier	22,9	1 aa
Mixed breed	26,2	5 aa

Table 1: signalment of dogs included in group G (gabapentin)

Group C		
Breed	Body weight (Kg)	Age (years)
Miniature Pinscher	4,4	1 aa
Toy Poodle	4,5	3 aa
Shibainu	7,8	1 aa
Pekingese	4,0	1 aa
French Bulldog	12,9	1 aa
Mixed breed	12	1 aa
German Shepherd	27,5	1 aa

Table 2: signalment of dogs included in group C (control)

Among the parameters assessed during the intraoperative period, no statistically significant differences were observed between the two groups; however, most physiological variables showed changes associated with the different phases of the surgical procedure.

The overall mean heart rate values at T0-T1-T2-T3-T4-T5-T6-T7-T8 were 59,8 beats per minute in the group G and 67,3 beats per minute in the group C. HR showed a statistically significant change

during the procedure ($p=0,015$), as analysis of individual subjects revealed a variation in this parameter over the course of surgery. However, there was no evidence of a significant difference in the pattern of change between the two groups ($p=0,390$). Not statistically significant within-group changes were also detected by Friedman test, with $\chi^2(8)=11,321$; $p=0,184$ in group G and $\chi^2(8)=10,846$; $p=0,211$ in group C. At the same time, also time $\times$ group interaction results not significant ($p=0,838$) (Table 4).

Respiratory rate also showed a significant change over time ($p=0,026$), with no significant difference in the pattern of change between the two groups ($p=0,640$) and the time $\times$ group interaction ($p=0,149$). The Friedman test revealed statistical significance only in the control group ($\chi^2(8)=16,661$; $p=0,034$); however, none of the post-hoc pairwise comparisons remained statistically significant after Bonferroni correction. The mean RR was 14,825 breaths/min in the group G and 15,476 breaths/min in the group C (Table 5).

Similarly, EtCO₂ also showed a significant change over time ($p=0,004$), with no significant difference between gabapentin and placebo groups ($p=0,511$); also, the time $\times$ group analysis results not significant ($p=0,918$). The Friedman test revealed a significance in both groups: group G ($\chi^2(8)=29,816$; $p<0,001$), group C ($\chi^2(8)=20,148$; $p=0,010$). The mean EtCO₂ was 37,127 mmHg in group G and 38,381 mmHg in group C (Table 6).

Blood pressure values showed some variations compared with the other parameters: the mean of SAP was found to be 116,587 mmHg in group G and 121,810 mmHg in group C. SAP showed a significant change over time ($p=0,004$); in contrast the group analysis results not significant ($p=0,682$), neither the time $\times$ group ($p=0,909$). The Friedman test revealed a statistical significance only in the gabapentin group ($\chi^2(8)=16,077$; $p=0,041$), without a significant Bonferroni correction (Table 7).

MAP was 79,968 mmHg in group G and 85,397 mmHg in group C. For this parameter, a variation over time was consistently observed ($p=0,046$), with no significant interaction between the two groups ($p=0,461$). However, the time $\times$ group interaction analysis revealed a trend, although this finding did not reach statistical significance ($p=0,076$). The Friedman test revealed a statistical significance only in the gabapentin group ($\chi^2(8)=16,077$; $p=0,041$), without a significant Bonferroni correction (Table 8).

Finally, DAP was the only parameter showing a different pattern, with a statistically significant time $\times$ group interaction ($p=0,019$). Higher values were observed in the gabapentin group during ovarian clamping. Particularly, at T5, PAD was 83,7 mmHg in the group G and 56,9 mmHg in the

group C; at T6, the corresponding values were 73,0 mmHg and 50,9 mmHg, respectively. In the comparisons between groups at individual time points, the difference at T6 was statistically significant ($p=0,044$), whereas T5 showed a trend toward significance ($p=0,060$) (Table 9).

Unlike all the other parameters, SpO₂ showed no statistically significant changes over time ($p=0,345$) or between groups ($p=0,386$), remaining stable and comparable between the two groups. The mean SpO₂ was 97,143% in group G and 97,921% in group C (Table 10).

Parameters	ANOVA RM	Friedman control	Friedman gabapentin	Synthesis
HR	Time $p=0,015$; group $p=0,390$; time×group $p=0,838$	$\chi^2(8)=10,846$; $p=0,211$	$\chi^2(8)=11,321$; $p=0,184$	Overall effect of time in the ANOVA, but no significant with-in group variation with the Friedman test.
MAP	Time $p=0,046$; group $p=0,461$; time×group $p=0,076$	$\chi^2(8)=8,882$; $p=0,352$	$\chi^2(8)=18,838$; $p=0,016$	Variation over time; Friedman test significant only in the gabapentin group. No significant pairwise comparisons after Bonferroni correction.
SAP	Time $p=0,004$; group $p=0,682$; time×group $p=0,909$	$\chi^2(8)=10,882$; $p=0,208$	$\chi^2(8)=16,077$; $p=0,041$	Effect of time; Friedman test significant only in the gabapentin group, with no significant Bonferroni-adjusted pairwise comparisons.
DAP	Time $p=0,182$; group $p=0,374$; time×group $p=0,019$	$\chi^2(8)=5,364$; $p=0,718$	$\chi^2(8)=20,530$; $p=0,009$	The only significant time×group interaction. Friedman test significant in the gabapentin group; T0-T5 significant after Bonferroni correction ($=0,046$).
EtCO ₂	Time $p=0,004$; group $p=0,511$; time×group $p=0,918$	$\chi^2(8)=20,148$; $p=0,010$	$\chi^2(8)=29,816$; $p<0,001$	Significant change over time in both groups; no significant pairwise comparisons after Bonferroni correction.
SpO ₂	Time $p=0,345$; group $p=0,386$; time×group $p=0,476$	$\chi^2(8)=13,916$; $p=0,084$	$\chi^2(8)=8,530$; $p=0,384$	No significant change over time or difference in trajectory between groups.
RR	Time $p=0,026$; group $p=0,640$; time×group $p=0,149$	$\chi^2(8)=16,661$; $p=0,034$	$\chi^2(8)=13,926$; $p=0,084$	Effect of time; Friedman test significant only in the control group, with no significant pairwise comparisons after Bonferroni correction

Table 3: statistical analysis of the intraoperative parameters

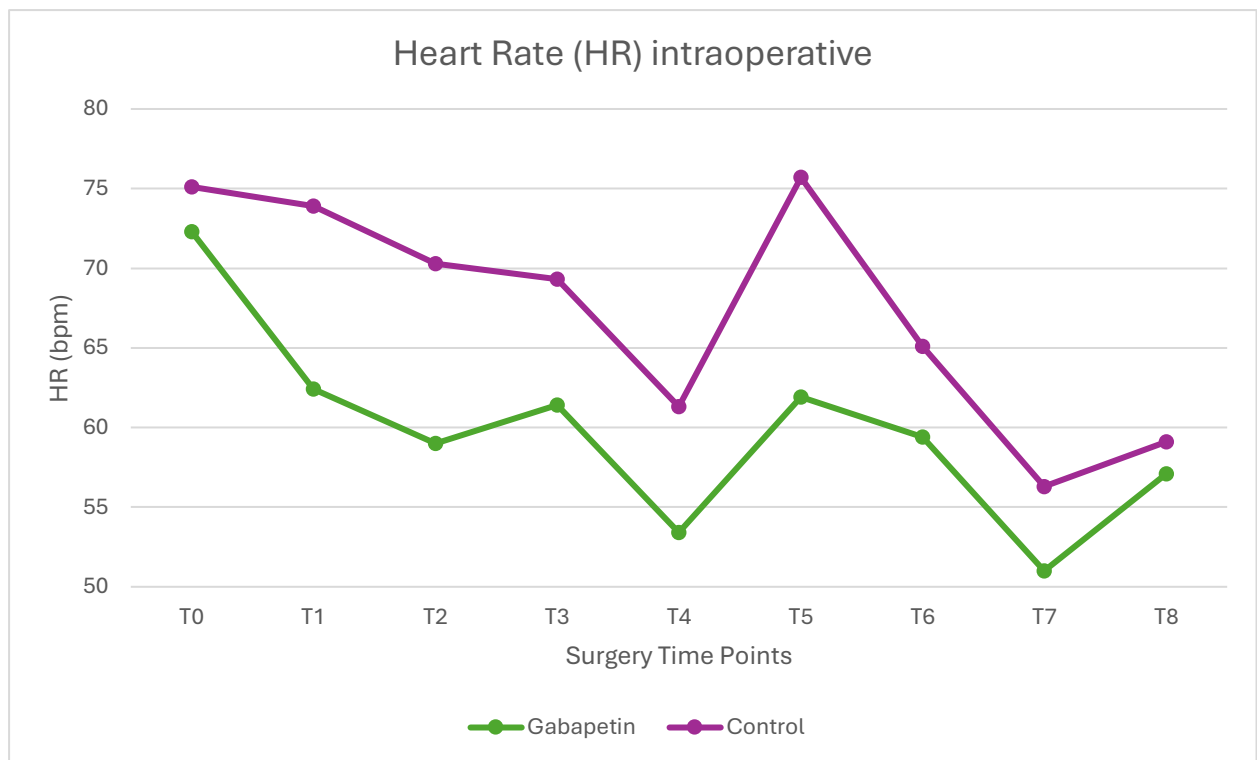


Table 4: mean HR over time, comparison between gabapentin and control groups

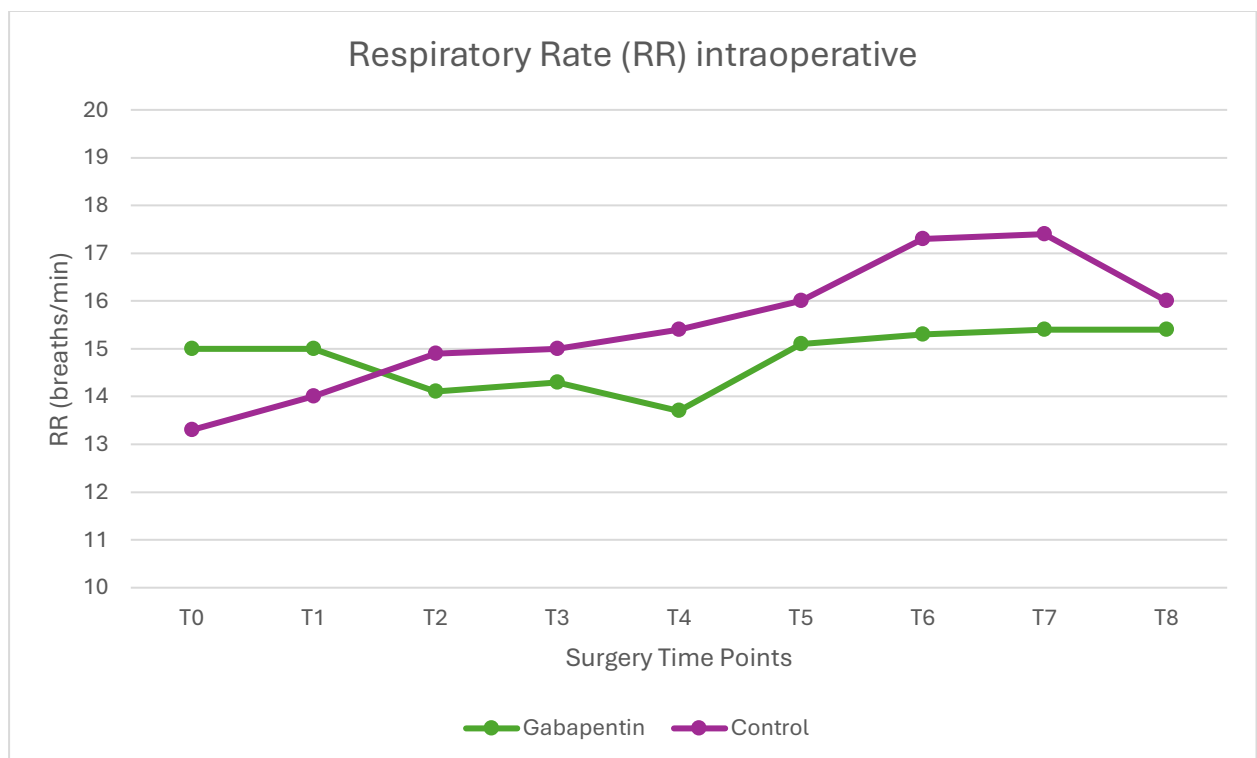


Table 5: mean RR over time, comparison between gabapentin and control groups

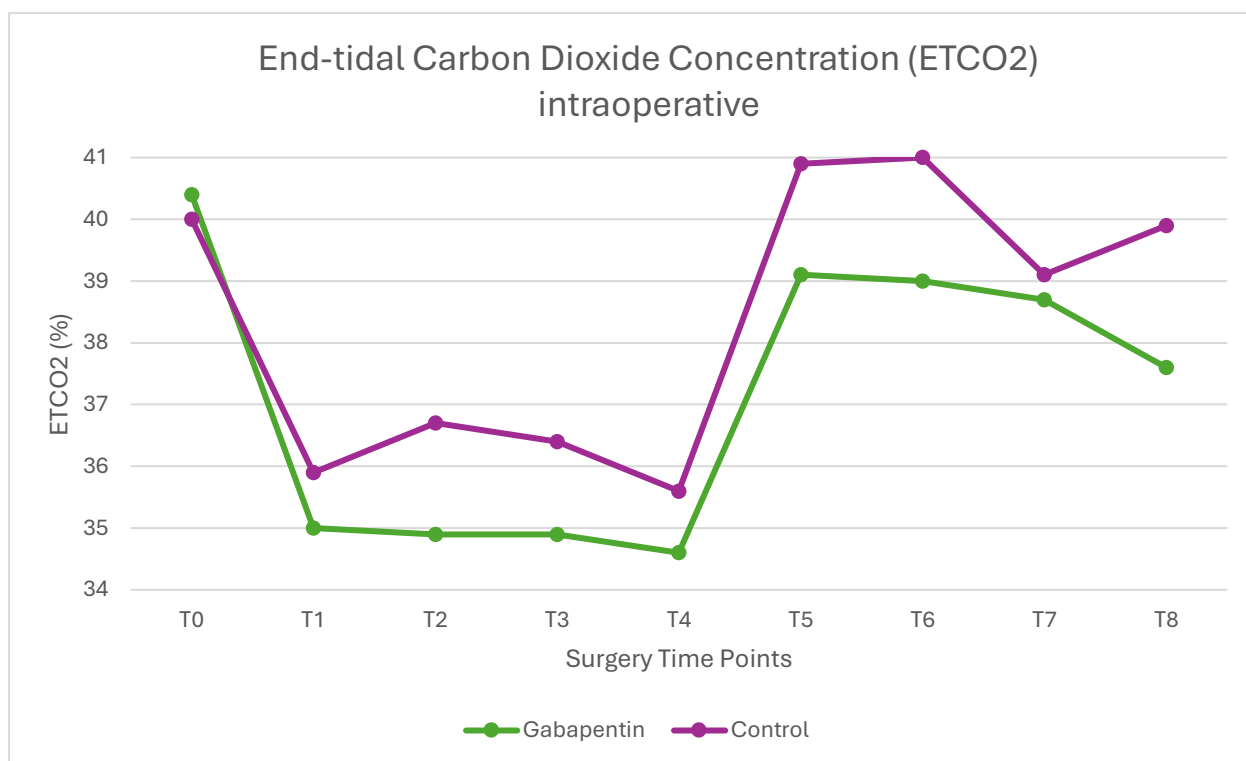


Table 6: mean EtCO2 over time, comparison between gabapentin and control groups

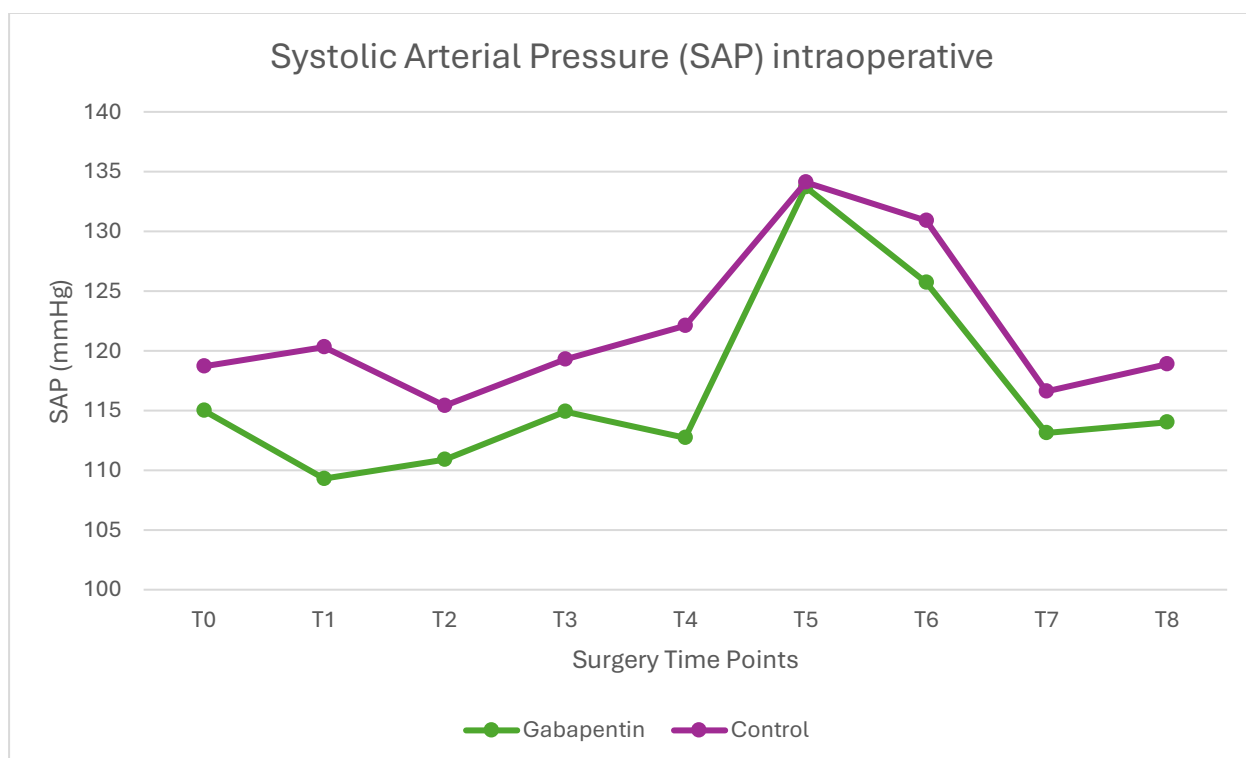


Table 7: mean SAP over time, comparison between gabapentin and control groups

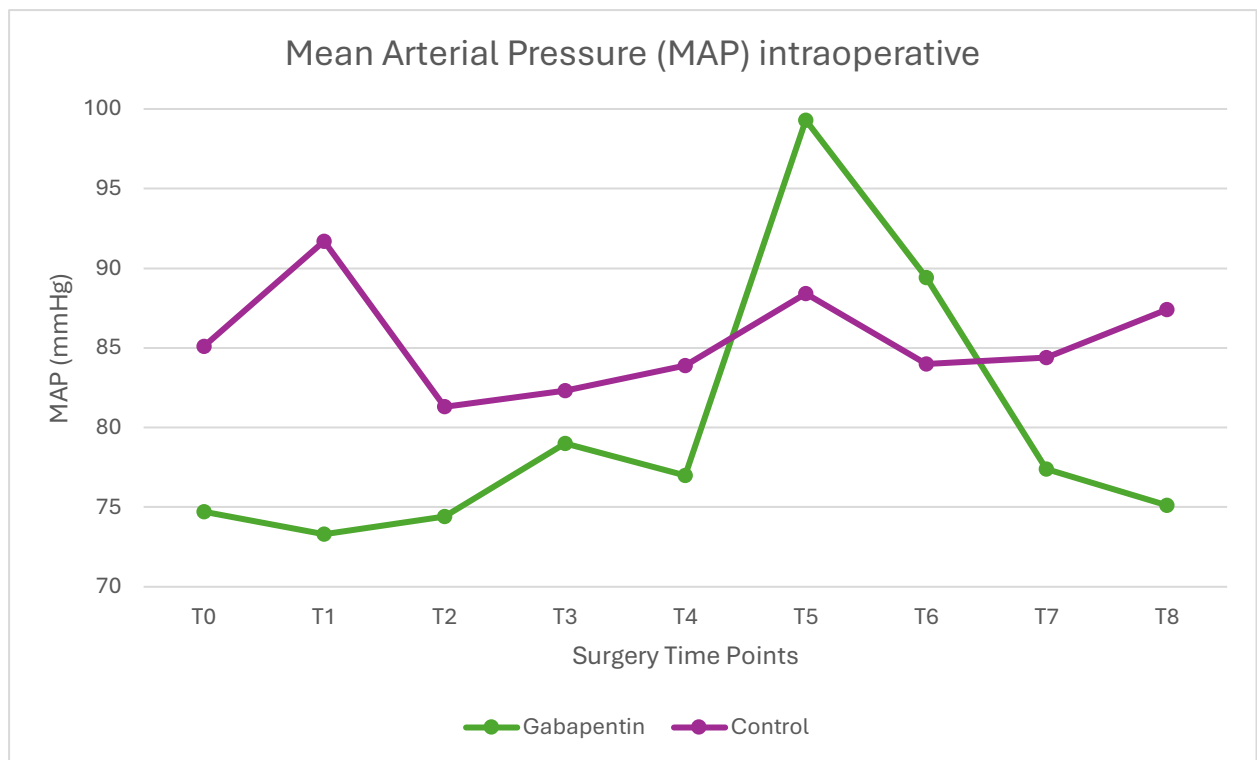


Table 8: mean MAP over time, comparison between gabapentin and control groups

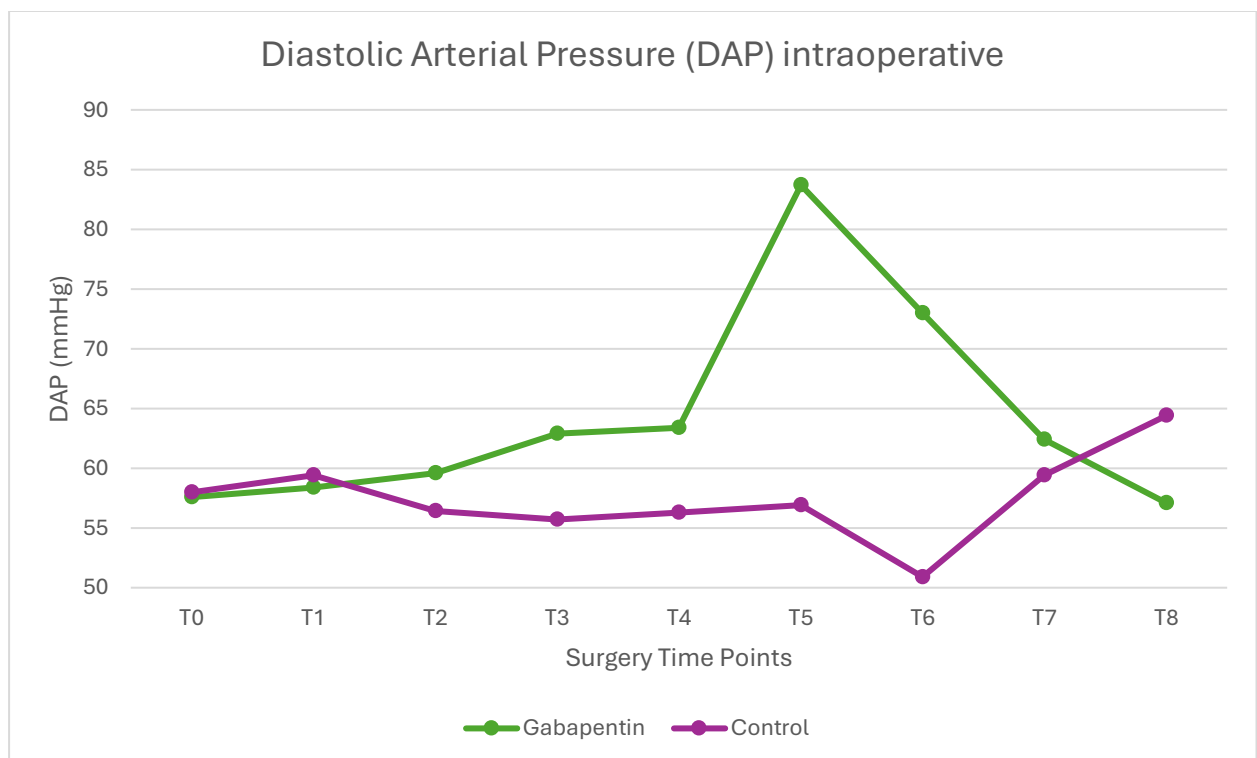


Table 9: mean DAP over time, comparison between gabapentin and control groups

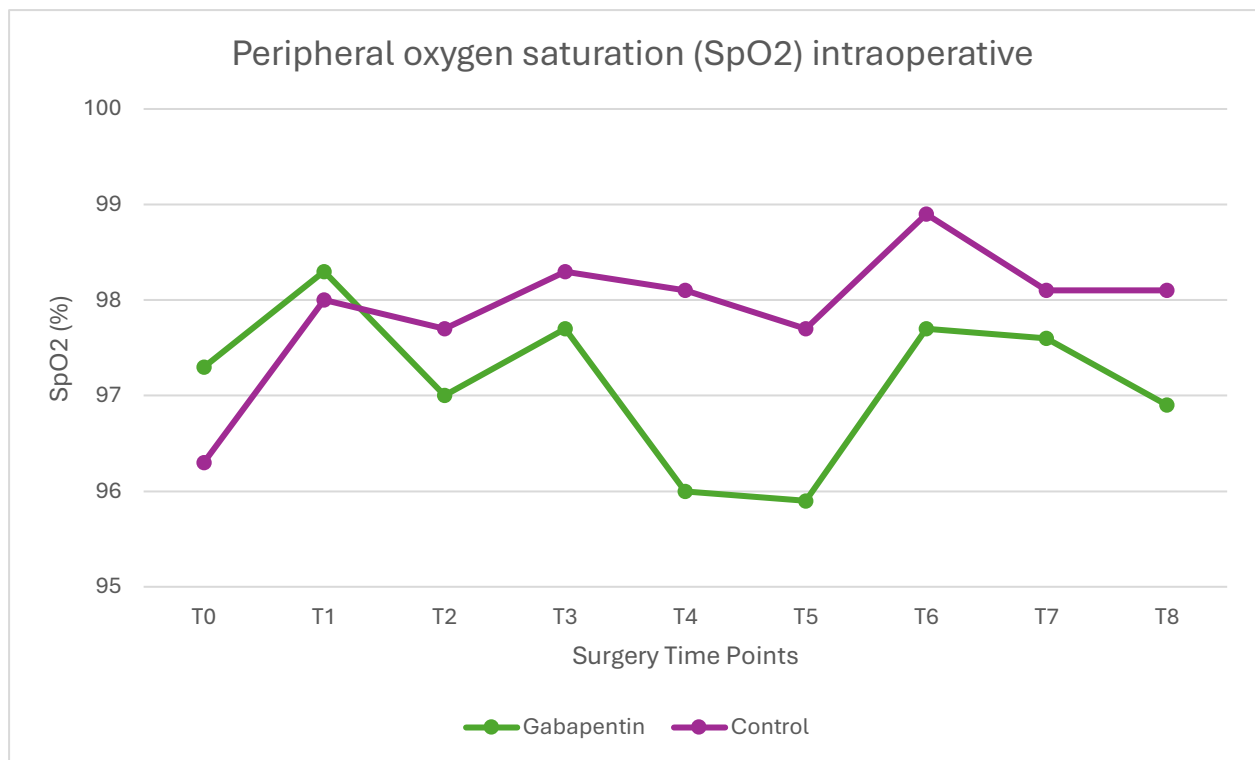


Table 10: mean SpO2 over time, comparison between gabapentin and control groups

Regarding the statistical analysis of the end-tidal isoflurane concentrations, the overall mean isoflurane concentration was comparable between the two groups: $1,154 \pm 0,190\%$ in the gabapentin group (median 1,122%) and $1,144 \pm 0,078\%$ in the control group (median 1,144%). No statistically significant differences were observed in the Mann-Whitney U test ($U=21.0$, $p=0,684$). Analysis of ETISO% revealed a significant effect of time ($p=0,004$), whereas neither the time×group interaction ($p=0,261$) nor the comparison between groups ($p=0,922$) reached statistical significance (Table 11).

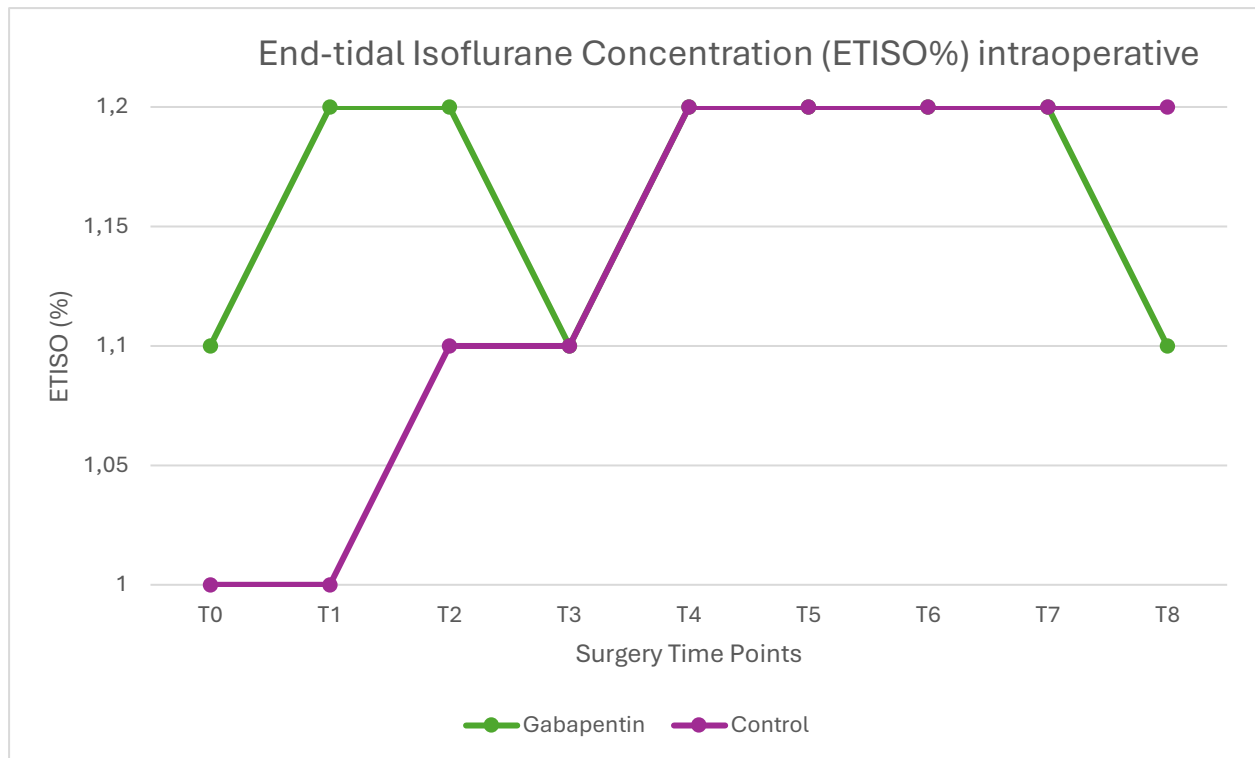


Table 11: mean ETISO% over time, comparison between gabapentin and control groups

The most relevant finding during the intraoperative period was the need for fentanyl rescue analgesia. Indeed, although this data did not reach a statistical significance between the two groups ($p=0,462$) and the number of boluses per subject also did not differ significantly between groups ($p=0,217$), the descriptive analysis nevertheless showed a lower overall number of fentanyl boluses in the group G compared with the group C. In the group C all 7/7 subjects received at least one fentanyl bolus, with a total of 13 boluses administered, whereas in group G, fentanyl was administered to 5/7 subjects, with a total of 8 boluses administered. The Friedman test revealed a significant temporal variation in bolus administration in both the control group ($\chi^2(8)=43,378$; $p<0,001$) and the gabapentin group ($\chi^2(8)=16,000$; $p=0,042$). However, following Bonferroni correction, none of the pairwise comparisons between individual time points reached statistical significance in either groups (Table 12).

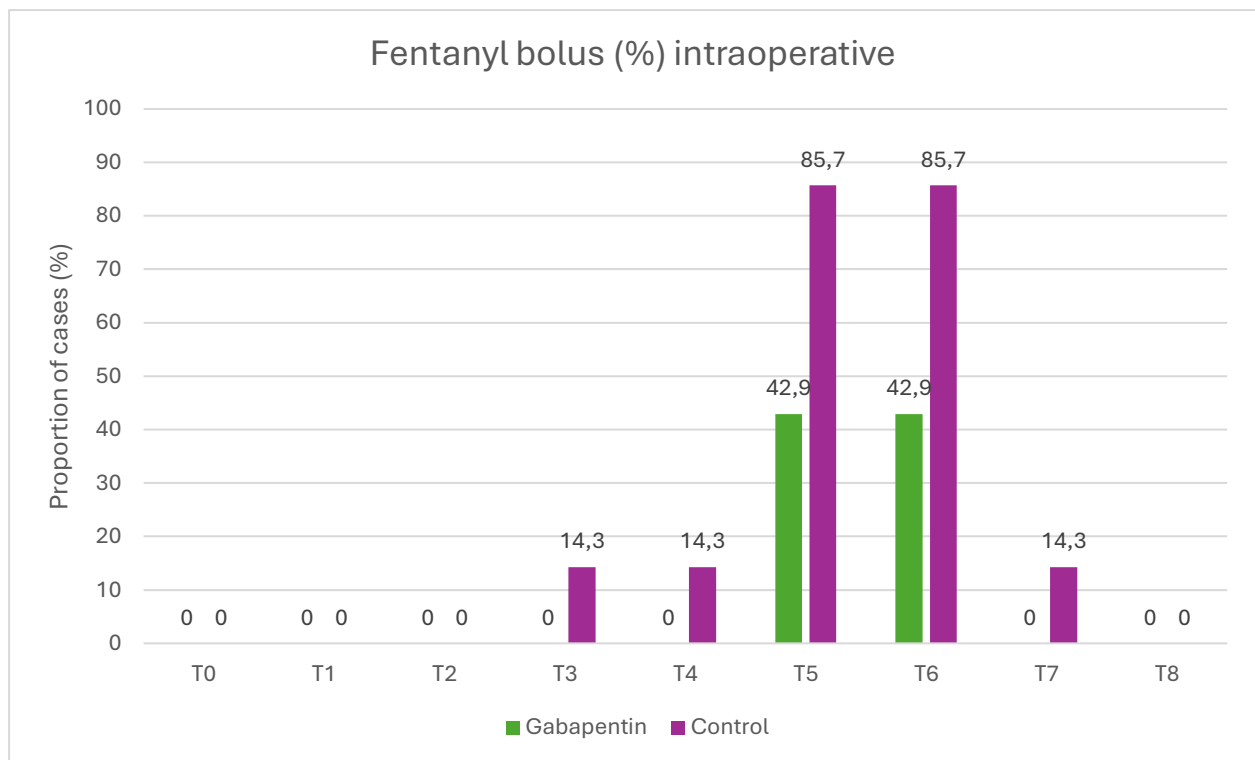


Table 12: percentage of fentanyl boluses over time in the gabapentin and control groups

Regarding postoperative physiological parameters, consistent with the findings observed for the intraoperative parameters, no significant differences were detected between the two groups; rather, the observed changes were related exclusively to the postoperative time point. Conversely, the data obtained from the Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF) revealed an interesting change in the observed trend.

It should nevertheless be noted that, although comparisons between the two groups at each individual time point were performed using all subjects available at that time point, data availability progressively decreased at later postoperative time points. For this reason, the analysis of the temporal trend within each group was limited only over the time points for which a sufficient number of subjects had complete measurements available.

For the Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF), the available data within 4 hours after the end of surgery (T1-T4) were considered for each group. The analysis was considered statistically valid only up to T4, as at T1-T2 and T3-T4, two subjects in the group C were excluded from further monitoring because their total GS scores exceeded the expected range. The Mann-Whitney comparison between the two groups showed no significant differences at T1 and T2, whereas significant differences emerged at T3 and T4. Specifically, mean Glasgow

Scale scores were lower in group G than in group C at both T3 and T4. Furthermore, the Friedman test performed on complete cases across T1-T4 showed no significant differences in group C ($\chi^2(3)=1,473$; $p=0,689$; $n=6$), whereas a significant difference was observed in group G ($\chi^2(3)=11,923$; $p=0,008$; $n=6$). In group G, post-hoc pairwise comparisons revealed a significant difference between T1 and T4 after Bonferroni correction ($p=0,044$). No pairwise comparison reached statistical significance in the group C (Table 13).

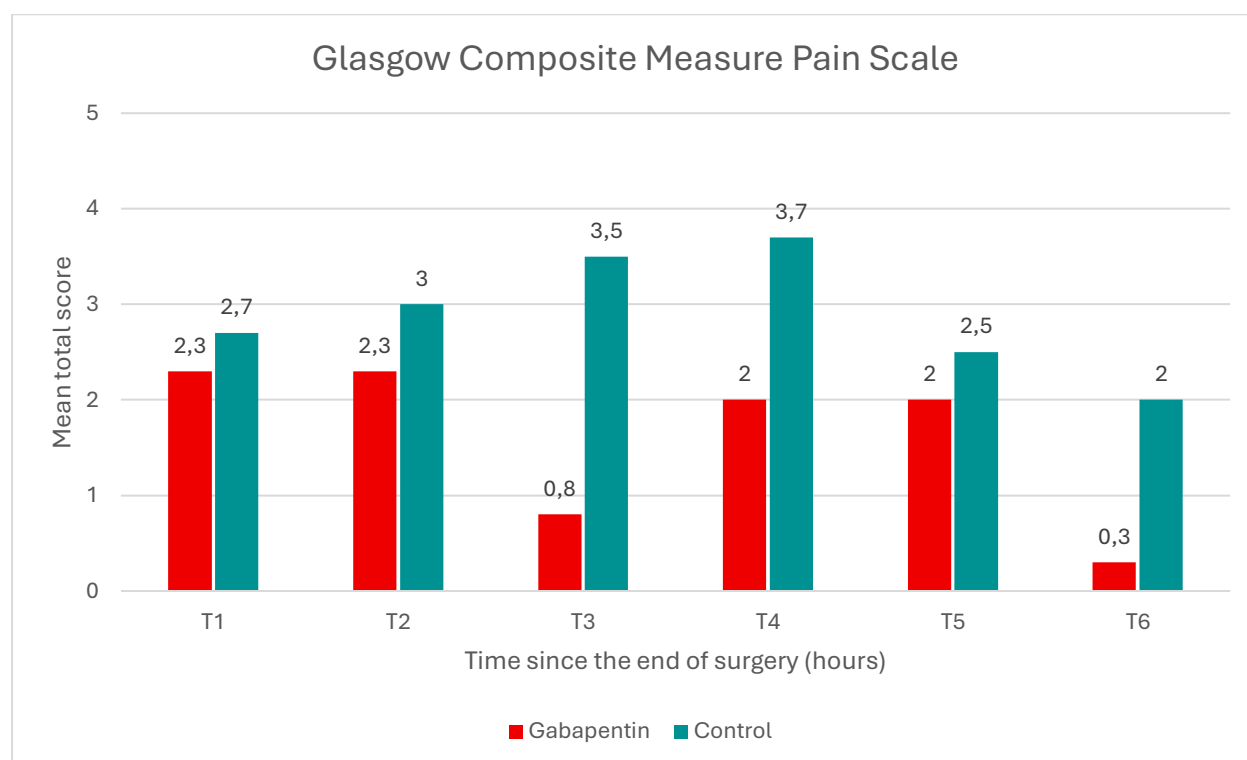


Table 13: mean CMPS-SF over time, comparison between gabapentin and control groups

Among physiological parameters, the comparison between groups from T0 to T4 revealed no significant differences in heart rate ($p \geq 0,423$). In contrast, the temporal trend was statistically significant in both groups, group G ($p=0,011$) and group C ($p=0,002$). In group G, the T0-T1 comparison was statistically significant (adjusted $p=0,014$). In group C, the T0-T3 (adjusted $p=0,007$) and T0-T4 (adjusted $p=0,005$) comparisons were statistically significant (Table 14). Similarly, no significant between-group differences in body temperature were observed at any of the individual time points ($p \geq 0,657$). However, body temperature varied significantly over the postoperative period in both groups: Friedman test showed a $p=0,006$ in group G and a $p=0,036$ in group C. In group G a significant change was observed during T1-T3 ($p=0,005$); while in group C during T1-T4 ($p=0,042$) (Table 15). The analysis of the respiratory rate parameter revealed no

statistically significant difference between groups from T0 to T3, whereas at T4 the exact significance was $p=0,048$, with higher ranks in group G. The Friedman test across T0-T4 showed a statistically significant difference in both group C ($\chi^2(4)=9,757$; $p=0,045$, $n=6$) and group G ($\chi^2(4)=12,250$; $p=0,016$, $n=6$). In the control group, no pairwise comparison remained statistically significant after Bonferroni correction; while, in the gabapentin group, the T0-T2 comparison remained statistically significant (adjusted $p=0,019$) (Table 16). Finally, regarding the arterial pressure parameters (SAP, MAP and DAP), no significant differences were observed between the gabapentin and control groups, as the substantial amount of missing data, particularly in T1-T2 control group, limited the statistical power of the analyses.

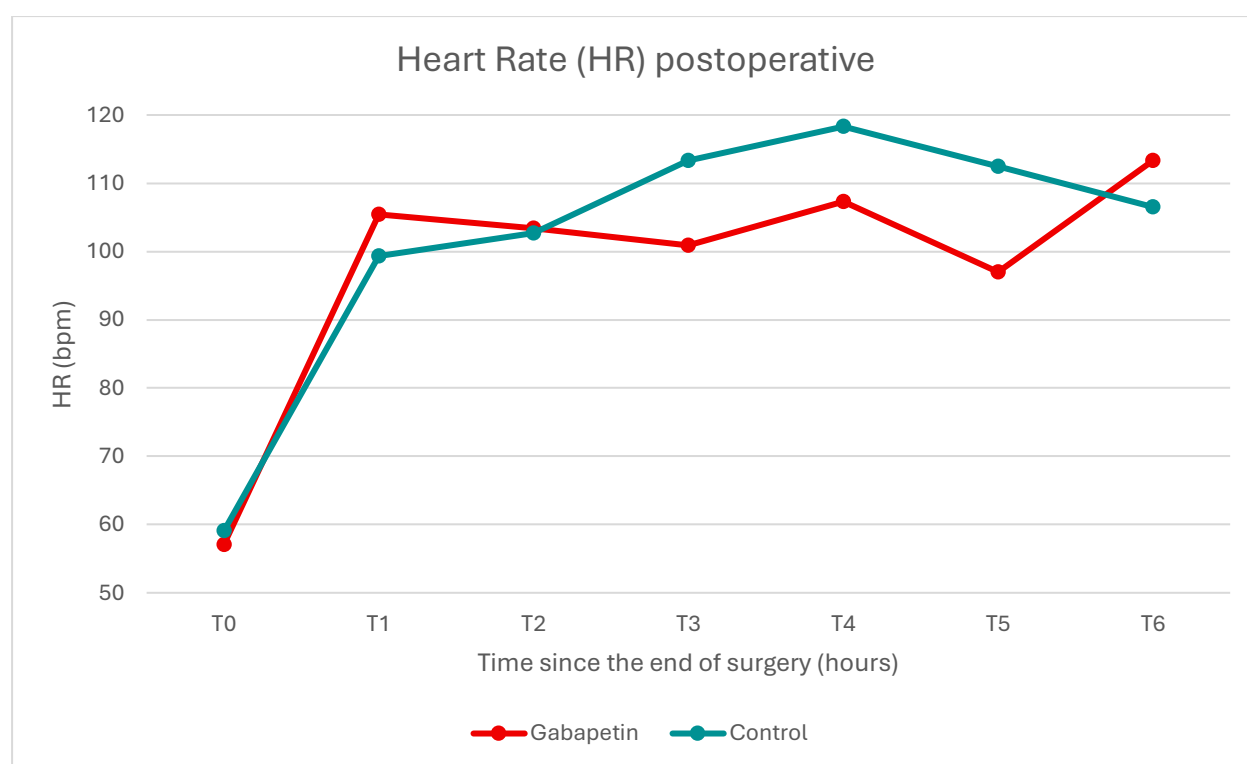


Table 14: mean HR over time, comparison between gabapentin and control groups

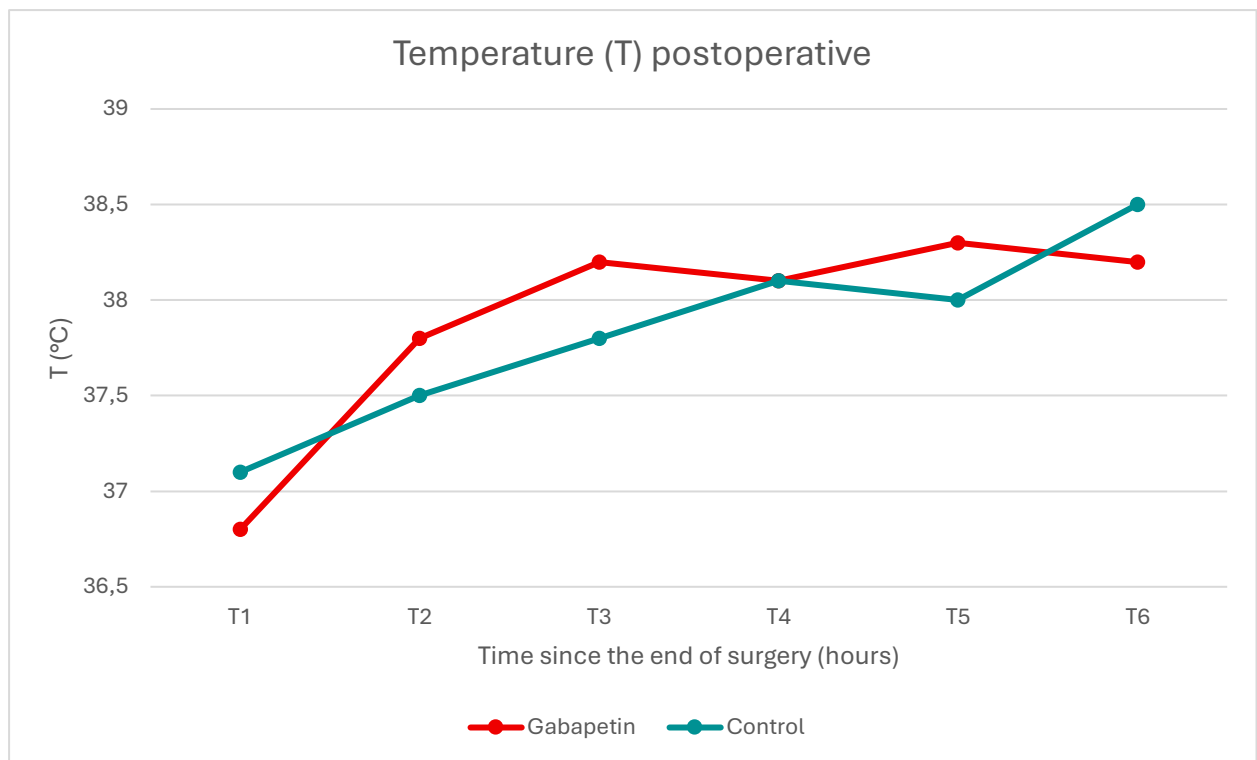


Table 15: mean T over time, comparison between gabapentin and control groups

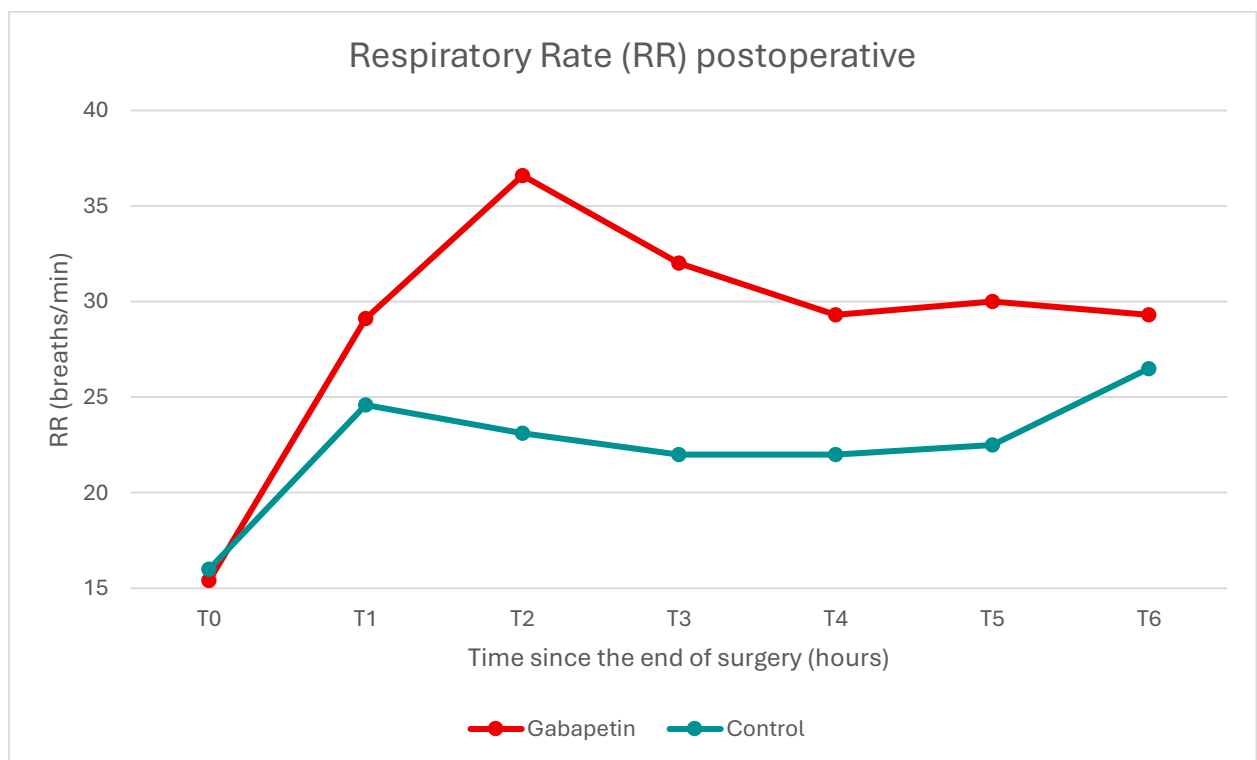


Table 16: mean RR over time, comparison between gabapentin and control groups

Discussion

The present study evaluated the effects of preoperative gabapentin administration in dogs undergoing laparoscopic ovariectomy, with particular focus on intraoperative opioid requirements, postoperative pain control, and inhalational anaesthetic consumption. Overall, the results suggest that preoperative administration of gabapentin may improve postoperative pain control, as shown by lower scores on the Glasgow Composite Measure Pain Scale – Short Form (CMPS-SF) during the early postoperative period and by the fact that no dogs required rescue analgesia. In contrast, no statistically significant effects were observed on intraoperative fentanyl requirements or end-tidal isoflurane concentrations. Although statistically significant differences were identified in some physiological parameters, particularly intraoperative diastolic arterial pressure and postoperative respiratory rate, these findings do not appear to reflect a direct effect of gabapentin and may instead be explained by other factors related to perioperative management and the characteristics of the animals included in the study.

The absence of statistically significant differences between the two groups should, however, be interpreted in light of the limited sample size. This study should therefore be considered a pilot study, and the results should be regarded as preliminary. The relatively small number of animals included may have limited the statistical power of the study and consequently the ability to detect potentially relevant differences between groups. Further studies involving a larger sample size are therefore required to confirm the present findings.

Moreover, as reported in previous studies, gabapentin incorporated in the pre-anaesthetic protocol, as part of a multimodal analgesic approach, appears to be a safe and well-tolerated option in dogs. In particular, Neogi et al. (2012) in their study on the effects of gabapentin premedication on hemodynamic stability during laparoscopic cholecystectomy in dogs reported that gabapentin did not interfere on hemodynamic stability, with none of the patients of either groups suffered from bradycardia or hypotension (Neogi et al., 2012). Similarly, in the present study, none of the dogs undergoing laparoscopic ovariectomy required rescue interventions in accordance with the predefined safety protocols for hypotension or bradycardia associated with hypotension during the surgery. Nevertheless, also this finding should be interpreted cautiously, particularly given the limited sample size.

The primary objective of the study was to determine whether preoperative administration of gabapentin could reduce intraoperative opioid requirements. In this regard, no statistically significant difference was detected between the two groups in the number of fentanyl boluses administered during surgery. Therefore, the present statistical analysis does not provide sufficient evidence to conclude that preoperative gabapentin administration reduces intraoperative opioid requirements. Nevertheless, the descriptive analysis revealed some differences that may be of interest. All dogs in the control group required at least one fentanyl bolus during the surgical procedure, whereas this was not the case in the gabapentin group. In addition, a total of 13 fentanyl boluses were administered to dogs in the control group, compared with 8 in the gabapentin group (Table 12). Although this difference did not reach statistical significance, the lower overall number of fentanyl boluses in the gabapentin group may suggest a potential reduction in intraoperative opioid requirements. This finding should therefore be interpreted as a possible trend rather than as evidence of a definitive opioid-sparing effect of gabapentin. The lack of statistical significance, together with the limited sample size, prevents firm conclusions from being drawn. However, the observed difference may justify further investigation in studies including a larger cohort, which would provide greater statistical power to determine whether the potential opioid-sparing effect of gabapentin is reproducible and clinically relevant.

Another objective of the study was to evaluate the effect of preoperative gabapentin administration on postoperative pain control. For this purpose, pain was assessed in both groups using the CMPS-SF, a validated multidimensional scale for the assessment of acute pain in dogs, based on behavioural and clinical parameters. The scale also allows the identification of an analgesic intervention threshold, above which additional analgesic treatment is considered warranted (Reid et al., 2007). The CMPS-SF Glasgow pain scores, analysed in this study, showed a different postoperative evolution between the two groups, with significantly lower pain scores in the gabapentin group at T3 and T4, corresponding to 3-4 hours after the end of the surgery (Table 13). Furthermore, two dogs in the control group exceeded the predefined analgesic intervention threshold, whereas this event was not observed in any of the dogs in the gabapentin group. Taken together, these findings may indicate a potential benefit of gabapentin in the management of postoperative pain and in reducing the need for additional opioid analgesia. The result is consistent with evidence from several studies in human medicine investigating the use of preoperative gabapentin for reducing pain scores and postoperative opioid consumption.

Indeed, several systematic reviews and meta-analyses have synthesized evidence from multiple randomized clinical trials to assess the efficacy of the drug following abdominal surgery. Among these, Kong et al. (2007), for example, conducted a systemic review including seven original randomized placebo-controlled trials. A significant reduction in postoperative opioid consumption, during the first 24 hours, was observed with gabapentin in six of the seven studies. Significant reductions in mean pain scores were also observed within the first 6 hours after surgery, both at rest (three of seven studies) and during activity (two of four studies) (Kong & Irwin, 2007). Similarly, Arumugam et al. (2016) reported a significant reduction in postoperative opioid consumption, particularly morphine, fentanyl, and tramadol, within the first 24 hours after mastectomy, spinal, abdominal, and thyroid surgeries (Arumugam et al., 2016).

On the other hand, the available evidence on the use of gabapentin in veterinary medicine is still limited, and only a few studies have investigated its efficacy in the perioperative management of pain in dogs. Among these, Crociolli et al. (2015) evaluated the effect of perioperative gabapentin administration in dogs undergoing mastectomy. Although postoperative pain scores, assessed using VAS and GCMPS, did not show statistically significant differences between the gabapentin and placebo groups at any of the assessment time points, dogs treated with gabapentin required significantly less morphine as rescue analgesia during the postoperative period, with a 44% reduction compared with the placebo group (Crociolli et al., 2015). This finding suggests that gabapentin may have contributed to better pain control by reducing the need for rescue analgesia, despite the absence of significant differences in pain scores.

Similarly, in our study preoperative administration of gabapentin was associated with better pain control during the first 3-4 hours of the postoperative period. Although the benefit of gabapentin in our study was mainly shown by lower pain scores, whereas in the study by Crociolli et al. (2015) it was reflected in a lower requirement for rescue morphine, both findings suggest a possible beneficial effect of gabapentin on postoperative pain control in dogs.

In the present study, intraoperative cardiorespiratory parameters were assessed, including heart rate (HR), respiratory rate (RR), systolic (SAP), mean (MAP), and diastolic (DAP) arterial blood pressure, peripheral oxygen saturation (SpO₂), and end-tidal carbon dioxide concentration (ETCO₂). While in the postoperative period, body temperature (T), HR, RR, SAP, MAP, and DAP were measured and recorded.

These physiological parameters were assessed as indirect indicators of intraoperative and postoperative pain. Painful stimuli can activate the autonomic nervous system and increase the

physiological response to stress, resulting in measurable changes in cardiorespiratory parameters. For this reason, changes in physiological variables have been proposed as potentially objective indicators of pain, although they are not specific to pain and may also be influenced by other factors (Cowen et al., 2015).

Based on this rationale, in the present study, the analysis of physiological parameters revealed no statistically significant differences in terms of pain perception between the gabapentin and control groups for most of the physiological parameters assessed during either the intraoperative or postoperative period, with the only exception of intraoperative DAP and postoperative RR. The absence of statistically significant differences between the gabapentin and control groups indicates that, overall, the two groups showed similar physiological responses and gabapentin administration did not result in significantly different physiological changes.

However, some parameters showed significant changes over time. This indicates that the physiological variables changed during the different phases of the perioperative period. Importantly, these changes over time were not associated with significant differences between the two groups. Therefore, changes in physiological parameters over time do not necessarily indicate an effect of gabapentin. Since similar changes were observed in both groups, they may instead reflect the normal physiological responses associated with anaesthesia, surgery, recovery, and the postoperative period.

As previously mentioned, two parameters showed significant differences between groups, in contrast to the overall pattern observed for the other physiological variables.

Specifically, intraoperative DAP analysis revealed a statistically significant time×group interaction, with higher values observed in the gabapentin group during ovarian clamping, particularly, at T5 and at T6 time points (Table 9). In the between-group comparisons performed at individual time points, the difference reached statistical significance at T6, whereas the difference at T5 showed a trend toward significance. However, this finding should be interpreted with caution, as the increase of DAP in group G should not necessarily be interpreted as a response to a painful stimulus in the absence of significant changes also in SAP and MAP values. Moreover, in the present study, T5 and T6 corresponded to the surgical time points associated with the greatest need for additional analgesic therapy. In the group C, 5/7 subjects required fentanyl administration at both time points, whereas 1/7 required it only at T5 and 1/7 only at T6. In the group G, fentanyl was administered at both T5 and T6 only in 1/7 subjects, at T5 in 2/7, and at T6 in 2/7 (Table 12). These findings suggest that subjects in the G group required lower amounts of

fentanyl during these two surgical time points. Previous studies in human medicine have compared the effects of gabapentin and fentanyl on haemodynamic responses. Parida et al. (2015) conducted a controlled clinical trial to compare the effects of a single preoperative dose of gabapentin with intravenous fentanyl administration on the haemodynamic response to tracheal intubation and laryngoscopy, and to determine whether their combined administration provided greater attenuation of this response. The results showed that post-intubation MAP was significantly higher in the gabapentin group than in the other groups, whereas no significant difference was observed between fentanyl and combined-treatment groups (Parida et al., 2015). Based on the findings of this previous study, it could be hypothesized that, in the present study, the lower amount of fentanyl administered at T5 and T6 in the G group may have influenced the arterial blood pressure values, particularly increasing DAP values, whereas highest amount of fentanyl maintained lower DAP values in the C group. This hypothesis should nevertheless be interpreted with caution, as numerous studies in both human and veterinary medicine have reported that gabapentin exerts a depressive effect on the haemodynamic responses caused by CO₂ pneumoperitoneum created for laparoscopic surgeries (Neogi et al., 2012). Therefore, the results of the present study do not allow us to determine with certainty whether the differences in DAP values were directly related to gabapentin administration, the different amount of fentanyl administered, or the interaction between these factors. Further studies involving larger sample sizes are needed to clarify the mechanisms underlying these haemodynamic changes during laparoscopic surgery.

The other physiological parameter that showed a statistically significant difference between groups was postoperative RR. The analysis of this parameter revealed no statistically significant difference between groups from T0 to T3, whereas at T4 higher ranks have been observed in group G (Table 15). An increase in RR could potentially indicate the presence of acute pain (Lamont, 2024); however, this finding appears to be inconsistent with the pain assessments obtained using the CMPS-SF during the postoperative time. Indeed, at T4, subjects in the gabapentin group had lower pain scores than those observed in the control group, with scores remaining below the established threshold for clinically relevant pain (Table 13). As an increased respiratory rate may also reflect stress or agitation, this finding could also be explained by the presence of a greater proportion of agitated subjects in the gabapentin group than in the control group. This interpretation is further supported by the initial sedation scores, as subjects in the group G exhibited a lighter degree of sedation at the time of assessment. Indeed, a mild degree

of sedation may result not only from an inadequate dose or administration of pre-anaesthetic agents but may also reflect a state of patient agitation. In fact, stress can be defined as the physiological and behavioural response of the organism to a perceived threat, involving the activation of the sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axes. For this reason, stressed animals may exhibit reduced sedation efficacy and an increased risk of anaesthetic complications (Riemer et al., 2021).

In addition to evaluating the effects of preoperative gabapentin administration on pain control and opioid requirements during both the intraoperative and postoperative period, the present study also investigated whether gabapentin could contribute to a multimodal anaesthetic approach by reducing amount of inhalational anaesthetic required during surgery. This assessment was based on the potential anaesthetic-sparing effect of gabapentin. When included in a multimodal anaesthetic protocol, gabapentin may reduce the requirement for inhalational anaesthetic agents, potentially limiting their dose-dependent adverse effects, such as hypotension associated with the vasodilatory effects of isoflurane (Johnson Brittney A. et al., 2019). To investigate this potential effect of gabapentin, end-tidal isoflurane concentrations were monitored throughout the intraoperative period. Analysis of this parameter revealed no statistically significant differences between the gabapentin and control groups, with the only significant effect being associated with time. Consequently, in the present study, these findings do not support a clinically relevant isoflurane-sparing effect of gabapentin when incorporated into a multimodal anaesthetic protocol. Nevertheless, given the limited sample size, this result should be interpreted with caution and warrants further investigation, particularly considering its discrepancy with previous studies reporting a MAC-sparing effect following gabapentin administration in dogs 2 hours prior to sedation (Aghighi et al., 2012); (Johnson Brittney A. et al., 2019).

The design of this study has some limitations. The small sample size in this type of clinical study may have limited its statistical power, preventing the detection of clinically relevant differences, particularly for intraoperative opioid requirement and isoflurane-sparing effect.

Another aspect to consider is the timing of gabapentin administration. In the present study, gabapentin was administered 30 minutes before induction of anaesthesia. This interval was selected to maintain a standardized protocol while remaining feasible within the routine clinical workflow. However, this factor may have influenced the effect of gabapentin during surgery.

Pharmacokinetic studies in dogs have reported that gabapentin reaches peak plasma concentrations approximately 1.3-1.5 hours after oral administration (Kukanich & Cohen, 2011). Therefore, at the time of induction and during the early stages of surgery, the drug may not have yet reached its peak plasma concentration. It is possible that the 30 minutes interval used in the present study limited the analgesic effect of gabapentin during the intraoperative period and contributed to the absence of a significant reduction in opioid requirements. However, the present study highlighted that the administration of gabapentin in close temporal proximity to the surgical procedure appeared to improve postoperative pain management, as reflected by lower CMPS-SF pain scores in the gabapentin group. Consequently, this finding may be clinically relevant for the early postoperative care of surgical patients, as improved pain control during this particularly stressful period may enhance patient comfort and facilitate postoperative management by the medical staff.

In conclusion, although the 30-minute interval was chosen to fit the clinical and operative schedule, future studies could investigate whether a longer interval between gabapentin administration and induction of anaesthesia results in a more pronounced intraoperative effect.

Conclusions

It can be concluded that, as part of a multimodal analgesic approach, the inclusion of gabapentin in the pre-anaesthetic protocol appears to be a safe and well-tolerated options in dogs undergoing laparoscopic ovariectomy, with no clinically relevant effects on haemodynamic and respiratory stability. Regarding intraoperative pain management, gabapentin did not appear to provide a clear additional analgesic benefit when incorporated into the anaesthetic protocol used in this study, as evidenced by the lack of a reduction in both opioid and isoflurane requirements. Nevertheless, the numerically lower number of fentanyl boluses observed during the procedure in dogs receiving gabapentin may represent a possible trend that should be further investigated. The main benefit of gabapentin was observed during the postoperative period, with lower CMPS-SF pain scores in the gabapentin group. Overall, these findings suggest that gabapentin may be a useful component of multimodal analgesia, particularly for improving early postoperative pain control. Further studies involving larger sample sizes will be necessary to confirm and strengthen these findings.

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