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Assessment of chronic pain in companion animals: development and concurrent validation of neurophysiological methods

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Assessment of chronic pain in companion animals: development and concurrent validation of neurophysiological methods

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Résumé

La douleur est une expérience complexe impliquant des composantes sensorielles et de perception (émotionnelle, affective, cognitive). Elle est associée au stress, de la souffrance et une dégradation de la qualité de la vie. Les affections douloureuses chroniques telles que l'arthrose et le cancer des os touchent les chats et les chiens. La douleur dans ces conditions implique de nombreux mécanismes affectant les systèmes nerveux périphérique et central, qui génèrent et entretiennent une douleur pathologique. Les tests sensoriels quantitatifs (TSQ) sont des outils pour quantifier la composante sensorielle de la douleur, qui peuvent aussi être utilisés pour éluder les mécanismes de la douleur impliquée. Les travaux initiaux sur les chats et les chiens souffrant d'arthrose ont permis de développer et de valider certaines méthodes de TSQ; cependant, quelques questions sont restées sans réponse chez les chats souffrant de l'arthrose, et cette méthodologie n'a pas été encore explorée chez les chiens atteints d'ostéosarcome.

Nos hypothèses de recherche étaient: 1) les chats arthrosiques sont affectés par des modifications neurophysiologiques caractéristiques de la sensibilisation centrale, pouvant être détectées par les TSQ et répondant à l'administration d'analgésiques à action centrale; et 2) l'ostéosarcome provoque une sensibilisation périphérique et centrale avec des mécanismes descendants d'inhibition de la douleur déficients chez le chien.

Nos objectifs étaient : 1) chez les chats souffrant d'arthrose, de fournir des évidences sur la thérapie basée sur les mécanismes neurophysiologiques à l'aide de TSQ; et 2) de tester la capacité d'un protocole TSQ à démontrer la sensibilisation périphérique et centrale chez les chiens atteints de cancer des os, y compris un test de modulation de la douleur conditionnée, et de tester l'efficacité d'un protocole d'analgésique palliatif par paliers chez ces patients.

En utilisant les TSQ statiques et dynamiques chez les chats arthrosiques, nous avons démontré que les analgésiques à action centrale tels que le tramadol peuvent renverser la sensibilisation centrale mesurée par la sommation temporelle de la douleur. Cet effet n'a pas été observé après l'administration d'analgésique à action périphérique tel que les antiinflammatoires non stéroïdiens comme le meloxicam. Ces résultats

soulignent l'importance d'une approche de traitement fondée sur les mécanismes de la douleur chronique.

Le protocole TSQ développé pour les chiens a révélé que ceux atteints de cancer des os manifestaient de l'hyperalgésie primaire et secondaire et de l'allodynie dynamique au brossage par rapport aux chiens en bonne santé. Un test de modulation de la douleur conditionnée pouvant être facilement appliqué a été mis au point et a démontré la capacité de différencier les chiens sains des chiens cancéreux. En utilisant cette méthodologie, il s'est avéré que cette dernière population démontrait un système descendant d'inhibition de la douleur déficient.

Ces études ont fourni des preuves des similitudes dans le profil sensoriel entre les malades humains et les animaux de compagnie affectés par l'arthrose, ainsi que les ostéosarcomiques. Les TSQ sont utiles dans la recherche vétérinaire sur la douleur et doivent être accompagnés des normes les plus strictes en matière de soins des animaux et de conception, de conduite et de compte-rendu des études.

Mots-clés : arthrose, cancer des os, chat, chien, douleur chronique, qualité de vie, sensibilisation centrale, sensibilisation périphérique, test sensoriel quantitatif.

Abstract

Pain is a complex experience involving sensory and perceptual components. It causes stress, suffering and decreased quality of life. Chronic painful conditions such as osteoarthritis (OA) and bone cancer affect cats and dogs. Pain in these conditions results from numerous mechanisms affecting the peripheral and central nervous systems which generate and maintain pathological pain in affected individuals. Quantitative sensory testing (QST) are means to quantify the sensory component of pain. In combination with observed analgesic efficacy, they can be used to study mechanisms of pain. Initial work on cats and dogs with OA has helped to develop and validate some QST methods; however, questions remained unanswered in cats with OA, and this methodology was not yet explored in dogs with bone cancer.

Our main hypotheses were: 1) osteoarthritic cats are affected by neurophysiological changes characteristic of central sensitization which can be detected by QST and the concomitant administration of centrally-acting analgesics; and 2) bone cancer in dogs causes peripheral and central sensitization with deficient descending modulating mechanisms.

Our main objectives were: 1) to provide evidence of mechanism-based therapy in cats with OA using QST; and 2) to test the ability of a QST protocol to provide evidence of peripheral and central sensitization in dogs with bone cancer including the development and validation of a conditioned pain modulation test, and to test the efficacy of a step-wise palliative analgesic protocol in these patients.

Using static and dynamic QST in osteoarthritic cats, we demonstrated that centrally-acting analgesics such as tramadol can reverse central sensitization as measured by facilitated temporal summation of pain, while the same is not observed when a peripherally-acting analgesic such as non-steroidal anti-inflammatory drug, meloxicam, is administered. These findings highlight the importance of mechanism-based approach for the treatment of chronic pain.

The QST protocol developed for use in dogs revealed that dogs with bone cancer are affected by primary and secondary hyperalgesia and brush allodynia when compared

with healthy dogs. A conditioned pain modulation test which can be easily applied into clinical practice was developed and demonstrated ability to differentiate between healthy and cancerous dogs. Using this methodology, the latter population was found to be affected by deficient descending modulating systems.

These studies provided evidence of the similarities in sensory profile between people and companion animals affected by OA- and bone cancer-related pain. The use of QST is valuable in veterinary pain research and should be accompanied by the highest standards of animal care and study design, conduct and reporting.

Keywords: cats, bone cancer, central sensitization, chronic pain, dogs, quality of life, quantitative sensory testing, osteoarthritis, peripheral sensitization.

Table of Contents

List of Tables

List of Figures

Liste of abbreviations

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ARRIVE: Animal Research: Reporting of In Vivo Experiments
BPI: Brief pain inventory
CBPI: Canine brief pain inventory
CKD: Chronic kidney disease
CMI: Clinical metrology instruments
CNS: Central nervous system
COX: Cyclooxygenase
CPM: Conditioned pain modulation
CSOM: Client-specific outcome measure
DNIC: Diffuse noxious inhibitory control
GABA: Gamma-aminobutyric acid
HRQoL: Health related quality of life
IV: Intravenous
LOAD: Liverpool osteoarthritis in dogs
MRI: Magnetic Resonance Imaging
NGF: Nerve growth factor
NK-1: Neurokinin-1
NMA: Night-time motor activity
NMDA: N-methyl-D-aspartate
NSAID: Non-steroidal anti-inflammatory drug
OA: Osteoarthritis
OSA: Osteosarcoma
PD: Pharmacodynamic
PK: Pharmacokinetic
PO: *Per os*
PVF: Peak Vertical Force
QoL: Quality of life
QST: Quantitative sensory testing
TENS: Transcutaneous electrical nerve stimulation
trkA: Tropomyosin receptor kinase A
TRPV: Transient receptor potential vanilloid
VAS: Visual analog scale

This dissertation is dedicated to all animals. To companion animals that deserve high-quality veterinary care. To captive animals that deserve our understanding of their physical and mental needs. To wild animals that deserve the protection of their habitat.

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Introduction

Pain is a personal, complex and multidimensional experience. The proper assessment of pain represents a unique challenge since it encompasses both sensorial (sensory-discriminative determinant or spatiotemporal properties) and perceptual (motivational-affective or emotional determinant, and cognitive-evaluative or central control determinant) experiences which can be affected by several genetic and environmental factors.[1] Pain intensity does not linearly correlate with the severity of the pathology; rather, it involves stress response and cognitive functions of the brain such as fear, memory, anxiety and distraction, in addition to sensory inputs. In fact, the recently proposed neuromatrix theory of pain places genetic contributions and neural-hormonal mechanisms of stress on a level of equal importance with the neural mechanisms of sensory transmission.[1] The similarities in physiology and pathophysiology of pain across mammalian species is quite remarkable; nevertheless, the complexity of the pain experience in animals is rather difficult to access due to our inability to evaluate their true perception of pain.[2,3]

In companion animals, pain negatively affects quality of life, delays recovery and induces behavioral changes that affect owner-companion animal bond. It causes unnecessary fear, anxiety and stress and may lead to sympathetic nervous system activation and altered food intake and metabolism.[4] For these reasons, the management of animal pain is a significant ethical and economic component in the modern practice of veterinary medicine. Pain is now considered as the 4th vital sign, and its assessment should be incorporated into the clinical evaluation of all animal patients.

In people, chronic pain is costly not only to the patient but also to the society. The economic burden of chronic pain is greater than that of cardiovascular disease and cancer combined.[5] Data from Statistics Canada from 2001 reveals that uncontrolled pain continues to be the single most common cause of disability among working-age adults.[6] Nearly ten years later, it is safe to estimate that this cost has only gone up, especially considering the aging of the population and increased incidence of chronic diseases.[5]

Despite the aforementioned expenditures and burden to the society, numerous reviews have highlighted a crippling failure to translate basic research into clinical practice

and the development of novel analgesics with proven efficacy and acceptable safety profile in humans.[7–11] It is now widely accepted that preclinical pain models do not properly predict the clinical efficacy of analgesics in humans. One of the main reasons for this is the over reliance on rodent models of induced disease. Thus, animal models of naturally occurring disease have been gaining increased attention from the pain community due to several advantages including the benefit for both animals and humans, the practice of the 3Rs (Replacement, Reduction and Refinement), in addition to greater applicability of animal research to humans for drug efficacy studies. Spontaneous painful disease in companion animals is common and may better reflect the complex genetic, environmental, temporal and physiological influences observed in people. Nevertheless, for a spontaneous disease model to successfully contribute to translational research, the outcome measures used to asses pain must be well developed and validated. The work in this thesis describes the development and concurrent validation of neurophysiological methods for the assessment of spontaneous chronic pain in companion animals in order to benefit animals and people within the concept of one health.

In the first chapter, a review of the literature is presented including a systematic review and meta-analysis. The second chapter presents three research studies that were performed to achieve the proposed objectives of this thesis. The third chapter discusses the findings of these studies and their limitations.

1 Literature review

The first two sections of this chapter present an overview of the current knowledge on pain pathophysiology, with a particular focus on osteoarthritis (OA) and bone cancer-related pain, as well as on quantitative sensory testing (QST) and their value on chronic pain assessment and the study of pain mechanisms.

In the third section, the manuscript of a systematic review and meta-analysis of QST in healthy and osteoarthritic cats entitled 'Quantitative sensory testing in feline osteoarthritic pain - A systematic review and meta-analysis' is included.

The forth section refers to current practices in cat chronic pain assessment and management with two published Chapters included in Appendix, and a short literature review on the particularities of pain assessment and treatment of chronic pain in dogs. Finally, this first chapter is concluded with a short summary and presentation of the research hypothesis and objectives.

1.1 Pathophysiology of pain in specific conditions

1.1.1 Overview of pain pathophysiology

In this section, the nociceptive pathway is used as a framework to present the mechanisms involved in each part of the process. The somatosensory system comprises all the structures implicated in the processing of nociceptive information from peripheral nociceptors to the spinal cord and higher structures of the central nervous system (CNS). The processing of nociceptive information occurs *via* four main accepted steps: transduction, transmission, modulation and perception.

1.1.1.1 Nociceptive transduction

Transduction is the first step in processing pain. It starts with the activation of nociceptors which are highly specialized structures that are present in the skin, muscles, joints and viscera, and that respond to noxious or potentially tissue-damaging stimuli.[12] The activation of nociceptors results in the opening of membrane ion channels allowing sodium and calcium ions to move down their respective concentration gradients with consequent membrane depolarization. If the stimulus is of sufficient intensity that it reaches the threshold for an action potential, a nerve impulse is generated. Mechanical, thermal or chemical stimulation may generate an action potential at the nociceptor level.[12,13] The skin is the most densely innervated tissue and it contains the widest variety of nociceptors.[14] A sensory unit comprises a single primary afferent and all its associated receptors. The receptive field comprises the area that the sensory unit collects information from.[15]

1.1.1.2 Nociceptors and cell surface receptors

Nociceptors can be classified into two subpopulations based on the content of receptor expression. Peptidergic nociceptors terminate in deep layers of the epidermis and release peptides such as substance P and calcitonin gene-related peptide. They also express the tropomyosin receptor kinase A (trkA) targeted by nerve growth factor (NGF). Non-peptidergic nociceptors terminate in superficial layers of the epidermis. They express the c-Ret neurotrophin receptor targeted by glial-derived neurotrophic factor.[13]

Nociceptors can be further subclassified according the response profile of the afferent: polymodal nociceptors characterize afferents that respond to mechanical, thermal and chemical stimuli; mechano-cold fibers respond to mechanical and cold stimuli; and mechanically insensitive afferents do not respond to mechanical stimuli. Also known by 'silent' nociceptors, mechanically insensitive afferents make up a substantial proportion of the nociceptor population. The acquisition of mechanosensitivity after tissue injury implicates them in the development and maintenance of hyperalgesic or hypersensitive states.[12–14,16]

There are three main cell surface proteins found in sensory neurons: ligand-gated ion channels (*e.g.* transient receptor potential channels and sodium channels (NaV1.8)), G-protein coupled receptors (*e.g.* B1 and B2 bradykinin receptors and EP1, EP3C and EP4 receptors for prostaglandin E_2) and receptors for neurotrophins (*e.g.* NGF family and glial cell line-derived family) or cytokines (*e.g.* immune cells, Schwann cells and fibroblasts).[12–14,16]

1.1.1.3 Nociceptive transmission

Transmission occurs when the nociceptive impulse travels from the primary afferent fiber (first order neuron) to the dorsal horn of the spinal cord. The first order neuron has peripheral and central axons. Peripheral axons are located in the skin, muscle, tendon and joints. Cell bodies from fibers innervating most of the body are located in the dorsal root ganglia. Cell bodies from fibers innervating the head are located in the trigeminal ganglia. Central axons are located in the CNS where they synapse with second order neurons including interneurons, neurons of ascending tracts, intersegmental neurons, projecting neurons and α-motor neurons involved in reflex withdrawal responses.[12,16]

Primary afferent fibers are pseudo-unipolar. This unique morphology allows the nociceptive signal to travel in both directions within the neuron. Thus, the majority of proteins synthetized by the cell bodies of primary afferents are distributed to both central and peripheral terminals.[13] This characteristic distinguishes primary afferents from the prototypical neuron in which the dendrite (the recipient branch of the neuron) is biochemically distinct from the axon (the transmission branch of the neuron). By having

both central and peripheral terminals biochemically equivalent, the nociceptor can send and receive messages from either end.

Peripheral somatosensory fibers can be divided into three types based on their structure, diameters and conduction velocity (Table I).[12–16]

Table I. Description of sensory afferents.

Fiber	Description	Diameter	Conduction velocity	Projections to spinal cord dorsal horn
Aβ	Large myelinated and rapidly conducting fibers involved in low threshold innocuous mechanical stimulation (*e.g.* touch).	More than 10 μm	30-100 m/s	Laminae III, IV and V
Aδ	Thinly myelinated and slowly conducting fibers primarily involved in nociceptive signaling (*i.e.* fast pain). Type I Aδ nociceptors respond to both mechanical and chemical stimuli but have relatively high heat thresholds (>50°C). Type II Aδ nociceptors have a much lower heat threshold, but a very high mechanical threshold.	2.0 to 6.0 μm	12-30 m/s	Laminae I and V
C	Unmyelinated and very slowly conducting fibers primarily involved in nociceptive signaling (*i.e.* slow pain). Most C fibers are polymodal and respond to heat and mechanical stimuli. A subgroup of C fibers called 'silent' nociceptors are mechanically insensitive but can become sensitized after tissue injury.	0.4 to 1.2 μm	0.5–2.0 m/s	Laminae I and II

1.1.1.4 Nociceptive modulation

The concept of pain modulation mediated *via* a central control was first proposed by Melzack and Wall[17] with the gate control theory. It proposed that the transmission of nerve impulses from afferent fibers to the spinal cord was modulated by a gating mechanism influenced by the relative amount of activity in large- and small-diameter fibers, as well as the nerve impulses descending from the brain.[17] Vast knowledge has progressed from this initial theory. Today, it is known that before pain is experienced or perceived, the signals are modulated at different levels of the peripheral and central nervous systems where the nociceptive message may be amplified or inhibited depending on the activated pathway and the nature of the neurotransmitters. In fact, the resulting output from the dorsal horn neurons pain signals reflects a complex interplay between excitatory and inhibitory inputs.[12,18,19] Yet, most modulation occurs within the dorsal horn of the spinal cord, the brain stem and several brain regions such as the thalamus, hippocampus, amygdala, prefrontal cortex, insular cortex, and anterior cingulate cortex.[20]

The spinal synapses between first and second order neurons can release excitatory and/or neuromodulatory neurotransmitters. Excitatory neurotransmitters include glutamate and aspartate, whereas vasoactive peptide, somatostatin, calcitonin gene-related peptide and substance P are neuropeptides modulating the signal.[12,16,19] Glutamate binds to several receptors on postsynaptic neurons including ligand-gated ion channels [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-methyl-D-aspartate (NMDA)] and G-protein coupled glutamate receptors. An intense, repeated, and sustained noxious stimulus with constant release of glutamate further activates NMDA receptors, among others, contributing to the phenomena of central sensitization.[18,21] Substance P binds to the neurokinin-1 (NK-1) receptors. Inhibitory neurotransmitters include gamma-aminobutyric acid (GABA) which binds pre-synaptically to $GABA_B$ and post-synaptically to $GABA_{A,B}$ receptors to decrease neuronal excitability. Enkephalin binds pre-synaptically to voltage-gated calcium channels. Glycine is primarily an inhibitory neurotransmitter although it participates in the activation of NMDA receptors as a co-agonist alongside glutamate. Opioid receptors (μ, δ and κ) and α-2 adrenergic receptors are located both in pre- and post-synaptic

terminals.[22] Opioids act pre-synaptically by decreasing calcium influx and the release of neurotransmitters and post-synaptically by opening potassium channels with consequent hyperpolarization. The latter leads to reduced generation of action potentials and activation of second order neurons.[12]

1.1.1.5 Ascending nociceptive pathways

Afferent fibers projecting within laminae I and V constitute the major output from the dorsal horn to the brain forming multiple ascending pathways. Three of the five major ascending pathways include the spinothalamic, spinoreticular and spinomesencephalic tracts.

The spinothalamic tract is the most prominent. It contains axons of projection neurons in laminae I and V and carries nociceptive information to the thalamic nuclei. This tract is particularly relevant to the sensory-discriminative component of pain (*i.e.* location, duration and intensity of the stimulus).[13,16]

The spinoreticular tract contains the axons of projection neurons primarily in laminae V, VII and VIII and it terminates both in the reticular formation and the thalamus.

The spinomesencephalic tract contains the axons of projection neurons in laminae I and V. This tract is particularly relevant to the affective-motivational component of pain (*i.e.* unpleasantness of the stimulus). Its projections terminate in the periaqueductal gray matter and parabrachial nucleus. Neurons of the parabrachial nucleus are the first to be activated by nociceptive inputs in the brain, and the central amygdala is one of its major targets of projection.[23]

The amygdala is a limbic brain region that plays a key role in emotional processing and in the emotional-affective dimension of pain. The plastic changes occurring in neurons connecting the parabrachial nucleus with the central amygdala, as well as the insula with the lateral amygdala have been suggested to contribute to the unpleasantness of pain.[24,25] For example, research with rodents involving sub-acute to chronic pain models reveal robust and large-magnitude parabrachial-amygdala synaptic potentiation, regardless of the type and etiology of pain.[25] The latter suggests that an

understanding of the synaptic changes of the neurons in the lateral parabrachial nucleus and central amygdala are key to understand the molecular and cellular mechanisms of the chronification of pain as an "emotional experience". In other studies with rodents, two independent subpopulations of neurons in the insular cortex were recently found to project to the lateral and central amygdala driving threat learning and fear-associated responses, respectively.[24] Strong excitatory synapses were formed between the two neuron populations and the two regions of the amygdala. This shows that the insular cortex is intricately involved in processing aversive somatosensory information. Finally, a distinct neural ensemble in the basolateral amygdala that encodes the negative affective/emotional qualities of pain has also been identified.[25] The basolateral amygdala is believed to mediate chronic pain unpleasantness by linking nociceptive inputs to aversive perceptions.

1.1.1.6 Descending modulatory pathways

The periaqueductal gray matter and rostroventral medulla are, among others, part of a descending pain-control system that modulates signaling *via* inhibitory and facilitatory mechanisms. Thus, descending controls can increase or decrease ascending nociceptive messages by involving an entire network of descending modulatory mechanisms that are activated simultaneously when a noxious stimulus occurs.

A mechanism of pain inhibition was first demonstrated in rats by Le Bars *et al.* in 1979 using the diffuse noxious inhibitory control (DNIC) paradigm.[26] The DNIC reflects an inhibition of nociceptive neurons (wide dynamic range neurons) in the spinal dorsal horn caused by a noxious stimulus applied distantly from the neurons' excitatory receptive field.[26,27] This paradigm relies on the concept of the spino-bulbo-spinal loop which refers to ascending nociceptive dorsal horn pathways that terminate in the parabrachial nucleus and periaqueductal gray. The rostroventral medulla then receives input from these higher centers (periaqueductal gray, parabrachial nucleus and insular cortex) and projects down back to the spinal dorsal horn resulting in facilitation or inhibition of the nociceptive message (Figure 1).[26–28]

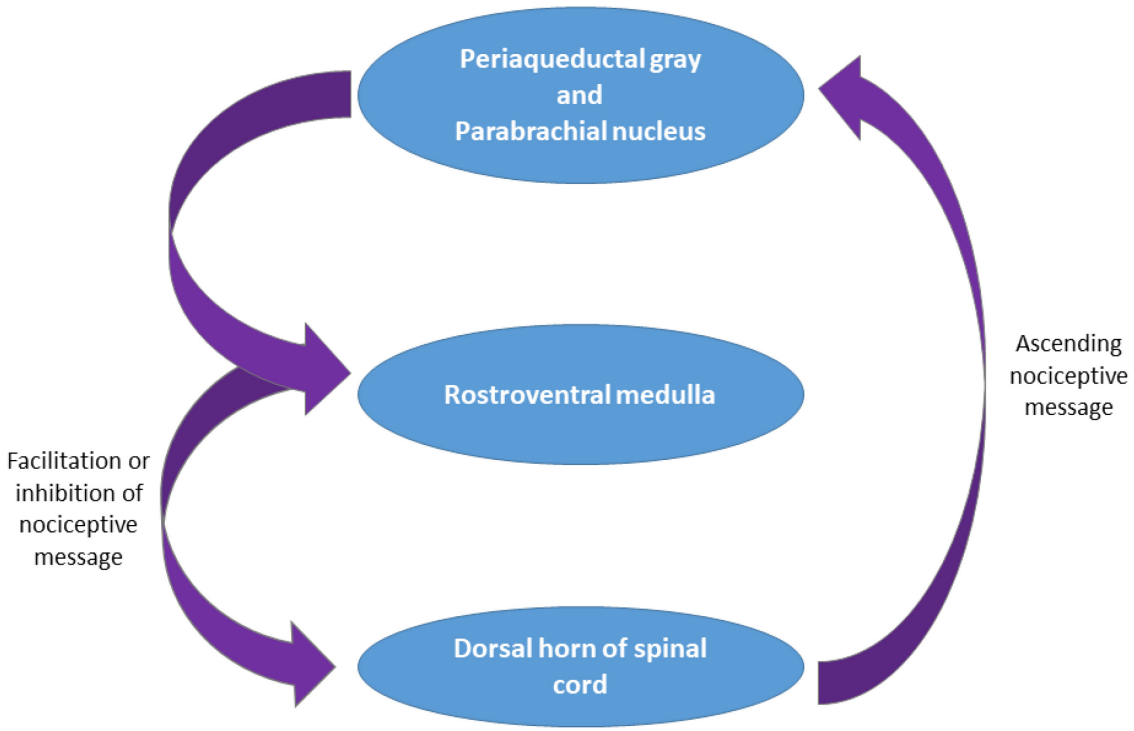

Figure 1. Simplified schematic of the spino-bulbo-spinal loop

In persistent pain conditions such as in inflammation and neuropathy, persistent nociception simultaneously triggers descending facilitation and inhibition.[29] For example, the descending serotoninergic pathway may exert inhibition or facilitation depending on the type and duration of pain, as well as on the expression of 5-HT receptors.[22] The activation of receptor 5-HT_1 results in antinociception, while activation of receptors 5-HT_2 and 5-HT_3 results in nociception.[20]

The dopaminergic pathway exerts antinociceptive effects *via* activation of D2 and D3 receptors and nociceptive effects *via* activation of D1.[20] The noradrenergic pathway seems to exert only antinociceptive effects *via* activation of α-1 and α-2 receptors.[22]

1.1.1.7 Pain perception

Perception of pain occurs when the nociceptive signal is integrated in the cerebral cortex and the stimulus is consciously perceived. Perception of noxious stimuli involves the incorporation of emotional attributes in the thalamus and cortical structures to these sensory signals.[30] Pain perception can be affected by numerous variables including past experiences, environmental and social contexts (Figure 2).[1] Interestingly, emotions also have powerful effects on pain perception, and it is generally accepted that negative emotions increase pain whereas positive emotions decrease pain.[31] The resulting pain experience is thus influenced by a strong interrelation between pain and emotion involving multiple mechanisms within the brain and spinal cord.

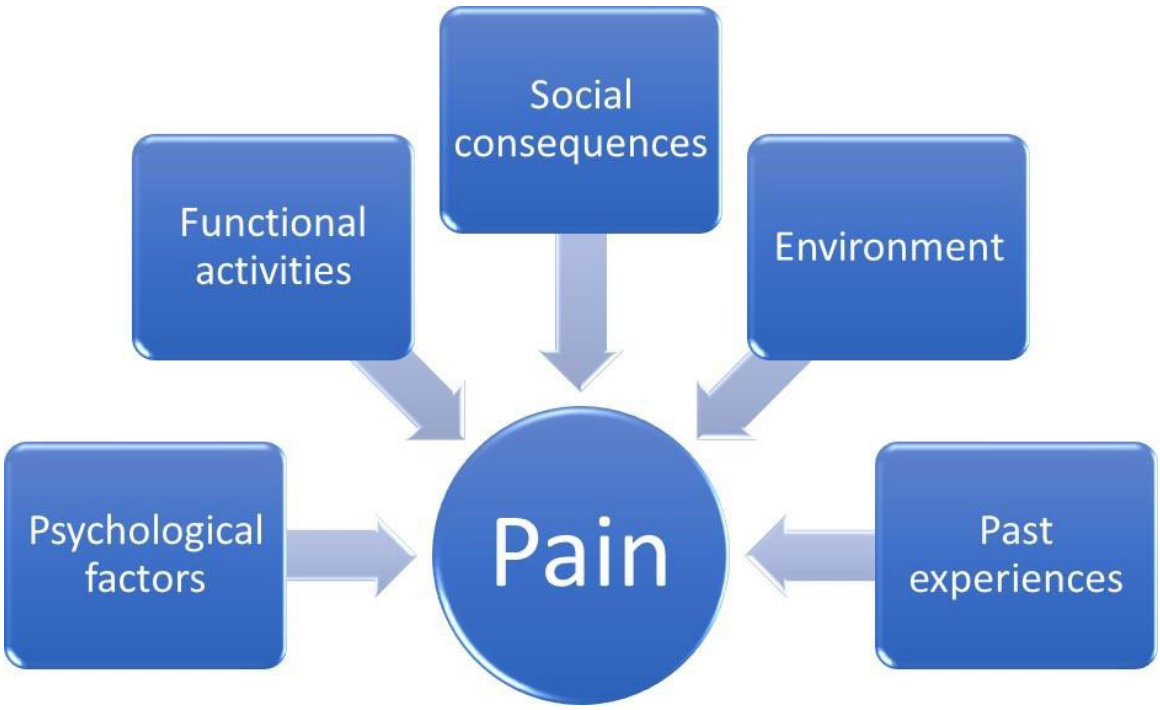

Figure 2. Perception of pain is influenced by numerous factors

1.1.1.8 Clinical pain

With such diverse population of nociceptors, channels and transducers, and numerous mechanisms that underlie nociceptor excitability, it becomes evident that the nociceptive pathway is extremely complex with potential for immense interindividual variability. Not only can these mechanisms differ among individuals, but the type of injury, time after injury, genetics, sex and history of the injured tissue also affect the pain experience (cognitive-evaluative determinant of pain). It explains how the pain profile can be so diverse among patients being affected by the same clinical condition. It also explains the difficultly in developing new therapeutic interventions for the management of pain and the varied response to therapeutic interventions.

Clinical pain can be categorized into four main groups. Nociceptive, inflammatory, neuropathic and (dys)functional pain. Nociceptive pain refers to physiological pain that serves a purpose (*i.e.* protection) and that usually subsides once the potential or actual tissue damage is no longer present (*i.e.* adaptive). Inflammatory pain involves the release of inflammatory mediators which sensitize nociceptors resulting in pain, in addition to redness, swelling, heat and loss of function. Neuropathic pain is defined as pain caused by a lesion or disease of the somatosensory nervous system.[32] Although the definition of (dys)functional pain varies in the literature, it can be considered as pain disorders for which no pathology or organic disease is encountered after comprehensive search to explain a pain symptom.[32] Some authors claim that cancer pain should be regarded as a fifth separate entity because of complex interactions between cancers and the somatosensory system.[33]

1.1.2 Osteoarthritis-induced peripheral and central sensitization

1.1.2.1 Introducing osteoarthritis

Osteoarthritis is a degenerative and inflammatory disease of synovial joints characterized by structural and functional changes in articular cartilage, subchondral bone, synovium and ligaments, as well as supporting musculature and fibrocartilaginous structures such as the meniscus in the knee joint.[34–36] Although not completely elucidated, the pathophysiology of OA involves inflammatory, biomechanical and metabolic components. Age, obesity, metabolic disease, sex, joint trauma and genetics are major risk factors for the development of OA in human beings,[37,38] and is suspected to be similar for companion animals.[39–42]

Recent evidence points to the importance of a systemic low-grade inflammation due to aging (*i.e.* inflammaging) and metabolic diseases (*e.g.* obesity, insulin resistance) causing the release of inflammatory mediators in the bloodstream which are deleterious for joint tissues. Along with biomechanical changes (following trauma or genetic structural disorder such as dysplasia), the latter events contribute to the process of joint degradation.[36,43,44] Therefore, we could distinguish different OA phenotypes: Aging [(ab)normal stress on (ab)normal joint]; Metabolic [abnormal stress on normal joint] and Biomechanic [abnormal stress on (ab)normal joint].

Osteoarthritis is the single most common cause of pain and disability in older adults. With a predicted increase in the size of the aging population and incidence of obesity world-wide, OA is now considered a "priority disease" by the World Health Organization.[45] It is estimated that 7 million Canadians (1 in 5) will be affected by OA in 2031.[46] The impact in the society is huge. Researchers emphasize the importance of prevention, since several environmental and lifestyle changes may reduce the incidence of OA.[36] In domestic animals, very little data is available regarding the costs associated with the disease. Nevertheless, with the expected increase in the life expectancy of dogs and cats, the prevalence of chronic painful conditions is also expected to rise.[47,48]

1.1.2.2 Joint innervation

Joints are innervated by Aβ, Aδ and C fibers. Free nerve endings are found in all joint structures (synovium, ligaments, fibrous capsule, adipose tissue, periosteum, meniscus) except in the cartilage.[49] C fibers with polymodal receptors represent the great majority of fibers innervating the joint. They can express various receptors including trkA for NGF, transient receptor potential vanilloid (TRPV) receptors which play a role in neuropathic pain,[50] inflammation receptors, as well as opioid and cannabinoid receptors.[51,52] Additionally, they can release various mediators including substance P, calcitonin gene-related peptide and neuropeptide Y.[53]

1.1.2.3 Peripheral sensitization

Joint pain results from peripheral and central inputs, with peripheral mechanisms prevailing at early stages of the disease and central mechanisms at later stages.[54] When joint inflammation develops, the release of inflammatory mediators such as prostaglandins and NGF renders peripheral nociceptors sensitized and hyperexcitable. This means that the threshold for activation is reduced and the magnitude of response after a given stimulus is increased.[34] A series of events take place including activation of 'silent' nociceptors and activation of neighboring nociceptors from undamaged healthy tissues expanding the receptive field. The ensemble of these phenomena are known by peripheral sensitization.[19,55,56]

The persistent recruitment of primary afferent fibers from inflamed joints significantly increases the input to the spinal cord which in turn, contribute to the development of central sensitization.

In OA, it is the chronic tissue damage and low-grade inflammation that produce and release inflammatory mediators in the joint (and elsewhere). This process not only activates innervating nociceptors but also promotes further joint destruction feeding onto this vicious cycle.[57] The microenvironment of the OA joint is one of great complexity with most cell types both producing and responding to inflammatory cytokines and chemokines and other mediators.[57] Another important component of joint pain is mechanical pain secondary to inflammation and consequent increased intra-articular pressure. While a normal joint has intra-articular pressures between 2 and 10 mmHg,[58]

the pressure in inflamed joints can rise up to 20 mmHg resulting in an increase of mechano-sensitivity of joint receptors characterized by mechanical hyperalgesia.

The clinical manifestation resulting from this phenomenon are primary and secondary hyperalgesia. Evidence from research indicates that primary and secondary hyperalgesia are handled differently by the descending control system.[29] It proposes that two spinal neuronal pools are affected differently by the descending system. For example, when the knee is inflamed, the spinal neuronal pool with predominant knee input is the primary pool and would be involved in primary hyperalgesia, whereas neighboring neuronal pools with predominant input from healthy tissues (*e.g.* foot), would be involved in secondary hyperalgesia.[29]

1.1.2.4 Central sensitization

Central sensitization is the increased responsiveness of nociceptive neurons in the CNS to their normal or subthreshold afferent input. It is expressed as pain hypersensitivity and sustained cerebral nociceptive inputs. These processes translate clinically to hyperalgesia (*i.e.* increased pain from a stimulus that normally provokes pain) and allodynia (*i.e.* pain due to a stimulus that does not normally provoke pain).[18–20] Three main cellular processes contribute to central sensitization: increased membrane excitability, facilitated synaptic strength and decreased inhibitory influences (*i.e.* disinhibition). Membrane excitability and facilitated synaptic strength result from changes in the threshold and activation of NMDA and AMPA receptors, trafficking of NMDA and AMPA receptors to the membrane, and alterations in ion channels to increase inward currents and decrease outward currents, among others.[18,59] These lead to long-term potentiation, spatial and temporal summation of perceived signals associated to extension of the sensitization (secondary) and facilitated sensitization (primary). Disinhibition results from the diminished activity of the descending inhibitory pathway and decreased inhibitory interneuron activity caused by decreased synthesis of inhibitory neurotransmitters (GABA and glycine) or loss of interneurons.[18,59] Intrinsic plasticity of dorsal horn neurons and alterations in the properties of low-threshold mechanoreceptive Aα afferents will contribute to sensory central sensitization. Finally, immune mediators released from spinal microglia and astrocytes can further enhance neuronal excitability and decrease inhibitory currents.[55] The aforementioned mechanisms are generally short-

lived and reversible; nevertheless, they can become pathologic in the face of sustained inflammation or nerve injury, such as in OA.[20]

1.1.2.5 Clinical pain

Osteoarthritis is a slowly progressing disease and it is unclear at which stage the joint becomes painful.[34] Clinical pain in patients with OA can have inflammatory and neuropathic components and features of peripheral and central sensitization. This results in a multitude of clinical presentations and raises the question of which tissues and pathological changes give rise to OA pain.[60] In order to clarify this question, three major factors contributing to OA pain have been proposed (Figure 3).[36,43,53]

It is interesting to note that the degree of joint damage does not correlate with clinical pain in people or domestic animals.[39,42,54,61] Nevertheless, synovitis is a recurrent feature of OA pathogenesis,[35,62] and a positive relationship between inflammatory changes in the joint and pain is now clear.[43,60,63,64] Furthermore, a neuropathic pain component may be predominant in individuals with minor joint changes but high levels of pain refractory to analgesic treatment, providing an alternative explanation for osteoarthritic pain perception.[65]

With so many distinct pain profiles, patients with knee OA can be stratified into six clinical phenotypes: 1) chronic pain in which central mechanisms (*e.g.* central sensitization) are prominent; 2) inflammatory (high levels of inflammatory biomarkers); 3) metabolic syndrome (high prevalence of obesity, diabetes and other metabolic disturbances); 4) bone and cartilage metabolism (alteration in local tissue metabolism); 5) mechanical overload; and 6) minimal joint disease characterized as minor clinical symptoms with slow progression over time.[66]

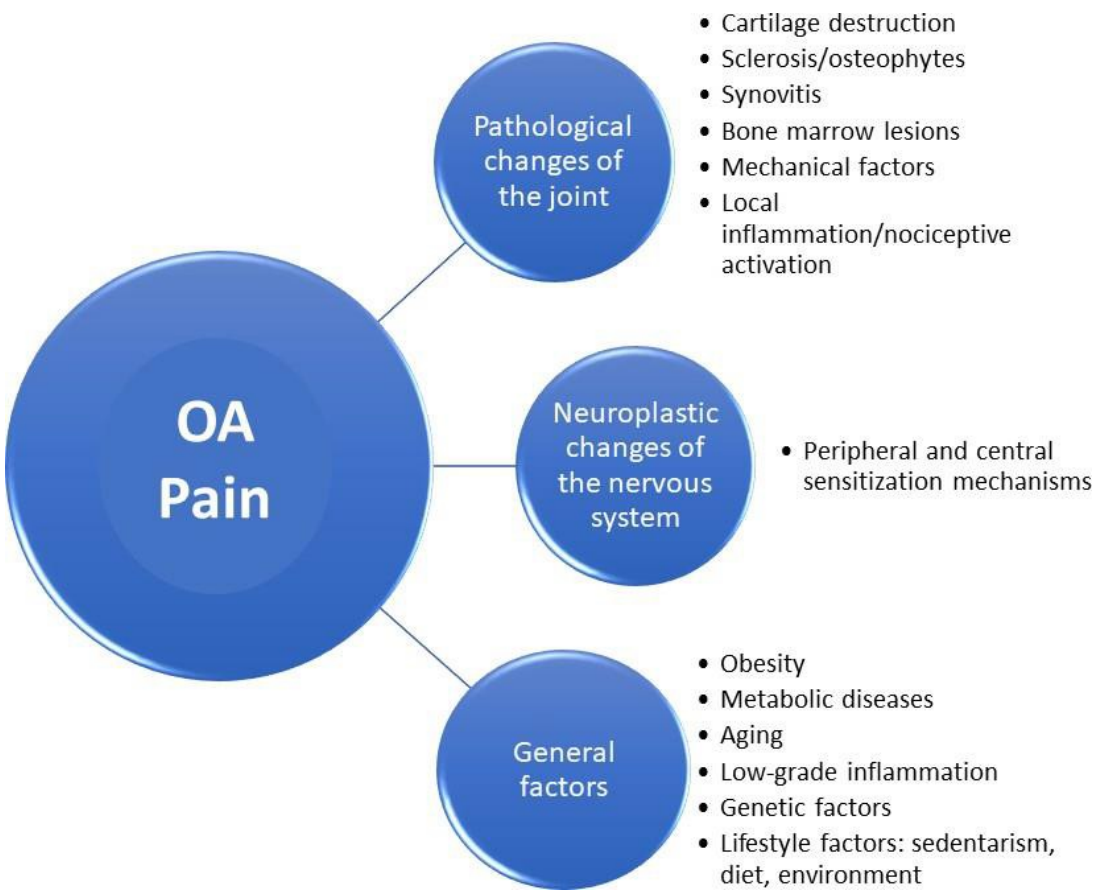

Figure 3. Factors contributing to osteoarthritis-related pain

Obesity-related metabolic factors, especially adipokines, contribute to OA development by inducing pro-inflammatory cytokines and degradative enzymes. This leads to cartilage matrix impairment and subchondral bone remodeling. Ectopic metabolite deposition and low-grade systemic inflammation can contribute to a toxic internal environment that exacerbates OA.[44] In fact, systemic factors that include altered lipid metabolism could explain the diversity of physiological changes in OA.[37] Aging-related pathophysiological changes and genetic predispositions are also major sources of variability in OA expression.

1.1.3 Osteosarcoma-induced peripheral and central sensitization

1.1.3.1 Introducing osteosarcoma

Osteosarcoma (OSA) is an aggressive and invasive malignant bone tumor causing osteolytic and proliferative changes. It is considered rare in people occurring in a bimodal distribution; it usually affects either children in the first two decades of life or patients older than 65 years.[67] In dogs, OSA seems to occur more frequently with incidence rates 27 times higher than in people, and with higher prevalence in large and giant breed dogs.[68–70] Regardless, OSA is the predominant bone cancer diagnosed in both human and canine patients.[67,71]

Osteosarcomas are bone forming tumors that typically originate within the medullary cavity and grow and proliferate invading surrounding soft tissues.[72] They are considered one of the most complex cancers in terms of molecular aberration with genetics definitely playing a role in the pathogenesis. No single driver gene can be pinpointed to be the cause of these tumors; rather, several oncogenic pathways cause genetic instability contributing to the development of osteosarcomas.[72] This might, at least in part, explain the marked heterogenic phenotype of OSA in both people and dogs. Tumors can be predominantly lytic (soft, fleshy and with areas of hemorrhage and necrosis), productive (hard consistency and variably grey in color), or a mix of both. It frequently transgresses the cortex at the same time that it grows within the medulla, rarely penetrating the joint.[70] The vast majority of OSAs originate in the metaphyseal regions of long bones with increased incidence in the appendicular skeleton (up to 80% in dogs and 90% in people).[73] In people, close to 50% of OSA occur in the region of the knee with decreasing incidence in the distal femur, proximal tibia and proximal humerus. In dogs, the thoracic limbs are affected twice as often as the hind limbs with decreasing incidence in the distal radius, proximal humerus, distal and proximal femur and distal tibia.[70,72,73] Prognosis are affected by the presence of metastasis which occurs most commonly in the lungs. In people, the 5-year event-free-survival is 27%, and 70%, for patients with and without metastatic tumors at diagnosis, respectively.[74,75] Dogs have usually poorer diagnostics because they are frequently diagnosed at advanced-stages of the disease. Following diagnosis of canine OSA, survival rate is commonly one year; for those that survive

beyond this period, 54% develop metastatic disease (median survival time: 243 days).[76]

Pain is one of the most feared and debilitating symptoms in patients with cancer affecting from 43 to 63% of patients at all disease stages.[77] When pain is present, it is moderate to severe in over one third of patients. Cancer pain is multidimensional. It is a consequence of a number of distinct mechanisms related to the direct physical effects of the tumor and its biochemical interactions with its host environment. In addition, pain may result as a consequence of therapies such as chemotherapy, surgery and radiation, diagnostic procedures, metastatic disease and concomitant painful diseases. This chapter will focus on primary bone cancer-induced pain.

1.1.3.2 Bone innervation

In contrast with the skin where a multitude of sensory fibers is present, the bone of fully-grown individuals has a restricted and unique innervation. There is a much greater percentage of $trka^+$ neurons innervating bone (close to 80%) *versus* the skin (close to 30%). Bones are largely innervated by thinly myelinated Aδ and peptidergic C fibers with greatest presence of these fibers in the periosteum, followed by the bone marrow and mineralized bone.[76] Bones have little to no innervation by Aβ fibers and non peptidergic C fibers since they are generally deeper structures located beneath the surface of the skin and do not require the same level of sensitivity as the skin. This means that most of the afferents innervating bone are only activated by injury or damage.[78]

1.1.3.3 Peripheral sensitization

Bone cancers such as OSA cause bone and soft tissue destruction resulting in the sensitization of primary afferents (Figure 4). As the tumor grows and cortical bone is destroyed, the periosteum becomes inflamed and disrupted. Tumor-induced tissue damage and abnormal osteoclast-mediated bone resorption activate TRPV receptor 1 and acid-sensing ion channels expressed on sensory afferents from bones. Both osteolytic and osteoblastic tumors induce a loss of the mechanical strength and stability of mineralized bone which further results in pathological bone fractures. This bone remodeling results in the distortion and consequent activation of mechanosensitive nerve fibers that innervate the bone.[79]

Stromal cells, such as mesenchymal, endothelial and a host of immune cells form what is called the host-derived stroma which interfaces directly with the tumor characterizing neuroimmune interactions between neoplastic cells and the host immune and nervous systems. Communications and interactions between these play a fundamental role in potentiating the growth and spread of tumors and in the generation and maintenance of cancer pain. The latter occurs *via* synthesis of a variety of chemical mediators that are released into the tumor microenvironment, further sensitizing primary afferents. These mediators include prostaglandins, NGF, endothelin and bradykinin.

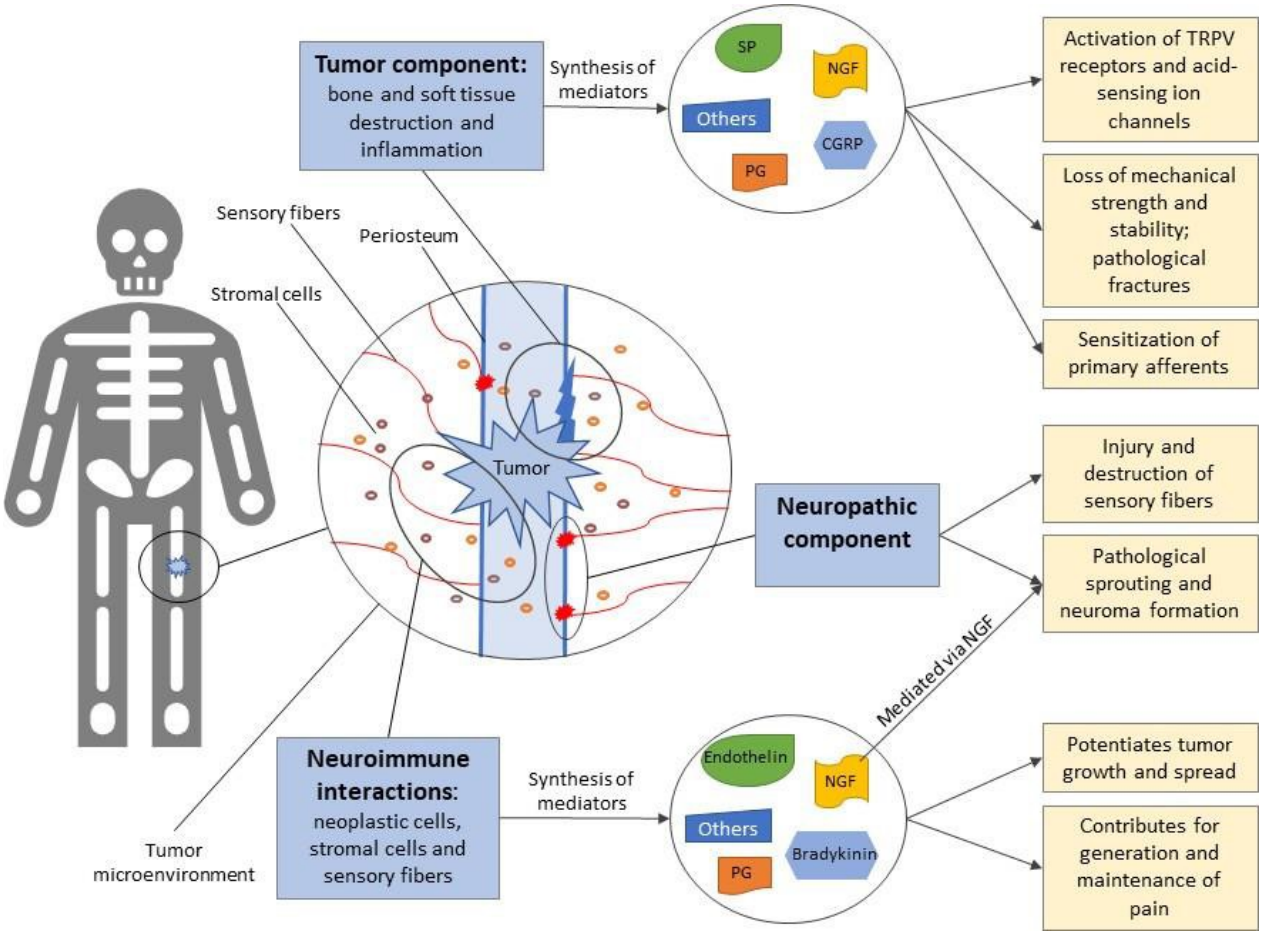

Figure 4. Components contributing to peripheral and central sensitization in patients with bone cancer

Nerve growth factor seems to be of particular importance in cancer-induced bone pain acting as an upstream regulator of pain. It influences neuronal growth, transmitter release and plasticity in the regions surrounding the tumor by modulating the sensitivity and increasing the expression of several receptors and ion channels that contribute to the increased excitability of nociceptors in the vicinity of the tumor.[33,78,79]

The neuropathic component of bone cancer pain is believed to be primarily associated with the injury and destruction of the distal processes of sensory fibers innervating the bone.[30,76] Another mechanism explaining the generation of neuropathic pain is mediated by NGF resulting in an active and pathological sprouting and neuroma formation by sensory and sympathetic nerve fibers that innervate the skeleton.[78,79]

1.1.3.4 Central sensitization

Cancer pain also causes significant pathological changes in the CNS that contribute to the generation and maintenance of pain.[80,81] Central changes are related with the plasticity of the somatosensory system after continuous and intense nociceptive input from primary Aδ and C fiber afferents innervating the tumor and surrounding tissues. Bone cancer induces upregulation of neuropeptides and transcription factors associated with neuronal damage resulting in simultaneous changes in the segments of the spinal cord that receive input from tumor-bearing tissues. Spinal cord changes are characterized by significant reorganization of both neuronal and supporting cell populations in the dorsal root ganglia and dorsal horn of the spinal cord.[80] In fact, these changes are particular to bone cancer pain and distinguishable from other persistent pain states with unique neuroplastic changes in the spinal excitatory synaptic transmission resulting in spinal sensitization and alterations in sensory modulation and transmission.[81]

1.1.3.5 Clinical pain

Pain from OSA is a mixture of inflammatory and neuropathic components with features of tissue destruction and central neurochemical changes. It is a result of periosteal anatomical disruption, local tissue destruction, changes in sensory innervation and release of pro-inflammatory signaling molecules from the growing tumor (Figure 4). The mechanisms that drive bone cancer pain evolve with disease progression and so does

the clinical presentation of patients.

People with bone cancer report a constant dull pain that gradually intensifies with time.[70] As bone remodeling progresses, severe spontaneous pain frequently occurs with possible episodes of breakthrough pain if microfractures occurs. In dogs, pain is also supposed to be severe with marked negative impact in the quality of life.[82,83]

1.2 Quantitative sensory testing

The quantification of sensory sensitivity allows researchers to make valid conclusions about pain mechanisms, patient phenotyping and response to therapy. Quantitative sensory testing is a psychophysical test used to quantify somatosensory function under normal or pathological conditions in which hypo- or hypersensitivity can occur.[3,84] It allows for the evaluation of Aβ, Aδ and C fibers activity, and their projection pathways to the brain, and ultimately contributes to our understanding of pain mechanisms. Sensory sensitivity can be quantified by QST using calibrated devices that produce mechanical, tactile, thermal, vibratory or electrical stimuli. Static QST focuses on the determination of sensory threshold, or the rating of a single stimulus, and the corresponding magnitude of pain. Dynamic QST focuses on the evaluation of pain modulation by means of the temporal and spatial summation of pain and conditioned pain modulation (CPM) paradigms.

1.2.1 Static QST

1.2.1.1 Definition and Background

Static QST involves the determination of an individual's pain threshold, or rating of a single stimulus, which is defined as the minimum intensity of a stimulus that is perceived as painful.[32] These psychophysical measurements employ the whole sensory axis, from peripheral sensors to the brain. They are primarily used in three distinct scenarios: 1) mechanistic studies to provide insights into the basal state of the nociceptive system in healthy individuals; 2) clinical studies for diagnostic and monitoring purposes; 3) pharmacological studies to evaluate the analgesic efficacy of new and existing compounds.[3]

Somatosensory testing has evolved from the initial work of Max von Frey who originally discovered discrete pain points on the human skin. He used horse and boar hairs of varying thickness to test mechanical sensitivity as well as other probes to localize spots sensitive to cold, warmth and pain. It was based on these observations that von Frey developed the theory of receptor sensitivity in the late nineteenth century. The foundation of the theory was that each sensory modality had a specific, minute, highly specialized

receptor.[85] Quantitative sensory testing has since evolved to a wide scope of stimuli and sophisticated methodology of testing protocols applied from basic science to clinical research.[3,30,86,87]

1.2.1.2 Procedures

Numerous QST protocols can be applied to skin or deeper tissues including muscles, bones and visceral organs.[3] The application of experimental painful stimuli to healthy individuals can be performed under normal conditions or under disease-simulating models. In the latter case, peripheral and/or central sensitization can be imposed experimentally by surgical procedures or by application of algogenic substances to specific tissues. The individual then develops a specific symptomatology including hyperalgesia or allodynia and these are considered a proxy for what is observed clinically allowing the study of the underlying mechanisms.[3] The use of electrical stimulation is unique as it bypasses the receptors and non- selectively stimulates afferent sensory fibers of all types. Yet, it is used for the same purposes of assessing the status of the somatosensory system and related neuroplasticity.[30]

Assessment of cutaneous sensitivity is done using chemical, thermal, electrical or mechanical (tactile) stimuli. Primary cutaneous hyperalgesia is assessed using heat and mechanical pressured which sensitize peripheral nociceptors. Secondary hyperalgesia is assessed using pinprick or stroking stimulation for which a positive response indicates central sensitization.[88,89] Models with predominantly primary hyperalgesic characteristics include topically applied capsaicin, controlled burn injury, UVB irradiation and continued electrical stimulation by intradermal wires.[3] Nevertheless, one should keep in mind that it can be very difficult to dissociate between peripheral and central components of sensitization as the former is usually the main driver of the latter.

Assessment of muscle hyperalgesia is done using endogenous or exogenous models. Endogenous models include ischemic and exercise-induced muscle pain. Exogenous models refer to external stimulation using mechanical, electrical and chemical modalities.[90] For example, muscle hyperalgesia can be induced after intramuscular injections of capsaicin, glutamate, or NGF.[3] Assessment of bone pain can

be done using pressure algometer that produces mechanical stimulus over the periosteum.[90,91]

Assessment of visceral pain is done in hollow organs and can be experimentally induced. For example, visceral hyperalgesia in the esophagus can be achieved using a probe that produces multimodal stimuli (cold, warm, electrical and mechanical) located in the lower part of the esophagus. Esophageal sensitization can also be induced by chemical stimulation using a perfusion of hydrochloric acid.[92] Visceral hyperalgesia in the bladder and bowels can be assessed using pressure manometry and balloon distension to study mechanosensitivity and pressure pain in these organs.[93,94]

1.2.1.3 Measures

Threshold determination (*e.g*. pain detection or tolerance threshold) or pain-magnitude rating (*e.g.* visual analogue scale) are usually the endpoint applied in static QST. Threshold determination is more indicative of the basal state of the somatosensory system with the advantage of involving a well-defined, stable and reproducible endpoint that can be applied to human beings and animals. Pain-magnitude rating is employed in humans or animals (by proxy) and requires a good set of standardized instructions that are neutral and should not bias the participant in any way. Regardless of the stimulus being used or organ being tested, four basic elements apply to all psychophysical methods (Table II).[30]

1.2.1.4 Interpretation

Distinct pathophysiological mechanisms of pain generation produce specific sensory abnormalities.[95,96] Even so that within the same condition, patients can present different sensory profiles.[97] The understanding of these differences can have a huge impact in the diagnosis of painful conditions and can be used as surrogate markers of pain mechanisms in order to develop a mechanism-based treatment.[95]

Table II. Components of quantitative sensory testing.

Component	Description
Subject	The subject who receives the stimulus. If it is a human, then detailed reporting of the perceived characteristics of the sensation produced by the stimulus is recorded. If it is an animal, then interpretation of behavior reactions is performed.
Stimulus	The stimulus must have well-defined physical properties that is produced using calibrated devices. Stimulus intensity, duration, and modality are controlled and do not vary over time. Stimulus can be applied to different tissues.
Examiner	An examiner who performs the delivery of the stimulus which is always standardized. If possible, the examiner is blinded to the condition of the patient and/or the numerical result of the QST.
Procedure	Standardized instructions or acclimation. If it is a human, then detailed instructions are given to guide the participant in what characteristics to attend to and what features to report. If it is an animal, then acclimation of the individual to the personnel and devices is performed over a period.

In the study of neuropathic pain, QST aides in the evaluation of positive and negative sensory neurological phenomena since hyper- and hyposensitivity are clinical features of patients with neuropathic pain (Table III). Thus, when pain, allodynia, hyperalgesia, hyperesthesia, and paresthesia are observed, a positive or excessive neural activity is diagnosed. When hypesthesia, anesthesia, hypoalgesia and analgesia are observed, a negative or deficient neural activity is diagnosed.[3,30]

In the study of musculoskeletal pain, particularly related to joint pain, hyperalgesia can be detected primarily at the affected site or at distal or remote locations which are observed with several QST modalities including mechanical (pressure, punctate, brush, vibratory), electrical, thermal and chemical stimuli.[79] Muscle pain can result in mechanical hyperalgesia, increased hyperalgesic reactions to cutaneous capsaicin and increased number of trigger points.

Table III. Quantitative sensory testing, fibers involved and interpretation of the pain profile according to stimulus' response.

Modified from Mücke *et al*. 2016[98]

QST type	Fibers assessed	Response to stimuli	Pain profile
Hot thermal stimuli	C, Aδ	Increased pain sensitivity	Heat hyperalgesia
Cold thermal stimuli	C, Aδ	Increased pain sensitivity	Cold hyperalgesia
Calibrated needle stimuli	C, Aδ	Increased pain sensitivity	Hyperalgesia to pinprick stimuli
Pressure algometer	C, Aδ	Increased pain sensitivity	Mechanical hyperalgesia
Brush, cotton swab to skin brushing	Aβ	Pain in response to non-nociceptive stimuli	Allodynia
Light cold stimuli	Aδ	Decreased sensitivity to non-painful stimuli	Cold thermal hypoesthesia
Light heat stimuli	C	Decreased sensitivity to non-painful stimuli	Hot thermal hypoesthesia
von Frey filaments	Aβ	Decreased sensitivity to non-painful stimuli	Mechanical hypoesthesia
Cold/heat stimulus	C, Aδ	Decreased sensitivity to painful stimuli	Thermal hypoalgesia
Calibrated needle stimuli	C, Aδ	Decreased sensitivity to painful stimuli	Mechanical hypoalgesia
Pressure algometer	C, Aδ	Decreased sensitivity to painful stimuli	Mechanical hypoalgesia

In the study of visceral pain, sensitization can occur locally in the affected viscera (*i.e.* visceral sensitization), in referred somatic areas (*i.e.* referred sensitization), or in other viscera (*i.e.* viscero-visceral sensitization).[3] Visceral sensitization can be seen for example in patients with esophagitis who have increased sensitivity to thermal and mechanical stimuli.[86] Referred sensitization is characterized by hyperalgesia in somatic tissues. This is explained by the 'convergence-projection theory' in which visceral and somatic afferents converge to the same neurons in the CNS.[90] An interesting example of referred sensitization from visceral pain is migraine which results in periorbital skin sensitivity. During migraine attacks, patients can develop cutaneous allodynia to thermal and mechanical stimuli within the referred pain area.[92] Finally, viscero-visceral sensitization is the co-existence of allogenic conditions in two internal organs with documented partially common sensory projections. This results in mutually enhancing pain symptoms from each organ such as in concomitant coronary artery disease and gallstones.[93] To further complicate things, the latter group of patients can also develop referred muscle chest and abdominal hyperalgesia indicating an involvement of viscero-visceral-somatic sensitization.[93]

All QST responses rely on the participant's perception, therefore a number of factors such as attention, cooperation, motivation and anxiety are known to influence results.[24,30] In order to reduce variability in results, standardized QST protocols have been proposed in people and dogs. Particularly in people, standardized QST protocols such as the German Research Network on Neuropathic Pain[99] may help to define very specific information regarding the function of the somatosensory system in specific neuropathic conditions. For example, specific areas of positive and negative sensory changes and its associated innervation can be pinpointed, and the kinds of fibers involved can be identified. Conceptually, a valid way to apply stimulus intensity rating is to use a reference point (unaffected site) against which stimulus in the affected site is rated.[76] Ultimately, it allows a wide assessment of the various fiber families in the peripheral nerve and their correlates in the CNS tract.[3]

1.2.2 Dynamic QST

1.2.2.1 Definition and Background

Dynamic QST focuses on the evaluation of pain modulation by means of the temporal summation of pain and CPM paradigms. It allows for the assessment of a complex course of pain processing by activating and measuring temporal and spatial summation as well as descending modulation of pain.

The temporal summation paradigm tests the activity-dependent facilitation of pain processing also known as spinal windup. It is the ability to integrate repetitive nociceptive input.[100] Hence, its test consists of the administration of a repetitive train of stimuli of constant intensity; increase in pain is expected along the series of stimulus. This occurs due to the summation of action potentials in the spinal cord resulting in amplification of pain as well as painful after-sensations that persist following cessation of the stimulus.[84,101] Spatial summation is the ability to integrate nociceptive input from larger areas than the expected primary site.[100]

The CPM paradigm tests the function of the descending pathways.[84,102] In people, this test is also called heterotopic noxious conditioning stimulation. When CPM is performed in animals, it is usually named DNIC. As previously presented in section 1.1.1.6, DNIC is defined as the inhibition of ascending spinal nociceptive neurons produced by a noxious stimulus applied at a location distant from the neurons' receptive field *via* a spino-bulbo-spinal loop mechanism.[26] The DNIC was first developed for use in rats and further adapted for use in people.[100] More recently, it has been validated for use in healthy dogs.[103]

Despite the confusion in terminology in the literature, it should be noted that the terms CPM and DNIC refer to different things. While DNIC refers to the mechanisms involved in pain inhibition, CPM refers to the test of its analgesic effects. In other words, the CPM can be regarded as the observable result of the activation of the DNIC. In this thesis, the term CPM will be applied in the context of the test of the DNIC in animals (*i.e.* the analgesic effects of DNIC are being evaluated in veterinary patients). Although similar descending pain inhibition mechanisms have been proposed between people and animals,[104] differences between species might exist, although they are not yet clear.

1.2.2.2 Procedures

Temporal summation tests can be performed using mechanical pressure, heat, cold and electrical stimuli repetition.[4] There are several different protocols and methodologies which complicates comparisons among studies. Nevertheless, temporal summation seems to consistently measure pain facilitation in people[54,105–108] and in animals with induced and natural pain conditions.[109–111] For example, one study reported the use of repetitive mechanical pressure over the tibia of healthy volunteers. The stimulus was repeated 10 times and volunteers rated the pain intensity using a visual analogue scale (VAS).[100] Another study reported the use of laser, thermode and electrical repetitive nociceptive stimulation in people after capsaicin-induced primary and secondary hyperalgesia model.[109] Temporal summation has also been performed in anesthetized healthy rats in which electromyographic responses were elicited by electrical stimulation.[110] In cats with OA-related pain, repeated mechanical pressure of subthreshold intensity of 4 Newtons has been reported to be the most reproducible and sensitive/specific. The stimulus was done around mid-radius/ulna for up to 30 repetitions or until a behavior response was observed (*e.g.* limb withdrawal).[111] Temporal summation testing has also been reported in dogs under general anesthesia.[112] Spatial summation tests can be done using thermal and mechanical stimulation, for example.[113] It is performed to mimic large areas of hyperalgesia as seen in pain conditions such as fibromyalgia.[114] This test is not frequently reported.

Conditioned pain modulation or DNIC testing consists of the evaluation of a pain threshold (*i.e.* test stimulus) followed by a second test stimulus either at the same time or immediately after a distant heterotopic stimulus of thermal, mechanical, electrical or chemical nature (*i.e.* conditioning stimulus). In most subjects, the pain intensity experienced with the second test stimulus is reduced during or after the conditioning stimulus due to activation of the inhibitory system.[102,115] The conditioning stimulus causes a decrease in pain perception induced by a different noxious stimulation given elsewhere in the body.[116] The testing procedures and reporting of CPM are greatly variable among studies and a standardization of protocols has been called in question.[102,115,117] The magnitude of CPM depends on how intense, when and where the conditioning stimulus is performed[118] and currently no gold standard CPM protocol has

been identified. It is also interesting to note that CPM induced by two concomitant conditioning stimuli was less efficient than when induced by a single one (cold pressor pain and muscle pain), which indicates that multilocational stimuli actually may interfere and disturb the balance between descending inhibition and facilitation.[119] In contrast, when the conditioning stimulus was a 60-second immersion of the hand in cold, hot or skin temperature, the conditioning pain modality did not seem to affect CPM test.[118] Finally, CPM testing can also be performed with the patient under general anesthesia as recently reported in dogs.[112]

1.2.2.3 Measures

In temporal summation the repeated stimulation of afferent C fibers from somatic tissues causes a frequency-dependent increase in neuronal excitability reflecting the phenomenon known as wind-up.[110] Long-lasting increases of excitability of primary afferents and spinal cord causes neural plasticity at spinal and supraspinal levels. Hence, the wind-up phenomenon achieved by temporal summation testing similarly resembles central sensitization and provides an indirect means to measure it. In fact, temporal summation provokes pain facilitation but also activates supraspinal mediated inhibitions. One offsets the other, and the resulting consequence reflects modulation of pain.[110]

In CPM testing, the descending pathways are assessed. As previously discussed, the descending modulation of pain has both inhibitory and facilitatory systems; the competition between them result in spinal modulation of pain.[29] In CPM or DNIC testing in people and animals it is not possible to dissociate between the two competing systems and only the net effect can be assessed. Thus, a reduction in inhibition may be related to deficiency of the inhibitory system or increased pain facilitation. Nevertheless, most studies have considered CPM testing as a means to primarily evaluate the inhibitory system; thus, most human studies have used the ‘‘pain-inhibits-pain’’ paradigm.[101]

1.2.2.4 Interpretation

Facilitated temporal summation of pain is detected when decreased pain threshold or elevated ratings of VAS are observed after repeated nociceptive stimuli. People with fibromyalgia and generalized diffuse musculoskeletal pain have lower pain threshold to

repeated electrical intramuscular stimulation when compared with healthy controls.[114] These patients are considered to have facilitated temporal summation of pain which in turn, is interpreted as an indication of central sensitization.[114] Similarly, in cats showing early behavioral response to repetitive sub-threshold stimuli, facilitated temporal summation was detected: cats with OA responded much earlier to repetitive stimuli when compared with healthy cats.[111]

A solid body of evidence indicates that CPM may be an important biomarker of chronic pain and predictor of treatment response. Patients with chronic pain have significantly reduced CPM effects when compared with healthy individuals.[54,105,117] In one study, people with knee OA and healthy controls were submitted to pressure pain threshold testing before and after ischemic compression of the arm as the conditioning stimulus. While healthy controls showed an increase in pressure pain threshold after the condition stimulus, patients with knee OA did not.[54] The lack of increase in pain threshold after the conditioning stimulus in patients with OA is interpreted as a lack of CPM/DNIC activation or deficient inhibitory mechanisms. This test was shown to predict mechanism-based treatment response in patients with painful diabetic neuropathy. Using contact heat as the test stimulus and immersion of the hand on hot water bath as the conditioning stimulus, the CPM effect was calculated as the difference (last minus first) in the pain scores of the two test stimuli. A negative value indicated efficient CPM. Patients with less efficient CPM revealed better analgesic response to duloxetine, a drug enhancing descending inhibitory mechanisms, than patients with normal (efficient) CPM.[97] It was concluded that pre-treatment CPM assessments predicted the efficacy of duloxetine highlighting the importance of pain pathophysiology in the clinical decision-making process.[97]

1.3 Quantitative sensory testing in feline osteoarthritic pain - A systematic review and meta-analysis

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1.3.2 Contributions from PhD candidate

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1.3.3 Abstract

Quantitative sensory testing (QST) is a psychophysical test used to quantify somatosensory sensation under normal or pathological conditions including osteoarthritis (OA). This study aimed to conduct a systematic review of studies using QST in healthy and osteoarthritic cats and to assess its ability to differentiate between those two populations.

Hierarchical models with random intercepts for each individual study extracted through the systematic review were fit to subject-level data, and QST measures were contrasted between healthy and osteoarthritic cats. Registration was done at Systematic Review Research Facility (#26-06-2017). Four bibliographic databases were searched; quality and risk of bias assessment were performed using pre-established criteria.

Six articles were included; most were of high quality and low risk of bias. Reporting guidelines were used in three studies. Punctate tactile threshold (n=70) and mechanical temporal summation (n=35) were eligible for analysis. Cats with OA have lower punctate tactile threshold and facilitated temporal summation of pain when compared with healthy cats. The effect of sex and body weight on sensory sensitivity remained inconclusive throughout all analyses. Due to the strong correlation between age and OA status, it remains difficult to assess the effect of OA on sensory sensitivity, independently of age.

Clear and transparent reporting using guidelines are amongst the most needed improvements in QST research in cats. Similar to people, centralized sensitization, manifested by lower threshold and facilitated excitability, is a feature of OA in cats. Research using QST in cats with natural OA is promising with potential to benefit feline health and welfare and improve translatability to clinical research.

1.3.4 Introduction

The quantification of sensory sensitivity allows researchers to make valid conclusions about pain mechanisms, patient phenotyping and response to therapy.[120] Quantitative sensory testing (QST) is a psychophysical test used to quantify somatosensory sensation under normal or pathological conditions in which hypo- or hypersensitivity can occur.[84,121] Static QST focuses on the determination of sensory thresholds or the rating of a single stimulus and the corresponding magnitude of pain. Dynamic QST focuses on the evaluation of pain modulation, such as the temporal summation of pain and conditioned pain modulation paradigms.[120,121]

Osteoarthritis (OA) is a degenerative disease of synovial joints.[35,36] In clinical research of OA, QSTs are largely used to quantify somatosensory sensation facilitating the characterization of a patient's phenotype.[3,120] Widespread hyperalgesia/allodynia demonstrated by decreased mechanical thresholds, facilitated temporal summation and decreased conditioned pain modulation has been reported in people with knee OA when they were compared with healthy controls.[54,61,117] In veterinary medicine, cats with naturally-occurring OA[111,122–124] and dogs affected by OA[87,125] or osteosarcoma[126] show similar sensory profile, particularly with deficient conditioned pain modulation.[126]

Similarities in physiology and pathophysiology of pain across mammalian species are quite remarkable[2] and animal models of pain have unquestionably contributed to our understanding of pathophysiology of pain; yet, the predictive value of animal models in clinical efficacy of analgesics in humans is still very limited.[10,11,127,128] The study of the somatosensory profile of domestic animals has been gaining increasing attention from the pain research community due to potentially improved translatability to clinical research in humans.[11,128] The literature on the use of QST in cats with OA has never been systematically reviewed.

The goals of this study were to conduct a systematic review of the use of QST in healthy cats and those with OA, and to assess the ability of QST to differentiate animals with naturally occurring OA from healthy controls (determining possible loss/gain of function in somatosensory processing) by means of an individual patient data meta-analysis. The specific research questions were: "What is the effect of OA on the sensory

profile of cats when compared with healthy controls by means of QST?" and "Does QST in cats with OA provide useful information that could be ultimately clinically applicable in human and veterinary medicine?"

1.3.5 Materials & Methods

The study protocol was registered at the SyRF (Systematic Review Research Facility) website on June 26th, 2017 (available at http://syrf.org.uk/protocols/). SyRF is an NC3Rs (National Centre for Replacement, Reduction and Refinement of Animals in Research)-funded initiative by CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) to support the application of evidence synthesis techniques to preclinical research. Findings are reported according to the PRISMA guidelines for systematic reviews[129] as well as the guidelines from the SyRF.[130]

Systematic literature search

Four bibliographic databases (Embase, PubMed, Web of Science and Global Health) were searched to identify studies published in peer-reviewed journals before February 12th, 2019. A systematic search strategy was developed using the following search terms: (arthritis OR osteoarthritis OR osteoarthrosis OR degenerative joint disease) AND (cat OR feline) AND (quantitative sensory testing OR somatosensory OR sensory OR sensitivity OR thermal OR mechanical OR von Frey OR electrical OR nociception OR hyperalgesia OR allodynia). Additional studies were identified by searching the references of the included articles. Reports were downloaded into Endnote X7.

Inclusion and exclusion criteria

Inclusion criteria were full-texts in English, French, Portuguese or Spanish using any type of QST for the evaluation of the somatosensory system in conscious cats deemed to be healthy or affected by naturally occurring OA. Three types of studies were included: longitudinal observational studies of the repeatability of QST, case-control studies and interventional trials. Exclusion criteria were reports of QST in chronic

painful conditions other than OA or models of acute pain (e.g. acute synovitis). One investigator (COT) screened study titles, abstracts, and where necessary full-text to determine study inclusion.

Assessment of Quality and Risk of Bias

Two investigators (BPM and COT) independently assessed quality and risk of bias for each included study; discrepancies and disagreements were resolved by a third investigator (ETR). The quality of the studies included in this systematic review was examined using a modified version of the criteria devised by Downs and Black, 1998,[131] as suggested by Suokas et al., 2012.[86] The latter version of the criteria was adapted herein so that different types of studies (case-control, repeatability and interventional) could be assessed (Table IV). In addition, studies were assessed for risk of bias using a modified version of the CAMARADES critical assessment tool as in previous studies (Table V).[132,133] Finally, studies were also evaluated for the mention of any reporting guidelines such as the ARRIVE (Animal Research: Reporting of In Vivo Experiments)[134] or CONSORT (Consolidated Standards of Reporting Trials).[135]

Data extraction and management

One investigator (BPM) independently extracted data using a standardized form. The form included year and journal of publication, study design type, setting, species, sample size, participants' sex, reproductive status (e.g. neutered or not), age and body weight, stimulus type and protocol, additional outcome measures, and where applicable, retest interval, reliability coefficient and intervention protocol. Setting was defined as research (laboratory animals) or clinical (client-owned animals). Stimulus type was defined as static or dynamic. Static QST included electrical, chemical, mechanical (sub-groups included mechanical pressure, punctate pinprick or tactile threshold sensitivity, and vibration) and thermal stimulus (hot and cold). Dynamic QST included brushing tactile sensitivity (brush-evoked allodynia), mechanical or thermal temporal summation, and conditioned pain modulation. Stimulus protocol included detailed description of the device, probe surface and/or temperature, profile stimulation (*e.g.* on-going increasing stimulus, gradually increased with steps, repeated stimulations, presence of safety cut-off, *etc*.) and anatomical location of the test.

Data Analysis

Authors of included studies were contacted by email and asked to provide subject-level data. For repeatability studies, only data from the first visit were selected. For interventional studies, only the baseline data (i.e. before intervention) were used. Measurement units were harmonized across studies prior to analysis.

For the punctate tactile outcome, we used a hierarchical linear model with a normal likelihood function. Each study was assigned its own random intercept centered on a global mean intercept which was itself given a diffuse normal prior, centered at zero with a large variance (100^2), appropriately encoding our a priori knowledge regarding the range of the outcome measurements. Covariate effects were given that same diffuse normal prior, and all standard deviation parameters were given exponential priors with 95% of their probability mass between 0 and 200, the range of outcome measurements. Three models were estimated, each differing from the others only in their covariates. The first model accounted for OA status, female sex, and body weight (per kg); the second model added to these age (per year) and the interaction between age and OA status; and the third model accounted only for age, female sex, and body weight. This was done to explore the potential multicollinearity between age and OA status. The main statistical estimate of interest was the mean difference in pain thresholds between healthy cats and cats with OA, while accounting for heterogeneity between the included studies and the other above-mentioned covariates.

Table IV. Criteria used for quality assessment.

Each item was score '1' if the response was 'yes' or '0' if the response was 'no' or 'unable to determine, unless stated otherwise. Adapted from previous studies.[84,129]

Category	Detailed criteria
Reporting	1. Does the study provide clear hypothesis and objectives related with the measurement of pain/sensory threshold using QST†: to detect differences in thresholds – why, for what purpose? If only hypothesis or objectives were reported, then score = 0.5. 2. Are the main outcomes to be measured clearly described in the Introduction or Methods section: thresholds and ratings? 3. Are the characteristics of the patients included in the study clearly described? • Inclusion criteria: yes = 0.5 • Exclusion criteria: yes = 0.5 4. Are the distributions of principal confounders in each group of subjects to be compared clearly described: patient demographics for age and gender either in a table or in text? 5. Are the main findings of the study clearly described by providing simple outcome data in text or graph format: numeric values (e.g. mean value) so that the reader can check the major analyses and conclusions? 6. Does the study provide estimates of the random variability in the data for the main outcomes in text or graph format: SE, SD or CIs for parametric data; inter-quartile range for non-parametric? 7. Have actual probability values been reported for the main outcomes (*e.g.* 0.035 rather than <0.05) except where $p < 0.001$? 8. If any of the results were based on 'data dredging', was this made clear (if no data dredging reported then answer yes)?
Validity	9. Were the statistical tests used to assess the main outcomes appropriate: to test reliability (reproducibility and repeatability) and validity (any difference between groups, interventions, etc.)?
Power	10. Were power calculations carried out for the primary outcome: threshold measurement?

†QST defined as inclusion of one or several of the following modalities: mechanical (e.g. pressure, punctate), thermal, electrical, chemical

Table V. Criteria used for risk of bias assessment.

Each item was scored '1' if it was reported 'satisfactorily' or '0' if 'not' or 'unclear risk of bias', unless otherwise stated. Adapted from previous studies.[130,131]

Risk of bias	Detailed criteria
Selection bias	1. Randomization: • Random sample of animals at inclusion (interventional trials, case-control and repeatability studies). Score = 0.5 • Random assignment of animals to treatment or control groups (interventional trials). Score = 0.5 • Random allocation of order of QST types or body location to be stimulated (case-control and repeatability studies). Score = 0.5
Detection bias	2. Blinding: • Keeping the persons who perform the experiment, collect data, and assess outcome unaware of: ▪ Treatment allocation (interventional al trials) ▪ OA status (case-control studies) ▪ Value of the QST measurement (repeatability studies)
Other sources of bias	3. Disease status: • Evidence that animals were evaluated in order to confirm the presence or absence of OA based on clinicians' or owners' assessments and radiographic imaging. If only one or the other were reported, then score = 0.5. 4. Conflict of interest: • Statement regarding potential conflict of interest 5. Approval of animal ethics committee: • Statement of compliance with animal welfare regulations

For the mechanical temporal summation outcome, we used a parametric proportional hazards model with a log link and a Weibull likelihood function, a flexible distribution commonly used in survival analyses. The appropriateness of our distributional and proportional hazards assumptions were assessed using a plot of the log of the negative of the log of the survival function (estimated with the Kaplan-Meier method) against the log of time (Figure 5).

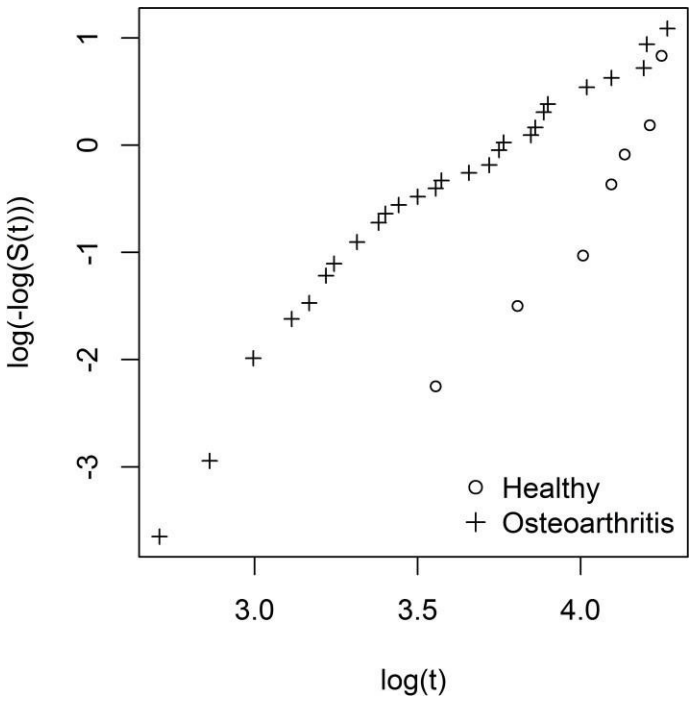

Figure 5. Diagnostic plot of time to temporal summation outcome

Using the Kaplan-Meier method, we estimated the survival function, S(t), stratified OA status. We then plotted log(-log(S(t))) against the log of time. The approximately straight, parallel curves indicate that our distributional (i.e., Weibull) and proportional hazards assumptions approximately hold—though, it is also apparent that toward the larger values of time, the lines seem to connect. Accounting for this discrepancy in our model is beyond the scope of what is reasonable given our small data set. The consequence is that our estimate of the effect of OA on temporal summation may be slightly biased toward the null.

Outcomes that reached the upper time-limit of the QST procedure were considered to be right-censored. Due to the imperfect hierarchical structure of the mechanical temporal summation data (i.e., subjects were not systematically nested within studies), both subject-specific and study-specific random intercepts were used (pooling studies 2 and 3 which shared subjects) along with a fixed intercept whose prior was normal and centered at zero with a large variance (1002), reflecting our ignorance regarding the baseline hazard for this outcome. Covariate priors were normal with 95% of their probability mass between log(1/10) and log(10), encoding our a priori knowledge that multiplicative effects of the covariates should be between 1/10 and 10 with high probability. Both subject-specific and study-specific random intercepts were given the

same normal priors centered at zero with standard deviation parameters which were themselves given exponential priors with 90% of their probability mass between 0 and log(10), reflecting our a priori knowledge that the average multiplicative deviation from the baseline hazard (by study or by subject) is very unlikely to be more than ten-fold. Finally, the Weibull distribution's shape parameter was given a diffuse exponential prior with 75% of its probability mass between 0 and 10. Three models were estimated which differed only in their covariates, as explained above for the punctate tactile outcome. The main statistical estimate of interest was the hazard ratio between cats with OA and healthy cats with respect to facilitated temporal summation, while accounting for heterogeneity between the included studies and the other aforementioned covariates.

There were no missing data, thus imputation was not required. All posterior distribution estimates were compared to their respective prior distributions to ensure that priors were not constraining estimated location or variability of posteriors, and posterior predictive checks were used to assess the appropriateness of model fits. All estimates were reported as posterior modes with their posterior highest density intervals. Computations were conducted using R statistical software[136] version 3.5 and WinBUGS[137] version1.4.

1.3.6 Results

Six articles met the inclusion criteria (Figure 6); static and dynamic QSTs were studied. Summary of studies' characteristics and QST modalities are available in Table VI.

Assessment of Quality and Risk of Bias

Assessment of quality and risk of bias of included studies are reported in Table VI. Most studies were of high quality. Power calculation was reported in one study.[138] Selection bias was detected in all articles due to unclear reporting of randomization.[111,122–124,138,139] Detection bias was detected in one article.[139] The number of evaluators performing the tests were rarely reported,[111,122,124,138] and the sex of the evaluator was never reported. Most studies clearly described the behavior response observed (*e.g.* withdrawal, escape behavior) for determination of the threshold during

QST testing and all of them reported a safety cut-off.[111,122–124,138,139] Veterinarian and radiographic examinations were used to determine the status of the cat (healthy *versus* OA) in all studies. Additional objective and subjective outcome measures other than QST were used in five studies including: kinetic analysis,[111,122–124,139] activity monitoring[111,122–124,138] and a clinical metrology instrument (Montreal Instrument for Cat Arthritis Testing for use by veterinarians (MI-CAT(V)).[138] All studies provided a statement related to conflict of interest and reported the approval of animal ethics committee. The ARRIVE guidelines were used in three studies.[111,122,138]

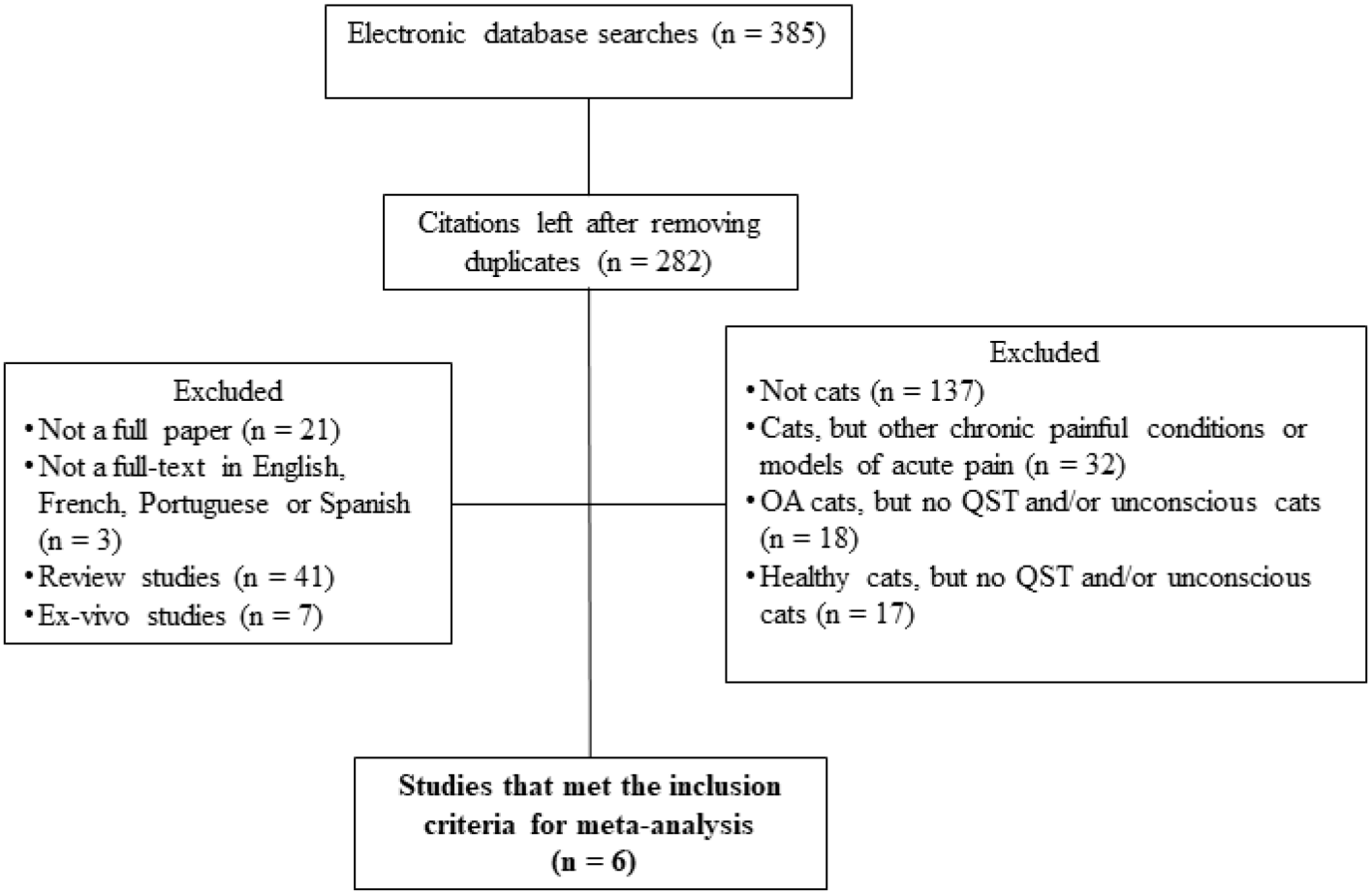

Figure 6. PRISMA flow diagram of study selection

Table VI. Summary of study characteristics, their main findings based on quantitative sensory testing (QST) and results from quality assessment and risk of bias of studies included in the systematic review.

Type of study	Setting	Population: N (male; female)	Test – re-test interval	Intervention	QSTs[†]	Results[†‡]	Quality assessment[¶] (%)	Risk of bias[¶] (%)	Refe-rence
Repeatability and Case-control	Clinical	Healthy: 14 (7;7) Natural OA: 7 (4;3)	2h to 26 weeks	N/A	1. Punctate tactile threshold (von Frey) 2. Thermal latency (hot) 3. Thermal latency (cold)	1. No inter-session difference. OA cats had lower threshold than healthy cats. 2. No inter-session difference. No difference between healthy and OA cats. 3. No inter-session difference. OA cats had lower frequency of thoracic paw lift than healthy cats.	85	40	[139]
	Research	Healthy: 4 (3;1) Natural OA: 10 (5;5)	7 days	N/A	1. Punctate tactile threshold (von Frey) 2. Mechanical temporal summation	1. No inter-session difference. OA cats had lower threshold than healthy cats. 2. No re-test done. OA cats supported lower number of stimulations than healthy cats.	85	10	[111]
Repeatability, Case-control and Intervention trial	Research	Healthy: 6 (3;3) Natural OA: 42 (18;24)	7 days	Meloxicam (0.025, 0.04 or 0.05 mg/kg) or placebo PO every 24h for 28 days	1. Punctate tactile threshold (von Frey)	1. No inter-session difference. OA cats had lower threshold than healthy cats. In OA cats receiving meloxicam or placebo, thresholds were not different over time.	85	10	[123]

Case-control and Intervention trial	Research	Healthy: 5 (not reported) Natural OA: 7 (not reported)	N/A	Gabapentin (10 mg/kg) PO every 8h for 30 days	1. Punctate tactile threshold (von Frey)	1. OA cats had lower threshold than healthy cats. In OA cats receiving gabapentin, thresholds increased over time. Such effect was not observed in healthy cats receiving gabapentin.	65	20	[138]
	Research	Healthy: 5 (3;2) Natural OA: 15 (8;7)	N/A	Tramadol (3 mg/kg) or placebo PO every 12h for 19 days	1.Mechanical temporal summation	1. OA cats supported lower number of stimulations than healthy cats. In OA cats receiving tramadol, there was an increase in the number of stimulations from baseline. Such effect was not observed in cats receiving placebo.	90	10	[122]
Intervention trial	Research	Natural OA: 15 (8;7)	N/A	Meloxicam (0.05 mg/kg) OTMS every 24h with tramadol (3 mg/kg) or placebo PO every 12h for 25 days	1. Mechanical temporal summation	1. Increased number of stimulations from baseline in cats receiving combined meloxicam and tramadol. Such effect was not observed in cats receiving meloxicam and placebo.	80	10	[124]

Abbreviations: OA, osteoarthritis; QST: quantitative sensory testing; N/A, not applicable; OTMS, oral transmucosal spray; PO, *per os*.
[†]The numbered list in column "QST" corresponds to the same numbers in column "Results"; [‡]Data retrieved from the articles included in this review and reported herein are subject to bias or error attributable to any misinterpretation or unclear reporting of the results; [¶]Higher values indicate higher study quality and higher risk of bias.

Data analysis

Data from individual cats from five studies were included in the meta-analysis done separately for punctate tactile threshold and mechanical temporal summation. Individual subject data could not be retrieved from one study.[139]

Data from punctate tactile threshold using electronic von Frey (Figure 7) were available from three studies.[111,123,138]

Figure 7. Punctate mechanical threshold testing in a cat with osteoarthritis

A) The cat is gently placed on a meshed cage and an electronic von Frey is used to apply pressure over the metacarpal and metatarsal pads. The stimulus is stopped as soon as a behavior response is observed. Note the left thoracic limb withdrawal and the cat looking at the device immediately after the stimulus. B) A close-up of the test with the electronic von Frey esthesiometer.

Two types of outcome were available for both OA and healthy cats: the mean value from tests done in all four limbs and the value from the most affected limb (i.e. the limb yielding the lowest threshold) (Table VII). Only the former was used for statistical analysis. Data from temporal summation were available from three studies (Figure 8).[111,122,124] Data were transformed from number of repetitions to time to event (seconds).

Table VII. Sample characteristics and descriptive statistics of subject-level data from healthy and osteoarthritic cats undergoing quantitative sensory testing (QST).

	Unit	QST type	Full sample	Osteoarthritis	Healthy
Number of subjects, N		Punctate tactile	70	56	14
		Temporal summation	35	25	10
Female sex, N (%)		Punctate tactile	36 (51%)	30 (54%)	6 (43%)
		Temporal summation	19 (54%)	16 (64%)	3 (30%)
Age, median (Q1 ; Q3)	Years	Punctate tactile	8 (5 ; 10)	10 (8 ; 11)	3 (2 ; 4)
	Years	Temporal summation	11 (6 ; 11)	11 (11 ; 12)	4 (2 ; 4)
Body weight, median (Q1 ; Q3)	Kg	Punctate tactile	5 (4 ; 6)	5 (4 ; 6)	5 (4 ; 6)
	Kg	Temporal summation	4 (4 ; 5)	4 (4 ; 5)	5 (4 ; 6)
Threshold (mean of four limbs), median (Q1 ; Q3)	Grams	Punctate tactile	127 (89 ; 154)	127 (86 ; 150)	145 (124 ; 170)
	Seconds	Temporal summation	44 (29 ; 60)	34 (22 ; 49)	61 (56 ; 69)
Threshold (most affected limb), median (Q1 ; Q3)	Grams	Punctate tactile	78 (56 ; 110)	68 (52 ; 99)	119 (92 ; 134)

Abbreviations: Q1, first quartile; Q3, third quartile

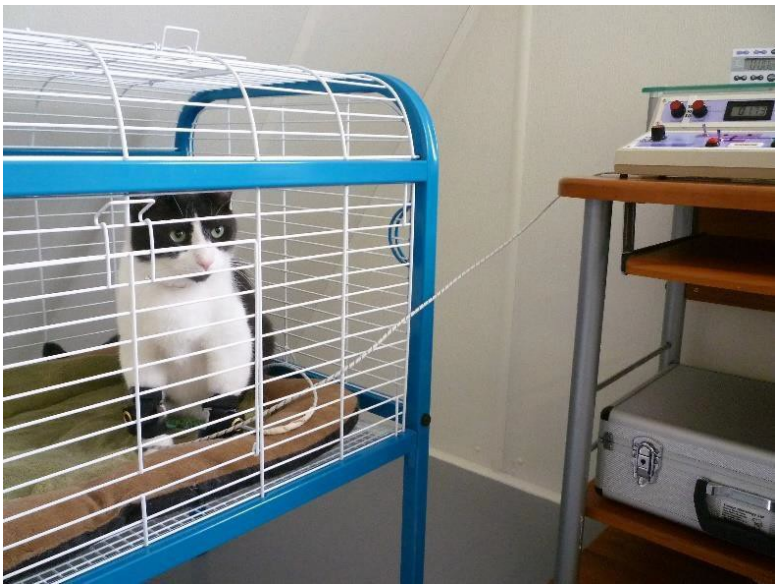

Figure 8. Temporal summation testing in a cat with osteoarthritis

The cat is gently placed on a cage with one elasticated band around each antebrachium. The band on the right limb is a 'dummy' and the band on the left limb is attached to an actuator that produces repeated subthreshold (4 Newtons) mechanical stimulation. The number of repetitions needed until a behavior response is observed such as limb withdrawal constitutes the 'threshold' for that cat.

Figure 9 presents the outcome measures for both of these QST approaches as a function of age and stratified by OA status. The most striking feature of these plots is that age and OA status appear to be very closely related, and especially for the mechanical temporal summation outcome. Thus, we expect to be difficult to separate the effect of OA from the effect of age.

As previously presented, three models including different covariates were estimated for punctate tactile threshold. The first model including OA status, sex and, body weight (and excluding age) was considered the most appropriate for answering our research question (Table VIII). Based on this model, cats with OA have a punctate tactile threshold (on average) decreased by approximately 30 grams when compared to healthy cats. Female sex and body weight did not appear to be associated with pain sensitivity thresholds. In the second model, OA and the interaction between age and OA appear to be highly collinear as suggested by the greatly widened estimate range compared to the

first model. In the third model, the effect of 8 years age difference appears to be approximately equivalent to the effect of OA as measured in the first model.

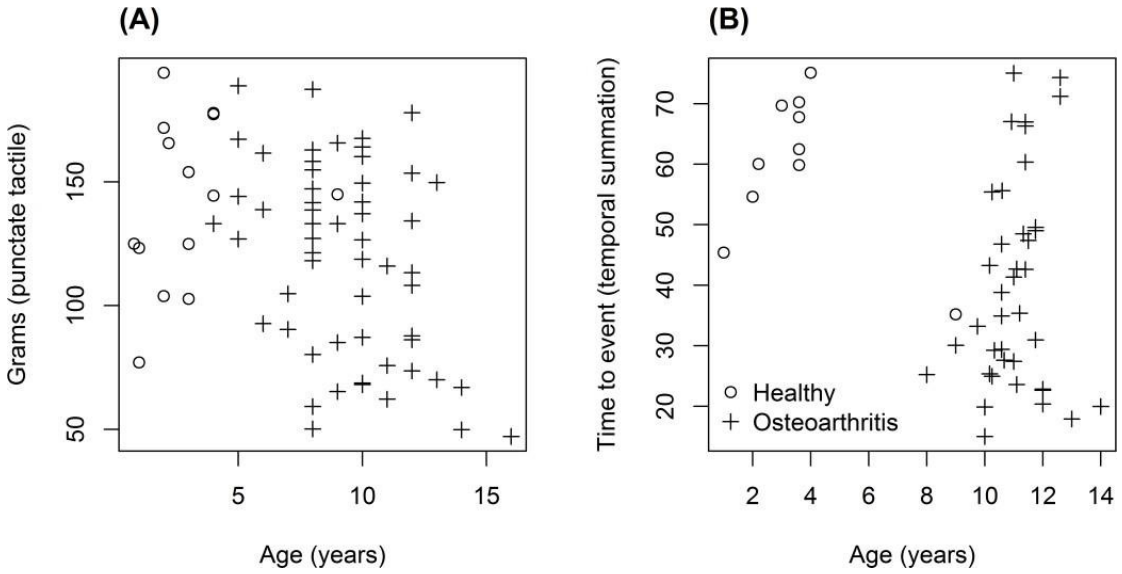

Figure 9. Scatter plot of outcome against age, stratified by OA status

In panel (A), we present the punctate tactile outcome, measured in grams, as a function of age. In panel (B), we present the temporal summation outcome, measured in seconds, as a function of age. Both plots are stratified by OA status, as indicated by the different symbols used in graphing the values. Both plots also show that there is little overlap in age distributions between OA status groups. This represents an important limitation of this study.

Three models were estimated for mechanical temporal summation. Similar to punctate tactile threshold. The first model was considered the most appropriate for the same reasons stated above (Table IX). Based on this model, cats with OA have about five times the hazard of facilitated temporal summation (*i.e.*, earlier response to the stimulus) when compared with healthy cats. The effects of sex or body weight on mechanical temporal summation were nearly null, similar to punctate tactile threshold. In the second model, the effect of age was essentially null. Furthermore, the effect of age among cats with OA seemed protective (*i.e.*, among osteoarthritic cats, the effect of OA on facilitated temporal summation decreases as they age). In the third model and similar to punctate tactile threshold, the effect of 8 years age difference is approximately

equivalent to the effect of OA, as measured in the first model.

Table VIII. Model estimates for punctate mechanical threshold, measured in grams, in healthy (n=14) and osteoarthritic (n=56) cats.

Results are reported as posterior modes and posterior highest density intervals as estimated from a hierarchical linear model with a normal likelihood function and weakly informative priors

Parameter	Most appropriate model	Additional exploratory models	
	Posterior mode[†] (95% HDI)	**Posterior mode[†] (95% HDI)**	**Posterior mode[†] (95% HDI)**
Sample mean	130 (49 ; 189)	137 (53 ; 197)	111 (38 ; 159)
Intercept, study 1	166 (136 ; 195)	172 (144 ; 202)	136 (126 ; 145)
Intercept, study 2	141 (112 ; 167)	144 (114 ; 180)	118 (97 ; 139)
Intercept, study 3	102 (80 ; 124)	111 (82 ; 141)	82 (67 ; 98)
Osteoarthritis [‡]	-34 (-64 ; -3)	-76 (-137 ; -24)	-
Age, per year[‡]	-	3 (-2 ; 8)	-4 (-6 ; -2)
Osteoarthritis * Age[‡]	-	-9 (-21 ; 0)	-
Female sex [‡]	-2 (-19 ; 17)	-3 (-20 ; 15)	0 (-19 ; 19)
Body weight, per kg[‡]	2 (-6 ; 10)	0 (-7 ; 8)	1 (-7 ; 9)
Within study SD, study 1	31 (25 ; 39)	30 (24 ; 38)	30 (25 ; 38)
Within study SD, study 2	32 (22 ; 52)	37 (24 ; 61)	36 (25 ; 57)
Within study SD, study 3	19 (12 ; 33)	15 (9 ; 27)	22 (15 ; 38)
Between study SD	29 (12 ; 118)	30 (12 ; 118)	26 (10 ; 106)

Abbreviations: SD, standard deviation; HDI, highest density interval.
[†]The mode is the value with the highest probability density, and the 95% HDI is the range of the probability density which meets the following criteria: (1) contains 95% of the density's total probability mass and (2) all points inside the HDI have higher density than all points outside it. For symmetric density functions, it is identical to the 95% credible interval (which is analogous to a 95% confidence interval). [‡]Age, sex, osteoarthritis status, and weight were centered at their respective means. All covariate effects are relative to the "average" subject.

Table IX. Model estimates for mechanical temporal summation, measured in seconds (time to event = response), in healthy (n=10) and osteoarthritic (n=25) cats.

Results are reported as posterior modes and posterior highest density intervals as estimated from a hierarchical generalized linear model with a Weibull likelihood function, a log link, and weakly informative priors

Parameter	Most appropriate model	Additional exploratory models	
	Posterior mode† (95% HDI)	Posterior mode† (95% HDI)	Posterior mode† (95% HDI)
Baseline log-hazard (time=0)	-13.55 (-18.72 ; -9.39)	-13.54 (-18.96 ; -9.39)	-12.97 (-18.29 ; -9.10)
Intercept (log-hazard), study 1	1.07 (-1.68 ; 4.14)	1.03 (-1.58 ; 4.12)	1.04 (-1.64 ; 4.07)
Intercept (log-hazard), studies 2 and 3	-1.05 (-4.11 ; 1.68)	-1.05 (-4.15 ; 1.54)	-1.00 (-4.10 ; 1.60)
Weibull shape parameter	3.52 (2.68 ; 4.59)	3.80 (2.86 ; 5.04)	3.50 (2.67 ; 4.64)
Osteoarthritis (HR) ‡	5.32 (2.18 ; 13.92)	1.87 (0.43 ; 9.97)	-
Age, per year (HR)‡	-	1.01 (0.80 ; 1.26)	1.22 (1.09 ; 1.40)
Osteoarthritis * Age (HR)‡	-	0.60 (0.35 ; 0.98)	-
Female sex (HR)‡	0.81 (0.37 ; 1.81)	0.70 (0.31 ; 1.65)	0.83 (0.37 ; 1.91)
Body weight, per kg (HR)‡	0.90 (0.61 ; 1.32)	0.93 (0.61 ; 1.38)	1.00 (0.66 ; 1.47)
Between-study SD	1.15 (0.43 ; 3.53)	1.17 (0.43 ; 3.52)	1.14 (0.43 ; 3.52)
Between-subject SD	0.35 (0.00 ; 0.92)	0.35 (0.00 ; 1.03)	0.45 (0.00 ; 0.97)

Abbreviations: SD, standard deviation; HR, hazard ratio; HDI, highest density interval.
†The mode is the value with the highest probability density, and the 95% HDI is the range of the probability density which meets the following criteria: (1) contains 95% of the density's total probability mass and (2) all points inside the HDI have higher density than all points outside it. For symmetric density functions, it is identical to the 95% credible interval (which is analogous to a 95% confidence interval). ‡Age, sex, osteoarthritis status, and body weight were centered at their respective means. Thus, all hazard ratios are relative to the "average" subject with baseline hazard.

1.3.8 Discussion

This systematic review and individual subject meta-analysis evaluated the quality of evidence from six studies about the somatosensory profile of healthy and osteoarthritic cats using punctate tactile threshold (n= 70) and mechanical temporal summation tests (n=35). Studies of QST in cats were generally of high quality and low risk of bias. Cats with OA are affected by punctate tactile hypersensitivity when compared with healthy cats (difference of approximately 30 grams), with no apparent effect of sex or body weight, independently of OA. For facilitated temporal summation of pain, the hazard is approximately five times greater for osteoarthritic than healthy cats, also with no apparent effect of sex or body weight, independently of OA. The use of QST in cats is feasible and appears to differentiate healthy from OA-affected individuals, suggesting gain-in-function for the somatosensory processing, but the confounding effect of age must be explored with the systematic sampling of a more diverse population of cats to be QST-tested.

Scientific quality of included studies

In this systematic review, lack of clear reporting was the main concern affecting scientific quality. Most common issues with reporting included lack of a clear description of both inclusion and exclusion criteria and the lack of power analysis. When it comes to study design, selection bias was the most common due to lack of randomization of animals or order of QST or body location. Reporting guidelines such as the ARRIVE help improve reporting standards by including key information that should be available in a manuscript to ensure that a study can be reviewed, scrutinized and reproduced.[134] Despite being endorsed by several highly- respected funding agencies and scientific journals, very little improvement in reporting standards were noted in pre-clinical trials.[140] Judged by the fact that only half of the included studies herein used reporting guidelines, it seems that their use is not yet widespread in veterinary medicine as well, further contributing to the lack of clear reporting observed in this systematic review. But such lack is also appealing for a critical revision of the ARRIVE guidelines.[141]

Pain assessment in animals can be affected by several factors including the sex of

the animal and the observer. For example, pain scores in cats following ovariohysterectomy were higher when given by a female when compared with a male observer.[142] Scores of facial grimacing or response to QSTs were decreased in male rodents when they were exposed to man' odors due to stress-induced analgesia.[143] In the studies included in this review, the sex of the observer was never reported, and it remains unknown if it affects QST assessment in cats. This may represent an important confounder that investigators need to consider and report.[143]

The phenotyping of included animals (healthy versus osteoarthritic) was performed using radiographs and subjective veterinary evaluation. It might be argued that the sensitivity of both methods to determine the presence or absence of OA is less than optimal. Although radiography is widely used for diagnosing OA, radiographic signs do not correlate linearly with clinical pain[61,107,144] and early structural joint changes might be missed.[145] Magnetic resonance imaging can provide the most comprehensive assessment of OA,[145] specifically in cats;[146] nevertheless, the procedure requires general anesthesia in animals and is much more costly. Assessment of OA-related pain in cats is quite challenging due to cats' natural behavior, small body size and ability to compensate for musculoskeletal conditions, particularly in presence of bilateral alteration.[147] Clinical metrology instruments for the diagnosis of OA in cats have been developed and partially validated.[138,148–151] None of the studies in this review used a validated clinical metrology instrument to phenotype cats. The relationship between QST and pain or disability in cats with OA has not been systemically investigated. Nevertheless, the use of clinical metrology instruments and other outcome measures including activity monitoring and kinetics provide us with a subjective/objective assessment of the pain burden in cats with OA. Affected cats are less active, have abnormal kinetics,[122,123] have difficulty in performing daily activities such as jumping, using the litter box or grooming and present with changes in socialization and temperament.[148,152,153]

The use of QST to study pain mechanisms

The use of QST provides interpretations of the underlying mechanisms involved with a patient's somatosensory profile.[82,118,119] Indeed, the individual pattern of somatosensory abnormalities at (distant of) the affected body area, *i.e.* the somatosensory profile, likely reflects altered functions in somatosensory processing; this might open a

window to understand the underlying mechanisms of pain generation. The results of this individual subject data meta-analysis seem to indicate that cats with OA are affected by centralized sensitization characterized by punctate tactile hypersensitivity and facilitated mechanical temporal summation of pain. No pattern with different combinations of abnormal signs (loss/gain of function) was detected, but a bias to gain-of-function could be present, or different conditions, such as feline diabetic neuropathy, could present mixed pattern.

Punctate tactile threshold is a static QST that helps to measure cutaneous hyperalgesia/allodynia. In cats with OA, we found that this threshold is decreased by approximately 30 grams, compared to healthy cats. This decrease by about 20% in average could be more accentuated when focusing on the most affected limb (the more sensitive one). Primary, secondary or distal punctate tactile/mechanical hyperalgesia/allodynia is also a feature in people with OA.[108,154] The exact anatomical location of OA was not reported in the included studies and it is unknown if punctate tactile tests were evaluating primary, secondary or distant hyperalgesia/allodynia. These tests were performed at metacarpal and metatarsal pads while the most common sites of radiographic OA in cats include the elbow, hip, stifle, tarsus, and lumbar and lumbosacral regions.[155–157] Thus, it might be argued that this test evaluated secondary or distant hyperalgesia/allodynia reflecting systemic altered pain processing and further reinforcing the evidence of centralized sensitization.[63] Interestingly, QST could facilitate distinction of local (primary) vs. referred manifestations. As in these osteoarthritic cats, the main site of stimulation was secondary, i.e. the paw pads, it is maybe representing assessment in the referred area or it may represent general sensitization. The fact, for example, that the sensitization would be bilateral, or extended to the four paws, would allow to distinguish both phenomena.[86]

Temporal summation is a dynamic QST used to measure central integration.[121] It evokes a complex course of pain processing by activating and measuring wind-up and temporal summation due to repeated neural firing.[158] In the present study, cats with OA had approximately five times the hazard of responding earlier to the repeated stimulus when compared with healthy cats. Facilitated temporal summation of pain is also a feature in people with OA,[54,108] demonstrating similar pain mechanisms in both species.

Conditioned pain modulation is another example of dynamic QST. Although there were no studies with this test in our review, similar mechanisms of decreased descending control between people and cats are likely to exist. Based on positron emission tomography of the cats' brain, researchers identified enhanced brain metabolism in the thalamus and periaqueductal gray matter of osteoarthritic cats suggesting an involvement of the descending pain modulatory system,[159] which was similar to what has been reported in human subjects with OA.[154]

Correlation between age and OA

Age and OA showed strong correlation and their effects on punctate tactile threshold and mechanical temporal summation were impossible to untangle. This is partly due to the fact that age was an inclusion criterion used in some of the studies included in this review. Younger cats generally comprised the healthy group and older cats comprised the OA group. For this reason, there was an absence of cats roughly between 4 and 8 years of age in the temporal summation studies which intrinsically introduced a bias to our data. When age and its interaction with OA were added to the statistical model, the effect of age was essentially null or inconclusive. Indeed, the effect of age among those with OA seemed protective for temporal summation (*i.e.*, a possible effect of desensitization in older cats). However, the confidence attached to these findings was low, particularly due to the gap in the age distribution. Furthermore, if we excluded OA from the model (assuming instead that the effect of OA was fully subsumed inside of the effect of age), we were able to estimate a reliably positive effect of age on sensitivity to pain. In fact, the effect of 8 years age difference was approximately equivalent to the effect of OA for both the punctate tactile threshold and mechanical temporal summation response. For these reasons, the model excluding age and its interaction with OA was considered to be the most appropriate, and the effect of age on OA in cats remains to be elucidated. The human literature is quite controversial on this topic with findings going in either direction. Some studies state that pain sensitivity decreases with aging while others that it increases with aging. There is a great deal of influence from stimulus modality and anatomical location,[160,161] and a recent systematic review and meta-analysis could not draw any strong conclusions regarding the effects of age in pain sensitivity.[161]

Correlation between sex or body weight and OA

Analysis was inconclusive on a possible effect of sex and body weight for pain sensitivity threshold and temporal summation. Increased sensitivity to pain of females has been overwhelmingly reported in individual studies across species, including greater incidence of chronic pain.[162] Yet, controversies still exist as these differences might be related to the type of sensory stimulus. For example, a systematic review reported strong evidence that women are more sensitive than men for thermal and pressure pain stimuli, but that such differences are not that clear regarding ischemic pain.[163] If one considers the ratio between the surface of the probe and the body weight of the cat, one might intuitively think that the bigger the cat, the less the pain, as there was no adaptation of the probe to the cat body weight. This seems to be a subject of relatively recent interest and again, the issue is not clear cut and different findings have been reported. For example, one study found hypoalgesia to electrical stimuli in obese patients when compared with controls,[164] whereas another study found hypersensitivity to pressure pain, but not thermal pain, in obese individuals.[165] Moreover, data regarding body mass index or body condition score from included cats was not available for analysis, and it is unknown if heavier cats were simply bigger or overweight or obese, making definitive conclusions hard to make. Both covariates could also be linked, as usually females are smaller than males, and both will require more information to conclude on any influence.

Study limitations

This study has several limitations. First, studies with different designs were assessed for risk of bias using the CAMARADES checklist which was primarily developed for assessment of intervention trials of pre-clinical models.[130] Adaptations to the original checklist were made in order to account for differences among study designs and animal population. Second, there were only two types of QST included in the meta-analysis. This reflects the dearth of available literature in the subject in cats. One study was not included in the meta-analysis because subject- level data were not available.[139] That study investigated thermal sensitivity of healthy and osteoarthritic cats using a custom-designed thermal platform which provided hot (40°C) and cold (7°C) stimuli and the frequency and duration of paw lifts was recorded. The study found no difference

between cats for hot stimulus, and increased sensitivity to cold stimulus.[139] Third, the effects of several other covariates such as anatomical location of tests, order of testing or position of animal during testing could not be evaluated, and their effects on QST testing in cats remain unknown. Forth, bias could have been introduced since we chose to select data from the first visit only in studies in which replicates were performed. This was done to avoid a possible confounding learning effect previously reported in dogs.[166] On the contrary, stress-induced analgesia could have occurred in the first testing session.[127]

The osteoarthritic cat as a model of spontaneous disease

The domestic cat has been long used as a model for research in neurosciences including Alzheimer's disease.[167] Lack of translation from pre-clinical models has been highlighted by several authors.[8,10,11,128] Questionable scientific quality of studies involving animal models including poor experimental design and lack of transparent reporting compromises and limits the interpretation that can be drawn from the study's findings. It is considered as the main contributor to the frequent failure of preclinical animal studies to translate into treatments for human disease.[10] Other contributing factors include: 1) the subject, which is normally comprised of young male rodents; 2) the assay, which is usually based on induced models of disease; and 3) the many environmental stressors, which can influence these tests.[10,11,128] Naturally occurring disease models mirror primary OA in humans; it is slowly progressing, and clinical symptoms can be variable.[168] Based on similar sensory profile, the present study further consolidates the domestic cat as a valuable model of naturally occurring OA. Provided that scientific quality and reporting are appropriate, it also has the potential to improve translatability to clinical research in people.

Conclusions

Clear and transparent reporting, including the use of reporting guidelines, are amongst the most needed improvements in QST research in cats. Cats with OA are affected by central sensitization characterized by punctate tactile hypersensitivity and facilitated mechanical temporal summation of pain. There exists a complex relationship between OA, age, and sensory sensitivity, and further research is needed to elucidate this puzzle. Provided that reporting standards are increased, QST use in cats with naturally

occurring OA could transform the predictive ability of preclinical studies, in addition to contributing with feline evidence-based medicine and welfare.

1.3.8 Conflict of interest statement and Acknowledgments

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1.4 Assessment and treatment of chronic pain in cats and dogs

An extensive literature review on feline chronic pain management is included in the Appendix of this thesis. The doctoral candidate contributed in editing both book chapters presented: Assessment and recognition of chronic (maladaptive) pain in cats (Appendix 1); and Treatment of chronic (maladaptive) pain in cats (Appendix 2).

1.4.1 Differences with cat chronic pain assessment

Assessment of chronic pain in dogs is similar to cats in that it relies on assessment by proxy. One of the main differences in OA-related pain is that dogs clearly develop lameness, something rarely detected in cats. Differences in pain assessment between both species are likely explained by profound differences in the domestication processes. Dogs have evolved with man over thousands of years and have been selected for certain social-cognitive abilities that facilitate communication. Dogs are able to interpret humans' behavior and react accordingly, whereas humans are able to interpret dogs' behavior in turn.[169,170] In contrast, cats have been domesticated much later than dogs and have not been genetically selected to be a companion for man or to work with man. They are generally more independent and tend to hide pain-related behaviors making pain measurement potentially more challenging in this species.[171]

Regardless of the species, clinical signs of chronic pain are often subtle and changes in behavior due to chronic pain may be so gradual that they may only be apparent to someone very familiar with the animal. It is interesting to note that rather than signs and symptoms, owners emphasize adherence to normal routine and displays of the dog-owner bond when evaluating their dog's QoL.[172] Things like sociability and companionship seem more important to them then body function, for example. Most commonly reported clinical signs of chronic pain in dogs include: changes in demeanor; aggressiveness; fearfulness; restlessness; lethargy; self- mutilation; reduced sociability; reduced willingness to play; altered posture; gait changes including stiffness, lameness or stumbling; muscle atrophy; hesitation, reluctance or refusal to perform activities such as jumping into a car; reduced general activity levels and changes in appetite, drinking,

urination and defecation.[4,171,173] The prevalence of chronic pain in dogs is not known; yet it can occur by itself or be associated with numerous conditions. A recent survey of veterinarians from the United Kingdom revealed that OA, dental and aural disease, vertebral and spinal cord conditions, neoplasia and skin conditions are considered important causes of chronic pain in dogs by the veterinarians.[174]

The ability for man and dog to communicate means that the dog is a good candidate for the development of instruments to measure pain that depend upon subjective judgement.[169] With this in mind, a few clinical metrology instruments have been developed and validated for use in dogs with chronic pain. The Canine Brief Pain Inventory (CBPI) was developed from the Brief Pain Inventory used in people. It comprises two main factors: pain severity and pain interference. Pain severity reflects pain intensity measured with four items: current pain, worst pain, least pain and average pain. Pain interference reflects how the pain interferes with the patient's function; it is measured with six items: general activity, enjoyment of life, ability to rise to standing, ability to walk, ability to run and ability to climb stairs. An additional third factor named QoL contains a single item pertaining to owner assessment of overall QoL.[175] The CBPI has been validated in dogs with OA and dogs with OSA.[175–177] When tested in dogs with OA, CBPI was able to detect treatment effect in dogs receiving NSAID or placebo treatment.[175] The Helsinki Chronic Pain Index is validated instrument for use in dogs with OA-chronic pain. It has demonstrated reliability and responsiveness. It consists of 11 questions with 5 possible answers each. The questions pertain to the dog's mobility, mood and demeanor. The owner chooses the answer best describing their dog in the previous week.[178] The Canine Orthopedic Index was designed to assess dogs with orthopedic disease. It is a valid and reliable instrument that consisted of 16 items divided within four domains including stiffness, gait, function and QoL. The instrument was initially validated in dogs with OA.[179,180] Finally, the Liverpool Osteoarthritis in Dogs (elbow) (LOAD) is a disease-specific clinical metrology instrument that was developed and partially validated to assess dogs with chronic OA of the elbow joint.[181,182]

The assessment of chronic pain invariably involves the assessment of QoL and health- related QoL (HRQoL) since chronic pain undoubtedly affects animal welfare. A clear difference among these components is hard to visualize and the assessment of one

usually correlates with assessment of the other. Health-related quality of life is the term given to those aspects of QoL that change with ill-health and medical treatment, and this seems to be more appropriate in conditions of chronic pain. A few HRQoL instruments have been developed and initially validated for use in dogs with cancer[172,183,184] and chronic pain.[185,186] A throughout review on available tools to measure QoL in dogs is available elsewhere.[187] It has been suggested that these instruments might be able to detect behavioral styles indicative of 'hidden' emotional or subjective states of dogs *via* structured interviews.[185]

All of the above described primarily the assessment of the affective or emotional component of chronic pain in dogs. The sensory aspect of chronic pain assessment was thus far only performed in the research setting. Nevertheless, interesting findings generally corroborate what is observed in people with clinical pain. For example, dogs with hip or stifle OA are affected by hyperalgesia in the affected joint as well at distal locations.[125,188–190] No information was available in dogs with OSA in the literature at the time of the presented work. Widespread hyperalgesia and decreased CPM would be features of dogs with naturally-occurring OA or OSA which would likely be indicative of central sensitization.

1.4.2 Specificities of dog chronic pain treatment

The major barriers for adequate treatment of chronic pain in dogs include difficulty with pain assessment, expense of drugs and owner compliance, which is similar to cats.[174] Treatment of chronic pain in dogs also involves a multimodal approach in which pharmacological and non- pharmacological therapies are combined. This section focuses on pharmacological approaches.

Probably, the most evident difference between dogs and cats in chronic pain treatment pertains to the use of tramadol. Although it seems to be widely used for the management of chronic pain, tramadol does not seem to be as effective in dogs[191,192] as it has been reported in cats.[193] This is likely explained by two main reasons: cats have a faster rate of formation of the active metabolite O-desmethyltramadol by the liver (3.9-fold), and cats have longer elimination half-life and higher concentrations of the active metabolite when compared with dogs.[194,195] In a recent clinical trial involving dogs with

OA that were administered tramadol, carprofen or placebo, tramadol administered for over 10 days did not seem to provide any clinical benefit based on force plate analysis and the CBPI.[191]

NSAIDs are the most widely used analgesics in veterinary medicine.[196] In OA, since there are no disease-modifying therapies with strong evidence of efficacy, management is based on relieving symptoms and improving function. Thus, NSAIDs remain as are the first-line pharmacological therapy in OA in dogs.[197] They have been used for the management of chronic

pain for decades with a robust body of evidence with generally good efficacy and relatively good safety profiles.[197,198] Although they are known for the potential for development of adverse effects, the incidence of serious adverse effects is low and usually associated with inappropriate administration.[196,198] However, the constraints on repeated administration is a major stress on what would be expected as reasonable compliance to treatment.[199,200]

Acetaminophen produces analgesic and antipyretic effects but has weak anti-inflammatory activity. It is believed that acetaminophen inhibits prostaglandin E_2 synthesis in the CNS *via* inhibition of cyclooxygenase (COX)-3 enzymes, a sub-form of COX-1, in the cerebral cortex, implicating a central mechanism of these drugs.[201] Acetaminophen is anecdotally used for management of chronic pain in dogs that are sensitive to NSAIDs or during wash-out periods between two different NSAIDs; however there is a lack of scientific evidence supporting its use for chronic pain management in dogs. Although both dogs and cats can develop acetaminophen toxicosis secondary to hepatotoxicity or methemoglobinemia, cats are much more susceptible because they have deficient glucuronidation. For this reason, acetaminophen is strictly contraindicated in cats.[202]

Grapiprant is a piprant licensed in various countries for management of pain and inflammation associated with canine OA. Piprants selectively blocks the EP4 receptor.[203] Hence, they produce analgesic and anti-inflammatory effects by selectively inhibiting a single prostanoid receptor without inhibiting other homeostatic functions usually managed by prostaglandins. Grapiprant has shown a good safety profile in research and

client-owned dogs.[204,205] In a clinical trial with osteoarthritic dogs being treated for 28 days, improved pain scores based on the CBPI were recorded when compared with placebo. Most common adverse-effects included vomiting, diarrhea and anorexia which generally resolved without treatment.[205] This drug is not presently labelled for use in cats.

Canine-specific anti-NGF monoclonal antibodies are showing promising results for the management of chronic pain in dogs and cats.[206] Dogs with chronic lameness were shown to have increased concentrations of NGF in synovial fluid of the shoulder, elbow and stifle joints when compared with healthy dogs.[207] These results further corroborate the involvement of NGF in OA inflammation and chronic pain. In two different clinical trials involving dogs with OA, treatment with anti-NGF monoclonal antibodies resulted in improved pain scores based on clinical metrology instruments.[208,209]

1.5 Summary and Research Hypothesis

This literature review introduces the basic pathophysiology of pain and mechanisms of pain in OA and bone cancer, as well as the use of QST to study these mechanisms and profile pain in affected patients. A systematic review and meta-analysis of QST in cats with OA was presented (currently under review at the journal *Osteoarthritis & Cartilage*). Some of the main differences of chronic pain assessment and treatment between dogs and cats were presented.

Currently in veterinary practice, diagnosis of chronic pain is based on a convergence of medical history, clinical signs and veterinary findings of physical examination. Treatment of chronic pain generally relies on a random choice of drugs for which little information might be available, except for NSAIDs. These approaches have several intrinsic limitations; nevertheless, they might be related with the lack of science to influence them otherwise.

Initial work on cats with OA and healthy cats has helped to develop and validate the use of tolerance threshold and temporal summation of pain to quantify sensory sensitivity in this species.[111,123] Moreover, the use of motor activity and kinetic analysis were used as surrogates for assessment of the pain burden of OA in cats in which response to meloxicam was observed.[123,149] These previous investigations certainly pioneered the research of chronic pain in cats and showed for the first time that cats with OA are affected by increased sensory sensitivity. They also showed that cats with OA have altered function characterized by decreased motor activity and peak vertical force (PVF).[111,123,149] At the same time, sensitized OA cats did not present any response to NSAID treatment (meloxicam) as tested with tactile von Frey esthesiometer static QST.[123] A cluster analysis on more to less sensitized OA cats showed that the degree of response to meloxicam could be related to the degree of sensitization.[123] However, the response of temporal summation to therapeutics had not been explored. The effect on motor activity and PVF after the administration of centrally-acting analgesics alone or in combination with peripherally-acting analgesics were also unknown. As research with QST developed, a need to systematically review and analyze the literature arouse, and this was conducted as presented on the third section of chapter 1.

Concerning dogs with OSA, little is still known regarding their pain profile. Previous studies have used them as a model of spontaneous bone cancer for validation of the CBPI.[175] It showed that normal dogs had significantly lower pain severity and interference scores than dogs with bone cancer. The CBPI reliably measured the same pain constructs in dogs as the BPI in people with bone cancer demonstrating its potential as a model with improved translatability to people.[175] Furthermore, dogs with bone cancer were used in the evaluation of therapeutic efficacy after intrathecal administration of resiniferatoxin and substance-P saporin,[210–212] none of which are available for use in veterinary clinical practice. The outcome measures used in these studies included time to unblinding (which meant the owner believed that their dog had an unacceptable level of discomfort and required an intervention), CBPI, veterinarian lameness assessment, VAS and/or motor activity. In general, dogs being treated with resiniferatoxin or substance-P saporin had better outcomes that those receiving conventional analgesic treatment.[210–212] Thus, all that was known about pain in dogs with bone cancer was that they are more painful than normal dogs and that pain improved with intrathecal resiniferatoxin or substance-P saporin. The sensory profile of dogs with cancer pain was unknown as was the response to a standardized pharmacological approach.

Cats with OA and dogs with OSA represent excellent models of spontaneous chronic pain that deserve to be further explored. They present with genetic diversity, share the same environment as people, and their disease occurs naturally over time, demonstrating to be models with higher fidelity to humans. A profound understanding of pain mechanisms and response to analgesics in dogs and cats with natural disease can only benefit animal and human health and welfare. In this dissertation, two studies performed in cats with OA and one study in dogs with OSA designed to address some of the shortcomings in our ability to fully utilize these models are presented in chapter 2 and discussed in chapter 3.

The main hypotheses were 1) osteoarthritic cats are affected by neurophysiological changes characteristic of central sensitization which can be detected by QST and the concomitant administration of centrally-acting analgesics; and 2) bone cancer in dogs causes peripheral and central sensitization with deficient descending modulating mechanisms.

The main objectives were 1) to provide evidence of mechanism-based therapy in cats with OA using QST; and 2) to test the ability of a QST protocol to provide evidence of peripheral and central sensitization in dogs with bone cancer including the development and validation of a conditioned pain modulation test, and to test the efficacy of a step-wise palliative analgesic protocol in these patients.

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2 Publications

The first objective, "*to provide evidence of mechanism-based therapy in cats with OA using QST*", was achieved with the first two articles presented in this chapter. The first study (Study I), entitled "Analgesic efficacy of tramadol in cats with naturally occurring osteoarthritis" was published on *PLoS ONE* (2017; 12(4): e0175565) by Beatriz P Monteiro, Mary P Klinck, Maxim Moreau, Martin Guillot, Paulo VM Steagall, Jean-Pierre Pelletier, Johanne Martel-Pelletier, Dominique Gauvin, Jerome RE del Castillo & Eric Troncy. In study I, we used response to mechanical temporal summation (RMTS) to study the efficacy of tramadol, a centrally-acting analgesic. This was the first study to evaluate the efficacy of a drug different from NSAIDs in cats with OA and it showed some degree of reversal of central sensitization after tramadol using RMTS. The second study (Study II), entitled "Analgesic efficacy of an oral transmucosal spray formulation of meloxicam alone or in combination with tramadol in cats with naturally occurring osteoarthritis" was published on *Veterinary Anaesthesia and Analgesia* (2016; 43(6): 643-651) by Beatriz P Monteiro, Mary P Klinck, Maxim Moreau, Martin Guillo, Paulo VM Steagall, Daniel K Edge, Jean-Pierre Pelletier, Johanne Martel-Pelletier, Dominique Gauvin, Jerome RE del Castillo & Eric Troncy. This study was performed as a follow up from the first study. It showed that using RMTS, only cats receiving meloxicam-tramadol improved, with no changes in meloxicam-placebo group. Along with study I, it points to a mechanism-based approach for the use of tramadol in cats with central sensitization.

The second objective, "*to test the ability of a QST protocol to provide evidence of peripheral and central sensitization in dogs with bone cancer including the development and validation of a conditioned pain modulation test, and to test the efficacy of a step-wise palliative analgesic protocol in these patients*", was achieved with the third article presented in this chapter (Study III) entitled "Pain characterization and response to palliative care in dogs with naturally-occurring appendicular osteosarcoma: An open label clinical trial". It was published on *PLoS ONE* (2018; 13(12): e0207200) by Beatriz P Monteiro, Louis-Philippe de Lorimier, Maxim Moreau, Guy Beauchamp, Jeffrey Blair,

Bertrand Lussier, Jean-Pierre Pelletier & Eric Troncy. This was the first study to perform a standardized procedure of QST in dogs with bone cancer pain. It showed that dogs with bone cancer are affected by primary and secondary hyperalgesia, brush allodynia and deficient descending modulation of pain in comparison with healthy controls. It also showed that a step-wise palliative analgesic protocol was not enough to control pain in bone cancer, at least for the subjective assessments, and the QoL of dogs with OSA. However, some objective outcomes, including QST and actimetry, demonstrated evidence of efficacy.

2.1 Analgesic efficacy of tramadol in cats with naturally occurring osteoarthritis

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2.1.1 Article identifiers

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2.1.2 Contributions from PhD candidate

Responsible for acclimation of cats to procedures and personnel; performing QST and PVF assessments; accelerometer-based activity monitor set up. Contributed with data management and interpretation of results. Drafted the manuscript which was further edited and finalized after co-authors' reviews.

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2.1.3 Abstract

Objectives: This study aimed to (1) compare outcome assessments in normal and osteoarthritic cats and (2) evaluate the analgesic efficacy of tramadol in feline osteoarthritis (OA), in a prospective, randomized, blinded, placebo-controlled, crossover design.

Methods: Twenty cats were included after clinical examination, blood work and full body radiographs were performed. In Phase 1, outcome assessments aimed to differentiate normal (n = 5; i.e. exempt of any radiographic and clinical sign of OA) from OA (n = 15) cats. In Phase 2, OA cats were treated twice daily with a placebo (PG: cornstarch 15 mg) or tramadol (TG: 3 mg/kg) orally for 19 days, with a 3-month washout period between treatments. Evaluations were performed in normal and OA cats at baseline and consisted of: 1) peak vertical force (PVF) after staircase exercise; 2) telemetered night-time motor activity (NMA); and 3) response to mechanical temporal summation (RMTS). After treatment, PVF, NMA and RMTS evaluations were repeated in OA cats. Data were analyzed with mixed model methods with an alpha-threshold of 5%.

Results: Phase 1: 1) PVF (% of body weight; mean ± SD) was higher in normal (59 ± 10.5) than in OA cats (50.6 ± 5.7) ($p = 0.005$); 2) NMA (no unit) was not different between groups; 3) RMTS (number of stimuli; median (range)) was higher in normal [29.5 (23.5±30)] than in OA cats [14 (8.5±28)] ($p < 0.0001$). Phase 2: PVF, NMA and RMTS presented a treatment effect ($p = 0.024$, $p = 0.008$ and $p = 0.018$, respectively). No clinically important adverse-effects were observed.

Conclusion: Outcome assessments such as kinetics (PVF) and evaluation of central sensitization (RMTS) are discriminant of OA status. Mobility measured by NMA was not discriminant of OA status, however it increased in OA cats with tramadol treatment. Nociceptive hypersensitivity quantified by RMTS was evident in OA cats and was responsive to tramadol treatment.

2.1.2 Introduction

Osteoarthritis (OA) is a degenerative disease associated with pathological changes of the synovial joint. The progressive deterioration of one or more components of the joint is associated with pain, inflammation, peripheral and/or central sensitization and decreased mobility, which ultimately impact activity and quality of life.[1–5] Radiographic evidence of OA is reported in up to 61% of cats older than six years of age[6] and in up to 90% of cats older than 12 years of age.[7] Similarly, the incidence of OA increases with age and is the leading cause of disability due to pain in both dogs[8] and humans.[9] In feline clinical practice, the signs of OA are very subtle and unspecific,[10] and client-based questionnaires and activity monitoring have been used to assess pain-induced behaviours in osteoarthritic cats.[6,11,12] In the research setting, OA- related outcome assessments have been recently validated to characterize functional disability and maladaptive pain secondary to central sensitization.[3,4,13,14] Indeed, brain functional imaging in cats with OA revealed sustained ascending nociceptive inputs and increased activity of the descending modulatory pathways, both consistent with central sensitization.[14] The analgesic treatment of OA in cats has been classically based on the use of non-steroidal anti-inflammatory drugs (NSAIDs) such as meloxicam.[2,3,11,15,16] These compounds seem to improve motor activity,[3,11] but not central sensitization.[3,5]

Tramadol is an analgesic used worldwide for its effects on improved physical function and good tolerability in humans with chronic OA pain.[17] Nevertheless, evidence of its efficacy in canine and feline OA are scarce. The mechanisms of action of tramadol have not been fully elucidated and to date, the majority of studies have focused on the activation of μ-opioid receptors and inhibition of monoamine reuptake as potential mechanisms.[18–20] The analgesic effects of tramadol are expected to be mostly related to the production of its active metabolite(s) such as O-desmethyl tramadol (M1), which binds to μ-opioid receptors with approximately 300- fold higher affinity than the parent compound.[19,21] However, the affinity of tramadol for the μ- opioid receptor is very low, approximately 10-fold less than that of codeine and 6000-fold less than that of morphine. Yet, the increases in pain thresholds induced by tramadol differ from those of other opioids in that they are only partially blocked by naloxone.[19] These latter findings

indicate that μ-opioid receptor activation is only one of the mechanisms of action of tramadol and M1. Other mechanisms of action include 1) inhibition of norepinephrine and serotonin reuptake (5-hydroxytryptamine (5-HT)), 2) inhibition of G-protein coupled receptors such as α2-adrenoceptor, NK1 receptors, muscarinic receptors and 3) inhibition of ion channels via nicotine acetylcholine receptors and N-methyl-D-aspartate (NMDA) receptors.[22] These mechanisms of action can increase the activity of the endogenous inhibitory system and decrease the transmission of pain likely explaining the central analgesic effects of tramadol.

In cats, the drug has high bioavailability after oral administration (93 ± 7%) and M1 follows tramadol's disposition profile.[23,24] Indeed, studies indicate that cats might have superior analgesic profile after tramadol administration when compared with dogs due to a longer elimination half-life and higher concentrations of M1.[23,25] Its effects in cats have been evaluated for the treatment of acute post-operative pain and in antinociceptive studies.[20,24,26] Tramadol is a low-cost outpatient oral analgesic that is potentially a viable option for the treatment of OA pain, however, its efficacy for the treatment of feline maladaptive pain is not known.

The authors hypothesized that 1) assessments of peak vertical force (PVF), night-time motor activity (NMA) and response to mechanical temporal summation (RMTS) would differentiate normal from OA cats, and that 2) in OA cats, tramadol treatment would improve assessments of PVF, NMA and RMTS when compared with placebo treatment in a prospective, randomized, blinded, placebo-controlled, crossover design study. Results of this study suggest

that cats with and without OA can be distinguished using objective outcome measures, and that cats with maladaptive pain seem to benefit from tramadol treatment, when compared with placebo treatment.

2.1.3 Materials & Methods

Animals and experimental protocol

The study was approved by the Institutional Animal Care and Use Committee (Comité d'Étique et d'Utilisation des Animaux - n° Rech-1482) and animals were

handled and housed according to the Canadian Council on Animal Care Guidelines. Furthermore, this study adhered to the guidelines for Research Ethical Issues of the IASP,[27] and the ARRIVE guidelines for reporting animal research.[28]

According to the inclusion criteria, experimental cats were selected based on normal physical and neurological examination and normal clinical pathology evaluations (complete blood count, serum total thyroxine (T4), serum chemistry profile and urinalysis). Whole body computed radiographs were performed under heavy sedation using medetomidine (0.02 mg/kg; Domitor 1 mg/mL, Zoetis Canada Inc., Kirkland, QC, Canada) and morphine (0.2 mg/kg; Morphine Sulfate Injection 10 mg/mL, Sandoz Canada Inc., Boucherville, QC, Canada), administered intramuscularly. Images were analysed by a board-certified radiologist.[13] Exclusion criteria included the administration of either an NSAID or a glucocorticoid within four or eight weeks, respectively, of the start of the study. An orthopaedic examination was performed in all animals. Two populations of cats were selected (n = 15 OA cats, and n = 5 normal cats). Geriatric cats (≥ 10 years of age) that presented radiographic signs of OA affecting at least one appendicular joint, and young adult cats (≤ 4 years of age) that did not present clinical or radiographic signs of OA, were assessed. For selection of OA cats, seventy-three cats from a colony of research animals normally used for investigations related to cognitive function were screened. None of the cats had experimentally induced orthopaedic disease. They also required being friendly and interested in human interaction, because they would be subject to constant handling during the study.

The cats were housed together in one large room with heat and humidity control, and with access to windows. Environmental enrichment was achieved with the use of toys, scratch posts, condos, bedding and covers. Cats were fed according to the food manufacturer's recommendations twice daily with a standard certified commercial cat food (Hill's Prescription Diet w/d Feline, CDMV, Inc., St.-Hyacinthe, QC, Canada). Fresh water was available ad libitum in fountains.

Prior to the beginning of the study, cats were acclimatized to the personnel, research facility and evaluation tools over 6 weeks. The study was divided into two phases. In Phase 1, OA (n = 15) and normal (n = 5) cats underwent three outcome

assessments: PVF, NMA and RMTS. The evaluators were blinded to the OA-status of the cat. In Phase 2, after baseline measurement, OA cats were randomized to receive one of the following treatments twice daily for 19 days by the oral route: placebo (15 mg cornstarch) (PG: placebo group; n = 15) or tramadol (3 mg/kg; Tramadol HCl, Gentès & Bolduc Pharmaciens, Inc., St.-Hyacinthe, QC, Canada) (TG: tramadol group; n = 14). Treatments were repeated in a crossover design after a three-month washout period. Tramadol capsules were prepared individually based on the body weight (BW) of each cat in an attempt to be as close as possible to a dose of 3 mg/kg. Placebo and tramadol capsules were identical, and the evaluators were blinded to the treatments. Phase 2 evaluations included NMA and RMTS and were repeated in the last day of treatment. Unfortunately, PVF evaluation was partially completed only during the Phase 2, for technical reasons (see below).

Measurement of PVF

The PVF was acquired using a floor mat-based plantar force measurement system (Walkway System WE4, Tekscan) and was managed using Walkway Research Software v.7.0. Calibration was performed prior to each measurement. The cats were coaxed using positive reinforcement (treats, clicker, brushing, etc.) to trot across the walkway at a comfortable speed (0.8–1.4 m s-1).[29] Speed was computed using the time and distance of a given trot. Only the four-foot strikes of the first stride of the trot were used for data comparisons. Among all kinetic gait parameters generated, only the maximal loading (i.e. PVF) was used.[3,13,30]

For each evaluation session, the cat was allowed 10 trot attempts across the mat in order to provide three valid sets of four-foot strikes that would be used for data comparison. Furthermore, a valid attempt was defined as one in which the cat trotted across the entire mat undisturbed, consistently, in a straight line, and at a constant speed. If three valid trots had not been achieved after a maximum of 10 trot attempts, the cat was released and data for that cat were not used for comparison. The PVF was recorded over approximately 3 minutes immediately after 60 seconds of stairs exercise that consisted of running up and down a 10- meter staircase.

Based on the PVF expressed as a BW percentage (% BW), the limb (thoracic or

pelvic) that yielded the lowest PVF value in baseline assessment was determined for each cat. The outcome was calculated by averaging the three valid attempts of the most affected limb of each cat. Unfortunately, the system was not operational for the second arm of the crossover and could not be repaired. In consequence, PVF assessment during the Phase 2 could be completed only for half of the OA cats.

Motor activity assessment

The NMA was assessed using a collar-attached accelerometer-based activity sensor (ActiWatch, Minimitter/Respironics, Bio-Lynx Scientific Equipment, Ville-St.-Laurent, QC, Canada) maintained in place from 14 days prior to beginning of Phase 1, until the last day of evaluation of Phase 2. The device was set to perform one activity intensity count every 2 minutes. The amplitude of each count was subsequently translated to a numeric value (from 0 to infinite) referring to the intensity count of MA. Based on previous research.[3,13] in order to avoid any interference caused by human interaction, only data from Friday, Saturday and Sunday evenings (from 17:00 to 06:58 hours) were used for NMA analyses. The collars were checked twice daily to ensure that the activity sensors were in place. For each period, the mean of the intensity counts was calculated for each cat and used for data comparison.

Response to mechanical temporal summation

Repeated mechanical stimuli of sub-threshold intensity at a fixed intensity (4 N), frequency (0.4 Hz) and duration (up to 30 stimulations over 75 seconds) were applied using a purpose-made device (Topcat Metrology Ltd; Cambs, UK). The mechanical stimulus was produced by a metallic pin (10 mm long) with a hemispherical tip (2.5 mm in diameter) mounted on a rolling diaphragm actuator that was placed on the cranial aspect of the right or left mid metacarpus, held by a narrow band around the limb. A 'dummy' device was placed on the contralateral limb. This pin would move back and forth perpendicularly to the skin, producing a pressure stimulus at each time (similarly to a repetitive mechanical nociceptive stimulation). The number of stimulations needed to elicit a behavioural response was recorded. Increases in the number of stimulations are considered to reflect decreases in central pain hypersensitivity. The evaluation protocol is described elsewhere.[4,5]

Adverse effects

Cats were monitored daily for any potential outwardly detectable treatment-induced adverse effects. Attitude (normal, depressed/sedated or euphoric) and pupil size (normal or dilated) were evaluated during treatment administration (every 12 hours). In addition, at any time that a clinical sign was witnessed by a staff member, the date, time, affected cat and clinical sign were recorded. Therefore, each time that a cat was noted to present with an abnormality, it was considered as 'one event' (i.e. if a cat was evaluated as 'euphoric' twice in one day, then two events of 'euphoria' were added to the total number of events). Clinical pathology evaluations, including complete blood count, serum chemistry profile, total T4 and urinalysis, were performed at baseline and repeated after the last treatment (day 19).

Statistical Analyses

According to hypotheses, analyses on PVF and NMA were two-sided, and those on RMTS were one-sided, using an α-threshold value of 0.05. Analyses were carried out using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA). The distribution of continuous data was assessed using the Shapiro-Wilk test (normal distribution) or kernel density estimation. The PVF and NMA data were assumed to be log-normally distributed, and the RMTS count data were assumed to be Poisson-distributed by nature. The Phase 1 comparisons between OA and normal cats were made using a generalised linear model for PVF and RMTS assessments, and an exact Wilcoxon-Mann-Whitney test for NMA assessments. The Phase 2 treatment effect was evaluated using a generalised linear mixed model for repeated measures with treatment groups, time and their interaction as fixed effects, and cat as a random effect. For each model, the homogeneity of variances was tested, and the best structure of the covariance was assessed using information criteria that measured the relative fit of the competing covariance model. Then, the treatment and time effects were assessed using likelihood modeling. More precisely, for PVF, it used a compound symmetry covariance structure test with BW, velocity and maximum number of attempts as covariates, NMA a paired t-test, and RMTS a signed rank test.

2.1.4 Results

Seven female and eight male cats were included in the OA group, and two female and three male cats were included in the normal group. All animals were neutered and completed the investigation, except for two OA cats that were withdrawn due to reasons unrelated to the study. During the wash-out period, one cat developed skin allergies that required further investigation, and another cat had to be euthanized due to stage III chronic kidney disease. In the first period of the crossover, these latter cats had been included in the TG (n = 8) and PG (n= 7), respectively. Thus, in the second period of the crossover, there were n = 6 cats in TG and n = 7 cats in PG. These exclusions resulted in available data for n = 14 cats in each TG and PG at the end of the study. In the OA group, cats had radiographic signs of OA in the unilateral hip (n = 4), bilateral hips (n = 2), shoulders (n = 3), elbows (n = 2), combination of tarsus and elbow (n = 1), or combination of shoulders and hips (n =3). At inclusion, the ranges of age and BW of OA cats were [10.17 – 11.75] years, and [2.91 – 6.05] kg, respectively. In the normal cats' group, respective ranges were [2.71 – 3.92] years, and [3.08 – 5.23] kg.

Findings of Phase 1 are presented in Table X. PVF data were not available for three OA cats due to reluctance to complete the assessment. PVF was higher in normal cats when compared with OA cats ($p = 0.005$). Data for NMA were not available for one normal cat due to a defective device (battery failure). Night-time MA was not different between groups. The RMTS was higher in normal cats when compared with OA cats ($p < 0.0001$).

After 19 days of treatment, PVF was collected for seven cats in TG, and six cats in PG (Figure 10), as a result of reluctance to complete the assessment for two cats, and technical failure of the system during the second arm of the crossover.

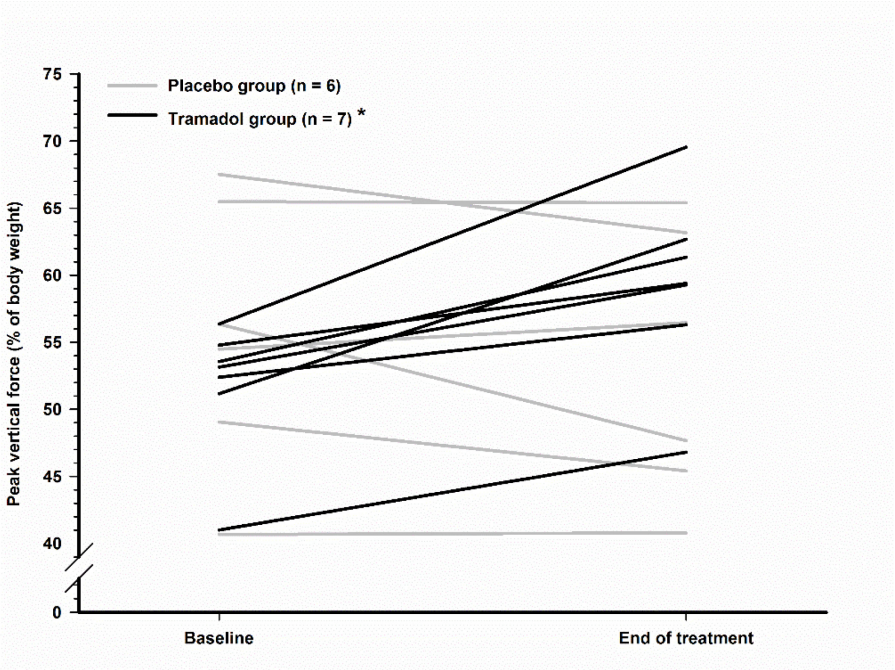
A pressure-sensitive mat was used for collection of data. Each value is the average of three valid attempts of the most affected limb of each cat. Cats with OA were randomly divided into two groups to receive either placebo (n = 6) or tramadol (n = 7; 3 mg/kg every 12 hours orally) and were re-evaluated after 19 days of treatment.

Table X. Peak vertical force (PVF), night-time motor activity (NMA) and response to mechanical temporal summation (RMTS).

Data from cats with and without naturally-occurring osteoarthritis (OA and normal cats, respectively).

	OA cats		**Normal cats**	
	n	**Mean (SD) or Median [Min – Max]**	***n***	**Mean (SD) or Median [Min – Max]**
PVF (% BW)	12	**50.6 (5.7)**	5	59.0 (10.5)*
NMA (no unit)	15	**47.8 (21.4)**	4	58.3 (38)
RMTS (number of Stimulations)	15	**14 [8.5 – 28.0]**	5	29.5 [23.5 – 30.0]*

*Significant between- and within-group difference.



* Significant between-group difference.

Figure 10. Individual values of peak vertical force before and after treatment in osteoarthritic cats

From baseline to end of treatment, PVF increased in all TG cats (p = 0.012), and this increase was considered important (> 10% of change percentage) in 5/7 cats, whereas none (0/6) of the PG treated cats showed improvement (p = 0.235). The range of percentage for the TG and PG were [+7.5% – +23.4%] and [-15.4% – +3.6%], respectively. The treatment effect was significant (p = 0.024), even with such a limited sample size. The NMA was significantly different between treatment groups at the end of treatment (p = 0.008) and activity only increased in the TG cats (Figure 11).

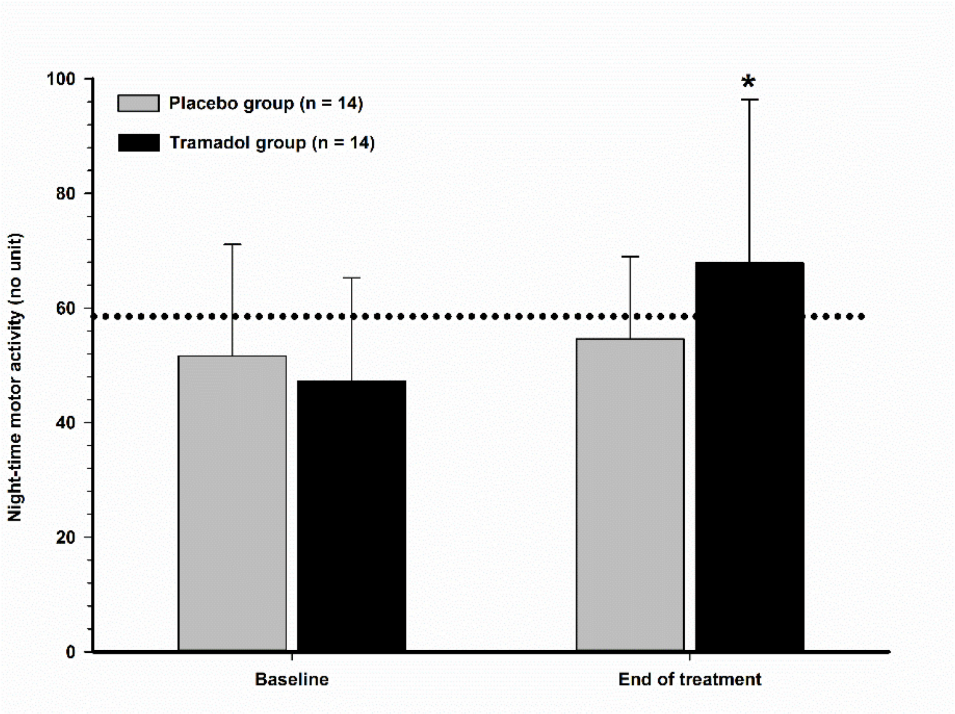


Figure 11. Night-time motor activity in cats with naturally occurring osteoarthritis

A collar-attached accelerometer device was used for collection of data. Cats with OA (n = 14) were randomly divided into two groups in a crossover design to receive either placebo or tramadol (3 mg/kg every 12 hours orally) and were re-evaluated after 19 days of treatment. Values are presented as mean (SD). The dotted line represents the averaged night-time motor activity (no unit) observed in normal cats during baseline evaluations. *Significant between- and within-group difference.

The RMTS was also significantly different between treatment groups at the end of treatment (p = 0.018) (Figure 12). The cut-off value (30 stimulations) was not reached in any OA cat at baseline. Six TG and three PG cats achieved the cut-off after treatment.

A mechanical device was used for collection of data. Cats with OA (n = 14) were randomly divided into two groups in a crossover design to receive either placebo or tramadol (3 mg/kg every 12 hours orally) and were re-evaluated after 19 days of treatment. Values are presented as mean (SD). The dotted line represents the number of mechanical stimuli (count) observed in normal cats during baseline evaluations. A significant between-group difference (MWW test) was found between normal cats and cats with osteoarthritis. *Significant between- and within-group difference.

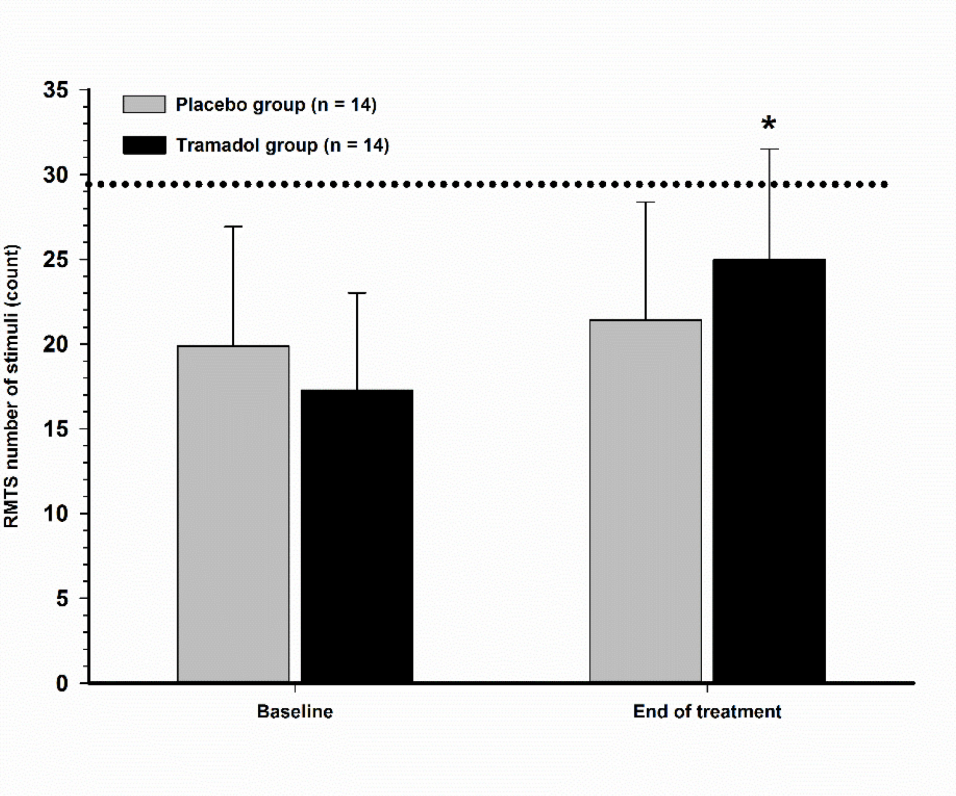


Figure 12. Response to mechanical temporal summation (RMTS) in cats with naturally occurring osteoarthritis

No adverse systemic effects were observed with either treatment based on clinical pathology evaluation. The following outwardly detectable adverse effects were recorded (treatment group: number of events): mydriasis (TG: 32; 20 events were related to n = 3 cats and PG: 3), mild sedation (described by observers as “depressed” or “overly quiet”) (TG: 32; 29 events were related to n = 2 cats), mild euphoria (TG: 6), polydipsia (TG: 1 and PG: 1), vomiting (TG: 8; 7 events related to n = 2 cats and PG: 1).

Although not systematically assessed, most cats allowed a stress-free pill administration with the use of a pet pillar device, with the exception of 3 cats, which displayed aversive and hiding behaviour (unknown frequency), hypersalivation (TG: 9 and PG: 2) and blood in the mouth (TG: 6 and PG: 1, all events related to the same cat) during pilling.

2.1.5 Discussion

Objective outcome measures such as PVF and RMTS were discriminant of OA status and revealed decreased biomechanical function and increased nociceptive hypersensitivity in OA cats. Night MA does not seem to differentiate between cats with and without OA, and perhaps a larger sample size would overcome the inter-individual heterogeneity observed. Treatment with tramadol demonstrated beneficial effects by increases in PVF, NMA and RMTS when compared to placebo treatment. There were no clinically important adverse-effects.

Assessment of PVF characterises biomechanical activity and is a valid and reliable tool for evaluating chronic pain in cats with OA.[3–5,13,29,30] Similar to previous studies evaluating cats with hip OA,[13] PVF values in Phase 1 were higher in normal cats when compared with OA cats. This may reflect altered biomechanics in OA pathology and/or decreased weight-bearing in affected limb secondary to pain. Nevertheless, it may also reflect neuromuscular aging-related changes to the musculoskeletal system such as decline in passive joint stability, ligament stiffness and muscle strength.[31] In fact, PVF values have been negatively correlated with age in feline patients.[13] It would have been ideal to have PFV data of all OA cats during Phase 2 in order to clarify whether the differences observed in Phase 1 are related to age only or OA status. Unfortunately, this was not possible due to technical limitations and we only have PVF data for a few cats in the first treatment period. It has to be noted that the most affected limb PVF, as used, was touching either thoracic or pelvic limb, increasing the intra-group variability in collected data. Indeed, in several quadrupeds, the normal weight distribution is higher in the thoracic (55% for cats) than in the pelvic (45% for cats) limbs.[29] The center of mass being closer to the thorax contributes to this imbalance.[32] Consequently, the range of values for the thoracic PVF is higher than for the pelvic PVF, and the intra-group

variability of the most affected limb PVF, when both thoracic and pelvic limbs are considered, is higher than the one restricted to PVF of thoracic or pelvic limbs alone, in OA-affected cats. Therefore, it is understood that the unfortunately limited sample size in Phase 2, evaluating treatment effect, had greatly reduced the power of inferential analysis. It is difficult to ascertain with great confidence that changes in PVF assessment are related to a treatment effect and not a type I error. Furthermore, placebo- treated cats may show exercise-induced improvement in PVF[3,5] due to all the necessary acclimation and training involved with PVF assessment in the species.[13,30] In these previous studies and the study herein, cats required a minimum of four weeks of training which inherently increased their physical activity. Thus, improvements in PVF assessment in placebo-treated cats might reflect the benefits of exercise alone in the management of OA.

Accelerometer-based MA provides an objective assessment of functional disability related to OA-associated maladaptive pain. This method has been largely used as an outcome to evaluate mobility and the impact of chronic pain in the quality of life of cats in the research and clinical settings.[3–5,11–13,33] Accelerometer-based MA had very good intra-class correlation coefficient indicating a good reliability of the test.[3] We had hypothesised that NMA of normal cats would be higher when compared with OA cats in Phase 1, as it has been previously reported,[13] however we could not find such a difference. One must take into consideration that this assessment is closely related to the individual's natural behaviour[3–5,11–13,33] and it may be that cats in the normal group were naturally less active. In addition, the number of cats in each group was unequal (5 *versus* 15 cats) and a large intra-group variability might have existed which would reduce the power to find a statistical difference. We speculate that if the number of individuals were similar, a difference would have been detected and would have corrected a type-II statistical error. Nevertheless, assessment of NMA revealed a clear treatment-effect, which is in agreement with other reports with tramadol,[5] or the NSAID meloxicam,[3,5,11,12] or even a veterinary OA therapeutic diet.[33] The mechanism by which this phenomenon takes place is unclear, but most likely it reflects an analgesic effect of tramadol and consequent increased mobility. In humans, tramadol is recognised for increasing mobility.[17] Considering that exercise is one of the most important pillars in OA-treatment and directly reflects quality of life, it seems reasonable to say that by increasing MA,

tramadol could play an important role in improving the quality of life of osteoarthritic cats.

Central sensitisation is expressed as pain hypersensitivity characterised by decreased tactile (von Frey) threshold,[3,4] decreased RMTS,[4] sustained cerebral nociceptive inputs (secondary somatosensory cortex) and increased activity of descending modulatory pathways (thalamus and periaqueductal gray matter)[14] in cats with OA. Temporal summation is considered to be an important tool for the study of maladaptive pain as it reflects the early phase of central sensitisation ("wind-up"). The latter is an intrinsic part of the early neuroplastic changes in the central nervous system.[34] This phenomenon is potentially reversible and tramadol has been used for this purpose in animals and humans.[35,36] The RMTS was lower in OA when compared with normal cats in Phase 1, which was similar to another study.[4] In OA cats, RMTS increased from 14 stimulations at baseline evaluations to 25 stimulations after tramadol treatment and a difference between TG and PG was found. These findings would infer that OA and normal cats present different neuro-sensitivity profiles and that OA cats are affected by central sensitisation, which in turn is translated by low RMTS. Thus, tramadol might reduce central sensitisation, alone (this study) or in combination with meloxicam,[5] in affected animals by means of RMTS, which was not observed in placebo-treated cats.

No clinically important adverse-effects were recorded. Mydriasis, sedation, euphoria and vomiting may be expected in cats after tramadol treatment.[37] Sedation was recorded mostly in the same n = 2 cats in TG, but did not seem to be important enough to affect activity as measured by NMA assessment. Indeed, as observers described sedation as cats being "overly quiet", the latter may be related to the personality of those 2 individual cats. Hypersalivation and blood in the mouth were more frequent in TG and are likely related to reluctance to pill administration. Tramadol is bitter tasting and may cause hypersalivation and retching if the capsule breaks open and the animal tastes the drug.[37] The bitter tasting of tramadol may become an obstacle to the treatment of some individuals as observed herein. Furthermore, the presence of blood in the mouth might also be related to the presence of gingivitis in these cats that would make them more likely to develop gingival bleeding after administration of the medication.

Antinociceptive studies suggest that a dosing regimen of 4 mg/kg administered

orally four times daily would be ideal.[24] In the study herein, because long-term treatment would be administered and no data was available for clinical cats, a dose of 3 mg/kg was chosen. The drug was administered every 12 hours to simulate intervals of administration that are commonly prescribed to owners for convenience and increased compliance. Moreover, it was the same dose used in a previous study,[5] where tramadol was combined to meloxicam oral transmucosal spray.

Based on PVF and RMTS, normal and OA cats display different biomechanics and central sensitisation profile. Assessment of NMA was not discriminatory of OA-status. Treatment with tramadol increased weight-bearing, mobility and decreased central sensitisation based on PVF, NMA and RMTS in cats with naturally occurring OA. To the best of our knowledge, this is the first demonstration of the benefits of tramadol treatment in cats with OA. Long-term tramadol therapy of up to 19 days seems safe and most common adverse-events are mydriasis, sedation and euphoria. These results are encouraging for promoting tramadol as a treatment for pain in osteoarthritic cats.

2.1.6 Conflict of interest statement and Acknowledgements

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2.2 Analgesic efficacy of an oral transmucosal spray formulation of meloxicam alone or in combination with tramadol in cats with naturally occurring osteoarthritis

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2.2.2 Contributions from PhD candidate

Responsible for acclimation of cats to procedures and personnel; performing QST and PVF assessments; accelerometer-based activity monitor set up. Contributed with data management and interpretation of results. Drafted the manuscript which was further edited and finalized after co-authors' reviews.

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2.2.3 Abstract

Objective: To evaluate the analgesic efficacy of meloxicam oral transmucosal spray (OTMS) alone and with tramadol in cats with osteoarthritis (OA).

Study design: Randomized, blinded study.

Animals: Fifteen geriatric cats weighing 4.5 ±1.0 kg.

Methods: Healthy cats with OA were randomly administered a placebo (every 12 hours orally) and meloxicam OTMS (approximately 0.05 mg/kg every 24 hours) (group M, n = 7), or tramadol (3 mg/kg every 12 hours orally) and meloxicam OTMS (group TM, n = 8) for 25 days. Evaluations performed before treatment (D0) and at week 3 (W3) consisted of peak vertical force, motor activity and response to mechanical temporal summation of pain (RMTS). Data were analyzed with mixed models and Fisher's exact test.

Results: Mean ± standard deviation peak vertical force (percentage of body weight) increased significantly in both groups ($p = 0.02$), from 47.7 ± 6.5% to 60.5 ± 9.4% in group M, and from 51.8 ± 5.0% to 64.1 ± 6.5% in group TM, with no difference between groups. Motor activity increased in M (from 43 ± 12 to 56 ± 13; $p = 0.02$), but not in TM. The number of stimulations from RMTS increased in TM only. Cut-off values were reached in a larger number of cats (n = 5) in TM than M (n = 1) ($p < 0.05$). Gastrointestinal adverse effects were self- limiting in six cats, including five in TM.

Conclusions and clinical relevance: Meloxicam OTMS had similar effects on peak vertical force, motor activity and pain sensitization as previously reported for oral meloxicam in OA cats. The tramadol–meloxicam combination provided no evident benefit over meloxicam alone, except for central hypersensitivity (assessed with RMTS). Further assessment of the potential toxicity of the combination is required prior to clinical use. Gingival administration was well accepted overall.

Keywords: analgesia, degenerative joint disease, feline chronic pain, meloxicam, osteoarthritis, tramadol.

2.2.2 Introduction

Osteoarthritis (OA) is characterized by a pathological change in the joint. It is associated with pain, inflammation, peripheral and/or central sensitization and decreased mobility, which impact on activity and quality of life.[1–5] Radiographic evidence of OA has been observed in up to 61% of cats aged >6 years[6] and in up to 90% of cats aged >12 years.[7] Signs of OA are very subtle and unspecific in clinical practice. Client-based questionnaires and activity monitoring have been used to assess pain-induced behaviors in cats with OA.[6,8] In the research setting, outcome assessment measures to characterize functional disability and maladaptive pain secondary to central sensitization have been validated.[4,5,9,10]

Traditionally, the treatment of OA-related symptoms such as pain and inflammation has been based on the use of non-steroidal anti-inflammatory drugs (NSAIDs) such as meloxicam.[2,4,8,11,12] Meloxicam is a COX-2 preferential NSAID with high oral bioavailability, efficacy, palatability and good tolerability.[2,4,8] Meloxicam improves motor activity,[4,8] but does not change von Frey hypersensitivity assessments in cats.[4] A new oral transmucosal formulation of meloxicam has been approved by the US Food and Drug Administration (FDA) for the control of pain and inflammation associated with OA in dogs, but not in cats.[13]

Tramadol is a centrally acting analgesic that produces l-opioid receptor activation and serotonin and noradrenergic reuptake inhibition.[14–16] The analgesic effects of tramadol result from its active metabolites, such as O-desmethyl-tramadol.[15] The drug has high bioavailability after oral administration and O-desmethyltramadol follows tramadol's disposition profile in cats.[17,18] Use of the combination of tramadol and meloxicam has been recently reported in a case series,[19] but the efficacy of the combination in the treatment of feline maladaptive (chronic) pain is unknown.

The aim of this pilot study was to evaluate peak vertical force, motor activity and response to mechanical temporal summation of pain (RMTS) after administration of tramadol– meloxicam, and meloxicam alone. The oral transmucosal spray (OTMS) of meloxicam was used in this study. The study hypothesis was that in cats with naturally occurring OA, the co- administration of tramadol and meloxicam would provide similar

or increased peak vertical force, motor activity and number of stimulations from RMTS in comparison with the administration of meloxicam alone.

2.2.3 Materials & Methods

Animals and experimental protocol

The study was approved by the Institutional Animal Care and Use Committee (no. 1757) of the University of Montreal and animals were handled and housed according to the Canadian Council on Animal Care Guidelines.

According to the inclusion criteria, experimental cats were selected based on normal neurologic examination and normal clinical pathology evaluations [complete blood count, serum total thyroxine (T4), serum chemistry profile and urinalysis]. The cats were older than 10 years of age, with normal physical examinations except for clinical signs of OA (pain at joint palpation), and radiographic evidence of OA affecting at least one appendicular joint. Cats were sedated for radiology by intramuscular administration of medetomidine (0.02 mg/kg; Domitor 1 mg/mL; Zoetis Canada, Inc., QC, Canada) and morphine (0.2 mg/kg; Morphine Sulfate Injection, 10 mg/mL1; Sandoz Canada, Inc., QC, Canada). Whole-body digital radiographs were analyzed by a board-certified radiologist.[9] Exclusion criteria were administration of an NSAID or a glucocorticoid within 4 weeks or 8 weeks, respectively, of the start of the study. Fifty-seven cats from a colony of geriatric research animals normally used for investigations related to cognitive function were screened. None of the cats had experimentally induced orthopedic disease. The cats were assessed as described. They were required to be friendly and to be interested in human interaction because they would be subject to constant handling during the study. Fifteen cats met the inclusion criteria and were included in the study.

The cats were housed together in one large room in which heat and humidity were controlled, and with access to windows. Environmental enrichment was achieved with the use of toys, scratch posts, condos, bedding and covers. Food was offered in the morning and in the afternoon (Hill's Prescription Diet w/d Feline; CDMV Siege Social, QC, Canada) and fresh water was continuously available in water fountains.

Prior to the beginning of the study, cats were acclimatized to the personnel,

research facility and evaluation tools over 6 weeks. Afterwards, animals were randomized to the administration of one of the following treatments for 25 days: 1) placebo (15 mg every 12 hours orally; cornstarch) and meloxicam OTMS (approximately 0.05 mg/kg every 24 hours; OroCAM Oral Transmucosal Spray, 0.25 mg/spray; Abbott Animal Health, Inc., IL, USA) (group M, n= 7); or 2) tramadol (3 mg/kg every 12 hours orally; Tramadol HCl; Gentès & Bolduc Pharmaciens, Inc., QC, Canada) and meloxicam OTMS as for group M (group TM, n = 8). Tramadol capsules were prepared individually based on the body weight of each cat in an attempt to remain as close as possible to a dose of 3 mg/kg. Placebo and tramadol capsules were physically identical. Although all spray bottles contained meloxicam OTMS, the evaluator was told that some spray bottles contained water. Therefore, the evaluator remained blinded to all treatments throughout the study period.

Outcome measures

Measurements of peak vertical force, motor activity and RMTS were evaluated at baseline (D0) and between 21 and 25 days of treatment administration (W3).

Measurement of peak vertical force

Peak vertical force was assessed using a pressure sensitive mat (Walkway System WE4; Tekscan, Inc., MA, USA) and was managed using commercial software (Walkway Research Version 7.0; Tekscan, Inc.). Calibration was performed before each measurement. Cats were trained to walk across the mat at a trotting speed (0.8–1.4 m/second) using a clicker and positive reinforcements such as treats and brushing.[20] Only peak vertical force data for the first strike of each of the four limbs were used for the purposes of data comparison.[4,9,21] In each evaluation, the cat was allowed up to 10 trials across the mat in order to provide three valid trials that would be used for data comparison. A valid trial was defined as one in which the cat moved across the mat in a trotting gait in a straight line and at a constant speed. If three valid trials had not been achieved after a maximum of 10 attempts, the cat was released and data for that cat were not used for comparison. Peak vertical force was recorded over approximately 3 minutes immediately after 60 seconds of stair exercise which consisted of running up and down a 10 m staircase. The limb (thoracic or pelvic) that yielded the lowest peak vertical force in

baseline assessments was used for comparison over time.[4,21]

Motor activity assessment

Motor activity was assessed by means of a small activity sensor (ActiWatch Minimitter/Respironics; Bio-Lynx Scientific Equipment, Inc., QC, Canada) that was secured to the cat's collar using tape. Cats wore the device for 14 days before beginning treatment and until the last day of evaluation (day 25). The device was set to perform one activity intensity count every 2 minutes, and this count was transformed into a numerical value that ranged from 0 to infinity. In order to avoid any interference caused by human interaction, only data from Friday, Saturday and Sunday evenings (17.00–06.59 hours) were used for analysis.[9] The collars were checked twice daily to ensure that the activity sensors were in place. For each period, the mean of the intensity counts was calculated for each cat.

Response to mechanical temporal summation

Response to mechanical temporal summation was performed using a device that produces sub-threshold mechanical stimuli at a fixed intensity (4 N), frequency (0.4 Hz) and duration (up to 30 stimulations for a total of 75 seconds) (Topcat Metrology Ltd, UK). The stimulus was applied cranial to the mid-metacarpal area using a metallic pin (10 mm long) with a hemispherical tip (2.5 mm in diameter). This pin would move back and forth perpendicularly to the skin, producing a pressure stimulus at each time (similarly to a repetitive mechanical nociceptive stimulation). The device was held by an elastic band placed around the forearm, and a 'dummy' device was placed on the contralateral limb. The stimulus was stopped when a behavior response of aversion (vocalization, limb withdrawal, walking backwards) was observed. The number of stimulations applied until a response occurred was recorded. Increases in the number of stimulations were considered to reflect decreases in pain hypersensitivity. Further details of the evaluation protocol have been previously published.[5]

Adverse effects

Cats were monitored daily for treatment-induced adverse effects. Attitude (normal, depressed or euphoric) and pupil size (normal or dilated) were evaluated during

treatment administration (every 12 hours). Appetite was evaluated during feeding times (early mornings and late afternoons). In addition, at any time that a clinical sign was witnessed by a staff member, the date, time, affected cat and clinical sign were recorded. Therefore, each time that a cat was noted to present with an abnormality was considered as 'one event' (i.e. if a cat was evaluated as 'depressed' twice in 1 day, then two events of depression were added to the total number of events). Clinical pathology evaluations were repeated after the last treatment (day 25) for comparison with baseline values.

Statistical analysis

According to hypotheses, analyses on peak vertical force and motor activity were two- sided, and those on RMTS were one-sided, using an a-value threshold of 0.05. Analyses were carried out using SAS Version 9.3 (SAS Institute, Inc., NC, USA). The distribution of continuous data was assessed using the Shapiro–Wilk test (normal distribution) or kernel density estimation. Data on peak vertical force and motor activity were assumed to be log-normally distributed, and data on the numbers of stimulations were assumed to be Poisson-distributed by nature. Group effect was evaluated using a generalized linear mixed model for repeated measures with groups, time and their interaction as fixed effects, and cat as a random effect. For each model, the homogeneity of variances was tested, and the best structure of the covariance was assessed using information criteria that measured the relative fit of the competing covariance model. Then, the treatment and time effects were assessed using likelihood modeling. More precisely, peak vertical force, using the cat's body weight and trial velocity as covariates, was analyzed using a compound symmetry covariance structure test, motor activity using a paired t test, and RMTS using a signed rank test. The proportions of cats reaching the cut-off value of 30 stimulations at W3 were compared with a Fisher's exact test.

2.2.4 Results

Seven female and eight neutered male cats were included. All cats had radiographic signs of OA in the unilateral hip (n = 4), bilateral hips (n = 2), shoulders (n = 3), elbows (n = 2), combination of tarsus and elbow (n = 1), or combination of shoulders and hips (n = 3). At inclusion, the cats' age range was 10.17–11.75 years, and

body weight range was 2.91–6.05 kg. All animals except one cat in TM completed the study. Meloxicam was administered at a dose range of 0.04–0.085 mg/kg.

Peak vertical force

At W3, peak vertical force data were not available for two cats in M and one cat in TM as a result of their reluctance to complete the assessment. Peak vertical force increased significantly in both groups in comparison with baseline (D0) values ($p = 0.02$) (Table XI). Peak vertical force values increased from D0 to W3 in all 11 cats. There were no significant differences between the groups (Figure 13).

Motor activity

Data from one device used in TM were not available as a result of technical problems (battery failure). Motor activity at W3 in comparison with D0 increased in M ($p = 0.02$), but not in TM ($p = 0.47$). No significant differences were detected between groups.

Response to mechanical temporal summation

The number of stimulations significantly increased from D0 to W3 in TM ($p = 0.048$), with individual values for all seven cats increasing (Figure 14). In M, increasing and decreasing numbers of stimulations were measured in equal numbers of cats (Figure 14). The cut-off value of 30 stimulations was not reached in any cat at baseline. At W3, one of seven and five of seven cats reached the cut-off value in M and TM, respectively ($p = 0.048$).

Adverse effects

No adverse effects that could be attributed to the treatments were detected in the clinical pathology tests. Adverse effects (number of events) observed during the study were mydriasis ($n = 2$), depression ($n = 22$), decreased appetite ($n = 10$), hypersalivation ($n = 3$), and vomiting ($n = 10$). These clinical signs were observed in five cats in TM. In one cat, 18 and 10 events of depression and decreased appetite, respectively, were recorded. Diarrhea was recorded once in one M cat. One TM cat became lethargic and anorexic with signs of upper respiratory congestion and ocular mucous discharge, and

was withdrawn from the study at 9 days. Specific behaviors observed during the 350 administrations of meloxicam OTMS were shaking the head with avoidance (10 instances in M, 15 in TM), shaking the head and gagging (two instances in TM) and running away (one instance in M). Fourteen of these events were displayed by the same TM cat, such that 50% of negative behavior was related to one cat. Consequently, the rate of acceptance of the administration of the meloxicam OTMS was approximately 92%.

Table XI. Peak vertical force, night-time motor activity and number of stimulations in response to mechanical temporal summation (RMTS).

Parameter	GM			GTM		
	n	**D0**	**W3**	***n***	**D0**	**W3**
Peak vertical force (% of body weight)	5	47.7 ± 6.5	60.5 ± 9.4*	6	51.8 ± 5.0	64.1 ± 6.5*
Motor activity (no unit)	7	42.8 ± 12.3	56.3 ± 13.0*	6	39.1 ± 24.8	41.1 ± 20.8
RMTS (number of stimulations)	7	17 ± 7	20 ± 7	7	16 ± 3	28 ± 3*

Data from cats with naturally occurring osteoarthritis at baseline and following 25 days of treatment with meloxicam oral transmucosal spray (approximately 0.05 mg/kg every 24 hours, group M) alone or with tramadol (3 mg/kg every 12 hours orally, group TM). Data are expressed as the mean ± standard deviation.*Significantly different from D0 within the group.

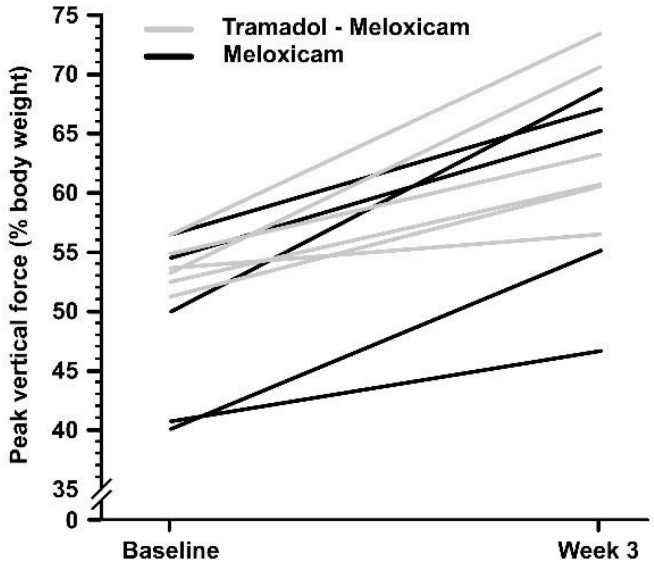

Figure 13. Individual values of peak vertical force evaluated using a pressure-sensitive mat

Data from cats with naturally occurring osteoarthritis before (baseline) and after administration of meloxicam oral transmucosal spray (approximately 0.05 mg/kg every 24 hours) alone or with tramadol (3 mg/kg every 12 hours orally) for 25 days (week 3).

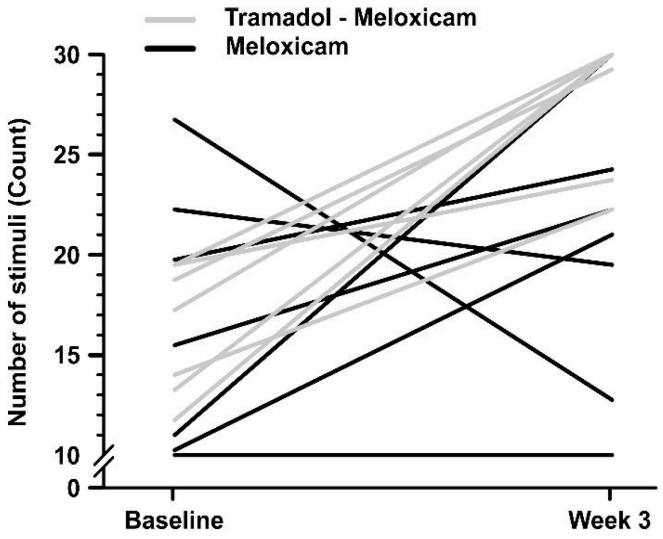

Figure 14. Individual values for the response to mechanical temporal summation (RMTS)

Data from cats with naturally occurring osteoarthritis before (baseline) and after administration of meloxicam oral transmucosal spray (approximately 0.05 mg/kg every 24 hours) alone or with tramadol (3 mg/kg every 12 hours orally) for 25 days (week 3).

2.2.5 Discussion

Treatment with a meloxicam OTMS resulted in similar beneficial effects on peak vertical force and motor activity, and no change in pain sensitization, as have been previously observed with oral meloxicam in cats with OA.[4] The results also showed that the addition of tramadol to meloxicam OTMS did not further improve peak vertical force or motor activity. However, RMTS measurements may suggest that tramadol reduced central sensitization. There was a higher prevalence of adverse effects in cats administered tramadol-meloxicam than in cats treated with meloxicam alone. Gingival administration was well accepted overall.

Assessment of peak vertical force is a valid and reliable tool for evaluating chronic pain in cats with OA.[4,5,9,10,21] Previously published research revealed that evaluating only the limb that yielded the lowest peak vertical force limited the dispersion of the data. In addition, performing the evaluation following stair climbing optimized the sample and effect sizes.[21] In this study, no significant difference in peak vertical force values was detected between the groups. However, significant within-group differences were observed in both groups. A similar improvement in peak vertical force after meloxicam oral treatment was observed in cats with OA-related pain.[21] It may be that tramadol does not have an effect on peak vertical force in cats, or that the improvement is subject to a ceiling effect and that differences between the present groups could not be detected. This cannot be confirmed because this study did not include a group of cats administered tramadol alone. Additionally, it has been reported that cats with OA show an exercise-induced improvement in peak vertical force measurements.[4] In humans, exercise is strongly encouraged for the management and improvement of OA.[22] It is difficult therefore to exclude the possibility that exercise itself improved peak vertical force values in this study.

Accelerometer-based motor activity provides an objective assessment of functional disability related to OA-associated maladaptive pain. This method has been largely used as a tool to evaluate mobility and the impact of chronic pain on the quality of life of cats in research and clinical settings.[4,5,8,9,23] It has a very good intraclass

correlation coefficient, indicating that the test has a good level of reliability.[4] The present results are similar to those of previous studies in which motor activity increased after treatment with meloxicam in cats with OA-related pain.[4,8] However, it is unclear why motor activity increased in M, but not in TM. The present authors speculate that, firstly, a lack of statistical power (favoring a Type II statistical error, particularly in TM) may have played a role, given the high intra-group variability. The motor activity assessment is related to the individual's natural behavior, which renders group comparisons difficult. It has been suggested that in assessments of motor activity data, each cat should be used as its own control.[4,5,9,23] Secondly, tramadol may have produced sedation, which is recognized as a clinical side effect of tramadol treatment in cats,[24] and this may have influenced motor activity. Thirdly, the tramadol–meloxicam combination induced some gastrointestinal adverse effects, which may have altered motor activity. Fourthly, the addition of a group administered tramadol alone would have potentially clarified these issues. Finally, it is not possible to know if an increase in the dose of tramadol would have changed these results. Antinociceptive studies suggest that a dosing regimen of 4 mg/kg every 6 hours would be ideal.[18] However, treatments were administered every 12 hours for convenience, increased compliance and to simulate the intervals of administration that are commonly applied at the study institution.

Central sensitization is expressed as pain hypersensitivity. In cats, this has been characterized by a decreased tactile (von Frey) threshold[4] and decreased number of stimulations in RMTS.[5] For the latter, OA cats exhibited an aversion response at a lower number of stimulations (6–12) than did non-OA cats (13–30). These findings reflect sustained cerebral nociceptive inputs and increased activity of descending modulatory pathways.[10] Temporal summation is considered to be an important tool in the study of maladaptive pain. It reflects the early phase of central sensitization ('wind-up') characterized by the neuroplastic changes that occur in the central nervous system.[25] This phenomenon is potentially reversible and tramadol has been used for this purpose in animals and humans.[26,27] In this study, tramadol may have reduced central sensitization as measured by RMTS. The baseline values for RMTS in both groups were higher than in a previous study,[5] suggesting that this population of cats presented with less central sensitization. A Fisher's exact test confirmed that cut-off values were reached in a larger

number of cats in TM than in M. All seven TM cats demonstrated an improvement in RMTS and the majority of them reached the cut-off value of 30 stimulations, which has been established as the normal value in healthy (non-OA) cats.[5] These findings are in agreement with those of a previous study showing that meloxicam alone does not change pain hypersensitivity as assessed by the tactile threshold using von Frey stimulation.[4]

Methods of drug administration, such as an OTMS, may increase owners' compliance with long term treatment of chronic conditions in cats. In general, the present findings for group M are in agreement with those of previous studies evaluating the effects of oral meloxicam in cats with OA.[4,8] It is important to highlight the fact that doses were similar among these studies; however, different formulations were used (OTMS versus liquid oral formulations). Pharmacokinetic data for the OTMS in cats are not available, but it seems reasonable to conclude that, at similar doses, both formulations produce comparable effects. Tramadol capsules were prepared to provide similar dosing for all animals. Although it is not expected to do so, whether the compound preparation altered the pharmacokinetic and/or pharmacodynamic profile of tramadol is unknown. Also unknown is whether the formulation would have any influence on the incidence of adverse effects.

In recent trials[1,2,28,29] meloxicam was found to be safe for the treatment of OA-related pain. In the present study, the incidence of serious adverse effects was low. One cat in TM was removed from the study for supportive treatment. This animal also developed clinical signs of upper respiratory disease, which may be unrelated to treatment administration. Of note, most of the cats that developed adverse effects were in group TM. Based on the pharmacology of each analgesic and its potential for the development of adverse effects, most adverse events might be expected to have resulted from NSAID administration. If this assumption was correct, occurrences of vomiting and diarrhea would have been similar in both groups. Nevertheless, it appears that the tramadol–meloxicam combination may have increased the prevalence of gastrointestinal adverse effects. Serotonin–norepinephrine reuptake inhibitors (such as tramadol) have been claimed to potentially increase the risk for gastrointestinal ulcers, both when administered alone and in combination with NSAIDs, in humans[30,31] and in animals.[24,32] However, the mechanisms related to such interactions remain unknown. When the ex vivo effects of

tramadol and indomethacin, alone and in combination, were investigated in the gastric mucosa of dogs, no interaction between the two drugs was observed.[33] It is difficult to make conclusions from the results of the present study about the incidence of adverse effects or even to extrapolate these data to the target population as most of the events were related to one TM cat (18 and 10 events of depression and decreased appetite, respectively) and four TM cats (one or two transient episodes of vomiting). Mydriasis was rarely observed, and dysphoria and constipation were never recorded. The primary goal of the study was not to compare the safety of the treatments, although clinical and laboratory test evaluations were included. Evidence is lacking to link increased gastric adverse events with the co-administration of tramadol and NSAIDs in cats. This issue remains to be elucidated.

In conclusion, based on peak vertical force and motor activity assessments, meloxicam OTMS revealed efficacy comparable with that previously reported for oral liquid meloxicam in cats with OA.[4,8] The addition of tramadol to OTMS did not further improve peak vertical force or motor activity in comparison with meloxicam alone in cats with naturally occurring OA. However, tramadol may have reduced central sensitization when measured by RMTS. Further studies are warranted to evaluate drug interactions-induced adverse effects after tramadol- meloxicam and OTMS in cats.

2.2.6 Conflict of interest statement and Acknowledgements

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2.3 Pain characterization and response to palliative care in dogs with naturally-occurring appendicular osteosarcoma: An open label clinical trial

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2.1.2 Contributions from PhD candidate

Responsible for recruiting dogs and client communication with owners. Performed QST and static weight bearing assessments. Responsible for accelerometer-based activity monitors set up and data management. Contributed with statistical analysis and interpretation of results in collaboration with co-authors. Drafted the manuscript which was further edited and finalized after co-authors' reviews.

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2.3.3 Abstract

This study aimed to characterize bone cancer pain (quantitative sensory testing (QST), stance asymmetry index, actimetry, scores of pain and quality of life (QoL)) in dogs with appendicular osteosarcoma (OSA), and to evaluate a stepwise palliative analgesic treatment.

The pain profile of thirteen client-owned dogs with OSA was compared with seven healthy dogs. Dogs with OSA were then enrolled in a prospective, open-label, clinical trial. Outcome measures included: primary and secondary mechanical thresholds (MT), conditioned pain modulation (CPM), stance asymmetry index, actimetry (most and least active periods), visual analog scales and QoL. After baseline assessments, stepwise treatment comprised orally administered cimicoxib (2 mg/kg q 24h), amitriptyline (1–1.5 mg/kg q 24h) and gabapentin (10 mg/kg q 8h); re-evaluations were performed after 14 (D14), 21 (D21) and 28 (D28) days, respectively. Statistics used mixed linear models (α = 5%; one-sided).

Centralized nociceptive sensitivity (primary and secondary MT, and dynamic allodynia) was recorded in OSA dogs. Healthy dogs had responsive CPM, but CPM was deficient in OSA dogs. Construct validity was observed for the QST protocol. Asymmetry index was significantly present in OSA dogs. The CPM improved significantly at D14. When compared with baseline (log mean ± SD: 4.1 ± 0.04), most active actimetry significantly improved at D14 (4.3 ± 0.04), D21 and D28 (4.2 ± 0.04 for both). When compared with baseline, least active actimetry significantly decreased after treatment at all time-points indicating improvement in night-time restlessness. No other significant treatment effect was observed. Except for tactile threshold and actimetry, all outcomes worsened when gabapentin was added to cimicoxib-amitriptyline.

Dogs with bone cancer are affected by widespread somatosensory sensitivity characterized by peripheral and central sensitization and have a deficient inhibitory system. This severe pain is mostly refractory to palliative analgesic treatment, and the latter was only detected by specific and sensitive outcomes.

2.3.4 Introduction

Cancer is the number one cause of mortality in dogs,[1] and pain is a common clinical feature leading to stress, suffering, and low scores of quality of life (QoL).[2,3] Osteosarcoma (OSA) is an aggressive and invasive neoplasm of the skeletal system that causes both osteolytic and proliferative changes. It is the most commonly diagnosed primary bone tumor in dogs[4,5] and is generally associated with a poor long term prognosis.[6] Bone cancer pain is characterized by peripheral and central sensitization with both nociceptive and neuropathic components.[7] In people, it is described as dull in character and constant in presentation, and bone remodeling results in severe spontaneous pain. Breakthrough pain is also a clinical feature characterized by episodes of extreme pain that can occur spontaneously or be induced by movement. These clinical signs tend to progress over time and patients become severely affected with altered function and QoL.[7,8] The actual incidence and characteristics of cancer pain in dogs remain unknown.[9] Nevertheless, given that dogs frequently present with advanced-stage cancer at initial evaluation and have similar cancer biology when compared with humans,[10] it is reasonable to presume that canine patients experience a similar pain profile.[2] Research aiming to further enhance our understanding of cancer pain in animals is clearly warranted. A recent survey of veterinarians in the United Kingdom revealed that 87% agreed or strongly agreed that cancer pain is underdiagnosed, and 66% disagreed or strongly disagreed with the statement "pain associated with cancer is easy to treat".[11] Under-diagnosis and under-treatment of cancer pain is also a reality in human patients, particularly when neuropathic pain is involved.[12]

The World Health Organization proposes a 3-stepwise palliative pharmacologic approach for the management of cancer pain.[13] Patients with mild pain are administered NSAIDs, and patients with moderate to severe pain are administered opioids in combination with adjuvant analgesics. In veterinary medicine, oral administration of opioids does not seem to produce significant anti-nociception.[14] Opioids are controlled drugs with limited availability worldwide and potential for abuse. For these reasons, veterinarians may be reluctant to prescribe opioids for outpatients.

Previous studies have evaluated the efficacy of intravenous administration of bisphosphonates,[15] and intrathecal administration of substance-P saporin,[16] and

resiniferatoxin.[17] Although efficacy was observed with these treatments, they can be relatively costly or are not readily available or require technical skills that make their use extremely limited. Therefore, this study proposed a stepwise palliative analgesic treatment including orally administered medications that are available and of low cost, namely cimicoxib, an NSAID,[18] amitriptyline, a tricyclic antidepressant drug,[19] and gabapentin, an inhibitor of voltage- dependent calcium channels.[20] None of these analgesics, to the best of the authors' knowledge, has been evaluated in dogs with OSA-related pain. The nature and intensity of OSA-related pain has not been characterized, nor has the response to a stepwise palliative analgesic treatment. This lack of knowledge impairs proper recognition and treatment of pain in dogs with bone cancer.[2,3]

The study objectives were to characterize OSA-related pain in dogs by evaluating their somatosensory system using different quantitative sensory testing (QST) applicable in clinical conditions, scores of pain and QoL, assessment of the level of activity and sleep disturbance, and to test the efficacy of a non-opioid stepwise palliative analgesic treatment.

The study hypotheses were that the somatosensory profile of OSA compared to healthy dogs could be characterized, with OSA dogs presenting peripheral and central sensitization, decreased activity, sleep disturbance and poor QoL scores, and that these outcome measures would significantly improve with treatment, indicating analgesic response after palliative analgesic therapy when compared with baseline values.

2.3.5 Materials & Methods

The study was approved by the Comité d'Éthique de l'Utilisation des Animaux (CÉUA Rech-1806), registered on the American Veterinary Medical Association (AVMA) Animal Health Studies Database (#AAHSD-000362 08/01/2016 to 04/17/2018) and is reported according to the ARRIVE guidelines. The individual in this manuscript has given written informed consent to publish these case details.

Animals and experimental protocol

The study was divided into two phases. First, healthy (n = 7) and OSA (n = 13) dogs were compared using a standardized QST protocol and an asymmetry index (AI)

measurement. Second, OSA dogs included in a prospective open-label clinical trial were evaluated using the same outcome measures, in addition to actimetry as well as pain and QoL scores.

Healthy client-owned dogs from the staff and students of the Faculty of Veterinary Medicine, Université de Montréal were selected for the first phase. Dogs were considered healthy if they had a normal physical examination and no history of trauma or any orthopedic or systemic disease. They also had to be good-tempered in order to accept manipulation and younger than 3 years of age. For selection of client-owned OSA dogs, the study was advertised to all referring veterinarians within a 100 km radius of the Centre Vétérinaire Rive-Sud (CVRS), where the clinical trial was conducted from August 2016 to April 2018. Inclusion criteria for OSA dogs were confirmed diagnosis of appendicular OSA by a board-certified oncologist using cytology or histopathology, or a very high suspicion of OSA based on signalment, history and radiographs (location and appearance of the bone lesion), and evidence of cancer-related pain. Dogs had to be good-tempered and conventional cancer treatments such as surgery, radiation, chemotherapy or bisphosphonates were not a viable option for the dog or had been declined by the owner. Dogs could not be receiving any analgesic at the time of inclusion. If the dog was being administered analgesics during screening, a wash-out period of 10 days for NSAIDs or steroids, and 2 days for centrally-acting analgesics such as tramadol or acetaminophen, was required. Dogs that presented with metastatic disease but were in good health could be included.

Dogs were excluded if they presented with severe concomitant disease, a survival time prognosis of less than one month, history of gastrointestinal disease, uncontrolled endocrine disease, history of NSAID intolerance, azotemia with chronic kidney disease IRIS stage 2 or higher,[21] or ALT greater than three times the upper reference limit. Dogs were not included if there was any question regarding the owner's compliance with treatment, knowledge of the dog's normal behavior or intellectual capacity.

Eligible dogs were included after a thorough discussion with the owner and an informed consent form had been signed. During the study, dogs were excluded if any adverse effects requiring intervention developed or if any medication outside the

protocol was administered.

Outcome measures

Standardized QST protocol

Evaluation of the somatosensory profile of dogs was performed using a QST protocol that was specifically designed for this study (dx.doi.org/10.17504/protocols.io.uwzexf6 and dx.doi.org/10.17504/protocols.io.uw4exgw).

Static QST, which typically assesses the sensory thresholds to or the rating of a single stimulus, included primary tactile threshold, and primary and secondary mechanical nociceptive thresholds (MTs). Potential primary tactile threshold was evaluated using an electronic von Frey anesthesiometer (with Rigid tip of 0.7 mm^2 surface, 28G, IITC Life Sciences Inc, Woodland Hills, CA, USA) with a 1000g internal load cell.[22] Briefly, gradually increasing pressure was applied perpendicular to the skin, dorsal to the right metacarpus (between metacarpal bones III and IV) in healthy dogs and perpendicular to the tumor in OSA dogs, until a behavior response indicative of a conscious perception of the stimulus was observed (e.g. paw withdrawal, reaching for the device, vocalization, etc.) (Figure 15).

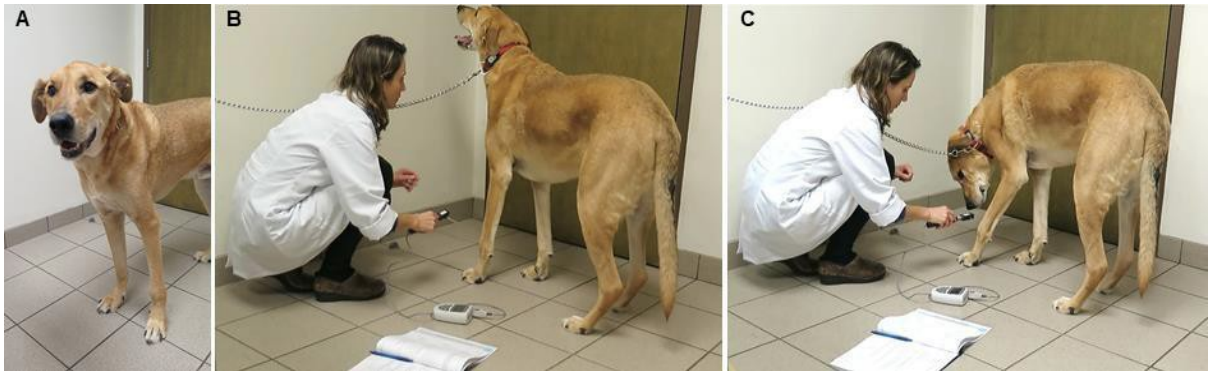

Figure 15. Primary tactile threshold test

(A) A 12-year-old neutered male mixed breed dog with OSA of the left distal radius. (B) Before the stimulus, the dog is bearing weight on the affected limb and looking around. (C) Behavioral response to the stimulus: the dog is looking at the stimulated area and withdrawing the weight from the limb.

The peak force was recorded, and a safety cut-off was defined at 500g. Mechanical nociceptive threshold was evaluated using a pressure algometer (Digital Force Gauge Series 3, Model M3-2, Mark-10, Copiague, NY, USA) with a 10N internal load cell connected to a blunt "W" shaped metal tip (primary MT) or a sharp pointed metal tip (secondary MT). Tests of MT were performed similarly to the primary tactile threshold tests and a safety cut-off was defined at 10N. Triplicate measurements of primary tactile threshold and primary MT were done with a 10-second interval between each test and the average was calculated. Tests of secondary hyperalgesia were performed over the paravertebral muscles (right and left sides) immediately caudal to the 11th and 13th vertebra in both healthy and OSA dogs.[23] Triplicate measurements were done at these four locations with 1-minute intervals between them, and the mean of the resulting 12 values was calculated for each time-point.

Dynamic QST, which assesses the response to a number of stimuli,[24–27] has gained increasing attention because it offers the opportunity to probe the central processing of incoming nociceptive signals.[24,26] In this study, dynamic QST included brush-evoked allodynia, and conditioned pain modulation (CPM). Brush-evoked allodynia was evaluated using apurposely- designed device with a soft bristle that was gently brushed along the hair growth up to three times or until a behavior response was observed. Dogs were defined as being affected by brush-evoked allodynia if a response was observed, and later evaluated as a proportion of positive responders. Referring to the phenomenon of "pain inhibits pain", CPM is the behavioral correlate of diffuse noxious inhibitory control (DNIC), where the presence of a second noxious stimulus (i.e., conditioning stimulus) decreases the pain perception from an initial noxious stimulus (i.e., test stimulus).[24,26] The test-retest stimulus were MT and the conditioning stimulus was ischemic pain (Figure 16), as adapted from protocols in humans.[28]

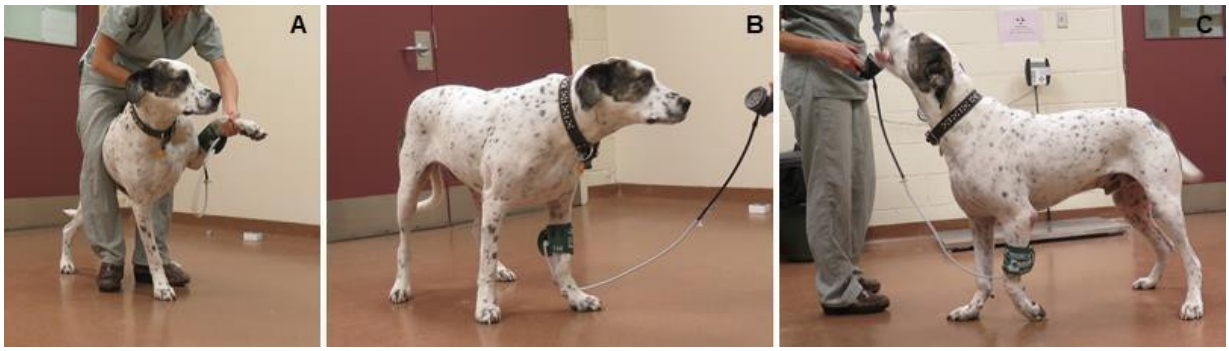

Figure 16. Conditioning stimulus (ischemic noxious model) for conditioned pain modulation (CPM) test in a healthy dog

(A) The dog's left thoracic limb is gently lifted and pressed/massaged from the paw towards the elbow or stifle joint. Then, a pressure cuff is placed around the mid-radius and inflated up to 200 mmHg. (B) The dog is encouraged to walk around the room for two minutes. (C) Note the left non-weight-bearing limb demonstrating the discomfort caused by the ischemia (conditioning stimulus).

In healthy dogs, the conditioning stimulus was done in the left thoracic limb, whereas in OSA dogs, it was done in the limb diagonal to the affected limb (i.e., if the tumor was in the left thoracic limb, the conditioning stimulus was done in the right pelvic limb). For the conditioning stimulus, the limb of the dog was gently lifted and pressed/massaged from the paw towards the elbow or stifle joint. Then, a pressure cuff was placed around the mid-radius or mid-tibia, inflated up to 200 mmHg, and the dog was encouraged to walk around the room for two minutes. Once the two minutes were complete, the retest-stimulus (i.e., MT) was immediately performed in quadruplicate (twice while the cuff was inflated and twice after cuff deflation) with 10-second intervals between each measurement. The mean of these four values was used as the MT post-conditioning stimulus. Data from the primary MT were used as the MT (pre-conditioning) test stimulus. The difference between retest and test MT, i.e., post- minus pre-conditioning stimulus was calculated (Delta CPM) and dogs were classified (Functional CPM rate) as having a functional (Delta CPM ≥ 0) or dysfunctional DNIC system (Delta CPM < 0).[29] The QST protocol was always completed in the same room and with the same staff to whom the dogs had been accustomed since the initial visit. The somatosensory

tests were performed while the dogs were in a standing position by one of two evaluators (BPM or LPdL). The latter was taken into consideration during statistical analyses. Dogs were allowed to rest between tests and were given food rewards once the QST protocol was completed.

Scores of pain and QoL

Pain was independently evaluated by the veterinary oncologist (LPdL) and the owner using a 10 cm visual analog scale (VAS) in which "0" corresponds to "no pain" and "10" corresponds to the "worst imaginable pain".[30] At each visit, the oncologist scored the VAS after the physical examination and the owner scored the VAS according to what he/she believed the dog had experienced on average in the last five days. The oncologist and the owners were not aware of each other's VAS scoring. Scores of QoL were done using a French language questionnaire specifically designed for the study (dx.doi.org/10.17504/protocols. io.uw5exg6 and dx.doi.org/10.17504/protocols.io.uw6exhe) according to previously published literature on the subject.[31–34] The QoL questionnaire included 19 items pertaining to three main domains: happiness (1 to 4), physical functioning (5 to 12), and quality of life (13 to 19). A 20th item was available to be added and scored if the owner believed that there was a specific behavior relevant to the dogs that had not been addressed in the questionnaire. In addition, the owners could choose not to score up to eight items they believed were irrelevant in their dog's case. Unscored items were then crossed out for the subsequent visits. The owners were also asked to choose the item they believed was the most relevant within each domain. Items could be scored using a 4- point Likert scale adapted for each item; thus, higher scores indicate higher impact from the disease in the dog's QoL. A final score was calculated as the percentage of the given scores divided by the maximum possible score according to the total number of scored items for each dog. At each time-point, the owners were systematically asked how the dog was doing since the last visit and were left alone in the room without further conversation. They were given the VAS and QoL questionnaire to be completed. Except for the baseline visits, owners were also given the previously scored QoL questionnaire since dependent interviewing has been shown to increase treatment effect sizes.[35] The same owner completed both the VAS and QoL questionnaire at all time-points.

Objective outcomes: Actimetry and asymmetry index

Actimetry was assessed using an accelerometry-based activity sensor attached to the collar throughout the entire study period (Actiwatch-64; Bio-Lynx Scientific Equipment, Inc., Montreal, QC, Canada). Actimetry (intensity of motor activity; no unit; from 0 to infinite) epoch was two minutes. Once the data were downloaded, the 1-hour (30 counts) intensity was summated. Then, the ten most and least active 1-hour periods were selected for each time-point of assessment. Finally, the average of these ten 1-hour periods was calculated, so that each dog had a mean actimetry value for its least and most active periods for each time-point. The most active periods reflected the level of activity while the dog was active. The least active periods reflected the periods in which the dog was sleeping and are a measure of sleep disturbance (i.e., the lesser the activity during the least active period, the better the sleep).[36]

Static weight bearing data were collected while dogs were standing over a pressure-sensitive mat (PetSafe Stance Analyzer, Model 300–2509, Vet therapy–Kruuse A/S, Langeskow, Denmark) with one paw in each of four quadrants. A series of eight data (proportion of body weight per paw) was collected during one minute at each time-point and its mean was calculated. Asymmetry index was calculated according to a previously published formula,[37] which accounts for the asymmetry in weight-bearing between two contralateral limbs. Thus, the greater the asymmetry, the higher the AI. In healthy dogs, AI was calculated for the thoracic limbs. In OSA dogs, AI was calculated for the affected limbs (i.e., thoracic or pelvic limbs depending on the anatomical location of the tumor).

Monitoring of adverse effects

Complete blood cell count, serum chemistry profile and urinalysis were performed at each time-point, except for Baseline 2. The owners were thoroughly educated as to which adverse effects to monitor and were instructed to contact the researchers if they were observed.

Treatments and assessment time-points

In the first phase, healthy and OSA dogs were evaluated twice for each outcome measure. Evaluation of the healthy dogs was done on the same day (morning and afternoon), whereas for the OSA dogs, Baseline 1 and 2 were separated with an interval

of 2 to 4 days in order to evaluate the repeatability of baseline assessments. After Baseline 2, all OSA dogs were prescribed treatment level 1 and were re-evaluated after 14, 21 and 28 days (D14, D21, and D28, respectively). At each re-evaluation, the treatment was changed to a higher level in a stepwise approach if at least one of the following conditions was observed: QoL scores did not decrease from the previous visit or were > 40%, or VAS scores from owners or the oncologist did not decrease or were > 4.0. Level 1 treatment comprised cimicoxib (Cimalgex chewable 8, 30 and 80 mg tables, Vetoquinol S.A, Lure, France) administered at 2 mg/kg orally every 24 hours. Level 2 treatment comprised cimicoxib (same dosage) in addition to amitriptyline (Apo-amitriptyline 10 and 25mg, Apotex, Toronto, ON, Canada) administered at 1–1.5 mg/kg orally every 24 hours. Level 3 treatment comprised cimicoxib (same dosage), amitriptyline (same dosage), in addition to gabapentin (Apo- gabapentin 100 and 300 mg, Apotex) administered at 8–10 mg/kg orally every 8 hours. Upon study completion, a long-term follow up was done by telephone interview.

Statistical analyses

Data were analyzed using SAS (version 9.3, SAS Institute, Inc., Cary, NC, USA). Data were tested for normality using the Shapiro-Wilk test. For comparisons between healthy and OSA dogs, data were analyzed using independent t-tests, Mann-Whitney or Fisher's exact tests

where appropriate. Pre- and post-conditioning stimulus MTs for CPM testing were compared with paired t-test. Repeatability of baseline measurements and the association between the VAS scores from owners and the oncologist or between the scores of VAS and QoL were calculated using intraclass correlation coefficient (ICC), kappa coefficient or Pearson's correlation coefficient. Results from ICC or kappa/Pearson statistics were interpreted using the Altman's classification (0.81–1.00 excellent; 0.61–0.80 good; 0.41–0.6 moderate; 0.21–0.4 fair and < 0.2 poor).[38] The average of both baseline measurements was calculated and used for subsequent comparisons over time. The effect of time and treatment on numerical variables was analyzed using a linear mixed model and analyses were one-tailed with regard to treatment efficacy hypothesis. Time was considered a fixed effect and dog a random effect for treatment. The QST evaluator, sex of the owner, anatomical location of the tumor (thoracic versus pelvic) and body weight were added to

the model as covariates where appropriate. Data from actimetry were log-transformed for analyses to normalize distribution. The effect of time and treatment on categorical variables was analyzed using the Cochran-Mantel-Haenszel test for repeated measures. Adjustments for multiple comparisons were done using the Benjamini-Hochberg sequential adjustment procedure. The level of statistical significance was set at 5%.

2.3.6 Results

Inclusion data

Seven healthy dogs were included. Twenty-eight OSA dogs were screened for eligibility and 13 met the inclusion criteria. Of these, 11 dogs completed the study. Individual characteristics of included OSA dogs are presented in Table XII; this data is consistent with previous studies on canine OSA. Fifteen dogs were not included in the study for the following reasons: being treated with other analgesics and compliance with the wash-out period required for inclusion would be too painful (n = 10), aggressiveness (n = 1), no signs of pain (n = 1), treatment with prednisone due to hyperadrenocorticism (n = 1), euthanasia before first visit due to rapid progression of disease (n = 1); unavailability of owner to be present at all required visits (n = 1).

Two dogs were excluded after baseline evaluations (one due to development possible of adverse effects and another due to euthanasia because of rapid progression of the disease). Baseline data from these two dogs were included in the data analysis. All data from one dog at D28 were excluded because the owner had not been able to administer the medications on the previous three days. Data for the QST protocol for one dog at D28 were not collected because the dog became aggressive during manipulation. Actimetry data for one dog and AI for another dog at D14, 21 and 28 could not be collected due to technical issues. The remaining data were all included in the analyses.

In the first phase, all QST tests and AI were significantly different between healthy and OSA dogs, except for primary tactile threshold (Table XIII).

Tableau XII. Individual characteristics of dogs with naturally-occurring osteosarcoma included in the study.

Breed	**Sex**	**Age** (years)	**Body weight** (kg)	**Anatomical location of the tumor**
Golden Retriever	Female	7.7	44.2	Left distal radius
Cane Corso	Male	7.8	64.5	Left distal radius
Great Dane	Male	4.7	70.8	Right distal tibia
Labrador Retriever X Great Dane	Male	8.9	54.9	Left distal radius
Newfoundland	Female	5.9	64.4	Right distal radius
Rottweiler	Female	9.0	41.1	Left proximal humerus
Siberian Husky	Male	11.5	22.4	Right proximal humerus
Rottweiler	Male	5.7	48.7	Right proximal tibia
Mastiff X Great Dane	Male	9.1	48.6	Right distal radius
Great Dane	Female	6.0	64.2	Right proximal humerus
Siberian Husky	Female	10.3	23.2	Right distal femur
Great Dane	Male	7.7	53	Right distal radius
Great Dane	Female	4.2	52.5	Right distal radius
	Mean ± SD	**7.6 ± 2.2**	**50.1 ± 14.7**	

Repeatability of baseline measurements

Repeatability for healthy dogs was good to excellent for primary tactile threshold, secondary MT, brush and functional CPM rate, and fair for primary MT, delta CPM and AI. In OSA dogs, ICC for primary tactile threshold, and primary and secondary MTs was 0.33, 0.50 and 0.91, respectively. The kappa coefficient for functional CPM and brush-evoked allodynia was 0.36 and 0.09, respectively. The ICC for the owners' scores of VAS and QoL were 0.58 and 0.87, respectively, and 0.90 for the oncologist's VAS score. The ICC for AI was 0.78.

Data from OSA dogs before and after treatment are reported in Table XIV. All OSA dogs received the three levels of treatment.

Standardized QST protocol (Table XIII)

Primary tactile threshold and primary MT did not change across time ($p = 0.15$ for both) and were not affected by anatomical location ($p = 0.31$ and 0.12, respectively) or the evaluator ($p = 0.35$ and 0.17, respectively). Secondary MT did not change across time ($p = 0.1$) and was not affected by the evaluator ($p = 0.38$) or the anatomical location ($p = 0.42$).

Response to brush-evoked allodynia did not change across time ($p = 0.36$). When MTs pre and post-conditioning stimulus for CPM testing were compared at each time point, a difference between them was observed at D14 ($p = 0.03$), but not at other time-points. Delta CPM did not change across time ($p = 0.07$) and was not affected by the evaluator ($p = 0.55$) or the anatomical location ($p = 0.71$). Compared to baseline, functional CPM rate increased significantly at D14 ($p = 0.016$), and slightly decreased subsequently. Except for primary tactile threshold and least active actimetry, all D28 outcomes worsened when gabapentin was added to cimicoxib-amitriptyline at D21.

Scores of pain and QoL (Table XIV)

Oncologist VAS score did not change across time ($p = 0.37$) and was not affected by the anatomical location of the tumor ($p = 0.56$). Owner VAS and QoL scores did not change across time ($p = 0.41$ and 0.47, respectively). Owner VAS score was higher in dogs whose tumors were in the pelvic limbs (least square mean ± SEM: 6.0 ± 0.6) when compared with the thoracic limbs (3.7 ± 0.3) ($p = 0.006$), and in dogs whose owners were women ($n = 8$, 5.8 ± 0.4) when compared with men ($n = 5$, 3.9 ± 0.5) ($p = 0.01$). Owner QoL score was not affected by anatomical location ($p = 0.09$), but women (65.1% ± 5.5) scored higher than men owners (45.7% ± 7.0) ($p = 0.04$). At first assessment, the most relevant items were “Willingness to play” (for 53.9% of respondents) in the “Happiness” domain, “Mobility (walking, trotting or running)” (38.5%) in the “Physical functioning” domain, and “My dog’s overall quality of life” (38.5%) in the “Quality of life” domain. Only three items, in the latter domain, were never used by respondents, namely “I am concerned about my dog’s general appearance”, “My dog seems dull or depressed, not alert”, and “My dog shakes or trembles”.

Tableau XIII. Quantitative sensory testing (QST) and asymmetry index measured by static weight bearing.

Outcome measure	Status	Mean ± SD	Frequency	*p*-value
QST **Primary tactile threshold** (grams) Lower value means sensitization	Healthy	297.6 ± 185.2	-	0.079
	OSA	198.4 ± 65.4	-	
Primary mechanical threshold (Newtons) Lower value means sensitization	Healthy	8.9 ± 0.6	-	0.012[a]
	OSA	6.6 ± 2.2	-	
Secondary mechanical threshold (Newtons) Lower value means sensitization	Healthy	9.8 ± 0.5	-	0.001[a]
	OSA	5.7 ± 2.5	-	
Brush-evoked allodynia (% of positive responders) High value means sensitization	Healthy	-	0	0.026[a]
	OSA	-	53.8	
Delta CPM (Newtons) Must be positive in normal conditions	Healthy	0.6 ± 0.6	-	0.014[a]
	OSA	-0.5 ± 1.5	-	
Functional CPM rate (%) Close to 100 in normal conditions	Healthy	-	85.7	0.035[a]
	OSA	-	38.5	
Asymmetry index (%) Close to 0 in normal conditions	Healthy	6 ± 2	-	0.000[a]
	OSA	30 ± 14	-	

Data from healthy dogs (n = 7) and dogs with naturally-occurring appendicular osteosarcoma (OSA) (n = 13).

CPM: conditioned pain modulation.

[a]Indicates *p*-values that are rejecting the null-hypothesis of the test (absence of difference between groups).

Tableau XIV. Outcome measures of dogs with naturally-occurring osteosarcoma (n = 13).

Data from before and after 14, 21 and 28 days of a stepwise palliative analgesic treatment in which they received cimicoxib (2 mg/kg PO q 24h), cimicoxib + amitriptyline (1-1.5 mg/kg PO q24h) and cimicoxib + amitriptyline + gabapentin (10 mg/kg PO q 8h), respectively

Outcome measure	Baseline	Day 14	Day 21	Day 28
Primary tactile threshold (grams)	178.1 ± 28.6	152.2 ± 30.2	190.25 ± 30.0	231.0 ± 33.1
Primary mechanical threshold (Newtons)	5.8 ± 0.6	4.7 ± 0.7	5.2 ± 0.6	4.7 ± 0.7
Secondary mechanical threshold (Newtons)	5.2 ± 0.8	4.7 ± 0.8	5.2 ± 0.8	5.1 ± 0.8
Brush-evoked allodynia (% of positive responders)	46.2	45.5	27.3	44.5
Delta CPM (Newtons)	-0.5 ± 0.5	1.2 ± 0.6	0.9 ± 0.6	0.6 ± 0.7
Functional CPM rate (%)	38.5	90.9[a]	81.8	75.0
Oncologist's VAS (no unit) Higher value means more pain	4.3 ± 0.4	4.3 ± 0.4	4.5 ± 0.4	5.0 ± 0.5
Owner's VAS (no unit) Higher value means more pain	4.5 ± 0.4	5.2 ± 0.4	4.8 ± 0.4	5.0 ± 0.4
Quality of life (%) Higher value means less QoL	51.3 ± 5.3	57.1 ± 5.5	56.0 ± 5.6	57.5 ± 5.5
Quality of life (most relevant item) (%) Higher value means less QoL	65.3 ± 28.1	66.7 ± 29.8	60.6 ± 25.0	73.3 ± 21.1
Log-transformed actimetry: least active (no unit)	2.3 ± 0.08	1.8 ± 0.1[a]	2.0 ± 0.1[a]	1.9 ± 0.1[a]
Log-transformed actimetry: most active (no unit)	4.1 ± 0.04	4.3 ± 0.1[a]	4.2 ± 0.1[a]	4.2 ± 0.1[a]
Actimetry: least active (no unit)[b] Lower value means less restlessness	224.4 ± 118.3	69.8 ± 19.8[a]	99.3 ± 38.2[a]	83.7 ± 34.0[a]
Actimetry: most active (no unit)[b] Higher value means more mobility	11039.7 ± 2964.9	20273.6 ± 6912.6[a]	16430.2 ± 3914.5[a]	14740.5 ± 4768.8[a]
Asymmetry index (%)	35.5 ± 4.2	40.8 ± 4.5	37.7 ± 4.5	40.9 ± 4.6

Data are presented as least square mean ± SEM unless otherwise stated. [a]Indicates a significant difference when compared with baseline. [b]Descriptive data presented as mean ± SD.

When considering only the most relevant item throughout the study, namely "My dog's overall quality of life", which was the 19th item in the questionnaire, it is interesting to note that the owners attributed higher score (65.3%) to their evaluation. Oncologist' and owner's VAS score correlations were moderate and good (Pearson's correlation coefficient = 0.44 and 0.77; p = 0.13 and p = 0.01) at baseline and D14, respectively, but poor (D21: 0.10; p = 0.78) and fair (D28: 0.32; p = 0.37), subsequently. Owner VAS and QoL scores were better correlated: Pearson's correlation coefficient = 0.89, 0.85, 0.59 and 0.72, at baseline, D14, D21 and D28 (p = 0.0001; 0.0009; 0.06 and 0.02), respectively.

Objective outcomes: Actimetry and asymmetry index

Compared to baseline, the least active actimetry decreased significantly at D14, D21 and D28 ($p < 0.0001$ for all). It was not affected by body weight (p = 0.48) or anatomical location (p = 0.94). The most active actimetry increased at D14 ($p < 0.0001$), D21 ($p < 0.0001$) and D28 (p = 0.0003). It was not affected by body weight (p = 0.25) or anatomical location (p = 0.0545). The effect of anatomical location was nearly significant, and it is interesting to note that dogs with tumors in the thoracic limbs (least square mean (log actimetry) ± SEM: 4.1 ± 0.03) were less active than those with tumors in the pelvic limbs (4.3 ± 0.05). Asymmetry index remained unaltered across time (p = 0.45) and was not affected by the evaluator (p = 0.96). Asymmetry index was higher in dogs with tumors in the pelvic (least square mean ± SEM: 48.2% ± 6.4) than in the thoracic limbs (29.3% ± 3.8) (p = 0.03).

Survival time

Data on the only surviving dog (male) at the time of this report were not included in the survival time analysis. Median (range) survival time was 43 (28–208) days since study inclusion. Survival time was weakly and negatively correlated with owner VAS and QoL scores from the last visit (Pearson's correlation coefficient = -0.04 and -0.13, respectively).

Monitoring of adverse effects

One male dog was withdrawn after three days of treatment with cimicoxib (level 1) due to development of liquid diarrhea with hematochezia and melena. Treatment was stopped, and the dog recovered within 24h. Another dog developed progressive

depression, anorexia and polyuria on D24. This dog was euthanized on D28 after data collection. Apart from these two dogs, no other clinically significant adverse effect was observed, except for increase in renal parameters over time for some dogs. Therefore, a linear mixed model for repeated measures was performed with values from creatinine, urea and urine specific gravity to qualify potential renal alteration. There was no significant change over time for creatinine or urine specific gravity ($p > 0.11$). Compared to baseline, urea values increased at D14 ($p = 0.026$), D21 ($p = 0.003$) and D28 ($p = 0.001$), but abnormal values (outside normal range [2.5–9.6 mmol/ L]) were present on 7 occasions, for 5 dogs. Other observed abnormalities included mild anemia (n = 2 occasions), increase in serum alkaline phosphatase (n = 1 occasion) and mild to moderate neutrophilia (n = 2 occasions). Overall, cimicoxib appeared to be well tolerated in this senior, cancer-bearing dog population.

2.3.5 Discussion

The profile of dogs with primary bone cancer was characterized, demonstrating widespread somatosensory sensitivity and dysfunction of the descending inhibitory nociceptive modulation (DNIC). This translated to a high degree of biomechanical alteration (as assessed by AI). Subjective pain assessment was on average about 50% of VAS and QoL scales. Construct validity of the proposed QST protocol was observed, based on the premise that if the outcome could actually measure sensory sensitivity, values between healthy and OSA dogs would be different. The responsiveness to analgesic palliative treatment was difficult to objectify: the responsiveness was present on actimetry and dynamic QST (indicating improvement in DNIC), more pronounced with cimicoxib alone, and did not change either static QST, or AI, or subjective assessments (VAS and QoL). The expected synergic addition of amitriptyline and gabapentin did not provide more analgesia, and could have even worsened the dogs' pain.

In the first phase, all static and dynamic QSTs were different between healthy and OSA dogs, except for primary tactile threshold. In order to further explore this potential lack of difference, we analyzed data from the OSA dogs (198.4 g ± 65.4, mean ± SD) in comparison with historical control of healthy dogs (n = 21; 403.4 g ± 135.6) ($p < 0.0001$). Thus, the absence of difference between healthy and OSA dogs for primary tactile

threshold is plausibly related to a type-II statistical error due to the small sample size (n = 7) of healthy dogs, especially if one considers their large SD observed for this outcome. Large variability in primary tactile threshold was also observed in recent studies comparing healthy and osteoarthritic dogs,[22] and cats.[39]

Dynamic QST brings another dimension to the neurophysiological phenotyping of animal pain. Brush-evoked allodynia QST was easy to complete and presented specific response (none of the healthy dogs responded to brushing), but moderate sensitivity with 54% of OSA dogs being positive responders (but the rate of sensitized OSA dogs could not be estimated). Although we cannot know the perceptual quality of this stimulation in animals, it is believed that the response to brushing is associated with dynamic mechanical allodynia. Indeed, in people with chronic pain, touch pleasantness elicited by brushing is decreased when compared with healthy individuals.[40] To the best of our knowledge, this is the first report of CPM in healthy dogs in comparison to dogs with chronic pain. Due to the lack of such literature in dogs, we opted to explore CPM effects in three different ways: by evaluating the difference between the pre- and post-conditioning stimulus MTs, by comparing the delta CPM between groups and with a binary classification of (dys)functional CPM rate. Regardless of the approach, descending inhibitory nociceptive modulation in the OSA dogs of our study was clearly affected in comparison with healthy dogs, concurring with a well-established finding in people with chronic pain.[29,41]

The AI was very different between healthy and OSA dogs, demonstrating a quasi-absence of imbalance in healthy dogs, and a 30% on average, contralateral report to the unaffected limb in OSA dogs. A previous study established an interval between 15.7–19.5% in which dogs with AI above this interval were affected by cranial cruciate ligament rupture differentiating them from healthy dogs.[37] It is interesting to note that dogs with OSA had nearly twice as much asymmetry than orthopedically-affected dogs, emphasizing the severe clinical presentation in the former population.

The ICC for QST assessments varied largely from poor to good.[38] Interpretation of these results is based on the assumption that the measures are stable,[42] which might not be the case in OSA patients. In addition, variability of the data might be related to stress,

learning,[43] or the evaluator's technique and interpretation. For example, previous research has shown that learning confounds algometric assessment in normal dogs since their thresholds decreased over time, with dogs anticipating the stimulus and reacting at lower thresholds.[43] Similar results with no replicate effect of QST measures have been reported in previous studies with dogs.[44,45]

In the second phase, with the exception of actimetry and dynamic QST, outcome measures (namely, static QST, AI, and subjective pain/QoL assessment) did not statistically change with treatment. Different explanations can be considered:

1. Nature of bone cancer pain: The efficacy of analgesic therapies might depend on the specific population of sensory neurons that innervate bone.[7] For example, primary afferent sensory neurons from the bone are restricted to specific A-delta and C-fibers.[46] In addition, recent research identified that canine OSA cells express and secrete nerve growth factor, endothelin-1 and prostaglandin E2 which potentially participate in malignant bone pain.[9] Finally, microfracture can result from progressive osteolysis by activated osteoclasts.[7] The sensitivity (responsiveness to treatment) of most assessment outcomes of this study could be too low, or the analgesic effect of the tested treatment could be too small, in view of such established chronic pain mechanisms.

2. Degree of neuropathic pain: In OSA, neuropathic pain can be caused by direct nerve damage or compression from the tumor, and by an active and pathological sprouting and neuroma formation by nerve fibers that innervate the skeleton.[7] A systematic review of studies in people reported a liberal estimate of the prevalence of neuropathic pain in patients with cancer to be 39%,[12] and the pharmacotherapy of neuropathic pain is challenging and frequently unsatisfactory in people.[47]

3. Rapid disease progression: In people, it is well known that the prevalence of pain increases with disease progression at a rate of 70–90% in patients with advanced disease.[47] The pain prevalence appears similar in canine cancer (75.2%).[33] Considering that most dogs in this study had locally advanced OSA, and that the median survival time was 43 days from study inclusion, it could be argued that the proposed analgesic approach was not enough to counteract the pain and evolution of disease.

4. Drugs and dosage protocol: The rationale of the proposed protocol

attempted to counteract peripheral and central mechanisms of pain by using centrally- and peripherally-acting analgesics. Cimicoxib is a cyclooxygenase (COX)-2 selective inhibitor ('coxib') licensed in Europe for the long-term management of pain and inflammation associated with osteoarthritis.[18] Amitriptyline is a tricyclic antidepressant drug that inhibits the reuptake of serotonin and norepinephrine in the central nervous system and is therefore expected to reinforce the descending inhibitory nociceptive modulation.[19] Gabapentin is an anticonvulsant drug with analgesic properties mediated via blockade of certain voltage-dependent calcium channels, and is therefore expected to inhibit the ascending nociceptive transmission.[20] Amitriptyline and gabapentin are the first line of treatment for neuropathic pain in humans.[48,49] It is possible that the dosage and treatment duration were not adequate to observe efficacy. Furthermore, it might be plausible that using orally administered analgesics is not enough to control bone cancer pain.

5. Small sample size: Calculation of sample size and power analysis could not be performed since there was no available data from the literature regarding pain in OSA dogs. A trend of effects was clearly observed for some of the outcome measures, such as primary tactile threshold, CPM, and brush-evoked allodynia (with the addition of amitriptyline); however, these were not statistically significant, due to a type II statistical error.

Regarding sensitive criteria able to detect a treatment efficacy, dynamic QSTs, and specifically CPM, are attractive. It was possible to include their use during clinical examination after a short period of learning. The baseline functional CPM rate was largely deficient in OSA dogs. It improved after two weeks of cimicoxib treatment and was maintained with the addition of amitriptyline and gabapentin, which supports the hypothesis of a central analgesic effect of the coxib drug. This reinforcement of a deficient descending inhibitory nociceptive modulation was more expected with the antidepressant drug, amitriptyline.[29] Indeed, at D21, the functional CPM rate in OSA dogs showed a trend to be improved, and the percentage of positive responders to brush-evoked allodynia to be decreased. Further validation and investigation of CPM in clinical trials may clarify the role of CPM in canine pain medicine.

Most and least active periods were affected by treatment. Activity monitoring is a non- invasive, valid and widely used outcome to assess spontaneous activity in dogs with chronic pain,[50] and their response to treatment.[51] In this study, it was hypothesized that

this pain syndrome would be associated with discomfort during rest (translated to increased 'low' activity) and during active motion (translated to decreased 'high' activity). Subsequently, the analgesic treatment, if efficient, would lead to a lower actimetry during least active periods (or, improvement in the sleep quality),[36] and to a higher actimetry during most active periods. Both results were observed to be significant in the present study, herein suggesting an improvement in sleep quality and more ease in active movement of the OSA dogs treated with cimicoxib. The addition of complementary analgesics did not result in additional improvement in these actimetry outcomes.

Scores of both VAS and QoL fluctuated over time with no improvement. One limitation of this study was the use of a non-validated clinical metrology instrument to assess QoL. However, based on a recent systematic review,[52,53] and the fact that there was no validated French language instrument, we designed the QoL questionnaire based on all available information.[31–34] It remains unknown whether the lack of improvement in QoL was real, or due to the lack of validity of the tool itself. Considering that some objective outcomes responded to analgesic treatment, the second hypothesis is more plausible. Finally, this study contributes to the psychometric validation of the QoL questionnaire, as some criteria appear to be excluded, and other presented some trend to respond to treatment. The deterioration in the most relevant (for owner) item throughout the study of the QoL scale from D21 to D28 evaluation is interesting, as the owners quoted highly the alteration for "My dog's overall quality of life", in addition to using it often in the scale, and is suggestive of a deterioration in the dogs' condition with the addition of gabapentin. The fact this item was the last one of the QoL questionnaire makes it attractive since it came after the owner thought about the previous items and it is a global QoL assessment criterion.

Another limitation of this study is the intrinsic bias from scores of pain and QoL for the owners and the clinician during post-treatment evaluations. Nevertheless, with the absence of any effect, including a placebo effect, it seems that observer bias was not relevant in this study. Furthermore, it would have been ethically unacceptable to include a placebo-control group in this clinical trial.

The results observed in this prospective open-label clinical trial highlight an

intriguing point about systematic use of multimodal therapies. At D28, after one week of gabapentin addition, it is obvious that, with the exception of primary tactile threshold and least active actimetry, and although not significant, all outcome measures deteriorated, when compared with D21. The decrease in delta CPM at D28 is significant compared to D14 and is suggestive of a deterioration in the descending inhibitory nociceptive modulation. Such pharmacological association is based on benefits observed in preclinical models,[54,55] but it is frequent for preclinical promises not to translate to similar clinical benefits,[56] and such observation warrants caution before recommending systematic multimodal analgesia protocols for canine cancer pain. It is possible that these findings were related with progression of the disease. Furthermore, the fact that most outcome measures did not improve in the present study raises an ethical concern regarding the palliative treatment of dogs with OSA. Further studies are unquestionably necessary to answer to this question.

The study results suggest that the aggravated bottom-up sensitization (widespread somatosensory sensitization) and the impaired endogenous descending pain inhibition (CPM) characterize canine patients with bone cancer pain similarly to people with severe chronic painful conditions.[7,8,28] This is the first report to characterize the nature of bone cancer pain in dogs and it suggests that neurophysiological pain assessment using standardized static and mostly dynamic QSTs are attractive to promote pain mechanisms-based analgesic therapy in veterinary patients.

2.3.6 Conflict of interest statement and Acknowledgements

There are no patents, products in development or marketed products to declare. We feel that our research brings new information to the cancer pain fundamental knowledge and has the potential to modify future endeavours in the research of treatment for cancer pain for veterinary patients. The authors declare to have no Competing Interests, but Dr Jeffrey Blair is currently working for the Vétoquinol SA funder of the study. This commercial affiliation does not alter our adherence to PLoS-One policies on sharing data and materials.

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3. General Discussion

Assessment of chronic pain in cats and dogs should aim at evaluating both the sensory- discriminative (spatiotemporal properties) and emotional (motivational-affective) aspects of pain. It would remain the cognitive-evaluative (central control) determinant of pain to be assessed,[1] but this would be quite challenging in animals. The sensory aspect of pain can be quantified using QST methodology which in turn helps to elucidate mechanisms (central control, in particular) of pain. At this time, QST assessment is only a reality in the research setting. Alternatively, in clinical practice, the veterinarian palpates areas of suspected pain and interprets the behavior response, which is quite subjective. The emotional aspect of pain can be thought of as the 'pain burden'. In other words, we are trying to assess how much pain affects the life of that animal including physical and mental states. For this, motor activity, force plate analysis, static weight bearing, as well as clinical metrology instruments (CMIs) and health-related quality of life questionnaires (HRQoL) are used in research. Assessment of motor activity, CMIs and HRQoL could and should be used in clinical practice. However, it is not known how well spread these tools are. This chapter will discuss the assessment of chronic pain in companion animals with greater emphasis on the use of QST in cats and dogs with spontaneous disease and their value as models for translational research.

3.1 Challenges of chronic pain assessment in cats

3.1.1 Assessing the somatosensory profile

The use of QST in cats is quite recent but it has provided enough information to demonstrate that cats with OA develop hyperalgesia, allodynia and facilitated temporal summation of pain. The first report of QST in cats used an electronic von Frey esthesiometer to test for punctate tactile hyperalgesia in the palmar and plantar aspect of cats' paws.[2] By comparing values from 39 cats with OA and 6 healthy cats, it showed that cats with OA had significantly lower values than heathy cats, and that the test had good repeatability over time.[2] They also identified a subpopulation of cats with OA that were classified as allodynic. Allodynia threshold was defined as 40g for the front paws and 50g for the hind paws, which was established based on the first quartile values of the OA cats. Hence, a cat was considered allodynic if at least one of its paws presented duplicate values under this fixed threshold. With these definitions, around 30% of cats were considered allodynic. Following this first period, cats with OA were administered meloxicam at 3 different doses for 4 weeks (0.025, 0.04 and 0.5 mg/kg, PO, every 24h). Interestingly, there were no significant improvements based on punctate tactile hyperalgesia in neither group.[2] By contrast, motor activity and kinetics improved under 0.025 and 0.05 mg/kg treatment, but not in the 0.04 mg/kg group which showed only a trend to improvement. The randomization was not controlled for QST-tested hypersensitivity and the group treated with 0.04 mg/kg had higher number of allodynic cats (42.5% of allodynic cats, compared to 27.5, and 17, in groups 0.025 and 0.05 mg/kg, respectively). Therefore, this makes sense if we think in terms of mechanism of pain. NSAIDs such as meloxicam are primarily peripherally-acting analgesics that reduce nociceptive input from the joint.[3,4] Thus, it provided local anti-inflammatory analgesia with consequent increase in activity (*i.e*. improved function). So, what explains the lack of effect based on von Frey esthesiometer? The von Frey was applied to the paws of osteoarthritic cats; thus, either distal or remote hyperalgesia was being evaluated if we consider that shoulders, elbow, hips and/or stifles were affected. If an improvement were to be seen with von Frey esthesiometer after meloxicam, it would indicate that the observed hyperalgesia was primarily due to peripheral sensitization. Since there were no improvements, particularly

including cats with allodynia, this indicates that the von Frey stimulation was performed in a referred area and cats were more likely to present with central sensitization. Although NSAIDs might have central effects,[5] they are not expected to reverse central sensitization.[6]

Following the previous study, those same authors set out to develop a dynamic QST protocol in an attempt to explore central pain processing. Again, by comparing 10 cats with OA and 4 healthy cats, they identified an optimal protocol to test mechanical temporal summation of pain. It showed that cats with OA respond earlier to the repetitive mechanical stimulus when compared with healthy cats (their RMTS was significantly and repetitively lower when compared with healthy cats).[7] They also showed that temporal summation was positively correlated with von Frey tactile threshold. Both these findings further support the idea that cats with OA are affected by central sensitization and that these tests are likely measuring the same phenomenon, at least partially. Presence of altered tactile threshold on more than one paw would favor the installation of a centralized sensitization. Addition of CPM (or DNIC) would complete the assessment of pain neurophysiology pathways, while it would allow to test the efficiency of descending inhibitory control system. Finally, in order to solidify this idea, we tested the responsiveness to treatment of these outcomes on centrally- or peripherally-acting analgesics. We designed a study in which tramadol (3 mg/kg PO every 12 hours) or placebo would be given to cats with OA for 19 days (Study I). We found that RMTS was improved in cats after tramadol but not after placebo.[8] Tramadol, in cats, likely produces most of its analgesic effects *via* activation of μ-opioid receptor and monoamine reuptake inhibition, hence primarily spinal mechanisms of action.[9] Based on this, and the observed response to tramadol, it indicates that these cats were indeed affected by central sensitization which was reversed, at least to some degree, by the drug. Another question arose from these findings. What if we combine NSAIDs and tramadol? A synergistic effect was to be expected. Thus, in our second study (Study II), cats with OA were randomized to receive meloxicam (0.05 mg/kg, oral transmucosal, every 24h) combined with either placebo or tramadol (3 mg/kg PO every 12 hours) for 25 days.[10] Mechanical temporal summation only improved in cats receiving the meloxicam-tramadol combination. Again, similar findings were observed in that treatment effect by RMTS

was only detected after tramadol, reiterating previous findings. The findings from these four studies provide evidence that cats with OA are affected by central sensitization which improves after tramadol, but not after meloxicam.

These previous studies had all been performed on laboratory cats affected by natural OA in a controlled environment. Those cats were acclimated to the facilities, personnel and tests for several weeks and its applicability in a clinical scenario was unknown. Most recently, a group from the UK evaluated the repeatability and ability of QST to differentiate healthy and osteoarthritic client-owned cats. Using 14 healthy and 7 osteoarthritic cats, they performed thermal (hot and cold) and tactile nociceptive stimuli using a temperature-controlled plate and manual and electronic von Frey esthesiometer, respectively.[11] All three QST were considered moderately repeatable. Although the interval between tests was generally of 2h for most cats, it greatly varied among 6 individuals from 4 to 26 weeks. The validity of these findings might be questionable as OA could have progressed over such periods. Thermal latency to cold stimulus and punctate tactile mechanical thresholds using the manual or electronic von Frey esthesiometer were different between osteoarthritic and healthy cats. No difference between these populations was found for thermal latency to hot stimulus.[11] Subsequently, another study investigated the feasibility of punctate tactile mechanical threshold using an electronic and a manual von Frey esthesiometer and compared the limits of agreement between the two.[12] It included 15 client-owned healthy cats that were evaluated twice with 1h interval by one examiner. The same protocol was repeated on the following day by a second examiner. Punctate tactile mechanical thresholds were applied to the upper lip and medial aspect of the stifle. These locations had not been explored before in cats. The study found that cats were much less cooperative on the second day and that the agreement between manual and electronic von Frey was fair. It should be noted that 4/15 cats were evaluated with the presence of the owners and were considered more cooperative. This might in fact add another confounding to the study.[12] Finally, a third study also evaluated punctate tactile mechanical thresholds in 13 healthy client- owned cats using an electronic von Frey esthesiometer and another algometer called SMALGO (Small Animal Algometer).[13] Inter-rater and inter-device reliability were studied. Stimulus was applied to the medial aspect of the stifle and the lumbosacral joint. Similar to the previous study,

cats seemed less cooperative on the second round of testing with consistently decreased thresholds. Inter-rater reliability was fair and inter-device reliability was good.[13]

The common observation of decreased cooperation with repeated testing is an interesting although not surprising observation. A visit to the veterinarian can be a stressful event to a cat,[14] and the constant manipulation and nociceptive testing likely surpassed the patience threshold of the individual. It even affected nociceptive testing resulting in decreased values. It is difficult to distinguish if values were lower because of simple lack of willingness to participate or decreased due to stress-induced analgesia. Cats in the research setting offer an obvious advantage from this aspect as they become quite accustomed to the testing protocol which is usually a minimally or stress-free activity in their day. One might suggest that this would be a better scenario to study pain mechanisms. This is especially true regarding temporal summation testing which might be quite challenging in clinical cats based on our observations of the cats' reactions on the first days of testing. Acclimation of cats to the temporal summation assessment is usually done over several (2-3) weeks and by the time the actual testing takes place, the cat had been in the testing cage at least 4-5 times. This might be a limitation of the applicability of this test in the clinic. By contrast, using client-owned cats does offer a few advantages. They share the same environment as owners and are ultimately the target population when investigating analgesics for the species. Both these factors are advantages for translatability and evidence- based practice, respectively. The use of von Frey and thermal testing seem to be feasible in client-owned cats and deserve more exploration as to their potential to detect treatment effects.

Our systematic review and meta-analyses described in chapter 1 included all of the above studies except for the two last which were published after the literature review. In general, the review found that studies were of high quality although they all had some degree of risk of bias. One disappointing finding that is not limited to QST research is the lack of use of reporting guidelines in half of the studies from the review and the two most recent studies. Reporting guidelines help to standardize and provide transparent reporting making it easier to compare studies and synthetize science. This topic will be further discussed below. Regardless, the main findings from our meta-analysis showed that 1) cats with OA have lower punctate tactile mechanical threshold and facilitated temporal

summation of pain when compared with healthy cats; 2) the effect of sex and body weight on sensory sensitivity remained inconclusive; and 3) due to the strong correlation between age and OA status, it remains difficult to assess the effect of OA on sensory sensitivity, independently of age. None of these findings are surprising and they similarly reflect what is reported in people with OA. Increased sensitivity to nociceptive testing as well as facilitated temporal summation of pain is reported in osteoarthritic human patients with strong level of evidence.[15–17] The effect of sex on OA is better elucidated in people and women are known to have increased sensitivity to pain when compared with men.[15,16,18] Although the evidence for the latter statement is overwhelming, controversies still remain as these differences could be related to the type of sensory stimulus used.[18] It is quite possible that female cats are similarly affected by increased sensory sensitivity. This will hopefully be clarified with large data from future studies using QST in cats. The relationship between body weight/size and sensory sensitivity is also unclear and controversial in the human literature[19,20] and deserves further exploration in human and veterinary medicine. Finally, the effect of age on OA is also quite controversial in people. While some studies state that pain sensitivity decreases with age, others state that it increases with age. Controversies seem to be related to the type of stimulus and anatomical location used for assessing sensory sensitivity.[21,22] As with any research question, high quality studies including large number of individuals will certainly help to elucidate these questions which remained unanswered.

3.1.2 Assessing the pain burden

The pain burden has been evaluated in cats using different outcome measures including kinetic gait analysis, motor activity, CMIs and HRQoL. The former two are used as objective measures of limb function and mobility and indirect markers of functional disability/ pain/ discomfort, whereas the latter two are used as subjective measures of pain and QoL.

Kinetic gait analysis provides information on the ground reaction forces produced during the gait cycle using force plates or pressure-sensitive walkways.[23] Several parameters can be measured such as mediolateral force, craniocaudal force and vertical force. The latter can be assessed using peak vertical force (PVF) and vertical impulse.

Assessment of PVF has been most widely reported in healthy and osteoarthritic cats.[2,7,8,10,24–27] A pilot study including 2 healthy and 4 osteoarthritic cats, and designed to describe structural changes associated with OA using magnetic resonance imaging (MRI) and computed radiographs, and to correlate these with PVF and motor activity, found that MRI scores of joint lesions were higher in cats with OA and that PVF was negatively correlated with MRI scores.[26] It was also found that PVF was positively correlated with motor activity indicating that these two measures are likely co-assessing physical function.[26] Different protocols for evaluating cats with OA have been evaluated and showed that data from the most affected limb (*i.e.* the limb yielding the lowest PVF value) should be used in order to limit the dispersion of the data. Ideally, testing should take place immediately following stair climbing exercise to optimize sample and effect sizes and preserve statistical power.[27] This protocol was subsequently reported in several studies.[2,7,8,10] One disadvantage of using only data from the most affected limb is the fact that data from fore- and hindlimbs are grouped together depending on which is the most affected limb of each cat, whereas it is known that cats bear more weight on forelimbs when compared with hindlimbs.[11,23,28] Hence, variability of the data might be increased as a result. In Study I, PVF was used to distinguish between healthy and osteoarthritic cats and to test for treatment efficacy.[8] Peak vertical force was higher in normal cats when compared with osteoarthritic cats demonstrating its ability to detect kinetic abnormalities induced by OA in cats. Similar findings had been previously reported.[7,8] Following treatment with tramadol or placebo, 5/7 and 0/6 cats with OA showed increases in PVF when compared with baseline values, respectively. This indicates that by centrally inhibiting pain, tramadol can improve mobility and function by decreasing lameness. The cat is less painful on its most affected limb and therefore, bears more weight on it. Improvements in function after tramadol have also been reported in people with OA.[29] In Study II, PVF increased over time in both groups with no difference between treatments (meloxicam-placebo and meloxicam-tramadol).[10] This indicates that NSAIDs also provide improvements in function by decreasing local joint inflammation, and that a potential additive effect by tramadol is not detectable. In other reports, PVF improved after meloxicam,[2] and it seemed to also improve over time with placebo treatment and physical exercise.[2,7] This brings an interesting point. Are the observed changes truly

related to the analgesic or to increase muscle mass and joint stability due to increased exercise? These cats required a minimum of 4-week training to learn how to walk over the mat on a straight line and constant speed which inherently increased their physical activity, which in turn, might have an effect detectable on PVF. There are no studies designed to answer this specific question and if one considers the low number of cats used in the aforementioned studies (< 12 cats/group), the level of confidence attached to these findings might not be that high. On this subject, a few studies looked into the feasibility and repeatability of PVF in untrained client-owned cats. In one study, 23 healthy untrained cats were encouraged to walk across a pressure-sensitive mat five times over a period of 30-45 minutes using positive reinforcement; 15/23 cats completed 3-5 analyzable walks.[28] In a different study, 14 healthy and 7 osteoarthritic untrained cats were similarly evaluated, although a maximum duration of the test was not reported.[11] Tests were repeated after 2h for most cats. It was found that PVF was lower in cats with OA for the hindlimbs on the first session only, and that PVF was not different for the forelimbs between healthy and osteoarthritic cats.[11] Those authors commented on the time-consuming characteristic of the test. Based on all these studies, a few conclusions can be made: 1) PVF testing requires considerable training. In untrained cats, it might not be feasible in close to half of them and the sensitivity of the test might be reduced;

2) PVF is generally able to differentiate healthy from osteoarthritic cats; and 3) PVF might be able to detect treatment efficacy from peripherally- and centrally-acting analgesics, although training and increased activity are possible confounding effects. Continued research including large numbers of untrained cats might help to elucidate this question.

Chronic joint pain is associated with decreased mobility in people, cats and dogs,[30–34] which implicates the need of objectively assessing mobility in affected patients. Accelerometers are the most common methods used to assess motor activity in cats.[7,8,10,35,36] They measure changes in acceleration by detecting low-frequency accelerations. Changes in acceleration (*i.e.* the amplitude of each count) are recorded and converted to 'activity counts' which is a unitless numerical value (from 0 to infinite) that is assessed every seconds to minutes depending on the epoch of device.[35,37] 'Activity counts' are considered arbitrary units since their measures vary depending on the device.

Actical®, the most commonly used device in veterinary medicine, uses digital integration for its measures in which 'activity counts' represent both the duration and intensity of acceleration.[37] Different devices might use time above threshold (the time acceleration exceeds a pre-set threshold) or zero crossing (the number of times acceleration crosses the no activity, or zero, point) to report as 'activity count'.[36] The device can be continuously attached to the collar of the cat remaining there for long periods and is generally well-accepted. Use of the device in the harness is also possible and data correlates well with collar-attached devices.[36] In fact, the latter seems to be more sensitive to eating and grooming which might increase activity counts. On the other hand, cats might take longer to adapt to harnesses than collars.[36] One of the early studies using accelerometer-based activity monitoring in cats found an overall correlation of 0.82 between data generated by the device and distance moved using simultaneous video analysis, indicating that activity monitors can be used as a surrogate measure of distance moved and activity in free-roaming cats.[36]

Activity monitors have been used as an important objective outcome measure for assessing analgesic treatments in cats with OA as well as in the validation process of CMIs.[2,24,38–41] Nevertheless, its ability to differentiate healthy from osteoarthritic cats is not evident. In Study I, night-time motor activity was not different between healthy and osteoarthritic cats,[8] which was in agreement,[2,7] or not,[26] with previous studies. Based on these findings motor activity seems to be a limited marker of disease in terms of sensitivity, despite the fact that decreased activity is a reported clinical sign in cats with OA. This lack of sensitivity might be related with the large individual variability in mobility among cats. Some being naturally less active or shyer than others. Cats included in these studies were laboratory cats housed together which could certainly affect their behavior due to dominances in the colony. In addition, only data from the night period without human interaction was used as this period showed increased activity of cats and increased ability to detect treatment effect in previous studies.[2,42] Another reason could be related to the approach to data analysis. In our studies, motor activity data was expressed as the average per-minute activity over that period which inherently omits a great deal of information from data distribution. Recently, actimetry data from 15 healthy and 83 osteoarthritic client-owned cats was evaluated using functional data analysis.[35]

Functional data analysis allows for the use of the entire profile of daily activity counts (over a 24-hour day), rather than summary values; hence, it avoids losing the richness of the information contained in the minute-by-minute counts and examines the dominant modes of variation of the data as a method for understanding the major sources of data variability.[35] In that study, although marked inter-cat variability was detected, osteoarthritic cats showed different patterns of activity from healthy cats. Activity and intensity were not always lower in osteoarthritic cats, but both the peaks and troughs of activity were less extreme than those of healthy cats. Interestingly, when only the mean value for each group was compared, no difference between them was found, highlighting the value of functional data analysis. That study also showed that cats exhibited a bimodal pattern of activity with a sharp peak in the morning and broader peak in the evening as well as differences between the activity pattern of cats during the weekdays and weekends.[35] These findings reflect the influence of human activity on the activity patterns of cats[43] as well as their normal behavior as diurnal or crepuscular, with peaks of activity at dawn and dusk.[44] Thus, the lack of difference between osteoarthritic and healthy cats in ours and other studies might be explained by 1) the approach to data analysis including use of average data and selection of night-time only data; 2) the fact that they are laboratory animals confined to a room in which many other cats also live; and 3) the relatively small number of animals in these studies and consequent reduced power of analysis.

By contrast with the lack of sensitivity to the presence or not of chronic pain, motor activity was able to detect treatment effect in our studies. In study I, night-time motor activity was significantly different between groups after treatment with tramadol or placebo, and it only increased in tramadol-treated cats when compared with baseline.[8] In study II, motor activity increased only after meloxicam-placebo but not after meloxicam-tramadol treatment.[10] Motor activity generally increased in other studies after treatment with meloxicam both in the research and clinical settings.[2,38,40,45] Considering the easiness with which this type of data is collected, its correlation with distance moved[36] and CMIS,[38,40] and its ability to detect treatment effects, and despite numerous possible influencing variables such as human interaction, housing type, access to outdoors, *etc.*, the use of motor activity seems to be a valid and applicable measure of activity in cats

with chronic pain. Nevertheless, clinical application will only be fully possible once reference ranges for what is normal or abnormal based on age and health status have been defined.

Clinical metrology instruments like pain scales and HRQoL questionnaires are a result of rigorous research to identify and validate key behaviors that are indicative of pain or QoL, respectively. Like all scientifically robust measures, these instruments undergo several steps of metrological validation to ensure that they measure what it is intended to measure (validity), produce consistent results when repeated over time (reliability), and can detect clinically relevant changes after administration of analgesics (sensitivity), among other criteria. Ideally, the instrument should be compared with a similar gold-standard tool, if one already exists.[46]

Clinical metrology instruments such as multidimensional pain scales take into consideration pain intensity and its impact in function. As previously discussed, there are currently three owner-based[38–40] and one veterinarian-based[41] CMIs that were developed and have been through various steps of validation for use in cats with OA. The veterinarian-based pain scale is the Montreal Instrument for Cat Arthritis Testing for use by veterinarians (MI- CAT(V)). The MI-CAT(V) was performed in the cats included in studies I and II, and this data was used as part of the validation process of the instrument demonstrating its ability to differentiate healthy from osteoarthritic cats.[41] Further validation performed with subsequent studies (unpublished confidential data, 2019) of a short-form of the MI-CAT(V) demonstrated its ability to detect treatment effects.

Assessment of QoL takes in consideration all aspects of a pet's life,[47] whereas HRQoL refers to the effect of a medical condition on the physical and emotional health of the individual.[48] Feline-specific HRQoL have been developed and partially validated for use in cats, which can be generic (*i.e.* used in any type of condition) or disease-specific. There are currently no HRQoL instrument developed specifically for OA. Nevertheless, most recently, a web-based generic HRQoL questionnaire (Vetmetrica) that measures the affective-motivational impact of chronic disease in cats has showed initial evidence to support its use in cats with OA. Not only it demonstrated ability to differentiate between healthy cats and those with chronic disease,[49] it was also able to differentiate between healthy cats, cats with mild and moderate/severe OA.[50] Thus, it

might be the first HRQoL tool applicable to cats with OA.

Health measurement scales such as CMIs and HRQoL are considered “living documents” as new studies help to further validate them by removing and/or adding new items. Thus, with the continuous study and validation of these scales, their use both in the research and clinical setting will help us understand the true burden of chronic pain in cats.

3.2 Challenges of chronic pain assessment in dogs

3.2.1 Assessing the somatosensory profile

The use of QST has been explored in dogs using primary nociceptive testing of tactile, mechanical, thermal and electrical modalities as well as CPM testing. A summary of these studies, their protocols and main findings are provided in Table XV. In fact, we are currently performing a systematic review and meta-analysis of studies using QST in healthy and osteoarthritic dogs, similar to our systematic review and meta-analysis in cats. Preliminary findings reveal that in general, static QST testing were repeatable and differentiated between healthy and osteoarthritic dogs, and that dogs with OA are affected by widespread increased sensory sensitivity.[51-64]

There is a potential to use mechanical and electrical QST to evaluate treatment effect of analgesics.[62– 64] Dynamic QST involving CPM testing is feasible in awake healthy dogs.[55] A recent study also reported use of CPM testing and temporal summation of pain in anesthetized dogs based on electrophysiological characteristics indicative of central sensitization.[65] Moreover, preliminary results from our review indicate lack of clear and transparent reporting and the use of reporting guidelines from a few articles similar to our findings in the systematic review and meta-analysis in cats. Uncertainties relating to whether the report adequately considered factors affecting scientific quality and risk of bias such as detection bias, phenotyping of patients, data collection and reporting also existed for some studies.

Despite this relatively extensive literature on the use of QST in healthy and osteoarthritic dogs, to our knowledge, there were no studies assessing QST standardized procedure in dogs with bone cancer pain. Thus, in study III, we aimed at evaluating primary and secondary hyperalgesia/ allodynia, brush allodynia and CPM in client-owned dogs that were healthy (n=7) or affected by spontaneous bone cancer (n=13) by means of a standardized QST protocol.[66] Repeatability for healthy dogs was good to excellent for primary tactile threshold, secondary mechanical threshold, brush allodynia and functional CPM rate, and fair for primary mechanical threshold and delta CPM. In dogs with OSA, repeatability was excellent for secondary mechanical threshold, moderate for primary

mechanical threshold, fair for primary tactile threshold and functional CPM, and poor for brush allodynia.[66]

When compared with studies evaluating healthy and osteoarthritic dogs, the repeatability of static QST was generally good with no difference between tests done a few hours to days apart,[51–57] whereas the repeatability of CPM testing in OA dogs has not been reported.[55]

In people, repeatability of static QST seems to be better than dynamic QST. This is likely explained by the complexity of the dynamic QST testing which is aiming to assess central processing and perhaps these phenomena are intrinsically more variable than primary nociceptive testing. For example, the repeatability of CPM was investigated in 42 healthy subjects on 3 occasions over 4 months.[67] Thermal (heat) and pressure pain threshold in the forearm and trapezius muscle, respectively, were used as the test stimulus. Immersion of the contralateral foot in cold water bath for 2 minutes was used as the conditioning stimulus. A significant linear decrease over time in CPM effect using both test stimuli was observed resulting in poor-to-fair reliability (ICC: 0.50 and 0.35 for heat and pressure pain threshold, respectively).[67] Another explanation for the low repeatability of CPM effect within and between studies is related to the vast array of testing protocols with differences in timing, stimulus modality, duration, intensity and location, which can all influence CPM responses. A recent systematic review of 10 studies performing CPM test in people showed that the most commonly used test stimulus was pressure pain threshold (n = 5), followed by contact heat pain (n = 3), whereas the most common conditioning stimulus was cold water immersion (n = 6) followed by water immersion (n = 3) and ischemic pain (n = 3).[68] In the latter review, the intrasession reliability of the CPM effect was good to excellent (ICC values ranging from 0.6 to > 0.75) in 3 studies, and the intersession reliability (retest intervals ranging from 2-28 days) was fair to excellent in 8 studies.[68] Poor intersession reliability was reported for the CPM effect in older adults with chronic pancreatitis and in young women across menstrual cycles.

Tableau XV. Summary of study characteristics and their main findings based on QST assessment in healthy and osteoarthritic dogs

Type of study	Population: N (male; female)	Test re-test interval	Intervention	QSTs [a]	Results based on QST assessment [a,b]	Reference
Repeatability	Healthy: 20 (11;9)	10 to 14 days (am and pm of each day)	N/A	1. Mechanical pressure	1. Significant effect of confounding variables (order, site, site order, time and day). Intact male dogs had higher thresholds than neutered males and spayed females	Coleman et al. 2014
	Healthy: 24 (12;12)	14 days	N/A	1. Punctate tactile 2. Mechanical pressure 3. Thermal latency (cold)	1. No inter-session difference 2. No inter-session difference. Significant positive correlations with body weight and age 3. No re-test done (lack of consistently quantifiable results)	Briley et al. 2014
	Healthy: 12 (7;5)	At least 15 min	N/A	1. Mechanical pressure	1. No significant effect of 'tip', 'rate', 'position' or 'site' on coefficient of variation. Wider tips were associated with higher thresholds. Thresholds increased with bodyweight and decreased with age	Harris et al. 2015
	Healthy: 25 (11;14)	7 days (2 or 3 visits)	N/A	1. Punctate tactile 2. Mechanical pressure 3. Thermal threshold (hot) 4. Thermal latency (cold)	1. No inter-session difference 2. No inter-session difference. Thresholds were lower in small dogs. Repeatability improved with operator experience 3. No inter-session difference. Thresholds were lower in small dogs and higher in young dogs 4. Higher percentage of response to cold stimulus in the last session	Sanchis-Mora et al. 2017
	Healthy: 16 (6; 10)	5 hours	N/A	1. Mechanical pressure 2. Thermal threshold (cold) 3. Electrical 4. DNIC	1. Replicate repeatability ranged from 26.8 to 88.4%. Values of the thoracic limbs were higher than pelvic limbs 2. Data could not be analyzed because results were inconsistent among dogs 3. Replicate repeatability ranged from 29.6 to 93.7%. No effect of anatomical site 4. Conditioning stimulus increased mechanical pressure demonstrating that the test is a valuable assessment of DNIC	Ruel et al. 2018

Case-control	Healthy: 15 (9;6) Natural OA: 11 (4;7)	N/A	N/A	1. Punctate tactile ɛrmal latency (cold)	1. No difference between healthy and OA dogs. Thresholds were lower in the ipsilateral than contralateral limb in OA dogs only. 2. OA dogs left the cold plate earlier and held their ipsilateral paw suspended for longer than healthy dogs	Brydges et al. 2012
	Healthy: 11 (6;5) Natural OA: 25 (13;12)	N/A	N/A	1. Punctate tactile 2. Mechanical pressure 3. Thermal latency (hot)	1. No difference between healthy and OA dogs 2. No difference between healthy and OA dogs 3. No difference between healthy and OA dogs	Freire et al. 2016
	Healthy: 23 (11;12) Natural OA: 31 (15;16)	N/A	N/A	1. Punctate tactile 2. Mechanical pressure 3. Thermal threshold (hot) 4. Thermal latency (cold)	1. OA dogs had lower thresholds at index joint and metatarsal site than healthy dogs 2. OA dogs had lower thresholds at index joint, tibial muscle and metatarsal site than healthy dogs 3. OA dogs had lower thresholds at index joint and metatarsal site than healthy dogs 4. OA dogs had lower threshold at index joint and metatarsal site than healthy dogs	Knazovicky et al. 2016
	Healthy: 28 (12; 16) Natural OA: 27 (10; 17)	N/A	N/A	1. Mechanical pressure	1. OA doges had lower thresholds in the stifle joint than healthy dogs. No differences were found in other anatomical locations	Harris et al. 2018
Repeatability and Case-control	Healthy: 14 (7;7) Natural OA: 7 (7;3)	2h to 26 weeks	N/A	1. Punctate tactile 2. Thermal latency (hot) 3. Thermal latency (cold)	1. No inter-session difference. OA cats had lower thresholds than healthy cats 2. No inter-session difference. No difference between healthy and OA cats 3. No inter-session difference. OA cats had lower frequency of thoracic paw lift than healthy cats	Addison & Clement 2017
	Healthy: 4 (3;1) Natural OA: 10 (5;5)	7 days	N/A	1. Punctate tactile 2. Mechanical temporal summation	1. No inter-session difference. OA cats had lower thresholds than healthy cats 2. No re-test done. OA cats supported lower number of stimulations than healthy cats	Guillot et al. 2014
	Healthy: 23 (not reported) Natural OA: 9 (not reported)	14 days	N/A	1. Thermal latency (hot)	1. No inter-session difference. OA dogs had longer thermal latency (took longer to lift paw off the plate) than healthy dogs	Williams et al. 2014

	Healthy: 23 (11;12) Natural OA: 31 (15;16)	7 days	N/A	1. Punctate tactile 2. Mechanical pressure 3. Thermal latency (hot) 4. Thermal latency (cold)	1. Thresholds were decreased in the re-test at the affected and metatarsal sites for OA dogs and at the metatarsal site for healthy dogs 2. No inter-session difference 3. Thresholds were increased in the re-test at the affected and tibial sites for OA dogs and at the tibial site for healthy dogs 4. No inter-session difference OBS: No difference between OA and healthy dogs for the replicate (test re-test) and site interaction effect on any of the QSTs	Knazovicky et al. 2017
Intervention trial	Natural OA: 44 (22;22)	N/A	Total hip replacement	1. Punctate tactile	1. Increased thresholds from baseline to 12 months post-operatively	Tomas et al. 2014
	Induced OA: 16 (not reported)	N/A	Tiludronic acid (2 mg/kg) or placebo SC on days 7, 21, 35 and 49 after cranial cruciate ligament transection	1. Electrical	1. Thresholds were lower at the affected site on days 28 and 56 post OA-induction in placebo-treated dogs and on day 28 in tiludronate-treated dogs as well as at a remote site on day 56 in placebo-treated dogs	Rialland et al. 2014
	Natural musculoske-letal disease: 64 (34;30)	N/A	Exercise restriction alone or with combined acupuncture and manual therapy or no treatment for 28 days	1. Mechanical pressure	1. Thresholds were higher over time in dogs treated with exercise restriction alone or with combined acupuncture and manual therapy. Such effect was not observed in dogs receiving no treatment	Lane et al. 2015

Finally, other aspects that can influence CPM/DNIC effects are attention and expectations.[69-71] For example, previous studies performed in young adults showed that a priori and manipulated expectations can enhance or block CPM analgesia. In other words, if the participants were told that pain would be decreased after the conditioning stimulus, pain ratings of the test stimulus were generally decreased. If they were told that pain would increase after conditioning stimulus, pain ratings would generally increase.[70,71] Taken together, findings from previous studies suggest that the reliability of CPM can vary due to numerous factors, some of which are difficult to control such as expectation and physiological variability of pain modulation over time (related or not to menstrual cycle in women). Other factors such as methodology of testing protocols can be manipulated. Indeed, the summary of a consensus meeting of researchers interested in CPM has been recently published to highlight the lack of uniform protocols for performing CPM which hinders our ability to compare and combine data from various studies performed in different laboratories and clinics as well as to offer suggestions of preferred methodologies and reporting standards.

Using a standardized QST protocol in study III, all tests were significantly different between healthy dogs and those with OSA, with exception of punctate tactile threshold (but close to significance, probably only a type-II statistical error), conferring construct validity of the protocol.[66] Despite not being significantly different between the two populations, the mean values of punctate tactile threshold were higher in healthy dogs (298 grams) than dogs with OSA (198 grams); yet the variability of the data was extremally large. Moreover, when additional analyses were done with historical controls, a difference was found.[66] We suspect that this is simply a lack of power from the study, particularly if one considers the difference observed with all other QST.

The use of brush allodynia was reported in our study for the first time in veterinary medicine, so it is hard to compare with other species. There is an intrinsic subjectivity with this test. By observing a response, how can we be sure that it relates to allodynia and not simply to the fact that the animal does not want to be brushed? To facilitate the interpretation of this test, we used it as a dichotomous variable in which ‘yes’ would be given if any response was observed after brushing for up to 3 times; otherwise ‘no’ would be given. In addition, since all other QST were pointing to the same direction (i.e. increased sensory sensitivity) and because none of the healthy dogs responded to brushing, we are confident that this test deserves further

exploring.

Testing the function of DNIC/CPM in dogs is quite recent in veterinary medicine. Before our publication there was a single study that had reported it in healthy dogs.[55] In that study, a feasibility score using a scale from 0 to 2 was given for each behavioral response of QST testing. A score of 0 was given when there was a lack of cooperation or when the observer lacked confidence in the data collected. A score of 2 corresponded to a combination of a clear response to the stimulus and strong confidence in the data collected. Concerning CPM testing, a difference between the test stimulus before and after the conditioned stimulus was detected only when responses receiving a score of 2 were used in the analysis.[55] If all CPM tests were to be included in data analysis (*i.e*. scores 0, 1 and 2), a CPM effect would not have been detected. In our study, a difference between the test stimulus before and after conditions stimulus was clear in healthy dogs, and this delta (after minus before) was significantly different between healthy and affected dogs. The protocols for CPM testing between theirs and our study were slightly different. For example, for the conditioning stimulus, Ruel et al.[55] used the same cuff with the same pressure, but pressure was maintained for 1 minute and the test stimulus performed after 3 minutes after cuff release, whereas in our study, pressure was maintained for 2 minutes while the dog was encouraged to move around, and the test stimulus was performed immediately after cuff release. It is possible that these minor differences in protocol activate the descending modulating systems differently. There is a clear relationship between the intensity of the conditioning stimulus and the strength of the resultant CPM.[72] Ischemia predominantly activates C-fiber following arterial occlusion with the inflated tourniquet cuff and evokes pain because of tissue metabolic changes including decreased PO_2 and increased PCO_2, lowered pH, decreased glycemia and increased lactate and potassium levels.[73,74] Thus is possible that by simply doubling the duration of the conditioning stimulus from 1 to 2 minutes and encouraging the dogs to walk around the room, we were able to activate the CPM effect more intensely. Another point likely to be affected by CPM methodology is the magnitude of CPM effect. The magnitude of CPM effect in people is approximately 29% (thus, a 29% increase in nociceptive threshold measured by the test stimulus after the conditioning stimulus), ranging from 10% to 55%.[75] If we consider our results in terms of magnitude of CPM effect, healthy dogs would have a CPM effect of 6.7% whereas dogs with OSA would have -7.6%, thus a negative CPM effect. From Ruel et al.[55], if we consider only data receiving a score of 2, this magnitude is

around 19.2%. Despite differences between these studies and a somewhat still questionable regard towards CPM testing in dogs, this approach does seem promising in veterinary medicine. It definitely shows a CPM effect in healthy dogs and an absent CPM effect in dogs with severe bone cancer pain. All that CPM testing requires is a pressure cuff which every practice already has and an algometer/esthesiometer which can be quite inexpensive considering medical equipment. It does not require any training for the dogs, and it could and should be incorporated into clinical practice in the future. The literature on CPM testing in dogs is quite young, yet further research will hopefully provide the level of scientific quality seen in people and help to guide therapy on a mechanism-based approach.

A palliative step-wise analgesic protocol poorly improved pain in dogs with OSA as measured by QST. The only observed effect was on functional CPM at day 14 (*i.e.* the frequency of dogs with a positive CPM delta after 14 days of cimicoxib treatment).[66] Considering the lack of effect for all other QST, we could question the real sensitivity of QST to detect a treatment effect. It might be suspected that the effect on functional CPM rate is not real and a consequence of a type I error, particularly because the treatment administered was a NSAID. However, it is also possible that the level of pain associated to OSA to be too elevated (as detected on visual analog scales and HRQoL), and then difficult to counteract with pharmacological treatment. Therefore, the functional CPM rate could be indeed a more sensitive outcome in all QST tested, and the fact that the brush allodynia decreased from 45.5% to 27.3% after one week of amitriptyline treatment is also interesting.

Our results question on the data interpretation for neurophysiological mechanisms of pain. It would be expected that primary tactile mechanical thresholds would increase with the administration of cimicoxib with the expected decrease in local inflammation. The centralized sensitization could have been so intense in OSA-associated pain that such effect was not apparent. As expected, brush allodynia and CPM effect would improve with the addition of amitriptyline to cimicoxib due to their mechanisms of action of reinforcing the inhibitory system and decreasing pain facilitation.[76] Moreover, visual analog scales (owner and oncologist), and HRQoL were not sensitive to treatment; yet, actimetry used to assess quality of sleep and mobility were. Surprisingly, the addition of gabapentin did result in a deterioration (albeit not significant) in all QST assessments, except primary tactile threshold. Antidepressants such as amitriptyline act by recruiting secondary downstream mechanisms as well as long-term

molecular and neuronal plasticity, whereas gabapentinoids such as gabapentin act by decreasing excitatory transmitter release and spinal sensitization.[76] Objective and subjective outcome measures worsened in the last assessment when the three drugs were onboard (cimicoxib, amitriptyline and gabapentin). Several reasons might explain this lack of effect including duration of treatment, severity of bone cancer pain, disease progression, sample size, *etc*. However, based on the fact that dogs were euthanized at median of 15 days after the end of the study due to disease progression, it might be speculated that the proposed protocol is simply not strong enough to counteract all of the pain mechanisms involved with bone cancer pain as previously described. In people, the clinical management of bone cancer pain is similarly inadequate.[77] The stepwise guidelines from the World Health Organization for cancer pain relief outline a treatment progression from non-opioid analgesics through strong opioids with adjuvant supplementation such as bisphosphonates and local radiotherapy to treat progressively worsening pain.[78] Despite these recommendations and the use of opioids, analgesic efficacy is limited to nearly 42% of patients.[79]

3.2.2. Assessing the pain burden

The pain burden in dogs can be assessed using similar objective and subjective outcome measures as used in cats. In study III, it was assessed using asymmetry index measured by static weight bearing, motor activity, scores of pain based on visual analog scales completed by the owner and the veterinary oncologist as well as scores of HRQoL. Except for asymmetry index which was done in healthy and affected dogs, these measures were only performed in dogs with bone cancer pain before and after treatment.

Assessment of function was performed using asymmetry index and motor activity. The former is much more limited as it assesses a single moment of the dog stepping over a pressure- sensitive platform (*i.e*. static weight bearing), whereas the latter can produce large quantities of data over long periods of time that can be approached in different ways. Assessment of asymmetry index showed that dogs with bone cancer tend to bear 30% less weight on the affected limb, whereas in dogs with OA, this contralateral compensation is less pronounced (around 16-20%).[80] In healthy dogs, no compensation should exist, and in fact asymmetry index in this population was 6% in our study, which must be considered as a normal noise variability in measurement. Asymmetry index did not change after treatment when

compared with baseline values. By contrast, motor activity was really the only outcome measure that improved in study III after a step-wise palliative analgesic protocol. Motor activity, its advantages and disadvantages have been previously discussed, and the literature is quite rich for this outcome in dogs.[25,34,63,81-83] Nevertheless, a different approach was used in study III as we attempted to evaluate the 'most' and 'least' active periods based on the premise that the 'most' active periods reflects true voluntary activity and the 'least' active periods reflect resting/sleeping periods in which little or no activity should be observed. Activity during the 'least' active period is considered restlessness likely related to pain and constant changing of position while trying to rest/sleep.[83] Based on this approach to the data, we observed increased activity for the 'most' active periods and decreased activity for the 'least' activity periods at all time-points after treatment when compared with baseline. These observations were interpreted as improved physical function and improved sleep quality, indicating at least to some degree, an analgesic effect from the pharmacotherapy utilized. Indeed, motor activity is considered a valid outcome assessment tool for documenting improved activity associated with treatment. For example, increased activity has been observed in dogs with OA after 21 days of carprofen administration.[84]

The HRQoL questionnaire used in study III was developed in French language based on previously published instruments. This questionnaire was not able to detect treatment efficacy in dogs with OSA and its validity for responsiveness remains unknown. Moreover, since we did not perform this questionnaire in healthy dogs to compare with dogs with OSA, construct validity also remains unknown. In retrospect, a better approach might have been to proceed with the translation of the CBPI to French because the CBPI had already been validated for use in dogs with bone cancer pain. The procedure of translation of a tool requires several steps of translation and back-translation in order to guarantee sematic and cultural equivalence.[85,86] Subsequently, the new CBPI in French language should be submitted by the same validation processes that the original version in English. For example, it should be tested in healthy dogs and in dogs with OSA as well as in dogs with OSA undergoing treatment to test for its construct validity and responsiveness. Other steps of validation would include testing the repeatability of the instrument over time and within different observers. The lack of treatment efficacy based on the HRQoL was not surprising due to the severity of pain in bone cancer. In people, bone cancer pain causes excruciating pain with negative impact in QoL and components

of neuropathic pain. It is often refractory to orally administered analgesics and challenging to manage in the clinical setting.[77,87,88]

Some therapies other than chemotherapy and radiation therapy have been investigated for their potential as analgesics in dogs with bone cancer by means of CMIs. Bisphosphonates are drugs that specifically inhibit osteoclastic activity and are considered standard therapy for various disease conditions of bone resorption in people including bone cancer for which they are first-line therapies.[89] In dogs with bone cancer, monthly intravenous administration of bisphosphonates seem to be a good option, particularly if combined with orally administered analgesics.[90-92] In two different studies, pamidronate was administered as a single-agent to dogs with OSA. It resulted in increased relative primary tumor bone mineral density,[90,91] and reduction of pain was observed in 12/43[90] and 4/10[91] dogs. It should be noted that when those studies were performed the use of validated CMI was not widespread and those authors utilized a 'cumulative pain index score' which is a non-validated CMI assessing painful vocalization, degree of lameness and changes in activity levels.[90,91] Multiple administrations of zoledronate to dogs with OSA also resulted in increased relative primary tumor bone mineral density. In 5/10 dogs, suppression of pathologic bone resorption was accompanied by subjective pain alleviation as assessed by pet owner perceived limb usage.[92] Intrathecal administration of resiniferatoxin and substance-P saporin have also been reported to provide analgesia based on CMIs;[93-95] however these treatments are not available for use in veterinary clinical practice. Finally, dogs with OSA were found to express and secret NGF.[96] Thus, therapies targeting NGF such as the canine specific anti-NGF antibodies might provide analgesia in bone cancer pain as it has been observed in dogs with OA.[97,98]

In conclusion, the proposed analgesic management in study III does very little for the dogs with the only real improvement documented by motor activity, allowing some concurrent validity of most promising dynamic QST assessment, such as functional CPM rate and brush allodynia. Cancer therapies such as chemotherapy, radiotherapy and amputation and adjuvant therapies such as bisphosphonates, should be considered.

3.3 Translational pain research

Animal models of pain have unquestionably contributed to our understanding of pathophysiology of pain, Yet, preclinical research with rodents is highly focused on the somatosensory component of pain with few possibilities to assess the pain burden in these animals. Hence, the predictive value of animal models in clinical efficacy of analgesics in humans is still very limited.[99–105] In fact, the likelihood of approval' (*i.e*. probability of reaching Food and Drug Administration approval from the current phase) from phase I clinical studies for analgesics is only 10.7%.[106] Changes into how preclinical research is designed, conducted and reported may improve translation to the changing landscape of clinical pain.[100]

3.3.1 Animal models

Animal models are comprised of three main components, the subject, the assay and the measure, all of which can greatly vary among different investigations and influence the outcome of the study. These are explained below:[103-105,107]

1) Subjects refers to the species, strain, genetic background, sex and age, as well as husbandry and manipulation procedures.

2) Assay refers to the etiology of the noxious model such as nociceptive (thermal, mechanical, chemical, electrical), inflammatory (algogen, sensitizing compound, inflammatory mediator) and neuropathic (surgical, chemical), as well as the body part (cutaneous, muscular, visceral).

3) Measure refers to the outcome including reflex hypersensitivity, spontaneous (avoidance behavior, gait, posture), operant (learned escape) and pain-affected behaviors (anxiety, attention, disability, social sleep).

3.3.1.1 Animal models of osteoarthritis (OA)-related pain

Animal models of OA can be spontaneous (naturally occurring, genetic models of disease) or induced (surgery, intra-articular chemical injection). Naturally occurring disease models mirror primary OA in humans; it is slowly progressing, and clinical symptoms can be variable and generally occur late in the disease process.[108] Genetically modified strains of

mice as models of OA are likely over simplistic as OA probably reflects the effects of many genes in addition to metabolic and environmental components. Induced disease is commonly achieved by surgery with the goal of causing joint instability, altered joint mechanics and inflammation to induce OA lesions. The progression of OA occurs rapidly which reflects posttraumatic OA more closely than primary OA (genetic, metabolic or aging-related phenotypes). Examples include transection of the (anterior or posterior) cranial or caudal cruciate ligament, meniscectomy, meniscotomy or destabilization of the medial meniscus, and medial or lateral collateral ligament transection.[109] Induced disease by intra-articular chemical injection causes widespread cell death or suffering and rapid joint destruction. Examples include monosodium iodoacetate, collagenase, carragheenin and Freund adjuvant. Rodents (mice, rats and rabbits) are normally used for investigations of basic pathophysiology and early screening of therapeutic interventions, whereas dogs are generally used for verification of findings from rodent models prior to human trials.[108]

3.3.1.2 Animal models of bone cancer pain

Prior to the years 2000, previous models involved the direct injection of cancer cells into the intramedullary cavity of the tibia or femur of mice.[110] However, since tumor cells could not be properly sealed into the medulla, tumors could develop in the surrounding tissues resulting in a large and highly variable extra-skeletal tumor mass.[111] More recently, models evolved with the injection of fibrosarcoma cells into the mouse femur followed by a seal using dental amalgam. This model produces a tumor that develops from the medullary cavity similar to spontaneous bone tumors. Thus, it allows for reproducible tumor development in the femur and the assessment of pain behaviors and molecular mechanisms involved in bone cancer pain.[112] This methodology is now applied to other bones using different cancer cell types including fibrosarcoma melanomas and mammary gland carcinomas in mice and rats.[111] There is now a diverse array of animal models of bone cancer pain using different host species, sites of injection, and cancer cell tissues of origin. All these variations sum up to at least 38 different models of bone cancer pain which were identified through a systematic review and meta- analysis in 2013.[113] They have allowed for the study of several pathophysiological mechanisms involved with tumor growth and pain generation and maintenance.

3.3.2 The issue of translation

Animal models of pain and the use of QST have certainly contributed to our understanding of nociception and pain mechanisms involved in the pathophysiology of chronic pain syndromes such as OA-related pain, bone cancer pain and neuropathic pain.[100] Despite unquestionable contributions and vast research in the field, there has been limited success in translating basic science data to develop truly novel analgesics with proven efficacy and acceptable safety profile in humans to a point that it is now widely accepted that preclinical pain models do not properly predict the clinical efficacy of analgesics in humans.[102,103,107] Failures are normally related to a lack of translation in efficacy and safety profile from animal models to humans. A classic example is the NK-1 receptor antagonist for substance P. In preclinical studies, this class of drugs had been shown to attenuate nociceptive responses in subjects with central sensitization secondary to inflammation or nerve damage.[114] Nevertheless, in human trials, it failed to exhibit efficacy in a variety of clinical pain states.[114] The lack of translation was later explained by differences in the physiology of substance P among species, as well as differences between clinical pain and the experimental noxious stimulus.[114] The reasons for the lack in translation from animal models to humans are certainly multifactorial. Although limitations of current animal models including the subject, assay and measures seem to play a major role, questionable scientific quality of published literature certainly has part of the blame. These will be briefly discussed herein.

Subjects: Animals used in preclinical pain research are generally young adult, male, healthy mice and rats often genetically identical, which clearly do not reflect the true population normally affected by chronic pain such as older adults with possible concomitant diseases and with a higher prevalence of female individuals.[115] Indeed, qualitative sex differences in pain and analgesic sensitivity have been reported and now it is widely accepted that female rats and mice are generally more sensitive than males;[18] both sexes should be included in animal models. Interestingly, husbandry and how the animals are manipulated can also influence studies' findings. Modulation of pain sensitivity by "empathy" has been reported in mice that had visual contact with cage mates.[116] When familiar mice were given noxious stimuli of different intensities, their pain behavior was influenced by the cage mate's status (painful or not).[116] Thus, social factors need to be taken in consideration. Another

aspect that contributes to the lack of translation of analgesic efficacy from rodents to clinical research pertains to differences in pharmacokinetics between species.[117] For example, most analgesics require higher doses to be efficacious in animals when compared to humans.[117]

Assay: It has been suggested that the limitations and unsuitability of experimental models is also a result from the lack of interaction between clinicians and basic scientists and lack of pathophysiological studies performed on patients with clinical pain.[100,102,118] There is a clear mismatch of lesions performed in animal models and diseases studies in clinical research. For example, peripheral nerve injury models are considered the "gold standard" for the pre- clinical assessment of new drug therapies for neuropathic pain. Nevertheless, only a small part of clinical research involves patients with peripheral nerve injury.[102]

Measures: Most studies using animal models predominantly evaluate nociceptive threshold testing from evoked pain as the main outcome used to triage analgesics.[100,107] A few problems result from this approach. First, these tests are generally activating a singular nociceptive modality which is quite different from clinical pain syndromes.[118] Evoked withdrawal responses measure not pain itself but rather the hypersensitivity (allodynia and hyperalgesia) that often accompanies pain.[107] Clinical pain syndromes present with continuous or paroxysmal spontaneous pain which are mediated by pathophysiological mechanisms different from those that mediate hypersensitivity states measured by evoked pain.[107] Second, there is no way to characterize the quality (shooting, stabbing, lancing) or intensity of pain. Similarly, sensory loss is quite challenging to be detected in animals and have not been reported in rodents.[119] Third, endpoints such as latency of hind-paw withdrawal or intensity of mechanical stimulus resulting in withdrawal are applied as measures of treatment effect in chronic pain states irrespectively of underlying differences in pathophysiology.[120] This is particularly relevant if one considers that dynamic allodynia (*e.g*. clothing brushing against the skin), rather than static allodynia (*e.g*. nociceptive testing with von Frey esthesiometer) is usually the symptomatology presented by patients.[107] Fourth, considering that chronic pain syndromes result in dramatic impacts in QoL including sociability, motivation, sleep quality, *etc*., this sphere of pain has to be somehow included in pain assessment.[121] For this, there has been an increasing focus to develop animal models that, in addition to reflex testing, investigate pain-related behaviors such as operant

responses which evoke cerebral processing and are more likely to reflect clinical pain.[118]

Reporting quality of published literature: There is clearly a lack of published negative data both from animal studies and clinical trials in which only true positive studies tend to be published. Thus, it is difficult to assess if animal models can detect false positives or false negatives because these studies are never made available to the scientific community.[100] In addition, there is growing concern that poor experimental design and lack of transparent reporting contribute to the frequent failure of preclinical animal studies to translate into treatments for human disease.[113] To help improve reporting standards, the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were developed consisting of a 20 items checklist including the key information that should be available in a manuscript to ensure that a study can be reviewed, scrutinized and reproduced.[122] Despite the fact that these guidelines were endorsed by several highly-respected funding agencies and scientific journals, very little improvement in reporting standards were noted.[123–125] A recent systematic review including 41 studies using animal experimental models undergoing a treatment intervention for rheumatology investigated the scientific quality of these studies using the ARRIVE guidelines. The systematic review found poor reporting of key study design principles in the majority of studies including unclear or no randomization (83%) and lack of sample size calculation or allocation method (100%).[126] Another systematic review and meta-analysis of studies of animal models of bone cancer pain found that studies reporting measures to reduce bias reported smaller differences in behavioral outcomes between tumor-bearing and control animals, and that studies presenting a statement of conflict of interest reported larger differences in behavioral outcomes.[113] Finally, a recent review assessed the influence of the endorsement or not of the ARRIVE guidelines by journals dedicated to laboratory animals, animal behavior and animal welfare.[125] Those authors found that journal support for the ARRIVE guidelines did not result in a meaningful improvement in reporting quality, contributing to ongoing waste in animal research.[125] Such results, as well as those observed in our systematic review of QST in OA cat pain are appealing for a critical revision of the ARRIVE guidelines.

3.3.3 New perspectives in pain research

The refinement of animal models to the most predictive of clinical translation is one of the current focus in pain research. More useful and sophisticated animal models are being developed to contribute to the advance of both preclinical and clinical research. The accuracy and relevance of data can be improved by choosing subjects and conditions that have more in common with the target population, epidemiologically and contextually, including both sexes, multiple strains, older animals, and with attention to manipulation and social factors.[105] Ideally, animal models should closely match the disease of clinical interest. With this in mind, animal models of naturally occurring disease have been gaining increased attention from the pain community due to several advantages including the benefit for both animals and humans, the practice of the 3Rs (Replacement, Reduction and Refinement), in addition to greater applicability of animal research to humans.[99,101,127] They provide potential advantages in all three components of animal models, although questions pertaining to scientific quality also exist in the veterinary literature.

Subjects: The pet population presented to the veterinary clinic with chronic pain is generally of older age and of both sexes, although females might be overrepresented as seen in our systematic review and meta-analysis, and with large genetic variability.[31,60] They can present with high prevalence of obesity and sedentarism and be affected by several co-morbidities.[128] They have distinct lifestyles, including the food they eat, the environment they live in, the pattern of physical activity and socialization, *etc*. Obvious differences set aside, the clinical scenario described above reflects in most aspects, the clinical scenario a human practitioner faces in terms of the affected population. Hence, pet-animals with natural disease represent obvious advantages over induced models (Tables XVI and XVII).

Table XVI. Comparison among spontaneous and induced models of osteoarthritis (OA) in cats in terms of to their potential for translational research.

Based on published literature. [99,101,108,129-134]

Disease model	Advantages	Disadvantages
Spontaneous OA (client-owned)	• Mimics primary OA • Microscopically, macroscopically, physiologically and symptomatically analogous to the human condition • Generally associated with similar risk factors (age, obesity, sedentarism) • Similar sensory profile when compared with people with OA (mechanical hyperalgesia and allodynia; facilitated temporal summation of pain) • Similar brain-imaging characteristics when compared with people with OA • Documented altered kinetics • Allows for assessment of quality of life, pain severity and interference by proxy using clinical metrology instruments • Similar clinical symptomatology and therapeutic management focused on pain and function • High prevalence: affects more than 90% of geriatric cats with half of them showing clinical signs • Possibility to recruit research cats with naturally-occurring OA and bypass the difficulties associated with clinical trials involving client-owned cats • Opportunity of high standard of care to enrolled subject	• Usually more expensive and time-consuming than induced models • Subjects are recruited from a pool of potential candidates and their availability depends upon their caretaker's availability and willingness to participate • Presence of concomitant diseases* • Greater variability including genetic background, food and water intake, exposition to environmental toxins/allergens* • Requires large number of subjects to achieve an appropriately powered study design (due to large variability)

Sodium urate synovitis (purpose-bred)	• Chemically-induced • Mimics acute OA • Rapid induction • Joint changes consistent with acute inflammation • Documented altered kinetics for a few days, returning to normal afterwards* • Subjects are easily recruited from research facilities and are always available for assessment • Uniform population* • Allows for euthanasia and collection of post-mortem tissue samples • Time for study completion is dictated by the researchers • Reversible: disappearance of pain-related behaviors after a few days minimizing suffering	• Requires intervention to induce disease • Healthy animals with no concomitant disease* • Initiating event, disease mechanism and many of the pathological joint changes differs from primary OA • Unknown sensory profile • Translation of therapeutic outcomes from induced models cannot be assumed • Single joint OA* • Raises ethical concerns
Anterior cruciate ligament transection (purpose-bred)	• Surgically-induced • Mimics post-traumatic (secondary) OA in the early period (after 4-5 months) and definite OA at long-term (after 26 months) • Similar joint changes with joint space narrowing, cartilage and subchondral degradation and osteophyte formation • Documented altered kinetics between 1-18 weeks, returning to near normal afterwards • Subjects are easily recruited from research facilities and are always available for assessment • Uniform population* • Allows for euthanasia and collection of post-mortem tissue samples • Allows to study the effect of joint instability • Time for study completion is dictated by the researchers	• Requires intervention to induce disease • Healthy animals with no concomitant disease* • Requires months to years for development of definite OA • Unknown sensory profile • Single joint OA* • Raises ethical concerns

*Might be considered an advantage or disadvantage depending on the perspective.

Table XVII. Particularities of rodents (rats and mice) and cats as animal models of osteoarthritis (OA) research.

Based on published literature.[100,135-142]

Particularities	Rats and mice	Cats
Enhanced brain imaging in chronic pain states	• Periaqueductal gray • Amygdala • Thalamus • Primary and secondary somatosensory cortex • Cingulate cortex	• Periaqueductal gray • Thalamus • Secondary somatosensory cortex
Pain assessment	• Reflective measures using stimulus-evoked responses is most predominantly used • Spontaneous measures: kinetics, kinematic and spontaneous activity; and pain-induced behaviors such as liking, biting, vocalization.	• Evoked pain • Spontaneous measures are widely used: kinetics, level of activity, behavioral assessments including quality of life assessment by proxy
Pharmacology	• In general, require higher doses of analgesics when compared with people (especially mice) • Highly efficient in acetylation (rats) • Good function of biliary excretion (rats and mice) • 'Fu-corrected intercept method': a scaling system that uses the allometric exponent of clearance and the ration of fu between rats and humans is available	• Deficient hepatic glucuronidation due to mutation in the UGT1A6 gene • Intermediate function of biliary excretion • No scaling methods between cats and humans available
Effect of obesity in OA	• High fat diet induced OA changes such as increased proteoglycan loss, decreased musculoskeletal performance	• Undocumented relationship between obesity and joint changes, although clinical observations are evident

Assays: Chronic painful syndromes such as OA, bone cancer pain, intervertebral disc disease, diabetes-induced neuropathy, idiopathic cystitis, orofacial pain syndrome, among others, spontaneously occur in companion animal patients with strikingly similar pathophysiological mechanisms as observed in people.[101,108,143-146] Companion animals often share the human environment, exposing them to similar epigenetic, and other risk and disease-modulating, factors.[101] Progression of disease occurs over long periods of time and companion animals often live to old age, permitting the study of the effects of such factors over the long term, as well as of the secondary effects of pain or painful disease over time.[101] These naturally occurring painful disease 'models' may better reflect the complex genetic, environmental, temporal and physiological influences present in humans.[99,101]

Measures: Pain assessment in companion animals involves assessment of both somato-sensory and emotional-affective aspects of pain and a lot more can be assessed in pet animals when compared with rodents in terms of pain burden. Both static and dynamic QST testing have been developed and validated for use in dogs and cats allowing for the assessment of nociceptive thresholds and central processing.[2,7,55,60,66] In people, chronic pain causes mood changes, social isolation, decreased activity, and several negative mental states.[121,147] Similar symptomatology is observed in companion animals, and assessment of QoL and welfare including activity, sociability, self-care, motivation, mood, *etc*., are performed using validated CMIs and HRQoL questionnaires.[41,45,148,149] The ability to assess Qol in pet animals is a huge advantage. These instruments are completed by a proxy (*i.e*. the caretaker), similar to the situation in pediatric patients.[95] Chronic pain also affects the quality of sleep in people and painful OA is often associated with restlessness.[117,150,151] Sleep disturbances can be assessed in animals using the aforementioned CMIs and HRQol as well as using objective assessments such as accelerometer- based activity monitors. Preliminary findings in dogs with OA and bone cancer have showed promising results in this respect.[66,79] Furthermore, mobility is used as a measure of health and wellbeing in people[151] and patients with OA present with limited physical function.[152] Mobility impairment is also a feature of dogs and cats with OA which improves with the administration of analgesics. The use of accelerometer-based activity monitors have been validated in dogs and cats representing an objective measure of mobility, activity and function.[25,35]

Reporting quality of published literature: The questionable scientific quality and reporting standards of studies involving animal models compromises and limits the interpretation that can be drawn from the study's findings and prevents peers from replicating and building on those findings.[100,103] Unfortunately, this problematic is not limited to preclinical studies involving rodents. In our systematic reviews and meta-analysis of QST in dogs and cats (unpublished data), the use of reporting guidelines was reported in only 3/6 and 1/14 articles involving cats and dogs, respectively. This was reflected by unclear reporting of inclusion and exclusion criteria, lack of descriptive information from included animals, and even lack of clear description of hypotheses and objectives. Power calculation was reported in 1/6 and 3/14 studies with cats and dogs, respectively. Uncertainties relating to whether the report adequately considered factors affecting scientific quality and risk of bias such as detection bias, phenotyping of patients, data collection and reporting existed for some studies. Finally, assessment and risk of bias ranged from 65-90% and 10-40% in cat's studies, and from 75-100% and 10-70% in dog's studies, respectively. Thus, improvements in the reporting standards of veterinary literature are required. It has been suggested that the issue is that the use of reporting guidelines such as ARRIVE need actually to be enforced by journals and manuscripts should be submitted with the actual checklist indicating in which page that specific information is reported. For example, this approach has resulted in substantial improvement in the reporting of risks of bias in *in vivo* research published in Nature journals.[153] A change in editorial policy from veterinary journals might yield similar results. Improving experimental design, study quality and reporting of veterinary studies are warranted in order to guarantee both the practice of evidence-based medicine in companion animals and enhance translatability to human studies.[99,103]

Several advantages exist when using veterinary patients with spontaneous disease. For example, OA is biomechanically, structurally, histologically, genomically and molecularly similar between dogs and people.[108] Dogs with naturally occurring OA are affected by widespread somatosensory sensitivity by means of QST, demonstrating similarities of the clinical scenario between dogs and humans with OA.[15,16,60] Osteoarthritis impacts QoL and sleep disturbances in both dogs and people.[83,121] Cats have been historically used in the field of neurosciences[154,155] and are also great candidates for the study of pain as similarities in the pathophysiology of OA and pain profile of osteoarthritic cats have been reported.[2,7,8,156,157] Cats

and people with OA are affected by mechanical hyperalgesia and allodynia as well as temporal summation of pain when compared with healthy controls.[2,7,16,158,159] Positron emission tomography (PET) imaging in cats with natural OA revealed increased brain metabolism in the secondary somatosensory cortex, thalamus and periaqueductal gray matter, which are also implied in humans and rodents with chronic pain.[157]

Another example is bone cancer pain in dogs which is also quite similar in people with respect to genetic diversity, DNA, physiology, cellular structure, and molecular features.[144,146] Canine patients with natural bone cancer can yield valid data applicable to humans by means of metrology instruments of pain. The completion by proxy of the CBPI resulted in reliable measures of the same pain constructs as seen in humans with bone cancer using the Brief Pain Inventory.[127] Translation of efficacy of treatments in dogs with cancer pain is also promising and clinical trials in canine patients are enabling the translation of innovative personalized therapeutics, with accurate predictive safety and efficacy, to human clinical trials.[103,146,160] Finally, it has been proposed that models of companion animal spontaneous disease can be useful in two ways. First by acting as a bridge between rodent preclinical and human clinical studies by testing drugs for efficacy prior to human clinical studies. Second, by providing tissue from naturally occurring disease states which can be used to study the neurobiology of pain in the natural disease state.[99]

There are certainly limitations and important consideration on the use of companion animals for improving translatability to humans. For example, even though QST testing can provide a great deal of sensory information in companion animals, interpretation of tests remains subjective as they rely on behavior response, and the perceptual qualities or physical properties of a nociceptive stimulus cannot be assessed. Although CMIs and HRQoL instruments can be used to assess pain burden, assessment of the complex pain-associated psychological symptomology such as depression and pain catastrophizing is non-existent in animals. Recruitment of companion animal patients can also be a challenge. A recent survey involving small animal practitioners revealed that more than 80% of them indicated that they spend no time in clinical research, although a high proportion were likely to recommend clinical trial participation to clients for their pets when those trials involved treatments licensed in other countries, novel treatments, respected investigators, or sponsoring by academic institutions.[161] A total of 28% of respondents indicated that they did not usually learn about such clinical trials

and most of them believed that their recommendation of a trial was important to their client's willingness to participate.[161] Similarly, when cat owners were asked about their motives to participate or not in a clinical trial, the most influential factors were trust in the organization, benefit to the cat and veterinarian recommendation.[162] The availability of 'Free Services' was also considered influential in the decision-making process.[162] Given the importance of recommendations of veterinarian practitioners in the recruitment of patients for clinical trials, the authors of these two articles highlighted that efforts should be increased to raise practitioner awareness and education of clinical trials for which patients might qualify.[161,162]

The use of companion animals in clinical trials should strictly respect ethics and animal use guidelines with clear end-point for terminal of trial. Animal welfare should always be considered a priority. Legal constraints to the use of companion animal for testing novel compounds can increase the complexity of the process but are essential if public confidence in the integrity of the veterinary profession is to be retained.[103]

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Conclusion

The use of static and dynamic QST in cats with OA has allowed us to further demonstrate that cats are affected by central sensitization which can be reduced with the administration of centrally-acting but not peripherally-acting analgesics. A systematic review and a meta-analysis of the literature reporting the use of QST in healthy and osteoarthritic cats have solidified our knowledge that OA results in centralized sensitization and facilitated temporal summation. Moreover, the use of a standardized QST protocol was examined for the first time in dogs with bone cancer in clinical practice, demonstrating the severity of pain experienced by these animals. A QST protocol showed that they are affected by primary and secondary hyperalgesia/allodynia, and brush allodynia. A conditioned pain modulation test was developed and validated in this population of dogs and allowed us to detect deficient descending pain modulation associated with bone cancer.

Outcome measures previously developed to examine the pain burden in cats and dogs with chronic pain were also investigated. The ability of accelerometer-based activity monitoring to detect treatment efficacy was testified by increases in activity in both species after analgesic treatment. Health measurement scales can provide extremely important information pertaining to pain, quality of life and welfare. However, they should be used based on a solid body of validation studies. Kinetics can provide valuable data, although their application requires substantial financial and time commitments.

The ability to assess both the somato-sensory and emotional-affective components of pain in companion animals with spontaneous disease revealed similarities between OA and bone cancer in animals and people. Thus, the use of animal models of spontaneous disease such as the ones reported herein could potentially transform the ability to predict efficacy in human trials due to improved translatability. This practice should be accompanied by the highest standards in animal care and study design, conduct and reporting in order to advance veterinary pain science and contribute to human pain science.

Appendix 1. Assessment and recognition of chronic (maladaptive) pain in cats

This is a book chapter written in collaboration with Pr. Duncan Lascelles on the assessment of chronic pain in cats from the recently published book 'Feline Anesthesia and Pain Management' edited by Paulo Steagall, Sheilah Robertson and Polly Taylor (2018; first edition) by John Wiley and sons. The challenges and recent advances on the clinical assessment of chronic pain in cats are discussed in a concise format which is presented predominantly in bullet points.

Appendix 2. Treatment of chronic (maladaptive) pain in cats

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Appendix 1. Assessment and recognition of chronic (maladaptive) pain in cats[a]

Beatriz Monteiro[1] and Duncan Lascelles[2]

Chronic pain reduces well-being, impacts on quality of life and induces behavioral changes that affect the owner-pet bond. The description of pain for an individual is personal and subjective in nature. Indeed, pain assessment in cats is a particular challenge that veterinarians face in patients with chronic conditions.

Cats do not always show clinical signs of pain in the way dogs do. In addition, due to their small body size and ability to compensate for chronic painful musculoskeletal conditions, owners may not notice subtle changes in behavior and thus will not seek help.

Cats of any age can be affected by maladaptive pain, although middle-age to older cats are over-represented. Pain is now considered as the 4th vital sign in veterinary medicine, and its assessment should always be incorporated into the clinical evaluation of all patients. Chapter 11 describes mechanisms of pain. Nevertheless, an overview of commonly used terms in this chapter is presented here.

Acute pain is usually self-limiting, characterized by inflammation and nociception, and associated with potential or actual tissue damage. In contrast, chronic pain is not associated with normal healing and it persists beyond the expected course of an acute disease process with no clear end-point. *Persistent postsurgical pain* is a condition where acute pain persists (abnormal healing) and has become maladaptive.

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[a] In: Feline Anesthesia and Pain Management, 2018. Eds: Paulo Steagall, Sheilah Robertson, Polly Taylor. First Edition. John Wiley & Sons, Inc. Hoboken, NJ, USA.

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Chronic pain should not be considered a symptom, but rather a disease in its own right since it may be present in the absence of a primary cause. This occurs due to the plasticity of

the CNS in which nociceptive signal processing within the spinal cord and higher centers is altered. This results in amplification of the signals, facilitated throughput, and even the generation of 'pain signals' (nociceptive input) from the CNS itself.

The end result of these CNS changes is spontaneous pain, hyperalgesia (increased response to painful stimuli) and allodynia (pain from normally non-painful stimuli). Additionally, pain may persist even after complete resolution of the primary cause of pain. These features are not specific and may be present in distinct types of pain (Box 1). In chronic painful conditions, there is often an imbalance between the excitatory and inhibitory control of nociceptive input with consequent facilitation of excitatory transmission and reduced inhibitory transmission of pain. For example, there is decreased activation of the endogenous analgesic system and its ability to control painful stimuli.

Box 1. Contributors to chronic (maladaptive) pain

- Inflammatory pain is associated with tissue injury, and direct stimulation and sensitization of nociceptors following activation of the inflammatory cascade. These changes result in the cardinal signs of inflammation (heat, pain, redness, swelling and loss of function).
- Neuropathic pain is caused by a lesion or disease of the somatosensory system peripherally or centrally. The somatosensory system includes all structures allowing the perception of sensory information coming from the skin and the musculoskeletal system. The diagnosis of neuropathic pain includes assessment of nerve fibers involved in nociception/proprioception (Aβ, Aδ, C) using techniques such as QST, advanced imaging and somatosensory evoked potentials.
- Functional pain is not associated with any detectable inflammatory or neuropathic etiology. It is characterized by neurophysiological dysfunction with no detectable structural, metabolic or immunological causes.

Clinical chronic pain is generally a mixture of different 'types' of pain – inflammatory, neuropathic and functional pain. In many of these cases, the degree of pain does not necessarily correlate with the pathology due to neurobiological changes; these include altered:

- Transduction in the periphery
- Processing at the level of the spinal cord
- Descending inhibitory control

For these reasons, treatment of maladaptive pain can be difficult. For example, cancer patients may be affected by inflammatory and neuropathic pain, whereas cats with feline interstitial cystitis may present with a combination of inflammatory and functional pain. In both situations there may be varying contributions to the pain state from peripheral, spinal cord and higher center changes.

Inflammatory pain results from the release of nociceptive sensitizers (e.g. PGE2, nerve growth factor) and nociceptive activators. It is commonly associated with surgery-induced acute (adaptive) pain; however, several chronic painful conditions have a component of inflammatory pain. Degenerative joint disease (DJD), cancer, stomatitis, periodontal disease, pancreatitis, gastritis, inflammatory bowel disease, interstitial cystitis, are some examples (Figure 1). A 'new' category of inflammatory pain is referred as 'neuroimmune inflammation' in which glial and immune (toll-like receptors) cells can dramatically amplify pain leading to the development and maintenance of neuropathic pain. Therefore, it is believed that some cases of neuropathic pain might be, at least in part, produced by an inflammatory component.

Figure 1. A tumor affecting the plantar aspect of the left pelvic limb of a cat

The tissue necrosis caused by the tumor results in inflammatory pain. This cat presented with the classical signs of inflammation including heat, redness and swelling of the area, pain at gentle palpation, and decreased function due to lameness of this limb. Neuropathic pain may also be present if a lesion of the somatosensory system occurs (e.g. compression of a nerve).

The development of *neuropathic pain* involves complex mechanisms including sensory aberrant ectopic activity, neuroplasticity, impaired endogenous inhibitory modulation and activation of the microglia, among others. Causes of neuropathic pain include nerve compression, infiltration by cancers, or following amputations involving nerve resection, or associated with intervertebral disk impingement on the spinal cord or nerve roots. However, growing evidence indicates that neuropathic pain is also present in many other conditions such as DJD. In humans, neuropathic pain is generally considered to cause severe and long-lasting pain which is less responsive to treatment with analgesics, and to cause greater mobility impairment when compared with other types of maladaptive pain. In cats, the hallmarks of a neuropathic component of chronic pain are:

- Malfunctioning and deterioration of the somatosensory system (i.e. abnormal processing of sensory information)
- Hyperalgesia
- Allodynia
- Spontaneous Pain

However, these signs are not limited to neuropathic pain and can be observed in functional pain in which the etiology cannot be identified and neurobiological changes are not clearly related to disease or a lesion of the somatosensory system. In veterinary medicine, this terminology is new; however, functional pain can be seen in cats with interstitial cystitis, intestinal bowel disease, and possibly orofacial pain syndrome.

The terms “chronic” and “maladaptive” pain are used interchangeably in this chapter, and the reader should understand that they both refer to a mixture of inflammatory, neuropathic and functional pain. Understanding the origin or cause of pain, which is not always known but underlies a particular condition, aims to help the veterinarian in directing what treatments should be used.

Common causes of chronic pain

Some chronic painful conditions have already been mentioned and the following list is not exhaustive. The clinician should consider that any pathology has the potential to cause maladaptive pain.

- Degenerative joint disease (DJD) and osteoarthritis (OA)
- Persistent postsurgical pain (e.g. limb or tail amputation, thoracotomy, chronic pain syndrome after onychectomy)
- Dental and oral disease (e.g. gingivitis, periodontitis, stomatitis)
- Feline orofacial pain syndrome
- Ocular conditions (e.g. corneal disease, uveitis and ulcers)
- Gastrointestinal conditions (e.g. megacolon, constipation, ileus, inflammatory bowel disease)
- Urogenital conditions (e.g. interstitial cystitis)
- Dermatological conditions (e.g. otitis, severe pruritus, burns, chronic wounds)
- Trauma
- Neoplasia (e.g. feline injection-associated sarcoma; oral squamous cell carcinoma)
- Chemotherapy-induced neuropathy and radiation-induced skin burns
- Diabetes-induced neuropathy
- Chronic kidney disease
- Feline hyperesthesia syndrome

Osteoarthritis (OA) refers to non-inflammatory pathological changes in synovial joints that may be primary and idiopathic, or secondary to trauma or abnormal developmental features. Degenerative joint disease (DJD) is a general definition that encompasses OA and inflammatory arthropathies of synovial joints (including immune-mediated arthropathies), degenerative changes to fibrocartilaginous joints, and *spondylosis deformans* of the vertebral column. The term DJD is used in this chapter since there is little information on the etiology of DJD and the histological characteristics in cats, making it difficult to sub-categorize the disease.

Clinical Signs

Table I below shows common clinical signs and behavioral changes associated with chronic pain in cats. These changes are generally subtle and may be inconsistent since signs can improve and/or deteriorate over time ("waxing and waning" effect). These clinical signs can be used concomitantly to assess quality of life.

Table I. The most common clinical signs and changes in behavior associated with chronic pain in cats

Clinical Signs	Behavior Descriptors
Decreased mobility	Decreased ease and fluidity of movement compared with the past Difficulty when getting up or moving around after resting
Decreased ability to perform activities	Difficulty in performing routine activities such as playing with toys, hunting, or jumping up and down Instead of jumping directly onto a high vertical surface, the cat prefers to do this in stages using intermediate surfaces Instead of jumping directly down, the cat reaches down to shorten the jump or finds an intermediate surface to decrease the height of the jump
Isolation	Decreased interaction with owners and other pets Prefers to be alone
Depression	Lack of interest towards things that would normally interest the cat (toys, birds out of the window, scratching posts, etc.)
Irritation	Complains/vocalizes/growls when picked up or handled Appears "grumpy" or short-tempered
Mood alteration	Change in temperament/demeanor (sadness, irritation, aggressiveness)
Decreased or increased grooming	Hair coat is not well groomed and can be greasy. Nails are dirty Increased grooming with possible alopecia at specific areas of the body indicating abnormal sensitivity (e.g. pain, numbness or tingling; hyperesthesia syndrome)† May resent being groomed
Inappropriate use of litter box	Difficulty in getting into or out of the litter box Inappropriate urination/defecation around the house
Appetite alteration	Decreased or increased appetite and/or water ingestion
Sensitivity to touch†*	Resents simply being touched or petted
Acute and intermittent intense pain behaviors†	Suddenly screams, looks at a region of the body and starts licking it without a known cause Returns to normal behavior shortly after these episodes

†Observed in cats with neuropathic pain *Observed in cats with allodynia

Age-associated behavioral changes may overlap with chronic pain and it is important to differentiate between the two with a rigorous and thorough clinical history and examination. Chronic pain must be ruled out before assuming that behavior changes are age-related only. Geriatric cats are likely to have DJD with or without chronic kidney disease. This population of cats should be closely examined for signs of pain.

The clinical signs depend on the primary cause of chronic pain, if there is one. In most cats, the signs are non-specific and behavioral changes are subtler in nature when compared with dogs. For example, lameness is easily observed in canine patients with DJD. In cats, this is not commonly detected and these individuals will normally show altered mobility and activity patterns that may only be noted in the home environment. Therefore, owners are crucial in the assessment and recognition of chronic pain and quality of life (QoL) in cats (Box 2).

Box 2 - The role of the owners in assessment and recognition of chronic pain and quality of life in cats

There are currently no fully validated pain scoring systems for veterinarians to assess chronic pain in cats. Thus, most of the assessment is based on owner-reported signs. Client communication is important in the diagnosis and the first clinical consultation is usually time- consuming when interviewing owners about the cat's behavior at home.

Owners can normally detect deterioration of pain relief rather than efficacy *per se*, and therefore, the clinician must careful plan how to ask specific questions. The cat will be unlikely to display chronic pain-induced behavioral changes in the examination room. In many cases, the primary reason for the consultation is not actually related to pain. Nevertheless, the potential of a chronic pain component in the primary complaint must always be considered. Interestingly, owners will often notice changes in behavior in additional cats other than the one first presented for evaluation following treatment of chronic pain.

Implications for Quality of Life

In humans, the association between chronic pain and deleterious psychological effects is well recognized. These patients generally self-report suffering, anxiety, impaired mobility, depression and isolation. It may be reasonable to assume that cats are similarly affected. Indeed, comparable behaviors are often perceived by their owners which impact the cat's health and welfare. A general QoL assessment can be made during a consultation and physical examination and include detailed history and investigation of specific clinical signs (Table X).

Step-wise approach for assessment and recognition of chronic pain in cats in the clinical setting

- Discussion with the owner regarding behavioral indicators of chronic pain.
- Low-stress physical examination. A quiet room without 'hiding places' in which the cat can get lodged is recommended. A surface that is soft and non-slip such as a yoga or baby's changing mat should be used. Restraint should be minimized unless absolutely necessary.
- A thorough evaluation focusing on body condition, the oral cavity, eyes, ears, skin and fur, paws and claws should be performed. Changes in grooming should be recorded (Figure 2). Thoracic auscultation and gentle abdominal palpation may reveal abnormalities and pain. Close monitoring of the cat's body language and facial expressions are important during assessment. The cat's temperament and resistance to manipulation should also be noted. The cat's responses to painful stimuli will be affected by where they are (home versus clinic) and with whom (owner versus veterinarian). The presence of the owner in the examinationroom may or may not be helpful. If a body part is known to be painful, it should be the last to be examined. However, this must be balanced with the cat's tolerance to examination and manipulation. It may be necessary to divide the examination into several short periods.

- Painful stimulation may elicit one or more of the following behaviors
- Body tension, resisting manipulation
- Vocalization (hiss, growl, howl)
- Attempt to escape (avoidance)
- Aggression (lash out, bite and/or scratch)
- Hypersensitivity reactions (muscle and/or skin fasciculation, and twitching)

- An orthopedic examination should follow, especially in geriatric cats that may be affected by DJD. Few cats will be willing to walk around the examination room and even if they do, lameness is unlikely to be seen. They may be encouraged to jump up and down, and over objects. Owners can be invited to submit "home videos" to the veterinarian to help with diagnosis.
- Palpation of all joints and long bones is performed. Passive movements of joints (flexion and extension) might be performed under sedation if needed, however, the latter is likely to affect behavioral responses to pain. Notably, it must be remembered that passive movement of joints does not accurately mimic their loading and movement during ambulation, just as in humans and dogs.
- Neurological examination may help to identify cases of neoplasia and neuropathic pain, by revealing spinal conditions that can be a source of chronic pain.
- Laboratory hematology is of limited value in diagnosing pain but may point to underlying disease processes.
- Radiographs may confirm the presence of DJD, neoplasia or megacolon, for example, but radiographic findings rarely correlate with pain in cats.
- Other advanced imaging or laboratory evaluation may be useful on a case-by-case basis, but as with radiography, imaging findings do not correlate with pain.

The concept of analgesic challenge

An "analgesic challenge" can be useful when chronic pain is suspected but not clear. In these cases, analgesics may be administered or prescribed; a decrease or resolution of clinical signs often confirms the diagnosis of chronic pain. In general, chronic pain is difficult to treat (compared with acute pain), and a lack of improvement with a single analgesic (e.g. NSAID) may not completely rule out the presence of chronic pain. An adjuvant analgesic may be added to the treatment.

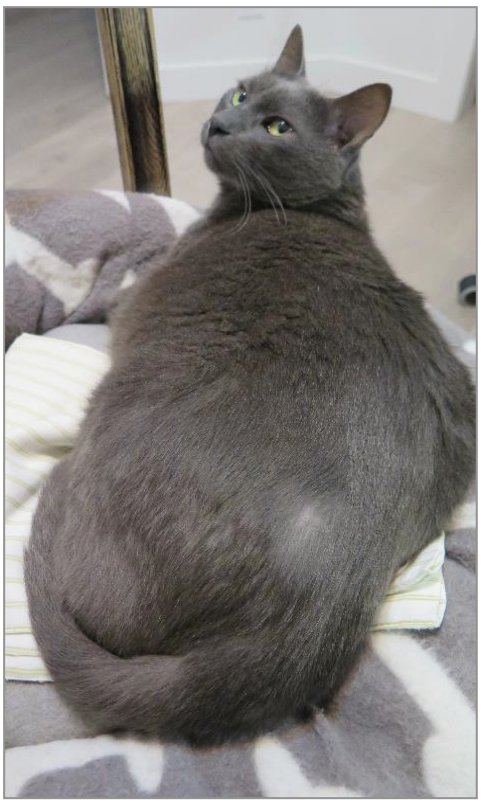

Figure 2. A 12-year-old male (neutered) cat with chronic lumbar pain.

This cat resents being groomed and performs excessive grooming and self-plucking resulting in alopecia in the lumbar region. These behaviors are described to have had a "sudden onset", possibly indicating a neuropathic pain component. On physical examination, the cat reacts adversely to a light touch and stroking the area, confirming the presence of allodynia

Degenerative joint disease (DJD) and Osteoarthritis (OA)

DJD is the progressive destruction of one or more components of a joint. For this reason, the pain results from a combination of inflammatory, neuropathic and functional components, albeit in unknown relative amounts. Plasticity of the nociceptive transmission system results in increased sensitivity, hyperalgesia and allodynia. It is likely, as in humans, that the drivers of pain are a combination of peripheral sensitization, central sensitization and decreased function of the endogenous analgesic system. In geriatric cats, the prevalence of DJD may be as high as 60-90%, and DJD is recognized as one of the main sources of chronic pain. In the axial skeleton, the lumbar and lumbosacral regions are most affected whereas in the appendicular skeleton DJD is more prevalent in the hip, followed by stifle, tarsus and elbow (Figure 3).

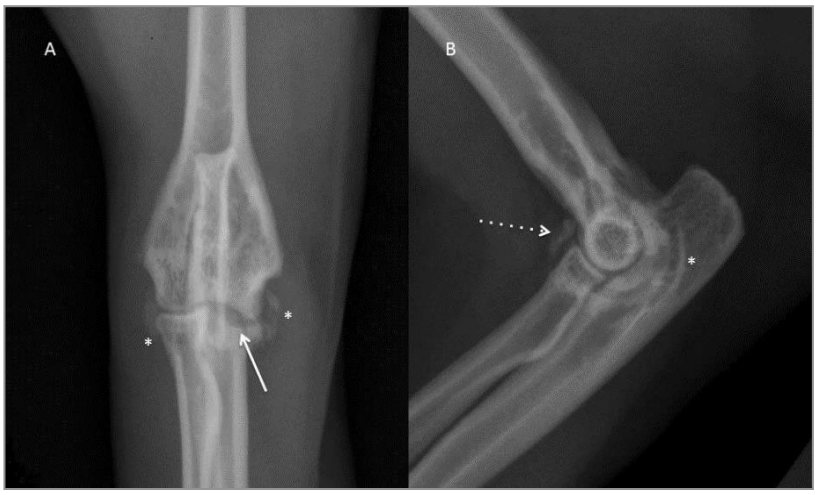

Figure 3. Radiographs of a cat with degenerative joint disease

Cranio-caudal (A) and lateral (B) radiographs of the elbow of a 10-year-old female (spayed) cat with osteoarthritis. Characteristic features are joint-associated mineralization (*), which on the medial aspect may be indicative of medial epicondylitis (where the ulnar nerve can be completely enclosed and flattened between the epitrochleo-anconeus muscle and new bone formation within the humeral head of the flexor carpi ulnaris muscle). The unevenly increased humero-ulna joint space (solid arrow) is probably associated with osteochondromatosis (osteochondral bodies within the joint which have resulted in erosion of the joint surfaces). Although the presence of a sesamoid in the supinator muscle (dotted arrow) is a normal feature, its enlargement and uneven appearance is associated with osteoarthritis of the elbow joint.

Clinical signs

Impaired mobility is reported by owners and can include:

- Decreased mobility (decreased daily distance moved, less jumping, decreased height of obstacles, stiffness, and problems walking up and down stairs)
- Altered movement such as a decreased fluidity of movement
- Less grooming
- Hiding and decreased or altered socialization with other pets and household members
- Changes in litter box use

These changes are too subtle to be detected by the clinician or only relate to the home environment. Cats often “freeze” in the hospital environment due to stress. The impact of stress-induced analgesia is well described in prey species, and although not formally investigated in cats, it may be a component of their normal physiology. Hence, owners provide a more reliable

perspective of the cat`s every-day behavior. Although there are no pain scales for cats with DJD-related pain that have undergone complete validation, some owner-based and one veterinarian-based clinical instruments have been developed specifically for cats. The Feline Musculoskeletal Pain Index (FMPI) and the Client Specific Outcome Measures (CSOM) are the tools that have been studied most and investigate abnormalities related to mobility, and self- maintenance, as well as social and exploratory behaviors. Questions are addressed towards the following specific behaviors: walking and running; playing and chasing objects; ability to jump up or down; height of jump; climbing and descending stairs; interaction with other pets; eating, grooming and sleeping habits.

The FMPI consists of a series of 17 questions. Its ability to detect cats with musculoskeletal pain and consequent effects of treatment has been demonstrated. It is available for use in clinical practice and is constantly being updated

(https://cvm.ncsu.edu/research/labs/clinical-sciences/comparative-pain-research/clinical- metrology-instruments).

The CSOM consist of 3 to 5 activities that are unique to an individual cat and chosen by the owner following discussion with the veterinarian. These specific activities including time and place (e.g. ability to jump onto the bed last thing at night) and are graded according to the degree of impairment and followed over time.

The Montreal Instrument for Cat Arthritis Testing (MI-CAT(V)) has been developed for use by veterinarians. It focuses on mobility impairments that can be evaluated at a distance. It has undergone preliminary validation and reliability testing where gait and body posture allowed for distinction between cats with and without DJD.

Orthopedic examination and goniometric measurements

Orthopedic examination in feline patients should consist of careful palpation of every appendicular joint as well as the axial skeleton while watching for pain responses. Minimal restraint is applied. Additionally, each appendicular joint should be evaluated for crepitus, effusion and thickening as these are other indicators of joint disease, although they do not necessarily relate to pain. Goniometry can be performed, but may prove to be difficult in awake cats. The temperament should also be assessed since a ‘grumpy’ cat may be behaving that way

due to chronic pain. Body condition score should be assessed and recorded for further monitoring.

Clinical signs versus radiographic signs

Radiographic signs of DJD do not necessarily correlate with clinical signs of pain. In cats, the best agreement between radiographic DJD and pain was found for the elbow and the lumbar and lumbo-sacral areas. In contrast, cats may have clinical evidence of DJD-induced pain without radiographic signs. This may be explained by lack of detail in radiographs that would enable detection of early changes of the joint. In humans, it has been recently suggested that pain may be more closely related to structures not assessed by radiographs, and magnetic resonance imaging is considered a more sensitive indicator of disease-induced pain. However, it is clear that as DJD progresses with ageing, radiographic severity becomes greater. Box 3 describes the progression of DJD and the importance of treating pain and increasing mobility while minimizing pathological changes associated with the disease.

Box 3 – The progression of degenerative joint disease

DJD-associated pain starts at the peripheral joint and result in decreased ability to perform daily activities and decreased mobility. This initiates musculoskeletal deterioration due to decreased use and altered body carriage. Additionally, as explained in the text, this nociceptive input (pain input) into the system can result in sensitization and pain (somatosensory system deterioration).

Heightened pain results in further negative effects on the musculoskeletal system (muscle deterioration, trigger point development, muscle pain) which can in turn result in a greater burden of pain as a result of decreased joint support. Thus, there is concurrent deterioration of the musculoskeletal system and the somatosensory system.

Pain also has negative effects on cognitive function, and on affective systems, resulting in heightened fear, anxiety and poor sleep. These changes in turn feed back and heighten pain (e.g. anxiety amplifies pain, and pain in turn heightens anxiety).

The inability to perform daily activities, resulting pain and deterioration of the musculoskeletal system, also drives negative affective changes through decreased and altered interactions with the cat's environment. These two-way relationships between pain, central sensitization, musculoskeletal deterioration and congnitive/affective deterioration are illustrated in Figure 4.

With so many complex changes occurring, and often involving multiple joints, staging ofthe DJD patient may seem daunting. However, "staging" is probably best performed by assessing the overall impact on the cat. Based on activity and mobility, a simple staging of the impact of DJD is proposed below, and in the future, treatment recommendations will take into account the stage of DJD.

Stage 1. Early signs of activity impairment

Stage 2. Intermittent signs of activity impairment

Stage 3. Obvious activity impairment and some decrease in mobility

Stage 4. Loss of mobility

In the future, treatment recommendations will take into account the 'stage' of DJD, and the stages will be sub-defined once central sensitization, cognitive decline and affective deterioration can be measured.

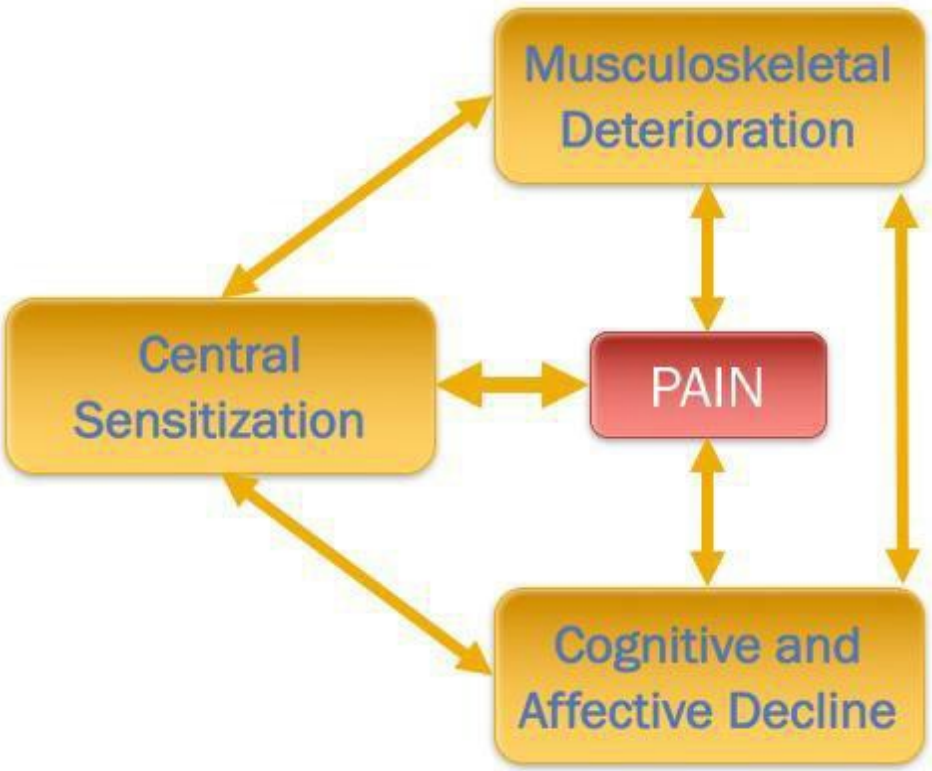

Figure 4 . The progression of degenerative joint disease

Neoplasia

Cancer is one of the main causes of mortality in veterinary patients, and pain is commonly a major component of the disease. Cancer-related pain may be caused by various mechanisms, with the location of the cancer as well as the type dramatically influencing the clinical signs. Inflammatory pain can be caused by tumor growth and destruction of adjacent tissues and structures (Figure 5). Visceral pain can be caused by visceral distension, and neuropathic pain can be caused by nerve compression or a primary tumor involving the nervous system with consequent lesions in the somatosensory system. Recent studies reveal that the tumor itself can contribute to the generation of pain via neuroimmune mechanisms. Even if the chronic pain is controlled, acute exacerbation of pain may occur if the tumor grows rapidly or becomes necrotic. Clinicians should also consider that patients with cancer are commonly affected by other painful co-morbidities such as DJD or chronic kidney disease. Additionally, the cancer treatment itself may cause chronic pain such as persistent postsurgical pain, chemotherapy-induced neuropathy and radiotherapy-induced pain.

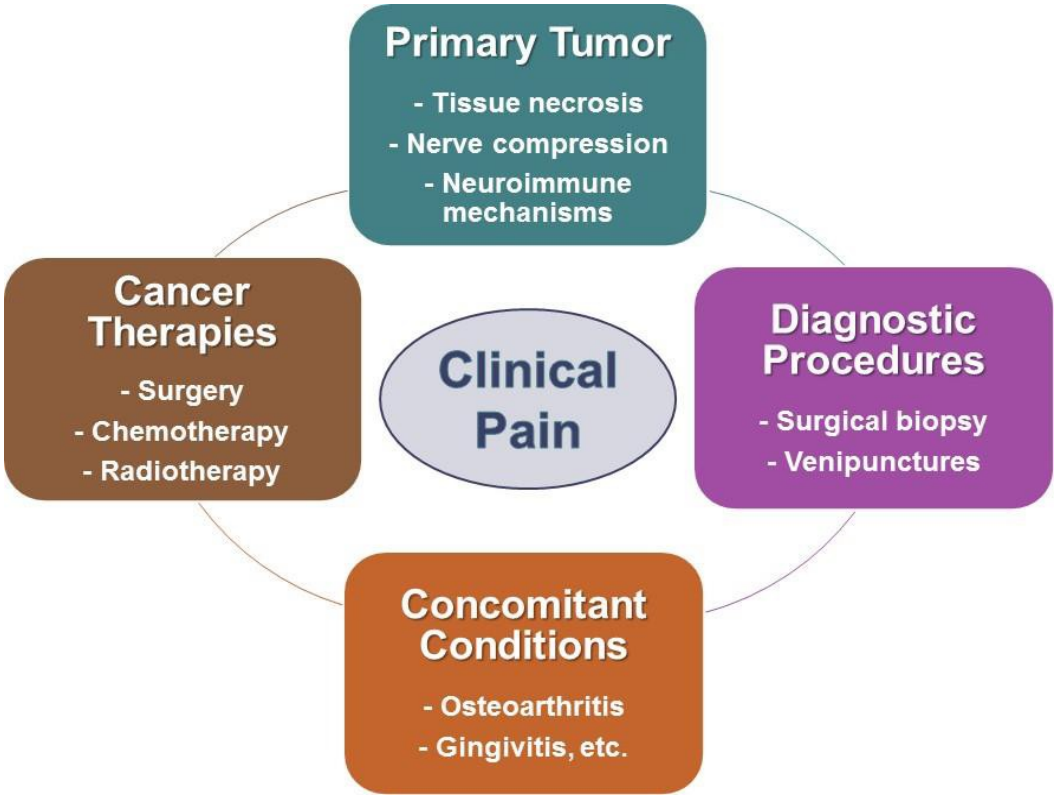

Figure 5. Multidimensional nature of pain in patients with cancer

Persistent postsurgical pain

In humans, persistent postsurgical pain is the second largest cause of chronic pain after OA. Sensitization of the nervous system due to nerve injury induced by insufficient perioperative analgesia and extensive tissue injury are thought to be the main causes of persistent postsurgical maladaptive pain. Although this process can occur following any surgery, amputation and thoracotomy are classic examples of surgical procedures where extensive nerve damage is unavoidable. Spontaneous ectopic discharges from injured nerves result in significantly increased nociceptive input inducing neuroplasticity, central sensitization and spontaneous pain. Inflammation resulting from the surgical trauma results in additional nociceptive input.

Amputation

The incidence of chronic pain following amputation in feline practice is unknown, whereas in humans, the condition often referred to as "phantom pain" may affect up to 80% of amputees. Clinical signs may develop within the immediate post-operative period or years after the procedure, and they are similar to that described for neuropathic pain. Aggressive perioperative pain management is required for this procedure.

Chronic pain syndrome after feline onychectomy

Feline onychectomy (i.e. the amputation of the third phalanx of each digit) is performed only in North America. A chronic pain syndrome can develop as a result of this procedure. A discussion of the ethical issues associated with this procedure is beyond the scope of this chapter. Diagnosis of the syndrome is based primarily on clinical signs and behavioral changes after ruling out DJD or other complications that could be corrected surgically. Behavioral changes associated with the syndrome include

- Chronic lameness
- Licking and chewing at the feet
- "Walking as if on hot coals"
- Shaking and "flicking" of the paws
- Aversion to the feet being touched
- Reluctance to use the feet to play with toys or to cover urine/feces with litter

- Periods of suddenly sitting still
- Spontaneous vocalization “for no apparent reason”

Periodontal disease

Periodontal disease characterized by inflammation of the gingiva or periodontal tissues is common in feline practice and is a source of chronic pain. Assessment of pain is difficult in these cases, but it maybe assumed that if inflammation is present, pain is present. Dysphagia is the most common clinical sign and some cats will frequently ‘paw against their mouth’ during and after eating. Spontaneous vocalization with escape behavior may be observed. These behaviors normally disappear once the dental disease is treated (Figure 6*).*

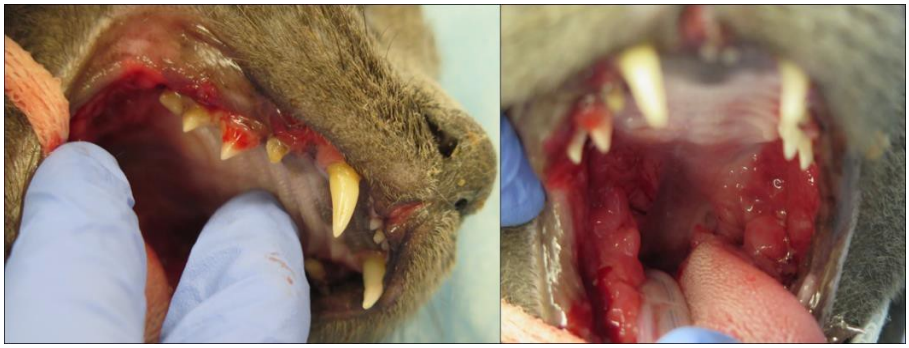

Figure 6. Cat with severe gingivostomatitis.

This cat showed classical clinical signs of oral pain including pawing at the mouth while eating. These signs resolved completely following full-mouth teeth extractions. The cat is reported to have become friendlier after the procedure indicating an affective and emotional component of oral pain.

Feline Orofacial Pain Syndrome

Feline Orofacial Pain syndrome is a chronic condition with acute episodes (acute on chronic pain). The etiology is not fully understood but it is suspected to be a neuropathic pain disorder analogous to trigeminal neuralgia in humans. Nevertheless, discomfort appears to be limited to the oral cavity and lips and no other areas of trigeminal nerve innervation. The presentation is usually unilateral and the diagnosis is made by exclusion. Burmese cats appear predisposed. Affected cats should be assessed for concomitant oral lesions and possible

environmental stressors, for example social incompatibility in a multi-cat household. Clinical signs may be triggered by mouth movement and include:

- Exaggerated licking and chewing movements
- Pawing at the mouth
- Anorexia or decreased appetite
- Mutilation of the tongue, lips and buccal mucosa (severe cases)

Gastrointestinal conditions

Any chronic disease affecting the gastrointestinal system and causing visceral distension, such as megacolon or constipation should be considered painful. Inflammatory bowel disease is characterized by chronic intestinal inflammation and may occur concomitantly with pancreatitis or cholangiohepatitis. These inflammatory diseases have the potential to cause chronic abdominal pain. Assessment of these patients is based on the clinical history and response to abdominal palpation.

Interstitial cystitis

Feline interstitial cystitis (FIC) is recognized as a chronic pain syndrome. Cats and humans are affected by this condition which has many shared features between the species. The process by which chronic pain develops is not fully understood. Clinically, it has been noted that interstitial cystitis impacts on the QoL. In cats, several factors such as obesity, confinement, sedentary lifestyle, inter-cat aggression and decreased water consumption have been proposed as potential contributors to development of the disease. Assessment of patients with FIC should comprise detailed history, consideration of possible environmental stressors, physical examination, urinalysis and urine culture, as well as assessment of QoL. Although non-specific for FIC, the most commonly observed clinical signs include:

- Dysuria
- Pollakiuria (excessive frequent urination)
- Hematuria (presence of blood in the urine)
- Periuria (urination in inappropriate locations)
- Over-grooming around the perineum
- Behavioral changes

Diabetes-induced neuropathy

Prevalence of feline diabetes mellitus is increasing because of obesity and sedentary lifestyles; older neutered male cats are most frequently affected. Diabetes-induced neuropathy is a type of neuropathic pain characterized by demyelination of motor and sensory nerve fibers. It affects approximately 10% of cats with this disease. Humans report numbness, tingling or allodynia, and emotional consequences include depression and decreases in QoL. It is now well recognized that diabetes mellitus in cats closely resembles human type-2 diabetes. It is reasonable to conclude that similar pain-induced behavior changes occur in cats. Clinical signs are related to neuropathic pain and functional disability. These may include

- Aversion to touching of the extremities
- Licking of the feet leading to discoloration of the fur (light-colored cats)
- Pelvic limb weakness and difficulty in ambulating
- Impaired ability to jump

Further Reading

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Appendix 2. Treatment of chronic (maladaptive) pain in cats[a]

Beatriz Monteiro[1] and Eric Troncy[b1]

Chronic (maladaptive) pain causes suffering and QoL in cats. Treatment often involves a *multimodal approach* including both administration of analgesic drugs and application of non-pharmacological therapies. The environment in which the cat lives is also important. It should be predictable, provide comfort, mental and physical stimulation, and interactions with family members. Continuous '*tender loving care'* will play a role in the cat's welfare and reinforce the human-animal bond.

Appropriate assessment of chronic pain is paramount for adequate treatment (chapter 14). Normally, identification of a primary cause will facilitate diagnosis and treatment (*e.g.* gingivitis, otitis). In some cases, a primary, single cause or condition cannot be identified (*e.g.* interstitial cystitis) and in others there is no cure (e.g. degenerative joint disease (DJD), some cancers). Pain and discomfort may eventually become severe and refractory to standard analgesic treatment. The following principles should be considered when treating chronic pain in cats, and may explain why response to treatment varies greatly among individuals:

"Clinical medical" reasoning is the thought-process involved in identifying the etiology and structural anatomical sources of pain. It requires an understanding of the pathophysiology of the painful condition to allow for a "mechanism-based" therapeutic approach. The mechanism and type of pain (e.g. nociceptive, neuropathic, inflammatory, mixed) is potentially identified and an analgesic drug chosen based on its mechanism of action. However, this is not always easy and it is often necessary to choose a therapy and assess the response before deciding on a longer-term treatment.

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"Psychosocial" reasoning is related to the owners' emotions, behaviors, attitudes and coping skills related to their pet's pain. The owner's perceptions can sometimes be extrapolated as the cat's perceptions, by proxy.

Client communication: a crucial component of treatment for chronic painful conditions

Owners play an important role in the diagnosis and treatment of chronic pain, because "trial and error" is often required in terms of finding a strategy that works best for managing the cat's pain (Box 4). The emotional burden of chronic pain for both the cat and the owner must not be underestimated. The owner-cat bond can be challenged, especially when grooming and elimination habits have changed. Euthanasia is a treatment option and should be considered in cases of poor prognosis, scheduling and financial constraints and lack of response to therapy.

Box 4 – Planning the treatment protocol with the owner

The owner must understand that complete resolution of clinical signs is rarely achieved and that the main goal of treatment is to improve comfort and QoL. A long-term treatment protocol should be based on reasonable expectations with the owners during the first consultation. Treatment should take into account ease of drug administration, palatability, scheduling and the cat's toleration. Owners are integral to the treatment process and are responsible for:

- Enriching the environment
- Frequent administration of analgesic drugs
- Monitoring efficacy and adverse-effects

Challenges in the treatment of chronic pain in cats

The following list includes some of the major challenges faced when treating chronic pain in cats:

- Difficulties in recognizing and assessing chronic pain in cats, resulting in inappropriate and ineffective treatment
- Fear of adverse effects with long-term administration of analgesic drugs
- Lack of pharmacokinetic (PK) and pharmacodynamic (PD) cat-specific data
- Individual variability in response to treatment
- Ineffective response to solely drug therapy
- Treatment cost
- Owners' compliance, time-commitment and expectations

Pharmacological therapy

Recommended doses for the most commonly used analgesics for treatment of chronic pain in cats are given in Tables II and III. Some of these recommendations are based on personal experience and anecdotal reports due to the lack of large, robust, prospective clinical studies.

Drug administration

• The oral route (PO) is most convenient for long-term treatment.

• Most medications are well tolerated if given with canned food. However, the unpalatability of some analgesic medications (e.g. tramadol and amitriptyline) may limit their clinical use (Box 5) (Figure 15).

• NSAIDs such as meloxicam and robenacoxib are highly palatable and well tolerated.

• Medications administered by the oral route may be available as pills, tablets, and liquid or spray formulations.

• The subcutaneous route is a potential route of administration although few injectable options exist for long-term treatment.

• Sustained release formulations of some drugs are now available and may be convenient for long-term treatment; however, data on safety and efficacy are lacking in cats.

Table II. The most common pharmacological options used in the multimodal treatment of chronic pain in cats. Drug regulations, labelling and scheduling vary among different countries.

Drug name	Treatment protocol	Observations
Amantadine	3-5 mg/kg PO every 12 to 24h	Information on safety and efficacy is not available
Amitriptyline	1-4 mg/kg PO every 12 to 24h	AE: Sedation GR: Doses may be started at the low end and slowly increased. Variable efficacy
Buprenorphine (1.8 mg/mL)	0.24 mg/kg SC every 24h up to 3 days	SE: Few studies indicate efficacy (antinociception) and safety AE: Euphoria has been observed (rolling, kneading with thoracic paws and purring) GR: This high-concentration formulation can be used "off-label" in cats with severe chronic pain or when pain flares up
Fluoxetine	0.5-1.5 mg/kg PO every 24h	Information on safety and efficacy is not available
Gabapentin	5-10 mg/kg PO every 8 to 12h	SE: Efficacy in feline DJD following 3 weeks of treatment (10 mg/kg PO every 8h). AE: Sedation and ataxia GR: Doses should be gradually increased to avoid profound sedation, specially in cats with renal disease. Higher doses have been reported and are generally limited by the severity of adverse-effects Important: Treatment should not be stopped abruptly. It must be tapered down gradually to avoid breakthrough pain
Gapiprant	2 mg/kg PO every 24h	SE: Efficacy data is not available. Few reports indicate a good tolerance following 28 days of administration AE: Unknown
Meloxicam	0.1 mg/kg PO once, followed by 0.05 mg/kg PO every 24h	SE: Dose-dependent efficacy and good tolerance in cats with feline DJD AE: See Box 15.2 GR: Administration of the minimum effective dose should be attempted in long-term treatment

Pamidronate	1-2 mg/kg IV every 28 days	SE: Efficacy studies are not available. Few reports in cats with bone-invasive squamous cell carcinoma or metastatic bone tumor indicate good tolerability AE: Gastrointestinal signs and neutropenia have been observed only in cats undergoing concomitant chemotherapy
Piroxicam	1 mg/cat (~0.3 mg/kg) PO every 24h	SE: Some reports describe its administration in cats with various carcinomas and sarcomas with unclear efficacy and acceptable tolerance AE: See Box 15.2. Vomiting, melena and anemia have been reported in long-term treatment GR: Administration every 2-3 days should be attempted in long-term treatment. Piroxicam can be chosen over other NSAIDs for its anticancer activity; however, it should not be used in other chronic painful conditions
Robenacoxib	1-2.4 mg/kg PO every 24h	SE: Efficacy and good tolerance in cats with feline DJD AE: See Box 15.2 GR: Administration of the minimum effective dose should be attempted in long-termtreatment
Selegiline	0.1-0.2 mg/kg PO every 24h	Information on safety and efficacy is not available
Tramadol	2-5 mg/kg PO every 8 to 12h	SE: Efficacy in feline DJD following 2 weeks of treatment (3 mg/kg PO every 12h) AE: Sedation, vomiting, euphoria and constipation may occur

PO: oral administration; AE: Adverse-effects; SE: Scientific evidence in chronic pain; GR: General recommendations

Table III. Suggested treatment for some chronic painful conditions in cats including "off-label" regimens. Non-pharmacological options should be considered for all cases

Condition	Suggested pharmacological treatment	Suggested non-pharmacological treatment	Comments
DJD-related pain	NSAID Tramadol Gabapentin Antidepressants	Physical activity and weight control Implement environmental enrichment Chondroprotective agents Physical therapy and rehabilitation, Acupuncture, TENS	In mild cases, analgesics are avoided As severity increases, more adjuvants are added
Cancer-related pain	NSAID as needed Gabapentin if neuropathic pain Antidepressants Amantadine Buprenorphine in the case of breakthrough pain	Any non-pharmacological treatment may be applicable depending on the case	Treat primary cause if possible
Periodontal disease	NSAID as needed Gabapentin if there is nerve involvement	Provide soft food; add water to the kibble	Treat the primary cause if possible
Interstitial cystitis	NSAIDs None in acute cases Amitriptyline in chronic cases	Reduce environmental stressors and implement environmental enrichment	Concomitant urinary tract infection should be investigated and addressed
Chronic pancreatitis	Low-dose NSAIDs (contra-indicated if concomitant inflammatory intestinal disease) Gabapentin Tramadol	Nutritional management: highly digestible diet with high fiber and limited fat Administration of pancreatic enzyme supplement	Treat primary cause if possible Consider the use of proton pump inhibitors

Chronic wounds	NSAIDs Adjuvants if needed: tramadol, amitriptyline	Bandages and wound care	Treat primary cause if possible (i.e. surgery, antibiotics, etc.)
Chronic otitis	NSAIDs Gabapentin if there is nerve involvement	Ear cleaning	Treat primary cause
Feline orofacial pain syndrome	Gabapentin until resolution of clinical signs; consider adding amitriptyline if not successful NSAID Antidepressants Amantadine	Reduce environmental stressors and implement environmental enrichment	Oral lesions (*e.g.* fracture tooth or tongue mutilation) may be present and should be addressed
Diabetes-induced neuropathy	Gabapentin until resolution of clinical signs; consider adding amitriptyline if not successful	Weight control	Diabetes must be carefully monitored
Chronic pain syndrome after feline onychectomy	Amantadine for 3-4 weeks NSAID: doses should be gradually decreased every 4 days for a total of 15-20 days Gabapentin until resolution of clinical signs; consider adding amitriptyline if not successful Buprenorphine for 2-4 days	Provide soft surfaces in areas where the cat likes to walk around Check for the preferred type of litter box Reduce environmental stressors and implement environmental enrichment	Radiographs should be performed to check if there is presence of bone fragments and the need of surgery Similar recommendations in cases of persistent post-surgical pain such as amputations and thoracotomies

NSAID: non-steroidal anti-inflammatory drug; DMOAs: disease-modifying osteoarthritic agents; TENS: transcutaneous electrical nerve stimulation

Box 5 – The issue of medicating cats

Common methods of oral delivery of medications

Medicating cats for chronic conditions can be quite challenging. The most common methods used by owners include force-pilling or hiding the medication within a highly palatable food (Figure 7). Force-pilling is made by placing the medication deep in the oral cavity with either the fingers or the use a pet-pillar device. However, cats generally resent being restraint for force-pilling and have a natural discriminatory ability when it comes to eating, resulting in difficulties to administer the medication.

Possible consequences of force-pilling

Although some owners and cats can be trained for a stress-free administration of medications using force-pilling, this method can be an additional environmental stressor for other cats. It can disturb the human-animal bond and therefore, it should be avoided. This is important in conditions in which stress plays a role in the pathophysiology of pain (*e.g.* idiopathic cystitis). In addition, force-pilling may result in the medication becoming trapped in the esophagus causing esophagitis or esophageal stricture.

What do owners think?

A recent e-survey involving owners of cats showed that medications that are labelled for use in the species and formulations such as solutions and suspensions are more likely to be accepted by the cat when compared with non-labelled medications (odds ration: 4.9) and formulations such as tablets or capsules (odds ration: 4.8), respectively. Adverse-effects and the "individual behavior of the cat" (*e.g.* resisting medication, salivation) were the most common reasons for lack of compliance to treatment.

Cat-friendly medications

As medications that are developed for cats are made available, acceptability and compliance is expected to increase. Indeed, an award called "Easy to Give" is yearly offered by the International Cat Care to pharmaceutical companies that produce medications in a format that is easy for owners to administer.

General recommendations

- Pilling should always be followed by positive reinforcement (*e.g.* treats, petting).
- If force-pilling is absolutely necessary, it should be followed by the oral administration of approximately 3 mL of water or by offering a small amount of soft food.
- Unpalatable medications (*e.g.* tramadol, amitriptyline) may be inserted into small capsules or compounded in palatable flavors.
- The website of the International Cat Care (www.icatcare.org) has detailed recommendations and step-by-step videos teaching how to administer oral medications to cats.
- Veterinary technicians are valuable in discussing these issues with owners and emphasizing the importance of a stress-free administration of medications.

Opioids

- Although opioids are the cornerstone of acute pain management efficacy has not been demonstrated for chronic pain. Orally administered opioids have low bioavailability, and most are controlled drugs with potential for diversion.
- A highly-concentrated formulation of buprenorphine (1.8 mg/mL, Simbadol®) is available in the USA. It may provide up to 24 hours of postoperative analgesia in cats (0.24 mg/kg) and is labelled for 3 consecutive days of use. This formulation is not licensed for long-term administration but could be an "off-label" option for cats with severe chronic pain as part of a multimodal approach, or when pain flares up ("acute on chronic" pain).

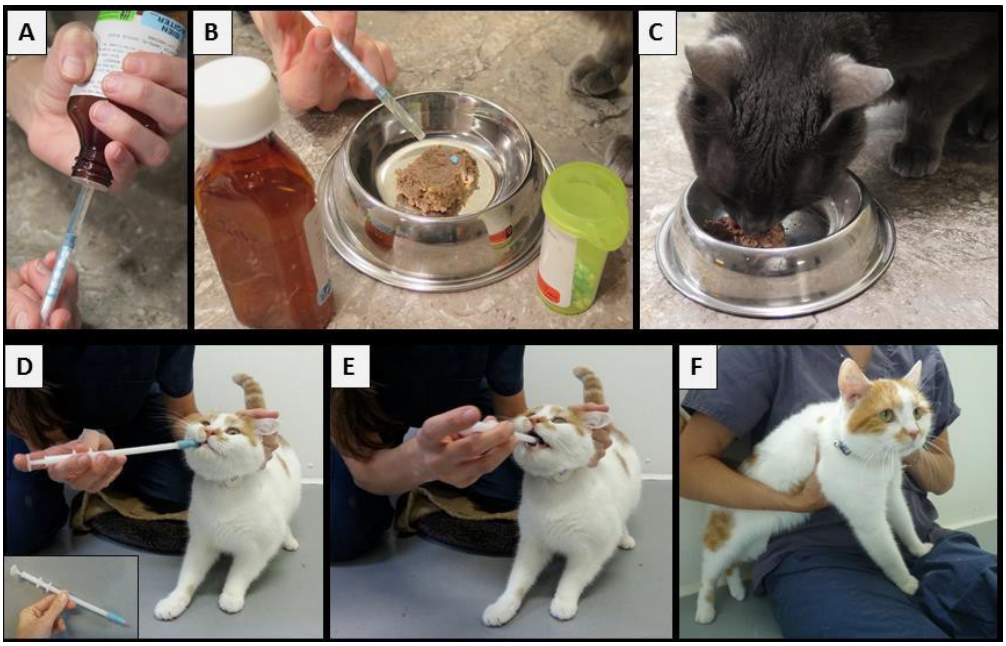

Figure 7. Different methods for administering medications to cats

The top pictures show the preferred method, without any physical contact. The bottom pictures show the method "force-pilling" with the use of a pet-piller device. In some cats this latter approach can be quite stressful. Note how the white cat depicted in these pictures tries to scape the administration by walking backwards. Top pictures: Gabapentin compounded in a palatable liquid formulation is withdrawn (A). A pill of amitriptyline is hidden within a tasty soft food and liquid gabapentin is added to the mixture (B). A cat eats his meal with hidden medications (C). Bottom pictures: Pet-pillar device prepared with a capsule of tramadol (D; corner). The medication is administered by placing one hand in the cat's neck while the other hand gently inserts the device through the side of the cheek pouch (D). The device is advanced as the cat opens the mouth and the medication is administered by pushing onto the plunger (E). The cat is petted immediately afterwards for positive reinforcement (F).

Local anesthetics

- Transdermal lidocaine patches may be an option for some types of pain (e.g. subcutaneous mass, chronic wound). The PK of a 700 mg lidocaine patch has been described in healthy cats with intact skin, and systemic uptake is minimal.
- Local anesthetic techniques may be applied in cases of severe, postsurgical or breakthrough pain in the hospital setting.

Non-steroidal anti-inflammatory drugs (NSAIDs)

- NSAIDs act by inhibiting expression of cyclooxygenase (COX) enzymes in cell membranes and have both peripheral and central actions.
- Meloxicam has high bioavailability following PO and SC administration.
- Robenacoxib has moderate and high bioavailability following PO and SC administration, respectively.
- Piroxicam has high bioavailability and a long terminal half-life following PO administration.
- NSAIDs accumulate in inflammatory exudate, explaining why the analgesic effects may last for up to 24 hours after a single dose despite relatively short half-lives.
- Many NSAIDs are metabolized in the liver via glucuronidation making them potentially toxic. Meloxicam utilizes oxidative pathways and has been successfully used long-term in cats.
- Meloxicam and robenacoxib are excreted predominantly via the biliary route (fecal), with a minor contribution from the kidneys (urine).

Clinical Use

- Indications: treatment of inflammatory pain (e.g. DJD-related pain, cancer pain, gingivostomatitis, otitis, interstitial cystitis, chronic wound).
- NSAIDs are the first-line of treatment for cats with DJD. These drugs decrease pain and improve activity, mobility and QoL in this population.
- Labelled recommendations for meloxicam and robenacoxib vary among countries. In the USA meloxicam is labelled for treatment of acute pain (single injectable dose), whereas in others, including Europe and Australia, it is labelled for treatment of acute pain and chronic musculoskeletal disorders (0.1 mg/kg on the first day, followed by 0.05 mg/kg PO every 24h; continued dosing). Robenacoxib is labelled for treatment of acute pain only, with a maximum duration of treatment in the USA of 3 days and 6 days in other countries (including Europe and Australia).

- Despite labeling variations, both meloxicam and robenacoxib have undergone several investigations in the research and clinical settings. Meloxicam has been shown to be safe and effective for treatment of chronic inflammatory pain in cats (DJD). Robenacoxib has been shown to be safe in cats with DJD, although efficacy studies are lacking.
- NSAID-induced adverse effects should not be underestimated. Guidelines for safe administration are provided in Box 6. The Association of American Feline Practitioners (AAFP) and the International Society of Feline Medicine (ISFM) have published an extensive review on the subject as well as client brochures for owner education (http://www.catvets.com/guidelines/practice-guidelines/nsaids-in-cats).
- In general, the lowest effective dose should be administered while monitoring for adverse effects. For example, meloxicam was safe, palatable and effective when administered to cats with DJD at 0.01-0.03 mg/kg for up to 9 months. Adverse effects included vomiting and diarrhea in a small number of individuals. No significant changes in serum creatinine were detected.
- Long-term safety of meloxicam and robenacoxib have been reported in cats with concomitant DJD and chronic kidney disease. (Box 7).

Clinical use in cancer pain

- NSAIDs also have anticancer properties, especially in tumors overexpressing COX-2. Their administration in cats with cancer should always be considered, unless otherwise indicated. Common use of NSAIDs in oncology include squamous cell carcinoma, adenocarcinomas (e.g. nasal, mammary, anal gland, vulvar), transitional cell carcinoma, pulmonary carcinoma, soft tissue sarcoma, fibrossarcoma and osteochondrosarcoma.
- Piroxicam is a non-selective NSAID with chemopreventative and antitumor effects. This drug is commonly administered with other cancer therapies. Piroxicam can be used for both its analgesic and anticancer effects in oncology patients; however, their use might result in increased incidence of adverse-effects when compared with meloxicam and robenacoxib. Piroxicam is not labelled for use in cats.
- Chronic administration of piroxicam (0.3 mg/kg PO every 24h) for at least one month was generally well tolerated in cats with cancer. Gastrointestinal clinical signs were more prevalent in cats receiving concomitant chemotherapy.
- NSAID-induced adverse effects are observed when dosage regimens are not respected.

Box 6 - Guidelines for the safe use of NSAIDs in cats

NSAID-induced adverse effects are thought to be related to their COX-1 inhibition. In general, COX-1 activity has cytoprotective effects in the gastrointestinal tract (secretion of gastric mucous and production of bicarbonate), kidneys (maintenance of renal blood flow under hypotensive conditions) and platelet aggregation (production of thromboxane). The most common clinically detectable adverse effects include anorexia, vomiting, diarrhea and lethargy. Hepatic adverse-effects are thought to be an idiosyncratic reaction to specific drugs rather than intrinsic hepatotoxicity. The following should be considered when NSAIDs are administered on a long-term basis:

- Patient selection
    - Complete history
    - Check for preexisting diseases
    - Check for current medication and possible interaction
- Client communication
    - Hand-outs for owner education and monitoring of clinical signs
    - What to expect: possible clinical signs such as anorexia, depression, vomiting and diarrhea
    - How to proceed: stop medication if clinical signs develop and contact the veterinarian
- Monitoring
    - Via telephone interview or physical examination during re-evaluation
    - Haematology: although not specific, liver and renal enzymes may be monitored for trends
- Dosage may be tailored to the individual, and the minimum effective dosage should be given for long-term therapy
- A minimum wash-out of 5-7 days is recommended when switching between NSAIDs to prevent inadvertent NSAID overdose. Analgesia can be provided using a different class of drug during this period.

Box 7 - Cats with chronic pain and concomitant chronic kidney disease (CKD): what`s the story?

Rational

NSAIDs are generally contra-indicated in cats with concomitant renal disease due to the inhibition of both COX enzymes and consequent negative effects on the maintenance of renal perfusion (vasodilation of renal artery) under circumstances of hypovolemia or hypotension. However, it is not uncommon that cats with chronic painful conditions are concomitantly affected by CKD. In fact, approximately 70% of cats with DJD are also affected by CKD.

Controversy

Growing evidence indicates that meloxicam and robenacoxib can be safely administered to cats with chronic painful conditions and stable CKD. It has been hypothesized that improved mobility and overall QoL subsequent to pain relief result in better appetite and increased water consumption. Another possibility is that NSAIDs may have a direct anti-inflammatory effect on the kidneys, leading to reduced deterioration of renal function.

Evidence in cats with chronic pain

Retrospective case-control study: cats with CKD receiving meloxicam (0.02 mg/kg/day) for a median duration of 467 days, and cats with CKD not receiving a NSAID. The cats in the first group (NSAID) showed smaller increases in blood creatinine concentration over time when compared with the cats in the second group (no NSAID). There was no difference between groups in urine specific gravity.

Retrospective study: cats without and with stable CKD (IRIS stages II and III) which had been under continuous treatment with meloxicam (0.02 mg/kg/day) for at least 6 months. There was no difference between groups in longevity.

Prospective clinical study: cats with CKD and cats without CKD receiving robenacoxib (1-2.4 mg/kg/day) or placebo treatment for 28 days. There was no difference between groups in the frequency of reported adverse events, body weight changes, serum and urine chemistry, or hematological variables.

Evidence in healthy normovolemic cats

Prospective study: cats with surgically-induced decreased renal mass and consequent CKD (IRIS stage 2 and 3) receiving meloxicam, acetylsalicylic acid or placebo treatment for 7 days. There was no decrease in glomerular filtration rate following any of the treatments.

Evidence in humans

Systematic review: population of 12,418 study subjects and 23,877 controls. The study aimed to evaluate the role of NSAIDs in the development of nephropathy. Eight out of 9 reports failed to identify an increased risk of chronic renal impairment with heavy NSAID consumption.

Conclusions

The administration of NSAIDs to cats with concomitant chronic painful conditions and CKD can be performed provided their overall clinical status is stable. Moreover, if these cats undergo anesthesia, maintenance of normal hydration, circulating blood volume and blood pressure are important.

Contra-indications

- History of gastrointestinal disease
- History of NSAID intolerance
- Uncontrolled renal or hepatic disease (Box 15.4)
- Anemia
- Coagulopathies
- Hypovolemia or hypotension
- Concurrent corticosteroid administration including topical treatments
- Administration in close temporal relationship with other NSAIDs

Tramadol

- Tramadol has a dual mechanism of action. It has weak affinity for μ-opioid receptors and acts as a serotonin and noradrenergic reuptake inhibitor. The latter effects are related to activation of the endogenous descending inhibitory pain pathways which modulate central sensitization.
- In humans, the analgesic effects are primarily due to the active metabolite, O-desmethyl-tramadol which has an opioid effect.
- In cats, tramadol has high bioavailability (93 ± 7%) after oral administration; O-desmethyl-tramadol follows tramadol's disposition profile and both are dose-dependent.
- The analgesic profile of tramadol in cats is thought to be superior to that in dogs, due to a longer elimination half-life and higher concentrations of the active metabolite. The rate of formation of O-desmethyl-tramadol from tramadol in cat liver is faster than that in dogs (3.9 fold).
- The terminal half-life of O-desmethyl-tramadol after IV (2 mg/kg) and oral (5 mg/kg) administration in cats is 261 ± 28 and 289 ± 19 min, respectively.
- An injectable formulation of tramadol is available in some countries; however, the oral route is preferred for long-term administration.
- Tramadol is bitter, often results in profound salivation and is resented by most cats.

Clinical Use

- Indications: conditions characterized by central sensitization (e.g. DJD-related pain, cancer-related pain). Tramadol has a synergistic effect when administered with NSAIDs and is normally administered as part of a multimodal protocol.
- Antinociceptive studies suggest that a dosing regimen of 4 mg/kg, PO, every 6h would be ideal. However, such dosage regimens may decrease owner compliance. The magnitude and duration of antinociception appear to be dose-dependent in cats; higher doses produce prolonged and sustained antinociception.
- Sedation maybe observed and dose adjustments can be done accordingly. However, clinical experience suggests that sedation normally subsides following the first days of administration.
- In cats with DJD-related pain, tramadol (3 mg/kg, PO, every 12h) increased activity and mobility, and decreased central sensitization when compared with placebo.
- Further studies may clarify the ideal dose and dosing interval for treatment of chronic pain. Based on currently available PK and PD data, 2-5 mg/kg, PO, every 8 to 12 h is recommended.
- Tramadol may be partially antagonized by naloxone in cases of intoxication (administered IV to effect).
- In many countries, tramadol is now a controlled substance (usually schedule IV).

Contra-indications

- Tramadol must not be administered in combination with serotonin reuptake inhibitors including monoamine oxidase inhibitors and tricyclic antidepressants due to the risk of serotonin toxicity.
- Serotonin toxicity is a clinical syndrome characterized by autonomic dysfunction, neuromuscular abnormalities and changes in mentation that may occur when multiple serotonergic agents are administered, or in cases of overdose.
- The diagnosis of serotonin toxicity is based on the exposure to serotonergic agents associated with clinical sings. Possible clinical signs in cats include: change in mental status, ataxia, agitation, tremor, myoclonus, seizures, hyperreflexia, tachycardia, hypertension, hyperthermia, nausea, vomiting, diarrhea and abdominal pain.

- The treatment of serotonin syndrome involves the cessation of administration of serotonergic medications and supportive treatment including fluid therapy, analgesics and 5-HT2 receptor antagonists (e.g. cyproheptadine, chlorpromazine). The long-term prognosis of serotonin toxicity is good to excellent.
- Toxicity has been reported in a cat following tramadol overdosing (80 mg/kg) due to a prescription error resulting in serotonin syndrome. This cat developed neurological signs (agitation followed by severe depression), hypersalivation, hypertension, tachycardia, tachypnea, constipation and abdominal pain. Treatment comprised fluid therapy, buprenorphine, cyproheptadine and microenema and the cat fully recovered within 7 days.
- Some reports in humans and cats suggest that co-administration of tramadol and NSAIDs may increase the risk of adverse intestinal effects. In addition to the gastrointestinal effects of NSAIDs, serotonin is known to increase gastric acid secretion and decrease platelet aggregation. The former would contribute to gastric mucosal lesions, and the latter affect mucosal healing. Available veterinary literature does not make clear recommendation and concern may be irrelevant in cats; adverse effects have only been seen in humans when tramadol was taken with non-preferential COX inhibitors (e.g. indomethacin).

Gabapentin

- Gabapentin was developed as an anticonvulsant and produces analgesia by reducing neuronal excitability and blocking calcium channels. It decreases the release of excitatory neurotransmitters and increases concentrations of GABA, an inhibitory neurotransmitter.
- The drug has high oral bioavailability in cats with a mean half-life of 2.8 hours. In humans, gabapentin is eliminated unchanged by renal clearance.

Clinical use

- Indications: neuropathic pain, for instance persistent postsurgical pain (e.g. amputation, chronic pain syndrome after feline onychectomy), feline orofacial pain syndrome, diabetes-induced neuropathy, DJD-related pain, cancer-related pain.
- Administration of gabapentin (10 mg/kg PO every 12h) for 2 weeks in a clinical trial in cats with clinical and radiographic signs of DJD, improved owner-assessed mobility scores.

However decreased activity, measured with accelerometers, was observed when compared with placebo, probably resulting from sedation, a known potential side-effect of gabapentin.

- In research cats with DJD-related pain, gabapentin (10 mg/kg PO every 8h) decreased central sensitization when compared with placebo treatment.
- Recommended dosage regimens have not been determined. Doses up to 50 mg/kg PO every 12h have been reported anecdotally. This emphasizes the need for individualized therapy based on the degree of pain, response to therapy and development of adverse-effects.
- Although adverse-effects such as sedation generally subside within a few days of gabapentin administration, it may be reasonable to initiate treatment at low doses and increase it slowly (e.g. weekly) until an analgesic response is observed. This approach may avoid profound sedation/ataxia which could be discouraging for some owners. Moreover, this approach should be used in cats with renal disease that may have impaired clearance and increased incidence of adverse-effects.
- Discontinuation of treatment for chronic conditions should be done gradually to avoid breakthrough pain. This may be achieved by slowly decreasing the dose/intervals of administration over the course of a few weeks.
- An analgesic trial of 3-6 weeks is recommended and may be required before any effect is apparent.

Contra-indications

- No serious contra indications have as yet been reported for cats.

Amitriptyline

- Amitriptyline is a tricyclic antidepressant. It produces analgesia via serotonin and norepinephrine reuptake inhibition, NMDA antagonism and blockade of calcium channels.
- A PK study in cats (5 mg/cat PO or transdermal) revealed poor transdermal absorption when compared with oral administration.

Clinical use

- Indications: neuropathic pain; it may ‘boost’ the endogenous descending inhibitory pathways and reverse central sensitization.

- In cats with chronic interstitial cystitis that had failed other treatments, administration of amitriptyline (10 mg/day PO every 24h) for 12 months completely resolved the clinical signs in 9/15 patients during treatment. Response to treatment was usually observed after 7 days. Reported adverse-effects included sedation, weight gain and decreased grooming. However, another study reported conflicting results. Cats with acute interstitial cystitis treated with amitriptyline (5 mg/day PO every 24h) for 7 days did not show any benefit when compared with placebo.
- Since interstitial cystitis is a syndrome resulting from several different mechanisms rather than a disease with a single cause, some cats may still benefit from treatment with amitriptyline (in association with environmental enrichment).
- The emotional component of depression in chronic pain may be targeted by amitriptyline, although efficacy data are not available for cats.
- Other examples of tricyclic antidepressant drugs include imipramine and clomipramine.
- There are anecdotal reports describing the use, in cats, of other antidepressants such as serotonin-specific reuptake inhibitors (fluoxetine), serotonin and norepinephrine reuptake inhibitors (venlafaxine and duloxetine) and monoamine oxidase inhibitors (selegiline), but information about these medications is very limited.
- An analgesic trial of 3-6 weeks is recommended and may be required before any effect is apparent.

Contra-indications

- Amitriptyline should be administered with caution in combination with drugs that affect serotonin uptake or metabolism due to the risk of serotonin toxicity; this includes monoamine oxidase inhibitors and tramadol.

Amantadine

- Amantadine was originally used as an antiviral agent. The drug is a NMDA receptor antagonist and increases dopamine concentration in the CNS.
- Amantadine (5 mg/kg PO and IV) has high oral bioavailability in cats. Its efficacy has not been documented in this species but it shows potential for pain therapy.

Clinical use

- Indications: conditions characterized by central sensitization (e.g. DJD-related pain, cancer-related pain).
- Amantadine should be administered in combination with other analgesics (i.e. multimodal analgesia) including opioids, NSAID, tramadol and amitriptyline. It is not used as a 'stand-alone' treatment for chronic pain.
- Amantadine significantly improved physical activity when compared with placebo in dogs with OA that were treated concurrently with meloxicam. This might be an option when pain is refractory to NSAID therapy.
- An analgesic trial of 3-6 weeks is recommended and may be required before any effect is apparent.

Contra-indications

- Currently unknown

Emerging analgesic modalities

- Anti-NGF monoclonal antibodies: Nerve growth factor is pronociceptive and may contribute to peripheral and central sensitization. Species-specific antibodies that target NGF are currently under investigation for treatment of DJD in cats. Early reports indicated adequate safety and efficacy for up to 6 weeks after a single SC injection in cats with naturally-occurring disease.
- Stem cell therapy: Stem cell therapy have well-documented anti-inflammatory and immunomodulatory effects. Mesenchymal stem cells can differentiate into a variety of cell types. They are collected from the bone marrow or adipose tissue for example, isolated and transplanted to tissues in need of regeneration. This therapy has been under investigation in humans with DJD, albeit several applications exit such as gingivostomatitis, a severe oral inflammatory disease. A recent study involving a small number of cats with severe refractory gingivostomatitis reports the IV administration (2 injections, one month apart) of autologous mesenchymal stem cell therapy. Oral biopsies and clinical evaluation before and after treatment showed complete remission or substantial clinical improvement in 5/7 cats. The impact of pain in these cats was not systematically evaluated, although it may be presumed

that pain and discomfort were reduced due to reduced inflammation. Further research may confirm the applicability of this treatment in the management of pain in cats with chronic inflammatory conditions such as DJD or gingivostomatitis.

- Piprants: They are prostaglandin receptor antagonists (PRA), a new class of selective compounds. What differentiates them from NSAIDs is that they act further down in the inflammatory cascade by selectively antagonizing the prostaglandin E2 EP4 receptor; thus, maintaining normal activity of COX enzymes. The EP4 receptor has been identified as the primary receptor involved with pain and inflammation in DJD. Gapiprant is a new analgesic and anti-inflammatory drug in the piprant class that is labelled for the control of pain and inflammation associated with DJD in dogs (Galliprant®). This drug was safe and effective when administered long-term in the target population (2 mg/kg PO every 24h, for 28 days). Moreover, the daily administration of gapiprant of doses up to 15 mg/kg for 28 days was well tolerated in research cats. Efficacy and safety data in cats with DJD may be available in the near future.

Other analgesics

- Pregabalin: an anticonvulsant with a mechanism of action similar to gabapentin. Data from cats are not available but it has potential benefit for treatment of maladaptive pain. Cost could be a limitation.
- Tapentadol: a centrally acting analgesic with a dual mechanism of action (μ opioid receptor agonist and noradrenaline reuptake inhibitor). In humans, it is often used for treatment of chronic pain as it has fewer adverse-effects than opioids. In cats, tapentadol produced short-term (approximately 2 hours) thermal antinociception (50 mg/cat); however, it was reported to be highly unpalatable which could be a limitation in the clinical setting.
- Codeine: its analgesic effects appear to depend on metabolites such as codeine-6- glucuronide, norcodeine and morphine. Codeine did not produce thermal antinociception in cats. Its role in the treatment of chronic pain remains unknown. Some codeine formulations are combined with acetaminophen (paracetamol) which is contra-indicated in cats due to the risk of toxicity.
- Maropitant: a central inhibitor of emesis and a NK-1 receptor antagonist that blocks the effects of substance P, a major pain neurotransmitter. In sevoflurane-anesthetized cats, the

administration of maropitant decreased the minimum alveolar concentrations of sevoflurane during ovarian ligament stimulation. Similar findings are reported in dogs. Although maropitant is effective in treating or preventing vomiting, and has anesthetic-sparing effects to visceral stimulation, its analgesic effects per se remain controversial.

- Bisphosphonates (e.g. pamidronate, zoledronate, tiludronate): these inhibit bone resorption due to osteoclast activity and produce analgesia for bone cancer pain. Some studies reveal a good therapeutic profile in dogs with osteosarcoma, especially when combined with NSAIDs. Tiludronate has shown some efficacy in experimental OA in dogs. Although there are no studies investigating the analgesic effects of bisphosphonates in cats, its administration is justifiable in terms of cancer biology. In a retrospective pilot study involving cats with invasive bone cancer, the administration of pamidronate (1-2 mg/kg IV every 21 to 28 days) was feasible and safe; no discomfort was observed during or after IV administration and no adverse-effects were not observed, except in cats undergoing concomitant chemotherapy.

Non-pharmacological treatment

The benefits of non-pharmacological treatment should not be underestimated. Although there is less scientific evidence on the efficacy of these techniques in veterinary patients, their importance in treatment of chronic pain in humans has been increasingly recognized. These techniques should be used in combination with pharmacological treatment (integrative medicine) with the ultimate goal of improving QoL.

Physical activity and weight control

Physical deconditioning results from a sedentary lifestyle leading, among other changes, to a decrease in lean body mass. Physical deconditioning makes a major contribution to chronic pain and is therefore an important therapeutic target. It may also be a consequence of chronic pain and the whole process becomes a vicious cycle. Sedentary lifestyle and obesity are now common in animals and treatment should include changing the attitude towards physical activity. Regular exercise is beneficial for both physical and mental health of the patient and for treatment of chronic pain.

Environmental enrichment

- A critical component in treatment of chronic pain as it increases activity and decreases pain.
- Environmental enrichment is low-cost and easy to implement. Owners should be encouraged to be creative and allow cats to choose their preferred environmental enrichment.
- Detailed description of all environmental needs of cats is published elsewhere (http://www.catvets.com/guidelines/practice-guidelines/environmental-needs-guidelines). Owners should provide multiple environmental resources that stimulate interest and physical activity such as scratching, playing, hunting and chasing (Figure 8).
- Scratch posts (vertical, horizontal, diagonal) should be available in different parts of the house. These must provide good stability, have a wide base and allow the cat to stretch fully. Owners must be educated that scratching behavior is part of the nature of the cat, and scratch posts may increase the level of activity and at the same time decrease destruction of household items.
- Toys (wrapping tapes, artificial prey, feathers, etc.) should be offered. These should stimulate hunting behaviors such as stalking, chasing, pouncing and biting. They should be rotated around the house to maintain interest.
- Food can be hidden within different toys to encourage foraging and hunting behavior.
- Elevated surfaces, ramps and stairs offer opportunities for playing and jumping.

Weight Control

- Obesity negatively affects musculoskeletal health due to increased loading forces on joints, but also due increased circulating pro-inflammatory cytokines (i.e. chronic low-grade inflammation). The latter is a metabolic consequence of obesity since adipose tissue is capable of secreting cytokines and pro-inflammatory proteins and plays a role in the development of DJD.
- Weight control can be achieved with restricted-calorie diets and increased physical activity.

Figure 8. Commercially available and homemade options for environmental enrichment

Observe how these provide excellent opportunities for playing and exercising, resulting in increased physical activity, weight control, and mental stimulation. Food is hidden within some toys to stimulate hunting behavior. Scratch posts are important for scratching and stretching. Other toys or condos stimulate running or jumping.

- Research in dogs shows that decreased food intake is associated with decreased progression of OA. A weight control program in dogs with OA decreased lameness and improved objective variables measured using kinetic gait analysis. Similarly, decreased kinetic measures have been observed with weight gain.
- Cats may be at increased risk of hepatic lipidosis if weight loss occurs too quickly. Therefore, a nutritional dietary program should be set up and be carefully monitored.

Physical therapy, Massage, Acupuncture and Transcutaneous electrical nerve stimulation (TENS)

These modalities are said to increase blood flow to the areas of attention as well as increasing the activity of descending inhibitory pain pathways.

Physical therapy and Massage

- Cats with chronic pain may have muscle wasting, joint stiffness and body tension. Physical therapy aims to prevent and treat these conditions in a controlled fashion. Stretching and exercises to enhance joint range of motion and muscle strength are performed.
- Several techniques are available, including passive range of motion, treadmill activities, water exercise (Figure 9) and stair exercise. These should be performed or supervised by individuals with appropriate training in physical therapy and rehabilitation.
- Research in pediatric patients indicates that massage significantly reduces the degree of stress, pain and discomfort.

Figure 9. A 15-year-old female cat undergoing physical therapy session (water treadmill) for management of degenerative joint disease

This cat was overweight and the exercise helped with weight loss and muscle strength, contributing to the multimodal management of her chronic pain.

- Massage techniques as well as passive range of motion exercises can be taught to owners and may be used to alleviate muscle pain. A rubber brush (also known as "zoom groom") canbe used for massaging and as an aid with grooming in cats that are unable to do this.

Acupuncture

- Acupuncture needles are inserted into anatomically predefined points (acupoints) which may be also stimulated by digital pressure (acupressure) or electric current (electro-acupuncture).
- The mechanism of action is not fully understood but may be related to the effects of neuromodulation due to neuronal gating and release of endogenous opioids. Acupuncture can indeed be antagonized by naloxone.
- Acupuncture has been recognized by the US National Institutes of Health as a modality for treatment of chronic pain. It inhibits nociceptive transmission and inflammation, produces muscle relaxation, and decreases joint compression. It also restores blood flow, improving oxygen and nutrient distribution to the affected site. The efficacy of acupuncture has been demonstrated in human and canine patients with chronic pain.
- Acupuncture treatment is minimally invasive and generally well tolerated by most patients (Figure 10); some cats become relaxed and may sleep during treatment.
- In chronic pain states, 3-5 sessions are required before clinical improvement can be detected, and as seen with pharmacological treatment, not all cats will respond. Acupuncture should be performed cautiously in cats with neuropathic pain since needle insertion can induce pain if allodynia and central sensitization are present.
- Acupuncture can be recommended in a range of maladaptive conditions such as DJD-related pain, intervertebral disc disease and secondary muscle stiffness, radiation-induced neuropathic pain, bone cancer pain and visceral pain secondary to intestinal bowel disease.
- Treatment should be performed by certified, trained acupuncturists.
- As with pharmacological treatment, not all cats will respond.

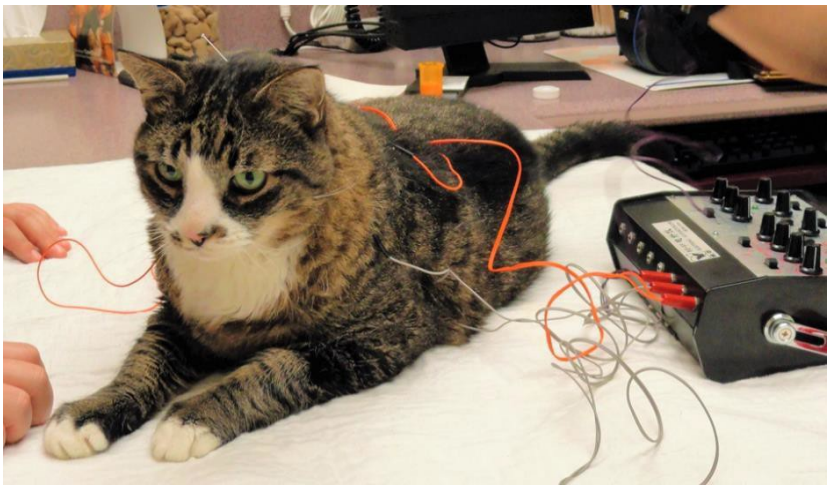

Figure 10. A cat undergoing electro-acupuncture therapy session for management of chronic pain.

Needles are inserted in acupoints and connected to an electric current. Observe how the cat is relaxed (body position and facial expression) and accepts well the treatment.

Transcutaneous Electrical Nerve Stimulation

- Electrodes (patches) are placed over the shaved skin of a painful area and electrical impulses are used to stimulate the area resulting in analgesia, increased blood flow and decreased muscle stiffness.
- Protocols include sensory-level TENS (high-frequency low-intensity) and motor-level TENS (low-frequency high-intensity). A combination of both protocols (i.e. mixed-frequency) is usually applied to prevent adaptation and accommodation to a set protocol.
- The mechanism of action of TENS is not fully understood, but conditioned pain modulation (i.e. the CNS modulates or "filters" its response to incoming electrical activity) may be involved. In this case, stimulation of cutaneous structures may reduce transmission of nociceptive input from the spinal cord to the brain utilizing the gate mechanism.
- TENS may be used in cats with maladaptive pain such as chronic musculoskeletal pain and neuropathic pain.
- TENS is contra-indicated in patients with a pacemaker and over open wounds or healing skin.

Degenerative joint disease (DJD)

Treatment of DJD-related pain should employ a multimodal approach and is dependent on the severity of the disease and feasibility of the treatment (Figure 11).

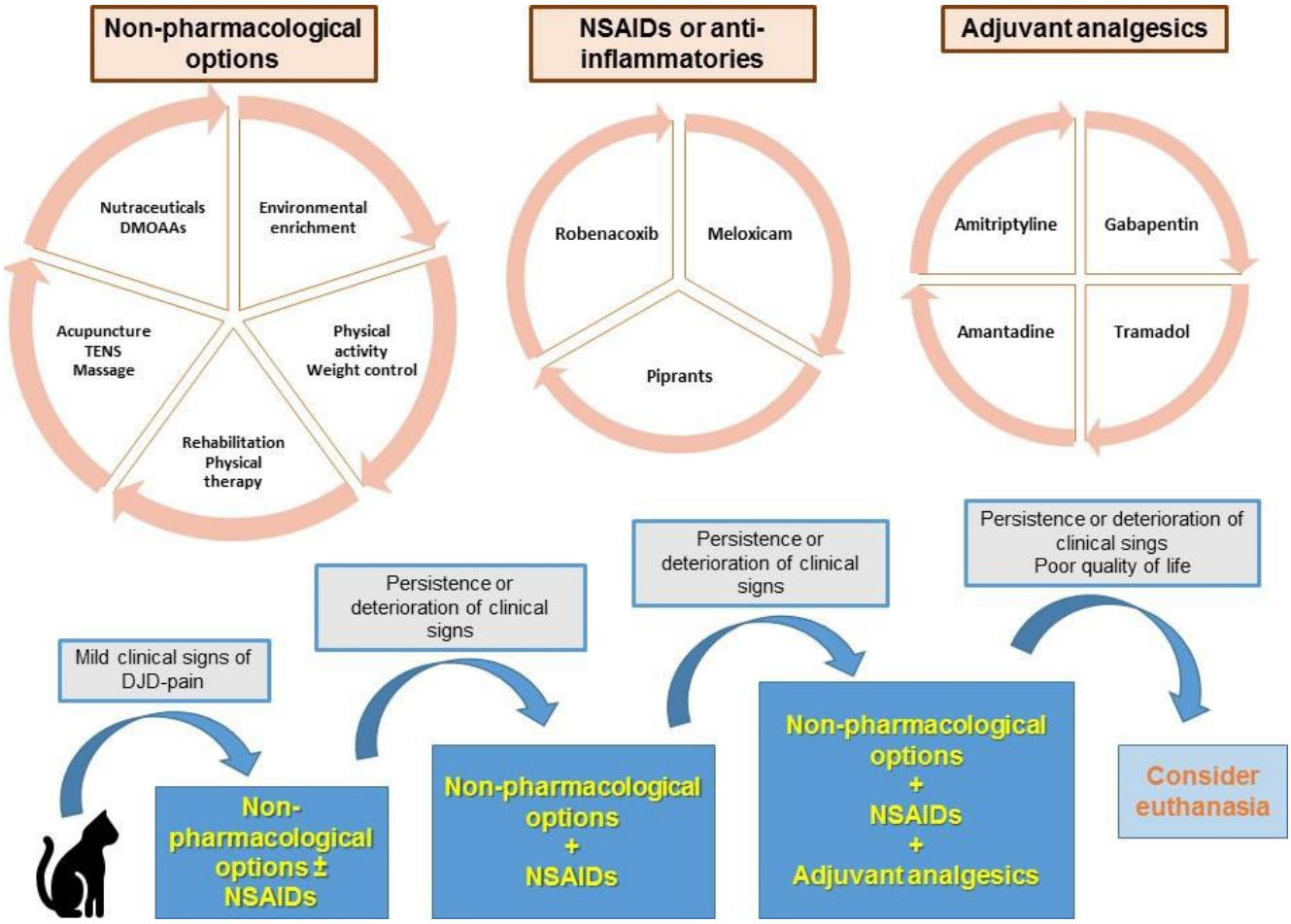

Figure 11. Multimodal approach for the management of degenerative joint disease (DJD) in cats based on the severity of clinical signs

Treatment options are added according to the progression of the disease. Ideally, cats with mild DJD should be managed without the administration of analgesics. As the disease progresses, non-steroidal anti-inflammatories (NSAIDs) are added to the treatment. Finally, in cats with severe DJD, adjuvant analgesics are added to the protocol. Several options (pharmacologic and non-pharmacologic) can be used in a in a "trial and error" manner.

DMOAAs = disease-modifying osteoarthritic agents; TENS = transcutaneous electrical nerve stimulation.

Analgesics

DJD produces pain secondary to joint inflammation, amplified nociceptive input and central sensitization. Clinically, cats with DJD show decreased activity and QoL.

- Long-term therapy with NSAIDs increases activity and mobility without adverse effects
- Some cats with DJD may have central sensitization and allodynia. Pain becomes refractory to treatment with NSAIDs and non-pharmaceutical therapies. Drugs such as gabapentin, amantadine, tramadol or amitriptyline may be considered. With the exception of tramadol and gabapentin, most adjuvant analgesics have not been systemically investigated in cats with OA-related pain. Nevertheless, they have been widely used with apparent good therapeutic and safety profiles.

Physical activity and weight control

The role of increased activity and weight control in the treatment of DJD-related pain cannot be overemphasized. Increased mobility is beneficial for bone and joint health, muscle tone and consequent joint stability. In humans, an intensive lifestyle intervention characterized by weight loss and exercise may prevent development of joint pain in patients at risk.

Nutraceuticals

Nutraceuticals are products derived from food sources that are purported to provide extra health benefits other than nutrition. They are administered orally and are increasingly being used

- Cats with DJD that were fed a supplemented joint diet were more active than cats fed a standard diet. The supplemented diet was high in fish oil-derived eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and was supplemented with green-lipped mussel extract and glucosamine with chondroitin sulfate.
- Recent reviews on the use of nutraceuticals in dogs and humans recommend their use in management of DJD-related pain despite the lack of high-quality studies.
- Cats with mild DJD may benefit from these diets in combination with weight control and environmental enrichment. Cats with moderate to severe DJD may also require treatment with analgesics.

Chondroprotective agents

Chondroprotective agents, or disease-modifying osteoarthritic agents, are precursors of cartilage matrix. They favor matrix synthesis and repair articular cartilage. They are generally considered most effective in the earlier stages of OA.

- Glucosamine and chondroitin sulfate: these are popular chondroprotective agents. However, there is limited information on their efficacy in both humans and animals. Administration of oral chondroprotective agents for 30 days was safe in cats based on clinical pathology. In cats with DJD-related pain, treatment with glucosamine and chondroitin sulfate was not superior to meloxicam. Although, there are no known contra-indication to their administration, nutraceuticals can become expensive for long-term treatment, and this must be taken into consideration in the treatment protocol since their efficacy is questionable.
- Polysulfated glycosaminoglycan (PSGAG): inhibits certain catabolic enzymes that are overexpressed in DJD-joints due to chronic inflammation. Known by the commercial name of Adequan®, this drug is FDA approved for use in dogs and horses with strong evidence of efficacy. Little is known about its use in cats, but general recommendations include SC administration (1-5 mg/kg every 4 days for 6 doses).
- Pentosan polysulfate: a semi-synthetic glycosaminoglycan that inhibits and modulates pro-inflammatory mediators. Known by the commercial name of Cartrophen Vet® and available in many countries, this drug is labeled for the management of DJD in dogs with unclear evidence of efficacy. In cats with interstitial cystitis, a few reports describe its use with no clinical success. In cats with DJD, its use is anecdotal (3 mg/kg SC every 5-7 for 4 doses) and its analgesic effects remain to be investigated.

Summary

There is no ‘one size fits all’ treatment for every maladaptive condition. Treatment should be tailored to each individual and may be changed and adapted according to the acceptance by the patient, response to therapy, development of adverse effects and patient- owner compliance. There is little scientific evidence for most of the treatment options presented in this chapter. A treatment plan will be decided in conjunction with the owner considering all available pharmacological and non-pharmacological options.

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