ABSTRACT

ADRIAN, DEREK EVAN. Maladaptive Pain in the Cat. (Under the direction of Dr. B. Duncan X. Lascelles and Dr. Mark G. Papich).

Chronic pain affects over 40% of adult humans in the United States. Few new and effective treatment options are available for these chronic pain sufferers because of a high failure rate (90%) of new therapeutics, perhaps partly stemming from the use of inappropriate, induced animal models. Degenerative joint disease (DJD), a chronic inflammatory and painful condition of the joints, may affect 90% of the 94+ million pet cats in the United States. An estimated 40% of those cats with radiographic evidence of DJD experience pain and disability secondary to the condition. This high prevalence of a naturally occurring painful disease, combined with the cat’s shared environment with humans, makes the cat an attractive model for analgesic research. Developing the cat as a model requires filling gaps in our knowledge of effective analgesics and their pharmacokinetics relevant to clinical use, and developing and refining tools for measuring chronic pain in cats.

First, we identified these gaps in our knowledge by reviewing the current status of tools to measure chronic (or maladaptive) pain, and pharmacological data surrounding potential analgesics. These gaps include our ability to detect or monitor pain and disability, in our knowledge regarding analgesic medications, and an inconsistent application of the currently available tools in clinical research.

Next, we assessed our ability to detect and measure treatment-associated improvements in pain and disability via a randomized, double-masked, clinical trial evaluating the efficacy of a COX-2 selective non-steroidal anti-inflammatory drug (NSAID), robenacoxib, for the treatment of DJD-associated pain in cats. Our aim was to identify gaps in the translational cat model, and improve treatment options available to veterinarians. We detected modest improvements in both activity and owner assessments of pain and disability. Given that NSAIDs are the mainstay of treatment for DJD across species, we expected more significant results. Although several explanations for our results exist, this study reinforced our observations that the current approaches to measuring chronic pain in cats require refinement and improvement, which includes the need for development of a novel, objective measure.
Our next goal was to determine the current prescribing practices of veterinarians for the treatment of chronic musculoskeletal pain in cats, to guide future research into potential analgesics of both veterinary and translational interest. A veterinarian-distributed survey collected data on respondent demographics, medications prescribed, and typical dosing regimens. Gabapentin, a medication without either evidence of analgesic efficacy in veterinary species or pharmacokinetic data relevant to the typical dosing regimen, was prescribed by 71.0% of respondents. Because gabapentin is used for maladaptive pain conditions in people, understanding its efficacy in the naturally occurring chronic pain cat model would benefit veterinary medicine, and would increase our understanding of the translational utility of the cat model.

We then investigated the pharmacokinetics of gabapentin in cats because data for long-term dosing was needed. We determined the effects of twice daily oral dosing for two weeks on the drug’s pharmacokinetics, and evaluated the systemic absorption of a transdermal preparation. Repeated oral dosing did not significantly affect drug kinetics; therefore, adjustments are not necessary for chronic administration. We also determined that the transdermal preparation used was poorly absorbed and therefore not a viable dosing strategy.

Our final study aimed to develop and evaluate the feasibility, repeatability, and sensitivity of a novel, objective measure of nociception in cats. The nociceptive withdrawal reflex (NWR) test is used in human analgesic research to investigate both central mechanisms of nociception, as well as medications affecting these central processes. We found testing was feasible, and occasionally repeatable; however, we were unable to detect differences between healthy and DJD-affected cats. Future exploration of the NWR requires higher-powered studies.
Maladaptive Pain in the Cat

by
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A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Comparative Biomedical Sciences

Raleigh, North Carolina
2019

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DEDICATION

This dissertation is devoted to my wife, my family, and my friends, for their continued support during this research.
Derek Adrian is originally from Durham, North Carolina. He earned a Bachelor of Science in Zoology from North Carolina State University in Raleigh, NC to prepare for veterinary school. He also obtained his Doctorate in Veterinary Medicine from North Carolina State University College of Veterinary Medicine (NCSU CVM). Following this, he worked as both a general practitioner in veterinary private practice and as a clinical trial investigator at the NCSU CVM. He remained at the NCSU CVM for his graduate studies. His academic interests include pharmacology, chronic pain and degenerative joint disease in companion animals, translational medicine, clinical research, and analgesics.
ACKNOWLEDGMENTS

I would like to thank my committee co-chairs, Duncan Lascelles and Mark Papich, for their continued mentoring, guidance, teaching, and time during my research. Without their dedication to me, I would not have been able to complete these works. My other committee members, Ronald Baynes, Sam Jones, and John Harris all heavily contributed to my research, representing the diversity of backgrounds, experience, and knowledge I found necessary to draw on during these years. Their willingness to read numerous drafts, analyze unending amounts of data, and direct me both personally and educationally were vital to my progress.

Robert Reed and Jane Owens provided mentoring and teaching throughout my training and career, which I am thankful for.

I would also like to thank both Andrea Thomson and Lyndy Harden-Plumley for their repeated assistance during these projects- their skills and knowledge were invaluable. I must also thank the entirety of the Translational Research in Pain lab, including Morika Williams, Constanza Meneses-Evans, Bea Belda-Lopez, Masataka Enomoto, Carrie Muller, Margaret Gruen, Samuel Chiu, Laura Minema, Jonathan Hash, Rachel Meyers, Lauryn Braxton, and Kayla Freeman for their collaboration, feedback, and comradery.

I must also thank those that assisted in my various projects, including Delta Dise, Jim Yeatts and Danielle Mzyk for their expertise and guidance with pharmacokinetic analysis and mass spectronomy. I must also thank the entirety of the Central Procedures Lab and Laboratory Animal Research for their care and protection of the cats that participated in my studies, and for their assistance coordinating my research. I thank Janice Harvey and Brigid Troan for lending me their histopathology expertise. I am also thankful for Jonathan King, Rudolph Parrish, Stephen King, Steven Budsberg, and Gabriella Sandberg, who were all instrumental in our clinical trial. I also thank Jo Murrell and Vicki Simmonds for their training and guidance regarding the NWR test. This research also would not have occurred without all of the cats, cat owners, and veterinarians that participated- Thank you.

Lastly, I must thank all of my friends and family who supported me throughout these endeavors. I must especially thank Mandesa, who pushed me to be my best both personally and professionally all of these years.

Without all of you, I could never have achieved so much.
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Chapter 1 Foreword

The Unmet Need of Chronic or Maladaptive Pain

Chronic pain is a major economic and public health crisis in the United States, affecting over 40% of adults and costing more than $600 billion annually.\textsuperscript{1,2} Unfortunately, new and effective treatment options for chronic pain in humans are lacking because of a high failure rate (90%) among therapeutics entering Phase 1 studies.\textsuperscript{3} This includes medications such as Substance P/NK-1 antagonists that were promising in their originating animal models, but failed to translate into efficacy in humans.\textsuperscript{4} Poor fidelity or face validity of the animal models and pain assessment tools used during research and drug development may contribute to this failure rate.\textsuperscript{5-7} For example, peripheral nerve ligation in the rodent and its resulting hypersensitivity to evoked pain is used to simulate neuropathic pain conditions. Yet the majority of neuropathic pain patients suffer from spontaneous pain,\textsuperscript{8} and the major sensory change is hypoesthesia.\textsuperscript{9} Similarly, acute animal models of osteoarthritis (OA) often include injection of an inflammatory or toxic substance into the joint space, whereas chronic, traumatic, or idiopathic etiologies are more common in human patients.\textsuperscript{10-12} Therefore, to evaluate treatment for chronic pain, a high fidelity model for both drug development and mechanistic exploration of the condition is needed.

Patients with chronic pain may develop secondary central changes in nociception that lead to facilitated or enhanced pain processing, termed central sensitization (CS).\textsuperscript{13} Central sensitization is caused by increased synaptic efficiency or decreased inhibition of primary afferent neurons in the dorsal root ganglion or dorsal horn neurons of the spinal cord, effected by cytokines (e.g., prostaglandins), neurotransmitters (e.g., Substance P) and glial cells.\textsuperscript{13} Other patients with chronic pain may have deficient Endogenous Analgesic Systems (EAS).\textsuperscript{14,15} The EAS sends descending signals that tonically or dynamically dampen noxious input, controlling the amount or intensity of pain signals reaching the brain. Identification and treatment of these central nociceptive changes is therefore important for clinical management of chronic pain in these subpopulations.

Translational chronic pain research could be improved with spontaneous, or non-induced, animal models.\textsuperscript{16} For example, OA in dogs and cats appears to closely mirror the biomechanical, histological, and molecular features of the condition in humans.\textsuperscript{11,17-20} Animal models would better represent the variability and pathophysiology seen in humans with chronic pain conditions, in contrast to the uniform presentation and histologically disparate features of induced models.
Degenerative Joint Disease (DJD), a chronic inflammatory disease of the joints that is inclusive of OA, affects approximately 90% of all 94+ million pet cats in the United States. Of these cats, an estimated 40% experience secondary pain and disability (unpublished findings from 21). Because DJD and chronic pain are both common and spontaneous in cats, the use of this model reduces risks inherent to induced models. Furthermore, people share their environment with their pet cats, and both species are self-motivated in their activities. There is extensive research that has developed tools assessing pain and disability secondary to DJD in the cat. These tools include owner-based assessments termed clinical metrology instruments (CMIs), and collar-mounted activity monitors that record acceleration and movement data (“accelerometers”; AMs). Novel study designs have also been implemented to improve the ability to separate treatment and placebo responses. Recently, research has aimed to detect signs of CS or altered nociception in cats, including the use of Quantitative Sensory Testing (QST).

The Spontaneous Feline DJD-Associated Pain Model

Subjective Assessment Tools

Questionnaires termed Clinical Metrology Instruments (CMIs) have become heavily used in veterinary chronic pain research. These questionnaires are completed by owners to evaluate the patient’s perceived pain and disability, or impact of disease (e.g., DJD) on the patient’s daily activities. Two questionnaires have been developed and used in multiple placebo-controlled clinical study in client-owned cats – the Feline Musculoskeletal Pain Index (FMPI) and the Client Specific Outcomes Measure (CSOM).

The FMPI is comprised of 17 standardized activities, and asks the owner to rate their cat’s ability to perform each on a 5-point Likert-type scale with options from “Normal” to “Not at all.” An option for “Don’t know or not applicable” also exists for activities that the owner is unable to assess, or for activities that are not relevant to the cat (e.g., Climbing stairs for a cat housed in a single-level residence). The CMI includes two questions for the owner to rate their cat’s preceding and current pain levels. Whereas the FMPI activities are standardized and unchanging, the CSOM is tailored to each individual cat/owner situation. The CSOM requires owners to select three activities that are important for their cat’s quality of life. The cat’s ability to perform each activity or task is rated by owners on a 5 point scale ranging from “No Problem”
to “Impossible.” These two CMIs may be complementary or even synergistic in a study, by providing both global (FMPI) and individualized (CSOM) assessments of the patient’s pain or disability.

A tool focused on determining changed activities or behaviors noticed by owners with DJD-affected cats, and categorized the findings into four domains: mobility, activity, grooming, and temperament. This tool asked owners to rate any abnormalities on a severity scale of 1 (mild) to 10 (severe), and provided examples of activities for owners to consider during the assessment. The Montreal Instrument for Cat Arthritis Testing, for use by caretaker/owner (MI-CAT[C]) requires further refinement and validation.

Most feline DJD research has included global assessments of quality of life (QoL) following treatment phases. These global measurements of QoL are included because pain impacts quality of life in addition to disease-specific metrics (e.g., activity counts, jumping) in humans, and therefore should also be measured in analgesic trials for a complete assessment of treatment effects. There is a strong association between unfriendly temperaments and the presence of DJD in cats. Treatment with an analgesic drug is associated with improved temperament. The description of “Grumpy on contact with [“other cats” or “other animals including owner”]” was included in its questionnaire, leading to the speculation that it may be possible to simplify the measure into patient happiness, and maintain responsiveness. However, these QoL outcome measures are not validated in the cat.

Assessment of treatment effects using CMIs is especially affected by the high placebo response in feline analgesic research, highlighting the importance of tool validation with placebo controlled studies. Only the FMPI and CSOM have been validated and used in multiple studies.

Validation is especially important for these subjective tools, to ensure that the questionnaires are reliable and measure the appropriate parameters. Table 1F.01 below summarizes placebo-controlled, blinded studies that have been used to assess both CMI and therapeutic. Validation of a CMI does not make it infallible, nor does it convert a subjective measure into an objective one. Indeed, the FMPI and CSOM are still prone to the significant placebo responses observed in feline arthritis research. One method to combat this risk of placebo response is to take each outcome measure into consideration as part of a whole picture—should a CMI indicate improvement in a cat that is supported by a concomitant
increase/improvement in activity measures, the researcher may be more confident that the effect seen is a true treatment effect. This has been termed “criterion validation,” assuming that activity, being an objective measure, is the “gold standard.” This is probably not true, and this approach is better termed cross-validation. The current status of cross-validation of feline chronic pain CMIs is summarized in Table 1F.02, indicating which studies have shown a concurrent improvement in both CMIs and activity measures.
Table 1F.01: Studies validating CMIs in feline analgesic research.

<table>
<thead>
<tr>
<th>Study</th>
<th>CMI</th>
<th>Face Validity and Item Generation</th>
<th>Cronbach’s α</th>
<th>Concurrent Validity</th>
<th>Test-Retest Repeatability</th>
<th>Responsiveness Validity</th>
<th>Extreme Group Validation</th>
<th>Criterion Validity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lascelles²³</td>
<td>CSOM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Zamprogno⁴²</td>
<td>FMPI</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Benito²⁴</td>
<td>CSOM</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>FMPI</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Gruen³¹</td>
<td>CSOM</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>FMPI</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Gruen³³</td>
<td>CSOM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>FMPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Klinck³⁶</td>
<td>MI-CAT(C)</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

CMI = Clinical Metrology Instrument; CSOM = Client Specific Outcomes Measure; FMPI = Feline Musculoskeletal Pain Index; MI-CAT (C) = Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/owner.
Table 1F.02 – Feline Outcome Measure Cross Validation.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Activity</th>
<th>FMPI</th>
<th>CSOM</th>
<th>QOL</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lascelles</td>
<td>Meloxicam</td>
<td>+</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>Serial</td>
</tr>
<tr>
<td>Lascelles</td>
<td>Diet</td>
<td>+</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>Parallel</td>
</tr>
<tr>
<td>Gruen</td>
<td>Meloxicam</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Parallel</td>
</tr>
<tr>
<td>Gruen</td>
<td>Anti-NGF Ab</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>N/A</td>
<td>Parallel</td>
</tr>
<tr>
<td>Adrian</td>
<td>Robenacoxib</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Parallel</td>
</tr>
<tr>
<td>Guedes</td>
<td>Tramadol</td>
<td>+</td>
<td>N/A</td>
<td>+*</td>
<td>**</td>
<td>Crossover</td>
</tr>
<tr>
<td>Guedes</td>
<td>Gabapentin</td>
<td>+ (decreased)</td>
<td>N/A</td>
<td>+*</td>
<td>**</td>
<td>Crossover</td>
</tr>
</tbody>
</table>

Table includes previously reported studies utilizing multiple outcome measures. Activity = Activity Monitors. FMPI = Feline Musculoskeletal Pain Index. CSOM = Client Specific Outcome Measures. QOL = Quality of Life. Design = Study design employed.

+ = significant treatment effects observed.
- = significant treatment effects not observed.
* = Significant effects seen after modification of original CSOM scores.
** = Data not analyzed for significance or not sufficiently reported.
The Placebo Response in Feline DJD

While placebo-controlled double-blinded studies have become standard practice, feline DJD research has experienced a dramatic placebo response rate.41 This meta-analysis found placebo effect sizes as high as 1.93 (95% CI 1.16-2.7), with a corresponding treatment over placebo effect size of -0.35 (-0.98 to 0.28). There are several potential explanations for this placebo effect. A caregiver placebo response can occur when an owner reports improvement following a placebo intervention. Suggested causes for this caregiver placebo response include the “better care effect,” where caregiver ratings on subjective measures are improved by better access to healthcare and more frequent follow-up.41 The owner’s desires to please the investigator or for a trial to be successful can also cause the caregiver placebo response. Subjects may also experience apparent improvement because of a “regression towards the mean,” or the natural waxing (when owners seek enrollment) and later waning of clinical signs of pain and disability. An owner’s expectations of improvement following administration of a treatment (active drug or placebo), and increased observations of the cat and subsequent altered interactions can also result in the placebo response.41

Alternative study designs have emerged as one way to diminish the placebo response. Inclusion of a baseline period between study screening and enrollment may reduce the impact of regression towards the mean. One alternative to the classic parallel group design is inclusion of a masked washout period. It was hypothesized if both the treatment and placebo responses were similar with initiation of a medication or placebo, that owners would be more sensitive to deterioration following masked withdrawal of the investigational drug. One feline analgesic study was able to demonstrate this significant deterioration response in cats previously receiving the NSAID meloxicam.26 This is similar to the RW portion of the Enriched Enrollment Randomized Washout (EERW) study design used in human medicine.45

Objective Assessment Tools

Activity is affected by chronic pain in humans,46 and activity monitors (AMs) have been used and reported in veterinary osteoarthritis research in recent years and offer an objective method to measure activity.38,47-49 Most commonly used devices are either pedometers or accelerometers. While pedometers report a step count for the selected time period, accelerometers instead report a non-standardized “activity count,” which prevents direct
comparison of outputs between studies using different monitors. However, several early studies have evaluated the correlation between AM output and actual patient movement or behaviors, generally showing AMs as an acceptable proxy for movement.\textsuperscript{47,50} Although inter-device variability,\textsuperscript{50} and effects of patient conformation\textsuperscript{51} have been observed in dogs, this has not been reported for cats. Investigators have hypothesized and tested the theory that DJD-associated pain and disability produces decreased activity in cats as detected by AMs, and activity can be improved or restored to normal levels with an effective analgesic.\textsuperscript{23,26,37,43} While these studies have used changes in activity counts as an outcome measure, it is not known what changes are anticipated after administration of an effective analgesic. It is also possible that effective analgesics could decrease activity, or result in increased activity via other drug effects (e.g., opioid-associated increases in activity in cats). For example, in the aforementioned studies with gabapentin and tramadol in cats with chronic osteoarthritis,\textsuperscript{37,38} treatment with tramadol increased activity and gabapentin decreased activity, yet both manuscripts reported effectiveness.

Determining the optimal methods to use to analyze activity data has been hampered by a relative lack of understanding of the factors influencing activity in cats. Cat activity can be affected by human presence/interaction, as demonstrated in both research-housed and privately-housed cats.\textsuperscript{47,48,52} Variation in owners’ schedules can result in day to day and weekday to weekend effects on cat activity in both normal and DJD-affected cats.\textsuperscript{48} Cats show a more pronounced crepuscular pattern of activity during weekdays correlating with owner departures or arrivals, which becomes muted during weekends when owner presence is more constant.\textsuperscript{48} Because of high inter-cat variability in activity, some studies have focused on the individual’s percentage change in activity compared to baseline, instead of using summary values (e.g., mean activity counts/period time), or absolute value changes.\textsuperscript{26,43,53} Cats spend a significant portion (up to 70-80%; observation) of the day inactive, suggesting that perhaps analysis should focus on periods of activity, instead of analyzing total daily activity. Research-purpose cats with naturally occurring OA (OA-research) were reported to be less active than healthy cats during the nighttime period,\textsuperscript{54} with significant increases in activity in these OA-research cats following administration of meloxicam or tramadol alone, but not in combination.\textsuperscript{28,29}

Another approach to data analysis is functional data analysis, which compares patterns of activity as a whole, rather than collapsing the data down to individual values or time periods.\textsuperscript{48} The study found that while total average activity was not significantly different between healthy
and DJD-affected cats, healthy cats exhibited more pronounced peaks of activity at times, compared to a smoother pattern in DJD-affected cats. Future methods of activity data analysis may focus on time spent in specific “states” of activity (e.g., high vs low intensity). Finally, there is interest in developing algorithms or devices that can detect specific activities (e.g., climbing stairs, jumping onto furniture, or sleeping) or differences in activity signatures between normal and DJD-affected cat. Some manufacturer’s websites claim the ability to perform this analysis, without evidence or validation to support the claim.

Measures of Somatosensory System Function

Quantitative Sensory Testing

In people, QST has demonstrated utility for detecting alterations in pain processing and sensitivity that can accompany knee and hip osteoarthritis. This modality applies a thermal (hot or cold), mechanical, chemical, or electrical stimulation to a patient, recording outcomes such as the threshold for pain detection, or the stimulus intensity or duration required to elicit withdrawal of the tested limb. The underlying presumption is that central sensitization, or maladaptive pain, results in lowered thresholds or reflex latencies, and higher sensitivity. Phenomena such as facilitated temporal summation can also be investigated, by delivery of repeated instead of single stimuli. However, QST is ultimately subjective, as its outcome measures require either subject-reported pain, or observer-determined withdrawal response. Patients can be categorized, or phenotyped, based on their responses to the various QST stimuli. A study evaluating the NSAID etoricoxib in patients with OA found treatment-related effects on QST outcomes.

The growing evidence and use of QST in human medicine has led to an interest in the modality in veterinary species, including cats. Early work in OA-research cats showed reduced mechanical thresholds when compared to normal cats, as well as shorter latencies of response to mechanical temporal summation (RMTS). Two additional studies in OA-research cats have evaluated RMTS, motor activity, and gait analysis, focusing on treatment effects of tramadol alone or administered with meloxicam. While meloxicam treatment increased motor activity (including nighttime activity) and gait metrics, treatment with tramadol (alone or in combination with meloxicam) resulted in increased nighttime activity and reduced sensitivity to RMTS. It is undetermined whether excitation secondary to tramadol’s opioid effects contributed to the
results, though mydriasis and euphoria (common opioidergic adverse effects) were noted in several cats. Finally, repeatability and discriminability of both thermal (hot and cold) and mechanical threshold testing in client-owned cats with OA compared against normal controls has been reported.\(^{30}\) While the group found only moderate repeatability between testing sessions, the results did indicate lower mechanical thresholds in OA-affected cats. The results also demonstrated a lower frequency and shorter duration of paw lifts to cold stimulation in cats with forelimb OA. This effect was not seen in cats with hind limb OA, nor were there any differences seen with hot plate stimulation.

The Nociceptive Withdrawal Reflex Test

The Nociceptive Withdrawal Reflex (NWR) test is similar to QST, in that the response to a noxious stimulus is measured. However, the response of interest with NWR is an electromyogram (EMG) recorded from a withdrawing muscle, making the modality truly objective.\(^{59}\) Limb withdrawal is elicited in response to a stimulus, which is typically direct electrical activation of local nerves.\(^{59}\) The withdrawal reflex was first described as a flexion reflex to noxious stimuli in decerebrate cats.\(^{59}\) The test/reflex includes both flexion and extension reflexes occurring to effect limb withdrawal.\(^{60,61}\) The movement produced can be described as the result of recruitment of a collection of reflexes required to withdraw a limb from a noxious stimulus, dependent on the site of the insult. The NWR test measures the EMG response of one or more of these reflexes. Typically, the area under the curve (AUC) or integral of the rectified (absolute value) response is used to measure the EMG response. The distinct conduction velocities and subsequent separation of motor responses of the myelinated A and unmyelinated C neurons also allows focused evaluation of differential effects of treatments or pathologies on either neuron population.\(^{62-64}\) The “early” response seen on EMG tracings (latency varies by species) correlates with Aβ (non-noxious and noxious) and Aδ (noxious) fiber stimulation, while the “late” or second response is produced by C fiber stimulation.

NWR was first described over a century ago in spinalized or decerebrate dogs and cats. It is used in CNS-intact animals and humans, whether awake, sedated, or anesthetized.\(^{15,65-69}\) The NWR can diagnose and measure CS.\(^{68,70,71}\) Increased synaptic efficiency or altered neuronal excitability seen with CS facilitates the reflex arc, and can result in decreased response thresholds, or increased EMG area in response to supra-threshold stimuli. Temporal summation
(TS), or the phenomenon where an increasing response is observed when a stimulus of unchanging intensity is repeatedly delivered, can be facilitated with CS, resulting in a greater total EMG area to fewer stimuli in affected subjects, and increased pain in response to repeated stimuli, compared to the non-CS state.

The modality can also explore the functionality of the endogenous analgesic system via Conditioned Pain Modulation (CPM) or Diffuse Noxious Inhibitory Control (DNIC) testing, where a “conditioning” stimulus is delivered to a remote limb prior to or during delivery of the original NWR testing stimulus. Both DNIC and CPM include a testing stimulus (e.g., noxious heat, cold, chemical, electrical, or mechanical stimuli) measured before and after application of a conditioning stimulus (any of the above stimuli) at a distant body site/limb from the testing stimulus. Human and animal testing uses different terminology because of the previous research and available information. DNIC was used in both animals and people originally because of the similarity of testing paradigms, despite the differences in recording sites and patient manipulations (e.g., spinal transection was only performed in animal subjects). Separate terms are now used because animal-based research explored and elucidated specific mechanisms and pathways, whereas human research evaluated the net clinical effects of numerous pathways. The term CPM will be used in this thesis because of the clinical and translational nature of the research in spinally-intact cats. With an intact EAS, the conditioning stimulus is expected to result in a reduced or ablated response to the NWR testing stimulus due to conditioning stimulus-activated descending analgesic mechanisms projecting to the level of the spinal cord.

The NWR test has explored differences between normal and affected individuals, including subjects with acute induced pain and inflammation, those with chronic OA, and even human patients with cluster headaches, by measuring CS and EAS. People with pain (acute or chronic) have facilitated withdrawal reflexes or facilitated nociceptive processing (CS), in addition to less functional EAS. NWR testing may be more invasive or require more advanced equipment than QST, but it objectively evaluates both spinal (CS) and supraspinal (EAS) mechanisms that contribute to nociception. NWR testing examines the pathophysiology associated with pain conditions, and can help define the pain phenotype of the individual.

An individual’s “pain phenotype,” or the underlying pathophysiology of their chronic pain, can affect the response to treatment. Gabapentinoids, which include gabapentin and
pregabalin, are effective for treating some patients with CS, such as those with fibromyalgia.\(^{13,80}\) The gabapentinoids exert their action via binding to and inhibiting of trafficking of the α2δ-1 subunit of voltage-gated calcium channels. These channels become upregulated in the dorsal root ganglion and dorsal horn of the spinal cord in models of neuropathic pain, which coincides with facilitated pain transmission and central sensitization.\(^{81,82}\) Increased activity of α2δ-1 subunits on Ca\(^{2+}\) channels produces greater calcium influx in response to an action potential, leading to greater neurotransmitter release and increased synaptic transmission of signals.\(^{83}\)

Patients with a dysfunctional EAS may be less capable of muting or blunting nociceptive signals. The EAS consists of projections from the rostral ventral medulla and dorsolateral pons, descending to the dorsal horn of the spinal cord, exerting its actions via α2 adrenoreceptors and 5-HT-ergic receptors in the superficial layers of the dorsal horn.\(^{84}\) Duloxetine, a serotonin and norepinephrine reuptake inhibitor, has been successfully used for treatment of chronic pain conditions because it potentiates this EAS system.\(^{85}\) EAS efficiency and response to duloxetine is correlated as measured using the NWR test, with EAS-deficient patients experiencing a greater improvement in their reported pain.\(^{86}\)

Thus, it becomes fully apparent that a patient’s “pain phenotype” should be considered when treating chronic or maladaptive pain, because the underlying phenotype may determine which analgesic drugs will be most effective. NWR testing has been proposed as one method to define the individual patient phenotype, and hence was an important focus of our work.

_Treatment of Chronic Maladaptive Pain in Cats with DJD-Associated Pain_

The following manuscript served as a literature review of chronic or maladaptive pain in the cat. The manuscript focused on tools for the condition’s detection and measurement, and treatment options (excluding NSAIDs) from a veterinary clinical perspective.
Chapter 1: Introduction - Chronic maladaptive pain in cats: A review of current and future drug treatment options
Chronic maladaptive pain in cats: A review of current and future drug treatment options
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Abstract

Despite our increasing understanding of the pathophysiology underlying chronic or maladaptive pain, there is a significant gap in our ability to diagnose and treat the condition in domestic cats. Newer techniques being used to identify abnormalities in pain processing in the cat include validated owner questionnaires, measurement of movement and activity, and measurement of sensory thresholds and somatomotor responses. While some data are available evaluating possible therapeutics for the treatment of chronic pain in the cat, most data are limited to normal cats. This review details our current understanding of chronic or maladaptive pain, techniques for the detection and measurement of the condition and the associated central nervous changes, as well as an overview of the data evaluating potential therapeutics in cats.

Keywords: Maladaptive pain; Cats; Analgesia; Central plasticity; Chronic pain
Introduction

While cats have become a very popular pet worldwide— with an estimated 75+ million in the US alone, the assessment and treatment of pain in cats has lagged behind that of dogs. Though this knowledge gap is diminishing, most information on pain control in cats exists regarding peri-operative analgesic use, with chronic pain conditions still being undiagnosed and under-treated. Chronic pain situations typically don’t have easily identifiable inciting incidents and the behavioral changes develop slowly and are often subtle. This makes measurement of chronic or long-standing pain conditions difficult, and although recent progress has been made in the development of tools to assess chronic pain, our ability to measure chronic pain lags behind that of acute pain in veterinary species. The relative lack of validated methods of chronic pain assessment contributes to our inability to assess efficacy of analgesics for the alleviation of such pain in cats. This review details our current understanding of chronic or maladaptive pain, techniques for the detection and measurement of the condition and the associated central nervous changes, as well as an overview of the data evaluating potential therapeutics in the cat.

Methods

The literature review was performed by searching on several databases, including PubMed, CAB Abstracts, and Google Scholar. Specific search for medications of interest were based on: personal experience, use, or knowledge, anecdotal reports of use or efficacy, recommendations and guidelines for the treatment of pain in cats, medications being currently researched, etc. Keywords used included: pain, chronic pain, maladaptive pain, feline, feline pain, osteoarthritis, degenerative joint disease, analgesics, pharmacokinetics, efficacy, etc.

Chronic Maladaptive Pain

Chronic pain has been defined in human medicine as any pain that lasts more than 3-6 months, but the relevance of this timeline to veterinary species with considerably shorter lifespans should also be considered. Different disease conditions like cancer may also affect the timeline, as it may not be prudent to “delay” treatment, or pathologies where the normal healing and recovery period is expected to be much shorter. This difficulty in clearly demarcating the transition from acute to chronic pain has led to a growing realization that previously termed
acute and chronic pain are actually on a continuum, and alternative definitions may be more useful in the context of understanding pain and how to treat it. Recently, the terms ‘adaptive’ and ‘maladaptive’ have been suggested as terms that better describe pain (Figures 1 and 2). Adaptive pain encompasses both nociceptive and inflammatory pain. Nociceptive pain is only activated by high-threshold noxious stimuli, including stimuli that cause tissue injury. Inflammatory pain occurs after tissue damage and produces heightened sensitivity of the tissue associated with a classical inflammatory response. Both of these types of pain are considered protective, or ‘adaptive’ pain in that they serve to sense and/or avoid actual or potential tissue damage. These typically have an easily identifiable cause (surgery, injury, etc.), and are reversible. Maladaptive pain, on the other hand, is not protective, and is primarily due to plastic changes in the pain processing system. It can be further divided into neuropathic pain, which is pain resulting from direct damage to neural tissue, and functional pain, where there are no neural lesions or inflammation, and pain is driven by dysfunction or malfunction of the nociceptive system. Classically, neuropathic pain is thought of as resulting from gross, obvious damage to the spinal cord, or obvious damage to peripheral nerves such as with peripheral nerve sheath tumors or surgical trauma. However, increasingly it is recognized that many diseases, such as osteoarthritis (OA) and cancers, may involve a degree of peripheral neuropathy via either direct damage to nerve endings present in the tissues, or via increased innervation that accompanies joint remodeling and angiogenesis. This explains the neuropathic pain-like symptoms reported in many human patients with OA. Similarly, the obvious example of functional pain is phantom limb pain or fibromyalgia—there is no evidence of a peripheral lesion or inflammation, yet there is increased sensitivity to stimuli and spontaneous pain. Yet increasingly, it is recognized that many conditions, such as OA, have a component of functional pain—changes in the central nervous system function that heightens sensitivity or results in spontaneous pain. It has been previously suggested that there is a central or maladaptive drive to pain in a significant portion (20-40%) of human patients suffering from osteoarthritis-associated pain. This underscores the importance of understanding the driving factors of a patient’s pain, as one patient may suffer from multiple types.

Central to the concept of maladaptive pain is the phenomenon of central plasticity (also referred to as central sensitization), initiated through cellular wind up. While wind-up is a neuron’s increasing response/output resulting from repeated, identical stimuli, central plasticity
is the global response that lasts autonomously after the conditioning (original) stimulus has been discontinued, or is sustained with low level nociceptor input from the periphery \(^{13}\). This results in a stronger painful reaction to a less intense (hyperalgesia) or previously innocuous stimulus (allodynia), and increases in the receptive fields of neurons, or the region of tissue that a neuron functionally innervates and responds to stimuli in. Central plasticity is driven by changes at various levels of the sensory transmission axis – primary afferent fiber, spinal cord and higher centers. In general, the processes driving central plasticity are a combination of increased neuronal excitability, facilitated synaptic transmission and decreased inhibitory influences \(^{13}\). However, as well as a gain in function, some processes are down-regulated (loss of gain) and so the term central plasticity is preferred over central sensitization.

Clinical long-standing pain (chronic pain) is a complex mixture of adaptive (inflammatory) and maladaptive (neuropathic, functional) pain. It is likely that different neurobiological processes are responsible for the different components of long-standing pain, but it is also likely that there is tremendous overlap. Most information about the processes involved in the maladaptive component of long-standing pain have been derived from work in rodents, using models of neuropathic pain. A multitude of mechanisms play varying roles in maladaptive pain states, and a laudable clinical goal would be to be able to understand the mechanisms responsible for pain in an individual, and so make informed choices about analgesics. Currently it is impossible to predict the mechanisms responsible for the pain state in individual patients, however, progress is being made in this area, with recent studies in humans testing the function of the endogenous analgesic mechanisms to predict response to analgesics \(^{86,102}\).

Most chronic diseases that are associated with pain consist of several different pain components, including both an active, sustained inflammatory component (as in degenerative joint disease, gingivostomatitis, and others) as well as the maladaptive pain with associated neuronal changes and sensitization \(^{13,103,104}\). Although it is not easy to clinically recognize inflammatory versus maladaptive pain states, there is increasing recognition that many common long-standing diseases are associated with central plasticity, and so maladaptive pain. Indeed, it was recently shown that dogs with OA have measureable central sensitization indicative of maladaptive pain \(^{105}\). Commonly occurring diseases that are possibly associated with a component of maladaptive pain in the cat include osteoarthritis and degenerative joint disease, interstitial cystitis, gingivostomatitis, diabetic neuropathy, cancers, ocular pathology (including
glaucoma, chronic anterior uveitis), dermatological conditions (including chronic infections, burns, slow-healing wounds, secondary effects of radiation therapy), and others \textsuperscript{106}. 
**Figure 1.01: Schematic illustration of Adaptive Pain.**

**Nociceptive Pain** - A noxious stimulus (red starburst) activates high-threshold nociceptive primary afferent sensory neurons (red/yellow line) with cell bodies in the dorsal root ganglion (DRG), and termination in the dorsal horn (DH). Here, the afferent signal is transmitted to the second order neuron via mono- or multi-synaptic processes, and crosses over to the other side of the spinal cord, then transmitted to the brain via ascending tracts in the spinal cord (red arrow), where it is interpreted as a warning of actual or potential tissue damage. There is tonically active descending inhibition (green line) from the CNS (channeled via the rostro-ventromedulla) that helps control whether the information from the primary afferent neuron is blocked at the level of entry into the DH of the spinal cord.

**Inflammatory Pain** - Local tissue damage results in release of inflammatory mediators, recruitment of inflammatory cells and further release of inflammatory mediators. These mediators either sensitize sensory nerves, or directly stimulate them, resulting in a lowering of thresholds in sensory nerves and generation of action potentials (nociceptive signals). These signals are carried by afferent neurons (red line) with cell bodies in the DRG and terminals in the DH. As before, ascending fibers carry the nociceptive input to the brain along ascending tracts (red arrow), and descending inhibitory signals (green line) may dampen down the input at the level of the spinal cord. The increased sensitivity in the periphery associated with inflammatory pain following tissue damage promotes protection of the area, allowing it to heal.
Adaptive Pain

Noxious stimuli (e.g. Heat)

Inflammation & Tissue Damage

Tissue damage

Nociceptor sensory neuron

Early warning system

Adaptive, high threshold pain

PROTECTIVE

Tenderness promotes repair
Figure 1.02: Schematic illustration of Maladaptive Pain.

**Neuropathic Pain**—Physical damage to nervous system tissue (e.g. in this case, a tumor - yellow circle) results in very abnormal activation of nociceptor sensory neurons – they become activated in response to previously sub-threshold stimuli (blue circle). The subsequent pathway is as described in ‘Adaptive’ pain, but at the level of the Dorsal Root Ganglion (DRG) and the dorsal horn of the spinal cord there are changes (nervous system plasticity) resulting in amplification of the signals and facilitation of throughput of the signals. Additionally, the tonically active descending inhibition is less effective (illustrated as a dashed green line), which again facilitates the signals being transmitted from the periphery to higher centers. Hypersensitivity (increased pain from a stimulus that normally provokes pain) and allodynia (pain due to a stimulus that does not normally provoke pain) occur as a result of these changes, and in addition, spontaneous pain can occur due to abnormal activity in the nervous system (e.g. generated at the site of nervous system injury). A hallmark of ‘neuropathic pain’ is the presence of actual physical damage to part of the nervous system and it is this that drives the changes in the way the system functions.

**Functional Pain**—In Functional Maladaptive pain, the nervous system is grossly normal – there is no physical damage of the system. However, the functioning of the system is abnormal. This abnormal central processing results from repeated input to the system, causing nervous system plasticity (changes in neurons and changes in the way supporting elements [e.g. microglia] communicate with neurons) and thus amplification and facilitation of the processing of nociceptive information. Under these conditions, a previously sub-threshold stimulus (blue circle) activates a physically normal nociceptor (red line) but abnormal central processing in the spinal cord or brain (inset) results in the stimulus being interpreted as painful. As with neuropathic pain, descending inhibition may be defective (dashed green line). Hypersensitivity (increased pain from a stimulus that normally provokes pain) and allodynia (pain due to a stimulus that does not normally provoke pain) occur as a result of these changes, and in addition, spontaneous pain can occur due to abnormal activity in the nervous system.
Maladaptive Pain

Neuropathic pain

Normal low threshold stimuli

Nervous system structural damage (e.g., peripheral nerve damage)

Neural lesion (e.g., damage, tumor)

Abnormal central (spinal cord or brain) processing

Nociceptor sensory neuron

Maladaptive low-threshold pain

Hypersensitivity & Allodynia
Diseased state of the nervous system

Functional pain

Physically normal nervous system

Abnormal central (spinal cord or brain) processing
It is important to clarify that chronic pain can exist on a continuum, and can in fact be comprised of multiple driving mechanisms. In some chronically painful conditions, the driving condition may start and remain as inflammatory pain, with an easily understood coupling of peripheral disease with degree of pain. In these painful conditions, nonsteroidal anti-inflammatory drugs (NSAIDs) are expected to be effective. However, it is likely that in many cases, the ongoing nociceptive input into the nervous system, along with damage to nerve endings as a result of the peripheral disease process can cause changes in the central nervous system and therefore produce maladaptive pain. It is this maladaptive component that makes chronic pain difficult to treat. Hence the search for novel, non-NSAID therapies that can be used along with, or in place of, NSAIDs. At this moment, we are limited in that we cannot clinically differentiate between maladaptive pain, and pain with a purely inflammatory drive.

**Assessment of Chronic Pain in Cats**

Recently, progress has been made in the assessment of chronic pain in the cat using owner questionnaires, called Clinical Metrology Instruments (CMIs). The two most studied CMIs are the Client Specific Outcome Measures (CSOM) and Feline Musculoskeletal Pain Index (FMPI) \(^{23,24,31-33,44}\).

The objective measurement of movement or activity also have been developed as methods to assess the impact of chronic/maladaptive pain and its treatment. Recently, activity monitors that record changes in acceleration associated with movement have been used as an objective outcome measure of mobility in cats \(^{26,44,54}\). Cats are fitted with a small accelerometer on a collar or harness, and allowed to move about normally in their home environment. This the tool can discriminate between normal and affected research cats \(^{54}\), and can even be used to show treatment effects in client-owned affected cats \(^{23,26,27,43,44}\).

To understand the mal-function of the somatosensory system present with maladaptive pain, methods evaluating sensorimotor function are needed. Quantitative sensory testing (QST) involves the measurement of the stimulus (mechanical, thermal hot/cold, etc.) strength or frequency of application required to elicit a withdrawal or response (e.g. head turn, limb withdrawal) by the patient, with the end of the test usually determined by observation of the response. It is useful for semi-objectively assessing changes in sensation, especially in relation to central plasticity, and its associated allodynia, hyperalgesia, enhanced temporal summation (an
increasing response to repetitive stimuli), etc. While QST is in its early development in cats, it can discriminate between healthy, non-affected cats, and those with OA. Other methods such as the measurement of Nociceptive Withdrawal Reflexes (NWR) have been explored in dogs, but not yet in cats. NWR Testing measures the magnitude of the withdrawal responses to various stimuli using EMG. This modality can evaluate the threshold to elicit withdrawal, in addition to the effect on withdrawal latency and magnitude after delivering repeated stimuli (temporal summation). Data produced are objective, as opposed to the semi-objective QST methodology. NWR testing is proposed to measure central plasticity and associated changes in pain processing, and affected patients are expected to have lower thresholds and higher or stronger EMG responses.

Overall, our ability to accurately measure chronic pain is limited, and our ability to measure the maladaptive component of this pain is even more restricted. As a result, diagnosis and treatment of the disorder often involves “trial and error” on the part of the clinician.
Treatment of Maladaptive Pain in Cats

In North America, there are no drugs approved for long-term use in cats with maladaptive pain, and only one NSAID (meloxicam) is approved for long-term use in some parts of the world. Despite recent information suggesting that NSAID therapy can partly reverse central plasticity, it is generally accepted that the maladaptive component of pain conditions is poorly responsive to NSAIDs. Because there are also concerns around the potential for adverse effects from NSAIDs, interest in alternative drug therapy has emerged. Currently, drug choices are based on experience in people, or because of their activity on mechanisms shown to be important in rodent models of maladaptive pain. Medications that have been suggested for use in cats for the treatment of maladaptive pain are gabapentin, tramadol, amantadine, amitriptyline, tapentadol, flupirtine and anti-nerve growth factor antibodies (Table 1). This review outlines what is currently known about non-NSAID drug treatments that may be effective for chronic or maladaptive pain in cats.
Table 1.01: Potential therapeutics and their mechanisms for the treatment of maladaptive pain in cats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism*</th>
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<tbody>
<tr>
<td>NSAIDs</td>
<td>COX 1 and/or 2 antagonism</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>$\alpha_2\delta$-1 subunit of voltage-gated calcium channels</td>
</tr>
<tr>
<td>Tramadol</td>
<td>$\mu$-opioid receptor agonism, norepinephrine and serotonin reuptake inhibition, $\alpha$-$2$ adrenergic receptor antagonism</td>
</tr>
<tr>
<td>Amantadine</td>
<td>NMDA antagonism</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Serotonin, norepinephrine, dopamine reuptake inhibition</td>
</tr>
<tr>
<td>Flupirtine</td>
<td>G-protein-regulated, inwardly rectifying $K^+$ channel agonism, NMDA antagonism</td>
</tr>
<tr>
<td>Tapentadol</td>
<td>$\mu$-opioid receptor agonism, norepinephrine reuptake inhibition</td>
</tr>
<tr>
<td>Maropitant</td>
<td>Neurokinin 1 receptor antagonism</td>
</tr>
<tr>
<td>Grapiprant</td>
<td>Prostaglandin E4 receptor antagonism</td>
</tr>
<tr>
<td>Frunevetmab</td>
<td>Anti-body against Nerve Growth Factor</td>
</tr>
</tbody>
</table>

* Generally accepted mechanism of action. There may be differences in the cat for some of the drugs dependent on metabolism

**Gabapentin**

Gabapentin is an analogue of the neurotransmitter $\gamma$-Aminobutyric acid $^{108,109}$. Gabapentin exerts its effects on voltage-gated calcium channels, which are found on excitatory cells such as neurons. These channels respond to depolarization currents by allowing the influx of calcium ions $^{83}$. Four subunits of calcium channels have been identified, the main pore-forming $\alpha_1$ subunit, and the accessory $\alpha_2\delta$, $\beta$, and $\gamma$ subunits $^{83}$. Models of neuropathic pain have demonstrated an increase in the $\alpha_2\delta$-1 subunit in dorsal root ganglion (DRG) and dorsal horn neurons $^{81,82}$. This subunit and binding target for gabapentin is responsible for guiding or trafficking of $\alpha_1$ subunits and therefore pore assembly, indicating a vital role of the $\alpha_2\delta$-1 subunit.
in altered neuronal excitability and pain processing \(^{81-83,109}\). This binding results in a decrease in the influx of calcium ions in response to an action potential, and therefore decreased neurotransmitter release or neuronal excitability. Gabapentin has been advocated for the treatment of neuropathic pain in veterinary species because of experience treating neuropathic pain in humans \(^{80,108,110,111}\). In people it is only approved for post herpetic neuralgia, and as an adjunctive therapy for partial onset seizures, which are undocumented syndromes in animals.

The pharmacokinetics of oral (10 mg/kg) and intravenous (4 mg/kg) gabapentin in 6 adult spayed female cats has been described \(^{112}\). While bioavailability varied greatly (range: 49.6 – 118.3\%), potentially partially due to ad libitum feeding, the half-life after oral administration was approximately 3 hours (177 ± 25 min), with peak concentrations (C\(_{\text{max}}\)) values ranging from 4.6–10.6 µg/mL \(^{112}\). Previously reported data and modeling suggests a half maximal effective concentration (EC\(_{50}\)) ranging from 1.4 and 16.7 µg/mL for treatment of hyperalgesia in the rat \(^{110,113,114}\) and an EC\(_{50}\) of 5.4 µg/mL was estimated for humans with neuropathic pain \(^{115}\). The authors then suggested a dosing regimen of 8 mg/kg every 6 hours for an antihyperalgesia effect in the cat \(^{112}\). However, caution is urged when extrapolating effective concentrations of the drug in cats based on pharmacokinetic-pharmacodynamic data from other species. There is also a current lack of information on pharmacokinetics after repeated dosing. Minimal or no plasma protein binding has been reported in other species, however this should be confirmed in the cat \(^{116}\).

Currently, there are no clinical studies evaluating the efficacy of gabapentin in chronic pain conditions in cats. In a study evaluating the effects of gabapentin on nociceptive thermal thresholds in research cats \(^{117}\), six female spayed adult cats received four dosages of oral gabapentin: 0 (placebo), 5, 10, and 30 mg/kg in a crossover design. Peak plasma concentrations ranged from 6.3 ± 1.3 µg/mL for the 5 mg/kg dosage, to 25.5 ± 8.6 µg/mL after administration of 30 mg/kg. Despite these plasma concentrations, there was no significant effect on thermal thresholds. This is not unexpected as the mechanism of action gabapentin suggests it would only show efficacy when α2δ\(_{-1}\) subunits are expressed in an abnormal, hyperalgesic state.

Several case studies describing the use of gabapentin exist \(^{118,119}\). One report details chronic use of gabapentin in three cats, following road trauma (two patients) or for musculoskeletal pain (one patient) \(^{118}\). Another case report details chronic gabapentin use after traumatic incidents \(^{119}\). In these case reports there was no objective or validated assessment of
response. These individual uncontrolled case reports may not be helpful because of the high placebo effects in owner reports\textsuperscript{26,41}. Additional research evaluating safety and efficacy treating chronic or maladaptive pain is necessary before treatment recommendations should be made.

**Tramadol**

Tramadol is an opioid-like drug that exerts its effects via many different mechanisms of action including very weak \(\mu\)-opioid effects, norepinephrine and serotonin reuptake inhibition, and binding of \(\alpha_2\) adrenergic receptors in the pain pathway\textsuperscript{120,121}. The drug is formulated with mixed enantiomers, each with slightly different effects. The first metabolite, M1 (o-desmethyltramadol), may be responsible for the majority of the analgesic effect in humans through opioidergic actions\textsuperscript{122,123}.

The pharmacokinetics of oral (5mg/kg) and intravenous (2mg/kg) tramadol in cats has been described\textsuperscript{124,125}. Oral bioavailability was reported as high, at 93\% \(\pm\) 7, with a terminal half-life of 4.82 \(\pm\) 0.32hr for M1\textsuperscript{124}. The mean M1 \(C_{\text{MAX}}\) values after IV dosing were 0.37 and 0.81\(\mu\)g/mL\textsuperscript{124,125}. Both studies found a ratio of tramadol: M1 of \(\geq 1\), which contrasts with dogs which do not appear to produce the M1 metabolite\textsuperscript{126}. While more data needs to be collected about minimum effective concentration, the pharmacokinetic data collected so far is promising.

There have been two studies evaluating the efficacy of tramadol either alone, or in combination with meloxicam, in research cats with naturally occurring chronic OA-associated pain\textsuperscript{28,29}. In the first study, tramadol (3 mg/kg orally every 12 hours) was compared against placebo in fifteen meloxicam-treated cats (oral transmucosal preparation, 0.05 mg/kg every 24 hours) with radiographically confirmed OA\textsuperscript{28}. Peak vertical force (PVF, expressed as \% bodyweight), accelerometer-based motor activity (MA), and response to mechanical temporal summation (RMTS- determined by the number of subthreshold stimuli required for response) were measured at baseline, and after 21-25 days of treatment. The group found that while both cohorts showed improvement in PVF, cats receiving only meloxicam showed improvement in motor activity, and only cats receiving both meloxicam and tramadol showed improvement (increase) in RMTS.

In the second study, fifteen cats with radiographically confirmed OA, and five cats without OA were randomized to receive either placebo or tramadol (3 mg/kg PO every 12 hours) for 19 days, with a crossover following a 3-month washout period\textsuperscript{29}. Outcome measures again
included PVF, MA, and RMTS, though the PVF data set was incomplete due to technical problems. The group found that both PVF and RMTS were able to discriminate between normal and affected cats at baseline. They also found significant within and between-group increases in all outcome measures in OA-affected cats after treatment with tramadol \(^{29}\). Mydriasis, sedation, hypersalivation, vomiting, and stomatorrhagia were observed in cats receiving tramadol \(^{28,29}\). It is suspected that the reported bitter taste of the medication is responsible for the latter observations.

While additional research in a larger cohort of client-owned cats would be ideal, the pharmacokinetic data, and recent work suggesting that tramadol may help with maladaptive components of chronic pain is encouraging. Aversion to administration of medication may present a problem with clinical use, and may require compounding or reformulation.

\textit{Amantadine}

Amantadine is used both as an antiviral medication (via unknown mechanism) in human medicine, as well as for treatment of Parkinson’s, due to its modulatory effects on CNS dopamine concentrations \(^{127}\). Amantadine has also been described as an N-methyl-D-Aspartate (NMDA) antagonist \(^{128}\), resulting in its evaluation as an analgesic \(^{129}\). The NMDA receptor, and its ligand, glutamate, have long been implicated in the development and maintenance of central plasticity, via increased and sustained excitation of neurons and subsequent alterations of gene and receptor expression \(^{103,130}\). Blockade of these receptors with NMDA antagonists has been shown to both prevent the development of central plasticity, as well as treat the condition in affected animals \(^{131,132}\).

Amantadine’s use in cats stems from anecdotal reports of efficacy \(^{133}\), or from demonstrated efficacy in dogs when used in conjunction with the NSAID meloxicam \(^{33}\). In this latter study, amantadine was evaluated in dogs with OA that were not fully responsive to NSAID therapy (maladaptive pain was suspected, though not specifically assessed for), and found to be beneficial \(^{33}\). While not indicative of amantadine’s efficacy as a sole analgesic, these data suggested promise when used as a part of multi-drug therapy, or in NSAID refractory cases.

The pharmacokinetics of amantadine in six healthy adult female spayed cats has been described \(^{134}\). Treatment groups included either 5 mg/kg administered orally or an IV infusion of 0.5 mg/kg*min for 10 minutes. Oral absorption of the drug was complete. The terminal half-life was calculated as 5.8 hours and 5.4 hours for IV and oral administration, respectively. Time to
maximal concentration ($T_{\text{max}}$) after oral administration ranged between 1.5 and 5 hours, with a $C_{\text{MAX}}$ of $1.1 \pm 0.1 \ \mu\text{g/mL}$. Subsequent research aimed to evaluate amantadine’s effect on oxymorphone-induced thermal antinociception $^{135}$. A constant rate infusion (CRI) targeting the 1100 ng/mL $C_{\text{max}}$ or an equivalent volume of saline were administered in combination with increasing oxymorphone CRI concentrations ranging from 0 to 0.4 $\mu\text{g/mL}$, chosen to approximate clinically relevant doses/concentrations $^{135}$. Overall, there was no effect of amantadine on thermal thresholds, however, similar to gabapentin, amantadine may require changes present in the maladaptive pain state to exert appreciable effects. As no data exist for minimum effective concentrations, no dosing recommendations were made. The current recommendation is 3-5 mg/kg PO once daily according to other sources, likely derived from the work in dogs.

Amantadine’s mechanism of action makes it an attractive candidate for further evaluation in cats. However, clinical data showing efficacy of amantadine is currently lacking.
Amitriptyline

Amitriptyline is a tricyclic antidepressant (TCA) that exerts its effect by inhibiting reuptake of the neurotransmitters serotonin, norepinephrine, and to a lesser effect, dopamine. It has also been shown to inhibit histamine (H1) release from mast cells in vitro. While its use in veterinary medicine has been limited primarily to behavioral disorders, research in humans has demonstrated an analgesic effect in those suffering from interstitial cystitis (a urinary bladder disease with a chronic, neurogenic pain component), and the drug is commonly used to treat neuropathic pain.

Due to the similarity of interstitial cystitis in humans, and idiopathic cystitis (IC) in cats, both proposed to have a neurogenic or neuropathic pain component, amitriptyline has been evaluated for efficacy in IC. Fifteen client-owned cats with severe, recurrent IC received 10 mg PO once daily, for up to 12 months in a non-blinded study. The number of cats reported to be free of clinical signs of disease at six and twelve months were eleven and nine, respectively. No changes in the cystoscopic examinations were apparent. It is thought that the clinical improvement, combined with the lack of changes in cystoscopy findings, indicates that amitriptyline’s efficacy is limited to treatment of the pain and discomfort associated with the disorder. However, urinary retention secondary to amitriptyline’s anti-cholinergic effect is another possibility. While placebo-controlled studies have evaluated amitriptyline’s efficacy for the treatment of feline lower urinary tract disease (an umbrella term which includes IC), no benefit against placebo was appreciated. However, these studies evaluated resolution of clinical signs of urinary disease after short term administration of the drug, so placebo-controlled data evaluating clinical signs of pain after long-term administration is still necessary.

The effect of amitriptyline on segmental inhibition, a physiological process that reduces the transmission of pain signals, was evaluated in 21 adult anesthetized cats. The genders and breeds of the cats are not reported. The segmental inhibition of wide dynamic range neurons, which populate the dorsal horn and respond to all somatosensory inputs, was significantly increased by IV doses of 1.0 – 4.0 mg/kg of the drug, though no effect was seen on responsiveness to low-threshold mechanoreceptors. This may be beneficial with maladaptive pain, where amitriptyline may be able to help correct the dysfunctional inhibitory processes of the CNS that have been demonstrated in models of maladaptive pain.
There are currently no data on the pharmacokinetics of amitriptyline in the cat, which would be important for making dosing recommendations. The drug’s reported bitter taste, and potential side effects such as reduced grooming, sedation, and weight gain may limit its utilization. Validated, and if possible, objective measures should be used to establish efficacy for other chronic or maladaptive pain conditions in the cat before making treatment recommendations.

**Flupirtine**

Flupirtine is an aminopyridine drug, which is classified as a selective neuronal potassium channel opener (SNEPCO). The mechanism of action is via interaction with G-protein-regulated, inwardly rectifying K+ channels (GIRKs), a class of potassium channels separate from the voltage-gated family. Activation of GIRKs by flupirtine results in stabilization of the membrane potential by generation of a hyperpolarizing current, and thus, decreased neuronal excitability. Flupirtine also indirectly inhibits the NMDA receptor due to its role as an oxidizing agent at the receptor’s redox site, which maintains the magnesium block on the NMDA receptor.

Flupirtine has historical use in Europe for a range of painful conditions in humans, including chronic pain, migraines, musculoskeletal back pain, myofascial pain, and for postoperative pain. Opioid-sparing effects have also been demonstrated. Unfortunately, acute hepatotoxicity (some cases requiring liver transplants) has been reported in humans.

Six healthy mixed breed adult cats (3 male, 3 female) received single doses of flupirtine at 5 mg/kg IV and PO in one study. The calculated bioavailability was 39.3 ± 9.7%, with a TMAX of 2.78 h ± 0.77 after oral administration of the drug. The elimination half-life was reported as 13.67 ± 4.43 hours after oral dosing, compared to an intravenous elimination half-life of 11.31 ± 2.24 hours.

Some data exist for efficacy of the drug in an electrical tooth pulp model in dogs and cats, which revealed an ED50 of 3.5 mg/kg PO for dogs, and 3.0 mg/kg for cats. Unfortunately, the remaining evidence of efficacy is limited to non-companion animal models, including efficacy in different models of pain in rodents. Flupirtine’s novel mechanism of action makes it an attractive candidate for evaluation, though the drug’s current availability in only European and Asian countries is a limitation.
**Tapentadol**

Tapentadol is part of a new class of drugs known as MORphine receptor agonist-Noradrenaline Reuptake Inhibitors (MOR-NRI), and shares structural similarities with tramadol\(^{152,153}\). Tapentadol’s MOR affinity is 50-fold less than that of morphine, which appears to translate to a decrease in the typical opioid associated adverse effects such as pruritus, vomiting, decreased GI motility, and diarrhea\(^{153}\). It also only exists as a single enantiomer, and only the parent compound exerts the MOR-NRI affects, in contrast with tramadol. Some aspects of the drug, including its weak antimuscarinic effect, poor oral bioavailability, and weak 5-HT3 antagonism may impair its utility\(^{154}\).

Tapentadol’s disposition after IV, IM, and SC administration (5 mg/kg) in six healthy adult mixed-breed cats has been characterized\(^{155}\). Bioavailability was high, at 93.93 ± 9.91% and 90.01 ± 6.52% for IM and SQ administration, respectively. Terminal half-life was calculated to be 2.93 ± 0.86 hours, 2.28 ± 0.85 hours, and 2.05 ± 0.6 hours for IV, IM and SQ respectively. Side effects were similar to those previously reported in dogs (salivation, panting, etc.), though agitation was also seen in some cats, as is typical with opioids. There are some data evaluating the efficacy of orally administered tapentadol on thermal antinociception in cats\(^{156}\). Six healthy adult cats (4 females, 2 males) received either placebo, IM buprenorphine (0.02 mg/kg) or tapentadol (25 mg or 50 mg) orally in a randomized crossover study. Tapentadol was found to have a significant effect on skin thermal thresholds at 1 and 1-2 hours (25mg and 50mg, respectively) when compared to baseline, but not when compared to placebo. This is contrasted to buprenorphine’s efficacy at 1 and 2 hours when compared against placebo. No pharmacokinetic data was collected or reported.

Currently, only parenteral routes of administration have been evaluated, with no data on potential efficacy in the cat. These data (oral pharmacokinetics and analgesic efficacy in the cat) are needed before any treatment recommendations can be made.

**Maropitant**

Maropitant is potent and selective neurokinin-1 receptor (NK-1R) antagonist that functions as a central and peripheral anti-emetic\(^{157}\). This receptor is also shared by the ligand Substance P (SP), which has been studied for its role in inflammatory and nociceptive pathways\(^{158}\). It is likely the knowledge that maropitant may have NK-1 antagonist activity, the known role
of Sup-P/NK-1 in pain and FDA approval for maropitant (Cerenia) in veterinary species that has led to interest in evaluating the drug for analgesic effects.

The pharmacokinetics of maropitant administered both intravenously and orally (1 mg/kg) and of the drug administered subcutaneously (1 mg/kg) was evaluated in four mixed breed cats. Oral bioavailability of the drug was low at 50%, while subcutaneous administration resulted complete absorption. Across the different routes of administration, the half-life varied: 16.5, 13.1, and 17.1 hours for IV, oral, and subcutaneous administration respectively. T_{MAX} values ranged from 2-3 hours for oral administration, and 0.5 -2 hours after subcutaneous administration, with corresponding C_{MAX} values of 156 ng/mL and 269 ng/mL respectively. Variability for these reported values were quite high, likely due in part to the small sample size.

The anesthetic-sparing effects of intravenous maropitant (1 and 5 mg/kg) was evaluated in ten female cats using an ovarian stimulation model previously developed in the dog. The study found a significant Minimum Alveolar Concentration (MAC) reduction effect of both the 1 mg/kg and 5 mg/kg doses of maropitant, but they were not different from each other. However, it is important to note that MAC reduction cannot be assumed to translate into analgesia, as demonstrated by midazolam.

While initial pharmacological data are available, there is currently insufficient data for pain therapeutic recommendations to be made due to lack of efficacy data. Additionally, it is important to note NK-1 receptor antagonists have failed clinical trials for multiple painful conditions in humans. Possible causes for this include parallel pathways in the transmission of pain which reduce the importance of any one ligand or receptor, as well as a mis-interpretation of anxiolysis as analgesia in pre-clinical animal data.

**Future Medications in Development**

*Grapiprant*

Grapiprant is a selective prostaglandin E receptor 4 (EP4) antagonist that is part of a new class of drugs, the piprants, which work by blocking prostaglandin E2 (PGE2) receptors. Research has indicated that the EP4 receptor is important in mediating pain associated with both rheumatoid and osteoarthritis, as well as inflammation in general. However, this mechanism
of action can be considered similar to traditional NSAIDs, and so grapiprant may not be efficacious for pain syndromes with a primarily maladaptive drive.

While pharmacokinetic and clinical data of efficacy is available for dogs with osteoarthritis, only safety and toxicokinetic data is available in cats\textsuperscript{163-165}. Grapiprant was administered to 24 healthy cats at doses ranging from 0 mg/kg to 15 mg/kg PO once daily for a 28 day period, with pharmacokinetic sampling occurring on Days 0 and 27. The half-life was quite variable, ranging from 2.08 ± 0.51 hours in the 3 mg/kg male cat group on Day 0, to 14.12 hours in the 3 mg/kg female cat group on Day 27. The reason for this wide disparity is unknown, but potentially related to the formulation (gel capsules) and prolonged residence in the GI tract of some cats. Significant accumulation was not seen, and there did not appear to be a relation between dosage and exposure. Minor clinical pathological abnormalities were reported, including changes in clotting times and hemoglobin, but not considered clinically relevant. Most importantly however, no GI or renal abnormalities were observed, in contrast to the concerns associated with the use of COX-inhibiting NSAIDs in cats. While the drug is still in early stages of evaluation in the cat, its potential as an anti-inflammatory, analgesic drug with an apparently good safety profile is encouraging. In the future, it is hoped that more robust pharmacokinetic and pharmacodynamic data become available, particularly studies of clinical efficacy in chronic pain conditions.

\textit{Anti-Nerve Growth Factor Antibodies}

There has been a recent interest in inhibiting Nerve Growth Factor (NGF), a protein that regulates the growth, maintenance, and survival of neurons in the developing animal\textsuperscript{166,167}. It is known that NGF levels are increased within the joints of humans and dogs affected by osteoarthritis\textsuperscript{168,169}, where it can act to increase sensitivity and excitability of nociceptors, in addition to stimulating the growth of new nerve fibers into inflamed tissue\textsuperscript{166,167}. NGF has its actions via binding to a specific tyrosine kinase receptor (TrkA)\textsuperscript{166}. The resulting signaling cascade eventually produces changes in the transient receptor potential vanilloid receptor 1 (TRPV1) cation channel, which increases both the TRPV1 channel’s excitability, as well as the production of other pro-excitation proteins\textsuperscript{166}. NGF is also known to activate mast cells, whose cellular products can increase sensitization of neurons\textsuperscript{167}.
Both dog-specific and cat-specific (ranefetmab and frunevetmab, respectively) monoclonal antibodies against NGF have been developed, evaluated in pilot trials\textsuperscript{43,170}, and demonstrated efficacy. Cats were required to have both chronic musculoskeletal disease and pain, based on physical, orthopedic, and radiographic examination, as well as owner-assessed pain or mobility impairment. Cats received a single injection of either 0.4 mg/kg or 0.8 mg/kg SC and demonstrated significant improvement compared to placebo in both objective measures of activity (accelerometer data) and subjective measures (veterinarian and owner assessments). In cats, the beneficial effect on activity was observed for 6 weeks. This is the first study that demonstrated an owner-assessed significant positive effect compared to placebo for chronic pain in cats. No adverse effects were reported. Currently, the role of anti-NGF antibody in treating central processes is unclear – we know that the biologic acts peripherally, but the robust efficacy suggests that there may be some modulation of central processes as well.

Pharmacokinetic data for NV-02 (the felinized antibody) is available for doses ranging from 2 mg/kg to 28 mg/kg SQ in 8 healthy cats\textsuperscript{171}. T\textsubscript{MAX} had a reported range of 1.9-4.3 days, and the half-life ranged from 7 to 15 days.

The data available for the felinizied anti-NGF antibody, including a long duration of action with few adverse effects, is promising. This biologic would be beneficial for patients in which oral administration of medications is not possible, or in patients where NSAIDs are not indicated. More pharmacologic and efficacy data is needed.

On a related note, development of anti-TNF\textsubscript{α} biologics have been reported, which may also be efficacious in maladaptive pain states, given the role of TNF\textsubscript{α} in maladaptive pain\textsuperscript{172}. However, insufficient information for discussion is available at this time.

**Conclusion**

Much is known about the neurobiology of chronic and maladaptive pain in rodent models, but conditions associated with maladaptive pain in cats have been recognized. Despite the interest in maladaptive pain in cats, assessing and measuring the pain still remains challenging and this hinders the assessment of putative analgesics. Treatment of chronic pain states in the cat thus remains a challenge, as only NSAIDs have extensively been clinically evaluated for long-term analgesia efficacy and safety. However, there are several drugs with mechanisms of action that make them attractive for the treatment of maladaptive pain, including
gabapentin, tramadol, amantadine and others. More data on the pharmacokinetics and pharmacodynamics of these drugs in cats is needed to guide treatment.

Declaration of Interest

Dr. Lascelles is a paid consultant for Aratana Therapeutics (grapiprant) and Nexvet (anti-NGF antibodies), and has received research funding from Nexvet. Drs. Adrian, Papich, Baynes, and Murrell have no conflicts of interest to disclose.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Chapter 1 Afterword

Since publication of our review paper, 173 additional relevant data have become available. Most notably, two studies evaluating the efficacy of gabapentin or tramadol for the treatment of OA in cats were published. 37,38 In the first study, tramadol was administered orally to 24 cats with OA at dosages of 0, 1, 2, and 4 mg/kg twice daily in a randomized crossover design for five days, with a two day washout period. The results showed significant improvements in activity and owner assessments at the 2 mg/kg dose, though these improvements were not seen when cats received 4 mg/kg. These doses also resulted in adverse effects such as dysphoria, sedation, decreased appetite, and diarrhea. The study’s activity data analysis included only comparisons of group total activity means and Area Under the Curve of activity, and not comparisons of percent changes from baseline, or partitioning of data by photoperiod. The extent of opioid-mediated excitation’s impact on the increased activity is unknown. Tramadol’s use should be limited until analgesic efficacy can be confirmed, and adverse effects are minimized.

In the second study, gabapentin was administered orally to 20 cats with OA at 10 mg/kg twice daily for two weeks in a randomized crossover-design study. Activity analysis was performed in the same manner as in the tramadol study.37 As expected, many cats experienced sedation during gabapentin administration, which appears to have translated into significantly decreased activity counts when compared against placebo treatment. This decreased activity is contrary to the general recommendation of exercise for chronic musculoskeletal pain in humans. 174 However, there were improvements on owner assessments of their cat’s pain and disability. It is unknown whether the reported sedation would have long-term effects on perceived patient comfort or quality of life. Analgesic efficacy should be demonstrated via additional outcome measures before further use. This could include more advanced activity data analysis, or collection of QST or NWR data.

Finally, one study evaluated the pharmacokinetics of pregabalin administered orally to six cats at a dose of 4 mg/kg. 175 Elimination half-life was reported as 10.4 ± 2.6 hours, with peak concentrations of 8.3 ± 1.6 µg/mL at 2.9 ± 1.2 hours. The authors noted that concentrations remained above previously reported therapeutic concentrations (seizure control) for 17.6 ± 6.2 hours. Sedation was noted in four of the six cats, and the authors suggested, without supporting evidence, that twice-daily administration of a lower dose (1-2 mg/kg) may achieve effective concentrations while avoiding adverse effects.
Chapter 1 Concluding Remarks

The preceding introduction and manuscript summarized the current literature surrounding chronic/maladaptive pain, its measurement, and its treatment in cats. The high prevalence of spontaneous DJD and its associated pain in cats, their shared environment, and self-motivation of activity make the cat a potential translational model for chronic pain in humans. However, while veterinary medicine has refined and developed multiple tools for measuring chronic pain and disability associated with DJD in cats, further refinement of the model is necessary. This includes demonstrating the ability to detect analgesic efficacy via high-quality clinical trials, generation of additional pharmacological data on medications with translational interest, and further development of newer modalities such as QST and NWR/CPM for assessing central changes in nociceptive processing.
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Chapter 2: Evaluation of the efficacy and safety of robenacoxib for the treatment of degenerative joint disease-associated pain and inflammation in cats: A randomized and masked clinical trial
Introduction

Outcome measure development and validation for DJD in cats is difficult because of a dearth of analgesic data, resulting in outcome measures being tested against therapies of unknown efficacy. It is unknown whether an outcome measure fails to detect a treatment effect because it is insensitive or invalid, or because the treatment used failed to produce a therapeutic effect. For the treatment of DJD, NSAIDs have the most robust evidence of efficacy, and with the exception of gabapentin, tramadol, and a monoclonal antibody, are the only medications that have been evaluated in the cat. The efficacy data for gabapentin and tramadol are minimal and were not published at the time of our study, and the anti-NGF monoclonal antibody is not available for use. The need for further development of outcome measures to advance DJD-associated pain in cats as a translational model, and for efficacy evaluation of new or previously untested analgesics for chronic pain in cats, led to the planning of the study evaluating the COX-2 selective NSAID robenacoxib, presented below.

Robenacoxib was selected for this study because of its previously demonstrated analgesic, anti-hyperalgesic, anti-inflammatory, and anti-pyretic actions in either kaolin-injection or acute musculoskeletal/surgery pain models in the cat. Pre-clinical research has also demonstrated a favorable safety profile with a wide therapeutic index, which may stem from the drug’s prolonged residence in inflamed tissue and short systemic half-life.

We included outcome measures developed for client-owned cats – objective activity monitoring and several subjective assessment tools. Activity monitoring is an objective outcome measure used in several previous studies. We also included several CMI (see Chapter 1), due to their previously demonstrated utility and validity in feline analgesic trials. These CMI included the CSOM, for a more individualized owner assessment of the cat’s pain and disability, as well as the FMPI, as a more standardized global assessment. Despite their lack of validation in the feline literature, we also included owner assessments of the cat’s QoL and temperament, because of the impact chronic pain can have on QoL, and their demonstrated correlation with the presence of DJD in cats (see Chapter 1).

This study was funded by Elanco Animal Health to evaluate the efficacy (and safety) of robenacoxib in cats with painful DJD. However, this study also presented an opportunity to evaluate the responsiveness of the QoL and temperament assessments, as well as an opportunity
to further refine and assess outcome measures like AMs and CMIs. We hypothesized that we would detect treatment-associated increases in activity (measured objectively by AMs), and improvement in: subjective owner-perceived mobility impairment and pain, temperament, happiness, and QoL. We further hypothesized that we would observe deterioration in outcome measures following masked discontinuation of robenacoxib, as compared against cats that continued to receive the medication. Finally, we hypothesized that we would observe no significant differences in adverse event rates between robenacoxib and placebo treatments. This work will provide a thorough understanding of veterinary medicine’s current capabilities to detect treatment-associated improvements in pain and disability in cats, and would expose any gaps and weaknesses in need of further research.

Materials and Methods

This study was approved by the Animal Care and Use Committees at North Carolina State University College of Veterinary Medicine (NCSU CVM; protocol 14-009-O), Novartis Animal Health (protocol GSO-13-007), and the University of Georgia College of Veterinary Medicine (UGA CVM; protocol CR-447). Written owner consent was granted in each case after verbal discussion of the study.

The manuscript was prepared after consultation of the CONSORT checklist for reporting of parallel-group randomized trials.¹²

Study Design

This study was conducted in compliance with Good Clinical Practices and was a double-blind, placebo-controlled, randomized study with three groups (Table 2.01).¹³ Treatment group notation indicates the order of treatments (Placebo [“P” or “p”] or Robenacoxib [“R”]) received by each cat, during the Baseline (BL), and Treatment periods (T1 and T2) with all cats receiving a known placebo during BL (denoted as \( p \)). Study days were defined in relation to enrollment (“Day 0”), with Day -14, Day 0, Day 21, and Day 42 involving site visits by the owner, cat or both (Figure 2.01). A minimum of 20 cats per group were originally planned, based on previous work. The number was increased to 30 cats per group midway into the study, as newer data became available.⁷ Cats were randomized in a 1:1:1 ratio (via permuted block randomization with block size of three) according to pre-determined randomization tables for each site.
Randomization and medication dispensing were performed by pharmacy personnel not involved in patient assessment or data collection. All people involved in the study were blinded to the treatments until after the database was locked, with the exception of the statistician and local Sponsor’s Representative.

Cats were client-owned with naturally occurring DJD-associated pain and owner-assessed mobility impairment. Subjects were recruited using advertising to owners and veterinarians. All study-related costs were covered by the study, as were any recruitment incentives.

Patient screening and data collection were performed at both the NCSU CVM and UGA CVM study sites. Cats were screened for eligibility and enrolled similarly to previous DJD-associated pain studies in cats performed by the authors.14-16 On the day of screening (Figure 2.01), cats underwent physical, orthopedic, and neurological exams. Blood and urine samples were obtained for hematology (CBC), serum biochemistry, urinalysis with sedimentation, and serum T4 analysis at an external laboratory (Antech Diagnostics, Southaven, MS, USA). Complete axial and appendicular orthogonal radiographs were obtained under sedation and were reviewed by a board-certified veterinary radiologist.

Table 2.01: Treatment group designations and treatments by period.

<table>
<thead>
<tr>
<th>Group and sequence</th>
<th>2 week baseline period</th>
<th>3 week treatment period 1 (T1*)</th>
<th>3 week treatment period 2 (T2*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (pPP) (N=36)</td>
<td>Placebo</td>
<td>Placebo</td>
<td>Placebo</td>
</tr>
<tr>
<td>2 (pRR) (N=37)</td>
<td>Placebo</td>
<td>Robenacoxib</td>
<td>Robenacoxib</td>
</tr>
<tr>
<td>3 (pRP) (N=36)</td>
<td>Placebo</td>
<td>Robenacoxib</td>
<td>Placebo</td>
</tr>
</tbody>
</table>

p and P = placebo; R = robenacoxib; N = Number of patients allocated to the specified treatment group.

*Treatment periods T1 and T2 were blinded.
Figure 2.01: Study design and timeline.

Study visit day designations are in reference to enrollment day (Day 0). Treatment groups: p = placebo during baseline period; P = Placebo during treatment periods; R = Robenacoxib; sPE = Screening Patient Evaluation, including physical, neurological, and orthopedic exams, lab work submission, and radiographic evaluation for signs consistent with DJD; PE = Physical Exam only; CMI = Clinical Metrology Instruments (Owner Questionnaires); ODD = Owner’s Daily Diary – review of diary to ensure adequate documentation of dosing, and to assess for unreported adverse events; ePH = Exit Patient Evaluation, including physical, neurological, and orthopedic exams, and lab work submission; UGA CVM = University of Georgia College of Veterinary Medicine

Inclusion/exclusion criteria:

Cats were required to have at least moderate owner-assessed mobility or activity impairment (CSOM ≤ 6, see below), evidence of pain during orthopedic evaluation of at least two joints or spinal segments, with radiographic changes associated with DJD in at least two joints or spinal segments identified to have pain. The same investigator at each site performed orthopedic pain assessments. Cats were also required to be at least one year in age and between 2.5 and 12.0 kg in weight (to allow dosing of the test article) and to be healthy and not currently receiving analgesic or anti-inflammatory medications (including potential analgesics). Treatment with dietary supplements (e.g., glucosamine chondroitin, fatty acids) was allowed, as long as
treatment was continued during the study, and had been initiated greater than 28 days before study screening. Cats with controlled diabetes, hyperthyroidism, or stable chronic kidney disease (CKD; international renal interest society (IRIS) stages 1 and 2) were allowed to participate. Cats were required to live indoors or have limited outdoors access to avoid AM loss.

Cats that met enrollment criteria entered an approximately 2-week acclimation/baseline period. The final 7 days of BL provided the baseline AM data, to avoid confounding effects of the visit to the study site, sedation medications, and acclimation to the collar or harness. This period also served as an additional screening step to determine an owner’s ability to administer medication (unmasked placebo, see below) and keep daily records of dosing and patient behaviors.

Following BL, cats were enrolled into the blinded portion of the study and were randomized to one of three treatment group (Table 2.01) to receive daily a minimum oral dosage of 1 mg/kg (range 1-2.4 mg/kg) of robenacoxib (supplied as 6 mg tablets) or an equivalent number of placebo tablets. Both the robenacoxib and placebo were supplied in packaging identical to the commercially available formulation (Onsior®) in aluminum blister pack cards of 6 tablets each. Robenacoxib (expiry 2 October 2016) was supplied by Novartis Animal Health (NAH) in the same formulation as that used for marketing. The placebo (original expiry 18 June 2015 extended to 5 August 2017 following retesting) was also supplied by NAH, formulated to be identical to the robenacoxib tablets but without active ingredient.

Owners were instructed to administer the tablet directly into the cat’s mouth or mixed with a small amount of food (one third or less of the daily food ration).

All study visits and assessments were performed as noted in Figure 2.01.

Outcome Measures and Statistical Model

Data were analyzed on both intent-to-treat (ITT) and per-protocol (PP) data sets. The ITT data set, which was the same as the safety data set, consisted of data from all randomized animals that received at least one dose of study medication after day 0 (n=109 cats). For the PP data set (n=104), data from 5 cats with substantial protocol deviations were excluded. The data was analyzed with a repeated measures analysis of variance (ANOVA). A mixed-effects linear model was used for continuous response variables and a generalized mixed-effects linear model was used for categorical response variables.
Responses were in each period for each of the three treatment groups. Most analysis used baseline-adjusted responses (i.e., arithmetic or relative change), with some analyses using unadjusted responses. The efficacy hypotheses were tested using three contrasts (termed C1, C2, and C3) were evaluated using the group- and period-specific least-squared means. C1 compared the placebo versus robenacoxib treated cats during T1 (i.e., placebo versus robenacoxib over three weeks). C2 compared the placebo versus robenacoxib responses during T1 and T2 combined (i.e., placebo versus robenacoxib over six weeks), and C3 compared changes in responses after discontinuation of robenacoxib for T2 (i.e., deterioration), using groups pRR and pRP.

**Primary Outcome Measure**

The primary outcome measure was the change from baseline in activity. Physical activity was measured on a per minute basis by the Actical® AM device. Devices were configured for each patient using the vendor-supplied reader and software, Actical® 3.10 (Respironics Inc.). Patient weight and estimated height were entered, and battery life assessed. The epoch (i.e., length of time for which activity is accumulated and recorded as an observed value) was set to 1 minute, and recording started at the upcoming midnight (00:00 or 12:00AM local time). The AM device was placed on a non-breakaway collar or harness, and placed upright on the patient’s ventral neck.

Activity values for each hour were summed for use in statistical analysis. Mean hourly activity levels were computed for each cat over the last seven days of BL (“Day 0 mean”) and over the first 20 days of the T1 and T2 separately (“Day 21 mean” and “Day 42 mean”, respectively).

At the time of study protocol development, the research on functional data analysis and treatment-associated increases in night time activity were not yet published. Additionally, it was not fully appreciated that cats are inactive for the majority of the day (>70% of the time), with peaks of activity in the mornings and evenings, necessitating the use of complex models, time-restricted data sets, or other data partitions. Publication of these data during the course of our work led us to partition the data in various ways for analysis, in addition to a simple comparison of overall changes in activity which was planned at the start of the study. To test for treatment effects on actual physical activity, a subset of the data was formed by excluding zero
per-minute activity values. Among-cat analysis used hourly sums, whereas within-cat analysis used average per-minute values generated by dividing the hourly sum by 60 (zero observations included) or by the number of non-zero minutes (zero observations excluded). This allowed for direct measurements of changes of activity over the active times. Data were also partitioned into “day time” (08:00 to 20:00) or “dusk to dawn” (20:00-08:00) sets, because of positive effects seen on nighttime activity in other studies. These data subsets allowed for combinations of total values and non-zero only values, as well as entire day, daytime, or dusk-to-dawn comparisons. Based on the previous findings of high inter-cat variability and the bimodal pattern of activity, analyses focused on percent change in activity relative to baseline and dusk-to-dawn activity data.

Results were analyzed using mixed-effects analysis of variance (ANOVA; MIXED procedure of SAS® software). The response variables used in these analyses were the arithmetic change from baseline and the relative (percent) change from baseline. Significance tests were conducted for the fixed effects. Among-subject analyses of activity were tested at a two-sided 0.05 level of significance. No adjustments for multiple comparisons were made in order not to inflate the type II error and because many of the analyses can be assumed to be correlated (i.e., not independent), such as C1 and C2, and dusk-to-dawn versus entire day activity.

Group 2 (pRP) cats received robenacoxib then placebo in T1 and T2, respectively, allowing for within-cat analysis. The cumulative distribution functions (CDFs) of hourly activity data for both treatment periods were examined and tested for significant differences. The nonparametric two-sample Kolmogorov-Smirnov test statistics (D+ and D−) of the maximum observed differences between the robenacoxib and placebo distribution functions were computed and the associated $P$ values were calculated for each cat individually. $D+$ corresponds to stochastically higher activity levels for robenacoxib compared to placebo, while $D−$ corresponds to the opposite case. The proportion of cats demonstrating significantly higher levels of activity under robenacoxib ($P < 0.05$) was compared with the proportion that had significantly higher activity under the placebo using McNemar’s test (two-tailed $P < 0.05$), assessing whether it was more likely that a cat would improve while on robenacoxib than otherwise. We calculated the number needed to treat (NNT) for one cat to benefit. This was calculated as the inverse of the difference between the proportion of cats improved with robenacoxib compared with placebo. Effect sizes (ES) were calculated for CSOM and FMPI endpoints as the ratio of the least squares
Secondary Outcome Measures

Our secondary outcome measures were owner-based assessments of mobility impairment and pain, using the FMPI (Appendix A) and CSOM questionnaires (Appendix B), plus questions about temperament, happiness and QoL (Appendix C). These assessments were completed on all study visits.

The CSOM asked owners to select three activities (either from the FMPI or self-generated) that their cat had difficulty performing, and rate the cat’s ability to perform the task over the past week. Owners assigned an integer score from 0 to 4 (0 = impossible, 4 = no problem) for each activity, with a CSOM total score ranging from 0-12. An adjusted score was calculated as the difference between the score at Days 21 or 42 and baseline/Day 0. An inverted score (iCSOM) was also calculated to align scores of 0 with no pain or disability and 12 with maximum pain or disability.

The FMPI questionnaire had owners rate their cat’s ability to perform 17 set activities on an integer scale of 0 to 4 (0 = not at all, 4 = normal); with a total FMPI score ranging from 0-68. Adjusted values, termed “nominal scores”, were used to maintain the 68-point scale when some questions were unanswered or not-applicable. The sum of scores for answered questions was adjusted by multiplying by 68 (4 times the number of questions answered). Two final questions assess patient pain levels on a visual integer scale from 0 to 100mm, with 0 representing “severe pain” and 100 representing “no pain”. The owner’s mark on the line was measured and converted to a number and analyzed separately.

At each study visit, the cat’s current QoL and temperament were rated by the owner, as were QoL, temperament, and happiness in comparison to before the preceding treatment period. Each outcome was rated on a 5-point Likert-type scale. Current QoL was rated from “poor” to “excellent.” Temperament ratings included “Unfriendly/unsocial,” “Shy,” “Independent,” “Friendly/social,” and “High attention seeking.” Both QoL and temperament compared to before were rated from “much worse” to “greatly improved.” Happiness was rated from “Much more unhappy” to “Much more happy” compared to before. This form is available in Appendix C. The responses were summarized into frequency distributions for analysis.
ANOVA was performed using the SAS® GLIMMIX procedure for CSOM total score (unadjusted) and the change in total score relative to baseline (adjusted). The SAS® MIXED procedure was used for the FMPI nominal score. Cats that exhibited a negative change in CSOM total score of at least two units from Day 21 to Day 42 were deemed ‘deteriorators’ (as previously reported), and the proportions were compared between the pRR and pRP groups.

Happiness, QoL and temperament responses were analyzed using a generalized mixed linear model for ordered categories, implemented using the SAS® GLIMMIX procedure, which modeled the probabilities of levels of the response variable having lower ordered values. For these analyses, odds ratios (OR) were calculated and expressed so that values greater than 1 indicated the occurrence of more positive outcomes associated with the robenacoxib treatments.

All secondary outcome measures were tested at a two-sided 0.05 level of significance.

Safety Measures

Owners were instructed to immediately report any adverse events (AE) to the investigators. Adverse events were defined as any observations in the cat that were deemed unfavorable and unintended that occurred during the study period, whether they were considered treatment-related or not. Serious adverse events (SAE) were defined as AEs that were fatal or life-threatening, required veterinary intervention, or were considered clinically serious by the investigators. Owners were required to bring their cat to the study site or local veterinarian if an SAE was suspected. Adverse events, including changes in clinical pathology assessments, were evaluated for differences in occurrences or rates between groups. Body weight and CBC variables were analyzed using ANOVA. Frequency data (e.g., for AEs) were analyzed using Fisher’s exact test.

All safety measures were tested at a two-sided 0.05 level of significance.

Results

Recruitment and prescreening at the NCSU CVM occurred between 19 May 2014 and 31 July 2016, over approximately 115 weeks. Recruitment and prescreening at the UGA CVM occurred between 31 March 2016 and 01 September 2016, over approximately 22 weeks, to supplement patient recruitment at NCSU CVM. Of approximately 270 enquiries, 179 cats were deemed pre-eligible, scheduled for, and attended a screening appointment. Of the 179 cats
screened, 70 were deemed ineligible for participation, and therefore 109 cats were enrolled (Figure 2.02). Patient numbers were assigned according to study site and order of enrollment (e.g., LAS-01 for NCSU CVM, BUD-01 for UGA CVM).

Patient demographic data for all subjects (ITT group, n=109) are shown in Table 2.02. There were no statistical differences between groups for body weight, age, or gender. Breed was not analyzed statistically.

Efficacy data analyses are presented on a PP basis, which were largely similar to analysis by ITT.

Ten enrolled cats were removed from the study before Day 42. Reasons for early withdrawal included AEs (with gastrointestinal signs being most common), recurrence of historical disease processes, or withdrawal of owner consent (Table 2.03). Five enrolled cats (LAS-11, 26, 37, 38, and 103) were excluded from the PP analysis because of serious deficiencies in dosing (n=5), or early withdrawal because of AEs in four cases (LAS-11, 26, 37, 38).
Figure 2.02: Patient recruitment and enrollment flowchart (CONSORT flowchart).

N = number of patients; Treatment groups: p and P = Placebo; R = Robenacoxib; ITT = Intent to Treat analysis; PP = Per Protocol analysis.
Table 2.02: Patient demographic data.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (n=36)</th>
<th>Group 2 (n=37)</th>
<th>Group 3 (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (47.2%)</td>
<td>17 (46.0%)</td>
<td>12 (33.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>19 (52.8%)</td>
<td>20 (54.1%)</td>
<td>24 (66.7%)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11.6</td>
<td>11.1</td>
<td>11.8</td>
</tr>
<tr>
<td>SD</td>
<td>3.41</td>
<td>2.90</td>
<td>3.00</td>
</tr>
<tr>
<td>Range</td>
<td>3 – 17</td>
<td>3 – 15</td>
<td>2 – 17</td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.70</td>
<td>5.34</td>
<td>5.76</td>
</tr>
<tr>
<td>SD</td>
<td>1.37</td>
<td>1.50</td>
<td>1.68</td>
</tr>
<tr>
<td>Range</td>
<td>3.89 – 9.19</td>
<td>3.16 – 10.7</td>
<td>3.25 – 9.75</td>
</tr>
<tr>
<td><strong>CKD Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iris Stages I or II</td>
<td>12 (33.3%)</td>
<td>5 (13.5%)</td>
<td>9 (25%)</td>
</tr>
<tr>
<td>No CKD</td>
<td>24 (66.7%)</td>
<td>32 (86.5%)</td>
<td>27 (75%)</td>
</tr>
</tbody>
</table>
### Table 2.02 (continued)

<table>
<thead>
<tr>
<th>Breed Category</th>
<th>Count</th>
<th>Percentage</th>
<th>Count</th>
<th>Percentage</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Domestic MH</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2.78%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Devon rex</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2.78%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic LH</td>
<td>4</td>
<td>11.1%</td>
<td>5</td>
<td>13.51%</td>
<td>3</td>
<td>8.33%</td>
</tr>
<tr>
<td>Domestic MH</td>
<td>1</td>
<td>2.8%</td>
<td>1</td>
<td>2.70%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Domestic SH</td>
<td>21</td>
<td>58.3%</td>
<td>28</td>
<td>75.7%</td>
<td>29</td>
<td>80.6%</td>
</tr>
<tr>
<td>Maine coon</td>
<td>2</td>
<td>5.56%</td>
<td>1</td>
<td>2.70%</td>
<td>1</td>
<td>2.78%</td>
</tr>
<tr>
<td>Manx</td>
<td>2</td>
<td>5.56%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oriental SH</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2.70%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Persian</td>
<td>1</td>
<td>2.78%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ragdoll</td>
<td>2</td>
<td>5.56%</td>
<td>1</td>
<td>2.70%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rex mix</td>
<td>1</td>
<td>2.78%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Siamese</td>
<td>1</td>
<td>2.78%</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2.78%</td>
</tr>
<tr>
<td>Siamese mix</td>
<td>1</td>
<td>2.78%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Gender and breed are presented as count (percentage of treatment group total). Treatment Groups 1-3 – lettering designates the medications the patient received during the three treatment periods; p and P = placebo; R = robenacoxib; n = number of patients. SD = Standard Deviation. MH = Medium Hair; LH = Long Hair; SH = Short Hair
Table 2.03: List of early study withdrawals

<table>
<thead>
<tr>
<th>Patient</th>
<th>Reason for Withdrawal</th>
<th>Day (period) of Withdrawal</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAS-11</td>
<td>AE - Neurological</td>
<td>Day 1 (T1)</td>
<td>2 (pRR)</td>
</tr>
<tr>
<td>LAS-23</td>
<td>Withdrawal of Owner Consent</td>
<td>Day 19 (T1/T2)</td>
<td>2 (pRR)</td>
</tr>
<tr>
<td>LAS-26</td>
<td>AE – Gastrointestinal - emesis</td>
<td>Day 4 (T1)</td>
<td>1 (pPP)</td>
</tr>
<tr>
<td>LAS-28</td>
<td>AE – Gastrointestinal - emesis</td>
<td>Day 23 (T2)</td>
<td>2 (pRR)</td>
</tr>
<tr>
<td>LAS-37</td>
<td>AE – Gastrointestinal – emesis</td>
<td>Day 11 (T1)</td>
<td>2 (pRR)</td>
</tr>
<tr>
<td>LAS-38</td>
<td>AE – Gastrointestinal - emesis</td>
<td>Day 15 (T1)</td>
<td>1 (pPP)</td>
</tr>
<tr>
<td>LAS-40</td>
<td>AE – Integumentary</td>
<td>Day 25 (T2)</td>
<td>3 (pRP)</td>
</tr>
<tr>
<td>LAS-63</td>
<td>AE – Gastrointestinal – emesis</td>
<td>Day 22 (T2)</td>
<td>1 (pPP)</td>
</tr>
<tr>
<td>LAS-86</td>
<td>AE – Gastrointestinal - emesis</td>
<td>Day 16 (T1)</td>
<td>3 (pRP)</td>
</tr>
<tr>
<td>LAS-87</td>
<td>Recurrence of Behavioral Disorder - defecation</td>
<td>Day 22 (T2)</td>
<td>2 (pRR)</td>
</tr>
</tbody>
</table>

Table includes reason for withdrawal, study day of withdrawal, and treatment group. LAS = patient identification code corresponding to patients enrolled at the NCSU CVM. AE = Adverse event. T1 = Treatment period 1; T2 = Treatment period 2; p and P = placebo; R = robenacoxib.

**Primary Outcome**

There was high variability observed between cats for the activity endpoint, with individual mean hourly activity values ranging ten-fold from 427.4 to 4793.6. Across all methods of data partitioning, cats receiving robenacoxib showed greater increases in activity compared to placebo (Tables 2.04 and 2.05). However, analysis of total activity failed to show significance for either arithmetic or percent change from baseline (Table 2.04).
Collectively 82.4% of AM values were zero. As such, subsequent analysis focused on non-zero values or the dusk-to-dawn period or both (Table 2.05). Analysis of entire day non-zero activity and dusk-to-dawn total activity values showed greater activity increases in robenacoxib-treated cats. However, these changes were not significant following either three or six weeks of treatment, nor was a significant deterioration following discontinuation of treatment detected. Analysis of non-zero dusk-to-dawn values revealed significant increases of approximately 11% for both three (C1) and six (C2) weeks of treatment ($P = 0.045$ and $P = 0.040$, respectively). No significant deterioration (C3) was detected.

Group $p_{RP}$ contained 35 evaluable cats for within-cat analysis (example in Figure 2.03). Significantly more cats showed robenacoxib-associated increases in activity when considering entire day non-zero activity (10:2 cats [28.6%:5.7%], $P = 0.021$) and dusk-to-dawn total activity (9:2 cats [25.7%:5.7%], $P = 0.035$) (Table 2.06). These ratios corresponded to NNT values of 4.4 and 5.0 cats, respectively. Significant effects were not detected in the other data partitions.

**Table 2.04: Contrasts of total activity data between treatment groups.**

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Arithmetic change</th>
<th>Relative change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
</tr>
<tr>
<td>C1</td>
<td>116.4</td>
<td>80.4</td>
</tr>
<tr>
<td>C2</td>
<td>75.1</td>
<td>87.7</td>
</tr>
<tr>
<td>C3</td>
<td>-23.2</td>
<td>60.3</td>
</tr>
</tbody>
</table>

Data are presented as either arithmetic or percentage change from baseline. Positive values indicate increased activity, whereas negative values indicate decreased activity. Estimate = change from baseline in hourly activity counts with robenacoxib relative to placebo. C1 = contrast 1 = treatment group $p_{PP}$ compared against groups $p_{RR}$ and $p_{RP}$ following 3 weeks of treatment ($p$ and $P =$ placebo; $R =$ robenacoxib); C2 = contrast 2 = treatment group $p_{PP}$ compared against group $p_{RR}$ following 6 weeks of treatment; C3 = contrast 3 = treatment group $p_{RP}$ compared against $p_{RR}$ for change in activity between weeks 3 and 6 of treatment; SE = standard error.

*$P$ values test whether the contrast is significantly different from zero.
Table 2.05: Analysis of activity data subsets.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Arithmetic change</th>
<th>Relative change (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td>P value</td>
<td>Estimate</td>
<td>SE</td>
</tr>
<tr>
<td>Non-Zero Values Entire Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>141.2</td>
<td>82.3</td>
<td>0.089</td>
<td>6.61</td>
<td>4.46</td>
</tr>
<tr>
<td>C2</td>
<td>93.5</td>
<td>89.8</td>
<td>0.30</td>
<td>4.47</td>
<td>4.90</td>
</tr>
<tr>
<td>C3</td>
<td>-40.5</td>
<td>61.7</td>
<td>0.51</td>
<td>0.33</td>
<td>3.15</td>
</tr>
<tr>
<td>Total Activity Values Dusk-to-Dawn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>140.7</td>
<td>90.5</td>
<td>0.12</td>
<td>9.97</td>
<td>5.62</td>
</tr>
<tr>
<td>C2</td>
<td>165.7</td>
<td>98.8</td>
<td>0.097</td>
<td>11.6</td>
<td>6.10</td>
</tr>
<tr>
<td>C3</td>
<td>8.16</td>
<td>67.9</td>
<td>0.90</td>
<td>2.06</td>
<td>4.41</td>
</tr>
<tr>
<td>Non-Zero Values Dusk–to-Dawn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>158.9</td>
<td>94.8</td>
<td>0.097</td>
<td>10.70</td>
<td>5.29</td>
</tr>
<tr>
<td>C2</td>
<td>172.9</td>
<td>103.9</td>
<td>0.099</td>
<td>11.9</td>
<td>5.74</td>
</tr>
<tr>
<td>C3</td>
<td>-2.41</td>
<td>68.7</td>
<td>0.97</td>
<td>1.74</td>
<td>4.18</td>
</tr>
</tbody>
</table>

Analysis used arithmetic change and relative change (percentage) for the sub-groups: non-zero activity across the whole day; all activity over the dusk-to-dawn time period; and non-zero activity over the dusk to dawn time period. Positive values indicate increased activity, whereas negative values indicate decreased activity. Estimate = change from baseline in hourly activity counts with robenacoxib relative to placebo; SE = standard error. The contrasts were: C1 = contrast 1 = treatment group pPP compared against groups pRR and pRP following 3 weeks of treatment (p and P = placebo; R = robenacoxib); C2 = contrast 2 = treatment group pPP compared against group pRR following 6 weeks of treatment; C3 = contrast 3 = treatment group pRP compared against pRR for change in activity between weeks 3 and 6 of treatment.

* denotes significance at 0.05 level
Figure 2.03: Example Cumulative Distribution Function

Example (n=1; LAS-31), demonstrates a rightward shift of activity during treatment with robenacoxib, as compared against treatment with placebo. This indicates that the activity counts were higher while receiving robenacoxib. P values from the Kolmogorov-Smirnov test were <0.0001 for the hypothesis that activity with robenacoxib > placebo, and 1.0 for placebo > robenacoxib.
Table 2.06: Analysis of within-cat activity data (N=35).

<table>
<thead>
<tr>
<th>Group</th>
<th>N (% of total) of cats with activity R &gt; P</th>
<th>N (% of total) of cats with activity P &gt; R</th>
<th>P value</th>
<th>Difference in response rates (R – P)</th>
<th>NNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Values</td>
<td>8 (22.9%)</td>
<td>2 (5.71%)</td>
<td>0.058</td>
<td>17.2%</td>
<td>5.83</td>
</tr>
<tr>
<td>Entire Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Zero Values</td>
<td>10 (28.6%)</td>
<td>2 (5.71%)</td>
<td>0.021*</td>
<td>22.9%</td>
<td>4.37</td>
</tr>
<tr>
<td>Entire Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dusk-to-Dawn Values</td>
<td>9 (25.7%)</td>
<td>2 (5.71%)</td>
<td>0.035*</td>
<td>20.0%</td>
<td>5.0</td>
</tr>
<tr>
<td>Non-Zero Values</td>
<td>6 (17.1%)</td>
<td>1 (2.86%)</td>
<td>0.059</td>
<td>14.3%</td>
<td>7.0</td>
</tr>
<tr>
<td>Dusk-to-Dawn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis shows the number and percentage of cats in which hourly activity counts were significantly higher during either placebo (P) or robenacoxib (R) treatment. NNT = number needed to treat.
* denotes significance at 0.05 level

Secondary Outcomes

CSOM total score comparisons demonstrated significantly greater improvement in robenacoxib-treated cats for both unadjusted (total scores) and baseline-adjusted (e.g., Day 42 score – Day 0 score) scores following six weeks of treatment (C2 = 1.40 [P = 0.010] and C2 = 0.94 [P = 0.044], respectively) (Table 2.07). This corresponds with effect sizes of 0.33 and 0.26, respectively. All other CSOM contrasts failed to achieve significance.

No significant treatment effects on FMPI scores (nominal or adjusted) were detected (Table 2.07).

Analysis of temperament and happiness compared to before the most recent treatment showed significant improvement following six weeks of treatment with robenacoxib compared with placebo (OR = 4.53 [P = 0.0039] and OR = 2.73 [P = 0.021], respectively), while all other comparisons failed to show significance (Table 2.08).

The CSOM data allowed for calculation of improvement in pain and disability by treatment group (e.g., PRR vs PPP). Using the iCSOM, cats in group PRR at week six (baseline
iCSOM=7.00) showed an average improvement of 3.53 points, compared against improvement of 2.28 points in cats in the pPP group (baseline iCSOM = 7.56). These correspond with improvements (decreases in pain and disability) of 50.4% in group PRR and 30.2% in group PPP, or an improvement over placebo of 19.2%.
Table 2.07: Analysis of the CSOM and FMPI assessments

| Owner Assessment | Estimate | SE  | t Value** | Pr > |t| | Effect Size |
|------------------|----------|-----|-----------|-------|----|-------------|
| CSOM Total Score |          |     |           |       |    |             |
| C1               | 0.49     | 0.52| 0.95      | 0.34  |    | 0.09        |
| C2               | 1.40     | 0.53| 2.62      | 0.010*|    | 0.33        |
| C3               | 0.67     | 0.54| 1.25      | 0.21  |    | 0.16        |
| CSOM Total Score Adjusted for Baseline | | | | | | |
| C1               | 0.23     | 0.46| 0.50      | 0.62  |    | 0.05        |
| C2               | 0.94     | 0.46| 2.04      | 0.044*|    | 0.26        |
| C3               | 0.60     | 0.54| 1.13      | 0.26  |    | 0.14        |
| FMPI Nominal Score |       |     |           |       |    |             |
| C1               | -0.66    | 2.36| -0.28     | 0.78  |    | -0.03       |
| C2               | 4.65     | 2.58| 1.80      | 0.075 |    | 0.18        |
| C3               | 0.43     | 1.77| 0.25      | 0.81  |    | 0.03        |
| FMPI Nominal Score Adjusted for Baseline | | | | | | |
| C1               | -2.66    | 1.86| -1.43     | 0.15  |    | -0.12       |
| C2               | 0.75     | 1.96| 0.38      | 0.70  |    | 0.04        |
| C3               | 0.75     | 1.77| 0.42      | 0.67  |    | 0.04        |

Estimates show the effect of robenacoxib relative to placebo. Positive values indicate owner-assessed improvements, whereas negative values indicate worsening or deterioration; SE = Standard Error. C1 = contrast 1 = treatment group pPP compared against groups pRR and pRP following three weeks of treatment (p and P = placebo; R = robenacoxib); C2 = contrast 2 = treatment group pPP compared against group pRR following six weeks of treatment; C3 = contrast 3 = treatment group pRP compared against pRR for change in activity between weeks three and six of treatment.

* denotes significance at 0.05 level

** Student’s t statistic for testing whether the contrast equals zero; two-tailed test
Table 2.08: Analysis of owner based assessments of quality of life (QoL), temperament, and happiness.

<table>
<thead>
<tr>
<th>Owner Assessment</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality of Life Compared to Before Most Recent Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>1.17</td>
<td>0.47</td>
<td>2.94</td>
</tr>
<tr>
<td>C2</td>
<td>2.09</td>
<td>0.87</td>
<td>5.04</td>
</tr>
<tr>
<td>C3</td>
<td>2.33</td>
<td>0.59</td>
<td>9.19</td>
</tr>
<tr>
<td>Temperament Compared to Before Most Recent Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>1.41</td>
<td>0.50</td>
<td>3.99</td>
</tr>
<tr>
<td>C2</td>
<td>4.53</td>
<td>1.65</td>
<td>12.5</td>
</tr>
<tr>
<td>C3</td>
<td>3.44</td>
<td>0.82</td>
<td>14.4</td>
</tr>
<tr>
<td>Happiness Compared to Most Recent Visit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>1.61</td>
<td>0.64</td>
<td>4.06</td>
</tr>
<tr>
<td>C2</td>
<td>2.73</td>
<td>1.17</td>
<td>6.38</td>
</tr>
<tr>
<td>C3</td>
<td>1.47</td>
<td>0.39</td>
<td>5.59</td>
</tr>
</tbody>
</table>

The odds ratios show the effect of robenacoxib relative to placebo. Positive values indicate improvement, whereas negative values indicate worsening or deterioration. C1 = contrast 1 = treatment group pPP compared against groups pRR and pRP following three weeks of treatment (p and P = placebo; R = robenacoxib); C2 = contrast 2 = treatment group pPP compared against group PRR following six weeks of treatment; C3 = contrast 3 = treatment group pRP compared against pRR for change in activity between weeks three and six of treatment. For QoL, temperament, and happiness, the tests are effectively comparing the categorical response probability distributions applicable to the “treatment arm”–“treatment period” combinations represented in the contrasts. An odds ratio greater than 1 indicates a higher likelihood of better outcomes for the robenacoxib treatment.

* denotes significance at 0.05 level
**Safety Measures**

Adverse events were generally mild and self-limiting, typically involving the gastrointestinal tract (Table 2.09). None of the rates of occurrence of AEs were significantly different between cats receiving placebo and robenacoxib. The proportion of cats with pre-existing chronic kidney disease experiencing at least one AE during the study was not significantly different between treatment groups \((P = 0.88)\).

No clinically relevant hematological, chemistry, or urinalysis differences between groups were observed. Several differences were observed between treatment groups at study exit. Select hematological, chemistry, and urinalysis data, including all statistically significant results, are available as supplementary material in Appendix D (Tables D.01, D.02, and D.03, respectively).
Table 2.09: Summary of adverse event frequencies

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>T1 Placebo</th>
<th>T1 Robenacoxib</th>
<th>P value</th>
<th>T2 Placebo</th>
<th>T2 Robenacoxib</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 36</td>
<td>n = 73</td>
<td></td>
<td>n = 69</td>
<td>n = 34</td>
<td></td>
</tr>
<tr>
<td>Emesis</td>
<td>7</td>
<td>10</td>
<td>0.57</td>
<td>2</td>
<td>4</td>
<td>0.091</td>
</tr>
<tr>
<td>Lethargy</td>
<td>5</td>
<td>3</td>
<td>0.11</td>
<td>2</td>
<td>1</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Anorexia</td>
<td>4</td>
<td>3</td>
<td>0.22</td>
<td>0</td>
<td>1</td>
<td>0.33</td>
</tr>
<tr>
<td>Intestinal Disorder NOS*</td>
<td>2</td>
<td>1</td>
<td>0.25</td>
<td>1</td>
<td>2</td>
<td>0.26</td>
</tr>
<tr>
<td>Pruritus</td>
<td>0</td>
<td>1</td>
<td>&gt;0.99</td>
<td>1</td>
<td>1</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Inappropriate Urination</td>
<td>1</td>
<td>2</td>
<td>&gt;0.99</td>
<td>0</td>
<td>1</td>
<td>0.33</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>1</td>
<td>&gt;0.99</td>
<td>3</td>
<td>0</td>
<td>0.55</td>
</tr>
<tr>
<td>Renal Insufficiency</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>2</td>
<td>2</td>
<td>0.60</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>1</td>
<td>0</td>
<td>0.33</td>
<td>1</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>Anemia NOS</td>
<td>0</td>
<td>1</td>
<td>&gt;0.99</td>
<td>2</td>
<td>0</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Intestinal Stasis</td>
<td>1</td>
<td>1</td>
<td>&gt;0.99</td>
<td>1</td>
<td>0</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

Table includes clinical signs for both placebo and robenacoxib treatment periods. T1 = Treatment period 1; T2 = Treatment period 2; NOS = Not Otherwise Specified; n = total number of patients. There were no significant differences in rates of AE occurrence between treatments.

Discussion

We showed that cats receiving robenacoxib would show increases in AM-measured physical activity when compared to placebo. Robenacoxib treatment was associated with owner-assessed decreases in pain and disability, and improvements in temperament, QoL, and happiness. This study is the first report of owners being able to detect the beneficial effects of an NSAID over placebo in a parallel-group design study. However, detection of clinically relevant
improvements in mobility or pain relief in cats with DJD or OA remains a challenge, despite the combined use of validated tools and novel study designs in the current study.\textsuperscript{7,8,21,22}

Treatment-related improvements in activity were detected following three weeks of treatment. We observed increases of approximately 5\% and 10\% with robenacoxib over placebo for total and dusk-to-dawn activity, respectively. Robenacoxib’s 5\% increase in total activity over placebo compares favorably with the previously reported increase of 3.32\% over placebo in total activity in cats with DJD receiving the NSAID meloxicam for three weeks.\textsuperscript{14} However, Gruen et. al. evaluated meloxicam administered at 0.035mg/kg daily - less than the maintenance dose of 0.05mg/kg daily approved in the EU. It is important to note that the current study did not evaluate the treatment effects of meloxicam. In human medicine, patients with end-stage (pre-joint replacement) knee and hip OA spent approximately 2.9\% less of their day in movement-related activities when compared with matched controls.\textsuperscript{23} While these data across studies are not directly comparable, it seems that a difference of approximately 3\% in daily activity (counts or time) is clinically relevant.

Treatment effects on owner-based outcome measures were significant following six weeks of treatment. This lag may be due to delays in owners noticing or learning to detect changes, time required for cats to “un-learn” their learned avoidance or fear of activities,\textsuperscript{24,25} or other unknown factors. We failed to detect any significant differences between groups using the FMPI questionnaire.

The current data did not support the second hypothesis that the study would detect a deterioration in outcome measures following discontinuation of robenacoxib, compared to cats that continued to receive the medication. The only other study that measured this response showed that cats deteriorated withdrawal of the NSAID meloxicam.\textsuperscript{7} Robenacoxib’s prolonged residence time in inflamed tissue\textsuperscript{6} may be one reason we did not see significant deterioration in the three weeks after cessation of therapy. Finally, our study may have been insufficiently powered to detect deterioration.

The iCSOM score calculations showed an improvement (reduction in pain and disability) of 19.2\% over placebo. The human literature has described reductions of pain of 15\%, 33\%, and 50\% to correlate with the minimal clinically important difference (MCID), “much better” improvement,\textsuperscript{26} and “very much improved,”\textsuperscript{27} respectively. The MCID of veterinary species is unknown. Effect sizes calculated from the activity data ranged from 0.084 (total activity C2, $P =$
0.50) to 0.26 (non-zero dusk-to-dawn C2, \( P = 0.042 \)). Baseline-adjusted CSOM scores indicated an effect size (for treatment over placebo) of 0.85 when comparing groups \( \rho PP \) and \( \rho RR \) following six weeks of treatment. While no other effect size data (CSOM or otherwise) are available for dogs or cats with DJD treated with NSAIDs, these results compare favorably against the effect size of 0.37 reported for humans with knee OA treated with NSAIDs. The NNTs calculated from group \( \rho RP \) (range 4.4-7 cats; Table 2.06) also compare favorably with NNTs for NSAIDs in humans of 3-13 and with those in dogs of 4-8.

Our CSOM results were similar to previous reports of a significant caregiver placebo effect in feline analgesic studies. We report placebo effect sizes of 1.40 and 1.68 following three and six weeks of treatment, respectively (Table 2.10). This placebo effect may be due to owners altering their interactions with the pet due to expectations of treatment-related improvement. Future exploration of placebo effects and mitigation strategies would be welcomed.

Table 2.10: Comparison of placebo effect sizes across feline CMSD/DJD studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Placebo Effect Size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet(^7)</td>
<td>1.93 (1.16 to 2.70)</td>
</tr>
<tr>
<td>Nutraceutical(^a)</td>
<td>1.71 (1.12 to 2.30)</td>
</tr>
<tr>
<td>FMPI(^14)</td>
<td>0.97 (0.16 to 1.78)</td>
</tr>
<tr>
<td>Low-dose meloxicam(^5)</td>
<td>1.05 (0.50 to 1.60)</td>
</tr>
<tr>
<td>Anti-NGF Antibody(^9)</td>
<td>1.20 (0.25 to 2.16)</td>
</tr>
<tr>
<td>Robenacoxib (3 weeks)</td>
<td>1.40 (0.95 to 1.85)</td>
</tr>
<tr>
<td>Robenacoxib (6 weeks)</td>
<td>1.68 (1.21 to 2.15)</td>
</tr>
</tbody>
</table>

Reproduced with permission from\(^{21,2}\). Placebo effect sizes were calculated using Client Specific Outcomes Measure data from respective studies, calculated using Cohen’s d for placebo group:

\[
\text{Mean Score}_{\text{Placebo}} - \text{Mean Score}_{\text{baseline}} / \text{Pooled standard deviation}
\]

Superscripts 5, 7, 9, and 14 correspond to citations in the main text. a. Data from an unpublished study evaluating a nutraceutical in cats, which appears in\(^{21}\). FMPI = Feline Musculoskeletal Pain Index; NGF = Nerve Growth Factor.
Our results are similar to previously published safety of robenacoxib in healthy and OA-affected cats (with and without CKD)\textsuperscript{5,33}. Most AEs were self-limiting and did not require medical intervention. Cats with IRIS stage 1 or 2 CKD were no more likely to experience an AE, which is important given the increasing prevalence and comorbidity of CKD and OA with age\textsuperscript{34}. However, the study design was optimized for efficacy rather than safety assessment, with other studies available for full safety analysis\textsuperscript{5,33}.

This study has several limitations. The observed high inter-cat variability relative to sample size is likely responsible for the lack of statistical significance in several comparisons. The within-cats analysis of activity data was based on CDFs of a single group rather than a full crossover design, with data partitions that may not be equally applicable to all cats. This analysis evaluated each cat in a binary sense as having or not having a significant improvement under robenacoxib compared to placebo.

The outcomes of QoL, temperament, and happiness are not validated in veterinary research, and their meanings were not standardized in this study. This may have resulted in assessments that were not directly comparable owner to owner. While these data have been used as supportive of other validated measures (AM and CSOM), the results should not stand alone, and may be of questionable overall validity.

Our findings may not be generalizable to the entire population of cats with DJD-associated pain. The majority of enrolled cats were from a single study site, presenting study site and investigator bias. Furthermore, while some comorbidities were allowed by the inclusion/exclusion criteria, cats were required to be overall healthy, meaning findings may be different in the general population of cats with mobility impairment. Lastly, inclusion criteria required that cats be moderately to severely impaired, meaning that results may be different in cats with milder impairment.

Overall, these data suggest that robenacoxib has utility for the treatment of DJD-associated pain in cats when given long term. We detected significant robenacoxib-associated improvements in both objective and subjective outcome measures following six weeks of treatment. However, many data were not significant, and activity data required partitioning to achieve significance. No deterioration following masked discontinuation of robenacoxib was detected, which underscores the lack of a significant effect seen at three weeks. While significant effects on patient temperament and happiness were detected, these measures were poorly defined.
and have not been previously validated. Finally, rates of adverse events between treatment groups were not significantly different, which agrees with previously published reports of the drug’s safety.

This study examined the capabilities various assessment tools to diagnose, measure, and detect treatment-associated changes in pain and disability secondary to maladaptive pain in cats. The difficulties we experienced in demonstrating efficacy of an NSAID show that further refinement of pain assessment tools, and development of a novel, objective measure of chronic pain in cats, are necessary.
References


Chapter 3 Foreword

In order to guide research with both translational and veterinary clinical impact, we set out to determine which medications are currently used in clinical practice for the treatment of chronic musculoskeletal pain in cats. What follows is a report of the methods and findings.
Chapter 3: Prescribing practices of veterinarians in the treatment of chronic musculoskeletal pain in cats
Prescribing Practices of Veterinarians in the Treatment of Chronic Musculoskeletal Pain in Cats

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Keywords: Cats, musculoskeletal pain, analgesics, surveys and questionnaires
Abstract

Objectives

Despite the high prevalence and increasing awareness of chronic musculoskeletal pain in cats, approved treatment options are completely lacking in the United States, and few other options have sufficient safety and efficacy data. Knowledge of current prescribing practices should inform future research of putative therapies. We aimed to determine which drug and non-drug therapies were being used by general practitioners for the treatment of musculoskeletal pain in cats and to understand demographic influences on prescribing practices.

Methods

We distributed a survey to 36,676 veterinarians who were members of the Veterinary Information Network in January 2017. Within 3 weeks, 1,056 practitioners completed the survey. The survey included demographic and background information, questions on prescribing frequency and dosing regimen of 13 drugs and non-drug therapies and questions on preferred medication formulations and dosing frequencies. Descriptive statistics were used and χ² testing to evaluate relationships between demographic variables and prescription practices.

Results

Gabapentin was prescribed most frequently (71% of respondents), followed by joint supplements (67.8%), meloxicam (64.0%), opioids (62.6%) fish oil (62.1%) and polysulfated glycosaminoglycans (61.9%). Years in practice appeared to influence prescribing habits, with practitioners graduated for >20 years prescribing glucocorticoids more frequently than other age groups (p=0.0002), while recent graduates (<1 year) reported prescribing therapies less frequently across all categories.

Conclusions and relevance

These results show a contrast between therapies prescribed by practitioners and what is supported by evidenced-based literature. Future research evaluating the safety and efficacy of gabapentin should be prioritized.
Introduction

Estimates of the population of pet cats in the United States exceed 94 million, compared to approximately 90 million dogs.\(^1\) Despite this, the ability of veterinarians to assess and treat pain in these patients has lagged behind that of dogs. Despite increasing interest and research to bridge this information gap, most information on pain control in cats focuses on perioperative pain and analgesia.\(^2\sim5\) Less information exists about conditions resulting in chronic pain; consequently, more cats with these conditions remain undiagnosed and under-treated.\(^6\sim8\) The gradual onset and subtle behavioral changes seen in chronic pain situations, coupled with cats’ abilities to mask clinical signs likely contributes to this disparity, making identification and measurement of chronic pain problematic.

Degenerative joint disease (DJD), which encompasses osteoarthritis (OA), commonly produces chronic musculoskeletal pain in cats, with an estimated ~60% to ~90% of all cats having radiographic changes consistent with the disease.\(^9\sim12\) Of cats with radiographic evidence of DJD, an estimated 40% have DJD-associated pain (unpublished author observations from patient exams).\(^9\)

Whereas OA includes degenerative changes of synovial joints, DJD is inclusive of degenerative changes in synovial, cartilaginous, or fibrous joints.\(^13\) When degenerative changes of both the appendicular and axial skeleton are being considered, DJD is the more correct term. For ease of understanding, the term chronic musculoskeletal pain/arthritis pain was used in the survey.

In order to guide future research of new analgesics for feline DJD, investigators should have a better understanding of what agents are currently being used and how they are being utilized. Although consensus statements and guidelines on treatment and management of chronic pain in cats exist,\(^4\sim14\) investigators have only evaluated the efficacy of a few suggested therapies appropriated. None of these are approved for the treatment of DJD in the United States. Only the non-steroidal anti-inflammatory drug (NSAID) meloxicam\(^15\), one ‘joint support diet’\(^16\) and a novel therapy (anti-nerve growth factor antibody) have undergone placebo-controlled trials and shown evidence of efficacy.\(^17\) Furthermore, data on current prescribing practices can help inform future research and ensure clinical relevancy, but are currently lacking.
In order to determine which drug and non-drug therapies were being used by veterinarians for the treatment of DJD-associated pain in cats, we performed a survey through the Veterinary Information Network (VIN).

**Materials and Methods**

Survey development involved a collaboration between experts in the fields of veterinary pharmacology, veterinary orthopedics, feline medicine, as well as survey development and distribution. The survey focused primarily on pharmacologic therapies and orally administered supplements, and excluded non-drug therapies such as acupuncture, stem cell or platelet rich plasma therapy, surgery, rehabilitation and laser therapy. The survey underwent several revisions with the final version comprising 107 questions, including questions regarding demographics/background, questions on 13 specific therapies and several options that allowed for other therapies to be entered as free text. Demographic and background information included years in practice, type of practice/species seen, frequency of presentation of suspected feline chronic musculoskeletal pain/arthritis cases, frequency that treatment was recommended for these and the frequency with which owners elected to treat the suspected chronic musculoskeletal pain/arthritis. Data were also collected as to whether the practitioner routinely used lean weight or true body weight (or ‘depends on therapy’) to calculate dosages.

For each of the therapies included in the survey, practitioners were instructed to indicate their typical maintenance dose (clinically relevant ranges on a mg/kg or mg/lb basis, where applicable), dosing frequency, treatment duration, preferred formulation and the percentage of cats with DJD-associated pain prescribed the therapy. In order to facilitate completion, questions were blocked by therapeutic agent, which allowed respondents to bypass questions pertaining to therapies they did not use. Figure 3.01 shows the general survey flow and logic.

At the end of the survey, respondents were instructed to indicate their ideal therapeutic formulation and frequency of administration, followed by formulations and dosing frequencies that were considered acceptable. Final comments were also permitted at the end of the survey. Survey logic requiring participants to select an answer before moving to the next question was not implemented. The full survey is available as supplementary material (Appendix E).
Survey logic was used to allow respondents to bypass questions regarding medications the respondent indicated they did not prescribe.

The survey was distributed electronically via email to 36,676 clinicians, all belonging to an online community and information service for veterinarians (VIN). Individual gender and nationality demographic information was not collected during the survey, but was available through the VIN database. The survey remained open from 17 January 2017 to 9 February 2017. Results were automatically recorded into an online database and transferred to a spreadsheet program for easier data management after the survey was closed (Excel, Microsoft Corporation).

Statistical analysis involved the use of statistical software (JMP Pro 13, SAS). Summary descriptive statistics are presented when most appropriate. $\chi^2$ testing for independence was used to evaluate relationships between number of years in practice or proportion of feline caseload and therapies prescribed, or between ideal medication formulation and ideal frequency of administration. Fisher’s exact test was used to evaluate for any relationships between prescription of one therapeutic agent and the concurrent prescription of gabapentin. Sided-tests were used, based on individual hypotheses: a right-sided test was used when a positive correlation between the therapy and gabapentin was suspected, while a left-sided test indicates a suspected negative correlation. Significance was set at a P-value of 0.05 or less, before any Bonferroni corrections.

Results

Of the 36,676 veterinarians receiving the survey via email, 1,056 completed the survey (defined as the respondent navigating through the final question or the survey logic ending the
survey), for a completed response rate of 2.9%. Two respondents indicated that they either never recommended treatment for chronic musculoskeletal pain in cats, or clients never elected to administer the selected treatments, resulting in no further collected information. Therefore, the maximum total number of responses per question is 1,054.

Most respondents were female, at 77.3%. Respondents were also primarily from the United States of America and Canada (77.0% and 13.6%, respectively), with some respondents residing in Australia (2.9%), the United Kingdom (1.3%) and New Zealand (1.1%). The level of clinical experience represented by the survey respondents ranged from less than one, to more than 20 years in practice. All experience groups, excepting the “less than one year” group, were well-represented (more than 150 respondents); those with the most experience (“20+ years of practice”) represented over 40% of all respondents (number of responses = 440). More than 70% of respondents indicated that cats represented 25-50% of their caseload. A small subset of respondents (6.2%) indicated that they practiced in feline-only clinics. Demographics of respondents completing the survey are summarized in Table 3.01.
Table 3.01: Respondent demographic information including gender, nationality, years of experience and feline portion of caseload.

<table>
<thead>
<tr>
<th>Respondent Gender</th>
<th>Respondents = 1037</th>
<th>Percent of Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>235</td>
<td>22.7%</td>
</tr>
<tr>
<td>Female</td>
<td>802</td>
<td>77.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respondent Nationality</th>
<th>Respondents = 1042</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States of America</td>
<td>802</td>
<td>77.0%</td>
</tr>
<tr>
<td>Canada</td>
<td>142</td>
<td>13.6%</td>
</tr>
<tr>
<td>Australia</td>
<td>30</td>
<td>2.9%</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>14</td>
<td>1.3%</td>
</tr>
<tr>
<td>New Zealand</td>
<td>11</td>
<td>1.1%</td>
</tr>
<tr>
<td>Other</td>
<td>43</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How long have you been in clinical practice?</th>
<th>Responses = 1054</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1 year</td>
<td>32</td>
<td>3%</td>
</tr>
<tr>
<td>Between 1 and 5 years</td>
<td>183</td>
<td>17.4%</td>
</tr>
<tr>
<td>Between 6 and 10 years</td>
<td>160</td>
<td>15.2%</td>
</tr>
<tr>
<td>Between 10 and 20 years</td>
<td>239</td>
<td>22.7%</td>
</tr>
<tr>
<td>More than 20 years</td>
<td>440</td>
<td>41.7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Approximately what percentage of your patients are cats?</th>
<th>Responses = 1039</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 25%</td>
<td>121</td>
<td>11.6%</td>
</tr>
<tr>
<td>Between 25% and 50%</td>
<td>751</td>
<td>72.3%</td>
</tr>
<tr>
<td>Between 51% and 75%</td>
<td>97</td>
<td>9.3%</td>
</tr>
<tr>
<td>Between 76% and 99%</td>
<td>6</td>
<td>0.6%</td>
</tr>
<tr>
<td>100% (Feline Only)</td>
<td>64</td>
<td>6.2%</td>
</tr>
</tbody>
</table>

Questions are presented as they appeared in the survey
Additional background questions collected data on the frequency of cases suspected to have chronic musculoskeletal pain/arthritis, how often respondents recommended treatment of this pain, and finally, how often respondents believed that owners followed these recommendations. Approximately 90% of respondents indicated they saw suspected cases of chronic musculoskeletal/arthritis pain in cats at least monthly, with 46.8% indicating that they saw suspected cases weekly and 15.8% indicating they saw cases daily. While only 26% of respondents indicated that they recommended treatment for pain in all of these suspected cases, 33.6% indicated they recommended treatment in more than 75% of cases, and 80% of respondents indicated they recommended treatment in at least 50% of these cases. However, respondents mostly believed that only 25% to 50% of owners followed these treatment recommendations. Full background information on frequency of cases seen, frequency of practitioner treatment recommendations and frequency of owners believed to be following treatment recommendations can be seen in Table 3.02.
### Table 3.02: Additional respondent demographic information.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Responses</th>
<th>Percent of Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>167</td>
<td>15.8%</td>
</tr>
<tr>
<td>Weekly</td>
<td>494</td>
<td>46.8%</td>
</tr>
<tr>
<td>Monthly</td>
<td>285</td>
<td>27.0%</td>
</tr>
<tr>
<td>Quarterly</td>
<td>95</td>
<td>9.0%</td>
</tr>
<tr>
<td>Yearly</td>
<td>15</td>
<td>1.4%</td>
</tr>
<tr>
<td>Never</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Responses</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never or 0%</td>
<td>1</td>
<td>0.1%</td>
</tr>
<tr>
<td>Less than 25% of cases</td>
<td>65</td>
<td>6.2%</td>
</tr>
<tr>
<td>Between 25% and 50% of cases</td>
<td>136</td>
<td>12.9%</td>
</tr>
<tr>
<td>Between 50% and 75% of cases</td>
<td>225</td>
<td>21.3%</td>
</tr>
<tr>
<td>Between 75% and 100% of cases</td>
<td>354</td>
<td>33.6%</td>
</tr>
<tr>
<td>100% of cases</td>
<td>274</td>
<td>26.0%</td>
</tr>
</tbody>
</table>
Seventy one percent of respondents reported prescribing gabapentin (Neurontin; Pfizer) for treating DJD-associated pain in cats. Slightly fewer (67.8%) prescribed joint supplements, meloxicam (Metacam; Boehringer Ingelheim, 64.0%), opioids (62.6%), fish oil (62.1%) and polysulfated glycosaminoglycans (PSGAGs; Adequan; Elanco, 61.9%). Very few respondents reported prescribing other NSAIDs (e.g., aspirin and carprofen, 5.6%) or triamcinolone (1.4%). Full data on percentage of respondents prescribing individual therapies are presented below in Table 3.03.

Years in practice and patterns of prescribing (Table 3.04) were compared. Opioids, PSGAGs and prednisolone, several ‘other’ options, and some groupings of therapies by class or mechanism of action (e.g., ‘Any Opioids’ or ‘Any Steroids’) showed an association. In general, practitioners with between six and 20 years of experience were most likely to prescribe some sort of therapy, and those with less than one year were least likely to prescribe any given therapy. Practitioners with more than 20 years of experience were most likely to prescribe glucocorticoids (Table 3.05).

Subsequently, relationships between percentage of cats seen in the practice and prescribed therapies (Table 3.06) were explored. There appeared to be little relationship between

<table>
<thead>
<tr>
<th>How frequently do clients elect to administer the recommended treatments?</th>
<th>Responses = 1055</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never or 0%</td>
<td>1</td>
<td>0.1%</td>
</tr>
<tr>
<td>Less than 25% of cases</td>
<td>277</td>
<td>26.3%</td>
</tr>
<tr>
<td>Between 25% and 50% of cases</td>
<td>359</td>
<td>34.0%</td>
</tr>
<tr>
<td>Between 50% and 75% of cases</td>
<td>278</td>
<td>26.4%</td>
</tr>
<tr>
<td>Between 75% and 100% of cases</td>
<td>120</td>
<td>11.4%</td>
</tr>
<tr>
<td>100% of cases</td>
<td>20</td>
<td>1.9%</td>
</tr>
</tbody>
</table>
the percentage of cats seen in the practice and prescription of individual therapies. In some instances, clinicians with high feline caseloads (76-99%) prescribed certain therapies at the highest frequency. However, this should be interpreted cautiously given the sample size for this group (number of responses = 6). That said, a positive relationship existed between the proportion of cats seen in the practice and the prescribing practices for PSGAGs. Similarly, practices seeing a higher proportion of cats reported higher prescribing frequencies of gabapentin, joint supplements, opioids and steroids (Table 3.07). A comparison of feline-only practitioners against all other respondents, revealed similar results (Table 3.08).
Table 3.03: Rank order percentages of respondents indicating that they prescribed a medication for the treatment of chronic musculoskeletal pain/arthritis in cats.

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Percent Respondents Prescribing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabapentin</td>
<td>71.0%</td>
</tr>
<tr>
<td>Joint Supp</td>
<td>67.8%</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>64.0%</td>
</tr>
<tr>
<td>Opioids</td>
<td>62.6%</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>62.1%</td>
</tr>
<tr>
<td>PSGAGs</td>
<td>61.9%</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>50.0%</td>
</tr>
<tr>
<td>Joint Diets</td>
<td>41.6%</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>34.4%</td>
</tr>
<tr>
<td>Tramadol</td>
<td>34.0%</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>8.6%</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>7.5%</td>
</tr>
<tr>
<td>Amantadine</td>
<td>6.9%</td>
</tr>
<tr>
<td>Other NSAID</td>
<td>5.6%</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>1.4%</td>
</tr>
<tr>
<td>Median number prescribed per practitioner</td>
<td>4</td>
</tr>
</tbody>
</table>

Median number of prescribed therapeutics is also included. NSAID = non-steroidal anti-inflammatory drug; PSGAGs = polysulfated glycosaminoglycans; Supp = supplement
Table 3.04: $\chi^2$ test for independence, showing where prescription of therapeutics varied significantly with years in practice

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>$\chi^2$ P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>0.003*</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>0.005*</td>
</tr>
<tr>
<td>NSAID Other</td>
<td>0.001†</td>
</tr>
<tr>
<td>Any NSAID</td>
<td>0.838</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>0.086</td>
</tr>
<tr>
<td>PSGAGs</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>0.195</td>
</tr>
<tr>
<td>Joint Support Diet</td>
<td>0.036*</td>
</tr>
<tr>
<td>Joint Supp Other</td>
<td>0.001†</td>
</tr>
<tr>
<td>Any Oral Joint Supp</td>
<td>0.010†</td>
</tr>
<tr>
<td>Tramadol</td>
<td>0.191</td>
</tr>
<tr>
<td>Opioids</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Any Opioid</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Amantadine</td>
<td>0.237</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.003*</td>
</tr>
<tr>
<td>Amantadine OR</td>
<td>0.010*</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.010*</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>0.004*</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>0.111</td>
</tr>
<tr>
<td>Any Steroid</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

*Denotes significance at $P = 0.05$

† Denotes significance at $P = 0.05$ with Bonferroni correction ($n = 20$)

NSAID = non-steroidal anti-inflammatory drug; PSGAGs = polysulfated glycosaminoglycans; Supp = supplement; Amant = amantadine; Amit = amitriptyline
### Table 3.05: Prescribing rates of therapeutics by years in practice.

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Years in Practice</th>
<th>Less than 1 year</th>
<th>1 to 5 Years</th>
<th>6 to 10 years</th>
<th>10 to 20 years</th>
<th>Greater than 20 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam*</td>
<td></td>
<td>46.9</td>
<td>56.3</td>
<td>59.4</td>
<td>70.7</td>
<td>66.4</td>
</tr>
<tr>
<td>Robenacoxib*</td>
<td></td>
<td>53.1</td>
<td>59.6</td>
<td>56.9</td>
<td>45.2</td>
<td>45.9</td>
</tr>
<tr>
<td>Other NSAIDs†</td>
<td></td>
<td>6.3</td>
<td>1.1</td>
<td>3.8</td>
<td>4.2</td>
<td>8.9</td>
</tr>
<tr>
<td>Any NSAID</td>
<td></td>
<td>84.4</td>
<td>85.2</td>
<td>85.6</td>
<td>83.3</td>
<td>82.3</td>
</tr>
<tr>
<td>Gabapentin</td>
<td></td>
<td>54.8</td>
<td>71.0</td>
<td>76.9</td>
<td>73.2</td>
<td>68.9</td>
</tr>
<tr>
<td>PSGAGs†</td>
<td></td>
<td>34.4</td>
<td>50.3</td>
<td>58.8</td>
<td>67.4</td>
<td>66.8</td>
</tr>
<tr>
<td>Fish Oil</td>
<td></td>
<td>50.0</td>
<td>62.5</td>
<td>67.8</td>
<td>64.8</td>
<td>59.3</td>
</tr>
<tr>
<td>Joint Support Diet*</td>
<td></td>
<td>25.0</td>
<td>45.2</td>
<td>50.0</td>
<td>41.4</td>
<td>38.6</td>
</tr>
<tr>
<td>Other Joint Supp†</td>
<td></td>
<td>41.9</td>
<td>65.5</td>
<td>75.8</td>
<td>72.5</td>
<td>65.3</td>
</tr>
<tr>
<td>Any Oral Joint Supp†</td>
<td></td>
<td>75.0</td>
<td>90.7</td>
<td>92.5</td>
<td>90.4</td>
<td>85.7</td>
</tr>
<tr>
<td>Tramadol</td>
<td></td>
<td>31.3</td>
<td>30.6</td>
<td>41.9</td>
<td>34.7</td>
<td>32.3</td>
</tr>
<tr>
<td>Other Opioids†</td>
<td></td>
<td>46.9</td>
<td>72.1</td>
<td>73.1</td>
<td>64.9</td>
<td>54.8</td>
</tr>
<tr>
<td>Any Opioid†</td>
<td></td>
<td>59.4</td>
<td>79.2</td>
<td>84.4</td>
<td>76.6</td>
<td>69.1</td>
</tr>
<tr>
<td>Amantadine</td>
<td></td>
<td>3.1</td>
<td>4.4</td>
<td>5.0</td>
<td>8.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Amitriptyline*</td>
<td></td>
<td>3.1</td>
<td>2.2</td>
<td>6.9</td>
<td>6.3</td>
<td>10.9</td>
</tr>
<tr>
<td>Amant or Amit*</td>
<td></td>
<td>6.3</td>
<td>5.5</td>
<td>11.3</td>
<td>12.6</td>
<td>15.5</td>
</tr>
<tr>
<td>Prednisolone†</td>
<td></td>
<td>9.4</td>
<td>15.3</td>
<td>19.4</td>
<td>37.7</td>
<td>48.0</td>
</tr>
<tr>
<td>Methylprednisolone*</td>
<td></td>
<td>6.3</td>
<td>3.3</td>
<td>6.3</td>
<td>7.9</td>
<td>12.3</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Any Steroid†</td>
<td></td>
<td>59.4</td>
<td>79.2</td>
<td>84.4</td>
<td>76.6</td>
<td>69.1</td>
</tr>
</tbody>
</table>

*Denotes significance at P = 0.05
† Denotes significance at P = 0.05 with Bonferroni correction (n = 20)

NSAID = non-steroidal anti-inflammatory drug; PSGAGs = polysulfated glycosaminoglycans; Supp = supplement; Amant = amantadine; Amit = amitriptyline
Table 3.06: Therapeutic prescription frequency by percentage of feline patients.

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Percentage Feline Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Less than 25%</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>58.7</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>45.5</td>
</tr>
<tr>
<td>NSAID Other</td>
<td>4.1</td>
</tr>
<tr>
<td>Any NSAID</td>
<td>78.5</td>
</tr>
<tr>
<td>Gabapentin*</td>
<td>62.8</td>
</tr>
<tr>
<td>PSGAGs†</td>
<td>66.9</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>58.3</td>
</tr>
<tr>
<td>Joint Support Diet</td>
<td>43.5</td>
</tr>
<tr>
<td>Other Joint Supp*</td>
<td>57.9</td>
</tr>
<tr>
<td>Any Oral Joint Supp</td>
<td>86.0</td>
</tr>
<tr>
<td>Tramadol</td>
<td>33.1</td>
</tr>
<tr>
<td>Other Opioids*</td>
<td>47.1</td>
</tr>
<tr>
<td>Any Opioid*</td>
<td>62.8</td>
</tr>
<tr>
<td>Amantadine</td>
<td>5.8</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>5.8</td>
</tr>
<tr>
<td>Amant and Amit</td>
<td>10.7</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>33.9</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>10.7</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>2.5</td>
</tr>
<tr>
<td>Any Steroid*</td>
<td>62.8</td>
</tr>
</tbody>
</table>

*Denotes significance at P = 0.05
† Denotes significance at P = 0.05 with Bonferroni correction (n = 20)

NSAID = non-steroidal anti-inflammatory drug; PSGAGs = polysulfated glycosaminoglycans; Supp = supplement; Amant = amantadine; Amit = amitriptyline
**Table 3.07: $\chi^2$ test for independence showing where prescription of therapeutics varied significantly with percentage of feline patients**

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>$\chi^2$ P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>0.057</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>0.256</td>
</tr>
<tr>
<td>NSAID Other</td>
<td>0.424</td>
</tr>
<tr>
<td>Any NSAID</td>
<td>0.637</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>0.005*</td>
</tr>
<tr>
<td>PSGAGs</td>
<td>0.001†</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>0.427</td>
</tr>
<tr>
<td>Joint Support Diet</td>
<td>0.219</td>
</tr>
<tr>
<td>Joint Supp Other</td>
<td>0.008*</td>
</tr>
<tr>
<td>Any Oral Joint Supp</td>
<td>0.183</td>
</tr>
<tr>
<td>Tramadol</td>
<td>0.179</td>
</tr>
<tr>
<td>Opioids</td>
<td>0.006*</td>
</tr>
<tr>
<td>Any Opioid</td>
<td>0.044*</td>
</tr>
<tr>
<td>Amantadine</td>
<td>0.851</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.213</td>
</tr>
<tr>
<td>Amant OR Amit</td>
<td>0.246</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.721</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>0.716</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>0.480</td>
</tr>
<tr>
<td>Any Steroid</td>
<td>0.044*</td>
</tr>
</tbody>
</table>

*Denotes significance at $P = 0.05$  
† Denotes significance at $P = 0.05$ with Bonferroni correction ($n = 20$)  
NSAID = non-steroidal anti-inflammatory drug; PSGAGs = polysulfated glycosaminoglycans; Supp = supplement; Amant = amantadine; Amit = amitriptyline
Next, associations between the prescription of one therapy and the concurrent frequency of gabapentin prescription were examined. Prescription of almost every therapy correlated positively with prescription of gabapentin, excepting meloxicam, (no relationship) and certain steroids (Table 3.09) – in other words, respondents who prescribed gabapentin more frequently also prescribed other therapies more frequently.

Respondents were asked whether they calculated therapeutic doses based on true or lean body weight. Approximately half indicated that their calculations depended on the drug, while the remainder skewed towards total body weight (Table 3.10).

For most therapies, doses and frequencies used long-term appear to be similar to label dosages regardless of label indications (e.g., robenacoxib [Onsior; Elanco], which is only approved for perioperative pain in the United States, yet is used for chronic musculoskeletal pain), reports and reviews in the veterinary literature, 15,18,19 and veterinary formularies (Table 3.11).20 Duration of treatment varied by therapy, with the joint supplements generally being used for longer durations than NSAIDs or glucocorticoids. Respondents prescribed most therapies to less than 25% of cats with DJD. Respondents indicated that they prescribed a few therapies to 26%-50% of cats with DJD (e.g., gabapentin, fish oil, PSGAGs), while they prescribed joint supplements to 51%-75% of cats with DJD.

Finally, practitioners were asked about their perceived ideal and acceptable therapeutic formulations and dosing frequencies. The results indicate that respondents considered liquid therapies dosed once daily most ideal. Almost all formulations were deemed acceptable, and more than 40% of respondents accepted any dosing frequency less than three times daily (Table 3.12). There was a relationship between ideal formulation and ideal frequency of administration (p < 0.0001). The responses for parenteral (subcutaneous [SQ] or intramuscular [IM]) formulations appeared to contribute the most to this relationship, with responses clustering around less frequent administration (e.g. once monthly) when compared against oral routes of administration (e.g. once daily). Additionally, clinicians with 10 or fewer years preferred liquid formulations, while clinicians with 10 or more years preferred parenteral formulations (p = 0.007). When asked about what other formulations would be considered acceptable, answers included ‘transdermal preparations’, ‘whatever the owner/cat would tolerate’, ‘compounded chews/treats’ and mentions of ‘alternative medicine’ techniques.
Table 3.08: $\chi^2$ test for independence showing where prescription of therapeutics varied significantly between feline-only and all other practitioners.

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>$\chi^2$ P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>0.057</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>0.796</td>
</tr>
<tr>
<td>NSAID Other</td>
<td>0.375</td>
</tr>
<tr>
<td>Any NSAID</td>
<td>0.600</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>0.003*</td>
</tr>
<tr>
<td>PSGAGs</td>
<td>0.001†</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>0.342</td>
</tr>
<tr>
<td>Joint Support Diet</td>
<td>0.397</td>
</tr>
<tr>
<td>Joint Supp Other</td>
<td>0.018*</td>
</tr>
<tr>
<td>Any Oral Joint Supp</td>
<td>0.851</td>
</tr>
<tr>
<td>Tramadol</td>
<td>0.118</td>
</tr>
<tr>
<td>Opioids</td>
<td>0.296</td>
</tr>
<tr>
<td>Any Opioid</td>
<td>0.936</td>
</tr>
<tr>
<td>Amantadine</td>
<td>0.427</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.040*</td>
</tr>
<tr>
<td>Amantadine OR Amitriptyline</td>
<td>0.039*</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.283</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>0.484</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>0.321</td>
</tr>
<tr>
<td>Any Steroid</td>
<td>0.936</td>
</tr>
</tbody>
</table>

*Denotes significance at $P = 0.05$

† Denotes significance at $P = 0.05$ with Bonferroni correction ($n = 20$)

NSAID = non-steroidal anti-inflammatory drug; PSGAGs = polysulfated glycosaminoglycans; Supp = supplement
Table 3.09: Fisher’s Exact Test of effect of a therapeutic’s prescription on gabapentin prescription frequency.

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Fisher’s Exact P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>0.813 R</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>&lt;0.001 R †</td>
</tr>
<tr>
<td>NSAID Other</td>
<td>0.016 L *</td>
</tr>
<tr>
<td>Any NSAID</td>
<td>0.044 R *</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>N/A</td>
</tr>
<tr>
<td>PSGAGs</td>
<td>&lt;0.001 R †</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>&lt;0.001 R†</td>
</tr>
<tr>
<td>Joint Support Diet</td>
<td>0.028 R*</td>
</tr>
<tr>
<td>Joint Supp Other</td>
<td>0.009 R*</td>
</tr>
<tr>
<td>Any Oral Joint Supp</td>
<td>&lt;0.001 R†</td>
</tr>
<tr>
<td>Tramadol</td>
<td>&lt;0.001 R†</td>
</tr>
<tr>
<td>Opioids</td>
<td>&lt;0.001 R†</td>
</tr>
<tr>
<td>Any Opioid</td>
<td>&lt;0.001 R†</td>
</tr>
<tr>
<td>Amantadine</td>
<td>&lt;0.001 R†</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.001 R†</td>
</tr>
<tr>
<td>Amant OR Amit</td>
<td>&lt;0.001 R†</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.029 L*</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>0.002 L†</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>0.674 L</td>
</tr>
<tr>
<td>Any Steroid</td>
<td>1.000 L</td>
</tr>
</tbody>
</table>

*Denotes significance at P = 0.05; † Denotes significance at P = 0.05 with Bonferroni correction (n = 19)
L Denotes the hypothesis that gabapentin is prescribed more when drug or non-drug oral therapeutic is NOT prescribed
R Denotes the hypothesis that gabapentin is prescribed more when drug or non-drug oral therapeutic IS prescribed
NSAID = non-steroidal anti-inflammatory drug; PSGAGs = polysulfated glycosaminoglycans; Supp = supplement; Amant = amantadine; Amit = amitriptyline
Table 3.10: Practitioner therapeutic dose calculation methods.

<table>
<thead>
<tr>
<th>How do you calculate dosing?</th>
<th>No. of responses = 1053</th>
<th>Percent of Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always on actual body weight</td>
<td>267</td>
<td>25.4%</td>
</tr>
<tr>
<td>Always on lean body weight</td>
<td>167</td>
<td>15.9%</td>
</tr>
<tr>
<td>It depends on the drug</td>
<td>585</td>
<td>55.6%</td>
</tr>
<tr>
<td>Other</td>
<td>34</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

One hundred and sixteen respondents specified prescribed therapies other than those populating the survey. Responses included joint supplements and sources of Omega 3 fatty acids (despite being included as choices earlier in the study; number of responses = 33), acupuncture (number of responses = 26), traditional Chinese medicine and/or herbs (number of responses = 18), laser therapy (number of responses = 18), homeopathic treatments (number of responses = 11), maropitant (Cerenia; Zoetis, number of responses = 7) and cannabidiol-containing products (number of responses = 4). Two-hundred and twenty-five respondents provided final comments at the end of the survey. These responses commonly included recommendations of other therapies, including acupuncture (number of responses = 54), laser therapy (number of responses = 49), Traditional Chinese Medicine (number of responses = 3), Assisi loops (number of responses = 2) and others. We did not include these responses in any statistical analysis.
Table 3.11: Summary data of typical dosing regimen (including dose, frequency, duration and formulation), as well as percentage of patients prescribed the therapeutic.

<table>
<thead>
<tr>
<th>Drug or Non-drug Therapeutic</th>
<th>Dose amount</th>
<th>Dosing Frequency</th>
<th>Dosing Duration</th>
<th>Most Common Formulation</th>
<th>Percent Patients Prescribed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meloxicam</strong></td>
<td>0.02-0.04 mg/kg (&lt;0.02 to &gt;0.1 mg/kg)</td>
<td>Q 24 hours (Q12 to Q72h; PRN or Other)</td>
<td>15-30 days (1d to &gt;120d)</td>
<td>Liquid</td>
<td>Less than 25% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td><strong>Robenacoxib</strong></td>
<td>1 -2.4 mg/kg (&lt;1 to &gt;2.4 mg/kg)</td>
<td>Q 24 hours (Q12h to Q72h; PRN or Other)</td>
<td>4-7 days (1d to &gt;120d)</td>
<td>Tablet</td>
<td>Less than 25% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td><strong>Other NSAID</strong></td>
<td>N/A</td>
<td>N/A</td>
<td>61-120 days (1d to &gt;120d)</td>
<td>Tablet</td>
<td>Less than 25% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td><strong>Gabapentin</strong></td>
<td>5.1-10 mg/kg (&lt;5 to &gt;20 mg/kg)</td>
<td>Q 12 hours (Q12h to Q48h; PRN or Other)</td>
<td>61-120 days (2-3d to &gt;120d)</td>
<td>Compounded</td>
<td>26% to 50% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td><strong>PSGAGs</strong></td>
<td>5 mg/kg (&gt;5 to &gt;5 mg/kg)</td>
<td>Q14 days (Q24h to Q30d; Other)</td>
<td>&gt;120 days (1d to &gt;120d)</td>
<td>N/A</td>
<td>26% to 50% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td><strong>Fish Oil</strong></td>
<td>50.1 – 100 mg/kg (&lt;25 to &gt;400 mg/kg)</td>
<td>Q 48 hours (Q12h to Q48h; PRN or Other)</td>
<td>&gt;120 days (11-14d to &gt;120d)</td>
<td>Liquid</td>
<td>26% to 50% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td><strong>Joint Diets</strong></td>
<td>N/A</td>
<td>N/A</td>
<td>&gt;120 days (1d to &gt;120d)</td>
<td>N/A</td>
<td>Less than 25% (&lt;25% to All Cats)</td>
</tr>
</tbody>
</table>
Table 3.11 (continued).

<table>
<thead>
<tr>
<th>Joint Supp.</th>
<th>N/A</th>
<th>Q 24 hours (Q24h to Q30d; Other)</th>
<th>&gt;120 days (1d to &gt;120d)</th>
<th>Capsule or Tablet</th>
<th>51% to 75% (&lt;25% to All Cats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tramadol</td>
<td>2.0-4.0 mg/kg (&lt;2 to &gt;4 mg/kg)</td>
<td>Q 12 hours (Q8h to Q24h; PRN or Other)</td>
<td>15-30 days (2-3d to &gt;120d)</td>
<td>Tablet</td>
<td>Less than 25% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td>Opioids</td>
<td>0.01-0.02 mg/kg (0.005 to 0.5 mg/kg)</td>
<td>Q 12 hours (Q8h to Q72h; PRN or other)</td>
<td>8-10 days (1d to &gt;120d)</td>
<td>Liquid</td>
<td>Less than 25% (&lt;25% to All Cats)</td>
</tr>
<tr>
<td>Amantadine</td>
<td>3.0-5.0 mg/kg (&lt;3 to &gt;5 mg/kg)</td>
<td>Q 24 hours (Q12h to Q24h)</td>
<td>31-60 days (2-3d to &gt;120 days)</td>
<td>Liquid</td>
<td>Less than 25% (&lt;25% to 51% - 75%)</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.5-1.0 mg/kg (&lt;0.5 to &gt;2.5 mg/kg)</td>
<td>Q 24 hours (Q12h to Q24h)</td>
<td>61-120 days (4-7d to &gt;120d)</td>
<td>Tablet</td>
<td>Less than 25% (&lt;25% to 51% - 75%)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.5-1.0 mg/kg (&lt;0.5 to &gt;2.0 mg/kg)</td>
<td>Q 24 hours (Q12h to Q72h; PRN or Other)</td>
<td>15-30 days (2-3d to &gt;120d)</td>
<td>Tablet</td>
<td>Less than 25% (&lt;25% to 76% - 99%)</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>10.0-20.0 mg/kg (&lt;10.0 to &gt;20.0 mg/kg)</td>
<td>Q 30 days (Q7d to Q60d; PRN or Other)</td>
<td>61-120 days (1d to &gt;120d)</td>
<td>N/A</td>
<td>Less than 25% (&lt;25% to 76% - 99%)</td>
</tr>
</tbody>
</table>
Table 3.11 (continued)

<table>
<thead>
<tr>
<th>Triamcinolone</th>
<th>One response:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.5 mg/kg (&lt;0.5 to 0.5-1.0 mg/kg)</td>
<td>15-30 days (1d to &gt;120d)</td>
</tr>
<tr>
<td>Remaining: PRN or Other</td>
<td>Tablet</td>
</tr>
<tr>
<td>Less than 25% (&lt;25% to 51% - 75%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median (range). Joint Supp = Joint supplements

Table 3.12: Ideal and acceptable therapeutic formulations and dosing frequencies, as indicated by respondents.

<table>
<thead>
<tr>
<th>What is your IDEAL treatment formulation for treating chronic musculoskeletal pain or arthritis in cats?</th>
<th>No. of responses</th>
<th>Percent of Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>72</td>
<td>7.1%</td>
</tr>
<tr>
<td>Capsule</td>
<td>27</td>
<td>2.7%</td>
</tr>
<tr>
<td>Liquid</td>
<td>653</td>
<td>64.1%</td>
</tr>
<tr>
<td>Parenteral (SQ or IM)</td>
<td>131</td>
<td>12.9%</td>
</tr>
<tr>
<td>Other</td>
<td>133</td>
<td>13.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is your IDEAL dosing frequency for medications used to treat chronic musculoskeletal pain or arthritis in cats?</th>
<th>No. of responses</th>
<th>Percent of Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrice Daily</td>
<td>2</td>
<td>0.2%</td>
</tr>
<tr>
<td>Twice Daily</td>
<td>45</td>
<td>4.3%</td>
</tr>
<tr>
<td>Once Daily</td>
<td>545</td>
<td>52.4%</td>
</tr>
<tr>
<td>Twice Weekly</td>
<td>103</td>
<td>9.9%</td>
</tr>
<tr>
<td>Once Weekly</td>
<td>155</td>
<td>14.9%</td>
</tr>
<tr>
<td>Twice Monthly</td>
<td>22</td>
<td>2.1%</td>
</tr>
<tr>
<td>Once Monthly</td>
<td>122</td>
<td>11.7%</td>
</tr>
<tr>
<td>Once Every Other Month</td>
<td>45</td>
<td>4.3%</td>
</tr>
</tbody>
</table>
Table 3.12 (continued)

<table>
<thead>
<tr>
<th>What formulations for treating chronic musculoskeletal pain or arthritis in cats would you consider acceptable?</th>
<th>No. of respondents = 1046; 3306 total responses</th>
<th>Percent Practitioners Considering Acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>793</td>
<td>75.8%</td>
</tr>
<tr>
<td>Capsule</td>
<td>579</td>
<td>55.3%</td>
</tr>
<tr>
<td>Liquid</td>
<td>1003</td>
<td>95.8%</td>
</tr>
<tr>
<td>Parenteral (SQ or IM)</td>
<td>732</td>
<td>69.9%</td>
</tr>
<tr>
<td>Other</td>
<td>202</td>
<td>19.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What dosing frequencies for medications used to treat chronic musculoskeletal pain or arthritis in cats would you consider acceptable?</th>
<th>No. of responses = 1048; 4749 total responses</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrice Daily</td>
<td>45</td>
<td>4.3%</td>
</tr>
<tr>
<td>Twice Daily</td>
<td>440</td>
<td>42.0%</td>
</tr>
<tr>
<td>Once Daily</td>
<td>934</td>
<td>89.1%</td>
</tr>
<tr>
<td>Twice Weekly</td>
<td>731</td>
<td>69.8%</td>
</tr>
<tr>
<td>Once Weekly</td>
<td>790</td>
<td>75.4%</td>
</tr>
<tr>
<td>Twice Monthly</td>
<td>609</td>
<td>58.1%</td>
</tr>
<tr>
<td>Once Monthly</td>
<td>698</td>
<td>66.6%</td>
</tr>
<tr>
<td>Once Every Other Month</td>
<td>502</td>
<td>47.9%</td>
</tr>
</tbody>
</table>

Table 3.12: Ideal and acceptable therapeutic formulations and dosing frequencies, as indicated by respondents.

Questions are presented as they appeared in the survey
Discussion

Our survey suggests that gabapentin is a very popular choice for treating chronic musculoskeletal pain in cats, both in terms of the proportion of respondents who prescribe gabapentin and the proportion of cats prescribed gabapentin. Joint supplements were similarly prescribed by a high proportion of respondents, and for a high proportion of cats. Similarly, despite the potential adverse events and the US Food and Drug Administration (FDA)-mandated ‘black box’ warning against using meloxicam beyond a single dose in the United States, many respondents reported prescribing NSAIDs. While the survey did not collect data on whether respondents were taking a multimodal approach in individual patients, half of the respondents prescribed at least four therapies (although not necessarily to each cat). Therapies with lower risk of adverse effects (true or perceived) had a longer median duration of treatment and higher median proportion of cats being prescribed the therapy (e.g., joint supplements, fish oil and PSGAGs, compared to NSAIDs, steroids).

We found differences in prescribing habits with years in practice. With few exceptions, respondents with less than one year of experience prescribed all therapies at the lowest rate. While speculative, reasons for this could be lack of comfort in treating the disease, concerns about off-label drug use, or simply due to fewer cases seen by that group. However, experience alone does not seem to correlate with an increased likelihood to prescribe therapies. While prescribing frequency increased as experience increased from one year up to 20 years, prescribing frequency decreased across all therapies seen in respondents with more than 20 years of experience. It is unclear whether this is related to the more recent emphasis on chronic pain in companion species, or other factors. One such factor might be practitioner gender – female physicians are perceived as more empathetic than their male colleagues are, and are more likely to prescribe pharmacologic agents as a first line of treatment for patients with lower back pain. Whether these relationships are present in veterinary medicine is unknown. Practitioner experience, age, gender and other factors all likely contribute to these differences in prescribing rates overall, and of specific drug classes like NSAIDs and steroids.

Major events and drug approvals can impact prescribing frequencies. The FDA released a ‘Boxed Warning’ on Metacam in late 2010. This might, in part explain the reported lower prescription frequencies of meloxicam by less experienced practitioners. Similarly, robenacoxib gained approval (in the US, for post-operative pain and inflammation for up to 3 days) in early
2011, which appears reflected by increased prescribing rates by less experienced veterinarians in our survey. Previous studies have shown that prescribing habits are closely associated with age of physician. It is worth noting that since development and distribution of the survey, the European Medicines Agency (EMA) has adopted a positive opinion in regards to long-term administration of robenacoxib. The drug’s new label indication now awaits final approval by the European Commission. This would not have been reflected in the results at the time of the survey. Differences in drug approvals or formulation availability in countries outside of the United States could influence prescribing habits. It remains unknown whether these relationships are real, or merely coincidental.

Our study highlights a disconnect between which therapies have clinical data to support their use, and which therapies are used most often. For example, most respondents prescribed gabapentin to between 26 and 50% of cats with chronic DJD-associated pain. However, no clinical data exist supporting use of gabapentin as an analgesic in cats, (or indeed in dogs) – indeed, the few studies examining analgesic effects of gabapentin found no effect. Similarly, many respondents reported prescribing joint supplements, with no clinical data supporting their use. We suspect that an ‘appeal to authority’ fallacy might contribute to this, whether from numerous anecdotal accounts of use and efficacy, or from perceived experts in the field. Additionally, the perceived high safety margin with joint supplements likely plays into ‘probably doesn’t hurt and might help’ thinking. Practitioners are also likely influenced by material they hear or read, and a search of Centre for Agriculture and Biosciences International (CABI) abstracts indicates a steady increase in the number of review articles and conference proceedings discussing chronic pain in cats, despite only a handful of ‘source evidence’ studies - clinical studies evaluating efficacy of analgesics in cats with chronic pain. Indeed, with a paucity of data or evidence, review articles focused on dogma proliferate.

Overall, this suggests increasing pressure on practitioners to identify and address chronic musculoskeletal pain due to DJD, without the appropriate research or data to support fully informed and evidence-based treatment selections.

There are, unfortunately, several issues that could result from this disconnect, relating to the risks of a lack of efficacy or unknown side effects. Perhaps most important is the risk of continued pain because of ineffective treatment, especially given that pain in cats can be difficult to detect, due to both a lack of validated assessment tools and a robust caregiver placebo effect.
Conversely, there exists the possibility of a beneficial placebo-by-proxy effect.\textsuperscript{34} This occurs when administration of a therapy (efficacious or not) causes a change in care-giver disposition/optimism, changed or increased interactions with the patient and resulting improvement in patient quality of life and activity. Finally, without safety data from chronic administration, clinicians cannot predict infrequent or rare adverse effects, some of which could be serious.

Respondents preferred once daily administration of a liquid by a wide margin (64.1% and 52.4% of responses, respectively). The preference for a liquid formulation is understandable, given the perceived difficulty of administering solid oral medications to many cats. Similarly, a liquid formulation may be preferable to parenteral routes of administration for owners that are uncomfortable using needles, or cats that resent injections. However, we could not understand the preference for once daily administration for oral routes of administration, especially given that we provided less frequent options including twice weekly, once weekly, and once every other month. It might be that respondents were concerned about owner compliance without a set daily routine, or about therapeutic toxicity or efficacy with once every other month administration. In contrast, the ideal dosing frequency for parenteral formulations clustered around less frequent administration.

Our study has several limitations. Perhaps foremost among these is the assumption that owners are following through with prescribed treatments. We cannot determine whether prescribed therapies were administered to the cats, and no data were collected on whether clients renewed prescriptions. There was also a limit in the number of questions that we could feasibly ask. While algorithms were used to attempt to reduce the number of questions any respondent answered (e.g., bypassing questions that were not relevant to individual practitioners), several respondents commented about the survey being too long or arduous. The survey length might have reduced the response rate. However, our response rate did not differ from most survey response rates for surveys administered to VIN members. Therefore, our responses might simply reflect the ‘survey-responsive’ pool of veterinarians who are VIN members. As with any convenience sample, the results, including any perceived trends by practice type or years in practice, might not represent the actual population. The questions pertaining to dose of therapies were not open-ended, precluding the calculation of true averages for dosing regimens. These data would be useful for researchers designing clinical trials or efficacy studies. Also, due to
limitations in length, we were unable to collect data on typical treatment regimens for a patient, or to see whether respondents commonly prescribed multimodal therapy. These data would also help researchers evaluating potential synergy between medications, so that clinically relevant combinations could be evaluated. We were also unable to collect data on perceived efficacy, reasons for discontinuation of a therapeutic, or other similar considerations. These data would also be beneficial for maximizing research impact.

The survey did not include non-pharmaceutical therapies, such as acupuncture, laser therapy, or other alternative medicine practices. While many respondents included this information in comments, the data were not collected in a way to allow analysis. The survey respondents were primarily female and from the US and Canada. This gender bias reflects VIN membership demographics (71% female) more so than the demographics of the veterinary profession. This means that important differences in prescribing habits as well as licensed indications between genders, countries, or regions could not be been appreciated. Finally, survey logic requiring a respondent to select an answer before moving on was not used. This means that partial or incomplete data might have been collected even within a given therapeutic agent.

Conclusions

Our study highlights the wide knowledge gap that exists when it comes to treating chronic musculoskeletal pain in cats. Research is required into efficacy of various therapies, and the pharmacokinetics and safety after chronic administration. Given our results, further research into gabapentin would appear most pertinent and pressing.

Acknowledgements

The authors would like to thank Dr. Mark. G. Papich for his assistance during survey development.

Supplementary Material (Appendix E)

Full survey distributed to VIN members investigating prescribing practices for chronic musculoskeletal pain in cats.
Author note
This paper was presented in part at the 2017 Annual College of Veterinary Medicine Research Forum on 22 September at the College of Veterinary Medicine, North Carolina State University, Raleigh, USA.

Funding
This research was funded by the Comparative Pain Research Program of the College of Veterinary Medicine, North Carolina State University, Raleigh, USA.

Conflict of Interest
The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
Chapter 3 Afterword

Gabapentin’s usage for maladaptive pain conditions in human medicine, combined with the preceding results, indicated that further research into the drug would be of both translational and clinical veterinary interest. The drug’s central mechanism of action allows for responsiveness testing of the objective measure of maladaptive pain in development (see Chapter 5). This responsiveness testing would require repeated oral administration of gabapentin; however, there was no pharmacokinetic data following this dosing regimen in the cat.
References


Chapter 4 Foreword

The following pharmacokinetic study was planned to allow for accurate determination of a repeated oral dosing regimen for our planned NWR testing, as well as any future clinical trials evaluating gabapentin in cats with maladaptive pain.
Chapter 4: The pharmacokinetics of gabapentin in cats
The pharmacokinetics of gabapentin in cats

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Running Head: Gabapentin Pharmacokinetics in the Cat

Key Words: Feline, transdermal, pain, compounded
Abbreviations:
NCSU CVM (North Carolina State University College of Veterinary Medicine)
GABA (γ-Aminobutyric acid)
USFDA (United States Food and Drug Administration)
IV (Intravenous)
SD (Standard Deviation)
BCS (Body Condition Score)
IACUC (Institutional Animal Care and Use Committee)
VAPs (Venous Access Ports)
USP (United States Pharmacopeia)
API (Active Pharmaceutical Ingredient)
SWFI (Sterile Water For Injection)
HPLC UV (High-Pressure Liquid Chromatography with Ultraviolet Detection)
min (minutes)
hr (hours)
UPLC MS:MS (Ultra Performance Liquid Chromatography with tandem Mass Spectrometry)
IS (Internal Standard)
r² (Coefficient of Determination)
LOQ (Limit of Quantitation)
LOD (Limit of Detection)
sec (seconds)
F (Bioavailability, or fraction absorbed unchanged)
AUC (Area Under the Curve for the concentration versus time profile)
K01 (absorption rate constant to the central compartment)
K10 (elimination rate constant from the central compartment)
K12 (distribution rate constant from the central (1) to peripheral compartment (2))
K21 (distribution rate constant from the peripheral (2) to central compartment (1))
V1 (apparent volume of the central compartment)
TLAG (lag time or delay for drug absorption following oral administration)
T₁/₂ (half-life)
α T₁/₂ (plasma or distribution half-life after IV administration)
β T1/2 (elimination half-life after IV administration)
CL (systemic clearance)
CMAX (peak concentration)
K01 T1/2 (absorption half-life after oral administration)
K10 T1/2 (elimination half-life after oral administration)
VSS (apparent volume of distribution at steady state)
TMAX (time to peak concentration)
EC50 (half maximal effective concentration)

All work was performed at the North Carolina State University College of Veterinary Medicine (NCSU CVM) in Raleigh, North Carolina, USA, except for external determination of compounded product strength, which was performed at Campbell University Pharmaceutical Education & Research Center in Lillington, North Carolina, USA.

The study was funded by the Veterinary Pharmacology Research Foundation Pharmacokinetics Grant (administered through the American Veterinary Medical Foundation), by the Comparative Pain Research Program, and by the Clinical Veterinary Pharmacy Residency Program at the NCSU CVM.

This paper was presented as an oral presentation during the Annual CVM Research Forum at NCSU CVM on 22 September 2017.

Both Dr. Baynes and Dr. Papich are on the Scientific Review Committee for the Veterinary Pharmacology Research Foundation, who awarded the primary grant for the research performed. Dr. Papich is the chair of the committee, and is also a member of the Board of Directors that awarded the funds.

Dr. Baynes' and Papich's relationship to the study and its investigators was fully disclosed to the Scientific Review Committee and the Veterinary Pharmacology Research Foundation. They recused themselves from any discussions pertaining to the grant. The authors have no other conflicts of interest to disclose.
Cefazolin sodium was used in an off-label manner during implantation of the jugular venous access ports.

All procedures were performed according to a protocol (16-187-O) approved by the NCSU CVM Institutional Animal Care and Use Committee.

The authors thank Mr. Jim Yeatts for assistance conducting drug analysis. The authors also thank Dr. Carrie Muller for her assistance during catheter implantation, drug administration, and sample collection.
Abstract

Background
Gabapentin is the most commonly prescribed medication for the treatment of chronic musculoskeletal pain in cats. Despite this common and chronic usage, clinically relevant pharmacokinetic data is lacking.

Objectives:
To evaluate the pharmacokinetics of clinically relevant dosing regimens of gabapentin in cats.

Animals:
Eight research-purpose mixed-breed cats.

Methods:
Cats were enrolled in a serial order, non-randomized pharmacokinetic study. Gabapentin was administered as an IV bolus (5 mg/kg), orally (10 mg/kg) as a single dose or twice daily for two weeks, or as a transdermal gel (10 mg/kg) in serial order. Serial blood samples were collected up to 48 hours. Plasma concentrations were determined using Ultra Performance Liquid Chromatography-Mass Spectrometry. Compartmental analysis was used to generate gabapentin time-concentration models.

Results:
After IV administration CL (median (range)) and terminal half-life were 160.67 mL/kg*hr (119.63-199.11) and 3.78 hr (3.12-4.47), respectively. After oral dosing, the terminal half-life was 3.63 hr (2.96-4.77), and 3.72 hr (3.12-4.51) for single and repeated dosing. TMAX, and CMAX, as predicted by the model were 1.05 hr (0.74-2.11), and 12.42 µg/mL (8.31-18.35) after single oral dosing, and 0.77 hr (0.58-1.64), and 14.78 µg/mL (9.70-18.41) after repeated oral dosing. Bioavailability after a single oral dose was 94.77% (82.46-122.83).
Importance:

Repeated oral dosing of gabapentin did not alter the drug’s pharmacokinetics, meaning no dose adjustments are necessary with long-term treatment. As prepared, the transdermal route is an inappropriate choice for drug administration. These relevant data are important for future studies evaluating potential efficacy of the medication for treating chronic pain states in cats.
Introduction

There are no approved medications for the treatment of chronic musculoskeletal pain in cats, and only a limited number of analgesic therapeutics with data about efficacy.\textsuperscript{1-5} Prior to the studies reported here, we surveyed veterinarians regarding pharmaceuticals and dietary supplements used for the alleviation of chronic pain in the cat.\textsuperscript{6} The most frequently prescribed therapy was gabapentin.

Gabapentin, an analogue of the neurotransmitter $\gamma$-Aminobutyric acid (GABA), is a medication commonly used in human medicine for chronic (maladaptive) pain conditions, such as diabetic neuropathy.\textsuperscript{7,8} The drug currently has United States Food and Drug Administration (USFDA) approval for postherpetic neuralgia, and as an adjunctive therapy for partial onset seizures in humans. It is proposed to alter trafficking of voltage-gated calcium channel subunits\textsuperscript{9,10} which have altered expression levels in rodent neuropathic pain models.\textsuperscript{11,12} No studies have been conducted and published assessing the efficacy of gabapentin for the treatment of pain in veterinary species, including cats.

The pharmacokinetics of single oral (10 mg/kg) and intravenous (IV; 4 mg/kg) doses of gabapentin in 6 adult spayed female cats has been described,\textsuperscript{13} but no other data are available on the pharmacokinetics of gabapentin in the cat. Due of this paucity of data relevant to the clinical use of the drug, the pharmacokinetics of gabapentin administered via intravenous, orally (both single and long-term), and transdermal routes were investigated in the cat.

Materials and Methods

\textit{Animals}

Healthy purpose bred male castrated (5) and female spayed (3) domestic shorthaired cats were used in this study. All cats were two years old. Mean body weight was 4.8 kg, with a
standard deviation (SD) of ± 0.8 kg, corresponding to a median body condition score (BCS) of 5 (4-9). Cats were housed in climate-controlled grouped housing (70°F with relative humidity between 30-70%) on a 12:12 light: dark cycle, with rotating enrichment toys and features. Cats were monitored at least twice daily for general health and wellbeing, with any abnormalities immediately reported to the staff veterinarian. Cats were fed according to their caloric needs, and allowed ad libitum access to water during the study. All study procedures were approved by the Institutional Animal Care and Use Committee at North Carolina State University (IACUC No. 16-187).

Instrumentation and Drug Administration

Venous access ports (VAPs; Companion Port 5, Norfolk Access Technologies) were surgically implanted into the right jugular vein and threaded to the junction of the cranial vena cava and right atrium of all cats as previously described. The port was placed at least two weeks prior to drug administration. This was to facilitate blood sample collection across the multiple phases of the study, and minimize stress to the cats.

On the respective day of drug administration, gabapentin was administered as an IV bolus (5 mg/kg; n=8) in a cephalic vein, as an oral capsule (10 mg/kg; n=7), or as a transdermal gel (10 mg/kg; n=7) applied to the interior ear pinnae. Compounded gabapentin products were prepared according to United States Pharmacopeia (USP) standards <795> and <797>, as appropriate, and placed in a brown bag to protect them from light. Gabapentin for intravenous administration was formulated by adding 0.3 g of gabapentin active pharmaceutical ingredient (API; Letco Medical) to 30 mL of sterile water for injection (SWFI) for a final concentration of 10 mg/mL. The syringe-to-syringe method was used to create a homogenous mixture. A bubble point test was used to assess a 0.2 μm filter, which was then used to sterilize the solution. The compound
was then placed in a 30 mL glass vial, and refrigerated until injection, approximately 1 hour. Gabapentin for oral administration was formulated into two different strengths (50 mg and 75 mg) by triturating gabapentin API with lactose USP (Letco Medical) via geometric dilution and placing the respective triturates into #3 size, clear capsules (Letco Medical). Gabapentin for transdermal administration was compounded into a 200 mg/mL preparation, using ethoxydiglycol (solvent; Professional Compounding Centers of America) gabapentin API, and transdermal base (Lipoderm; Professional Compounding Centers of America). The mixture was made homogenous by using the syringe-to-syringe method. Strength of the compounded preparations was measured at an external lab using High-Pressure Liquid Chromatography with Ultraviolet detection (HPLC UV; Campbell University Pharmaceutical Education & Research Center, Lillington, NC), and in an internal lab following the USP standards (HPLC UV; 600 Controller/Pump, 717plus Autosampler, 2487 Dual Absorbance Detector, Waters).16

On the morning of the procedure, the area overlying the VAP was numbed with a topical 4% lidocaine cream (LMX4, Ferndale Laboratories). After approximately 1 hour, the area was clipped if necessary, and cleaned with a diluted iodine solution. A 22g right angle Huber needle infusion set (Norfolk Access Technologies) was then inserted through the skin into the port hub, and patency was assessed using heparinized saline solution (BD PosiFlush, Becton, Dickinson and Company). Prior to all samples, a 2-3 mL “dump sample” was collected and set aside. The 2-3 mL sample was then collected, and the “dump sample” returned to the cat. The catheter was then rinsed with either normal or heparinized saline solution. Blood samples were collected at 1, 3, 9, 15, 30 and 60 minutes (min), and then at 2, 4, 6, 8, 12, and 24 hours (hr) after IV administration. After administration of a single oral dose, samples were collected at 5, 15, 30, 45, 60 and 90 min, followed by collection at 2, 4, 8, 12, and 24 hr. After repeated oral administration
(twice daily for 14 days) of the drug, additional samples were collected immediately prior to the second-to-last and last doses, as well as at 36 hours after the final dose. Samples were collected at 15, 30, 60, and 90 min, followed by collection at 2, 4, 8, 12, 24, 36, and 48 hr after transdermal application of the drug. Samples were immediately placed into K_{2}EDTA tubes (BD Vacutainer, Becton, Dickinson and Company) for processing. Samples were centrifuged at 3,000 g for 15 min at 5°C, and plasma was aliquoted into two separate cryotubes and stored at -80°C until analyzed by Ultra Performance Liquid Chromatography with tandem Mass Spectrometry (UPLC-MS:MS; Acquity UPLC, Xevo Triple Quadrupole Mass Spectrometer, Waters).

Cats received gabapentin by each route in a serial order of IV bolus, single oral, repeated oral (twice daily for 14 days around normal feeding times at 0:630-07:00 and 15:30-16:00), and then transdermal routes. Cats were fasted a minimum of 12 hours prior to dose administration on sampling days, and were allowed access to food after the 2 hr sample was collected. The single oral dose was administered without any food, and was immediately followed with a 5mL oral bolus of water. The multiple oral doses were administered with a small amount of canned food (a/d Critical Care, c/d Urinary Care, Hill’s Pet Nutrition), including on the sample collection day. The repeated oral phase was started immediately after the 24hr sample was collected for the single oral administration, and was continued for 14 days. Otherwise, a washout period of at least 3 weeks was observed between study phases (IV and single oral, multiple oral and transdermal).

**Gabapentin Analysis**

The concentration of gabapentin in feline plasma was quantified with UPLC-MS:MS analysis of extracted samples using prepared calibration standards. An initial stock solution of 3000 µg/mL was prepared by dissolving 15000 µg gabapentin reference standard in 5.0 mL of 50:50 acetonitrile:H_{2}O (ACN:H_{2}O) solvent. This was then serially diluted to create working
solutions of 0.5, 2.5, 5.0, 25, 50, 250, 500, and 1500 µg/mL. The plasma calibration curve was prepared by diluting the working solutions with drug-free feline plasma (extracted using EDTA, Equitech-Bio, Inc.) for a final calibration curve of 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 30.0, and 60.0 µg/mL. The plasma calibration curve was prepared fresh each day of analysis. A pregabalin (Lyrica, Pfizer) internal standard was used as a control for variability in extraction, injection, and ionization.\(^{17}\) Pregabalin internal standard was prepared by emptying and dissolving a 150 mg capsule in 30 mL of methanol, for an initial concentration of 5 mg/mL. This solution/suspension was vortexed for approximately 5 minutes, and then centrifuged at 10,000 rct for 10 minutes. Supernatant was removed and serially diluted to achieve a final concentration of 0.05 µg/ml. (B. Kukanich, personal communication, December 20, 2016).

Plasma concentrations of gabapentin API, m/z 172.1 → 154, and the internal standard (IS) pregabalin, m/z 160.2 → 142.1 were determined by UPLC MS MS. Pure analytical reference standard for pregabalin was not available at the time of assay validation, however, because previous reports have shown satisfactory results when using the finished product as an internal standard\(^{17,18}\) we used Lyrica as the internal standard. A subsequent comparison between the pregabalin finished product and API (USP) was made to validate results. The standard curve was linear from 0.05 to 60 µg/mL (Ultra Performance Liquid Chromatography with tandem Mass Spectrometry = 0.997 ± 0.003; n=3). A minimum of four replicates of 0.05, 0.5, 5, and 60 µg/mL were used to calculate intraday accuracies of 103.9%, 85.1%, 89.0%, and 101.6% respectively. Intraday precisions were 7.53%, 3.23%, 7.36%, and 7.71%, respectively. The pregabalin to gabapentin percent recoveries using either the API or the finished product were compared at spiked gabapentin concentrations of 0.05, 0.5, 5, and 60 µg/mL. With a minimum of three replicates at each concentration, the average ratios (API/finished product) were 1.08, 0.99,
1.02, and 0.99, respectively. The gabapentin limit of quantitation (LOQ) was determined to be 0.05 µg/mL, as it was the lowest concentration in the standard curve with acceptable accuracy (100 +/- 15%) and precision (<10%). The limit of detection (LOD) was 0.01 µg/mL, as the concentration was repeatedly measureable with a signal to noise ratio of at least 2, though variability precluded accurate quantification. Data points below the LOQ were excluded from analysis, due to the high coefficient of variability of concentrations below 0.05 µg/mL. Sample preparation involved combining 0.2 mL of plasma, 0.2 mL of pregabalin internal standard (0.05 µg/mL) and 0.8 mL methanol with 0.1% formic acid. The sample was then vortexed for 5 seconds (sec), and centrifuged at 15,000 g for 10 min. A volume of 1 mL of supernatant was removed and placed into a glass test tube to be evaporated for 50 min at 40°C. The precipitate was then reconstituted with 0.3 mL of 50:50 ACN:H₂O, vortexed for 30 s, and then filtered using 0.2 µm injection vials. A volume of 5 µL was injected for each sample.

Separation was achieved at 40°C using a phenyl column (2.1 x 100mm, 1.7 µm phenyl column, Waters), using mobile phase of A: methanol with 0.1% formic acid and B: 0.1% formic acid, at a flow rate of 0.4 mL/min.

Pharmacokinetic Analysis

All pharmacokinetic analyses were performed using commercially available software (Phoenix WinNonlin, Certara). Nonlinear least squares regression was performed on plasma gabapentin concentrations after IV or oral administration, but not after transdermal administration. Data for all routes of administration were weighted by the reciprocal of the square of the observed plasma gabapentin concentration. 1-, 2-, and 3- compartment models with elimination from the central compartment were fit to the data for IV administration. 1-, and 2-
compartment models with both first-order absorption in and elimination from the central compartment, with or without lag time were fit to the oral administration data. Models were selected based on observation of the residuals plots, visual inspection, and by use of the Akaike information criterion (AIC). 19 Bioavailability values (F) for single oral dosing were calculated by use of the following equation:

\[
F = \frac{AUC_{oral} \times dose_{IV}}{AUC_{IV} \times dose_{oral}} \times 100
\]

where \( AUC_{oral} \) and \( AUC_{IV} \) were the Area Under the Curve after oral and IV administration, respectively; and \( dose_{IV} \) and \( dose_{oral} \) were the dose for IV and oral administration, respectively. The parameters \( V1 \) (central volume of distribution), \( K10, K12, K21 \), were estimated by use of the model for IV administration, and \( V \) (volume of distribution), \( K01, \) and \( K10 \) were estimated for both oral administration models. Other pharmacokinetic parameters were calculated by use of standard pharmacokinetic equations. The Wilcoxon signed-rank test was used to compare pharmacokinetic parameters after single and repeated oral administration of gabapentin.

Results

The strength of the compounded formulations were reported as (mean ±SD, when appropriate) 99% (n=1), 99.7 ± 5.9% (n=3), 98.0 ± 3.5% (n=3), and 137% (n=1) of the labeled strengths for the IV, 50 mg capsules, 75 mg capsules, and transdermal gel, respectively. Internal lab strength measurements indicated strengths of 92.6%, 95%, and 94.1% for the IV, 50mg capsule, and 75mg capsule formulations, respectively (n=1).

The model that was the best fit for the plasma concentrations of gabapentin after IV administration was a 2-compartment model (Figure 4.01). The actual dose administered ranged from 4.90 to 5.11 mg/kg. Pharmacokinetic parameters are available in Table 4.01.
Figure 4.01: Gabapentin plasma concentrations after administration as an IV bolus (5 mg/kg).

Data are available from 8 cats (open diamonds) with modeled time concentration curve (using geometric mean of derived parameters; solid line). Samples were collected at 1, 3, 9, 15, 30, and 60 minutes, and then at 2, 4, 6, 8, 12, and 24 hours after IV administration. Data below the limit of quantitation are excluded.

A 1-compartment model with lag time ($T_{lag}$) was the best fit for both single oral and repeated oral data (Figures 4.02 and 4.03, respectively). One cat was removed from study prior to oral or transdermal dosing due to complications secondary to the VAP. Data for three time points after single oral administration were excluded for one cat because they were clearly outliers, based on the extrapolation from the preceding plasma concentrations, and comparison to the other values obtained at those time points. A model could not be fit to the trough of pre-dose
time points during repeated oral dosing. The actual dose administered ranged from 9.04 to 12.5 mg/kg for both phases of oral administration. Pharmacokinetic parameters are summarized in Table 4.01. Wilcoxon signed-rank tests reveal that only $T_{lag}$ were significantly different ($p=0.016$) between single and multiple oral dosing. The pharmacokinetic parameters calculated after single oral dosing were used to simulate the concentration time profile over the entirety of the multiple oral dosing regimen, with individual plasma values overlaid (Figure 4.04).
Table 4.01: Summary pharmacokinetics statistics after administration of gabapentin as a single IV bolus (5 mg/kg; n=8), a single oral dose (10 mg/kg; n=7) and repeated oral doses (10mg/kg; n=7).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV</th>
<th>Single Oral</th>
<th>Repeated Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (%)</td>
<td>*</td>
<td>94.8 (82.5-122.8)</td>
<td>*</td>
</tr>
<tr>
<td>K01 (1/hr)</td>
<td>*</td>
<td>5.24 (1.77-15.62)</td>
<td>7.06 (1.32-11.46)</td>
</tr>
<tr>
<td>K10 (1/hr)</td>
<td>1.21 (0.87-1.67)</td>
<td>0.20 (0.14-0.23)</td>
<td>0.18 (0.15-0.22)</td>
</tr>
<tr>
<td>K12 (1/hr)</td>
<td>13.67 (8.35-25.46)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>K21 (1/hr)</td>
<td>2.82 (1.88-5.12)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>V1 (mL/kg)</td>
<td>129.09 (80.35-171.50)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>TLAG (hr)</td>
<td>*</td>
<td>0.45a (0.24-0.72)</td>
<td>0.21a (0.01-0.23)</td>
</tr>
</tbody>
</table>
Table 4.01 (continued)

<table>
<thead>
<tr>
<th></th>
<th>Median (Range)</th>
<th>*</th>
<th>*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (µg/mL)</td>
<td>32.77 (23.79-55.89)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>α (1/hr)</td>
<td>16.97 (11.15-31.74)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>α T₁/₂ (hr)</td>
<td>0.04 (0.02-0.06)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>B (µg/mL)</td>
<td>5.61 (4.21-6.80)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>β (1/hr)</td>
<td>0.18 (0.15-0.22)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>β T₁/₂ (hr)</td>
<td>3.78 (3.12-4.47)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>AUC (hr*µg/mL)</td>
<td>32.01 (24.61-42.38)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CL (mL/kg/hr)</td>
<td>160.67 (119.63-199.11)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CMAX (µg/mL)</td>
<td>*</td>
<td>12.42 (8.31-18.35)</td>
<td>14.78 (9.70-18.41)</td>
</tr>
<tr>
<td>K01 T₁/₂ (hr)</td>
<td>*</td>
<td>0.13 (0.04-0.39)</td>
<td>0.10 (0.06-0.53)</td>
</tr>
<tr>
<td>K10 T₁/₂ (hr)</td>
<td>*</td>
<td>3.53 (2.96-4.78)</td>
<td>3.90 (3.12-4.51)</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>5.17 (4.34 – 6.05)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>VSS (mL/kg)</td>
<td>804.57 (643.71-1049.90)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>TMAX (hr)</td>
<td>*</td>
<td>1.05 (0.74-2.11)</td>
<td>0.77 (0.58-1.64)</td>
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</tbody>
</table>

Results are presented as median (range). F, bioavailability, or fraction absorbed unchanged; K01, absorption rate constant to the central compartment; K10, elimination rate constant from the central compartment; K12, distribution rate constant from the central (1) to peripheral compartment (2); K21, distribution rate constant from the peripheral (2) to central compartment (1); V1, apparent volume of the central compartment; TLAG, lag time or delay for drug absorption following oral administration; A and B, and α and β = Coefficients and exponents, respectively, in the following equation used describe the drug disposition curve at time t: (A X e–αt) + (B X e–βt), where e is Euler’s number (2.7183); T₁/₂, half-life; α T₁/₂, plasma or distribution half-life after IV administration; β T₁/₂, elimination half-life after IV administration; AUC, area under the curve for the concentration versus time profile; CL, systemic clearance; CMAX, peak concentration; K01 T₁/₂, absorption half-life after oral administration; K10 T₁/₂, elimination half-life after oral administration; VSS, apparent volume of distribution at steady state, TMAX time to peak concentration

* Statistically significant difference (p=0.0052)
Due to erratic and poor transdermal absorption of gabapentin, the data was not modeled. Approximately 61% of collected samples had concentrations below the LOQ or LOD. The actual dose administered ranged from 9.68 to 10.05 mg/kg as labeled, or 13.26 to 13.77 mg/kg if corrected for the measured strength.

**Figure 4.02: Gabapentin plasma concentrations after a single oral dose (10 mg/kg)**
Data are available from 7 cats (closed circles) with modeled time-concentration curve (using geometric mean of derived parameters; solid line). Samples were collected at 5, 15, 30, 45, 60 and 90 min, followed by collection at 2, 4, 8, 12, and 24 hr after oral administration. Data below the Limit of quantitation are excluded.
Figure 4.03: Gabapentin plasma concentrations after repeated oral dosing (10 mg/kg)

Data are available from 7 cats (open circles) with modeled time-concentration curve (using geometric mean of derived parameters; solid line). Samples were collected at 5, 15, 30, 45, 60 and 90 min, followed by collection at 2, 4, 8, 12, 24, and 36 hr after oral administration. Data below the Limit of quantitation are excluded.

The only observed side effect during the study was mild-moderate sedation, noted during the 2 hours after IV administration. Oral administration of the compounded capsules was well tolerated by all cats, with the majority of cats freely consuming the capsule during the multiple/long term administration phase. Soft Elizabethan collars were placed on all cats after transdermal application of the drug, in order to reduce the likelihood of transfer of the transdermal gel to oral ingestion. Three cats removed these collars at various time points, though
they were not observed to have been actively grooming their ears and did not have correlating higher plasma drug concentrations than the other cats.

Figure 4.04: Gabapentin plasma concentrations after single (10 mg/kg; closed circles) and repeated oral dosing (10 mg/kg; open circles).

Data are available from 7 cats with modeled time-concentration curve (using geometric mean of derived repeated oral dosing parameters; solid line). Samples were collected at 5, 15, 30, 45, 60 and 90 min, followed by collection at 2, 4, 8, 12, and 24 hours after single oral dose administration. After the last repeated oral dose, samples were collected at 5, 15, 30, 45, 60 and 90 min, followed by collection at 2, 4, 8, 12, 24, and 36 hr. Data below the Limit of quantitation are excluded.
Discussion

This report demonstrated that repeated oral dosing does not significantly impact gabapentin pharmacokinetics, and that the drug has poor bioavailability when administered as the transdermal gel compounded for this study.

While our values for clearance and terminal half-lives are comparable to another report after a single IV bolus in cats, our results for the oral dose differ from data in that same report. Most notably, our maximum concentration ($C_{\text{MAX}}$) was approximately 50% greater than previously reported ($7.982 \pm 1.053 \, \mu g/mL; \, 4.638 - 10.550$), with a slightly longer half-life ($2.95 \pm 0.42 \, \text{hr}; \, 2.52 - 3.52$). These differences may be due to our reported higher bioavailability, higher dosages in some individuals, and differences in sampling sites and times. Ultimately, the reasons for the discrepancies between studies are undetermined.

There are no data for cats that describe the optimum plasma drug concentrations for gabapentin. While previously reported data and modeling suggests a half maximal effective concentration (EC50) ranging from 1.4 and 16.7 $\mu g/mL$ for treatment of hyperalgesia in the rat and 5.4 $\mu g/mL$ for the treatment of neuropathic pain in man, we cannot determine if these values will apply to cats. Calculation of the average plasma concentration after multiple doses results in a value of 6.11 $\mu g/mL$, with median trough concentrations of 2.55 and 1.49 $\mu g/mL$ for the second-to-last and last dose respectively. This would suggest that the current prescribing practices (10 mg/kg administered twice daily) would be insufficient to maintain plasma concentrations associated with efficacy in other species. Modeling to determine a potential dose and dosing interval, using a targeted minimum concentration of 5.4 $\mu g/mL$ and $C_{\text{MAX}}$ of 16.7 $\mu g/mL$ (based on the EC50 reported in man and rat, respectively) results in a suggested dose of approximately 8 mg/kg at an interval of 6 hours. Our data also suggest that gabapentin (as prepared in this study) has minimal transdermal absorption, and is not an appropriate route of
administration. The transdermal vehicle was chosen because of its drug delivery properties, and its common use in practice.\textsuperscript{24,25} These data are relevant given the increasing interest in compounding of transdermal preparations for cats.\textsuperscript{24-26}

There were several sources of potential bias or error in our study. Treatments were administered in a serial order, without randomization. While clearance, volume of distribution, and other pharmacokinetic parameters are not expected to change significantly in healthy adult cats over period of 3 weeks (washout period), unpredictable or uncontrollable factors could have affected our results. Additionally, we wanted to compare the first and last doses in a contiguous dosing regimen in order to make the results more clinically relevant, and so the oral dosing (single and repeat) was set up serially. During the repeated oral dosing phase, gabapentin capsules were administered around the cats’ normal feeding times. This was done to emulate how owners are likely to time dosing. This resulted in varied inter-dose intervals rather than rigid 12-hour intervals, which could affect factors like accumulation. However, the accumulation ratio (AR) calculated using the median K10 after a single oral dose and a 12 hour dosing interval indicates minimal accumulation (AR = 1.09), which was similar to the value obtained when comparing the AUCs of the two dosing regimens (AR = 1.05). Any other factors potentially impacting the pharmacokinetics after repeated dosing (i.e. induction or inhibition of metabolism) would still have occurred, if present. Data points below the LOQ were excluded from analysis due to the high variability observed during assay validation. This results in modeled time-concentration curves that appear to poorly fit the data at later time points, and could affect parameters calculated by the terminal elimination phase. However, the models show good fit at preceding time points, and evaluation of residual plots, AIC values, and visual examination indicated that alternative models or weighing factors were unsatisfactory.
While the strengths of the IV solution and oral capsules were within the USP’s acceptable range of $\pm$ 10%, our transdermal gel was not. This resulted in higher than intended doses being administered, though absorption was still poor. It is possible that the transdermal preparations lacked adequate homogenization, which could have affected both drug kinetics during the study, and subsequent strength analysis. Cephalic catheters for IV bolus administration were placed either ipsi- or contralateral to the sampling ports, which could affect the detected plasma concentrations. This risk may be reduced by the placement of the catheter in the cranial vena cava/right atrium, but the risk remains. The use of a finished product (Lyrica) as our internal standard added a confounding factor to our results. We chose this internal standard due to its previous use in the veterinary literature, however, the use of an analytical grade standard would have eliminated this risk. Subsequent comparison of the assay using either the finished product or API revealed satisfactory agreement. Cats were fasted prior to drug administration, and compounded capsules were administered either with a water bolus (single oral dose) or with a small amount of food (multiple/long term dosing). It is known that gastrointestinal transit time (and therefore, absorption kinetics) are different between the fed and fasted state, as well as among different food particle sizes.\textsuperscript{27} The type of meal affects gabapentin absorption in people.\textsuperscript{28} There was also not an even distribution of sexes, and one cat would have been classified as obese. Finally, our research cats do not represent the “target” population of (typically older) cats with chronic or maladaptive pain.
Chapter 4 Afterword

We confirmed gabapentin’s short half-life in the cat, and the potential need for more frequent administration to maintain plasma concentrations. However, targeted concentrations and dosing recommendations have been based on effective concentrations reported in rats and humans, and may not be appropriate in the cat. Furthermore, our plasma data do not reflect the drug’s distribution to effector compartments like the spinal cord, and rodent modeling data may again be irrelevant to the cat. It is clear that methods to assess gabapentin’s efficacy are needed, and in particular, methods to measure central sensitization due to gabapentin’s mechanism of action as an “anti-hyperalgesic” drug. This is the focus of the work described in Chapter 5.

A recent report regarding oral administration of pregabalin in cats indicates that twice daily dosing may be appropriate, positioning pregabalin as an attractive candidate for future research. It is important to note that the preceding research was performed in young, healthy cats, rather than the target population of older cats with clinical symptoms of DJD. Clearance or other pharmacokinetic parameters may be affected by age-related changes in efficiency, or by concomitant disease processes like chronic kidney disease. Pharmacokinetic data in these populations would be important for future clinical use.
References


Chapter 5: Pilot studies developing and evaluating the Nociceptive Withdrawal Reflex test in normal and degenerative joint disease-affected cats
Introduction

Spontaneous animal models of pain with relevant and validated outcome measures can be used to study translational chronic pain.\textsuperscript{1} Feline medicine also will benefit from the development of objective measures of chronic or maladaptive pain in the cat. Our objective for this study was to use the nociceptive withdrawal reflex (NWR) test in cats to address these shared needs. The NWR can provide direct evidence of central sensitization\textsuperscript{2-4} and evidence of a deficient endogenous analgesic system (dEAS),\textsuperscript{5-7} both of which occur in, and contribute to, chronic pain states. The NWR is an objective test when electrical currents are used as a test stimulus, and electromyography (EMG) recordings are used as an outcome measure. This modality also allows for fine tuning of input, and accurate analysis of output in relation to each other. The tests can be used for diagnosis of maladaptive pain, and evaluation of putative analgesics with central mechanisms of action.

The NWR test can diagnose the presence of CS,\textsuperscript{3,8,9} by detecting withdrawal responses at lower stimulation intensities (decreased response thresholds), or increased EMG responses to stimuli above this threshold. These increased EMG responses can also be seen with Temporal Summation (TS), which can be facilitated with CS.\textsuperscript{10}

EAS function is assessed with the NWR test by measuring the change in an EMG response from a baseline stimulus (test stimulus) to the response following or during application of a distant noxious stimulus (conditioning stimulus).\textsuperscript{2,7,11-13} In normal individuals, the conditioning stimulus activates the EAS which sends descending signals to inhibit pain, resulting in analgesia. This is measured in the NWR test as a decreased EMG response to a second, post-conditioned test stimulus. In individuals with a dysfunctional EAS, the EMG response following the conditioning stimulus is not decreased, and may even increase.\textsuperscript{2,4,13}

Validation of NWR testing in humans is still in its early stages, but two studies have reported excellent inter-session test-retest reliability of NWR thresholds, and one study has reported excellent repeatability of the threshold to elicit TS in patients with chronic lower back pain.\textsuperscript{5,11} There was excellent inter-session repeatability of the NWR threshold using a cold conditioning stimulus in human patients with chronic lower back pain,\textsuperscript{11} but in another study, there was moderate repeatability of reflex magnitude at a predetermined stimulation intensity using a hot water bath conditioning stimulus in healthy humans.\textsuperscript{12} The NWR test appears to be discriminatory in humans with knee OA,\textsuperscript{14} with similar findings of discriminatory ability in OA-
affected dogs. Repeatability assessment and standardization have not been performed in dogs to date.

Our first hypothesis was that the NWR test would be feasible in both normal and DJD-affected cats, with good to excellent repeatability between sessions. Our second hypothesis was that DJD-affected cats would demonstrate signs consistent with CS or dEAS in comparison to normal cats. The third hypothesis was that gabapentin administration to DJD-affected cats would result in a “return to normal” of stimulus responses in DJD-affected cats. In order to test these hypotheses, our first aim of the study was to determine the feasibility and repeatability of NWR testing in cats. The second aim was to collect preliminary data regarding any separation of normal and DJD-affected cats based on NWR responses or NWR measures of CPM. The third aim was to collect preliminary data on the effects of gabapentin on NWR test results in cats with DJD.

Materials and Methods

All study procedures occurred at the North Carolina State University College of Veterinary Medicine (NCSU-CVM), and were approved by the Institutional Animal Care and Use Committee at NCSU (IACUC No. 16-186).

Control Animals

Control animals used in this study were normal, healthy, purpose-bred domestic shorthaired cats. Cats were group-housed on a 12:12 light: dark cycle with climate control set at 70°F with relative humidity between 30-70%. Cats had access to rotating enrichment toys and features. Monitoring occurred at least twice daily for general health and wellbeing, and any abnormalities were immediately reported to the staff veterinarian. Cats had ad libitum access to water, and were fed according to their caloric needs. Control cats were confirmed free of clinical signs of pain and DJD, with minimal radiographic changes associated with the disease.
Client-Owned Animals DJD-Associated Pain

Client-owned cats with DJD-associated pain and owner-assessed mobility impairment were recruited for the study using advertising to owners and veterinarians. All research-related activities were paid for by the study.

Screening and eligibility procedures were similar to previous feline DJD-associated pain studies.16,17 Cats underwent physical, orthopedic, and neurological exams on the day of screening, at least two weeks prior to NWR testing. The screening visit also included the collection of blood and urine samples for analysis, a hematology profile, serum biochemistry profile, urinalysis with sedimentation, and serum T4 analysis, performed in-house (NSCU-CVM). Complete axial and appendicular radiographs were obtained under sedation and were reviewed. Study inclusion required clinical and radiographic evidence of DJD in at least two joints or spinal segments, in addition to owner-assessed mobility or activity impairment (CSOM > 6, see Chapters 1 and 2). Eligible DJD-affected cats were required to be at least one year of age, and not currently receiving anti-inflammatory or analgesic medications (proven effective or otherwise). Cats were allowed to receive dietary supplements (e.g., fish oils, glucosamine-chondroitin, or polysulfated glycosaminoglycans) as long as the dosing regimen was stable and continued during the study. Cats with controlled hyperthyroidism, or stable chronic kidney disease (CKD; up to IRIS stage 2) were allowed to participate in the DJD-associated pain (DJD-pain) group.

The CSOM questionnaire was repeated at every study visit to ensure persistent and stable disability in the patient, as well as to monitor treatment effects of gabapentin.

Cats that met these criteria entered a minimum 2-week baseline period to serve as a washout period for any post-sedation analgesic or other drug effects.

Testing

The testing procedure was modeled on previous work in the dog and adapted to the cat.15,18 Preliminary work established that cats sedated with acepromazine alone would not tolerate the NWR test, in contrast to previous reports in dogs.18-20 On the day of testing, cats were premedicated with 0.1 mg/kg acepromazine administered either intravenously (IV) or intramuscularly (IM). A cephalic catheter was then placed using a clean technique, with a preference for placement in the right cephalic vein. Cats were heavily sedated for NWR testing
and EMG recording with 2 mg/kg alfaxalone (Alfaxan®; Jurox Inc., North Kansas City, MO, USA), followed by a 2 mg/kg/hr constant rate infusion (CRI), delivered using a syringe pump (Medfusion 3010a; Smiths Medical MD, Inc., St. Paul, MN, USA), and titrated to effect. Sedation depth was considered appropriate when the patient made no gross conscious movements, pupils were constricted and central or slightly ventral, and ear flick and palpebral reflexes were present and brisk. Cats were placed with their hind end in right lateral recumbency and front end partially in sternal recumbency. Heat supplementation was provided via padding and a circulating warm water blanket placed under the patient. Supplemental heating was also provided through re-usable sodium acetate heating pads and/or a heating lamp. Patient heart rate, ECG, respiratory rate, body temperature, and SpO2 were monitored during testing. Supplemental “flow-by” oxygen was provided at a rate of 2L/min or greater, with normal saline as a maintenance fluid according to current guidelines.

The fur overlaying the left cranial tibial muscle, the left plantar tarsus, and the lumbosacral spinal segment was clipped. Two disposable 27 gauge subdermal needle electrodes (12mm Stainless Steel Needle, Natus Neuro, Middleton, WI, USA) were inserted perpendicularly into the left cranial tibial muscle 15mm apart along the long axis of the muscle. Another two electrodes were inserted intradermally/subcutaneously immediately proximal to the left metatarsal pad. The electrodes were placed 10mm apart, traveling from the lateral to medial aspect. The negative (-) electrode was placed most proximal, while the positive (+) electrode was placed most distal, closest to the metatarsal pad. Ground leads from the cranial tibial recording electrodes were secured to an alligator clip placed on the skin immediately overlying the lumbosacral spinal region. This grounding lead was periodically moistened with 70% ethanol to maintain conductivity. Figure 5.01 shows the full patient and equipment setup.

Cranial tibial recording electrodes were connected to the (+) and (-) terminals of a 4-channel differential amplifier (DP-304A, Warner Instruments, Hamden, CT, USA). Both high pass (10 Hz) and low pass (1 kHz) filters were applied. The signal was amplified (1k), before being transmitted to an analogue to digital converter (Powerlab 4/35, AD Instruments, Oxford, UK). Labchart 8 software (AD Instruments, Oxford, UK) running on a laptop computer was used for EMG recording and electrical stimulation management. Both raw EMG and rectified EMG traces were recorded. Stimulating electrodes were connected to the (+) and (-) terminals of a stimulus isolator (FE180, AD Instruments, Oxford, UK) connected to the Powerlab 4/35 using
Labchart 8 software. Figure 5.01 shows how the equipment was connected. When possible, recording equipment power supplies were connected to a separate set of outlets from the monitoring or heating equipment, to avoid electrical interference.

Recordings were monitored for excessive interference in the signal, which was addressed with adjustments to the electrode positioning or grounding. Occasionally supplemental heating equipment had to be moved or turned off for testing because of excessive AC interference. Prior to testing, a single testing stimulus (10 mA at 1 ms duration) was applied to the paw, using a grossly visible “toe twitch” and a stimulus-associated EMG recording to confirm both stimulation and recording electrode placement. At approximately 30 minutes post-induction, depth of sedation was assessed and verified to be appropriate. If findings were satisfactory, testing commenced. The depth of sedation was regularly assessed to ensure this was as stable as possible. If adjustment of the alfaxalone CRI was necessary, recordings were only made after a 10-minute waiting period to allow for stabilization at the new depth. Testing was performed in the order of: threshold determination, response to temporal summation, CPM measurement, and stimulus response curve generation.
Figure 5.01: Schematic of patient and equipment setup for Nociceptive Withdrawal Reflex recording.

A. Patient setup for NWR testing. The patient was placed in right lateral recumbency, with the head laying to the left of the image. The fur immediately overlying the left cranial tibial muscle (yellow dashed lines), the left plantar tarsus, and the lumbosacral area was clipped. EMG recording electrodes (yellow lines) were inserted into the cranial tibial muscle 15 mm apart (yellow asterisks). Positive (red +) and negative (black -) stimulation electrodes were placed 10mm apart, just proximal to the left metatarsal pad. Grounding leads ( ) from recording electrodes were attached to the patient in the lumbosacral region. An ECG lead (blue diamond) is also visible on the right hind paw pad.

B. Stimulation electrode placement in the plantar hind paw. The positive (red +) and negative (gray -) electrodes were placed approximately 10mm apart, in a lateral to medial direction just under the skin, with the positive electrode just proximal to the metatarsal pad.

C. Equipment setup for NWR testing. Full equipment information available in the body of the text.
Protocol Development

Development of the final NWR testing protocol occurred across three testing sessions in normal cats, with the testing paradigms employed at each session outlined in Figure 5.02.

The first testing session focused on initial protocol development and finalization, where multiple stimulation profiles for the TS and CPM profiles were evaluated. During the first testing session, threshold determination was discontinued if a cat’s threshold was above 6.0 mA, as any threshold at or above this value would not allow for 2x threshold testing during the TS profile. The TS protocol for the first testing session of normal cats consisted of eight stimulations for four cats, with two cats receiving 12 stimulations, both using 1ms pulse width stimulations delivered at either 1 or 2 Hz. The CPM testing paradigm was still undergoing refinement during the first session. The protocol for the SRC was unchanged across testing sessions.

The second testing session in normal cats focused on repeatability of results. During the second testing session, the NWR threshold was determined without any cutoffs. The second session’s TS profile consisted of 12 stimulations delivered at 2 Hz, because it more consistently elicited TS in the first session.

The third session in normal cats aimed to determine whether it was possible to consistently elicit the late response, or C-fiber mediated response (100-500ms after stimulation), because of a lack of late responses in earlier sessions. The NWR threshold was again determined without any cutoffs. Although the third sessions’ TS profile consisted of 12 stimulations, we also explored 2 to 5ms pulse width stimulations, as well as train of five (e.g., 12 x (1ms pulse width x 5 stimulations at 100 Hz) at 2 Hz; To5) stimulation in place of single stimulations. Although the SRC profile was unchanged, it was preceded by these significantly greater TS stimulation intensities during this third session.
Figure 5.02: Study Timeline of NWR Testing Sessions.

Normal cats underwent three testing sessions for protocol refinement and determination of repeatability. DJD-pain cats underwent two testing sessions using the final protocol, with an optional third testing session following administration of gabapentin. CPM = Conditioned Pain Modulation; GBP = Gabapentin. Text in red indicates NWR test data that was not used in final analysis.

* Indicates differences from final protocol sufficient to exclude data from analysis.

** Indicates differences from final protocol were minor, and did not preclude use in analysis.

*** Indicates no differences in protocol; however, differences in earlier testing protocol could have unwanted effect on data; therefore, these data were excluded from analysis.

Threshold determination

Stimulations of 1ms duration were administered at least 1 minute apart in a staircase method, starting at 1mA and continuing to 3, 5, 7, 9, and 10mA. A positive response was defined as an action potential (AP) with a root means square (RMS) amplitude at least 2x greater than the baseline signal. The comparison of AP and baseline was made contemporaneously using in-software functions in between stimulations. Following a positive response, the stimulus intensity was adjusted down in increments of 0.5 mA. Once responses were absent, the stimulus intensity...
was increased in 0.1 mA increments until a positive response was again detected. The NWR threshold was defined as the minimum stimulus intensity at which positive responses (AP) were elicited in at least two (with a preference for three) replicates.

Temporal Summation (TS)

A one minute resting interval between threshold determination and TS stimulation was observed. The TS stimulation profile consisted of eight (during protocol development in normal cats) or 12 (all subsequent testing) x 1ms stimulations at a frequency of 2 Hz. Temporal summation was first assessed at 2x Thr (if possible - the maximum stimulus intensity allowed by the program and equipment was 10mA). Otherwise, TS was assessed at 10mA for all cats. The TS profile was performed three times with a five-minute rest period in-between.

Conditioned Pain Modulation (CPM)

The same TS profile as described above was used for determination of the endogenous analgesic system’s function, using the three TS profiles as a baseline. This was followed by a TS profile during the application of the conditioning stimulus (repeated twice), and finally a post-conditioning TS profile (repeated twice in DJD cats). The conditioning stimulus consisted of application of noxious mechanical pressure to the dorsal aspect of the third metacarpus of the right paw with an electronic von Frey anesthesiometer (evF; Almemo 2450, IITC Life Science Inc., Woodland Hills, CA, USA). The pressure applied was just below the amount of force required to elicit limb withdrawal in each patient. This was determined by applying a steadily increasing amount of force to the third metacarpus, until twitching of the paw or withdrawal attempts were first perceived by the operator. The pressure was then slowly decreased until withdrawal attempts disappeared, with the resulting force used as a conditioning stimulus. This pressure was then applied for 30 seconds prior to, and during, the TS profile. This procedure was repeated after a 5-minute inter-test interval. A final TS stimulation profile was applied without the conditioning stimulus, as a “post–CPM” test. All CPM profiles were performed using both TS stimulation at 2x Thr (if applicable), and TS at 10mA (all cats).

Stimulus Response Curve (SRC)

The final stimulation sequence generated a stimulus response curve (SRC) at set stimulation intensities. To elicit responses at most stimulation intensities, and avoid overlap with NWR threshold data, To5 stimulations of 1ms pulse duration at 100 Hz were utilized for the SRC. These stimuli were delivered at sequential integer intensities from 0.1mA to 10mA, with a
1 minute inter-stimulation period. Two SRC were performed with a five-minute inter-test interval.

Immediately following the second stimulus response curve, a dose of 2mg/kg of robenacoxib (Onsior, Elanco Animal Health, Greenfield, IN, USA) was administered subcutaneously to the cat to control for any potential pain or inflammation. The alfaxalone CRI was discontinued, and the cat was placed in a warm, quiet cage for recovery. The cats were closely monitored during recovery until they were awake, fully responsive, mobile (some ataxia was deemed acceptable), and had normal vital signs. The cats were then returned to their colony or owner.

Testing for repeatability of responses occurred at least four weeks later using the same protocol detailed above.

Effects of Gabapentin

If owners consented, client-owned cats with DJD-associated pain continued into an optional aim of the study to gather preliminary data on the effects (if any) of gabapentin on patient mobility/comfort and NWR test results. Cats received gabapentin capsules compounded at the North Carolina State Veterinary Hospital Compounding Laboratory according to USP <795> standards22 (Gabapentin API titrated with lactose in #3 gel capsules, Letco Medical, Decatur, AL, USA). Gabapentin was dosed at approximately 10 mg/kg, to be administered two times daily for at least 30 days. Following this treatment, subjects returned for a third and final testing session. On the final testing day, gabapentin was administered approximately 30 minutes prior to acepromazine premedication, or one hour before sedation induction. The timing was chosen to align NWR testing and anticipated maximum plasma concentrations of gabapentin.23 The testing protocol otherwise proceeded without changes.

Data Extraction

Data were extracted from the recording software using a previously developed automated instruction set (macro) which compiled the rectified integral values of the 100 ms immediately following a stimulation (early response)18,24 or rectified integral values for the time period 100-500 ms following a stimulation (late response)18,24 into an in-software data pad (Figure 5.03). Baseline data were manually extracted by selecting either a 100ms or 400ms time period immediately preceding each stimulation set. These values were then transcribed into data
management software (Excel, Microsoft Corporation, Redmond, WA, USA). All data were normalized to baseline by subtracting the baseline integral value from the individual early or late response values. Any resulting negative values were set to zero.

Figure 5.03: Example EMG tracing.

Example EMG tracing (black line) from temporal summation stimulation shows A-fiber response (0-100ms post-stimulation, blue box) and C-fiber response (100-500ms post-stimulation, green box) and their respective areas extracted by the macro. The blue tracing indicates when an electrical stimulation was delivered. $V = $ volts.

Data Analysis

Threshold data required no further adjustment prior to analysis.

Linear regression analysis was applied to the TS data for each testing set. Slopes were calculated for scatterplots including either the first six stimulations only (TS6), or all stimulations (TSAll). To calculate “Maximum Summation” (TS Max) the maximum value from the final nine (or five) stimulations of each 10mA TS set was divided by the minimum value of the initial three stimulations of the set. This value was expressed as a percentage. Other methods for calculating summation used the average or median of the first three stimulations, to account for any uncharacteristically high or low initial responses. These calculations were termed
“Maximum Summation Average” (TS Avg) and “Maximum Summation Median” (TS Med) respectively. Additionally, the area under the curve of the TS sets (TS AUC) was calculated using the trapezoidal rule.

For determination of EAS activation, the average of the “CPM” data sets was divided by the averaged “pre-CPM” data. A 20% decrease (or increase) in response was considered of sufficient magnitude to indicate whether EAS activation or response facilitation occurred. A resulting value of less than 0.80 was determined to be indicative of Endogenous Analgesic System activation, a resulting value greater than 0.8 was interpreted as failure to induce the EAS, and a resulting value greater than 1.2 was further interpreted as indicative of the presence of facilitation. TS data at twice threshold stimulation intensity were not analyzed, as 25% of 2xThr TS data were missing because of high initial NWR thresholds.

Stimulus response curve data were also evaluated by linear regression. This plot was used to calculate an Area Under the Curve value utilizing the trapezoidal method. A linear trend line was inserted to calculate the slope of the total plot (all 11 stimulus responses) for comparison.

CSOM values for DJD-pain cats were summarized for each testing session. Analysis of gabapentin effects was performed using the same methods as described above.

Data analyses were performed using data management software (Excel) and statistical software (JMP 13). Each cat’s NWR Thr, TS, CPM, and SRC results were averaged across sessions (e.g., the averages of two total NWR Thr, six total TS at 10mA profiles, four total CPM and post-CPM profiles [two post-CPM profiles in normal cats], and four total SRC profiles) and these averages were used to determine group differences. Repeats were averaged within each session (e.g., the averages of three total TS at 10mA profiles each for sessions 1 and 2, two total SRC profiles each for sessions 1 and 2, no averages for NWR Thr) and were used to determine repeatability across sessions. All data sets were assessed for normality using the Shapiro-Wilk test. Significant differences between groups for normally distributed data were assessed using a 1-sided Student’s t-test, while non-normally distributed data were analyzed using the Wilcoxon Ranked Sums test. Repeatability within groups was assessed using both the Pearson’s and Spearman’s coefficients of correlation. Coefficient ranges of greater than 0.9, from 0.75 to 0.9, from 0.5 to 0.75, and less than 0.5 were used as indicative of excellent, good, moderate, and poor repeatability, respectively. Significance was set at a level of p<0.05 for all tests.
Results

Nociceptive Withdrawal Reflex testing with alfaxalone sedation was feasible in most normal, and all DJD-pain cats. Initial testing in normal cats included ten research-purpose domestic shorthaired cats. Of these ten cats, four did not undergo repeatability testing because of poor patient temperament. Within these four excluded cats, two were also excluded because of difficulties in eliciting and recording the reflex with resulting poor-quality data. Ultimately, six cats completed the study, consisting of five male castrated and one female spayed cat. The median body weight was 4.39 kg, and the median age was 2.5 years. Seven client-owned domestic shorthaired cats with DJD-associated pain were screened for eligibility. One cat was deemed ineligible because of a suspicion of previously undiagnosed neoplasia. Six client-owned DJD-pain cats were therefore enrolled in the study, with all cats completing the required two testing sessions. These six cats consisted of two male castrated and four female spayed cats. One cat in the DJD-pain GBP group was diagnosed with IRIS stage II CKD, with a second cat in this group diagnosed with stage I CKD. The remaining cats had no other relevant medical concerns. The median body weight for the DJD-pain group was 4.89 kg, with a median age of 10.5 years. Though enrollment criterion allowed for it, no cats were receiving analgesic medications, dietary supplements, joint-support diets. Baseline patient demographic data are available in Table 5.01.
Table 5.01: Baseline demographic information for normal and DJD-pain cats completing the study.

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<tr>
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<th>Normal (n=6)</th>
<th>DJD-pain (n=6)</th>
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<tbody>
<tr>
<td>Breed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSH</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>FS</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>2.5 (2-4)</td>
<td>10.5 (4-14)</td>
</tr>
<tr>
<td>Weight (kgs)</td>
<td>4.39 (4.12-5.0)</td>
<td>4.89 (4.05-8.09)</td>
</tr>
<tr>
<td>BCS (1-9)</td>
<td>5 (5-6)</td>
<td>5.5 (5-9)</td>
</tr>
<tr>
<td>CSOM (0-12)</td>
<td>N/A</td>
<td>6 (6-9)</td>
</tr>
</tbody>
</table>

Summary data are presented as median (range). DJD = Degenerative Joint Disease; N = number of patients; DSH = Domestic Shorthaired; MC = Male Castrated; FS = Female Spayed; BCS = Body Condition Score; CSOM = Client Specific Outcomes Measure; N/A = Not Applicable.

The testing paradigms differed across sessions in normal cats. Only A-fiber responses could be consistently elicited, so all data reported are from this early response. Threshold analysis used data from the second and third testing sessions, TS data analysis uses data from the first and second sessions, CPM analysis is only available for the second session, and SRC analysis was performed using the first and second sessions. The testing protocol was finalized before DJD-pain cats were enrolled, resulting in two sets of data for all outcomes in DJD-pain cats. A third set of data was available for DJD-pain cats that completed the optional gabapentin testing session. This data set was not included in repeatability calculations.

One cat in the DJD-pain group experienced an adverse event and went into cardiopulmonary arrest soon after the completing the third optional gabapentin session. Its data was still included in analysis. Otherwise, no adverse events were observed during the remaining 31 test sessions in normal cats, or the 14 remaining test sessions in DJD-pain cats.

Data obtained in this study were a mixture of normal and non-normal distribution. Table 5.02 includes the median and range for each outcome by group, as well as the Shapiro-Wilk W
Test for normality result, and the appropriate significance test’s value. Figures 5.03 is a visualization of the distribution of data by group for each outcome measure.

Table 5.02: Summary of results of NWR testing in both normal (n=6) and DJD-pain cats (n=6).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Summary Data</th>
<th>Normality</th>
<th>Significance</th>
<th>CPM</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr (mA)</td>
<td>S-W</td>
<td>t</td>
<td>W</td>
<td>P</td>
<td>S</td>
</tr>
<tr>
<td>Normal</td>
<td>2.8 (0.8 to 7.3)</td>
<td>0.081</td>
<td>0.58</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DJD-pain</td>
<td>3.6 (2.3 to 7.25)</td>
<td>0.081</td>
<td>0.58</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>TS Max (%)</td>
<td>Normal</td>
<td>373.6 (264.2 to 1303.2)</td>
<td>0.003</td>
<td>N/A</td>
<td>0.34</td>
</tr>
<tr>
<td>DJD-pain</td>
<td>311.0 (260.3 to 730.4)</td>
<td>0.003</td>
<td>N/A</td>
<td>0.34</td>
<td>0.92 (0.52 to 1.25)</td>
</tr>
<tr>
<td>TS Avg (%)</td>
<td>Normal</td>
<td>219.70 (175.52 to 497.48)</td>
<td>0.0019</td>
<td>N/A</td>
<td>0.34</td>
</tr>
<tr>
<td>DJD-pain</td>
<td>211.70 (157.43 to 255.58)</td>
<td>0.0019</td>
<td>N/A</td>
<td>0.34</td>
<td>1.06 (0.68 to 1.15)</td>
</tr>
<tr>
<td>TS Med (%)</td>
<td>Normal</td>
<td>196.79 (195.78 to 545.88)</td>
<td>0.0012</td>
<td>N/A</td>
<td>0.2</td>
</tr>
<tr>
<td>DJD-pain</td>
<td>200.01 (139.04 to 260.98)</td>
<td>0.0012</td>
<td>N/A</td>
<td>0.2</td>
<td>1.00 (0.72 to 1.23)</td>
</tr>
<tr>
<td>TS AUC (V.s)</td>
<td>Normal</td>
<td>0.040 (0.021 to 0.10)</td>
<td>0.11</td>
<td>0.93</td>
<td>N/A</td>
</tr>
<tr>
<td>DJD-pain</td>
<td>0.017 (0.0042 to 0.057)</td>
<td>0.11</td>
<td>0.93</td>
<td>N/A</td>
<td>0.96 (0.64 to 1.27)</td>
</tr>
<tr>
<td>TS 6</td>
<td>Normal</td>
<td>2.5e-4 (1.6e-4 to 1.1e-3)</td>
<td>0.027</td>
<td>N/A</td>
<td>0.2</td>
</tr>
<tr>
<td>DJD-pain</td>
<td>9.1e-5 (3.6e-5 to 8.5e-4)</td>
<td>0.027</td>
<td>N/A</td>
<td>0.2</td>
<td>1.05 (-0.47 to 2.31)</td>
</tr>
<tr>
<td>TS All</td>
<td>Normal</td>
<td>2.0e-4 (4.9e-5 to 4.0e-4)</td>
<td>0.2</td>
<td>0.83</td>
<td>N/A</td>
</tr>
<tr>
<td>DJD-pain</td>
<td>6.9e-5 (1.4e-5 to 4.2e-4)</td>
<td>0.2</td>
<td>0.83</td>
<td>N/A</td>
<td>0.90 (0.44 to 1.73)</td>
</tr>
</tbody>
</table>
Table 5.02 (continued)

<table>
<thead>
<tr>
<th>SRC AUC (V.s)</th>
<th>Normal</th>
<th>DJD-pain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.037 (0.017 to 0.126)</td>
<td>0.040 (0.006 to 0.080)</td>
</tr>
<tr>
<td></td>
<td>0.24 0.67 N/A</td>
<td>0.25 0.1</td>
</tr>
<tr>
<td></td>
<td>0.79 0.8</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SRC Slope</th>
<th>Normal</th>
<th>DJD-pain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.6e-4 (3.8e-4 to 1.9e-3)</td>
<td>6.8e-4 (1.5e-4 to 1.8e-3)</td>
</tr>
<tr>
<td></td>
<td>0.041 N/A 0.36</td>
<td>0.76 0.8</td>
</tr>
<tr>
<td></td>
<td>0.78 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Summary data are presented as median (range) when applicable. S-W = Shapiro-Wilk Test; t = Student’s t test; W = Wilcoxon test; P = Pearson’s coefficient; S = Spearman’s coefficient; CPM = Conditioned Pain Modulation, a measurement of Endogenous Analgesic System activation; DJD = Degenerative Joint Disease; Thr = Threshold; mA = milliamps; TS = Temporal Summation; Max Sum = Maximum Summation, calculated as previously discussed; Max Sum Avg = Maximum Summation Average, calculated as previously discussed; Max Sum Med = Maximum Summation Median, calculated as previously discussed; AUC = Area Under the Curve; V.s = Volts x Seconds (area of recorded electromyogram for a given interval); TS6 = The slope of the linear trend line of the first six stimulus responses for the set; TSAll = The slope of the linear trend line of all stimulus responses for the set; SRC = Stimulus Response Curve. Significance set at p = 0.05.

Threshold determination

Threshold data were normally distributed (Table 5.02, Figure 5.04). Though the median NWR threshold of DJD-pain cats was greater than the median normal cat threshold, the ranges were wide and no statistically significant differences were detected between groups.

Temporal summation

Temporal summation calculations, excepting the slope of all responses (TSAll), and area under the curve (TS AUC) were not normally distributed. No statistically significant differences were detected between groups. Temporal summation slopes were occasionally negative, because of high initial responses followed by later lower responses to stimulation.

Conditioned Pain Modulation

We were unable to reliably induce the EAS activation. When assessing for induction of the EAS, per cat CPM averages were created using the TS (Max, Avg, Med) and TS AUC calculations. Slope data were not included in this calculation because of occasional negative
values. The median CPM responses were 99.1% (range 68.7% to 173.4%) in normal cats, and 104.1% (72.2% to 108.5%) in DJD-pain cats. These per cat averaged CPM values indicated only two normal cats demonstrated \( \geq 20\% \) response reduction following the conditioning stimulus (CPM response), and only one DJD-pain cat showing a CPM response. Conversely, two normal cats appeared to demonstrate response facilitation with the conditioning stimulus, with no DJD-pain cats showing facilitation. Data were not further analyzed.
Figure 5.04: Comparison of Normal and DJD-Pain Cat Nociceptive Withdrawal Reflex Test Outcome Measures.

Distributions of scores are presented for the average, untreated values for all normal cats (n=6, blue), and all DJD-pain cats (n=6, dark green). DJD = Degenerative Joint Disease-affected cats; A: Thresholds for the NWR test. NWR = Nociceptive Withdrawal Reflex; mA = milliamps; Thr = Threshold. No significant group effect was detected. B-D: Measures of maximum summation in response to temporal summation stimulation. B: Maximum Summation, A measurement of the maximum increase in responses during temporal summation at 10mA. C: Maximum Average Summation, A measurement of the maximum increase in responses during temporal summation at 10mA, using the average of the first three stimulation responses as the minimum value. D: Maximum Median Summation, A measurement of the maximum increase in responses during temporal summation at 10mA, using the median of the first three stimulation responses as the minimum value. No significant group effects were detected. E-G: Response slopes. E: TS6, A measurement of the slope of the initial six responses to temporal summation stimulation at 10mA, calculated using the linear trend line. F: TSAll, A measurement of the slope of all responses to temporal summation stimulation at 10mA, calculated using the linear trend line. G: SRC Slope, A measurement of the slope of all responses in the Stimulus Response Curve (SRC), calculated using the linear trend line. TS = Temporal Summation, TS6 = Slope of the initial 6 responses to TS at 10mA; TSAll = Slope of all responses to TS at 10mA. No significant group effects were detected. H-I: Area Under the Curve (AUC) of responses. H: TS AUC, A measurement of total response (AUC) to temporal summation stimulation at 10mA, calculated using the trapezoidal rule. I: SRC AUC, A measurement of total response (AUC) to the Stimulus Response Curve, calculated using the trapezoidal rule. V.s = Volts x Seconds (area of recorded electromyogram for a given interval). No significant group effects were detected.
Stimulus Response Curve

Stimulus response curve (SRC) area under the curve (AUC) values were normally distributed, while SRC slopes were not. Figure 5.05 shows SRC data by group. EMG responses were positively correlated with stimulus intensity, with correlation coefficient values ($R^2$) of 0.93 and 0.92 for normal and DJD-pain cats, respectively. Visual inspection of the data revealed overlap between groups, with a subsequent failure to detect significant differences. All SRC slopes were positive, as opposed to the temporal summation slopes.

Figure 5.05: Stimulus Response Curves by Group

Data are presented as medians with interquartile ranges in the error bars for normal (n=6; blue) and DJD-pain (n=6; green) cats. Stimulations consisted of a train of 5 stimulus (5 x 1ms pulse width at 100 Hz) at the indicated stimulus strength. V = volts, a measure of the EMG response; mA = milliamps, a measure of stimulus strength.

Client Specific Outcome Measures

There was some variation from session to session in CSOM data, with scores clustering around 6 or 7.
Effects of Gabapentin

Three DJD-pain cats enrolled in and completed the optional gabapentin portion of the study (DJD-GBP). All three cats received gabapentin orally twice daily. The median actual dose received by each cat was 9.8 mg/kg. Patient demographic and dosing information are summarized in Table 5.03.

Table 5.03: Summary baseline data and results from DJD-pain cats that completed the optional gabapentin phase of the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age</th>
<th>Weight</th>
<th>BCS (1-9)</th>
<th>GBP Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DJD-pain GBP</td>
<td>1 MC, 2 SF</td>
<td>12</td>
<td>6.45</td>
<td>7</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4-14)</td>
<td>(5.05 to 8.09)</td>
<td>(6 to 9)</td>
<td>(9.8 to 12.4)</td>
</tr>
</tbody>
</table>

Data are presented as median (range). DJD = Degenerative Joint Disease; GBP = Gabapentin; MC = Male Castrated; SF = Spayed Female; BCS = Body Condition Score; CSOM = Client Specific Outcomes Measure.

There were no significant within-cat differences when comparing pre and post-gabapentin NWR results. However, administration of gabapentin did result in a significant improvement in CSOM scores. This disparity between clinical subjective and NWR results appears most pronounced when evaluating the response to temporal summation (Table 5.05 and Figure 5.06).

The DJD-GBP data were analyzed and visualized similarly to before, in Table 5.04 and Figure 5.05.
Table 5.04: Summary of results of NWR testing in DJD-pain cats (n=6), including results for cats completing the optional gabapentin portion of the study (n=3).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Summary Data</th>
<th>Normality</th>
<th>Significance</th>
<th>CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S-W</td>
<td>t</td>
<td>W</td>
</tr>
<tr>
<td>Thr (mA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJD-pain All</td>
<td>3.6</td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(2.3 to 7.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJD-pain Pre</td>
<td>2.6</td>
<td>0.59</td>
<td>0.11</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(2.3 to 7.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJD-pain GBP</td>
<td>6.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5 to 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS Max (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJD-pain All</td>
<td>311.0</td>
<td></td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>(260.3 to 730.4)</td>
<td></td>
<td></td>
<td>(0.52 to 1.25)</td>
</tr>
<tr>
<td>DJD-pain Pre</td>
<td>290.07</td>
<td>0.46</td>
<td>0.81</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(260.30 to 612.59)</td>
<td></td>
<td></td>
<td>(0.52 to 1.25)</td>
</tr>
<tr>
<td>DJD-pain GBP</td>
<td>930.28</td>
<td></td>
<td></td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>(111.36 to 1374.98)</td>
<td></td>
<td></td>
<td>(0.44 to 2.14)</td>
</tr>
<tr>
<td>TS Avg (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJD-pain All</td>
<td>211.70</td>
<td></td>
<td></td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>(157.43 to 255.58)</td>
<td></td>
<td></td>
<td>(0.68 to 1.15)</td>
</tr>
<tr>
<td>DJD-pain Pre</td>
<td>236.12</td>
<td>0.72</td>
<td>0.73</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(203.27 to 302.70)</td>
<td></td>
<td></td>
<td>(0.68 to 1.15)</td>
</tr>
<tr>
<td>DJD-pain GBP</td>
<td>373.40</td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>(84.70 to 615.65)</td>
<td></td>
<td></td>
<td>(0.36 to 1.22)</td>
</tr>
<tr>
<td>TS Med (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJD-pain All</td>
<td>200.01</td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>(139.04 to 260.98)</td>
<td></td>
<td></td>
<td>(0.72 to 1.23)</td>
</tr>
<tr>
<td>DJD-pain Pre-GBP</td>
<td>242.57</td>
<td>0.38</td>
<td>0.79</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(200.97 to 293.54)</td>
<td></td>
<td></td>
<td>(0.72 to 1.23)</td>
</tr>
<tr>
<td>DJD-pain GBP</td>
<td>488.08</td>
<td></td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>(87.28 to 800.57)</td>
<td></td>
<td></td>
<td>(0.22 to 1.07)</td>
</tr>
<tr>
<td>TS AUC (V.s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJD-pain All</td>
<td>0.017</td>
<td></td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>(0.0042 to 0.057)</td>
<td></td>
<td></td>
<td>(0.52 to 1.25)</td>
</tr>
<tr>
<td>DJD-pain Pre</td>
<td>0.017</td>
<td>0.0029</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.0042 to 0.057)</td>
<td></td>
<td></td>
<td>(0.86 to 0.97)</td>
</tr>
<tr>
<td>DJD-pain GBP</td>
<td>0.0037</td>
<td></td>
<td></td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>(0.0022 to 0.0070)</td>
<td></td>
<td></td>
<td>(0.84 to 1.22)</td>
</tr>
</tbody>
</table>
Table 5.04 (continued)

<table>
<thead>
<tr>
<th>TS 6</th>
<th>DJD-pain All</th>
<th>DJD-pain Pre</th>
<th>DJD-pain GBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.1e-5 (3.6e-5 to 8.5e-4)</td>
<td>7.50e-5 (3.60e-5 to 5.63e-4)</td>
<td>2.7e-5 (-9.3e-5 to 7.3e-5)</td>
</tr>
<tr>
<td></td>
<td>1.05 (-0.47 to 2.31)</td>
<td>1.19 (-0.47 to 2.31)</td>
<td>0.95 (0.75 to 1.31)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TS All</th>
<th>DJD-pain All</th>
<th>DJD-pain Pre</th>
<th>DJD-pain GBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.9e-5 (1.4e-5 to 4.2e-4)</td>
<td>6.67e-5 (3.32e-5 to 2.54e-4)</td>
<td>2.7e-5 (-4.0e-5 to 6.3e-5)</td>
</tr>
<tr>
<td></td>
<td>0.90 (0.44 to 1.73)</td>
<td>0.93 (0.44 to 1.73)</td>
<td>0.56 (0.39 to 0.75)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SRC AUC (V.s)</th>
<th>DJD-pain All</th>
<th>DJD-pain Pre</th>
<th>DJD-pain GBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.040 (0.006 to 0.080)</td>
<td>0.028 (0.0098 to 0.080)</td>
<td>0.0073 (0.0051 to 0.038)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SRC Slope</th>
<th>DJD-pain All</th>
<th>DJD-pain Pre</th>
<th>DJD-pain GBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.8e-4 (1.5e-4 to 1.8e-3)</td>
<td>6.8e-4 (2.5e-4 to 1.5e-3)</td>
<td>1.0e-4 (1.0e-4 to 9.0e-4)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CSOM</th>
<th>DJD-pain All</th>
<th>DJD-pain Pre</th>
<th>DJD-pain GBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.33 (5.67 to 8.33)</td>
<td>6 (6-6.67)</td>
<td>2 (1 to 4)</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Summary data are presented as median (range) when applicable. S-W = Shapiro-Wilk Test; t = Student’s t test; W = Wilcoxon test; CPM = Conditioned Pain Modulation, a measurement of Endogenous Analgesic System activation; DJD = Degenerative Joint Disease; DJD Pre = Averaged pre-gabapentin responses for cats completing the optional phase of the study (n=3); DJD-GBP = Responses following administration of gabapentin in cats completing the optional phase of the study (n=3); Thr = Threshold; mA = milliamps TS = Temporal Summation; Max = Maximum Summation, calculated as previously discussed; Avg = Maximum Summation Average, calculated as previously discussed; Med = Maximum Summation
Median, calculated as previously discussed; AUC = Area Under the Curve; V.s = Volts x Seconds (area of recorded electromyogram for a given interval); TS6 = The slope of the linear trend line of the first six stimulus responses for the set; TSAII = The slope of the linear trend line of all stimulus responses for the set; SRC = Stimulus Response Curve; CSOM = Client Specific Outcomes Measure. Significance set at p = 0.05.

**Repeatability**

Repeatability of outcome measures ranged from good to poor, with occasional differences ratings between the normal and DJD-pain groups (Table 5.02). For both groups, NWR thresholds and TSAII showed good repeatability. Only the normal cat group showed good repeatability for TS6, while only the DJD-pain group showed good repeatability for the SRC calculations (SRC AUC and SRC slope). Figure 5.06 includes visualizations of representative results from session to session, including individual cat responses and group averages.
**Figure 5.06: Treatment Effect of Gabapentin on NWR Test Outcome Measures.**

Distributions of results are presented for the average, untreated values for all DJD-pain cats (n=6, dark green), the average pre-treatment values for DJD-pain cats who completed the optional gabapentin portion of the study (n=3, light green), and the post-gabapentin treatment values for cats completing the optional portion of the study (n=3, gray). DJD = Degenerative Joint Disease-affected cats; Pre-GBP = Averaged pre-gabapentin treatment scores for cats completing the optional phase of the study; DJD-GBP = scores following gabapentin treatment in the same cohort; GBP = Gabapentin. **A:** Treatment effects on Client Specific Outcome Measures (CSOM) Scores, an owner assessment of patient pain and disability, with higher scores corresponding with greater pain or disability. A significant treatment effect was detected (p=0.02). **B:** Treatment effects on the threshold for the reflex. NWR = Nociceptive Withdrawal Reflex; mA = milliamps; Thr = Threshold. No significant treatment effect was detected. **C-E:** Treatment effects on measures of maximum summation in response to temporal summation stimulation. **C:** Maximum Summation, A measurement of the maximum increase in responses during temporal summation at 10mA. **D:** Maximum Average Summation, A measurement of the maximum increase in responses during temporal summation at 10mA, using the average of the first three stimulation responses as the minimum value. **E:** Maximum Median Summation, A measurement of the maximum increase in responses during temporal summation at 10mA, using the median of the first three stimulation responses as the minimum value. No treatment effects were detected. **F-H:** Treatment effects on response slopes. **F:** Treatment effects on TS6, A measurement of the slope of the initial six responses to temporal summation stimulation at 10mA, calculated using the linear trend line. **G:** Treatment effects on TSAll, A measurement of the slope of all responses to temporal summation stimulation at 10mA, calculated using the linear trend line. **H:** Treatment effects on SRC Slope, A measurement of the slope of all responses in the Stimulus Response Curve (SRC), calculated using the linear trend line. **I-J:** Treatment effects on Area Under the Curve (AUC) of responses. **I:** Treatment effects on TS AUC, A measurement of total response (AUC) to temporal summation stimulation at 10mA, calculated using the trapezoidal rule. **J:** Treatment effects on SRC AUC, A measurement of total response (AUC) to the Stimulus Response Curve, calculated using the trapezoidal rule. V.s = Volts x Seconds (area of recorded electromyogram for a given interval). No significant treatment effects were detected.

* indicates outcomes for which p<0.15.

** indicates outcomes for which p<0.05.
Figure 5.07: Representative graphs of inter-session repeatability of NWR results in normal and DJD-pain cats.

Results are presented as individual session averages for each cat, as well as group averages per session. Solid, blue lines represent normal cats (n=6), while dashed green lines represent DJD-pain cats (n=6). Group averages (Avg) are also presented as a solid dark blue line (normal cats) and dashed dark green line (DJD-pain cats).

A: Thresholds for the NWR test, an example of good repeatability in both groups. NWR = Nociceptive Withdrawal Reflex; mA = milliamps; Thr = Threshold.

B: Maximum Average Summation, A measurement of the maximum increase in responses during temporal summation at 10mA, using the average of the first three stimulation responses as the minimum value. This outcome measure showed poor repeatability in both groups.

C: SRC AUC, A measurement of total response (AUC) to the Stimulus Response Curve, calculated using the trapezoidal rule. This outcome measure showed good repeatability in the DJD group, compared to poor repeatability in the normal group. V.s = Volts x Seconds (area of recorded electromyogram for a given interval). No significant group effects were detected. mA = milliamps; NWR = Nociceptive Withdrawal Reflex; DJD = Degenerative Joint Disease-affected cats. See Table 5.02 for correlation coefficients of repeatability.
Because no significant differences between normal and DJD-pain cats were detected, post-hoc power analysis was performed using our results (Table 5.05). Power was generally poor, with only two outcome measures (TS Max Med Sum and TS AUC) exceeding 25% power.

**Table 5.05: Post-Hoc Power analysis using reported results.**

<table>
<thead>
<tr>
<th></th>
<th>Thr</th>
<th>TS Max</th>
<th>TS Avg</th>
<th>TS Med</th>
<th>TS AUC</th>
<th>TS6</th>
<th>TSAII</th>
<th>SRC AUC</th>
<th>SRC Slope</th>
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<tr>
<td>Normal</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>4.12</td>
<td>417.04</td>
<td>267.85</td>
<td>279.31</td>
<td>0.053</td>
<td>2.8E-04</td>
<td>1.4E-04</td>
<td>0.041</td>
<td>8.3E-04</td>
</tr>
<tr>
<td>Std Dev</td>
<td>1.88</td>
<td>201.99</td>
<td>121.16</td>
<td>136</td>
<td>0.036</td>
<td>3.4E-04</td>
<td>1.6E-04</td>
<td>0.032</td>
<td>6.6E-04</td>
</tr>
<tr>
<td>DJD-pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.85</td>
<td>567.54</td>
<td>207.77</td>
<td>199.53</td>
<td>0.026</td>
<td>4.0E-04</td>
<td>2.3E-04</td>
<td>0.051</td>
<td>9.8E-04</td>
</tr>
<tr>
<td>Std Dev</td>
<td>2.66</td>
<td>401.68</td>
<td>39.5</td>
<td>46.05</td>
<td>0.023</td>
<td>3.5E-04</td>
<td>1.3E-04</td>
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<td>6.9E-04</td>
</tr>
<tr>
<td>Power (%)</td>
<td>3.9</td>
<td>12.7</td>
<td>21</td>
<td>27.5</td>
<td>34</td>
<td>8.7</td>
<td>18.7</td>
<td>6.8</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Thr = Nociceptive Withdrawal Reflex Threshold; TS Max = 10mA Temporal Summation Maximum Summation, calculated as previously reported; TS Avg = 10mA Temporal Summation Maximum Average Summation, calculated as previously reported, TS Med = 10mA Temporal Summation Maximum Median Summation, calculated as previously reported; TS AUC = 10mA Temporal Summation Area Under the Curve, calculated as previously reported; TS6 = 10mA Temporal Summation Slope of the initial 6 stimulation responses, calculated as previously reported; TSAII = 10mA Temporal Summation Slope of All stimulation responses, calculated as previously reported; SRC AUC = Stimulus Response Curve Area Under the Curve, calculated as previously reported; SRC Slope = Stimulus Response Curve Slope, calculated as previously reported; DJD = Degenerative Joint Disease; Std Dev = Standard Deviation
Discussion

We hypothesized that DJD-pain cats would have lower reflex thresholds when compared to normal controls, similar to observations in humans with central sensitization.\(^2,13\) We further hypothesized that cats with DJD-associated pain would show greater temporal summation, less efficient Endogenous Analgesic System activation, and a greater total response to the Stimulus Response Curve. We did not detect any of these changes, and saw no separation between normal and DJD-pain cats in our results.

Without additional studies, the reason for our inability to detect differences between normal and DJD-pain cats is not known. While the initial stimulation profiles were modeled on human and dog research, protocol changes to stimulation intensities or frequencies based on preliminary findings may have affected our discriminatory ability.

One possibility for our failure to detect differences between groups is that cats with DJD-associated pain do not develop CS. It is also possible that the EAS is not present (whether efficient or deficient) in cats (normal or DJD-pain). However, this notion is contested by literature in both research-purpose and client-owned cats with naturally occurring OA that is suggestive of facilitated nociception and CS,\(^25-29\) with similar findings of CS with OA or DJD in other species.\(^6,15,18,30-32\) Furthermore, research in normal cats demonstrated the presence of the EAS via induction of CPM,\(^33\) with other studies confirming the presence of the EAS system in healthy subjects, and dEAS in chronically painful subjects of other species.\(^5-7,15,34-38\)

It is possible that the NWR parameters we measured demonstrate a large inter-cat variability, similar to the variability in activity data in cats seen in our study (Chapter 2) and others.\(^17,39\) Differences in these NWR parameters between normal and DJD-pain cats may be apparent only when comparing analgesic treatment-associated changes from baseline. Our results in DJD-pain cats receiving gabapentin suggested a potential treatment effect, supporting this speculation. However, data following gabapentin administration to normal cats were not collected, so comparison between groups of any changes from baseline is not possible.

The difficulty in recruiting cats for the study, and subsequent low power of our tests could have contributed to our inability to find significant results. Perceived benefit to the individual cat is important to owners considering participation in feline clinical trials, with owners less likely to participate in a study using experimental or unproven procedures, or studies where there is a risk of discomfort to their cat.\(^40\) Our recruitment difficulty may have resulted
from our primary aim of novel tool development and the potential for discomfort to the patient, with individual pain relief as a secondary aim. It is also possible that only a subpopulation of DJD-pain cats develop DJD-associated maladaptive pain, and we failed to recruit these cats. The sex imbalance of the recruited cats could have biased our results. We may have failed to recruit cats that were truly painful- our inclusion criteria relied on subjective assessments of pain by both veterinarian and owner. Recruiting cats that were “responders” in a previous feline analgesic trial would increase our confidence in the cat’s inclusion in the DJD-pain group. Without an objective method to detect CS or altered nociceptive processing, we were unable to screen for this specific cohort.

However, the NWR test is discriminatory for chronically painful conditions in other species. While we detected no significant differences in thresholds between normal and DJD-pain cats, dogs with untreated osteoarthritis-associated pain were found to have higher NWR thresholds than normal controls, in contrast to reports of lower thresholds with pain states in other species. Our sedation, patient positioning, electrode placement, and threshold determination protocols were similar or identical to the dog study. Rats with monoiodoacetate (MIA)-induced stifle OA had reduced reflex thresholds. This study also used alfaxalone sedation during recording, though mechanical pressure was used to elicit the reflex response. NWR testing in human patients with knee OA revealed decreased thresholds; however, subjects were awake in the study, and a train of 10 (1ms pulse widths x 10 stimulations at 200 Hz; To10) stimulations were delivered to the foot using surface electrodes. Studies performed in awake humans with cluster headaches (CH) or medication overuse headaches (MOH) found decreased NWR thresholds compared to controls using To5 stimulation. Combined, these studies suggest that differences in NWR thresholds between normal and DJD/OA-affected subjects can be detected in other species using methodologies ranging from identical to disparate to ours.

Similarly, we were unable to detect significant group effects on measures of TS in our subjects. Rats with MIA-induced OA showed more facilitated TS than normal controls only when analyzing the late response EMGs, using an 8 x 1ms 10mA 1 Hz stimulation profile, with early responses not showing significant differences between groups. TS is considered a primarily C-fiber mediated response, and early protocol development focused on eliciting a C-fiber mediated TS response by increasing pulse widths (e.g., 2ms, 5ms) and using To5
stimulations (e.g., 12 x (5 x 1ms at 100 Hz) at 2 Hz) in place of single stimuli. These efforts were still unable to consistently elicit a late response and were ultimately too noxious for use in non-terminal studies. TS can also be mediated by A-fibers; however, A-fiber mediated TS becomes quickly saturated at high stimulation intensities (e.g., 10mA), affecting the sensitivity of our measures of summation and TS slopes. We attempted to measure TS at lower stimulation intensities (2x Thr), but responses at lower intensities were not consistently elicited, and cats with thresholds above 5mA could not be tested in this manner because our equipment could not stimulate at intensities greater than 10mA. In dogs, measurement of both early and late responses demonstrated that OA was associated with greater TS of both.\textsuperscript{15} The stimulation profile used in the study in dogs\textsuperscript{15} consisted of 8 x 1ms 10mA stimuli delivered at a rate of 1 Hz, in contrast to our 12 x 1ms 10mA at a rate of 2 Hz. Although a 2 Hz stimulation frequency can elicit TS in both A\textsubscript{δ} and C fibers, a 1 Hz stimulation frequency preferentially elicits a C-fiber mediated TS response.\textsuperscript{42,43} The 2 Hz stimulation frequency was selected instead of a 1 Hz stimulation frequency during protocol development in normal cats because it more consistently elicited a TS response. However, in DJD-pain cats with CS, a 1 Hz stimulation frequency may have elicited a C-fiber TS response, resulting in significant group differences. Data was log-transformed in dogs before analysis using a general linear model. Examination of our log-transformed TS data (not presented) did not reveal any new significance or trends.

The methodology of TS evaluation using NWR in human studies is quite different, instead reporting the threshold for TS (TS Thr), or the stimulus intensity (mA) of a To5 (1ms pulse width x 5 stimulations at 200 Hz) repeated 5 times at 2 Hz required to elicit a facilitation of the reflex, defined as greater than 20µV for 10ms or longer.\textsuperscript{2,13} These studies reported a significant decrease in TS thresholds in patients with CH or MOH. It is possible that our predetermined stimulation strength (10mA) saturated the TS response in the normal cats, prohibiting any significant increases in TS elicited the DJD-pain groups. Therefore, determination of TS threshold may have allowed for separation of the normal and DJD-pain groups. However, just as we detected no differences between groups in NWR threshold, we may also have been unable to detect differences in TS threshold. Furthermore, TS Thr determination (with five-minute resting intervals) would have extended testing time beyond the approximately three hour point when stability of patient sedation and NWR responses deteriorated during protocol development. The use of a single stimulation in our study, contrasted with a To5, could
have affected our ability to detect group differences. However, our TS stimulus appeared to be supra-threshold and saturated responses for many cats, and a To5 stimulus may have further saturated responses.

We were unable to reliably induce or detect the effects of the EAS. Our conditioning stimulus may have been insufficiently noxious to elicit EAS activation in a heavily sedated cat, despite applying a pressure just below the operator-perceived paw withdrawal threshold. This failure to elicit a CPM response is likely due to the difficulties faced with matching and balancing the test and conditioning stimuli. The TS stimulus used resulted in saturation of responses, as previously discussed, which may have prevented detection of EAS activation. However, lower stimulus intensities did not elicit NWR or TS responses.

Our conditioning stimulus may not have been the optimal modality, compared to other conditioning stimuli like thermal hot or cold that are commonly used in human research. However, previous research in dogs indicated while noxious mechanical pressure could activate the EAS, noxious cooling of the limb did not (unpublished data). Eliciting CPM in dogs using mechanical pressure applied by a “bulldog clip” (a metal clip used to temporarily secure papers together) for 20 seconds discriminates between OA-affected and normal dogs. We selected noxious mechanical pressure as our conditioning stimulus for this reason. We initially assessed using binding clips as the conditioning stimulus during protocol development in normal cats, but the magnitude of the pressure applied by the clips was sufficiently noxious to alter the cats’ depth of sedation. We therefore selected the evF for mechanical pressure application, because of the device’s adjustable nature. Although we applied our conditioning stimulus for 30 seconds compared to the dog study’s 20 seconds, the conditioning stimulus duration does not have a significant effect on the extent of the CPM response in humans using pressure cuff occlusion. Other studies have detected CPM with using similar testing paradigms. Because the testing described in this paper was intended for clinical patients, it was not appropriate to use chemical irritants, such as mustard oil, capsaicin, and formalin, as conditioning stimuli. These methods are reserved for research animals such as rodent and rabbit models. The tissue damage and residual pain caused by these agents make them inappropriate for clinical use.

We were unable to detect differences in SRC outcomes between DJD-pain and normal cats. Canine subjects with OA pain that had not been administered analgesics showed similar SRC responses to normal controls, using the same stimulation profile as our study. The cats
recruited to our study had moderate DJD-associated pain, and would be expected to show evidence of CS. We expected the SRC would be sensitive for detection of CS because of the T05’s ability to elicit responses over a greater range of intensities (Figure 5.05); however, no studies have reported such discriminability of the SRC.

Although other NWR CPM studies in dogs\textsuperscript{15,18} and rats\textsuperscript{6} have used alfaxalone alone or with acepromazine for sedation, the drugs’ specific effects on our subjects are unknown. Administration of acepromazine and alfaxalone for sedation during NWR testing may have affected our ability to detect group effects. Alfaxalone is a centrally-acting neuroactive steroid that effects sedation (or anesthesia) and muscle relaxation via enhancement of the gamma (γ) aminobutyric acid type A (GABA)_A receptor’s inhibitory effects. This mechanism of action could inhibit nociception, and affect reflex arcs, as well as inhibit or decrease muscle contraction. However, alfaxalone was chosen for this study because of its previous use in the literature,\textsuperscript{6,18,49} and because alternative sedatives or anesthetic agents, such as isoflurane or opioids, have marked analgesic effects that would inhibit reflex arcs and muscle contraction to a greater degree than alfaxalone.\textsuperscript{50} Furthermore, alfaxalone has fewer side effects when compared to other anesthetic agents, is easily titratable, and allows for quick adjustment of the level of sedation. There were significantly increased mechanical and electrical thresholds, as well as decreased EMG responses to mechanical and electrical stimulation when comparing alfaxalone sedation to acepromazine sedation in dogs.\textsuperscript{18} Acepromazine is a centrally-acting dopamine (primarily D_2) and histamine receptor antagonist\textsuperscript{51} that is often used to produce tranquilization and sedation in veterinary species.\textsuperscript{52} Dopamine’s role in nociception and the EAS is complex, having both facilitatory and inhibitory actions depending on the concentrations and receptor,\textsuperscript{53} suggesting that acepromazine had the potential to affect our results. However, the use of acepromazine alongside alfaxalone did not preclude NWR recording or detection of group effects in dogs.\textsuperscript{15,18}

Although gabapentin is effective in people for treating some types of neuropathic pain syndromes with central sensitization, we failed to demonstrate any significant effects on NWR test results in three cats.\textsuperscript{54} This could be explained by the methodological considerations discussed above, and the low number of treated animals in our experiments- as we could not detect differences between normal and DJD-pain cats, we would not be expected to detect “improvement” or a “return to normal” with gabapentin administration. There was a significant
treatment effect for owner rated pain and disability, via the CSOM. This corroborates findings from a recent feline DJD clinical study, where gabapentin treatment was associated with owner-rated improvement.\textsuperscript{55} However, our lack of masking likely contributed to a placebo response. The presence of renal impairment in two of the DJD-pain GBP cats may have decreased drug clearance and also biased our results. However, CKD and DJD are highly co-prevalent in older cats, so these results may be more reflective of the target population.\textsuperscript{56} We did observe several trends (p<0.15) in our data that may suggest a treatment effect, although these trends need to be further explored with higher numbers of animals. These trends include the increased NWR thresholds, decreased TS6 and TSAll slopes, and decreased TS AUCs. All of these results could be interpreted as decreased nociceptive facilitation and reversal of CS.

Certain NWR testing paradigms were repeatable, with threshold and the slope of all temporal summation responses (TSAll) showing the highest repeatability in both groups. Repeatability of other measures differed between groups, such as good repeatability of the Stimulus Response Curve AUC and slopes for DJD-pain cats, but poor or mixed repeatability in normal cats. Repeatability should not rely on a single ICC value (Table 5.02 and Figure 5.07). For example, while NWR threshold data showed good repeatability in both groups there was considerable variability in the data, with thresholds increasing and decreasing between sessions in both groups. Our repeatability was likely affected by the subjective nature of sedation and differences in electrode placement between sessions.

NWR inter-session results appear to be repeatable in human subjects.\textsuperscript{3} However, the stimulation site can affect repeatability.\textsuperscript{57} Inter-session reliability of NWR CPM testing in humans ranges from moderate to excellent.\textsuperscript{11,12} Inter-session repeatability data for the NWR test are not available in dogs or rodents, as most study designs employ single testing sessions.\textsuperscript{6,15,18,19}

The data reported are subject to confounding effects because of the study design or modality, including the subjective nature of sedation depth monitoring and adjustment.\textsuperscript{18} Despite these limitations and our negative results, we were able to develop and demonstrate feasibility and repeatability of a protocol for NWR testing in cats.
References


Chapter 6: Future Directions
This research was designed to further develop the naturally occurring model of painful degenerative joint disease in the cat as a translational model for chronic or maladaptive pain in humans, and to advance our understanding of how to measure and treat chronic musculoskeletal pain in the cat.

Our literature review and research made it clear that veterinary medicine has made great strides in increasing our understanding of how to measure pain in this spontaneous model. However, data surrounding analgesics (proven efficacious or otherwise) is limited, and so future research is required to assess these drugs.

The findings in Chapter 2 indicated that more work is needed to refine the currently available outcome measures. For example, whereas the CSOM comparisons demonstrated robenacoxib’s efficacy as expected, the FMPI failed to detect significant improvement in treated cats. Refinement of the FMPI’s responsiveness may be assessed in a clinical trial using a medication with previously demonstrated efficacy. While robenacoxib’s efficacy was moderate, potentially impacting the FMPI’s sensitivity, an anti-NGF antibody, Frunevetmab, has previously demonstrated greater treatment-associated improvements in a feline clinical trial, with similar findings in other species. We propose that Frunevetmab should be planned as the positive control for a future study once the therapeutic becomes commercially available.

Our results in Chapter 2 demonstrated the need for refinement of our methods of analyzing the high-frequency, longitudinal, objective accelerometer data collected by activity monitors. Clinical trials should be designed that use functional data analysis to compare patterns of activity before and after treatment with a known efficacious analgesic, alongside the use of Hidden Semi-Markov modeling to determine whether the time spent in high or low activity states is altered by treatment. We propose a device or algorithm to be developed that can detect specific activity signatures in accelerometer data to compare frequencies of activities and characteristics of activity signatures (e.g., smoothness of activity, velocities, or durations) between cats with DJD-associated pain and normal cats. The ability to measure counts of specific activities will complement the CSOM or FMPI, adding an objective measure of any treatment-associated increases in the frequency or manner an activity is performed.

The activity data from Chapter 2 showed the utility of within-cat activity analysis. By comparing activity in cats receiving both robenacoxib and placebo, we demonstrated significant differences in the frequencies of cats with greater activity while receiving the active drug and
those with higher activity when receiving placebo. While we also aimed to detect deterioration following a masked placebo period, we were unable to replicate a previous report of this deterioration effect.\textsuperscript{5} Similarly, future feline analgesic studies should consider using a randomized crossover design so that all cats receive both active drug and placebo, improving the study’s power to detect treatment effects using within-cat analysis.

In the future, a follow-up to Chapter 3’s survey could be planned. While the initial survey collected a wealth of data, we were unable to determine whether veterinarians frequently prescribed multiple therapeutics to individual patients, and what these multi-modal regimens consisted of. Future surveys are also needed to track changes in prescribing habits as new medications and research become available. Furthermore, we were unable to collect data on the criterion that veterinarians use to determine when therapy should be initiated in a patient, and how (if) therapeutic efficacy is monitored. Knowing the answers to these questions could inform future clinical trials evaluating medication combinations, or could guide further tool refinement according to practitioner needs.

Following from Chapter 4’s results, future studies should evaluate the pharmacokinetics of gabapentin in the target population (e.g., cats with DJD-associated pain), including older cats with and without renal impairment. The dosing regimen produced from a clinical pharmacokinetic study could be tested for efficacy in a clinical trial. Centrally-acting medications should undergo similar pharmacokinetic and efficacy evaluation. Research in both client-owned and OA-research cats suggests that tramadol may be an effective therapeutic with the ability to reverse CS.\textsuperscript{6-8} Human research has demonstrated that duloxetine, a serotonin and norepinephrine reuptake inhibitor, is more efficacious in patients with dysfunctional EAS.\textsuperscript{9} Once tools for detecting and measuring CS in cats are more refined, these evaluations of tramadol and duloxetine would greatly improve the translational utility of naturally occurring painful DJD in the cat.

Although our negative results in Chapter 5 were disappointing, future research could continue to refine the NWR test. A new testing protocol should be developed to rectify the differences between our protocol and those used successfully in human and veterinary studies. Protocol development should also occur in DJD-pain cats. This would identify testing paradigms that elicit responses in subjects with suspected CS (using QST- see below), using reduced or even negative responses in normal cats as evidence of group effects. We also evaluated several
testing paradigms and multiple methods of data analysis. A future study could focus on refining a single test, such as TS, because of its potential responsiveness to treatment with gabapentin. This study could determine the optimal TS stimulation frequency (e.g., 1 or 2 Hz) and pulse width (e.g., 1ms or 2ms) in DJD-pain cats, to evaluate whether a C-fiber response can be elicited in the cohort. The study could also evaluate whether a lower pre-determined stimulation intensity (e.g., 5mA) is capable of eliciting the TS response in DJD-pain cats, to rectify our issue of saturated responses with 10mA TS. Following refinement of TS, we could then determine the optimal conditioning stimulus for use with the TS protocol, in order to continue assessment of EAS function. It may be that the less noxious testing stimulus evaluated in the first part of the study allows for detection of CPM using the same evF conditioning stimulus. We could also evaluate other mechanical conditioning stimuli, because of its known efficacy in dogs, or other application sites for the conditioning stimulus. An electrical conditioning stimulus (e.g., 10mA TS in a distant limb) may show utility, because of its precise and near-infinitely adjustable nature.

While unfortunately our research failed to demonstrate the NWR test’s discriminability between normal and DJD-pain cats, mechanical QST (mQST) is discriminatory in human research, and could be included in future studies to screen for suspected CS. Initial reports in research purpose cats with naturally occurring OA and client-owned cats suggest that mQST can discriminate between healthy controls and OA-affected cats. Furthermore, mQST was responsive to a centrally-acting medication (tramadol) in the research-purpose cats. Another study in client-owned cats with and without DJD-associated pain again found separation of groups by mQST methods, and reported moderate re-test repeatability at 2+ hours. Until other, fully objective measures of maladaptive pain in the cat are developed, mQST should be included in future clinical feline analgesic trials, especially those evaluating centrally-acting medications.

Because we were unable to achieve our goal of developing and validating the NWR test in cats with DJD-associated pain, future translational research should continue the pursuit of a novel measure of CS in the cat. While QST is a semi-objective measure of CS, the available data in humans and cats is promising. The next steps for development of QST in cats should include evaluation of the modality’s responsiveness in larger cohorts of client-owned cats. Because previous research suggests that QST is not responsive to meloxicam treatment effects, duloxetine, tramadol, or another centrally-acting medication would be most appropriate.
Research should focus on standardizing QST methodology, to allow for better comparison of results between studies, and determination of inter-session repeatability. Regardless of which novel measure of CS in the cat is further developed, practical implementation requires a “cage-side” test of the outcome measure that would not require advanced equipment not available in clinical practice. This could mean refining a single, most representative and reliable testing paradigm.

Finally, once relevant pharmacokinetic data is collected and these tools are refined, future work can focus on pharmacokinetic/pharmacodynamic (PK/PD) modeling in the target population of older, DJD-pain cats. This would include generation of dose-concentration-response curves, to evaluate the effects of several dosing regimens on test outcomes (e.g., QST or NWR results). Pain phenotypes can be defined in cats with naturally occurring painful DJD once the tools are available. These phenotypes could include peripheral and/or central pain (CS), with and without a functional EAS, and could even be further sub-phenotyped into specific sensory (e.g., mechanical or thermal) hyper- or hypo-algesia reported in humans. Predictability of phenotype-specific treatment responses and PK/PD models could then be developed using both peripherally (e.g., NSAIDs) and centrally acting medications (e.g., gabapentin, tramadol, duloxetine). Future somatosensory testing (QST or NWR) assessing treatment effects would benefit from collection of samples for PK analysis to verify therapeutic concentrations were achieved during testing. Ultimately, this ability to phenotype and predict treatment responses would be a major advancement in clinical veterinary medicine, optimizing treatment and minimizing unnecessary patient exposure to drugs and other therapeutics.

These future studies would greatly advance naturally occurring painful DJD in the cat as a model of chronic pain in humans, and would allow for use of the model in clinical, pharmaceutical, and mechanistic research.
References


APPENDICES
Appendix A: Feline Musculoskeletal Pain Index

NOTES TO ACCOMPANY THE FMPI CLINICAL METROLOGY INSTRUMENT

Conditions of use:
- The FMPI is designed as a Clinical Metrology Instrument (Questionnaire) for the assessment of Feline Musculoskeletal Pain. It can be used in clinical research studies, and also by practitioners for individual case assessment.
- Use of this questionnaire in a commercial setting (e.g., company funded clinical trials) requires the permission for use of the FMPI under license from North Carolina State University.
- The FMPI will be acknowledged in any publication or report by citing the appropriate reference.
- The FMPI will be used only in the form presented here, and the format, wording and order of the questions and responses will not be changed.
- The FMPI must not be given to others.
- The FMPI must not be sold in any form.

The FMPI is a questionnaire with appropriate readability, reliability and proven discriminatory ability. Full validity testing is continuing, and further versions of the FMPI may well take place in the future.

Instructions:
1. The following instructions should be read to owners by the operator each time the FMPI is administered.
   "This questionnaire asks you questions about your cat’s ability to do various activities compared to what you think a normal adult cat without mobility impairment would be able to do. Please read the questions carefully and place an ‘X’ in the appropriate box. ‘Normal’ is located here, and then there are various degrees of ‘abnormal’. If the activity does not apply, such as if you do not have stairs in your home, check this box on the far right. Owners should be encouraged to answer all questions at every evaluation and only select ‘Not applicable’ if the question or activity truly does not apply for their cat.

2. Upon completion of the questionnaire, the owner should return the questionnaire to the operator.

3. FMPI scores are calculated by assigning whole integer scores from 0 to 4, with 0 representing ‘not at all’, and 4 representing ‘normal’.

4. The total FMPI score is the sum of scores for each question. Higher totals indicate less impairment with a possible range of (0-68). For analysis, total score or percent possible can be used. Calculation of percent possible is performed by taking the total score for the cat and dividing by the total possible points (the number of questions answered multiplied by 4).

   FMPI%possible Score = (sum of Q1-17 scores) / (number of questions answered*4)

5. If repeat FMPI scores are acquired from an individual owner, they should not see their previous scores or responses prior to completing the questionnaire.

We welcome feedback on the FMPI. Please contact Dr. Duncan Lasseles using:
Duncan_Lasseles@ncsu.edu
The Comparative Pain Research Laboratory is very grateful to Morris Animal Foundation, Novartis Animal Health, and Boehringer Ingelheim Vetmedica Inc. for sponsoring the work that has led to the development and validation of the FMPI.
**FELINE MUSCULOSKELETAL PAIN INDEX**

Please take some time to complete the following questions.

Please mark the circle that best describes your cat’s ability to perform the following activities as compared to what you think a normal adult cat, without mobility impairment, would be able to do.

### 1. Walk and/or move easily?

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### 2. Run?

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### 3. Jump up (how well and how easily)?

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### 4. Jump up to kitchen-counter height in one try?

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Please rate your cat's ability to:

### 5. Jump down (how well and how easily)?

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### 6. Climb up stairs or steps?

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### 7. Go down stairs or steps?

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### 8. Play with toys and/or chase objects?

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### 9. Play and interact with other pets?

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Please rate your cat’s ability to:

10. Get up from a resting position?

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11. Lie and/or sit down?

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12. Stretch?

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13. Groom himself or herself?

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14. Interact with you and family members?

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Please rate your cat's ability to:

15. Tolerate being touched and/or held?

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16. Eat?

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17. Use the litter box (get in and out, squat, cover waste?)

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Appendix B: Client Specific Outcome Measures - feline

Client Specific Outcome Measures (CSOMf) - feline

Guide to setting up a 3-question CSOM for the evaluation of mobility impairment associated with Feline Degenerative Joint Disease (DJD)

Through the use of this Clinical Metrology Instrument, the severity of activity impairment caused by osteoarthritis/DJD is measured by the owner’s evaluation of very specific activities over time. The difference between this system and other clinical metrology instruments (e.g. FMPI) is that the activities being followed by each owner are unique. The system is a modification of a previously published system (Gingerich and Strobel 2003), which has been used successfully in the assessment of known analgesics (Cozzi and Spensley 2013) and putative analgesics (Lascelles et al. 2008) in dogs, and also used in cats to assess putative analgesics (Gruen et al. 2014; Lascelles et al. 2010; Lascelles et al. 2007; Gruen et al. 2015).

The CSOM is NOT an ‘off the shelf’ questionnaire – it needs to be created, and each instrument is unique to an individual cat & owner. To successfully use this instrument, it needs to created carefully, then completed the same individual on subsequent occasions. A higher score indicates more impairment (the owner is asked ‘how much difficulty has your cat had performing the following activities). The currently recommended scoring is based on 1 (normal) to 5 (impossible) for each question.

To create the activities to be followed, the investigator works with the owner to find suitable activities to evaluate. To start the thought process, the investigator discusses the cat’s activity with the owner, and discusses what mobility problems, or problems with activities the cat enjoys doing, that the owner is aware of. These discussions eventually lead to a highly defined and specific problem being identified, and the time and place that the problem seen is identified. Three such activities need to be defined.
1. Identifying the problematic activities
Owners are interviewed by a trained individual to identify at least three activities that his/her cat does not do as well anymore OR has stopped doing.

Example activities:

- Walking
- Running
- Pooping
- Crouching
- Rearing Up
- Jumping Up
- Lying down
- Getting up
- Grooming
- Jumping down
- Getting onto counters
- Going down stairs

Difficulty moving after long rest
Difficulty finding comfortable position
Use of litter box
Getting onto the bed
Playing with toys
Climbing stairs
Defecation
Interaction with human family members
Sleeping restfully
Playing with other animals
Getting up onto a high resting spot
Jumping down

Owners may either choose an activity from the list above or may modify or create one to better describe an activity that is adversely affected in their cat.

If the owner offers more than the prescribed number (3), then allow the owner to describe them all, record them, then ask again for owner to rank them as you start to determine the 3 activities to follow.

The owner should be encouraged to select the activities which are impaired AND most important to them and to the cat, and encouraged to select activities that have potential for improving with analgesic therapy. Other considerations include using:
- Everyday activities so the cat can be observed during these activities
- Activities that are difficult enough for the cat that the owners have to help them may be good activities. The owner must be willing to encourage the cat to perform these activities without help
- Activities that the cat has stopped doing all together (however, be careful not to include activities that the cat would never be able to do now, regardless of pain control)

To help the process of defining activities, the investigator can explore the cat’s activity by asking questions such as: How does it look when your cat jumps up onto the bed now compared to when it was a younger? Does he/she need to use front claws to help get up on things, when this was not the case before? Does he/she move around in their environment by making use of furniture that is at different levels, starting lower and gradually getting up higher? When he/she jumps down, does he/she hesitate or make a harsh landing? When he/she goes down the stairs, does he/she hesitate or does it take longer then previously?
It is best to try to identify a range of different activities – e.g. not all activities should be ‘jumping up……’

2. Constructing the description of the activity to be followed

Owners will then be asked to be very specific and to indicate both places and times when they see these activities impaired, e.g., “climbing house stairs last thing at night”, or “getting in and out of the litterbox.” The question needs to be constructed so that when asked to rate how problematic each activity is, the question can be answered. Avoid describing the difficulty:

   e.g.  Poor construction: ‘difficulty running’
         Good construction: ‘running easily across the kitchen first thing in the morning’

When constructing the CSOM activity, simple and understandable language must be used - not ‘vet-speak’

Do not mix two different activities into one, as in these following examples of Poor construction.

   Poor construction:
   a. Getting up normally after resting and stretching
   b. Going up and down stairs-one way is usually more difficult than the other
   c. Running and activity level

Make sure the wording is constructed properly. In the first example (a), we would not know whether to score for the getting up after rest or stretching part. In the second example (b), going upstairs and going downstairs should be considered two different activities. The last example (c), is asking about two activities and would be difficult to score using this scoring system.

   Better construction would be:
   a. Getting up normally after resting
   b. Ascending stairs without stopping
   c. Running while playing string

3. Specifying the time and place of the activity

However, note that in the above examples, there is no indication of the time and place that the activity takes place. If possible, the time and place the activity is/was observed should be incorporated into the question.

The examples above could read:

   a. Getting up normally after resting in the morning
   b. Ascending stairs last thing at night, at bedtime, without stopping
   c. Running while playing string in the evening after work
If the owner notices an activity occurring at two defined times of the day, then these can be incorporated into the same question. The owner must then score as an aggregate of the two time points.

4. Examples of good CSOM activities
Jumping off of bed without shortening the distance to jump down in the morning
Jumping onto bed without pulling itself up by the forelimbs in the evening
Jumping from floor to seat of the living room couch without struggling, in the evening
Getting up normally after lying down at any time of the day
Groom entire body after breakfast
Socializing and interacting with family in the evening

5. Rating the degree of impairment
An example CSOM form to be completed is shown below (and is available on this website [CSOM form]):

**How much difficulty has your cat had over the last week performing the following activities:**

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<th>Activity</th>
<th>No Problem</th>
<th>Mild Difficulty</th>
<th>Moderate Difficulty</th>
<th>Severe Difficulty</th>
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As seen from the above, owners will then be asked to rate how much difficulty has your cat had over the last week performing the following activities, where difficulty is rated as “no problem, mild difficulty, moderate difficulty, severe difficulty, impossible”

An owner is not permitted to put ‘no difficulty’ in the initial evaluation as this shows there is not room for improvement and that there is not a problem with this activity. After listening to an owner describe the problems their cat has, it is the role of the study personnel help the owner choose the correct level of impairment.

To help owners appropriately categorize their cat’s activity impairment as either no problem, mild, moderate, severe, or impossible the owners are provided definitions and general descriptions of each term as well as other similar words that might be used to describe that level of impairment.
**No Problem = 1**  
Definition: Able to perform without difficulty as a normal cat would do  
Description: difficult activity is no longer difficult  
Synonyms: easy, non-existent, like a spring chicken

**Mild = 2**  
Definition: far from extreme  
Description: owner can detect impairment whereas others might not  
Synonyms: slight, insubstantial, minor, small, weak

**Moderate = 3**  
Definition: not excessive or extreme  
Description: impairment easily detected by owner, others can observe impairment  
Synonyms: midway, modest, medium, intermediate

**Severe = 4**  
Definition: intensely or extremely bad or unpleasant in degree or quality  
Description: very obvious to any observer, condition requires evaluation or treatment  
Synonyms: extreme, serious, highly, great, large

**Impossible = 5**  
Definition: This activity cannot be done. If the cat always hesitates before jumping onto chair, it needs to be marked ‘impossible’ if the activity was worded “Jumping onto dining room chair without hesitating”  
Description: Not seen  
Synonyms: futile, hopeless, unattainable, no-way

An example of a completed CSOM:

<table>
<thead>
<tr>
<th>Activity</th>
<th>No Problem Difficulty</th>
<th>Mild Difficulty</th>
<th>Moderate Difficulty</th>
<th>Severe Difficulty</th>
<th>Impossible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jumping off of bed without shortening the distance to jump down in the morning</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Descending the main stairs in the morning, without hesitating</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Running while playing string in the evening after work</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

In this case, the score is 3 + 4 + 3 = 10
6. Scoring the CSOM.
The CSOM score is the addition of the scores for the 3 activities (see example above).

7. Review of the activities in the context of the clinical signs
The activities chosen will be reviewed in the context of the location of osteoarthritis (OA) or DJD, and either accepted, or the case may be rejected (in the case of a clinical study), or the owner re-questioned. If for example the chosen activities are all related to jumping down, which emphasizes forelimb function, and the OA/DJD is in the hind limbs only, the chosen activities for the CSOM should be considered questionable.
The following reviews our "example activities", and think about whether impairment of these activities relates more to fore, or hind limb OA, or could relate to either or both:

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>IMPAIRMENT RELATED TO OA OF FORE, OR HIND OR EITHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Playing/interacting with owners</td>
<td>Either</td>
</tr>
<tr>
<td>Playing with toys</td>
<td>Either</td>
</tr>
<tr>
<td>Playing with other pets</td>
<td>Either</td>
</tr>
<tr>
<td>Playing a specific game</td>
<td>Either</td>
</tr>
<tr>
<td>Walking</td>
<td>Either</td>
</tr>
<tr>
<td>Running</td>
<td>Either</td>
</tr>
<tr>
<td>Jumping up</td>
<td>Hind</td>
</tr>
<tr>
<td>Jumping down</td>
<td>Fore</td>
</tr>
<tr>
<td>Laying down</td>
<td>Usually Hind</td>
</tr>
<tr>
<td>Getting up</td>
<td>Fore (for first part of getting up); Hind for second part [most commonly Hind limb OA affects getting up]</td>
</tr>
<tr>
<td>Ascending stairs</td>
<td>Hind</td>
</tr>
<tr>
<td>Descending stairs</td>
<td>Fore</td>
</tr>
<tr>
<td>Difficulty moving after rest</td>
<td>Either</td>
</tr>
<tr>
<td>Difficulty moving after major activity</td>
<td>Either</td>
</tr>
<tr>
<td>Pouncing/crouching</td>
<td>Hind</td>
</tr>
<tr>
<td>Rearing up</td>
<td>Hind</td>
</tr>
<tr>
<td>Use of litter box</td>
<td>Hind (squat) Fore (entering box)</td>
</tr>
<tr>
<td>Jumping onto furniture</td>
<td>Hind</td>
</tr>
<tr>
<td>Jumping onto the bed</td>
<td>Hind</td>
</tr>
<tr>
<td>Jumping off the bed</td>
<td>Fore</td>
</tr>
<tr>
<td>Jumping off furniture</td>
<td>Fore</td>
</tr>
<tr>
<td>Grooming</td>
<td>Hind</td>
</tr>
<tr>
<td>Sleeping restfully</td>
<td>Either</td>
</tr>
</tbody>
</table>
8. Review of the activities to ensure a variety of activities are captured
The activities chosen will also be reviewed to ensure they are not all very similar. For example, 3
activities that are all related to jumping onto furniture would be considered too similar, and not
sufficiently different to capture any change in activity ability.

9. Environment in which the instrument is constructed and completed
The environment in which the instrument is constructed will affect the results, as will the way
the personnel interact with the owner completing the instrument.
Although not studied in detail, we recommend:
- that the pet not be present if it is playful or grabs the attention of the owner
- the environment be calm and neutral, such as a consultation/exam room, or quiet room
- the approach of the personnel be calm, and portray the sense of detail and seriousness
Appendix C: Quality of Life, Temperament, and Happiness Assessments

Quality of Life

Compared to before your cat received this most recent treatment, do you think your cat’s overall quality of life is (check one):

- Much worse
- Slightly worse
- No change
- Slightly improved
- Greatly improved

What is your cat’s overall quality of life today?

- Poor
- Fair
- Good
- Very Good
- Excellent

Temperament

Compared to before your cat received this most recent treatment do you think your cat’s temperament (mood, demeanor) is:

- Much worse
- Slightly worse
- No change
- Slightly improved
- Greatly improved

How would you describe your cat’s temperament (mood, demeanor) today?

- Unfriendly/unsocial
- Shy
- Independent
- Friendly/social
- High attention seeking

Compared to your last visit, would you say your cat is:

- Much more unhappy
- Slightly more unhappy
- No change in happiness
- Slightly more happy
- Much more happy

Owner/Agent
Signature: __________________________ Date: __________________________

Investigator
Signature: __________________________ Date: __________________________
Appendix D: Robenacoxib Safety Outcome Measures

Table D.01: Select hematological values by treatment group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>1 (pPP)</th>
<th>2 (pRR)</th>
<th>3 (pRP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>WBC* (1x10⁹/µL)</td>
<td>Pre</td>
<td>36</td>
<td>7.0</td>
<td>3.11</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>7.7</td>
<td>4.04</td>
</tr>
<tr>
<td>Absolute Neut* (/ul)</td>
<td>Pre</td>
<td>36</td>
<td>4756.5</td>
<td>2417.4</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>5013.1</td>
<td>3351.1</td>
</tr>
<tr>
<td>Absolute Lymph (/ul)</td>
<td>Pre</td>
<td>36</td>
<td>1515.1</td>
<td>968.2</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>1884.0</td>
<td>1482.8</td>
</tr>
<tr>
<td>%Lymph* (%)</td>
<td>Pre</td>
<td>36</td>
<td>22.0</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>25.1</td>
<td>12.3</td>
</tr>
<tr>
<td>%Neut* (%)</td>
<td>Pre</td>
<td>36</td>
<td>67.5</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>64.3</td>
<td>13.7</td>
</tr>
<tr>
<td>RBC (1x10⁶/CMM)</td>
<td>Pre</td>
<td>36</td>
<td>7.9</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>8.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>Pre</td>
<td>36</td>
<td>38.1</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>40.8</td>
<td>5.3</td>
</tr>
<tr>
<td>Hgb (g/dL)</td>
<td>Pre</td>
<td>36</td>
<td>12.1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>12.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Plt (1x10³/CMM)</td>
<td>Pre</td>
<td>36</td>
<td>237.0</td>
<td>100.8</td>
</tr>
<tr>
<td></td>
<td>Exit</td>
<td>36</td>
<td>238.8</td>
<td>93.3</td>
</tr>
</tbody>
</table>

Pre = Pre-enrollment time point; Exit = Study Exit time point; p and P = placebo; R = robenacoxib; N = number of patients; SD = Standard Deviation; WBC = White Blood Cells; Neut = Neutrophils; Lymp = Lymphocytes; RBC = Red Blood Cells; Hct = Hematocrit; Hgb = Hemoglobin; Plt = Platelets.

*Denotes statistically significant differences (Least Squares Mean difference, p<0.05) between pRP and pRR groups.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>1 (pPP)</th>
<th>2 (pRR)</th>
<th>3 (pRP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>36</td>
<td>3.5</td>
<td>0.30</td>
<td>36</td>
</tr>
<tr>
<td><strong>Globulin</strong></td>
<td>36</td>
<td>3.8</td>
<td>0.59</td>
<td>36</td>
</tr>
<tr>
<td><strong>TP</strong></td>
<td>36</td>
<td>7.3</td>
<td>0.59</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>0.48</td>
<td>36</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>36</td>
<td>129.2</td>
<td>47.90</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>37.90</td>
<td>36</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>36</td>
<td>31.6</td>
<td>14.48</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>36.0</td>
<td>36</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>36</td>
<td>56.6</td>
<td>24.42</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>36.0</td>
<td>36</td>
</tr>
<tr>
<td><strong>ALP</strong></td>
<td>36</td>
<td>23.1</td>
<td>14.16</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>36.0</td>
<td>36</td>
</tr>
<tr>
<td><strong>CPK</strong></td>
<td>36</td>
<td>377.8</td>
<td>442.72</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>36.0</td>
<td>36</td>
</tr>
<tr>
<td><strong>Trig</strong></td>
<td>36</td>
<td>66.8</td>
<td>56.19</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>36.0</td>
<td>36</td>
</tr>
<tr>
<td><strong>Lipase</strong>*</td>
<td>18</td>
<td>129.6</td>
<td>52.56</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.0</td>
<td>18.0</td>
<td>17</td>
</tr>
<tr>
<td><strong>Lipase</strong></td>
<td>15</td>
<td>20.6</td>
<td>10.78</td>
<td>15</td>
</tr>
<tr>
<td>(Precision PSL)</td>
<td></td>
<td>15.0</td>
<td>15.0</td>
<td>15</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td>36</td>
<td>186.9</td>
<td>49.23</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.0</td>
<td>36.0</td>
<td>36</td>
</tr>
</tbody>
</table>
Table D.02 (continued)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>1.6</th>
<th>0.38</th>
<th>36</th>
<th>1.5</th>
<th>0.30</th>
<th>36</th>
<th>1.5</th>
<th>0.37</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Creatinine (mg/dL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BUN (mg/dL)</strong></td>
<td>Pre</td>
<td>36</td>
<td>27.8</td>
<td>7.28</td>
<td>36</td>
<td>27.2</td>
<td>6.21</td>
<td>36</td>
<td>28.3</td>
</tr>
<tr>
<td>Exit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Magnesium (MEQ/L)</strong></td>
<td>Pre</td>
<td>36</td>
<td>1.9</td>
<td>0.23</td>
<td>36</td>
<td>1.9</td>
<td>0.22</td>
<td>36</td>
<td>1.9</td>
</tr>
<tr>
<td>Exit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Phosphorus (MEQ/L)</strong></td>
<td>Pre</td>
<td>36</td>
<td>4.2</td>
<td>0.78</td>
<td>36</td>
<td>4.1</td>
<td>0.63</td>
<td>36</td>
<td>4.4</td>
</tr>
<tr>
<td>Exit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Potassium (MEQ/L)</strong></td>
<td>Pre</td>
<td>36</td>
<td>4.3</td>
<td>0.46</td>
<td>36</td>
<td>4.2</td>
<td>0.48</td>
<td>36</td>
<td>4.3</td>
</tr>
<tr>
<td>Exit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sodium (MEQ/L)</strong></td>
<td>Pre</td>
<td>36</td>
<td>151.8</td>
<td>1.92</td>
<td>36</td>
<td>152.2</td>
<td>1.95</td>
<td>36</td>
<td>152.1</td>
</tr>
<tr>
<td>Exit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chloride (MEQ/L)</strong></td>
<td>Pre</td>
<td>36</td>
<td>118.8</td>
<td>2.49</td>
<td>36</td>
<td>119.0</td>
<td>2.54</td>
<td>36</td>
<td>117.9</td>
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<td>Exit</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pre = Pre-enrollment time point; Exit = Study Exit time point; p and P = placebo; R = robenacoxib N = number of patients; SD = Standard Deviation; TP = Total Protein; AST = Aspartate Transaminase; ALT = Alanine Transferase; ALP = Alkaline Phosphatase; Trig = Triglycerides; BUN = Blood Urea Nitrogen; * Denotes statistically significant differences (Least Squares Mean difference, \( P < 0.05 \)) between \( p_{PP} \) and \( p_{RP} \), and between \( p_{PP} \) and \( p_{RR} \) groups.
Table D.03: Select urinalysis results by treatment group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Time</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity</td>
<td>1 (pPP)</td>
<td>Pre</td>
<td>35</td>
<td>1.0</td>
<td>0.02</td>
<td>36</td>
<td>1.0</td>
<td>0.01</td>
<td>36</td>
<td>1.0</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exit</td>
<td>35</td>
<td>1.0</td>
<td>0.02</td>
<td>36</td>
<td>1.0</td>
<td>0.02</td>
<td>36</td>
<td>1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>pH</td>
<td>2 (pRR)</td>
<td>Pre</td>
<td>35</td>
<td>6.5</td>
<td>0.46</td>
<td>36</td>
<td>6.5</td>
<td>0.40</td>
<td>36</td>
<td>6.6</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exit</td>
<td>35</td>
<td>6.8</td>
<td>0.80</td>
<td>36</td>
<td>6.7</td>
<td>0.58</td>
<td>36</td>
<td>6.7</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Pre = Pre-enrollment time point; Exit = Study Exit time point; p and P = placebo; R = robenacoxib N = number of patients; SD = Standard Deviation. There were no statistically significant differences between groups.
Appendix E: VIN Survey

Treatment Approaches for Chronic Musculoskeletal Pain in Cats

Q1.1 How many years have you been in practice?
- Less than 1 year (1)
- Between 1 and 5 years (2)
- Between 5 and 10 years (3)
- Between 10 and 20 years (4)
- More than 20 years (5)

Q1.2 What types of patients does your practice routinely see (mark all that apply)?
- Dogs (1)
- Cats (2)
- Small mammals (3)
- Exotics (birds, reptiles, etc.) (4)
- Horses (5)
- Farm/food animal (6)

Q1.3 What percentage of your patients are cats?
- None or 0% (1)
- Between 0% and 25% (2)
- Between 25% and 50% (3)
- Between 50% and 75% (4)
- Between 75% and 100% (5)
- Feline only practice or 100% (6)

If None or 0% is selected, then skip to end of survey.
Q1.4 Approximately how often do you see cats with suspected chronic musculoskeletal pain/arthritis, whether based on patient history, exam, or radiographic findings?
- Never (1)
- Daily (2)
- Weekly (3)
- Monthly (4)
- Yearly (5)

If Never Is Selected, Then Skip To End of Survey

Q1.5 For those patients in Question 4 with suspected chronic musculoskeletal pain/arthritis, how often do you recommend treatment?
- Never or 0% of the time (1)
- Between 0% and 25% of the time (2)
- Between 25% and 50% of the time (3)
- Between 50% and 75% of the time (4)
- Between 75% and 100% of the time (5)
- Always or 100% of the time (6)

If Never or 0% of the time Is Selected, Then Skip To End of Survey

Q1.6 For those patients in Question 5 for whom you recommend treatment, how often do owners proceed with treatment?
- Never or 0% of the time (1)
- Between 0% and 25% of the time (2)
- Between 25% and 50% of the time (3)
- Between 50% and 75% of the time (4)
- Between 75% and 100% of the time (5)
- Always or 100% of the time (6)

If Never or 0% of the time Is Selected, Then Skip To End of Survey
Q2.1 Which of the following treatments do you use for chronic musculoskeletal pain/arthritis in cats (mark all that apply)?

- Metacam/meloxicam (1)
- Onsior/robenacoxib (2)
- NSAID Other (3)
- Neurontin/gabapentin (4)
- Adequan/polysulfated glycosaminoglycan (5)
- Joint supplements/Nutraceuticals/Dietary Supplements (6)
- Fish Oil (7)
- Joint Support Diets (8)
- Tramadol (9)
- Opioids (10)
- Symadine/amantadine (11)
- Amitril/amitriptyline (12)
- Prednisolone (13)
- Triamcinolone Acetonide (15)
- Depo-Medrol/methylprednisolone (14)
- Other (16) ____________________

Q2.2 Please rank the treatments in order of frequency of use (click and drag).

Q2.3 In general, how do you calculate your dosing?

- Based on true/"scale" weight (1)
- Based on lean weight (2)
- Other (describe) (3) ____________________
Q3.1 For Metacam/meloxicam, what is your typical prescribed dose (per administration) at maintenance?
- Less than 0.02 mg/kg (1)
- Between 0.02 mg/kg and 0.04 mg/kg (2)
- Between 0.041 mg/kg and 0.06 mg/kg (3)
- Between 0.061 mg/kg and 0.08 mg/kg (4)
- Between 0.081 mg/kg and 0.1 mg/kg (5)
- More than 0.1 mg/kg (6)

Q3.2 For Metacam/meloxicam, what is your typical dosing frequency?
- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q3.3 For Metacam/meloxicam, what is your preferred formulation?
- Liquid/commercial formulation (Metacam) (1)
- Tablet/commercial (describe) (2) ____________________
- Compounded/other (describe) (3) ____________________

Q3.4 For Metacam/meloxicam, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)
Q3.5 For Metacam/meloxicam, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q4.1 For Onsior/robenacoxib, what is your typical prescribed dose (per administration)?
- Less than 1 mg/kg (1)
- Between 1 mg/kg and 2.4 mg/kg (2)
- More than 2.4 mg/kg (3)

Q4.2 For Onsior/robenacoxib, what is your typical dosing frequency?
- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q4.3 For Onsior/robenacoxib, what is your preferred formulation?
- Tablet/commercial (Onsior) (1)
- Compounded/other (describe) (2) ____________________
Q4.4 For Onsior/robenacoxib, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q4.5 For Onsior/robenacoxib, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q5.1 What other NSAIDs do you prescribe to treat chronic musculoskeletal pain/arthritis in cats (please list all)?

Q5.2 What is your MOST PREFERRED other NSAID to prescribe?

Q5.3 For your MOST PREFERRED other NSAID, what is your typical prescribed dose (per administration)?
Q5.4 For your MOST PREFERRED NSAID other, what is your typical dosing frequency?

- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q5.5 For your MOST PREFERRED NSAID other, what is your preferred formulation?

- Tablet/capsule/chewable commercial (1)
- Liquid commercial (2)
- Compounded/other (describe) (3) ____________________

Q5.6 For all other NSAIDs, what percentage of the patients from Question 5 will be prescribed these medications?

- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)
Q5.7 For your MOST PREFERRED NSAID other, what is the average duration of treatment for each patient?

- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q6.1 For Neurontin/gabapentin, what is your typical prescribed dose (per administration)?

- Less than 1 mg/kg (1)
- Between 1 mg/kg and 5 mg/kg (2)
- Between 5.1 mg/kg and 10 mg/kg (3)
- Between 10.1 mg/kg and 15 mg/kg (4)
- Between 15.1 mg/kg and 20 mg/kg (5)
- More than 20 mg/kg (6)

Q6.2 For Neurontin/gabapentin, what is your typical dosing frequency?

- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________
Q6.3 For Neurontin/gabapentin, what is your preferred formulation?
- Capsule commercial (describe) (1) ________________
- Liquid commercial (describe) (2) ________________
- Compounded/other (describe) (3) ________________

Q6.4 For Neurontin/gabapentin, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q6.5 For Neurontin/gabapentin, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q7.1 For Adequan/polysulfated glycosaminoglycans, what is your typical prescribed dose (per administration)?
- Less than 5 mg/kg (1)
- Approximately 5 mg/kg (2)
- More than 5 mg/kg (3)
Q7.2 For Adequan/polysulfated glycosaminoglycans, what is your typical dosing frequency?

- Every day (1)
- Every 3-4 days (2)
- Every 7 days (3)
- Every 8-14 days (4)
- Every 15-30 days (5)
- Every 30+ days (6)

Q7.3 For Adequan/polysulfated glycosaminoglycans, what percentage of the patients from Question 5 will be prescribed this medication?

- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q7.4 For Adequan/polysulfated glycosaminoglycans, what is the average duration of treatment for each patient?

- 1 day (1)
- Between 1 and 3 days (2)
- Between 2 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q8.1 For joint supplements/nutraceuticals/dietary supplements, which products do you use?
Q8.2 What is your MOST PREFERRED joint supplement/nutraceutical/dietary supplement to prescribe?

Q8.3 For your MOST PREFERRED joint supplement/nutraceutical/dietary supplement, what is your typical prescribed dose and frequency?
- Product label dose and frequency (1)
- Other (describe) (2) ____________________

Q8.4 For joint supplements/nutraceuticals/dietary supplements, what is your preferred formulation?
- Capsule/tablet (1) ____________________
- Liquid (2) ____________________

Q8.5 For joint supplements/nutraceuticals/dietary supplements, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)
Q8.6 For your MOST PREFERRED joint supplement/nutraceutical/dietary supplement, what is the average duration of treatment for each patient?

- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q9.1 For fish oils, what is your typical prescribed dose (total omega-3 fatty acids per administration)?

- Less than 25 mg/kg (1)
- Between 25 and 50 mg/kg (2)
- Between 50.1 and 100 mg/kg (3)
- Between 100.1 and 200 mg/kg (4)
- Between 200.1 and 400 mg/kg (5)
- More than 400 mg/kg (6)

Q9.2 For fish oils, what is your typical dosing frequency?

- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________
Q9.3 For fish oils, what is your preferred formulation (please indicate name/brand)?
- Capsule commercial (1) ____________________
- Liquid commercial (2) ____________________

Q9.4 For fish oils, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q9.5 For fish oils, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q10.1 What are your preferred joint support diets (please list all)?

Q10.2 What is your MOST PREFERRED joint support diet?
Q10.3 For joint support diets, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q10.4 For joint support diets, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q11.1 For Ultram/tramadol, what is your typical prescribed dose (per administration)?
- Less than 2 mg/kg (1)
- Between 2 and 4 mg/kg (2)
- More than 4 mg/kg (3)
Q11.2 For Ultram/tramadol, what is your typical dosing frequency?
- Every 8 hours (1)
- Every 12 hours (2)
- Every 24 hours (3)
- Every 48 hours (4)
- Every 72 hours (5)
- As needed/PRN (6)
- Other (describe) (7) ____________________

Q11.3 For Ultram/tramadol, what is your preferred formulation?
- Capsule commercial (1) ____________________
- Liquid commercial (2) ____________________

Q11.4 For Ultram/tramadol, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)
Q11.5 For Ultram/tramadol, what is the average duration of treatment for each patient?

- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q12.1 Which opioids do you prefer to prescribe (please list all)?

Q12.2 What is your MOST PREFERRED opioid to prescribe?

Q12.3 For your MOST PREFERRED opioid listed above, what is your typical prescribed dose (per administration)?

Q12.4 For your MOST PREFERRED opioid, what is your typical dosing frequency?

- Every 8 hours (1)
- Every 12 hours (2)
- Every 24 hours (3)
- Every 48 hours (4)
- Every 72 hours (5)
- As needed/PRN (6)
- Other (describe) (7) ____________________

Q12.5 For your MOST PREFERRED opioid, what is your preferred formulation?

- Capsule/tablet commercial (describe) (1) ____________________
- Liquid commercial (describe) (2) ____________________
Q12.6 For your MOST PREFERRED opioid, what is your preferred route of administration?
- Per OS (1)
- Transmucosal (2)
- Injectable (SQ or IM) (3)
- Injectable (IV) (4)
- Other (describe) (5) ____________________

Q12.7 For all opioids, what percentage of the patients from Question 5 will be prescribed these medications?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q12.8 For your MOST PREFERRED opioid, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)
Q13.1 For Symadine/amantadine, what is your typical prescribed dose (per administration)?
- Less than 3 mg/kg (1)
- Between 3 and 5 mg/kg (2)
- More than 5 mg/kg (3)

Q13.2 For Symadine/amantadine what is your typical dosing frequency?
- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q13.3 For Symadine/amantadine, what is your preferred formulation?
- Capsule commercial (1) ____________________
- Liquid commercial (2) ____________________
- Compounded/other (describe) (3) ____________________

Q13.4 For Symadine/amantadine, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)
Q13.5 For Symadine/amantadine, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q14.1 For Amitril/amitriptyline, what is your typical prescribed dose (per administration)?
- Less than 0.5 mg/kg (1)
- Between 0.51 and 1 mg/kg (2)
- Between 1.1 and 2.5 mg/kg (3)
- More than 2.5 mg/kg (4)

Q14.2 For Amitril/amitriptyline, what is your typical dosing frequency?
- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q14.3 For Amitril/amitriptyline, what is your preferred formulation?
- Tablet commercial (1) ____________________
- Compounded/other (describe) (2) ____________________
Q14.4 For Amitril/amitriptyline, what percentage of the patients from Question 5 will be prescribed this medication?

- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q14.5 For Amitril/amitriptyline, what is the average duration of treatment for each patient?

- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q15.1 For prednisolone, what is your typical prescribed dose (per administration)?

- Less than 0.5 mg/kg (1)
- Between 0.5 and 1 mg/kg (2)
- Between 1.1 and 2 mg/kg (3)
- More than 2 mg/kg (4)
Q15.2 For prednisolone, what is your typical dosing frequency?
- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q15.3 For prednisolone, what is your preferred formulation?
- Tablet commercial (1) ____________________
- Liquid commercial (2) ____________________
- Compounded/other (describe) (3) ________________

Q15.4 For prednisolone, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)
Q15.5 For prednisolone, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q16.1 For Depo-Medrol/methylprednisolone acetate, what is your typical prescribed dose (per administration)?
- Less than 10 mg total (1)
- Between 10 mg and 20 mg total (2)
- More than 20 mg total (3)

Q16.2 For Depo-Medrol/methylprednisolone acetate, what is your typical dosing frequency?
- Once weekly (1)
- Every 2 weeks (2)
- Every 3 weeks (3)
- Every 4 weeks (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q16.3 For Depo-Medrol/methylprednisolone acetate, what is your preferred formulation?
- Tablet commercial (1) ____________________
- Compounded/other (describe) (2) ________________
Q16.4 For Depo-Medrol/methylprednisolone acetate, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q16.5 For Depo-Medrol/methylprednisolone acetate, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)

Q17.1 For triamcinolone acetonide, what is your typical prescribed dose (per administration)?
- Less than 0.5 mg/kg (1)
- Between 0.5 and 1 mg/kg (2)
- More than 1 mg/kg (3)
Q17.2 For triamcinolone acetonide, what is your typical dosing frequency?
- Every 12 hours (1)
- Every 24 hours (2)
- Every 48 hours (3)
- Every 72 hours (4)
- As needed/PRN (5)
- Other (describe) (6) ____________________

Q17.3 For triamcinolone acetonide, what is your preferred formulation?
- Tablet commercial (1) ____________________
- Compounded/other (describe) (2) ____________________

Q17.4 For triamcinolone acetonide, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)

Q17.5 For triamcinolone acetonide, what is the average duration of treatment for each patient?
- 1 day (1)
- Between 2 and 3 days (2)
- Between 4 and 7 days (3)
- Between 8 and 10 days (4)
- Between 11 and 14 days (5)
- Between 15 and 30 days (6)
- Between 31 and 60 days (7)
- Between 61 and 120 days (8)
- More than 120 days (9)
Q18.1 What other medications do you prefer to use for chronic musculoskeletal pain/arthritis in cats (please list all)?

Q18.2 What is your MOST PREFERRED other medication?

Q18.3 For your MOST PREFERRED other medication, what is your typical prescribed dose (per administration) and dosing frequency at maintenance?

Q18.4 For your MOST PREFERRED other medication, what is your preferred formulation?
- Tablet/capsule commercial (1) _________________
- Liquid commercial (2) _________________
- Compounded/other (describe) (3) _________________

Q18.5 For your MOST PREFERRED other medication, what percentage of the patients from Question 5 will be prescribed this medication?
- Between 0% and 25% (1)
- Between 25% and 50% (2)
- Between 50% and 75% (3)
- Between 75% and 100% (4)
- All or 100% (5)
Q18.6 For your MOST PREFERRED other medication, what is the average duration of treatment for each patient?

- 1 day (17)
- Between 2 and 3 days (18)
- Between 4 and 7 days (19)
- Between 8 and 10 days (20)
- Between 11 and 14 days (21)
- Between 15 and 30 days (22)
- Between 31 and 60 days (23)
- Between 61 and 120 days (24)
- More than 120 days (25)

Q19.1 Thinking of the ideal medication for treating chronic musculoskeletal pain/arthritis in cats, what is your preferred formulation?

- Tablet (1)
- Capsule (2)
- Liquid (3)
- Injectable (SQ or IM) (4)
- Other (describe) (5) ________________

Q19.2 Thinking of the ideal medication for treating chronic musculoskeletal pain/arthritis in cats, what is your ideal dosing frequency?

- Thrice daily (1)
- Twice daily (2)
- Once daily (3)
- Twice weekly (4)
- Once weekly (5)
- Twice monthly (6)
- Once monthly (7)
- Once every other month (8)
Q19.3 Which of the following formulations would you consider "acceptable" when considering treatment options for chronic musculoskeletal pain/arthritis in cats (please mark all that apply)?

- Tablet (1)
- Capsule (2)
- Liquid (3)
- Injectable (SQ or IM) (4)

Q19.4 Which of the following dosing frequencies would you consider "acceptable" when considering treatment options for chronic musculoskeletal pain/arthritis in cats (please mark all that apply)?

- Thrice daily (1)
- Twice daily (2)
- Once daily (3)
- Once every other day (4)
- Twice weekly (5)
- Once weekly (6)
- Twice monthly (7)
- Once monthly (8)
- Once every other month (9)