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Pain burden, sensory profile and inflammatory cytokines of dogs with naturally-occurring neuropathic pain treated with gabapentin alone or with meloxicam

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ABSTRACT

Canine neuropathic pain (NeuP) has been poorly investigated. This study aimed to evaluate the pain burden, sensory profile and inflammatory cytokines in dogs with naturally-occurring NeuP. Twenty-nine client-owned dogs with NeuP were included in a prospective, partially masked, randomized crossover clinical trial, and treated with gabapentin/placebo/gabapentin-meloxicam or gabapentin-meloxicam/placebo/gabapentin (each treatment block of 7 days; total 21 days). Pain scores, mechanical (MNT) and electrical (ENT) nociceptive thresholds and descending noxious inhibitory controls (DNIC) were assessed at baseline, days 7, 14, and 21. DNIC was evaluated using ΔMNT (after-before conditioning stimulus). Positive or negative ΔMNT corresponded to inhibitory or facilitatory pain profiles, respectively. Data from baseline were compared to those of sixteen healthy controls. ΔMNT, but not MNT and ENT, was significantly larger in controls (2.3 ± 0.9 N) than in NeuP (-0.2 ± 0.7 N). The percentage of dogs with facilitatory sensory profile was similar at baseline and after placebo (61.5-63%), and between controls and after gabapentin (33.3-34.6%). Pain scores were lower than baseline after gabapentin or gabapentin-meloxicam. Cytokine levels were not different between groups or treatments. Dogs with NeuP have deficient inhibitory pain mechanisms. Pain burden was reduced after gabapentin and gabapentin-meloxicam depending on the pain scoring instrument used.

Introduction

Neuropathic pain (NeuP) is caused by a lesion or disease of the somatosensory system [1]. Its diagnosis relies on sensory examination of nerve fibers involved in

nociception/proprioception for both loss (i.e. hypoesthesia and hypoalgesia) and gain of function (i.e. hyperalgesia and allodynia) via quantitative sensory testing (QST) [2]. In brief, QST is a psychophysical method that evaluates the somatosensory function from receptor to cortex using calibrated innocuous or noxious stimuli. It offers useful insight into the underlying pain mechanisms and the characterization of painful conditions [3]. For example, it is possible to stratify human patients with peripheral NeuP by categories of phenotypes using cluster analysis of their mechanical and thermal sensory profiles instead of a disease etiology-based classification [4]. Therefore, response to therapy can be predicted in precision or personalized medicine based on the specific patient sensory profile [5]. Additionally, changes in QST before and after the application of a conditioning stimulus provide useful information about the diffuse noxious inhibitory control (DNIC) as a representation of central descending modulatory pain mechanisms. The latter could predict people's response to drugs acting on central pain modulation [6]. It has been proposed that inflammatory cytokines play a role in the development and maintenance of NeuP and could be an avenue for future therapeutic options [7].

The diagnosis of NeuP in veterinary and cognitively-impaired human patients is a challenge. In companion animal medicine, the disease is diagnosed after appropriate physical, neurological and magnetic resonance imaging (MRI) examination, and clinical signs of pain and allodynia [8]. In dogs, NeuP can be caused by spinal cord disease, chronic musculoskeletal conditions and peripheral neuropathies, among others. Treatment recommendations for this disease in companion animals are mostly based on case-series, review articles, anecdotal reports and scientific evidence from humans. Gabapentinoids (e.g. gabapentin) and tricyclic antidepressants (e.g. amitriptyline) have been suggested as the first line of treatment of this disease [8]. Non-steroidal (NSAIDs) or steroidal anti-inflammatory drugs and antagonists of N-methyl-D-aspartate receptors (e.g. amantadine) have been also recommended [8]. Thus, a combination of a

NSAID (e.g. meloxicam) and gabapentin are often anecdotally used in the treatment of NeuP conditions that are refractory to therapy with gabapentin alone. However, the efficacy of these treatments for NeuP has not been systematically studied in veterinary medicine.

The aims of this study were to evaluate the pain burden, sensory profile and inflammatory cytokines of dogs with NeuP before and after treatment with placebo, gabapentin alone or gabapentin-meloxicam. The sensory (QST) and inflammatory profiles of dogs with NeuP at presentation were compared with a population of healthy controls. Pain burden was determined using clinical pain assessment tools (pet owner and veterinary assessments). The hypotheses were that NeuP presents different sensory profile (i.e. hypo- or hyperalgesia) when compared with healthy controls and that treatment with gabapentin alone or with meloxicam alters this profile. Finally, pain scores are expected to be lower after treatment with gabapentin or gabapentin-meloxicam when compared with baseline (initial presentation) and placebo using both owner and veterinary assessments. Finally, pro-and anti-inflammatory cytokine concentrations would be higher and lower, respectively, in dogs with NeuP than in controls. The serum concentrations of gabapentin were measured as an indirect method to assess treatment compliance.

Methods

Ethical statement

This study was approved by the local animal care committee of the Faculty of Veterinary Medicine, Université de Montréal (16-Rech-1835 and 16-Rech-1848) and was conducted between October 2016 and July 2018. The study is reported according to the CONSORT

guidelines for randomized, clinical trials [9]. This was a prospective, partially masked, randomized crossover clinical trial.

Animals

Thirty-two client-owned dogs were admitted to the veterinary teaching hospital (Centre Hospitalier Universitaire Vétérinaire) of the Université de Montréal. Dogs were recruited after physical and neurological examinations by a board-certified veterinary neurologist (H.L.M.R.). Owner's written consent was obtained for each patient.

Sixteen client-owned healthy control dogs (4.8 ± 2.1 years; 32 ± 16.7 kg; six males and ten females) were recruited simultaneously and their data were used for comparison. They were considered healthy based on history, physical, orthopedic and neurological examinations and did not received any analgesic treatment at least 30 days prior to recruitment. Exclusion criteria were the same as those described below for dogs with NeuP. Data for these individuals were previously reported as part of the validation of our methodology [10].

Inclusion and exclusion criteria

Inclusion criteria were based on specific body weight (≥ 4 kg), age (> 6 months) and the owner's option for medical management of NeuP. Dogs were included if the duration of painful clinical signs was ≥ 4 weeks and if a neurological lesion was found in the MRI consistent with the previous neurological examination and clinical signs of pain. Exclusion criteria included pregnancy, lactation, aggressive behavior, anxiety, history of pacemaker placement, systemic disease including chronic renal and hepatic disease, suspected immune-mediated disorders or any clinically relevant comorbidity, and significant changes in hematology and serum biochemistry analysis. Patients receiving treatments were weaned off medications at least 7 days (steroidal

anti-inflammatory drugs), 24 hours (gabapentin), 72 hours (NSAIDs) and at least 60 minutes (remifentanil) before the clinical trial had begun.

Treatments

Each dog was randomly allocated to treatment groups 1 or 2 (Table 1). Randomization was performed using balanced permutations (www.randomization.com). Each treatment was divided into three blocks of 7 days to include gabapentin or gabapentin-meloxicam (either first or third block) or placebo (always during the second block allowing a "wash-out" period between the first and third blocks). The total duration of the study was 21 days. Resting was recommended for all dogs (Fig 1).

Table 1. Treatment groups of a prospective, randomized, partially masked, placebo-controlled clinical trial in dogs with naturally-occurring presumptive neuropathic pain.

	1st block	**2nd block**	**3rd block**
Treatment group 1 **n = 16**	gabapentin (10 mg/kg every 8h, PO) + placebo tablets (every 24h, PO)	placebo capsules (every 8h, PO) + placebo tablets (every 24h, PO)	gabapentin (10 mg/kg every 8h, PO) + meloxicam (0.2 mg/kg PO followed by 0.1 mg/kg every 24h, PO)
Treatment group 2 **n = 16**	gabapentin (10 mg/kg every 8h, PO) +	placebo capsules (every 8h, PO) +	gabapentin (10 mg/kg every 8h, PO) +

	meloxicam (0.2 mg/kg PO followed by 0.1 mg/kg every 24h, PO)	placebo tablets (every 24h, PO)	placebo tablets (every 24h, PO)

Oral administration (PO)

Fig 1. Timeline of the study. Dogs were randomized to receive either treatment 1 or 2. Pain assessment and Quantitative Sensory Testing (QST) were evaluated after each block of treatment (7 days). Abbreviations: QST, quantitative sensory testing (including mechanical and electrical nociceptive thresholds and assessment of the descending noxious inhibitory controls); MRI, magnetic resonance imaging.

Treatments were placed in pill dispensers and given to owners one week at a time. The capsules of 50, 100, 300 mg and tablets of 600 mg of gabapentin, and tablets of 1 and 2.5 mg of meloxicam were used. Drugs were administered orally (PO) at a targeted dose of 10 mg/kg every 8 hours for gabapentin (gabapentin, Apotex®, Canada) and 0.2 mg/kg once followed by 0.1 mg/kg every 24 hours for meloxicam (Metacam, Boehringer Ingelheim Inc) (nearest whole capsule or fraction of tablet available). Placebo compounds of dextrose were administered in tablets and/or capsules so that owners were masked to the treatment. The board-certified veterinary neurologist who participated in the study design was masked to the first and third (active treatments), but not to the second block (placebo).

Quantitative sensory testing (QST)

QST was performed after physical and neurological examination and before the MRI at initial presentation (baseline, day 0) and following each treatment block (days 7, 14 and 21) (Fig 1).

Dogs were acclimated to the testing room for 10 minutes before the experimentation and had free access to water. For QST, they were positioned either in semi-sternal position or in lateral recumbency over a mat [10]. Nociceptive stimulations were stopped as soon as behavioral changes in response to stimuli were observed (looking at the probe, voluntary movement away from the probe, attempts to bite, etc.) [10].

The feasibility, intra- and inter-observer reliability, test-retest and sham-testing of our QST methodology have been previously reported [10]. Stimulation was applied to the dorsal aspect of the metacarpus and the plantar aspect of the metatarsus above the plantar pad bilaterally after clipping. The order of QST modality (electrical nociceptive thresholds, ENT; mechanical nociceptive thresholds, MNT), the limb and the side (right/left) of stimulation were randomized according to a random permutation generator (www.randomization.com). The observer graded each response to QST as poor (score 0), fair (score 1) or good (score 2) [10]. Replicates were obtained 60 seconds apart. If one of the responses received a score of 0 or 1, a third measurement was obtained 60 seconds later. Results with score 0 were not considered for statistical analysis. Outcome data for MNT and ENT were the mean of all measurements from all limbs, obtained with a score ≥ 1.

Electrical nociceptive thresholds — The stimulation was provided using a transcutaneous electrical nerve stimulator (TENS unit; Intelect® Vet two channel combo unit, Chattanooga, Guildford, Surrey, UK) in the VMS™ mode (View, Tempe, AZ, USA). The stimulation was delivered via two adhesive electrodes and consisted in a symmetrical biphasic waveform with a

100 µsec interphase. Settings were adjusted to a CC mode using a frequency of 200 Hz, phase duration of 20 µsec and a ramp of 0 seconds. The current was increased gradually until a behavioral response was observed, or until the cut-off of 150 mA was reached after 2 minutes.

Mechanical nociceptive threshold (MNT) and diffuse noxious inhibitory controls (DNIC) — For MNT, increasing pressure was applied perpendicular to the skin with an algometer (Bioseb, Vitrolles, France) with a flat tip of 3.5 mm diameter until a behavioral response was observed or the cut-off of 20 N reached.

The assessment of DNIC was based on the difference in MNT applied to one of the thoracic limbs before and after a conditioning stimulus. The conditioning stimulus was performed by placing an adult blood pressure cuff around the humerus and inflated it up to 200 mmHg for 60 seconds using a sphygmomanometer. After 3 minutes, the MNT was repeated on the same limb. The ΔMNT (after – before conditioning stimulus) was used as an outcome for the assessment of DNIC. When MNT was not obtained either pre or post-conditioning stimulus for a dog, ΔMNT was not recorded. The percentage of positive and negative ΔMNT was calculated for each group. The DNIC was applied to the "least affected thoracic limb". The latter was based on neurological examination and localization of the lesion on the MRI. Increases in MNT after the conditioning stimulus are expected in healthy individuals with functional DNIC (i.e. functional inhibitory conditioned pain modulation), based on the ''pain-inhibits-pain'' paradigm [11].

The board-certified veterinary neurologist had previous training in QST in dogs [10]. This individual was responsible for identifying behavioral changes associated with nociceptive stimulation. This observer was not aware of stimuli intensity during testing. Two other individuals (M.C.E., R.W.) were involved in the QST: one was responsible for mild restraint of dogs during testing whereas the other controlled the electrical stimulation as previously reported

[10]. They were also both responsible for randomization, recording nociceptive thresholds, preparation of the pill dispensers and compilation of results.

Pain assessment tools (questionnaires)

At each visit (days 0, 7, 14 and 21), dog owners were asked to complete the client specific-outcome measures (CSOM) [12] and the French version of the Canine Brief Pain Inventory (CBPI) [13,14]. To complete the CSOM, owners listed three activities that were impaired due to pain or that elicited pain (e.g. getting up from lying down, jumping into the owner's car). The degree of difficulty to perform each activity (no problem, mildly problematic, moderately problematic, severely problematic or impossible) was followed weekly. The CBPI assesses pain severity, interference of pain on function (locomotion) and the owner's global impression about the dog's quality of life ("overall impression"). For "interference", questions regarding the dog's ability to run and to climb stairs were excluded since resting was recommended during the study. Therefore, the sections "pain" ($CBPI_{pain}$) and "interference" ($CBPI_{interference}$) contained each four questions scored on a 10-point scale (higher scores corresponding to greater difficulties/pain). The "overall impression" ($CBPI_{overall\ impression}$) was graded as poor, fair, good, very good and excellent. Additionally, the short-form Glasgow Composite Measure Pain Scale [CMPS-SF] [15] was completed at each visit by the veterinarian. During the study, inadequate analgesia could be reported by the owners if they felt that clinical signs of pain persisted and were similar to presentation. In that case, a re-evaluation was scheduled at the earliest convenience and physical/neurological examination, pain scoring and QST repeated. If analgesic failure was observed with gabapentin-meloxicam during the first block, the dog was excluded from the trial. If it happened during the second block (placebo), the third block would start immediately. If it occurred during the third block, the study was finalized,

and the dog treated according to the clinician's discretion. If owners reported pain during the withdrawal period (before entering the study), dogs were hospitalized to receive an intravenous infusion (CRI) of remifentanil as needed to alleviate pain until the study could be started. Initial assessment would then be performed at least 60 minutes after the cessation of the administration of remifentanil. The choice of this drug as rescue analgesia was based on recent evidence that remifentanil was not associated with opioid-induced hyperalgesia in dogs and the convenience of its short half-life, allowing testing shortly after the cessation of the CRI and thus, minimizing the period without treatment of pain for the patient [16].

Serum concentrations of gabapentin and inflammatory cytokines

Blood was collected by venipuncture into a sterile 3 mL anticoagulant-free glass tube (Monoject Blood Collection Tube; Covidien Canada, Saint-Laurent, QC, Canada) at each visit (day 0, 7, 14 and 21). Samples were allowed to clot at room temperature for at least 30 minutes before being centrifuged at 3000 rpm for 10 minutes. Subsequently, serum was aliquoted and stored at -70°C in cryovials. Gabapentin was extracted from dog serum using a protein precipitation technique, separated by chromatography and then identified by mass spectrometry. (S1 Supplementary methods).

Serum samples were analyzed for concentrations of GM-CSF, IFN-γ, IL-2, IL-6, IL-7, IL-8, IL-15, IP-10, KC-like, IL-10, IL-18, MCP-1, and TNF-α using a pre-mixed Milliplex 13-plex Canine Magnetic Bead Panel (Millipore, Burlington, USA) according to the manufacturer's instructions. Acquisition was performed on the MAGPIX platform (Luminex®) and data analyzed using the MILLIPLEX Analyst 5.1 software (Upstate Group/Millipore). Standard curves and quality control checking were performed. Analytes with more than 50% out of range concentrations were excluded from statistical analyses. Cytokines of dogs with visible

inflammatory conditions (severe oral inflammatory disease, dermatological problems such as skin allergies and otitis) were excluded from the statistical analysis.

Statistical analysis

A mixed linear model was used to analyze ENT, MNT and ΔMNT with treatment as the main effect and sex, age and body weight as covariates and dog ID as random effect. A mixed linear model was also used to assess the effects of treatment order with treatments and treatment order as main effects and age, sex and body weight as covariates. Additionally, a linear model was used to compare ENT, MNT and ΔMNT between healthy controls and NeuP using age, sex and body weight as covariates. The level of statistical significance was set at 5 %. Incomplete questionnaires for pain assessment were excluded from the analysis. For the CSOM, responses were converted into a numerical scale ranging from 1 to 5, as previously described [12], with 1 = no problem, 2 = mildly problematic, 3 = moderately problematic, 4 = severely problematic, and 5 = impossible. The total CSOM score represented the sum of scores for each of the three activities.

Each section of the CBPI (namely $CBPI_{pain}$, $CBPI_{interference}$ and $CBPI_{overall\ impression}$) was analyzed separately. Grades for $CBPI_{overall\ impression}$ (poor, fair, good, very good and excellent) were translated to rank scores from 1 to 5 (poor: 1 to excellent: 5). Data for $CBPI_{overall\ impression}$ were analyzed with the Mantel-Haenszel chi-square followed pairwise comparisons using the sequential Benjamini-Hochberg procedure to adjust alpha levels. Data from CSOM, $CBPI_{pain}$ and $CBPI_{interference}$ and CMPS-SF were analyzed using a mixed linear model with treatment as the main effect and age, sex and body weight as covariates followed by Tukey's post-hoc tests when appropriate.

Serum concentrations of inflammatory cytokines were compared after $\log_{10}$ transformation. When measures obtained were out of range, they were replaced by the lowest value extrapolated by the software minus 0.01 in order to avoid missing data (and inherent bias). Cytokine analyses were performed using nonparametric test when the distribution of data was asymmetrical (TNF-α). Otherwise, linear models were used (GM-CSF, IFN-γ, IL-2, IL-6, IL-7, IL-8, IL-15, IP-10, KC-like, IL-10, IL-18, MCP-1). Comparisons between treatments were performed using mixed linear models for all analytes, except for TNF-α, where Friedman test was used. The association between concentrations of cytokines and pain scores was assessed with Spearman correlation for CMPS-SF and CBPI $_{\text{overall impression}}$ which displayed a non-normal distribution and represented ordinal data. Furthermore, considering the absence of treatment effect on cytokine levels, data from NeuP and controls were pooled together to increase the sample size and avoid repeated measures for these parameters. Mixed linear models were used to analyze the association of all cytokine concentrations (except TNF-α) and CBPI $_{\text{pain}}$, CBPI $_{\text{interference}}$ and CSOM, after $\log_{10}$ transformation of the data (normalization). Friedman test was used to analyze these associations for TNF-α which followed a non-normal distribution. When linear models were used, age, sex, and weight were considered as co-factors. For the associations with CBPI $_{\text{pain}}$, CBPI $_{\text{interference}}$ and CSOM, the control group was excluded because all data for CBPI were equal to zero and the CSOM was not part of the assessment of the control population.

Results

Animals

Three dogs were excluded for the following reasons: suspected immune-mediated disease of the central nervous system, mast cell tumor diagnosed on day 21, and significant serum levels of gabapentin measured during the placebo period (treatment error; Fig 2), respectively.

Fig 2. CONSORT Flow Diagram showing the flow of **a)** healthy dogs and **b)** dogs with neuropathic pain through the study.

Twenty-nine dogs completed the study (mean age ± SD: 6.6 ± 3.0 years and mean body weight ± SD: 27.0 ± 18.5 kg; 21 males and 8 females) (Figure 2). Breeds included Labrador Retriever (n = 4), Bernese Mountain Dog (n = 6), Poodle Toy (n = 1), Siberian Husky (n = 2), Golden Retriever (n = 1), Cavalier King Charles Spaniel (n = 5), Polish Tatra Sheepdog (n = 1), Wire Fox Terrier (n = 1), Boxer (n = 1), Pug (n = 1), Longhaired Dachshund (n = 1), Basset Hound (n = 1), Beagle (n = 1), Pomeranian (n = 1), mixed-breed (n = 2). Duration of pain prior to enrollment ranged from 1 to 60 months according to the owner's report with a median of 12 months. Pain-associated conditions diagnosed by MRI included spondylomyelopathies, lumbosacral syndromes, intervertebral disk disease with or without discospondylitis, Chiari malformations, congenital vertebral malformation, nerve sheath tumor and meningeal tumor. Dogs had at least one of the above lesions in the MRI. Dogs with NeuP were older than controls (P = .021) but there was no difference for body weight (P = .36). There were significantly more males in the NeuP group than in controls (72.4 % versus 37.5 %, P = .030).

Adverse reaction / Analgesic failure

One dog developed erythema associated with pruritus shortly after the treatment with gabapentin-meloxicam was initiated, which subsided after the meloxicam was stopped. Owners

306 reported a history of food allergy and it was believed that the erythema could be associated with
307 the palatable agent contained in chewable tablets of meloxicam. Other adverse effects were not
308 recorded with the other treatment blocks and the dog completed the study. Analgesic failure was
309 observed in one patient with nerve sheath tumor receiving gabapentin-meloxicam in the first
310 block. This dog was excluded from the study. Finally, recurrence of severe signs of pain
311 prompted a re-evaluation in one individual with osseous-associated cervical spondylomyelopathy
312 after 4 days into the placebo period.

313

314 **Quantitative Sensory Testing**

315 Mean ± SEM MNT and ENT did not differ between healthy controls and NeuP at initial
316 presentation (MNT: 10.4 ± 0.8 and 10.6 ± 0.6; $P = .86$ and ENT: 49.5 ± 6.7 and 48.8 ± 5.2; $P =$
317 .94, respectively). There was an effect of body weight on both modalities (MNT: $P < .0001$;
318 ENT: $P = .0055$) with higher thresholds observed in heavier dogs.
319 Mean ± SEM ΔMNT was significantly larger in healthy controls than in NeuP (2.3 ± 0.9 N and -
320 0.2 ± 0.7 N, respectively; $P = .045$). Body weight ($P = .47$), sex ($P = .88$) and age ($P = .076$)
321 were not associated with ΔMNT.
322 Treatment order did not influence ENT and MNT ($P = .20$ and $P = .80$, respectively). In NeuP,
323 ENT, MNT or ΔMNT were not affected by treatment ($P = .06$, $P = .94$ and $P = .21$,
324 respectively), and there was no association between ENT, MNT, ΔMNT and sex ($P = .22$, $P =$
325 .90 and $P = .99$) or age ($P = .12$, $P = .76$ and $P = .25$), respectively. Both ENT and MNT were
326 positively associated with body weight ($p < .0001$) but not ΔMNT ($P = .50$) (Table 2).

327 **Table 2.** Electrical and mechanical nociceptive thresholds (ENT and MNT, respectively) and
328 changes in mechanical nociceptive thresholds after application of a conditioning stimulus

329 (ΔMNT) in dogs with naturally-occurring presumptive neuropathic pain before and after each
330 treatment period.
331

	ENT (mA)	MNT (N)	ΔMNT (N)
Baseline	49.5 ± 3.4 (n = 29)	10.2 ± 0.5 (n = 29)	- 0.1 ± 0.6 (n = 27)
Placebo	42.3 ± 3.4 (n = 28)	10.3 ± 0.5 (n = 28)	- 0.9 ± 0.6 (n = 26)
Gabapentin	38.3 ± 3.4 (n = 28)	10.1 ± 0.5 (n = 28)	0.8 ± 0.6 (n = 26)
Gabapentin-meloxicam	39.7 ± 3.4 (n = 28)	10.3 ± 0.5 (n = 28)	0.5 ± 0.6 (n = 26)

332 Data shown as mean ± SEM after a mixed linear model to analyze ENT, MNT and ΔMNT with
333 treatment as the main effect and sex, age and body weight as covariates.
334
335 The percentage of positive and negative ΔMNT was calculated for each group (healthy controls
336 and NeuP) and after each treatment block. In healthy controls, 33.3% of the dogs had a negative
337 ΔMNT (i.e. facilitatory profile) whereas 66.7% showed a positive ΔMNT (i.e. inhibitory profile)
338 (Figure 3). The percentage of negative ΔMNT were as follows in NeuP: 61.5% of dogs had a
339 negative ΔMNT at initial presentation, 34.6% after gabapentin, 53.8% after gabapentin-
340 meloxicam and 63.0% after placebo; positive ΔMNT was recorded in 38.5% of NeuP at initial

341 presentation, 65.4% after gabapentin, 46.2% after gabapentin-meloxicam and 37.0% after
342 placebo (Fig 3).
343
344 **Fig 3.** Diffuse Noxious Inhibitory Control (DNIC) in the population of a) healthy dogs, b) dogs
345 with neuropathic pain at initial presentation, c) after placebo, d) after gabapentin-meloxicam and
346 e) after gabapentin alone. Negative values represent facilitatory while positive values represent
347 inhibitory conditioned pain modulation.
348
349 **Pain assessment tools**
350 The cumulative score for the CPBI severity and interferences domains were 0 for all
351 control dogs. The $CBPI_{overall\ impression}$ ranged from very good (n = 2) to excellent (n = 14). The
352 median (range) scores for CMPS-SF for control dogs were 0 (0 – 1) and were 5 (0 – 9) for NeuP.
353 The treatment order for NeuP did not significantly change the scores of CSOM (P = .07), $CBPI_{pain}$
354 (P = .064), $CBPI_{interference}$ (P = .15) and CMPS-SF (P = .58). There was no association
355 between sex and age for CSOM (P = .94 and P = .42, respectively), $CBPI_{pain}$ (P = .97 and P =
356 .80, respectively) and $CBPI_{interference}$ (P = .81 and P = .28, respectively).

357 *CSOM* **—** Treatment influenced CSOM scores (P < .0001). Higher scores (more difficult to
358 perform a given activity) were attributed by owners at presentation than after each treatment
359 including placebo (Table 3).

360 **Table 3.** Pain scores obtained in dogs with naturally-occurring neuropathic pain before and after
361 each treatment period. Data are presented as mean ± SEM for scores from Client Specific
362 Outcome Measures (CSOM), Canine Brief Pain Inventory ($CBPI_{pain}$ and $CBPI_{interference}$), and

363 short-form Glasgow Composite Measure Pain Scale (CMPS-SF). Data are presented as median
364 (range) for scores from CBPI $_{\text{overall impression}}$.
365

	CSOM	CBPI pain	CBPI interference	CBPI overall impression	CMPS-SF
Baseline	10.4 ± 0.7 (n = 25)	20.2 ± 1.8 (n = 28)	21.2 ± 1.8 (n = 28)	2.0 (1.0 – 4.0) (n = 29)	4.4 ± 0.5 (n = 24)
Placebo	**8.5 ± 0.7** (n = 24)	17.9 ± 1.8 (n = 27)	17.0 ± 1.8 (n = 27)	2.8 (1.0 – 5.0) (n = 28)	3.9 ± 0.5 (n = 19)
Gabapentin	**7.7 ± 0.7** (n = 20)	**15.7 ± 1.9** (n = 23)	16.4 ± 1.9 (n = 22)	**3.0 (2.0 – 5.0)** (n = 24)	**2.9 ± 0.5** (n = 18)
Gabapentin-meloxicam	**7.5 ± 0.7** (n = 24)	**14.7 ± 1.9** (n = 24)	**16.6 ± 1.9** (n = 24)	3.0 (1.0 – 5.0) (n = 24)	**2.5 ± 0.5*** (n = 18)

366 Data in bold are significantly different from results at initial presentation and the asterisk (*) marks
367 significant difference compared with placebo.
368

369 *CBPI $_{pain}$* — Treatment influenced CBPI $_{\text{pain}}$ (P = .002). These scores were higher (more painful)
370 at presentation than after gabapentin or gabapentin-meloxicam (Table 3).

371 *CBPI $_{interference}$* — Treatment influenced CBPI $_{\text{interference}}$ (P = .02). These scores were higher at
372 presentation (locomotion more severely affected) than after gabapentin-meloxicam (Table 3).

373 *CBPI $_{overall\ impression}$* — Treatment influenced CBPI $_{\text{overall impression}}$ (P = .0002). These scores were
374 higher (improved overall impression) after gabapentin than at presentation (Table 3).

375 *CMPS-SF* — Treatment influenced CMPS-SF scores ($P = .002$). These scores were higher at
376 presentation than after gabapentin and gabapentin-meloxicam and were higher after placebo than
377 gabapentin-meloxicam (Table 3). Pain scores were higher in male than female dogs ($P = .038$).

378

379 **Serum concentrations of gabapentin and inflammatory cytokines**

380

381 Mean ± SD dose of gabapentin was 11.05 ± 1.46 mg/kg (range: 8.62 – 14.49 mg/kg).
382 Most of the dogs included in this study had undetectable concentrations of gabapentin at
383 presentation and at day 14 (end of placebo period); minimal concentrations of gabapentin were
384 found in the serum of 5 dogs at presentation (≤ 0.11 µg/mL; four had received a dose of
385 gabapentin 24 to 48 hours before blood drawn) and 4 dogs at day 14 (< 0.26 µg/mL, except for
386 one dog that had concentrations of approximately 9 µg/mL and was excluded from analysis).
387 Concentrations of gabapentin in the first and third blocks ranged from 0.36 – 18.47 µg/mL. Mean
388 concentrations of gabapentin ± SD were 8.53 ± 3.07 µg/mL and 7.13 ± 5.09 µg/mL after
389 gabapentin alone or in combination with meloxicam, respectively.

390 Standard measure obtained for MCP-1 on one of the two plates used for the analysis was not
391 included in the quality control range provided by the manufacturer therefore, corresponding data
392 for MCP-1 were excluded. Two analytes (IFN-γ and IL-2) showed a proportion of results below
393 detection level (out of range) superior to 50% and were therefore not analyzed. Among the
394 population studied, 7 dogs were excluded from the cytokine analyses (chronic skin conditions: n
395 = 4; oral inflammatory disease: n = 2; femoro-tibial effusion: n = 1). Concentrations of cytokines
396 measured in controls and NeuP before treatment are summarized in Table 4. No differences were
397 found between groups. Significant effects of sex and body weight were found for some analytes

398 (Table 4 and 5). A significant correlation was found between MCP-1 concentrations and the

399 overall impression of the owners on their dogs' quality of life (Tables 6, 7).

400 **Table 4.** Cytokine concentrations (median and range) in pg/mL measured in healthy control dogs and in dogs with presumptive neuropathic pain

401 (NeuP) using the Milliplex Canine Cytokine Panel.

	Controls	**NeuP**	**p**	**Covariates effect**		
	n = 13; MCP-1: n = 11	n = 23; MCP-1: n = 11		P_{sex}	P_{age}	P_{weight}
GM-CSF	15.02 (0.56 – 219.95)	30.12 (0.56 – 240.47)	0.53	0.17	0.90	0.18
KC-like	417.23 (203.88 – 1,391.12)	668.54 (67.69 – 1,381.57)	0.54	0.56	0.34	0.56
IP-10	7.00 (1.42 – 34.35)	7.79 (0.65 – 62.87)	0.16	0.51	0.14	0.49
IL-6	6.16 (2.02 – 80.89)	8.79 (1.89 – 78.55)	0.15	**0.015**	0.67	0.06
IL-7	34.34 (3.36 – 187.41)	21.50 (1.11 – 133.66)	0.07	0.10	0.85	0.18
IL-8	2,504.34 (966.25 – 3,768.76)	3,311.17 (690.87 – 13,131.05)	0.35	0.75	0.11	0.47
IL-10	0.94 (0.33 – 162.04)	1.53 (0.33 – 44.96)	0.42	0.10	0.36	0.61
IL-15	47.85 (7.24 – 2,381.73)	47.85 (4.98 – 1,251.31	0.33	0.59	0.62	**0.013**
IL-18	25.32 (10.71 – 178.37)	24.15 (8.92 – 141.83)	0.06	0.17	0.49	0.19
MCP-1	205.98 (154.27 – 410.62)	259.17 (174.39 – 539.18)	0.52	0.41	0.07	0.10
TNFα	1.25 (0.05 – 59.87)	1.63 (0.05 – 43.02)	0.74	NA	NA	NA

402 NA = Data non available (nonparametric test). Data in bold are significant.

Table 5. Cytokine concentrations (median and range) in pg/mL measured in dogs with presumptive neuropathic pain (NeuP) before and after treatments of placebo, gabapentin, gabapentin-meloxicam using the Milliplex Canine Cytokine Panel.

	Baseline	**Placebo**	**Gabapentin**	**Gabapentin-meloxicam**	**p**	**Covariates effect**		
	n = 23; MCP-1: n = 11	n =22; MCP-1: n = 11	n = 22; MCP-1: n = 11	n = 20; MCP-1: n = 11		p_{sex}	p_{age}	p_{weight}
GM-CSF	30.12 (0.56 – 240.47)	20.74 (0.56 – 265.78)	35.16 (0.56 – 336.65)	16.81 (0.56 – 262.01)	0.73	0.45	0.78	0.06
KC-like	668.54 (67.69 – 1,381.57)	589.36 (80.65 – 1,596.31)	492.40 (41.48 – 1,520.10)	564.58 (46.36 – 1,570.21)	0.38	0.96	0.31	0.29
IP-10	7.79 (0.65 – 62.87)	5.96 (0.65 – 34.60)	6.27 (0.65 – 37.67)	7.32 (0.65 – 43.69)	0.73	0.96	0.17	0.25
IL-6	8.79 (1.89 – 78.55)	6.58 (2.02 – 86.76)	12.16 (2.35 – 100.41)	7.39 (2.35 – 79.68)	0.57	0.06	0.56	**0.035**
IL-7	21.50 (1.11 – 133.66)	16.03 (1.11 – 149.90)	18.66 (1.98 – 172.46)	13.98 (1.11 – 141.01)	0.25	**0.048**	0.99	0.10
IL-8	3311.17 (690.87 – 13,131.05)	3,462.72 (450.80 – 9,539.46)	3,335.68 (1,080.60 – 19,188.58)	3,276.29 (889.47 – 10,406.34)	0.99	0.78	0.06	0.99
IL-10	1.53 (0.33 – 44.96)	2.53 (0.33 – 44.96)	2.09 (0.33 – 75.93)	0.95 (0.33 – 51.84)	0.49	0.08	0.15	**0.015**
IL-15	47.85 (4.98 – 1,251.31)	21.05 (4.98 – 1,255.93)	48.06 (4.98 – 1,431.35)	32.11 (4.98 – 1,302.06)	0.52	0.72	0.64	**0.001**
IL-18	21.15 (8.92 – 141.83)	20.96 (9.49 – 158.79)	22.23 (8.92 – 186.90)	20.69 (7.71 – 149.13)	0.17	**0.032**	0.33	**0.006**
MCP-1	259.17 (174.39 – 539.18)	261.84 (176.16 - 409.52)	253.67 (159.41 - 401.28)	250.84 (163.47 – 492.68)	0.91	0.08	**0.048**	0.40
TNF α	1.63 (0.05 – 43.02)	0.92 (0.05 – 48.27)	2.30 (0.05 – 57.41)	0.29 (0.05 – 44.18)	0.23	NA	NA	NA

A nonparametric test was used to analyze TNF α, therefore it was not possible to test for the effect of sex, age and weight on the concentration of this analyte (NA = non applicable). Data in bold are significant.

Table 6. Results of the statistical analysis evaluating the association between cytokines concentrations and a) owners' perception of their dog's quality of life b) CMPS-SF**.**

	$CBPI_{overall\ impression}$ (n = 36)		**CMPS-SF** (n = 32)	
	Spearman's rho correlation coefficient	Significance (P value)	Spearman's rho correlation coefficient	Significance (P value)
GM-CSF	0.056	0.74	-0.027	0.87
KC-like	-0.092	0.59	-0.015	0.94
IP-10	0.091	0.59	-0.21	0.24
IL-6	-0.037	0.83	0.047	0.79
IL-7	0.15	0.37	-0.22	0.22
IL-8	-0.21	0.22	0.13	0.47
IL-10	-0.175	0.30	0.086	0.63
IL-15	0.27	0.11	-0.19	0.29
IL-18	0.18	0.29	-0.12	0.50
MCP-1	**-0.38**	**0.024**	0.31	0.08
TNF- α	0.118	0.48	-0.125	0.49

Data in bold are significant.

Table 7. Results of the statistical analysis evaluating the association between cytokines concentrations and a) Client Specific Outcome Measures scores b) Canine Brief Pain Inventory (section pain) scores c) Canine Brief Pain Inventory (section interference, locomotion) scores.

	CSOM		$CBPI_{pain}$		$CBPI_{interference}$	
	Slope (SEM)	p value	Slope (SEM)	p value	Slope (SEM)	p value
GM-CSF	0.000489 (0.00915)	0.96	-0.00143 (0.00389)	0.71	0.00155 (0.00322)	0.63
KC-like	-0.00072 (0.00644)	0.91	0.00232 (0.00297)	0.44	-0.00069 (0.00242)	0.78
IP-10	-0.00248 (0.00546)	0.65	0.00087 (0.00281)	0.76	0.000294 (0.0023)	0.90
IL-6	-0.0102 (0.00973)	0.30	-0.00125 (0.00417)	0.76	0.000642 (0.00353)	0.86
IL-7	0.00218 (0.00649)	0.74	-0.00116 (0.00321)	0.72	0.000762 (0.00267)	0.78
IL-8	0.000556 (0.00998)	0.96	-0.00207 (0.00416)	0.62	0.000965 (0.00356)	0.79
IL-10	0.000524 (0.0133)	0.97	0.0028 (0.00585)	0.63	-0.00248 (0.00488)	0.61
IL-15	-0.0151 (0.0131)	0.25	-0.00844 (0.00525)	0.11	-0.00454 (0.00445)	0.31
IL-18	-0.00225 (0.00499)	0.65	-0.00159 (0.00221)	0.47	0.00082 (0.00186)	0.66
MCP-1	-0.00367 (0.00418)	0.39	0.000263 (0.00203)	0.90	-0.00082 (0.00171)	0.63
TNF-α	0.0202 (0.03)	0.50	0.00252 (0.0107)	0.82	0.00385 (0.01)	0.70

Discussion

This study provides novel insights on the sensory profile and pain burden of dogs with naturally-occurring NeuP undergoing medical treatment. The functional assessment of DNIC in dogs with NeuP showed that ΔMNT remained mostly unchanged or even decreased (i.e. negative values, indicating a facilitatory profile) after the application of a conditioning stimulus. These values were significantly different than healthy controls that presented mean positive values for ΔMNT (i.e. inhibitory profile) [10]. This result suggests a dysfunctional DNIC in dogs with NeuP, which is consistent with previous results obtained by different methods of DNIC assessment in dogs suffering from osteoarthritis [17] and osteosarcoma [18] and in rodent models of NeuP [19]. Therefore, NeuP may present changes in the descending modulatory mechanisms of pain (facilitatory over inhibitory input) reinforcing the need for disease-modifying therapies that produce changes in central pain modulation (e.g. gabapentinoids). In addition, the pain burden was overall reduced with gabapentin or gabapentin-meloxicam depending on the pain scoring instrument used. This is particularly true when considering the results for CBPI using owners' assessment who were fully masked to treatments.

The assessment of DNIC using the percentage of positive and negative ΔMNT has been described in humans with fibromyalgia [11]. Following the activation of spinal cord neurons conveying nociceptive input, supraspinal descending controls are normally activated to produce an inhibitory effect at the level of the dorsal horn of the spinal cord. In healthy conditions, the expected outcome would be the attenuation of subsequent painful input [20]. Therefore, animals with a functional DNIC should show positive values of ΔMNT (i.e. inhibitory profile) after the application of a conditioning stimulus. Indeed, most of the healthy individuals showed an inhibitory profile. However, approximately a third of this population had ΔMNT negative values

448 (i.e. facilitatory profile). Similar findings have been reported in healthy dogs and humans
449 [11,18]. In this study, approximately 60% of dogs with NeuP had a facilitatory profile at
450 presentation and after the administration of placebo, which is approximately a 2-fold increase
451 when compared with the percentage of healthy dogs with the same sensory profile. On the other
452 hand, the percentage of dogs with facilitatory profile after gabapentin was comparable with
453 healthy controls. A similar effect has been found with pregabalin in human patients with
454 fibromyalgia [21]. This finding is consistent with recent research showing an activation of the
455 inhibitory system by increased activity of noradrenergic neurons located in the locus coeruleus
456 after the administration of gabapentin [22]. In our study, the DNIC function of NeuP was
457 regained after gabapentin. It is not clear why the same effect was not observed after the
458 administration of gabapentin-meloxicam where approximately 50% of NeuP continued to show a
459 facilitatory profile. However, despite being not statistically significant, there was a trend for
460 ΔMNT values to be negative at presentation and after placebo, and positive after gabapentin and
461 gabapentin-meloxicam. While DNIC and stress-induced analgesia are two endogenous analgesic
462 mechanisms that can be triggered by a noxious stimulus [23], the authors used a fear-free
463 approach to minimize stress-induced analgesia and we believe the results are indeed a reflection
464 of DNIC profile of these patients.

465 Central sensitization has been observed in patients with NeuP [24]. In animal models of NeuP
466 based on peripheral nerve injury, this phenomenon is commonly studied by measuring
467 nociceptive thresholds in a remote area from the injury [25]. For this reason, it was deemed that
468 using the 'less affected limb' for the assessment of the DNIC would provide a more accurate
469 value than using the 'most affected limb'. Also, ENT and MNT measured at the affected, but
470 also other limbs were averaged for each individual. Thresholds were expected to be overall lower
471 in NeuP than in controls due to potential for central sensitization. However, MNT and ENT were

not significantly different between the two populations and did not change after treatments in NeuP. This could be explained by the great individual variability of both QST modalities in dogs from different breeds, ages and body weight [10]. On the other hand, a recent study investigating NeuP in Cavalier King Charles Spaniels dogs reported higher MNT after the administration of pregabalin when compared with baseline or placebo treatment [26]. The different findings could rely on the homogeneity of the population studied (same breed and same underlying disease), different testing sites, technique or nociceptive threshold device. Finally, both ENT and MNT were influenced by body weight. A positive correlation between body weight and MNT has been described in healthy dogs [27]. Since our two populations (controls and NeuP) had similar body weight, this was not considered as a confounding factor in the present study.

The pain burden caused by NeuP in dogs was evaluated at presentation and after therapy using different pain scoring systems. The CBPI allowed the evaluation of NeuP in terms of comfort ($CBPI_{pain}$), function ($CBPI_{interference}$) and quality of life ($CBPI_{overall\ impression}$). The function was further assessed using the CSOM. These two methods of pain assessment (CBPI and CSOM) were used to investigate the pain burden in a familiar environment as perceived by owners who were masked to the treatment. A method of acute pain assessment (CMPS-SF) was used for the veterinarian's evaluation due to the possibility of an acute episode of pain related to the chronic underlying condition and the lack of valid pain assessment instruments to evaluate NeuP in dogs. A difference in the scores between males and females was recorded with the CMPS-SF. Considering that males were overrepresented in the NeuP group, this could represent a bias in our population. All instruments (CBPI, CSOM, and CMPS-SF) detected a positive effect of one or both active treatments compared with presentation. Gabapentin alone or in combination with meloxicam reduced pain scores as measured by CSOM, $CBPI_{pain}$ and CMPS-SF. Gabapentin exerts its analgesic effect through its action on supraspinal region to promote descending

inhibition of nociceptive stimuli [22], and it binds to the α_2-δ subunit of the voltage-gated calcium channels involved in the maintenance of mechanical hypersensitivity in rodent models of NeuP [28]. The CBPI $_{\text{overall impression}}$ showed an improved quality of life after the administration of gabapentin when compared with presentation. The same results were not observed for gabapentin-meloxicam. However, less than one third of dogs were classified with a "poor" or "fair" quality of life after gabapentin or gabapentin-meloxicam, whereas at least 50% of dogs were classified within these categories after placebo and at presentation. The combination of gabapentin and meloxicam was associated with improved activity using CBPI $_{\text{interference}}$ when compared with presentation, and when using CMPS-SF compared with placebo. These findings indicate a beneficial effect of meloxicam on mobility and locomotion of dogs with NeuP. Severe orthopedic conditions were used as exclusion criteria, yet the treatment with an anti-inflammatory drug may have helped with chronic conditions such as osteoarthritis that might have been concomitant with the neurological disease. Indeed, meloxicam is a non-steroidal anti-inflammatory drug, a preferential cyclooxygenase 2 (COX-2) inhibitor, used for the treatment of osteoarthritis in dogs [29]. An overexpression of COX-2 has been observed with peripheral NeuP [30]. This discovery was the rationale to focus on preferential or selective COX-2 inhibitors as potential therapeutic avenues for the management of NeuP, as well as previous studies suggesting potential benefits of this combination in people with therapy-related NeuP [31].

A significant improvement was found after placebo treatment using the CSOM. Resting was recommended as part of treatment and could have contributed to pain relief in this study. Additionally, a carry-over effect after the first week of treatments (gabapentin or gabapentin-meloxicam) cannot be ruled out especially considering the low concentrations of gabapentin detected on day 14 at the end of placebo administration. However, a significant effect was not observed for treatment order and it is unlikely that these small serum concentrations of

gabapentin would produce an analgesic effect in dogs with NeuP. It is also possible that a placebo effect existed with the CSOM, but not the CBPI where scores were not significantly different between initial presentation and placebo. This highlights how difficult chronic pain assessment in companion animals can be especially when validated tools specific for the assessment of NeuP are not available. It also demonstrates the importance of using different instruments for pain assessment involving both owners' and veterinarian's evaluations. Depending on the instrument used, research findings can have different outcomes. Finally, the veterinarian performing evaluations was masked to the first and third blocks (gabapentin or gabapentin-meloxicam), but not the second (placebo) block of treatments. Thus, the evaluation of the dogs after placebo treatment relied mostly on the unbiased owners' evaluation.

In the present study, serum concentrations of gabapentin were evaluated as an indirect assessment of owners' compliance to treatment administration and to report these concentrations for *posteriori* studies potentially correlating therapeutic levels with dosage regimens, sex, breed, age and the analgesic efficacy of gabapentin. The concentrations of gabapentin required to alleviate NeuP remain unknown. Based on pharmacologic modelling, the potency of gabapentin (EC 50) in rats for its anti-allodynic effect was reported between 1.4 to 16.4 µg/mL [32,33] and 5.35 µg/mL for the treatment of neuropathic pain in man [34]. In our study, dogs had concentrations ranging between 0.36 and 18.5 µg/mL but timing of blood collection could not be standardized due to owners' constraints for scheduling re-evaluations and time of drug administration. Given both veterinarian's and owners' positive outcomes, the dosage regimens for gabapentin were considered effective in the treatment of NeuP in dogs. However, there was a large range of concentrations showing significant individual variability that could impact the pharmacokinetics and potentially the pharmacodynamics of the drug in the clinical setting.

543 The concentrations of inflammatory cytokines measured in this study are consistent with
544 previously published data in healthy dogs [35], with large individual concentration variability,
545 especially considering individuals of different breeds and suffering from different neurological
546 pathologies. Therefore, the lack of significant differences between control and NeuP groups, or
547 between treatments in this study may reflect a type 2 error, more than an actual homogeneity of
548 these populations. A higher concentration of MCP-1 was associated with a worse appreciation of
549 the quality of life of their dog by the owner. These results corroborate previous findings in
550 humans where MCP-1 concentrations were positively associated with more severe fibromyalgia-
551 related pain when evaluated with the brief pain inventory [3]. Our results also suggest that future
552 investigations on inflammatory cytokines in canine NeuP should divide the population into
553 subgroups based on sex and body weight to better understand the disease.

554 The limitations of our study design including a partially masked evaluator and a bias towards the
555 placebo effect have been discussed. Some other limitations should be considered. Due to ethical
556 considerations in clinical pain research, dogs experiencing pain were immediately treated either
557 before (administration of remifentanil) or during the study (rescue analgesia), therefore
558 introducing a potential bias in the results. However, in the present study, these interventions were
559 minimal (exclusion during the first block with gabapentin-meloxicam, n = 1; four days of
560 placebo period instead of 7, n = 1) but it may have contributed to a mild overall improvement
561 observed after placebo or gabapentin. The initial assessment may also have been altered by the
562 administration of remifentanil in two dogs before the withdrawal period of 60 minutes. The drug
563 may have provided sustained analgesia reducing clinical signs of central sensitization in dogs
564 with NeuP before QST at initial presentation. Also, there is no definitive test to diagnose NeuP.
565 Therefore, inclusion criteria were determined to meet the most recent definition of NeuP by the
566 International Association for the Study of Pain: “pain arising as a direct consequence of a lesion

567 or disease affecting the somatosensory system". All dogs included had a long-term history of
568 pain and a confirmed neurological lesion found at MRI. Additionally, most dogs had delayed
569 paw placements or ataxia which indicated an involvement of the somatosensory system.
570 Recognition of NeuP remains a challenge in veterinary medicine and in non-verbal human
571 patients since it is characterized by the combination of sensory qualities that can only be self-
572 reported [37].

573 In conclusion, dogs with NeuP have changes in sensory profile characterized by a dysfunctional
574 DNIC compared with healthy controls. These results could be the expression of maladaptive
575 changes in favor of pain facilitation over inhibition in the central pain processing. This study
576 supports the use of gabapentin alone or in combination with meloxicam for the medical
577 management of NeuP in dogs due to improvements in the sensory profile and pain burden.
578 Depending on which pain scoring instrument, gabapentin alone or in combination with
579 meloxicam provided pain relief in client-owned dogs with naturally-occurring presumed NeuP.

580

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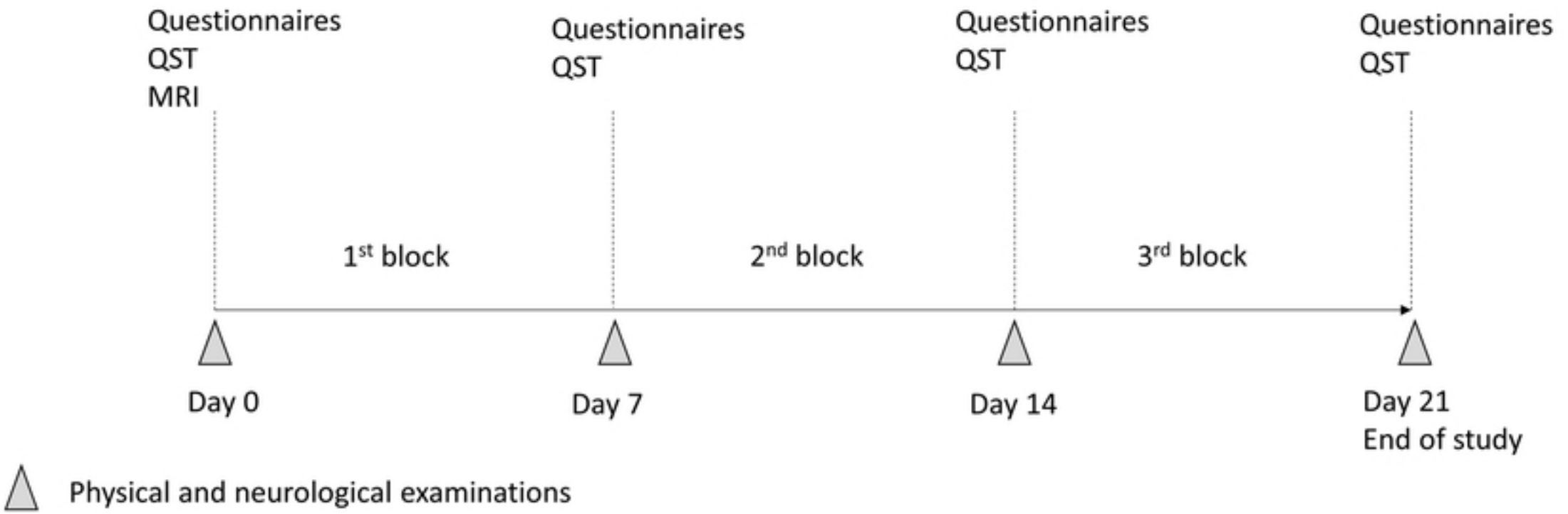
Supporting information

S1 Supplementary methods. Serum concentrations of gabapentin in dogs.

S2 Appendix — Dogs with neuropathic pain. A) Scores obtained with Scores obtained with Client Specific Outcome Measures (CSOM), Canine Brief Pain Inventory (CBPI pain and interference), short-form Glasgow Composite Measure Pain Scale (CMPS-SF) shown as mean ± SD ; B) Values of electrical and mechanical nociceptive thresholds and changes in mechanical nociceptive thresholds after application of a conditioning stimulus in dogs with naturally-occurring neuropathic pain before and after each treatment period. Data are shown as mean ± SD.

699 **S3 Database**


Questionnaires
QST
MRI
Questionnaires
QST
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QST
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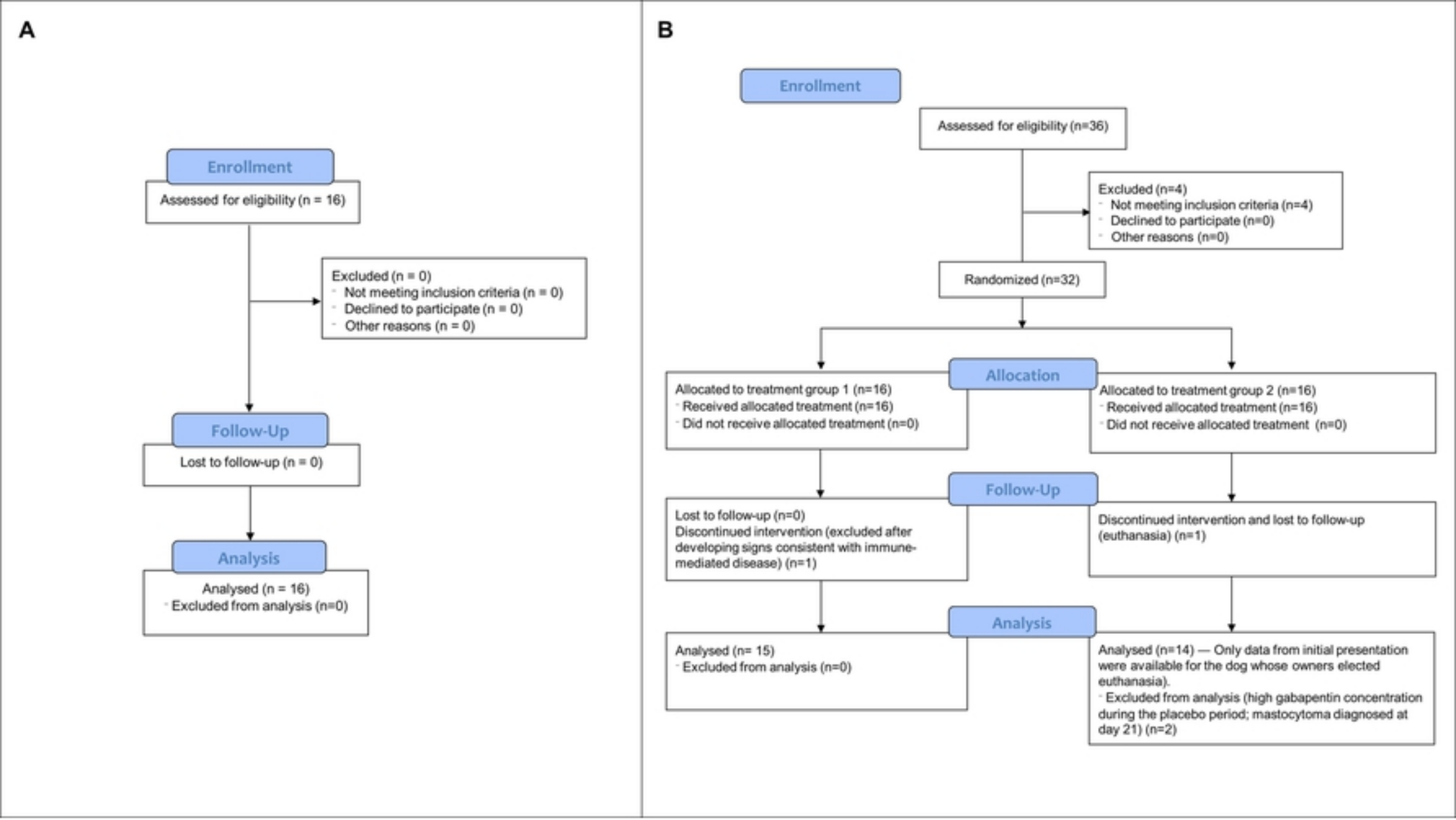
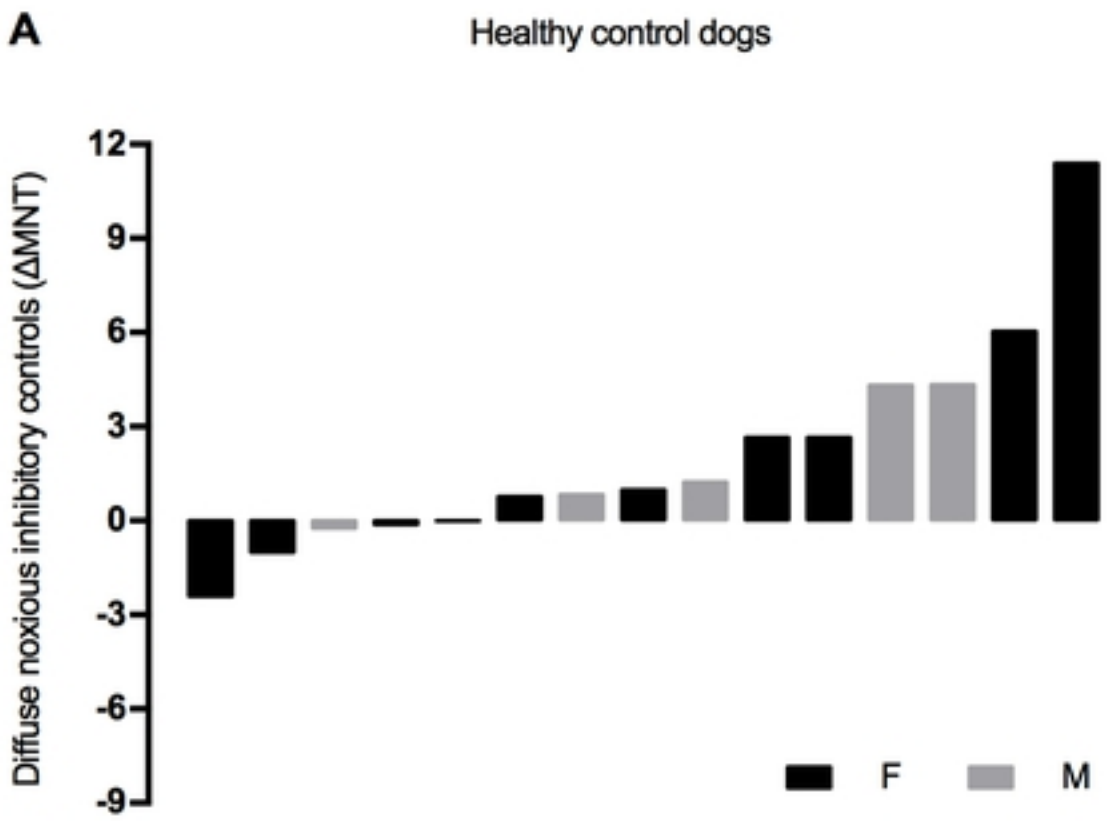
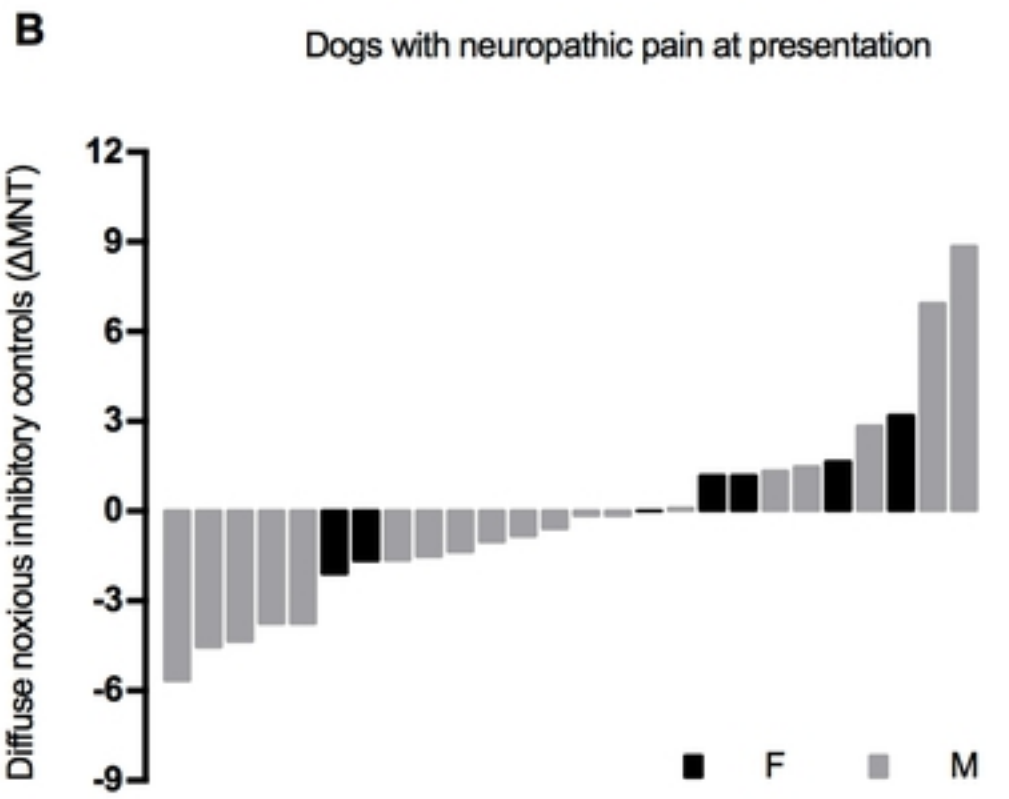
QST
1st block
2nd block
3rd block
Day 0
Day 7
Day 14
Day 21
End of study
Physical and neurological examinations

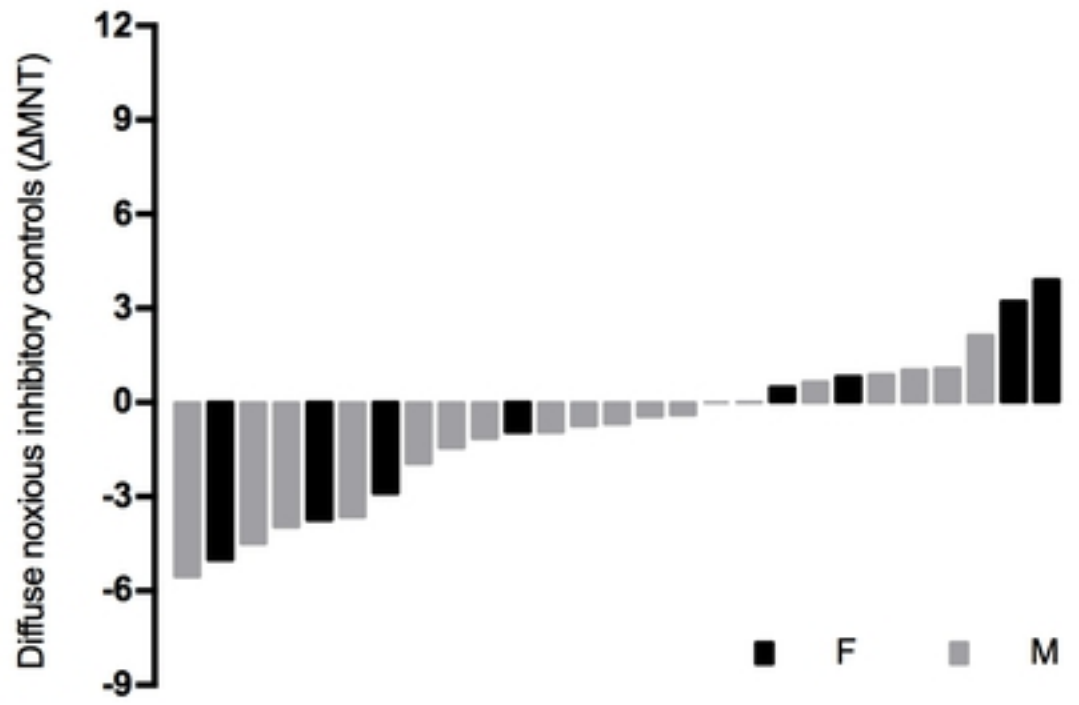
Figure

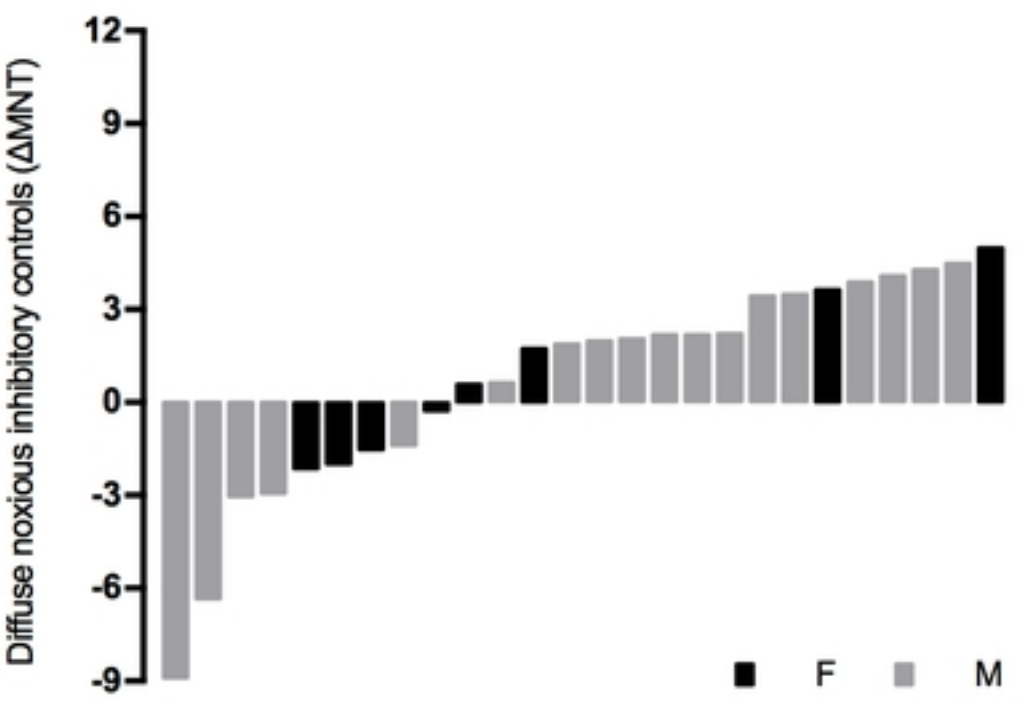

A
Enrollment
Assessed for eligibility (n = 16)
Excluded (n = 0)
Not meeting inclusion criteria (n = 0)
Declined to participate (n = 0)
Other reasons (n = 0)
Follow-Up
Lost to follow-up (n = 0)
Analysis
Analysed (n = 16)
Excluded from analysis (n=0)
B
Enrollment
Assessed for eligibility (n=36)
Excluded (n=4)
Not meeting inclusion criteria (n=4)
Declined to participate (n=0)
Other reasons (n=0)
Randomized (n=32)
Allocation
Allocated to treatment group 1 (n=16)
Received allocated treatment (n=16)
Did not receive allocated treatment (n=0)
Allocated to treatment group 2 (n=16)
Received allocated treatment (n=16)
Did not receive allocated treatment (n=0)
Follow-Up
Lost to follow-up (n=0)
Discontinued intervention (excluded after developing signs consistent with immune-mediated disease) (n=1)
Discontinued intervention and lost to follow-up (euthanasia) (n=1)
Analysis
Analysed (n= 15)
Excluded from analysis (n=0)
Analysed (n=14) — Only data from initial presentation were available for the dog whose owners elected euthanasia).
Excluded from analysis (high gabapentin concentration during the placebo period; mastocytoma diagnosed at day 21) (n=2)

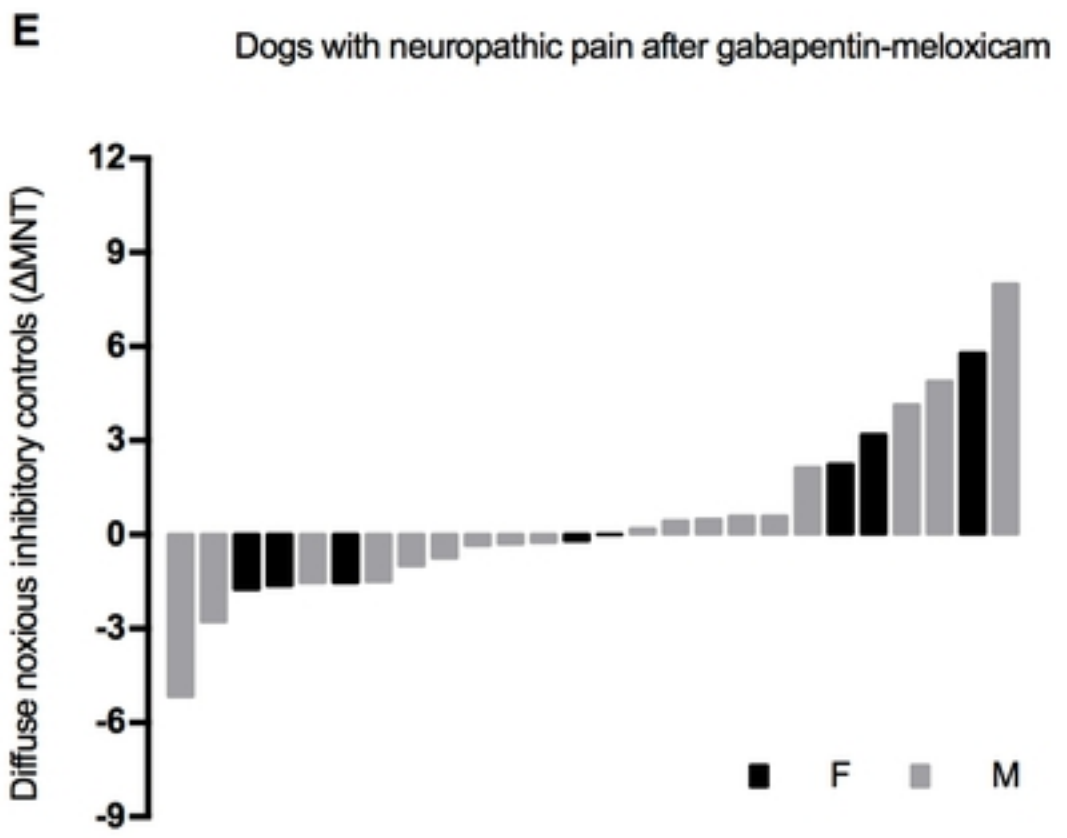
Figure











Figure

S1 Supplementary Methods

Gabapentin in dog serum

1. *Analytical Procedure*

1.1. Reagents

Gabapentin and $^{2}H_{6}$-gabapentin were purchased from Toronto Research Chemical (Toronto, ON, Canada). Drug-free dog serum was supplied by our laboratory. Formic acid was purchased form Sigma-Aldrich (St-Louis, MO, USA). Other chemicals, including, methanol, acetonitrile and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

1.2. Sample preparation

Using protein precipitation as sample preparation technique, gabapentin was extracted from dog serum. One thousand microliters of internal standard solution (100 ng/mL $^{2}H_{6}$-gabapentin in methanol) was added to an aliquot of twenty-five microliters of sample. The sample was vortexed for approximately 5 seconds and let stand for a period of 10 minutes, then centrifuged at 16 000 × *g* for 10 minutes. The supernatant was transferred into a clean 13 x 100 mm borosilicate tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The dried extract was re-suspended with 2000 µL of 0.1% (v/v) formic acid in water and transferred to an injection vial for analysis.

1.3. Chromatographic conditions

A gradient mobile phase was used with a Thermo Scientific Aquasil C18 analytical column (100 x 2.1 mm I.D., 5 µm) operating at ambient temperature. The initial mobile phase conditions consisted of 0.1 % (v/v) formic acid in acetonitrile and 0.1 % (v/v) formic acid water at a ratio of 5:95, respectively, and this ratio was maintained for 0.5 min. At 0.6 min, a step gradient was applied to a ratio of 95:5 and maintained for 2.9 min. At 3.6 min, the mobile phase composition was reverted to the original conditions and the column was allowed to equilibrate for 2.4 min for a total run time of 7.0 min. The flow rate was fixed at 200 µl/min and both compounds eluted at 3.1 min.

1.4. Mass spectrometric conditions

The mass spectrometer was interfaced with the UHPLC system using a pneumatic assisted heated electrospray ion source. MS detection was performed in positive ion mode, using selected reaction monitoring (SRM). In order to optimize the MS/MS parameters, standard solutions of gabapentin and $^{2}H_{6}$-gabapentin were infused into the mass spectrometer. The following parameters were obtained. Nitrogen was used for the sheath and auxiliary gases and was set at 50 and 15 arbitrary units. The HESI electrode was set to 3500 V. The capillary temperature was set to 350°C and the vaporizer temperature was set to 400°C.

Argon was used as collision gas at a pressure of 2.5 mTorr. The precursor-ion reaction for gabapentin and $^2H_6$-gabapentin were set at 172.2 → 137.3 and 178.3 → 143.2, respectively. The collision energy (E_{lab}) for both compounds was set to 15 eV. Total cycle time was set at 0.25 seconds. Peak width of Q1 and Q3 were both set at 0.7 FWHM.

2. *Chromatograms*

The mass chromatograms of the extracted blank serum sample did not show any significant interference from endogenous substances at the expected retention time of gabapentin or $^2H_6$-gabapentin.

3. *Analytical Qualification*

A stock solution of gabapentin was prepared by accurately weighing and dissolving the compound in water to obtain a final concentration of 0.5 mg/mL. A serie of standard working solution of gabapentin was obtained by mixing the standard stock solution and further diluting with water. Calibration standards were prepared by fortifying the dog serum with the standard working solutions at 5% (v/v) to enable concentrations spanning the following analytical range 0.10 to 25.0 µg/mL. The method is linear using a linear regression weighted 1/x analysis. $R^2 \geq 0.9988$ for the qualification batch.

4. *Sample Analysis*

During qualification, the method met all requirements of sensitivity, linearity, precision and accuracy within a batch. This assay is suitable for the analysis of gabapentin in dog serum. The correlation coefficient for gabapentin during all sample analysis batches was greater than $R^2 \geq 0.9997$. During all analytical batches, the accuracy ranged from 100.1 to 106.7 % and the precision observed was greater than 1.4 %. Samples were injected in duplicate. Dogs from the study with detectable concentrations of gabapentin at initial presentation and during the placebo period were repeated, the repeat results confirmed initial analysis.

ID	GROUP	SEX (M=male; F=female)	AGE (years)	WEIGHT (Kg)	BREED	Main lesions
C1	Control	MC	6.0	28.5	Siberian husky	NA
C2	Control	FS	9.0	31.0	Greyhound	NA
C3	Control	FS	6.0	5.5	Pinsher	NA
C4	Control	MC	2.0	8.0	French bulldog	NA
C5	Control	FS	7.0	16.4	Mix breed	NA
C6	Control	MC	3.0	29.4	Mix breed	NA
C7	Control	FS	4.0	30.5	Greyhound	NA
C8	Control	MC	5.0	33.0	Mix breed	NA
C9	Control	FS	9.0	26.9	Golden retriever	NA
C10	Control	FS	3.0	60.0	Mastiff	NA
C11	Control	FS	4.0	47.0	Dogue de Bordeaux	NA
C12	Control	FS	4.0	22.6	Mix breed	NA
C13	Control	MC	4.0	51.0	Rottweiller	NA
C14	Control	F	3.0	65.5	Newfoundland	NA
C15	Control	M	4.0	30.7	French spaniel	NA
C16	Control	FS	4.0	26.8	Mix breed	NA
1	Study group	M	8.0	35.0	Labrador retriever	IVDD (C)
2	Study group	MC	6.6	57.0	Bernese mountain dog	Cervical spondylomyelopathy
4	Study group	MC	10.4	38.8	Bernese mountain dog	Nerve sheath tumor (C6)
5	Study group	MC	5.2	56.0	Bernese mountain dog	Cervical spondylomyelopathy
6	Study group	FS	3.7	6.9	Cavalier king charles spaniel	Chiari-like malformation associcated with syringomyelia
7	Study group	MC	8.5	4.5	Poodle toy	IVDD (C, TH, L)
8	Study group	MC	3.6	51.0	Bernese mountain dog	Cervical spondylomyelopathy; IVDD (C)
9	Study group	MC	8.3	31.4	Siberian husky	LS stenosis; IVDD (Th,L)
10	Study group	FS	4.0	31.0	Golden retriever	Mild LS stenosis
11	Study group	MC	3.1	11.6	Cavalier king charles spaniel	Caudal occipital bone malformation syndrome; IVDD (Th, L)
13	Study group	FS	7.3	7.4	Cavalier king charles spaniel	IVDD (L, LS)
14	Study group	MC	5.1	53.0	Bernese mountain dog	Cervical spondylomyelopathy, IVDD (C)
15	Study group	MC	3.5	7.1	Pug	IVDD (Th, L)
16	Study group	MC	9.3	10.7	Wire fox terrier	IVDD (Th, L)
17	Study group	MC	8.0	38.7	Boxer	IVDD, discospondylitis
18	Study group	MC	6.0	57.0	Polish tatra sheepdog	Cervical spondylomyelopathy
19	Study group	MC	6.2	9.3	Cavalier king charles spaniel	Chiari-like malformation associcated with syringomyelia
20	Study group	MC	0.6	16.7	Labrador retriever	Congenital vertebral malformation (C)
21	Study group	MC	6.0	28.4	Labrador retriever	IVDD (L, LS), LS stenosis
22	Study group	MC	6.9	4.2	Mix breed	Cervical syringomyelia; IVDD (C, Th, L)
23	Study group	MC	10.9	4.2	Longhaired dachshund	IVDD (C, L)
24	Study group	FS	12.5	30.5	Basset hound	IVDD (Th, L)
25	Study group	MC	4.6	12.9	Pomeranian	Mild stenosis of the vertebral canal and foramina (LS)
26	Study group	MC	8.1	49.4	Bernese mountain dog	Cervical spondylomyelopathy; IVDD (C); syringomyelia; edema/gliosis (C)
27	Study group	FS	8.0	28.9	Labrador retriever	IVDD (Th, L)
28	Study group	FS	7.7	10.7	Cavalier king charles spaniel	Chiari-like malformation associcated with syringomyelia
30	Study group	MC	1.6	49.0	Mix breed	Cervical spondylomyelopathy and syringomyelia
31	Study group	FS	12.9	29.4	Siberian husky	Suspicion of meningeal neoplam
32	Study group	FS	5.9	12.9	Beagle	LS stenosis

NA = Non applicable
IVDD = Intervertebral disc disease
C = Cervical
Th = Thoracic
L = Lumbar
LS = Lumbosacral

ID	mg gabapentin/kg	mg meloxicam/kg	$[gabapentin]_{meas}$ (µg/mL)	SD	$[gabapentin]_{meas}$ when administered with meloxicam (µg/mL)	SD
1	11.42857143	0.1	NA	NA	NA	NA
2	10.52631579	0.100877193	3.614	0.019	3.362	0.052
4	10.30927835	0.090206186	NA	NA	NA	NA
5	10.71428571	0.098214286	11.934	0.191	18.473	0.137
6	14.49275362	0.09057971	8.584	0.057	10.183	0.029
7	11.11111111	0.111111111	5.076	0.022	0.837	0.002
8	9.803921569	0.098039216	13.321	0.074	2.245	0.007
9	9.554140127	0.095541401	10.92	0.068	4.31	0.035
10	9.677419355	0.096774194	9.194	0.275	13.771	0.02
11	8.620689655	0.086206897	8.265	0.005	11.21	0.077
13	13.51351351	0.101351351	2.706	0.002	0.359	0.007
14	11.32075472	0.094339623	8.191	0.058	13.556	0.012
15	14.08450704	0.088028169	10.714	0.072	9.001	0.14
16	9.345794393	0.093457944	4.904	0.011	3.874	0.061
17	10.33591731	0.096899225	10.125	0.033	4.347	0.036
18	10.52631579	0.096491228	8.214	0.052	10.533	0.061
19	13.69863014	0.102739726	2.582	0.006	5.103	0.02
20	11.9760479	0.089820359	5.383	0.033	3.262	0.002
21	10.56338028	0.088028169	13.275	0.667	17.995	0.222
22	11.96172249	0.09569378	8.76	0.032	5.563	0.074
23	11.96172249	0.09569378	9.128	0.008	8.45	0.007
24	11.47540984	0.098360656	9.449	0.055	3.545	0.027
25	11.9047619	0.099206349	7.264	0.075	10.875	0.1
26	10.12145749	0.096153846	8.422	0.036	7.046	0.003
27	10.38062284	0.095155709	13.657	0.004	NA	NA
28	9.345794393	0.093457944	10.508	0.006	7.659	0.096
30	10.20408163	0.102040816	6.473	0.024	7.797	0.004
31	10.20408163	0.102040816	11.415	0.006	1.339	0.007
32	11.19402985	0.093283582	8.21	0.054	0.777	0.003

ID	MNT (N)	Δ MNT	ENT (mA)	CBPI pain	CBPI interference	CBPI overall impression	CMPS-SF
C1	6.3625	-0.25	136.625	0	0	excellent	0
C2	11.9	-1.0125	35.75	0	0	excellent	0
C3	7.95	0.1333	18.8125	0	0	excellent	0
C4	7.525	1.25	44.6875	0	0	excellent	0
C5	7.15	11.4	23.5625	0	0	excellent	0
C6	12.575	4.3333	56.625	0	0	excellent	0
C7	15.35	-0.0125	59.125	0	0	excellent	1
C8	15.73333		35.9375	0	0	very good	0
C9	7.8	6.05	38.875	0	0	excellent	0
C10	16.8	2.6625	70.75	0	0	excellent	1
C11	17.4625	-2.425	45.875	0	0	excellent	1
C12	7.8125	0.7833	31.5	0	0	excellent	0
C13	15.375	0.85	69.125	0	0	very good	0
C14	10.975	2.675	60.75	0	0	excellent	0
C15	8.7	4.35	20.375	0	0	excellent	0
C16	8.15	1.0	41.375	0	0	excellent	0

CYTOKINE CONCENTRATIONS (pg/mL)

Treatment	ID	GM-CSF (14)	KC-like (61)	IP-10 (57)	IL-2 (29)	IL-6 (35)	IL-7 (36)	IL-8 (48)	IL-10 (63)	IL-15 (54)	IL-18 (67)	MCP-1 (74)	TNF-a (76)	Time point
None	C7	0.56	1116.49	1.42	0.31	2.02	5.72	3441.7	12.95	7.24	10.71	201.12	0.13	
None	C1	104.53	887.58	14.63	14.51	23.83	45.53	2967.64	0.94	158.5	59.6	251.02	8.97	
None	C16	7.2	250.98	5.39	1.35	4.88	35.18	966.25	2.37	21.85	13.5	.	0.05	
None	C6	19.32	417.23	27.71	13.78	6.16	14.56	1947.35	0.94	2381.73	25.32	154.27	0.13	
None	C8	15.02	649.41	6.06	0.31	6.68	34.34	1915.62	8.27	7.24	22.06	192.2	3.25	
None	C10	11.83	399.72	3.31	1.35	3.27	11.41	1513.03	0.33	47.85	24.75	.	1.25	
None	C2	0.56	1391.12	1.42	0.31	2.53	3.36	3768.76	0.94	7.24	10.71	205.98	0.13	
None	C5	0.56	449.13	8.69	0.31	4.07	7.2	2616.29	3.77	7.24	16.93	212.24	0.13	
None	C3	177.4	584.33	5.33	118.27	58.04	92.21	1596.01	0.94	585.26	116.75	410.62	25.38	
None	C9	134.78	203.88	23.09	61	29.97	137.86	1163.61	0.33	795.65	114.21	.	32.82	
None	C13	219.95	345.97	34.35	51.21	56.07	144.64	3091.28	162.04	1223.21	178.37	.	40.3	
None	C14	11.83	397.87	7	54.69	2.81	11.41	3085.17	4.55	30.46	49.2	.	0.05	
None	C12	144.41	375.55	13.67	40.82	80.89	187.41	2504.34	0.72	890.22	148.75	.	59.87	
None	14	5.57	668.54	2.01	0.31	4.07	10.28	1754.58	0.94	7.24	13.79	313.11	0.13	T0
G	14	3.76	578.57	1.43	0.31	4.07	10.28	1906.86	0.94	7.24	15.67	241.18	0.13	T7
GM	14	1.76	925.62	1.73	0.31	4.07	9.24	4424.68	0.95	7.24	15.04	258.15	0.13	T21
P	14	3.76	618.89	1.73	0.31	3.04	10.28	2975.76	0.94	7.24	15.04	219.98	0.13	T14
None	30	34.47	830.63	8.1	1.36	12.38	23.56	7553	20.29	39.13	23.55	.	29.36	T0
G	30	28.85	461.72	7.79	1.35	10.57	19.44	3209.96	3.23	21.85	21.75	.	23.74	T7
GM	30	11.83	319.17	7.32	1.35	4.65	11.41	1971.25	6.82	4.98	16.42	.	18.43	T21
P	30	11.83	379.05	5.55	1.35	4.65	11.41	2113.16	4.55	4.99	9.49	.	15.8	T14
None	16	16.47	787.7	16.01	2.68	45.24	27.12	12828.25	5.24	15.96	25.32	539.18	0.14	T0
G	16	39.09	726.99	27.79	4.63	14.65	40.51	3945.95	75.93	131.55	36.68	289.8	2.55	T7
GM	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	250.84	1.18	T21
P	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7.24	35.32	261.84	1.4	T14
None	27	38.28	308.3	13.38	1.35	7.84	21.5	13131.05	2.37	92.02	24.15	.	2.95	T0
G	27	39.88	238.7	10.36	1.35	6.93	16.39	3979.23	0.33	74.26	21.15	.	2.3	T21
P	27	44.62	370.94	10.5	1.35	6.48	16.39	2640.2	0.33	92.02	21.15	.	3.38	T14
None	11	44.07	631.39	1.42	8.04	22.74	31.91	6427	0.94	180.99	31.29	211.33	7.19	T0
G	11	35.29	473.11	1.42	3.32	18.41	23.6	4493.21	0.94	122.59	27.96	218.31	4.46	T7
GM	11	24.82	786.73	1.42	0.87	12.51	14.56	5881.61	0.94	91.27	21.41	217.59	2.31	T21
P	11	36.24	763.81	1.04	5.56	19.51	27.31	5260.88	5.28	170.56	27.6	176.16	5.13	T14
None	23	48.74	349.19	10.36	3.9	18.93	29.85	1522.46	16.35	254.9	67.74	.	4.04	T0
G	23	35.02	401.2	11.38	1.35	13.74	22.53	1626.78	18.31	163.86	74.55	.	2.3	T7
GM	23	18.6	407.11	10.67	1.35	7.39	13.39	889.47	10.56	74.26	52.05	.	0.05	T21
P	23	23.57	377.21	10.65	1.35	9.66	16.39	1446.41	11.51	100.93	52.37	.	0.44	T14
None	20	27.12	226.12	0.65	1.35	8.53	12.39	690.87	1.53	47.85	21.45	.	0.44	T0
G	20	121.11	259.38	4.37	65.92	62.38	86.29	1628.01	16.35	420.89	65.48	.	25.32	T21
GM	20	57.78	444.48	2.37	13.05	29.07	34.11	2124.99	4.11	154.83	34.52	.	8.32	T7
P	20	125.54	315.85	1.77	51.9	49.77	76.43	450.8	11.51	379.19	61.62	.	18.91	T14
G	10	8.91	456.09	2.55	0.31	4.07	6.21	1578.86	0.94	7.24	11.93	159.41	0.13	T21
GM	10	13.54	436.66	3.05	0.31	4.59	7.2	4630.17	0.94	7.24	16.3	163.47	0.13	T7
P	10	13.54	441.46	2.01	0.31	6.16	7.2	1948.93	0.94	7.24	12.55	182.7	0.13	T14
None	24	1.17	914.07	10.68	1.35	1.89	1.11	8875.45	4.11	4.98	10.05	.	0.05	T0
G	24	1.17	1520.1	0.65	1.35	2.35	1.98	19188.58	11.51	4.98	11.77	.	0.05	T7
GM	24	1.17	1404.83	0.65	1.35	2.35	1.11	7884.6	8.67	4.98	9.49	.	0.05	T21
P	24	1.17	1521.69	1.35	1.35	2.35	1.11	9539.46	8.67	4.98	11.19	.	0.05	T14
None	17	0.57	1381.57	13.63	0.31	3.04	4.29	5990.51	0.94	7.24	10.71	397.95	0.13	T0
G	17	0.56	1314.01	15.12	0.31	4.07	6.21	6688.03	0.94	7.24	17.56	310.02	0.13	T7
GM	17	2.79	1570.21	11.4	0.31	3.04	4.29	9879.82	0.94	7.24	7.71	302.78	0.13	T21
P	17	0.56	1104.25	12.44	0.31	2.02	3.36	6299.77	0.94	7.24	11.32	305.43	0.13	T14
None	31	15.99	707.25	14.23	1.35	4.42	16.39	3438.71	8.67	30.46	21.15	.	0.05	T0
G	31	10.36	773.26	14.65	1.35	2.81	12.39	3218.04	6.82	4.98	17	.	0.05	T21
GM	31	11.83	1432.59	15.2	1.35	3.27	15.39	10406.34	15.37	21.85	19.96	.	0.05	T7
P	31	10.36	814.37	0.65	1.35	5.11	12.39	4127.03	5.9	4.99	15.24	.	0.05	T14
None	26	110.42	203.78	0.65	283.2	26.37	27.74	3311.17	0.33	47.85	67.74	.	8.44	T0
G	26	91.87	264.36	0.65	268.52	22.77	24.6	1346.68	0.33	21.85	60.34	.	6.5	T21
P	26	101.87	589.47	0.65	238.29	21.98	21.79	2936.26	0.33	34.85	49.56	.	6.85	T14
None	7	240.47	865	6.96	86.09	78.55	133.66	4824.24	6.75	649.27	141.83	394.5	43.02	T0
G	7	336.65	1062.92	7.94	106.97	100.41	172.46	7173.64	8.27	951.99	186.9	401.28	57.41	T7
GM	7	262.01	1070.5	13.96	80.84	79.68	141.01	6628.77	34.27	704.2	149.13	492.68	44.18	T21
P	7	265.78	1106	9.98	96.7	86.76	149.9	6818.5	12.95	750.02	158.79	409.52	48.27	T14
None	2	0.56	843.56	5.33	0.31	3.04	3.36	6082.66	0.94	7.24	13.79	174.39	0.13	T0
G	2	0.56	511.69	4.9	0.31	3.04	3.36	3453.31	0.94	7.24	11.32	254.37	0.13	T7
GM	2	0.56	623.7	8.51	0.31	3.04	4.29	2375.88	0.94	7.24	12.24	261.35	0.13	T21
P	2	0.56	640.4	6.36	0.31	3.55	3.36	2813.92	0.94	7.24	9.5	268.33	0.13	T14
None	4	39.09	871.46	36.83	0.31	8.79	30.7	13089.03	22.63	95.74	44.2	310.15	2.55	T0
None	5	30.12	67.69	1.42	0.31	35.31	13.47	1194.57	0.94	15.96	24.01	200.53	1.63	T0
G	5	56.22	129.3	1.42	0.31	38.89	21.29	6428.14	0.94	77.89	35.32	174.62	4.21	T7
GM	5	35.92	46.36	1.42	0.31	31.47	12.39	1307.93	0.94	42.36	24.67	208.7	1.63	T21
P	5	45.31	80.65	1.42	0.31	33.66	15.66	1375.84	0.94	42.36	28.62	253.32	3.02	T14
None	13	5.57	661.44	19.42	0.31	3.04	11.86	2871.64	0.94	1251.31	19.8	216.86	0.13	T0
G	13	12.04	1297.4	19.57	0.32	48.57	13.47	3866.37	3.77	1431.35	20.76	253.67	0.13	T7
GM	13	15.02	1113.11	17.69	0.31	29.28	12.39	3436.54	2.33	1302.06	18.2	287.09	0.13	T21
P	13	13.54	1233.58	18.01	0.31	37.51	12.39	3949.67	5.24	1255.93	18.84	280.16	0.13	T14
None	9	28.81	112.56	1.73	2.68	11.44	17.88	2186.87	0.94	15.96	24.67	240.21	3.02	T0
G	9	35.29	41.48	1.42	7	14.11	17.88	1080.6	0.94	15.96	22.71	246.66	3.73	T21
GM	9	30.12	150.04	1.42	2.06	11.97	16.77	2155.02	0.94	15.96	21.41	222.19	3.25	T7
P	9	30.12	280.32	1.42	1.45	11.44	17.88	5009.14	0.94	7.25	20.76	177.23	2.31	T14
None	1	44.62	1082.75	62.87	1.36	14.19	34.11	1767.21	23.29	191.06	33.29	.	3.16	T0
None	25	1.17	1177.36	0.65	1.35	1.89	1.11	2820.64	3.23	4.98	8.92	.	0.05	T0
G	25	1.17	1415.13	0.65	1.35	2.35	1.98	2972.62	10.08	4.98	8.92	.	0.05	T21
GM	25	1.17	1448.88	0.65	1.35	3.27	1.98	4040.08	2.8	4.98	12.34	.	0.05	T7
P	25	1.18	1596.31	1.35	1.35	3.73	3.78	3963.64	5.45	4.98	10.05	.	0.05	T14
None	22	77.02	888.42	7.79	1.99	21.41	61.61	5241.38	44.96	485.98	56.51	.	7.18	T0
G	22	69.44	916.66	7.63	1.35	16.9	49.33	6504.75	31.42	383.82	50.78	.	5.37	T21
GM	22	82.63	1219.65	7.32	2.63	25.02	79.9	7155.72	51.84	616.85	68.71	.	8.78	T7
P	22	82.63	1182.62	7.16	1.36	22.77	72.99	6379.97	44.96	518.62	63.23	.	8.55	T14
None	21	49.76	129.79	13.74	1.35	20.06	40.57	2091.13	1.53	213.8	45.74	.	12.26	T0
G	21	73.25	97.48	16.17	8.44	34.92	77.01	4884.17	3.23	346.84	79.76	.	25.2	T21
GM	21	54.8	109.4	16.58	1.36	23.22	51.55	2913.92	0.34	245.75	59.06	.	16.52	T7
P	21	63.65	186.67	17.66	7.14	29.97	67.28	9102.32	4.11	282.4	71.95	.	20.59	T14
None	18	5.57	656.03	1.42	0.31	5.63	41.76	3237.16	0.94	7.24	16.3	259.17	0.13	T0
G	18	5.57	587.21	1.42	0.31	6.68	38.02	3202.9	0.94	7.24	13.17	395.77	0.13	T7
GM	18	11.83	258.18	0.65	1.35	7.39	38.41	2705.6	0.33	56.62	14.08	.	0.06	T21
P	18	3.76	345.59	1.42	0.31	4.59	35.56	2950.02	0.94	7.24	9.5	292.05	0.13	T14
None	28	59.75	230.19	40.85	1.35	5.57	13.39	1039.36	0.33	273.22	49.52	.	0.24	T0
G	28	60.24	381.45	37.67	1.35	5.11	13.39	1135.82	0.33	250.32	46.37	.	0.24	T7
GM	28	63.65	266.66	43.69	1.35	6.93	16.39	1123.23	0.33	273.22	53.96	189.21	0.44	T21
P	28	56.79	282.33	34.6	1.35	5.57	12.39	1913.35	0.33	241.18	42.6	.	0.05	T14

Time point

T0 = at initial presentation

T7 = after 7 days of treatment 1

T14 = after 7 days of placebo

T21 = after 7 days of treatment 2

Treatments

None = at initial presentation

G = gabapentin

GM = gabapentin + meloxicam

P = placebo

ID	Treatment	Time point	CSOM	CBPI pain	CBPI interference	CBPI overall impression (numerical score)	CMPS-SF	
1	None	T0		30	35	1	5	
1	GM	T7	11	26	24	3	5	
1	P	T14	12	16	12	2.5		
1	G	T21						
2	None	T0	10	19	16	2	5	
2	G	T7	4	5	4	4	0	
2	P	T14	3	4	4	5	0	
2	GM	T21	3	4	4		0	
4	None	T0	13	31	34	1	7	
4	GM	T7						
4	P	T14						
4	G	T21						
5	None	T0	11	30	34	1	6	
5	G	T7	9	24	28	2		
5	P	T14	10	23	23	2	4	
5	GM	T21	8	16	25	3	3	
6	None	T0	8	10	12	4		
6	GM	T7	7	22	10	1		
6	P	T14	7	12	4	4	1	
6	G	T21	7	5	4	5		
7	None	T0	7.5	12	12	3	4	
7	G	T7					1	
7	P	T14	6	11	10	4	1	
7	GM	T21	3	5	5	5	1	
8	None	T0	12.5	33	22	1	6	
8	GM	T7	11	18	25	2	2	
8	P	T14	11	31	28	1	6	
8	G	T21	9	22	24	2	3	
9	None	T0	8	15	20	3	8	
9	GM	T7	4	9	9	4	6	
9	P	T14	4	8	9	4	8	
9	G	T21						
10	None	T0	9	18	25	2	3	
10	GM	T7	9	16	20	2		
10	P	T14	8	22	27	2	2	
10	G	T21	5	10	11	3	1	
11	None	T0	11	17	17	2	7	
11	G	T7	8	8	8	3	3	
11	P	T14	7	8	10	3	5	
11	GM	T21	4	6	6	4	3	
13	None	T0	11	15	13	4	1	
13	G	T7	5.5	11	4	5	1	
13	P	T14	8.5	16	16	2	2	
13	GM	T21	4.5	5	5	4	0	
14	None	T0	15	33	34	1	8	
14	G	T7	8	20	24	3		
14	P	T14	7	14	15	3	5	
14	GM	T21	8	24	25	3	4	
15	None	T0	7	10	13	3	5	
15	G	T7	7	10	8	4	3	
15	P	T14	6	7	6	4	3	
15	GM	T21	6	6	6	5	4	
16	None	T0	15			1	8	
16	G	T7	14			2	6	
16	P	T14				1		
16	GM	T21				1		
17	None	T0	10	30	29	1	4	
17	G	T7	13.5	24	29	2	7	
17	P	T14	7	17	17	3	5	
17	GM	T21	14	28	36	1	5	
18	None	T0	13	25	33	1	1	
18	G	T7	3	21	14	3		
18	P	T14	13	33	29	1	4	
18	GM	T21	3	7	6	5		
19	None	T0	10	20	21	3	4	
19	GM	T7	9	24	18	3	3	
19	P	T14	11	29	23	2		
19	G	T21	7	15	9	4	0	
20	None	T0	15	36	34	1	9	
20	GM	T7	10	33			5	
20	P	T14	12	31	21	3		
20	G	T21		31	29	2		
21	None	T0	5	6	7	4		
21	GM	T7		4	28	4		
21	P	T14	3	7	20	4	4	
21	G	T21	5	5	14	4		
22	None	T0	8	15	17	3	5	
22	GM	T7	9	12	11	3	3	
22	P	T14	8	27	29	2	11	
22	G	T21						
23	None	T0	12.5	21	25	2	5	
23	G	T7	4	15	16	3	2	
23	P	T14		8	9	3	4	
23	GM	T21	3	4	4	3	2	
24	None	T0	12	19	24	2		
24	G	T7		9	21	3	2	
24	P	T14		23	27	2		
24	GM	T21						
25	None	T0		19	15	3		
25	GM	T7	9		29	2	5	
25	P	T14	9	18	12	2		
25	G	T21		21	18	2	3	
26	None	T0		27	33	2	6	
26	GM	T7	9	21	34	2		
26	P	T14	9	20	17	2		
26	G	T21		21	29	2		
27	None	T0	6	9	13	2		
27	GM	T7					1	
27	P	T14	8	18	21	2	1	
27	G	T21	7	20		3	3	
28	None	T0		17	10	3	3	
28	G	T7	8	11	8	4	5	
28	P	T14		13	10	2		
28	GM	T21	6	7	9	3	2	
30	None	T0	7	8	9	4	0	
30	G	T7	6	6	6	5	2	
30	P	T14	8	9	5	4		
30	GM	T21	7	9	7	4		
31	None	T0	12	22	25	3	8	
31	GM	T7	12	24	22	3		
31	P	T14	12	28	27	3	7	
31	G	T21	12	25	25	3	7	
32	None	T0	10.5	28	25	2	3	
32	G	T7	11	25	24	3	4	
32	P	T14	9.5	21	27	3	5	
32	GM	T21	8.5	17	18	3		

Time point
T0 = at initial presentation
T7 = after 7 days of treatment 1
T14 = after 7 days of placebo
T21 = after 7 days of treatment 2

Treatments
None = at initial presentation
G = gabapentin
GM = gabapentin + meloxicam
P = placebo