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Original Article

An investigation of mechanical nociceptive thresholds in dogs with hind limb joint pain compared to healthy control dogs

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Abstract

This study investigated the effects of osteoarthritis (OA) on somatosensory processing in dogs using mechanical threshold testing. A pressure algometer was used to measure mechanical thresholds in 27 dogs with presumed hind limb osteoarthritis and 28 healthy dogs. Mechanical thresholds were measured at the stifles, radii and sternum, and were correlated with scores from an owner questionnaire and a clinical checklist, a scoring system that quantified clinical signs of osteoarthritis. The effects of age and bodyweight on mechanical thresholds were also investigated. Multiple regression models indicated that, when bodyweight was taken into account, dogs with presumed osteoarthritis had lower mechanical thresholds at the stifles than control dogs ($P < 0.05$), but not at other sites. Non-parametric correlations showed that clinical checklist scores and questionnaire scores were negatively correlated with mechanical thresholds at the stifles ($P < 0.05$). The results suggest that mechanical threshold testing using a pressure algometer can detect primary, and possibly secondary, hyperalgesia in dogs with presumed osteoarthritis. This suggests that the mechanical threshold testing protocol used in this study might facilitate assessment of somatosensory changes associated with disease progression or response to treatment.

Keywords: Canine; Osteoarthritis; Nociception; Mechanical thresholds; Pain

Introduction

Canine osteoarthritis (OA) is a highly prevalent disease, estimated to affect 20% of dogs over 1 year of age (Johnston, 1997). The key clinical signs of canine OA are loss of mobility and pain (Innes et al., 2010). However, assessing the level of pain, thereby determining disease progression or response to treatment is difficult and subjective.

Mechanical threshold testing might provide a useful measure of pain associated with OA. Mechanical threshold testing is used to identify the point at which an animal responds to an increasing mechanical stimulus; the magnitude of the stimulus at this point represents the animal's nociceptive threshold (Le Bars et al., 2001). Mechanical threshold testing has been used to measure somatosensory changes in human OA patients; multiple studies have found that OA patients have lower mechanical thresholds (MTs) than healthy controls (Suokas et al., 2012). These differences might indicate sensitisation of nociceptive neurones, which can manifest as hyperalgesia (increased pain from a stimulus that normally provokes pain) or allodynia (pain due to a stimulus that does not normally provoke pain). Sensitisation can occur both at the affected site (i.e. an arthritic joint), indicating primary hyperalgesia or allodynia, or at areas remote to the affected site, indicating secondary hyperalgesia or allodynia (Curatolo et al., 2006; Pavlakovic and Petzke, 2010).

Despite the wealth of evidence in human medicine, there are few studies that have investigated the impact of canine OA on MTs. Tomas et al. (2014) found that unilateral total hip replacement, as a treatment for coxofemoral OA, resulted in increased MTs recorded from the affected pelvic limb 12 months after surgery compared to measurements made pre-operatively. Knazovicky et al. (2016) found that mechanical thresholds were negatively correlated with joint pain scores in dogs with coxofemoral and stifle OA.

In the present study, we investigated widespread secondary hyperalgesia (hyperalgesia occurring at anatomical locations completely remote from arthritic limbs) in dogs with osteoarthritis. We compared MTs in dogs exhibiting clinical signs of OA in the hind limbs with MTs in healthy control dogs with no clinical signs of OA using a pressure algometer which we had previously evaluated for this purpose in a pilot study (Harris et al., 2015). Mechanical thresholds were measured around joints with clinical signs of OA, at areas located distally to joints with OA and at areas completely remote from the site of OA, to investigate hyperalgesia associated with canine OA. We hypothesised that dogs showing clinical signs of OA would exhibit lower MTs than healthy control dogs at primary, distal and remote anatomical locations.

Materials and methods

Ethical approval and power calculations

The study was approved by the University of Bristol Ethical Review Group (UIN number UB/12/005, January 2013). We referred to ARRIVE guidelines (Kilkenny et al., 2010) while preparing this manuscript. Sample size calculations indicated, that to detect a clinically relevant difference (2N) in mechanical thresholds between groups (with a 95% confidence interval and 80% statistical power), 27 dogs per group would be required. The value 2N was extrapolated from a clinical study using the same algometer to measure postoperative hyperalgesia in dogs (Hunt et al., 2013).

Selection of study subjects

Twenty-seven dogs with presumed OA in one or more hind limb joints (OA group) and 28 healthy controls (control group) took part in the study. Dogs were recruited either by

e-mail or telephone; an email was sent to University of Bristol staff with a call for participants to volunteer their own dog if the dog 'showed signs of hind limb stiffness', and detailed the demographic inclusion criteria below. Clients at the University of Bristol Small Animal Practice were called if their clinical records indicated signs of hind limb OA; the remainder of the inclusion criteria were confirmed during the telephone conversation. Dogs could only be assigned to the OA or control group once they had arrived at the veterinary hospital and had undergone veterinary clinical examination. Inclusion criteria for the OA group were that dogs exhibited signs of pain on manipulation of at least one hind limb joint during clinical examination. Dogs without signs of orthopaedic disease were eligible for inclusion in the control group. Since radiographs were not obtained in any dogs, we defined OA presumptively, based on clinical signs and history. Therefore, any reference to OA in the text should be taken to mean 'presumed OA'. Demographic inclusion criteria for both groups were that dogs were neutered, > 2 years of age and > 4 kg in body weight. Dogs were excluded from the study if they had undergone orthopaedic surgery, had received analgesic medication within 72 h of the start of testing, or had clinical signs of another unrelated condition likely to cause pain or affect sensory processing. Concomitant non-pharmacological therapies, including dietary supplements such as glucosamine, were not considered to be a reason to exclude a dog from the study. Informed, written owner consent was obtained for all dogs.

Measurement of mechanical threshold

Mechanical thresholds were measured using a handheld pressure algometer (ProD-Plus; Topcat Metrology) with a hemispherical tip (the part of the device in contact with the dog's skin) 2 mm in diameter. The rate of application (the speed at which force was applied) was kept constant (2 N/s) by warning lights that turned on if the rate changed by 0.5 N/s.

Each dog underwent a single session of data collection at the University of Bristol School of Veterinary Sciences, Langford. The session was carried out in a quiet room. On arrival, dogs were weighed and received a clinical examination from a veterinary surgeon. Owners accompanied their dog during the initial clinical examination, but were then asked to leave for the rest of the study. An orthopaedic examination was then completed by the veterinary surgeon who had undertaken the initial examination. Four different veterinary surgeons carried out examinations on different dogs throughout the study.

Severity of osteoarthritis was measured using scores from an owner questionnaire adapted from the Helsinki chronic pain index (HCPI; Hielm-Bjorkman et al., 2009) and a specially designed clinical checklist (see Appendix: Supplementary material), which was completed by the surgeon performing the orthopaedic examination. Higher scores for both of these measures were interpreted as an indication of greater OA severity. A single researcher carried out all mechanical threshold testing. The researcher was present during the orthopaedic examination and therefore was not blinded to whether the dog had OA or was a healthy control dog.

All testing was carried out in the same room, in which dogs were familiarised for 5 min before data collection began. The mechanical threshold testing session was split into six blocks of testing; in each block, five anatomical sites (left and right radii, left and right stifles, and sternum) were tested once (Table 1). In three of the testing blocks, dogs were positioned in a sitting posture; in the other three blocks, dogs were lying laterally recumbent such that the limb to be tested was dorsal. In total, the algometer was applied to each dog 30 times. Dogs were minimally restrained throughout the procedure to allow a range of behavioural

responses. Computer randomisation was used to determine the order in which anatomical sites were tested in each block and the order of 'sitting' and 'lying' blocks.

For each application of the algometer (or 'test'), the tip was positioned in contact with the anatomical site and force was applied by pushing the algometer against the site perpendicularly to the skin surface. Application of force was immediately stopped if the dog exhibited a behavioural response (moving away, vocalising) to the stimulus. The force at which the dog responded appeared on the algometer screen and was recorded as the MT. If a pre-defined maximum cut-out force (13 N) was reached before the dog exhibited a response, the test was terminated to avoid tissue damage. If a MT could not be obtained because the test reached cut-out, or for any other reason (the tip became dislodged, the dogs was avoiding the algometer or the dog moved spontaneously), this was noted. After each test the experimenter moved straight onto the next anatomical site within the same testing block, whether or not a MT was obtained. A rest period between each block allowed at least 15 min between tests at the same site (Dixon et al., 2007). The duration of data collection was approximately 105 min for each dog. Dogs for the OA and control groups were recruited concurrently; therefore, the order of OA and control cases was pseudo-randomised.

Data analysis

All analyses were performed using SPSS version 21 (IBM). The MT data were pooled into three values for each dog (stifle MT, radius MT and sternum MT). These values were the averages of all MTs collected at these sites. This was justified because no statistically significant differences were found between MTs obtained at the left and the right radius, the left and right stifles, or when the dog was sitting or lying down during testing. All dogs with OA had clinical signs indicative of OA in at least one hind limb joint; therefore, the pooled

stifle MT values for each dog either represented primary MTs (for dogs with stifle joint OA) or distal MTs (for dogs with coxofemoral joint OA). The pooled radius and sternum MTs represented remote MTs. The total scores on the checklist and the owner questionnaire were used as measures of OA severity. Mann-Whitney *U* tests were used to compare MTs obtained from dogs with OA to control dogs. Spearman's Rank correlations indicated age and body weight had an effect on MT, therefore multiple regression models were constructed so that bodyweight and age could be taken into account in the statistical analysis.

Three independent variables were used to represent OA: (1) group allocation (i.e. whether the dog was in the OA or the control group); (2) checklist total score; and (3) owner questionnaire total score. The effect of each of these three independent variables was measured separately by adding them to a multiple regression model with either stifle, radius or sternum MT as the dependent variable. Age and weight were included in all nine models.

Results

Demographics and clinical evidence of osteoarthritis

The OA group comprised 27 neutered dogs (17 females, 10 males), with a mean age ± standard deviation (SD) of 9.6 ± 3.0 years, and mean weight ± SD of 27.8 ± 11.4 kg. The control group comprised 28 neutered dogs (16 females, 12 males), with had a mean ± SD age of 7.6 ± 1.7 years and a mean weight ± SD of 24.5 ± 11.4 kg. Dogs did not differ in weight ($P = 0.297$) or sex ($P = 0.660$), but OA dogs were older than control dogs ($P = 0.004$).

Although we aimed to recruit dogs with hind limb OA only, four dogs also expressed a pain response to manipulation of a forelimb joint (Table 2). Twenty of the 27 dogs with OA

exhibited pain on manipulation of the coxofemoral joint and seven exhibited pain on manipulation of the stifle.

Mechanical thresholds

Despite applying the algometer to each dog a total of 30 times, we could not always measure a MT; an average of 26 MT values were obtained per dog, representing a response rate of approximately 88%; 3% of tests reached the cut-out force, 6% were terminated because the dog was avoiding the algometer and 3% were terminated because the tip became dislodged. A Friedman's test indicated that MT did not change over repeated tests (P = 0.401).

Mechanical thresholds in dogs with OA differed only at the stifle joints from control dogs (P = 0.014); MTs are represented by median ± interquartile range (IQR; stifle: OA = 3.8 ± 2.2, control = 5.1 ± 2.1, P = 0.014; radius: OA = 4.5 ± 3.5, control = 5.1 ± 2.0, P = 0.201; sternum: OA = 5.3 ± 3.8, control = 5.9 ± 1.4, P = 0.270).

Dogs with OA had higher checklist total scores (P < 0.001) and questionnaire total scores (P < 0.001) than control dogs. Mechanical thresholds measured at the stifle correlated negatively with both the checklist (ρ = -0.277, P = 0.047) and the questionnaire (ρ = -0.293, P = 0.030); MTs measured at radii or sternum did not correlate with the questionnaire or checklist. Age correlated negatively with stifle MTs and positively with both the checklist and questionnaire scores, which meant that it was difficult isolate the effect of OA on MT from the effect of age (Table 3). These data were further examined using multiple regression.

Multiple regression models

Following square root transformations of the dependent variables (stifle, radius and sternum MTs), the assumptions of linearity, independence of errors, homoscedasticity, unusual points, normality and independence of residuals, and multicollinearity were met for all models. Regression models had effect sizes that ranged from 17 to 25 %; these effect sizes are considered medium according to Cohen's classifications for multiple regression (small = 2%, medium = 15%, large = 35%; Cohen, 1992).

When the effects of age and weight were taken into account, dogs with OA had lower MTs at the stifle joints than healthy controls (model 3). In all other models, there was no effect of OA on MT (Table 4). In model 7, which included checklist total score as an independent variable and sternum MT as the dependent variable, age affected MT. In all other models only weight affected MT.

Discussion

In our study, MTs measured at the stifles were lower in dogs with hind limb OA than healthy controls; this is consistent with findings from numerous studies of human OA (Suokas et al., 2012). Human studies also suggest that patients with OA often exhibit hyperalgesia at locations remote from the arthritic joint. For example, Arendt-Nielsen et al. (2010) found that patients with knee OA exhibited lower MTs at regions on the arm. However, in the current study we were unable to replicate this finding in dogs, as there were no significant differences in MTs at the sternum or radii between the two groups.

Only seven dogs with OA exhibited pain on manipulation of a stifle joint, which suggests that the majority of the dogs with OA were experiencing joint pain primarily in the hip. This suggests that some of the dogs which exhibited lower MTs at the stifle were

demonstrating secondary hyperalgesia at an area other than the affected joint. A similar finding was made by Knazovicky et al. (2016); dogs with OA in the coxofemoral or stifle joint exhibited lower MTs at sites located distally to the affected joints (the tibia and metatarsal joint) compared to healthy controls.

Radiographs were not included in our assessment of OA; we considered sedation an unnecessary risk for geriatric dogs. Instead, we considered the owner questionnaire and the checklist sufficient estimates of hind limb joint pain. The owner questionnaire was adapted from the HCPI designed by Hielm-Bjorkman et al. (2003), which discriminated between dogs with canine hip dysplasia and healthy controls, and showed high internal consistency and test-retest reliability (Hielm-Bjorkman et al., 2009). The checklist was developed through liaison with veterinary orthopaedic specialists. A limitation of this method was that orthopaedic examinations were not all undertaken by the same veterinarian, this meant that there was potential for inter-observer bias in assessment of the severity of OA; particularly for the subjective parts of the checklist, such as lameness scoring (Waxman et al., 2008). A further limitation is that the researcher carrying out the mechanical threshold testing was aware of which experimental group the dog was in, which had the potential to bias the measurements.

Two between-subject factors, bodyweight and age, covaried with MTs and OA severity. We observed similar correlations between MTs and both body weight and age in our pilot study (Harris et al., 2015) and also in previous studies by other researchers (Briley et al., 2014; Moore et al., 2013). Therefore, we used multiple regression to control for the possible confounding effects of body weight and age. Dogs with OA had lower MTs at the stifles than control dogs after accounting for weight and age. These findings suggest primary hyperalgesia at locations on an arthritic joint (in dogs with stifle joint OA) or secondary

hyperalgesia at areas on the same limb (in dogs with coxofemoral OA only). We could not detect a relationship between the checklist, or the questionnaire, and stifle MT after accounting for weight and age. This could be explained by the fact that two of the predictor variables in the model, age and OA severity, were correlated with each other. Since age and OA severity were both negatively correlated with MT at the stifle joint, the independent effects of age and OA severity on MT were difficult to separate, which may have led to neither having a significant influence on MT on their own. Weight, on the other hand, had an effect in all models; weight was not correlated with any other independent variables, so might not have suffered the same confounding effects.

Although the effect sizes of the models with age and weight included could be considered 'medium' according to Cohen (1992), the models only accounted for a maximum of 25% of variation in MT. This suggests that factors not accounted for in the model contributed to a large proportion of the variation in in MT; one source of variation could be between-subject factors. Using dogs from the clinical population, as opposed to a cohort of dogs bred for the purpose of study, may explain the high between-subject variability observed. However, this population reflects the real-world situation more closely than an experimental purpose-bred population of dogs.

The decision to include dogs exhibiting a pain response to manipulation of forelimb joints on examination allowed the sample size to remain above the level found appropriate in our power calculations. The fact that no significant effect of OA on MT was found at the radii in any of the statistical models, suggests that including these dogs did not affect our results.

Conclusions

In summary, the lower MTs measured in dogs with OA compared to healthy controls indicated that mechanical threshold testing was able to detect hyperalgesia at stifle joints with OA, or stifle joints located distally to a coxofemoral joint with OA. The observed effect of between-subject factors on MT indicates that strict case-control matching is important when comparing groups. The study suggests that mechanical threshold testing might provide an additional, objective measure of pain in dogs with OA.

Conflict of interest statement

This study was financially supported by Zoetis, but this organisation did not play any role in the study design, in the collection, analysis and interpretation of data, or in the manuscript writing or submission for publication. None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi: ...

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Table 1
Summary of a typical session.

Order	Block 1 (lying)	Block 2 (sitting)	Block 3 (sitting)	Block 4 (lying)	Block 5 (lying)	Block 6 (sitting)
1	Right radius [a]	Sternum	Left radius	Right radius	Left stifle	Left stifle
2	Right stifle [b]	Right radius	Sternum	Right stifle	Right stifle	Right radius
3	Left stifle	Left radius	Left stifle	Left radius	Sternum	Right stifle
4	Sternum [c]	Left stifle	Right stifle	Sternum	Left radius	Sternum
5	Left radius	Right stifle	Right radius	Left stifle	Right radius	Left radius

Dogs underwent a single session split into six blocks (three with the dog sitting and three with the dog lying down). The order of sitting and lying blocks was randomised for each dog, and the order in which the sites were tested was randomised for each block.

[a] For tests at the radii, the algometer was placed on the cranial aspect of the radius, approximately midway between the elbow and the carpus.

[b] For tests at the stifles, the algometer was placed on the lateral aspect of the stifle joint, on the bony prominence formed by the lateral condyle of the femur.

[c] For tests at the sternum, the algometer was placed on the proximal sternum, at the point where the forelimbs join the torso.

Table 2
Pain response to manipulation of joints in dogs with presumed osteoarthritis (OA) during clinical examination.

	Hind limbs			Pain response to manipulation of forelimbs	No clinical signs of OA in any joints on examination
Dog	Stifle(s) [a]	Hip(s) [a]	Tarsus/hock(s) [a]		
1	1	1	0	0	0
2	1	1	0	0	0
3	0	1	0	0	0
4	1	0	0	0	0
5	1	0	0	0	0
6	1	0	0	0	0
7	1	1	0	0	0
8	1	0	0	0	0
9	0	1	0	0	0
10	0	1	0	1	0
11	0	1	0	1	0
12	0	1	0	0	0
13	0	1	0	0	0
14	0	0	0	0	1 [b]
15	0	0	0	0	1 [b]
16	0	1	0	1	0
17	0	1	0	0	0
18	0	1	0	0	0
19	0	1	0	0	0
20	0	1	0	0	0
21	0	1	0	1	0
22	0	1	0	0	0
23	0	1	0	0	0
24	0	1	0	0	0
25	0	1	0	0	0
26	0	1	0	0	0
27	0	0	1	0	0
28	0	1	0	0	0
29	0	1	0	0	0
Total number of dogs:	7	22	1	4	2

[a] '1' in this column means at least one of these joints, left and/or right, were affected.
[b] Two dogs had no clinical signs of OA on clinical examination, despite owner reported signs (e.g. stiffness after exercise); these dogs were excluded from analyses.

Table 3
Correlations of dependent variables used to assess pain severity in dogs with presumed osteoarthritis.

	Median (IQR)		Correlations [c]			
Dependent variables	Control group	OA group	Checklist total score	Questionnaire total score	Weight	Age
Checklist total score [a]	0 (0)	11.0 (12.0)	-	0.815 **	0.226	0.493 **
Questionnaire total score [b]	3.6 (5.0)	20.6 (11.8)	0.815 **	-	0.111	0.445 **
MT both stifles	5.1 (2.1)	4.0 (2.2)	-0.277 *	-0.293 *	0.331 *	-0.342 *
MT both radii	5.1 (2.0)	4.8 (3.5)	-0.079	-0.218	0.463 **	-0.235
MT sternum	5.9 (1.4)	5.3 (3.7)	-0.088	-0.250	0.330 *	-0.284*

OA, osteoarthritis; IQR, interquartile range; MT, mechanical threshold.
[a] The checklist was a composite scoring system to standardise veterinary assessment of OA. All parts of the checklist could be categorised as follows: (a) mobility, an 11-point lameness score (0-10) adapted from Vasseur and Slatter (1993), and scales rating the dog's ability to stand up and sit down (0-3); (b) JA, number of joint abnormalities observed (e.g. crepitus, effusion); (c) JFS, joint function score (adapted from Impellizeri 2000); and (d) GS, global score of severity of the dog's disease by the clinician (none, mild, moderate, severe; 0-3).
[b] The owner questionnaire was adapted from Hielm Bjorkman (2009). Questions 1-12 ask owners to select one of five verbal responses, which were then ranked 0-4. Question 13 was a visual analogue scale (VAS) with a maximum score of 10. The questions could be categorised as follows: (a) mobility, e.g. 'rate your dog's willingness to walk' (nine questions); (b) vocalisation (one question); and (c) quality of life, e.g. 'rate your dog's mood' (three questions). The main adaptation to the original HCPI was the addition of two extra questions (questions 1 and 13) relating to quality of life.
[c] Untransformed variables were not normally distributed, therefore these correlations are non-parametric (Spearman's rank)
Significance of correlation: * $P < 0.05$; ** $P < 0.01$.

Table 4
Summary of different multiple regression models showing the impact of each independent variable on the dependent variable when other variables in the model are held constant.

Model	Dependent variable	Effect size [a]	Independent variables	B [b]	SE (B) [c]	*B* [d]	*P*
1	MT stifles	16%	Intercept	0.656	0.098		
			Checklist total	-0.004	0.003	-0.216	0.211
			Age	-0.011	0.010	-0.183	0.270
			Weight	0.005	0.002	0.350	0.015 *
2	MT stifles	20%	Intercept	0.721	0.093		
			Questionnaire total	-0.004	0.002	-0.221	0.157
			Age	-0.017	0.010	-0.252	0.091
			Weight	0.004	0.002	0.288	0.025 *
3	MT stifles	24%	Intercept	0.718	0.089		
			Group (OA or Control)	-0.099	0.045	-0.288	0.033 *
			Age	-0.017	0.009	-0.251	0.060
			Weight	0.005	0.002	0.302	0.016 *
4	MT radii	21%	Intercept	0.620	0.096		
			Checklist total	-0.001	0.003	-0.055	0.742
			Age	-0.012	0.010	-0.189	0.238
			Weight	0.006	0.002	0.465	0.001 **
5	MT radii	24%	Intercept	0.624	0.085		
			Questionnaire total	-0.003	0.002	-0.183	0.207
			Age	-0.010	0.009	-0.159	0.269
			Weight	0.006	0.002	0.455	0.000 **
6	MT radii	25%	Intercept	0.631	0.083		
			Group (OA or Control)	-0.056	0.042	-0.174	0.189
			Age	-0.012	0.008	-0.189	0.152
			Weight	0.006	0.002	0.454	0.000 **
7	MT sternum	18%	Intercept	0.799	0.091		
			Checklist total	0.002	0.003	0.119	0.483
			Age	-0.019	0.009	-0.338	0.042 *
			Weight	0.004	0.002	0.341	0.016 *
8	MT sternum	22%	Intercept	0.736	0.081		
			Questionnaire total	-0.004	0.002	-0.276	0.064
			Age	-0.008	0.008	-0.129	0.376
			Weight	0.005	0.002	0.387	0.003 **
9	MT sternum	17%	Intercept	0.765	0.082		
			Group (OA or Control)	-0.028	0.041	-0.092	0.505
			Age	-0.014	0.008	-0.243	0.081
			Weight	0.005	0.002	0.356	0.007 **

OA, osteoarthritis; MT, mechanical threshold.
* $P < 0.05$ level, ** $P < 0.01$.
[a] Effect size is estimated from the adjusted R2 of each model.
[b] B, unstandardised coefficient (how much the dependent variable varies with each independent variable).
[c] SE (B), standard error of B.
[d] B, standardised coefficient.