The Veterinary Journal 204 (2015) 82–87

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Influence of experimental protocol on response rate and repeatability of mechanical threshold testing in dogs

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A R T I C L E I N F O

Article history:
Accepted 5 February 2015

Keywords:
Canine
Degenerative joint disease
Nociception
Mechanical thresholds
Protocol evaluation

A B S T R A C T

Mechanical threshold (MT) testing is widely used to measure nociceptive thresholds. However, there has been little research into factors that contribute to the response rate and repeatability (collectively termed 'efficacy') of MT testing protocols. The aim of this study was to investigate whether the efficacy of a protocol using a hand-held algometer to measure MTs (N) in healthy dogs ($n = 12$) was affected by varying (1) the area over which force was applied (tip diameter), (2) rate of force application, (3) position of dog during testing, and (4) anatomical site of testing. The effect of these factors on MT and the impact of individual dog effects on both efficacy and MT were also investigated.

Overall, 3175/3888 tests (82%) resulted in a measurable response. The response rate was reduced by using wider tip diameters, testing at the tibia, and testing when the dog was lying down (compared to sitting upright). Wider tips were associated with higher, more variable MTs (mean ± standard deviation) with values of 4.18 ± 2.55 N for 2 mm diameter tips, 5.54 ± 3.33 for those of 4 mm, and 7.59 ± 4.73 for 8 mm tips. Individual dog effects had the most significant impact on efficacy and MT. The findings indicate that tip diameter, dog position, and anatomical site may affect both protocol efficacy and MTs, and should be taken into account when comparing different studies and in designing protocols to measure MTs in dogs. The predominant effect of the individual dog over other factors indicates that between-subject differences should always be accounted for in future studies.

Introduction

Mechanical threshold testing (MTT) is a method of non-invasively quantifying nociceptive thresholds in awake animals by measuring the magnitude of an increasing mechanical stimulus at which individuals respond (Le Bars et al., 2001). MTT is widely used in veterinary pain and analgesia research (see, for example, Lascelles et al., 1998; Slingsby et al., 2001; Kongara et al., 2009; Vinuela-Fernandez et al., 2011). However, there has been little research into the effect of protocol on the response rate and repeatability (collectively termed 'efficacy') of MTT. Previous studies have investigated the effect of protocol on mechanical thresholds (MTs); for example, MTs have been found to differ significantly between different anatomical locations in humans (Johansson et al., 1999), horses (Haussler et al., 2007), and dogs (Coleman et al., 2014). Although the feasibility and repeatability of MTT in dogs have been studied (Briley et al., 2014), the direct effect of protocol on the efficacy of MTT has not been investigated.

Degenerative joint disease (DJD) is highly prevalent in the canine population (Johnston, 1997), and is likely to impact on welfare. Associations between DJD and reduced MT are well established in human medicine (Hendiani et al., 2003; Arendt-Nielsen et al., 2010), and are also evident in dogs (Brydges et al., 2012; Tomas et al., 2014). MTT could be used to measure changes in somatosensory processing associated with DJD, and the effect of treatment; for example, Moss et al. (2007) observed increased MTs in human patients with knee osteoarthritis (OA) following joint mobilisation treatment.

The aim of the current study was to evaluate the effects of tip diameter (the part of the MTT device in contact with the skin), rate of force application, position of dog during testing, and anatomical site of testing on three outcomes: (1) the response rate of MTT (the proportion of tests where an MT could be measured), (2) the repeatability of MTT, and (3) MT. The ultimate aim was to develop a protocol for measuring MTs in dogs with DJD.

Materials and methods

Animals

Twelve healthy dogs were studied. They comprised five females (two neutered) and seven males (three neutered) with a mean (range) age and weight of 5.3 (1–13) years, and 20.6 (9–32) kg. Body condition scores (BCS) were 4/9 ($n = 2$), 5/9 ($n = 8$) and 6/9 ($n = 2$). Inclusion criteria were that subjects should not have any illness or injury likely to cause pain or affect normal behavioural responses, or be receiv-

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http://dx.doi.org/10.1016/j.tvjl.2015.02.008

Table 1
Summary of a typical session. Dogs underwent 12 sessions, each with a different combination of tip, rate and position. The order of sessions was randomised for each dog, and the order in which the sites were tested was randomised for each block.

Session 1 (tip = 2 mm, rate = 4 N/s, position = sitting)							
Block 1			Block 2			Block 3	
Test number	Site		Test number	Site		Test number	Site
1	Right radius		10	Right elbow		19	Right tibia
2	Left radius		11	Left radius		20	Right elbow
3	Left stifle		12	Sternum		21	Right radius
4	Left elbow		13	Left tibia		22	Left elbow
5	Sternum	15 min rest	14	Right radius	15 min rest	23	Sternum
6	Right elbow		15	Left stifle		24	Left stifle
7	Right stifle		16	Right tibia		25	Left tibia
8	Left tibia		17	Right stifle		26	Left radius
9	Right tibia		18	Left elbow		27	Right stifle

ing analgesic medication. The criteria were confirmed by consulting the owners, and informed owner consent was obtained for all dogs.

The study was approved by the University of Bristol Ethical Review Group (UIN number UB/12/005 – 17 February 2012).

Equipment

MTs were measured in Newtons, using a handheld pressure algometer (ProD-Plus, Topcat Metrology) with interchangeable, hemispherical tips of 2, 4 or 8 mm diameter. The rate of application was kept constant (2 N/s or 4 N/s) by warning lights that turned on if the device changed by 0.5 N/s above or below the set rate.

Data collection

A single researcher (LKH) carried out all data collection. Before testing, dogs were weighed and assigned a body condition score (Laflamme, 1997). Dogs underwent 12 randomly ordered study sessions (Table 1), one for every combination of protocol factors 'tip', 'rate' and 'position' (sitting or lying) (Table 2). Sessions were divided into three blocks and within each block the algometer was applied once to nine anatomical sites (Table 2) in a randomised order. There was a rest period between blocks to allow at least 15 min between tests at the same site (Dixon et al., 2007). Each session lasted approximately 45–60 min.

All testing was carried out in the same room, in which dogs were familiarised for 5 min before data collection began. Dogs were verbally encouraged to sit or lie down on a fleece mat on the floor. When lying, dogs were positioned in lateral recumbency such that the limb to be tested was dorsal. Dogs were minimally restrained throughout the procedure.

For each application of the algometer (or 'test'), the tip was positioned in contact with the anatomical site selected and force was applied by pushing the algometer against the site at a perpendicular angle to the skin surface (Fig. 1). Application of force was immediately stopped if the dog exhibited a clear behavioural endpoint (a deliberate reaction to the stimulus, such as withdrawing the limb). The force at which the animal responded appeared on the algometer screen and was recorded as the MT. If a pre-defined maximum cut-out force (2 mm = 13 N, 4 mm = 15 N, 8 mm = 20 N) was reached before the dog responded, the test was terminated in order to prevent tissue damage, and 'no response' was recorded. If an MT could not be obtained for any reason other than reaching the cut-out force, this was recorded as an 'unmeasurable outcome' (Table 3).

Depending on availability, most dogs underwent one or two sessions per day, often not consecutively, until all 12 sessions had been completed. Only one dog underwent three sessions in one day. A rest period of at least 1 h was allowed between sessions.

Data analyses

All analyses were performed using SPSS Statistics version 19.

Table 2
Summary of average mechanical thresholds (MTs).

Factor		Average MT – all 12 dogs included (mean ± SD)
Rate	2 N/s	5.8 ± 4.0
	4 N/s	5.8 ± 3.8
Tip	2 mm	4.18 ± 2.55[a]
	4 mm	5.64 ± 3.33[a]
	8 mm	7.59 ± 4.73[a]
Position	Sitting – *upright posture, hind quarters lowered.*	5.7 ± 3.9
	Lying – *lateral recumbency*	5.8 ± 3.9
Site	Right radius – *midpoint along the length of the right radius, dorsal aspect*	6.0 ± 4.1
	Left radius – *midpoint along the length of the left radius, dorsal aspect*	5.7 ± 4.0
	Right elbow – *lateral condyle of the right humerus*	5.7 ± 4.3
	Left elbow – *lateral condyle of the left humerus*	5.7 ± 3.9
	Right tibia – *midpoint along the length of the right tibia, lateral aspect*	5.6 ± 3.8
	Left tibia – *midpoint along the length of the left tibia, lateral aspect*	5.8 ± 3.8
	Right stifle – *lateral condyle of the right femur*	5.6 ± 4.0
	Left stifle – *lateral condyle of the left femur*	5.4 ± 3.7
	Sternum – *proximal sternum, at the point where the forelimbs join the torso.*	6.3 ± 3.8

[a] Tip diameter had a significant effect on MT (larger tips were associated with higher MT) $P < 0.05$.

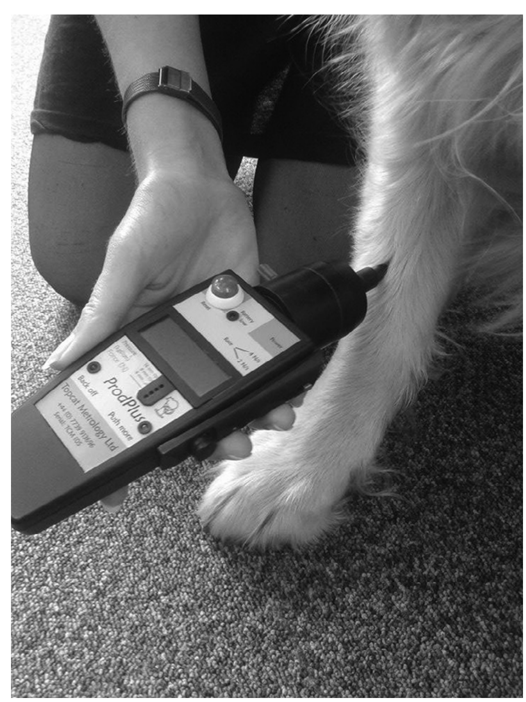

Fig. 1. Example of the algometer being applied to the radius of a dog in sitting position.