



The use of an electronic von Frey device for evaluation of sensory threshold in neurologically normal dogs and those with acute spinal cord injury

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ABSTRACT

The utility and inter-session repeatability of sensory threshold measurements using an electronic von Frey anesthesiometer (VFA) were assessed in a group of six neurologically normal dogs. Sensory threshold values obtained in neurologically normal dogs were compared to those of dogs with acute spinal cord injury (SCI) caused by intervertebral disc extrusion ($n = 6$) and to a group of neurologically normal dogs with cranial cruciate ligament rupture (CCLR; $n = 6$).

Sensory threshold values in neurologically normal dogs were 155.8 ± 37.7 g and 154.7 ± 67.2 g for the left and right pelvic limbs, respectively. The difference in mean sensory threshold values obtained for the group when two distinct testing sessions were compared was not statistically significant ($P > 0.05$). Mean sensory threshold values for the group with SCI were significantly higher than those for neurologically normal dogs at 351.1 ± 116.5 g and 420.3 ± 157.7 g for the left and right pelvic limbs, respectively ($P = 0.01$). A comparison of sensory threshold values for the group with CCLR and neurologically normal dogs was not statistically significant ($P > 0.05$). The modified dorsal technique for VFA described here represents a reliable method to assess sensory threshold in neurologically normal dogs and in those with SCI.

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Introduction

Dogs are increasingly recognized as an important pre-clinical animal model for the study of spinal cord injury (SCI) because of the high prevalence of spontaneous SCI in certain breeds (Olby et al., 2004; Levine et al., 2011). While the mechanism of injury frequently observed in dogs differs from that seen in humans with SCI, there are many similarities between the spinal cord pathology after acute SCI and in mediators of secondary injury (Smith and Jeffery, 2006). For these reasons, dogs serve as an attractive model by which to study clinical interventions that could improve sensory and motor outcome after SCI for canine and human patients alike.

Much attention has been paid to assessing motor recovery in dogs for the purpose of clinical trials, including the development of several locomotor scoring systems (Olby et al., 2001; Levine et al., 2009). However, little focus has been applied to quantitative sensory assessment in dogs. Improvements in sensory outcome are important, as they might result in decreased incidence of pressure sores and self-mutilation in canine patients. This could facilitate the detection of injury and decrease the incidence of allodynia or

paresthesia, thereby resulting in improved quality of life in humans with SCI.

Several methods to assess sensory threshold are currently employed in rodent models of SCI. These include the infrared plantar heat test, von Frey filament testing and more recently, an electronic method for von Frey anesthesiometry (VFA) applied to the dorsal surface of the paw (Hargreaves et al., 1988; Lindsey et al., 2000; Detloff et al., 2010). While all three techniques offer utility in sensory threshold evaluation, electronic VFA allows for more rapid assessment and better reproducibility compared to other methods (Tena et al., 2011), as well as providing an absolute value for sensory threshold, thus aiding in statistical evaluations.

Two previous studies have used electronic VFA to assess sensory threshold in neurologically normal dogs, but neither evaluated the procedure with the goal of quantifying sensory dysfunction (KuKanich et al., 2005a,b). These previous reports describe a technique applied to the thoracic limbs of standing dogs, which might not be feasible in dogs with acute SCI resulting in severe paraparesis or paraplegia. Additionally, most dogs with acute SCI have injuries involving the T3–L3 spinal cord segments, thus sparing the thoracic limbs. The present study was designed (1) to determine the utility and inter-session repeatability of a modified electronic VFA technique to assess sensory threshold in the pelvic limbs of neurologically normal dogs in a non-weight bearing position; (2) to compare results obtained in neurologically normal dogs

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with those obtained in dogs with SCI; and (3) to ascertain whether non-neurological disease which limits joint mobility (i.e. orthopedic disease such as cranial cruciate ligament rupture – CCLR) might confound the behavioral response used to assess sensory threshold using VFA.

Materials and methods

Animals

This study was approved by the IACUC and the Clinical Research Advisory Committee of The Ohio State University Veterinary Medical Center (OSU VMC). Dogs were enrolled in the study only after written consent from the owner was obtained.

Eighteen dogs were enrolled in the study: six were neurologically normal and free of orthopedic and neurological disease, six had acute SCI secondary to intervertebral disc extrusion (IVDE) and six had unilateral pelvic limb lameness due to cranial cruciate ligament rupture (CCLR). Dogs with IVDE had complete neurological examinations performed to document injury severity and were assigned gait scores ranging from 1 to 14 using the Olby spinal cord injury scale – OSCIS (Olby et al., 2001). Dogs with CCLR were confirmed to be neurologically normal prior to enrollment in the study on complete neurologic examination.

von Frey device

The electronic von Frey device (IITC Life Science) consisted of a load cell, handle, recording device and tip (Fig. 1). The system was supplied with a 1000 g probe and a rigid 0.8 mm diameter tip. An internal load cell was attached to the tip and connected to the recording device, allowing the maximum force applied to the limb to be digitally recorded. The system measured, stored and displayed the amount of pressure applied to a subject, from 0.1 to 1000 g.

Modified dorsal von Frey technique

Initially, various surfaces of the hind paws were evaluated for testing in neurologically normal dogs, as well as various positions for the subject. While the main carpal pad served as a stimulus point in the thoracic limbs in dogs previously studied (KuKanich et al., 2005a,b), use of the main tarsal pad was subjectively unreliable as a stimulus point for the pelvic limbs during preliminary testing. It also became apparent that acclimation to the testing environment and the dog's temperament affected the behavioral response observed. The following protocol was used for testing, as it produced subjectively consistent behavioral responses and repeatable sensory threshold readings in neurologically normal dogs.

A quiet room with minimal traffic was chosen for the testing site. A floor mat treated with dog appeasement pheromone (DAP, Ceva Animal Health) was placed in the center of the space. Additionally, a cloth treated with DAP was placed around each animal's collar at the start of the testing session. Dogs were allowed to acclimate to the testing space for 15 min prior to the start of sensory threshold measurements. During this time, they were allowed to roam freely about the space and investigate their surroundings. They were given attention by the investigators (petting, other positive interactions) only if the dog initiated the interaction.

After 15 min, the dog was placed in lateral recumbency on the mat and maintained in that position with the minimum amount of restraint necessary to do so. Dogs were placed in right lateral recumbency for testing of the left pelvic limb

and vice versa. Both pelvic limbs were evaluated and a coin toss determined which side was tested first. Dogs were maintained in lateral recumbency to acclimate for an additional 2 min prior to initiation of testing. The pelvic limbs were allowed to rest on the mat in a neutral position, which varied slightly between dogs. A rigid surface was placed behind the paw for support. The electronic von Frey anesthesiometry (VFA) probe was applied perpendicular to the dorsal surface of the metatarsus, approximately 50% of the distance between tarso-metatarsal and metatarso-phalangeal joints in the cutaneous autonomous zone of the peroneal nerve (Fig. 2). For this study, the probe was positioned on the skin between metatarsal bones IV and V for consistency.

All sensory threshold measurements were made using the VFA probe by the same operator (SM). The evaluator was blinded to the force reading on the VFA device until after a behavioral response was elicited. Steady pressure was exerted via the VFA probe handle and tip as shown in Fig. 1 until the dog made an escape movement (moved the limb away from the probe), vocalized, or the maximum weight of the probe (1000 g) was applied. A simple 'flinch' reflex on first touch of the device tip to the dorsal surface of the paw was not accepted as an endpoint. The maximum force applied by the VFA probe at the time the behavioral response occurred was recorded as the measured sensory threshold. A total of five trials were completed for each limb, allowing 30 s in between measurements. The same protocol was used for the dogs with acute SCI and CCLR.

Inter-session repeatability in neurologically normal dogs

To assess inter-session repeatability of the technique, the testing was repeated on the six neurologically normal dogs 1 week after initial testing. Results from the two sessions were compared.

Statistical analysis

Five threshold values were obtained for each pelvic limb in every dog evaluated. The highest and lowest values were excluded and the three middle values were averaged to assign each limb a sensory threshold. Sensory values are reported as a means \pm standard deviation (SD) for the left and right limbs for the group. For statistical comparison between left and right pelvic limbs and for comparison of neurologically normal dogs to those with SCI and to those with CCLR, a paired *t* test was used. Additionally, within the group of dogs with CCLR, the limb affected with CCLR was compared to the orthopedically normal limb. For assessment of correlation between clinical parameters and sensory threshold, Pearson's correlation coefficient was used. For all statistical analyses, $P \leq 0.05$ was considered significant.

Results

Neurologically normal dogs

The group of neurologically normal dogs consisted of three mixed-breed dogs and one each of the following breeds – Pit-bull,

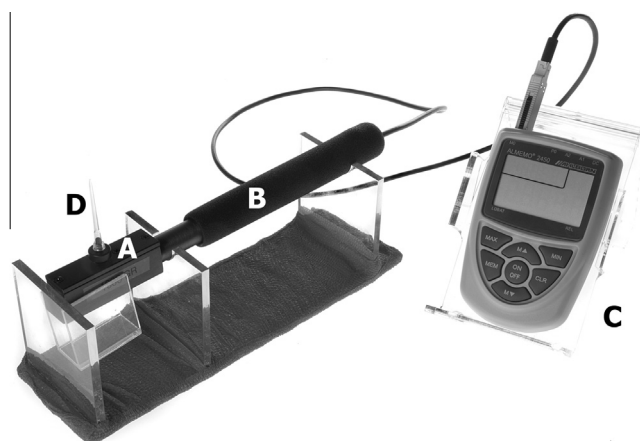


Fig. 1. The electronic von Frey device used in this study. The device consisted of a load cell (A), handle (B), recording device (C) and tip (D). The system measured, stored and displayed the amount of pressure applied to a subject, from 0.1 to 1000 g.



Fig. 2. Dog positioning for the modified dorsal von Frey technique for assessment of sensory threshold in the pelvic limb of dogs. The dog was placed in lateral recumbency and the limb was allowed to rest in a neutral position with a rigid surface placed behind the paw for support. The electronic probe was applied perpendicular to the dorsal surface of the metatarsus, approximately 50% of the distance between tarso-metatarsal and metatarso-phalangeal joints in the cutaneous autonomous zone of the peroneal nerve (inset).

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