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TITLE:

Assessment of Sensory Thresholds in Dogs Using Mechanical and Hot Thermal Quantitative Sensory Testing

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SUMMARY:

This work describes a standard protocol for mechanical and hot thermal quantitative sensory testing to evaluate the somatosensory system in dogs. Sensory thresholds are measured using an electronic von Frey anesthesiometer, pressure algometer, and hot contact thermode.

ABSTRACT:

Quantitative sensory testing (QST) is used to evaluate the function of the somatosensory system in dogs by assessing the response to applied mechanical and thermal stimuli. QST is used to determine normal dogs' sensory thresholds and evaluate alterations in peripheral and central sensory pathways caused by various disease states, including osteoarthritis, spinal cord injury, and cranial cruciate ligament rupture. Mechanical sensory thresholds are measured by electronic von Frey anesthesiometers and pressure algometers. They are determined as the force at which the dog exhibits a response indicating conscious stimulus perception. Hot thermal sensory thresholds are the latency to respond to a fixed or ramped temperature stimulus applied by a contact thermode.

Following a consistent protocol for performing QST and paying attention to details of the testing environment, procedure, and individual study subjects are critical for obtaining accurate QST results for dogs. Protocols for the standardized collection of QST data in dogs have not been described in detail. QST should be performed in a quiet, distraction-free environment that is comfortable for the dog, the QST operator, and the handler. Ensuring that the dog is calm, relaxed, and properly positioned for each measurement helps produce reliable, consistent responses to the stimuli and makes the testing process more manageable. The QST operator and handler should be familiar and comfortable with handling dogs and interpreting dogs' behavioral responses to potentially painful stimuli to determine the endpoint of testing, reduce stress, and maintain safety during the testing process.

INTRODUCTION:

Quantitative sensory testing (QST) assesses the responses elicited by externally applied stimuli; it is used to evaluate the function of the somatosensory system in humans and animals[1]. Mechanical stimuli in the form of punctate pressure or deep pressure are applied as a ramped stimulus. The sensory threshold is determined as the force that evokes a psychophysical response[1]. Hot or cold thermal stimuli can be used as a ramped stimulus or as a fixed intensity stimulus. The sensory threshold is determined as the temperature at which there is a response or the latency to respond to the stimulus. Punctate pressure sensory thresholds are measured using electronic von Frey anesthesiometers or von Frey hair filaments, deep pressure is measured using handheld pressure algometers, and thermal sensory thresholds are determined using a variety of contact thermode systems.

QST provides information about the functioning of both peripheral and central sensory pathways and can be used to evaluate alterations in these sensory pathways (algoplasticity) in various disease processes, particularly those that cause chronic pain[1]. Meissner's corpuscles detect punctate pressure, and the sensation is transmitted by Aβ afferent fibers at non-noxious levels and Aδ afferent fibers when the stimulus is of a noxious intensity[1,2]. Deep pressure is detected by Pacinian corpuscles and transmitted by C afferent fibers, noxious heat is detected by Ruffini corpuscles and transmitted by Aδ and C afferent fibers, and noxious cold is detected by Krause corpuscles and transmitted by C afferent fibers[1,2]. QST can be used to detect both inhibition (decreased sensitivity, hypoesthesia) and facilitation (increased sensitivity, hyperesthesia) of these receptors and pathways. In dogs, QST has been used to evaluate alterations in sensory thresholds secondary to acute spinal cord injury[3,4,5], Chiari-like malformation and syringomyelia[6], cranial cruciate ligament rupture[5,7], and osteoarthritis (OA)[8,9,10]. Additionally, some studies have used QST to assess pain alleviation provided by certain analgesics[6,11,12,13] and surgical procedures[14]. These studies have provided important insights into the mechanisms of pain sensation in dogs, such as evidence for peripheral and central sensitization after surgery and diseases causing chronic pain states such as cranial cruciate ligament rupture and OA. This information can help improve the detection and treatment of pain in dogs.

Validation studies of mechanical and hot thermal QST in dogs have shown good feasibility, repeatability, and reliability of QST results over time in normal dogs and dogs with chronic pain

from OA[8,9,15,16]. However, several studies have found poor repeatability and reliability of cold thermal and occasionally von Frey QST[1,15,17]. These studies used different equipment and methodology but provided evidence that mechanical and hot thermal QST is an accurate, semi-quantitative method of measuring sensory thresholds in dogs. However, attention to precise details, including the setting of the measurements, is critical to optimizing QST in dogs, necessitating a standardized protocol for QST. Sanchis-Mora et al. detailed a sensory threshold examination protocol (STEP) for mechanical and hot and cold thermal QST but encountered difficulty with dogs not responding to the cold thermal QST or the highest gram force von Frey filament used in the study[17]. The following protocol provides a standard method for mechanical and hot thermal QST in dogs; this protocol can assess sensory thresholds in normal dogs or dogs with various disease processes affecting the somatosensory system. The development of standardized protocols may allow for comparing results across studies and meta-analyses of data to improve the utility of QST in veterinary medicine.

PROTOCOL:

All procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

1. Room set-up and study subject acclimatization

1.1. Perform QST in a dedicated space where there is ample room for a QST operator, handler, and dog of any size to move about comfortably. Minimize potential auditory and visual distractions and use a white noise machine to block out ambient sound.

1.2. Place a large yoga mat or similar padding on the floor to ensure that the dogs are comfortable in lateral recumbency during testing.

1.3. Allow the dog at least 10 min to freely explore and acclimate to the room and become comfortable with the QST operator and handler. Offer fresh water *ad libitum* in the room, and give occasional food rewards.

1.4. Randomize the testing site (left or right side) by a coin flip. Clip an approximately 2 x 4 cm section of fur centered around the space between the dorsal surface of the third and fourth metatarsals halfway between the tarsometatarsal joint and the metatarsophalangeal joint. Clip an approximately 1 x 2 cm fur section on the lateral antebrachium just proximal to the antebrachiocarpal joint over the ulna.

2. Electronic von Frey anesthesiometer

2.1. Instrument set-up

2.1.1. Gently apply a rigid 0.9 mm von Frey tip to the load cell and ensure that the load cell is securely screwed into the handpiece. Connect the cord from the handpiece to the recording device through the M0 channel (**Figure 1A,B**).

2.1.2. Turn on the recording device and press the **MAX** button so that the device will record and display the maximum force achieved when the dog responds to the applied stimulus (**Figure 1C**).

2.1.3. Zero the instrument by pressing the **CLR** button.

2.2. Data collection

2.2.1. Place the dog in lateral recumbency for measuring thresholds.

NOTE: Dogs are placed in right lateral recumbency for measuring thresholds on the left limbs or placed in left lateral recumbency for measuring thresholds on the right limbs. If the dog will not willingly lay in lateral recumbency when given verbal cues, the QST operator and handler can manually lay the dog in lateral recumbency.

2.2.2. Apply minimal to moderate restraint as needed to maintain the dog in lateral recumbency and relatively still.

NOTE: The handler performs this step.

2.2.3. Apply the stimulus once the dog is calm and relaxed and the limb being tested is in at least 70% extension. Provide gentle manual support to the limb being tested to keep the limb off the floor and provide stable backing to apply force against while not preventing the dog from withdrawing the limb.

NOTE: The QST operator performs this step.

2.2.4. Apply the von Frey tip perpendicular to the skin of the area being tested. If the dog exhibits reflex movements (e.g., twitching of the paw or withdrawal of the limb before force being applied) from the sensation of the von Frey tip on the skin, allow the dog to relax the limb again before reapplying the von Frey tip. Take a measurement when the skin does not cause reflex movements by applying the von Frey tip.

2.2.5. Apply steadily increasing force with the von Frey tip (~20 g/sec) until the dog withdraws the limb, vocalizes, turns to look at the stimulus, or exhibits other movements or behavioral responses that indicate the conscious perception of the stimulus. Remove the stimulus when the dog withdraws the limb, or the maximum force is reached.

NOTE: Do not exceed 1,000 g of force.

2.2.6. Record the maximum force applied that is displayed on the recording device.

NOTE: If the safety cut-off of 1,000 g of force is reached, 1,000 g is recorded as the sensory threshold, and it is noted that there was no response before the safety cut-off.

2.2.7. Repeat the measurements for a total of five trials, allowing 1 min between each measurement (inter-trial interval). Zero the instrument between each step by pressing the **CLR** button.

2.2.7.1. Allow the dog to remain in lateral recumbency during the inter-trial intervals if they remain relatively calm and relaxed with no or minimal restraint. Otherwise, allow the dog to sit, stand, or move about the QST room to maintain their comfort and replace the dog in lateral recumbency before the subsequent measurement.

2.2.8. Record a feasibility score of 0–5 to indicate the ease with which the data was collected.

NOTE: Feasibility scores are as follows: 0 = no problem, 1 = mild difficulty, 2 = moderate difficulty, 3 = significant difficulty, 4 = extremely difficult, 5 = impossible. The rubric used for assigning feasibility scores is provided in **Table 1**.

2.2.9. Give the dog a 5 min break before starting measurements with the blunt probed pressure algometer.

3. Blunt probed pressure algometer

3.1. Instrument set-up

3.1.1. Ensure that the small blunt probe is securely screwed into the device (**Figure 2A**).

3.1.2. Turn on the recording device and press the **MAX** button to continue when prompted on the screen. Press the **UNIT** button until the unit is displayed as grams (g) at the top of the screen (**Figure 2B**).

3.1.3. Zero the instrument by pressing the **ZERO** button.

3.2. Data collection

3.2.1. Place the dog in lateral recumbency for measuring thresholds.

NOTE: Dogs are placed in right lateral recumbency for measuring thresholds on the left limbs or placed in left lateral recumbency for measuring thresholds on the right limbs. If the dog will not willingly lay in lateral recumbency when given verbal cues, the QST operator and handler can manually lay the dog in lateral recumbency.

3.2.2. Apply minimal to moderate restraint as needed to maintain the dog in lateral recumbency and relatively still.

NOTE: The handler performs this step.

3.2.3. Apply the stimulus once the dog is calm and relaxed and the limb being tested is at approximately 70% extension. Provide gentle manual support to the limb being tested to keep the limb off the floor and provide stable backing to apply force against, while not preventing the dog from withdrawing the limb.

NOTE: The QST operator performs this step.

3.2.4. Apply the blunt probe perpendicular to the skin of the area being tested (**Figure 2C**). If the dog exhibits reflex movements (e.g., twitching of the paw or withdrawal of the limb before force being applied) from the sensation of the blunt probe on the skin, allow the dog to relax the limb again before reapplying the blunt probe. Take a measurement when the application of the blunt probe to the skin does not cause reflex movements.

3.2.5. Apply steadily increasing force with the probe (~20 g/s) until the dog withdraws the limb, vocalizes, turns to look at the stimulus, or exhibits other movements or behavioral responses that indicate the conscious perception of the stimulus. Remove the stimulus when the dog withdraws the limb or the maximum force is reached.

NOTE: Do not exceed 2,500 g of force.

3.2.6. Record the maximum force applied that is displayed on the recording device.

NOTE: If the safety cut-off of 2,500 g of force is reached, 2,500 g is recorded as the sensory threshold, and it is noted that there was no response before the safety cut-off.

3.2.7. Repeat the measurements for a total of five trials, allowing 1 min between each measure (inter-trial interval). Zero the instrument between each step by pressing the **ZERO** button.

3.2.7.1. Allow the dog to remain in lateral recumbency during the inter-trial interval if they remain relatively calm and relaxed with no or minimal restraint. Otherwise, allow the dog to sit, stand, or move about the QST room to maintain their comfort and are replaced in lateral recumbency before the subsequent measurement.

3.2.8. Record a feasibility score of 0–5 to indicate the ease with which the data was collected.

NOTE: Feasibility scores are as follows: 0 = no problem, 1 = mild difficulty, 2 = moderate difficulty, 3 = significant difficulty, 4 = extremely difficult, 5 = impossible.

3.2.9. Give the dog a 5 min break before starting measurements with the hot thermal probe.

4. Hot thermal probe

4.1. Instrument set-up

4.1.1. Connect the thermosensory analyzer to the computer *via* the USB cable and ensure that the 16 x 16 mm thermode is connected to the analyzer. Turn on the analyzer.

4.1.2. Open the thermosensory analyzer software on the computer and select the **TSA II** analyzer from the startup menu. Click on **OK** on the pop-up warning for the analyzer self-test. Ensure that the thermode is not connected to the study subject during the self-test.

4.1.3. In the **TEST** tab (upper right-hand corner), under the **Select Patient** prompt (left-hand side of the screen), select the appropriate patient by double-clicking on the name in the list.

4.1.3.1. To create a new patient, click on the **PATIENTS** tab to the right of the **TEST** tab. Click on the **New Patient** icon in the lower left-hand corner and fill in the patient details (department, patient first and last name, ID, gender, and date of birth).

4.1.4. Under the **Select Program** prompt in the **TEST** tab, select the appropriate program by double-clicking on the program in the list.

4.1.4.1. To create a new program, single-click on the **PROGRAMS** tab to the right of the **PATIENTS** tab. Click on the **New Program** icon in the lower left-hand corner and fill in the program details.

NOTE: For this protocol, the program details are given in **Table 2**. A body site does not need to be selected under the **Select Body Site** prompt in the **TEST** tab.

4.1.5. Once the appropriate patient and program have been selected, click on the **Go to Test** prompt under the **TEST** tab. Single click on the **Pre-Test** button in the lower left-hand corner to calibrate the analyzer to the specified program.

4.1.6. Once the Pre-Test is complete, the **Pre-Test** button is replaced by the **Start** button, and the test window displays: Press Start button to start the test (**Figure 3A**).

4.1.7. Unwind the thermode cable and ensure that the thermode is easily accessible.

4.2. Data collection

4.2.1. Place the dog in lateral recumbency for measuring thermal latency.

NOTE: Dogs are placed in right lateral recumbency for measuring thresholds on the left limbs or placed in left lateral recumbency for measuring thresholds on the right limbs. If the dog will not willingly lay in lateral recumbency when given verbal cues, the QST operator and handler can manually lay the dog in lateral recumbency.

4.2.2. Apply minimal to moderate restraint as needed to maintain the dog in lateral recumbency and relatively still.

NOTE: The handler performs this step.

4.2.3. Apply the stimulus once the dog is calm and relaxed and the limb being tested is in approximately 70% extension. Provide gentle manual support to the limb being tested to keep the limb off the floor while not preventing the dog from withdrawing the limb. Also, hold and operate a stopwatch with the hand supporting the limb.

NOTE: The QST operator performs this step.

4.2.4. Apply the thermode to the skin of the area being tested (**Figure 3B**). If the dog exhibits reflex movements (e.g., twitching of the paw or withdrawal of the limb before heat being applied) from the sensation of the thermode on the skin, allow the dog to relax the limb again before reapplying the thermode. Take a measurement when the application of the thermode to the skin does not cause reflex movements.

4.2.5. Click on the **Start** button in the lower left-hand corner of the **TEST** tab to start the test.

NOTE: The QST operator signals to the handler to start the test (e.g., by nodding their head), and the QST operator simultaneously starts the stopwatch.

4.2.6. Remove the thermode when the dog withdraws the limb, vocalizes, turns to look at the stimulus, or exhibits other movements or behavioral responses that indicate the conscious perception of the stimulus or when the maximum latency is reached while simultaneously stopping the stopwatch.

NOTE: The QST operator performs this step. Do not exceed 20 s of thermode application or 49 °C of maximum thermode temperature.

4.2.7. Record the latency to withdrawal. If the safety cut-off of 20 s of thermode application is reached, record 20 s as the sensory latency, and note that there was no response before the safety cut-off.

4.2.8. Repeat the measurements for a total of five trials, allowing 1 min between each measure (inter-trial interval). Click on the **End Test** button, and then the **Pre-Test** button between each measurement to stop heating the thermode and recalibrate the thermode to prepare for the next application.

NOTE: The handler performs this step.

4.2.8.1. Allow the dog to remain in lateral recumbency during the inter-trial interval if they remain relatively calm and relaxed with no or minimal restraint. Otherwise, allow the dog to sit,

stand, or move about the QST room to maintain their comfort and replace them in lateral recumbency before the subsequent measurement.

4.2.9. Record a feasibility score of 0–5 to indicate the ease with which the data was collected.

NOTE: Feasibility scores are as follows: 0 = no problem, 1 = mild difficulty, 2 = moderate difficulty, 3 = significant difficulty, 4 = extremely difficult, 5 = impossible.

REPRESENTATIVE RESULTS:

Mechanical and thermal QST has been performed to detect sensory thresholds in both research and client-owned dogs under various clinical conditions, including normal, healthy dogs, dogs with chronically painful conditions such as OA, dogs with acute spinal cord injury, and to assess post-operative pain and effectiveness of analgesics. Though there is a growing body of work on QST in dogs, no normal range of values has been established for any testing modalities. However, several studies have assessed the feasibility and repeatability of mechanical and thermal QST in dogs, showing QST data as accurate measurements of sensory thresholds in dogs[8,9,15,16].

The values reported here are from a previously published data set of 23 normal dogs who were older than 2 years of age, weighed greater than 15 kg, had no abnormalities detected on orthopedic and neurologic examination, and had no history of impairment reported by the owner[10]. This group of dogs included 8 mixed breed dogs, 4 Labrador retrievers, 6 golden retrievers, and 1 each of: American Staffordshire terrier, Australian cattle dog, otterhound, Australian shepherd dog, and German shorthaired pointer. Mechanical and hot thermal QST data from these dogs, which represent typical data obtained for QST in dogs, are summarized in **Table 3** and graphically represented in **Figure 4, Figure 5, and Figure 6**. For getting the average QST value for each modality in each dog, the dog's highest and lowest values from the five trials of the QST modality are eliminated, and the remaining three values are averaged. The QST data from each modality was evaluated using repeated-measures mixed-effects models to determine the influence of covariates, including age, sex, body weight, and feasibility score. Then, the association between covariates and the QST threshold was evaluated using Wald tests[10]. This analysis showed no significant effect of age, sex, and feasibility score on the values of any of the QST modalities ($p > 0.05$) and a substantial impact of body weight on the values of hot thermal QST ($p = 0.006$), but neither of the other two modalities. There were not enough dogs of any breed to assess the effect of breed on the QST values.

When interpreting mechanical and thermal QST data, lower pressure thresholds and shorter latency times indicate greater sensitivity to the applied stimulus, while higher pressure thresholds and longer latency times indicate less sensitivity. A variety of clinical conditions have been shown to affect sensory thresholds in dogs. Though there is some inconsistency in the data, most studies report lower sensory thresholds (greater sensitivity, hyperalgesia) in dogs with OA both at the primary site of the joint(s) affected by OA and at secondary sites distant to the affected joint(s)[8,9,10]. All studies that have assessed sensory thresholds in dogs with acute thoracolumbar spinal cord injury report higher sensory thresholds (decreased sensitivity, hypoalgesia) in the pelvic limbs of these dogs[3,4,5]. Studies assessing post-operative pain in dogs

undergoing ovariohysterectomy have indicated lower sensory thresholds at the surgical site and at a distant secondary site in the pelvic limbs (distal tibia) that were alleviated by pre-and post-operative administration of analgesic medications[11,12]. Thus, the population of dogs being assessed and their medical history, including the chronicity of pain and administration of analgesic medications, should be considered when determining expected results and interpreting data.

Feasibility scores are used to indicate the ease with which QST data were obtained from each subject for each testing modality. Feasibility scores are assigned based on a 6-point scale (0–5). They are determined based on the dog's level of cooperation with testing, the amount of restraint needed to accomplish testing, and the clarity of the dog's reaction to the applied stimuli (**Table 1**). Increasing scores on the feasibility scale indicate the increasing difficulty of data collection, with scores of 0–2 considered easy data collection and 3–5 considered difficult data collection. Mechanical and hot thermal QST is generally well-tolerated in dogs. Studies have reported feasibility scores to show that most dogs have feasibility scores indicating easy data collection[8,10,15]. Feasibility scores also indicate the quality of data collected, as dogs who require significant restraint, are not cooperative, are sensitive to their feet being touched, or who have unclear or inconsistent reactions to the applied stimuli decrease the QST operator's confidence that the data collected truly represent the dog's sensory thresholds (versus being an indication of the dog's reaction to these factors).

FIGURE AND TABLE LEGENDS:

Figure 1: Electronic von Frey anesthesiometer. (**A**) Device set-up showing the rigid von Frey tip applied to the load cell and the cord from the handpiece connected to the recording device through the M0 channel. (**B**) Close-up of the von Frey tip attached to the load cell. (**C**) Close-up of the recording device showing the arrangement of buttons and displaying the current force (center), maximum force (upper left), and units (upper right).

Figure 2: Blunt probed pressure algometer. (**A**) Device set-up showing the small blunt probe attached to the recording device. (**B**) Close-up of the recording device showing the arrangement of buttons and displaying the maximum force (center) and units (top). (**C**) Application of the blunt probed pressure algometer to the dorsal metatarsal region of a dog. The tip is applied perpendicular to the skin.

Figure 3: Hot thermosensory analyzer. (**A**) Computer screen display when the analyzer is ready to start a test. The Start button is in the lower-left corner of the screen. (**B**) Application of the thermode to the dorsal metatarsal region of a dog. The QST operator also operates the stopwatch with the hand supporting the limb.

Figure 4: Electronic von Frey anesthesiometer sensory thresholds (g) data by body weight (kg). Bodyweight did not have a significant effect on sensory thresholds ($p = 0.905$).

Figure 5: Data for blunt probed pressure algometer sensory thresholds (g) by body weight (kg).

Bodyweight did not have a significant effect on sensory thresholds (p = 0.734).

Figure 6: Hot thermal probe sensory latency (s) data by body weight (kg). Bodyweight had a significant effect on sensory latency (p = 0.006).

Table 1: QST feasibility scoring rubric. Rubric used for evaluation of the ease with which mechanical and thermal QST data can be collected from dogs. Feasibility scores range from 0 = no problem to 5 = impossible.

Table 2: Program details for the hot thermal probe.

Table 3: Average and range of values of mechanical and hot thermal QST results in 23 normal dogs. The highest and lowest values of the five trials from each modality were excluded, and then the values of the remaining three trials were averaged for each dog. The overall average, standard deviation, and range were calculated from these individual averages. Thresholds for the von Frey and pressure algometer are reported in grams (g), and latency for the hot thermode is reported in seconds (s). All measurements were taken at the dorsal metatarsal region.

DISCUSSION:

It is crucial to the acquisition of accurate data—that reflects the dog's sensory thresholds—that the dog is as calm, relaxed, and positioned adequately as possible for each measurement. A previous study noted that agitation from restraint or distraction from factors within or outside the testing environment affected dogs' responses to the QST stimuli[16]. If the dog becomes agitated from recumbency or restraint or is distracted, the dog should be given time to settle before a measurement is taken; dogs who do not settle quickly should be given a short break from the testing procedure. Dogs who are overly anxious or become stressed from the testing procedure may exhibit stress-induced analgesia, causing a false increase in measured sensory threshold[9]. Anxious or stressed dogs may also become overly reactive to the stimuli or the testing procedure. They may appear to have decreased sensory thresholds. However, this is likely from the dog reacting to the stimulus's presence or the QST operator's actions instead of a noxious sensation from the stimulus. If a dog becomes anxious or stressed, the testing procedure should be terminated. For dogs who rest their limb flexibly or have muscle tension, the QST operator can gently extend the limb and hold it in extension until the dog relaxes, allowing for a more consistent withdrawal response. For dogs who exhibit reflex movements upon contact of the probes or thermode to the skin, the operator can briefly touch or very gently rub the skin of the testing area before contact or apply continuous contact of the probe or thermode without using force or heat to desensitize the skin to touch. Any touch or rubbing should be light and brief to prevent adaptation of the deeper sensory receptors being tested.

The QST operator and handler should be comfortable with handling and restraining dogs and familiar with their behavioral responses to potentially painful stimuli to acquire quality data and maintain the safety of the investigators and study subjects. Ideally, the QST operator and handler should be the same people for the duration of the collection of a data set to maintain consistency in the data. However, the effect of different handlers has not been studied. QST is a

psychophysical method of sensory threshold measurement and requires observation of behavioral responses to determine the endpoint of testing in non-verbal species[1]. In addition to withdrawal of the limb, dogs may exhibit vocalization, turning to look directly at the stimulus, or other movements that indicate the conscious perception of the stimulus[10]. Each dog's behavioral response to the QST stimuli should be observed closely to determine the endpoint for measurements. In the authors' experience, a small proportion of dogs will exhibit extreme reactions to the QST stimuli, including attempting to bite, even when they appeared calm and relaxed before applying the stimulus. The operator and handler should always be aware of the dog's behavior and anxiety level to ensure safe testing. The testing procedure should be terminated if a dog exhibits potentially dangerous behavior.

The testing sites described in this protocol were selected because they are areas where differences in sensory thresholds can be detected for various clinical conditions, including spinal cord injury[3,4,5], cranial cruciate ligament rupture[7], and osteoarthritis[10,16]. In addition, several studies have shown generally good feasibility of QST testing of sites in the distal limbs[8,9,15,16]. Using the same testing sites also allows for a better comparison of results between studies. Though most reports of QST in dogs have the dogs positioned in lateral recumbency, several studies have been conducted with the dogs standing or in other positions dictated by the dog as needed for their clinical condition[3,7,8,9]. Dogs may become stressed from the restraint required to keep them in lateral recumbency, and some dogs refuse to lay in lateral recumbency altogether. These dogs can be allowed to adopt alternative positions, such as sternal recumbency with the hips in lateral recumbency or standing, to reduce stress to the dog and produce consistent responses to the stimuli. Whether or not different positioning affects the response to QST stimuli or the sensory threshold or latency has not been reported.

Performing QST in dogs presents unique challenges that give rise to some limitations in the method. When measuring sensory thresholds in dogs and other non-verbal species, determining the endpoint of testing relies on the operator's observation of a behavioral response that they judge to indicate the conscious perception of the stimulus. In humans, differentiation of the thresholds of the first detection of the stimulus, first sensation of pain, and maximum tolerable pain can be made by verbal report[1]. It is unknown at what intensity of the stimulus that dogs respond and it is likely that different dogs will respond to varying levels of perceived stimulus intensity. Additionally, some dogs may react to the touch or constant contact of the probe rather than the intensity of the stimulus producing a noxious sensation. In humans, cognitive factors including attention, motivation, and cognitive impairment have been found to affect QST thresholds[18,] and similar factors may likely affect results in dogs. The characteristics of the study population should be considered when interpreting QST results and determining factors that may alter those results.

Several studies have reported good feasibility and repeatability using various equipment and methodology for mechanical and hot thermal QST in dogs[8,9,15,16,17]. Results in these studies suggest that pressure algometers may produce the most consistent results of the QST modalities. Most recent studies have used an electronic von Frey anesthesiometer that measures over a continuous range of force, making more accurate and precise measurements than the graduated

measurements of von Frey filaments. However, no direct comparison of the two methods has been performed in dogs[1]. A variety of equipment has been used to assess sensory heat thresholds in dogs. Equipment utilizing constant intensity or ramped heat stimuli, handheld thermodes, and an apparatus in which dogs stood on a glass plate heated by a light source have shown good feasibility and repeatability. However, each method has its limitations[8,9,16]. Some studies have found prolonged latency to respond to cold thermal stimuli in normal dogs[1,15,17], who often reach the safety cut-off time without responding, and report more significant variance and lower feasibility of cold thermal QST, all findings that mirror those in human studies[19]. These factors may limit the usefulness of the cold thermal modality in QST testing in dogs. Therefore, a protocol for cold thermal testing was not detailed here.

Though many QST studies have been performed in dogs to establish the validity of the method and compare the sensory thresholds of normal dogs and dogs with various disease states, no study to date has aimed to establish a normative data range of QST values for dogs. Most studies have had small sample sizes of normal dogs, making it difficult to determine whether characteristics of the dogs—such as body weight, age, sex, or breed—have a significant effect on sensory thresholds. Additionally, the methodology has significantly varied, making it difficult to compare and contrast different studies and has made it impossible to combine data. Large-scale studies of diverse populations of normal dogs are warranted to establish normative ranges of QST values and to elucidate better what factors affect sensory thresholds in normal dogs. Such studies should be performed using standardized, well-described, repeatable protocols for data collection. Establishing these baseline data will help better understand how sensory thresholds are affected by different disease states in dogs.

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The authors would like to thank Andrea Thomson, Jon Hash, Hope Woods, and Autumn Anthony for handling dogs for QST, Masataka Enomoto for his help screening dogs, and Sam Chiu for his contributions to establishing the protocol for hot thermal QST.

DISCLOSURES:
The authors have no conflicts of interest to disclose.

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Figure 1. Electronic von Frey anesthesiometer

Click here to access/download;Figure;Fig. 1 Electronic von Frey Anesthesiometer.pdf

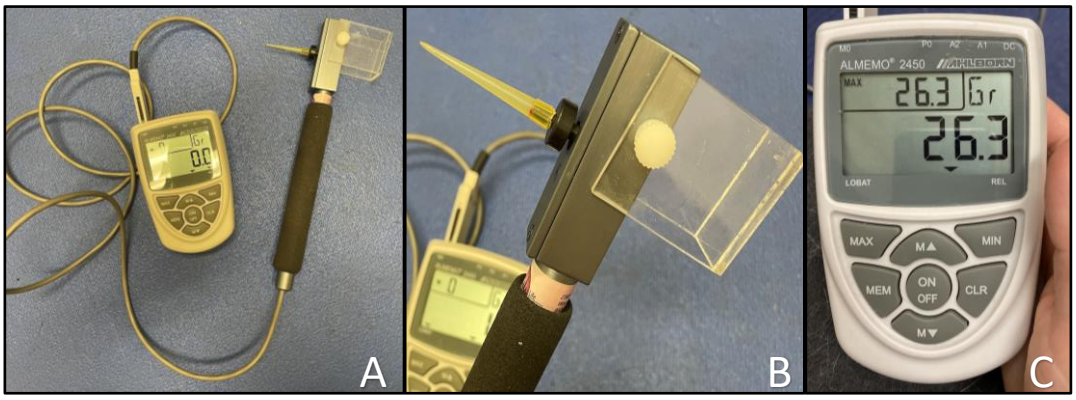


Figure 2. Blunt Probed Pressure Algometer

Click here to access/download;Figure;Fig. 2 Blunt Probed Pressure Algometer.pdf

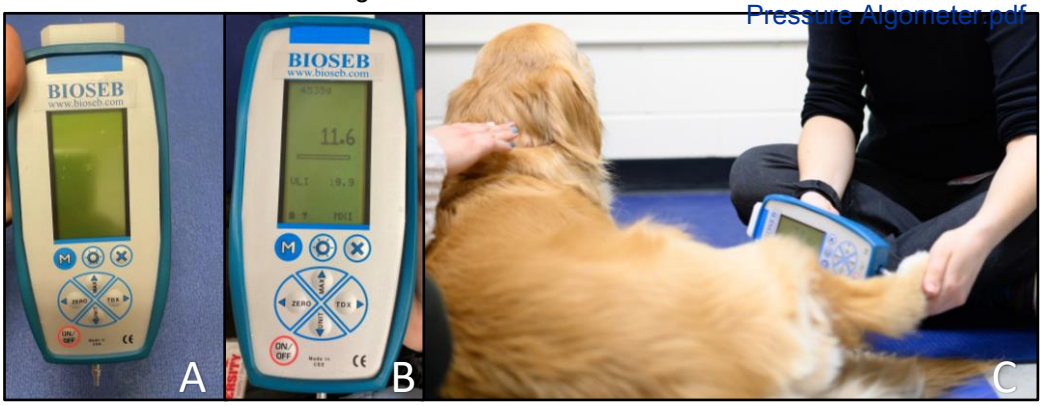


Figure 3. Thermosensory Analyzer

Click here to access/download;Figure;Fig. 3 Thermosensory Analyzer.pdf

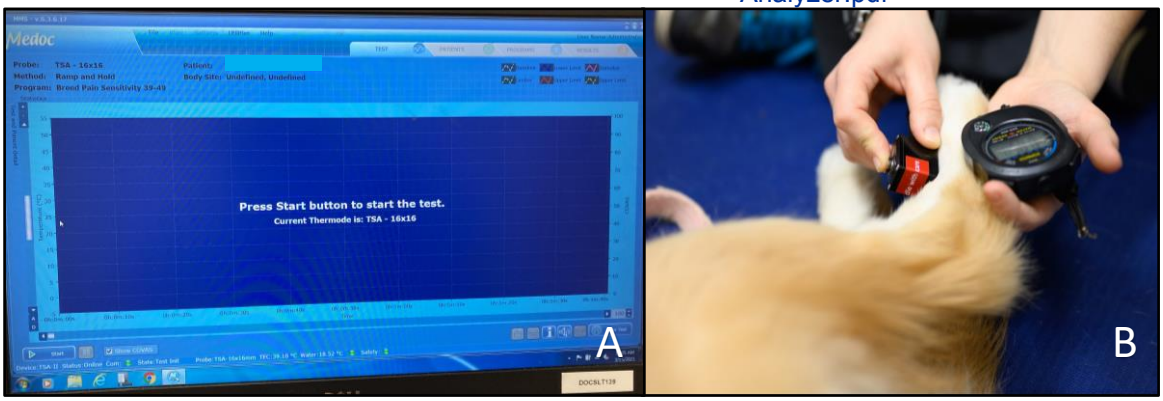


Figure 4. von Frey Thresholds

Click here to access/download;Figure;Fig. 4 von Frey Thresholds.pdf

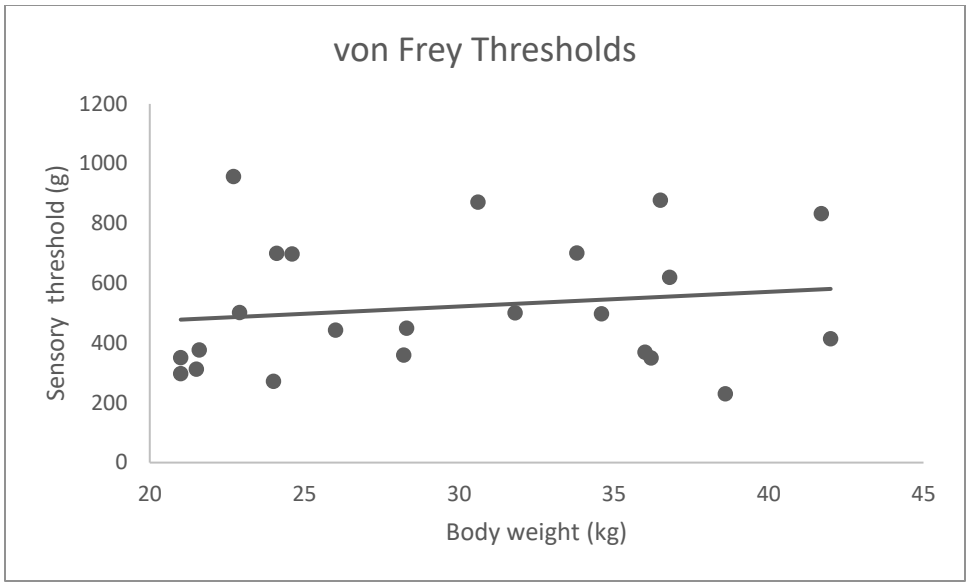


Figure 5. Pressure Algometer Thresholds

Click here to access/download;Figure;Fig. 5 Pressure Algometer Thresholds.pdf

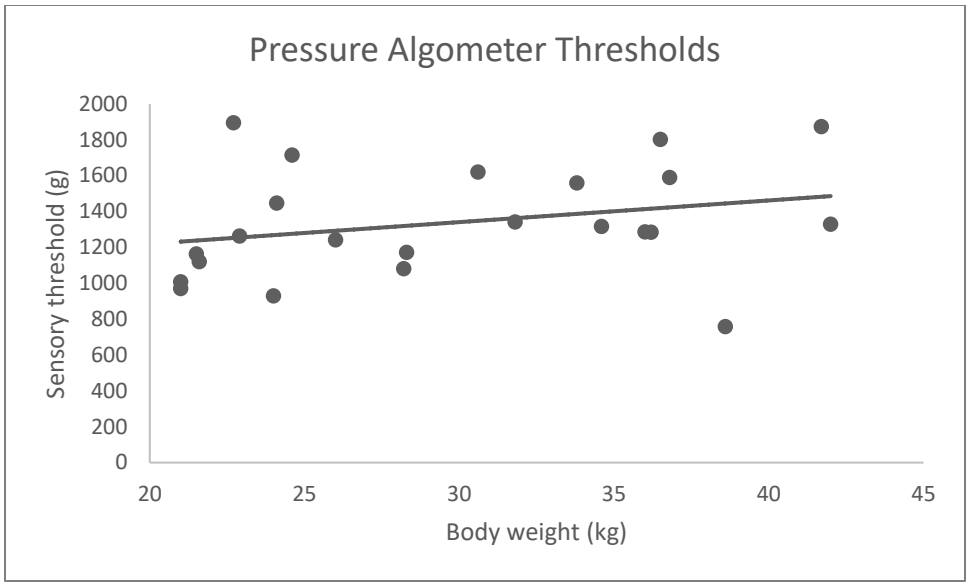
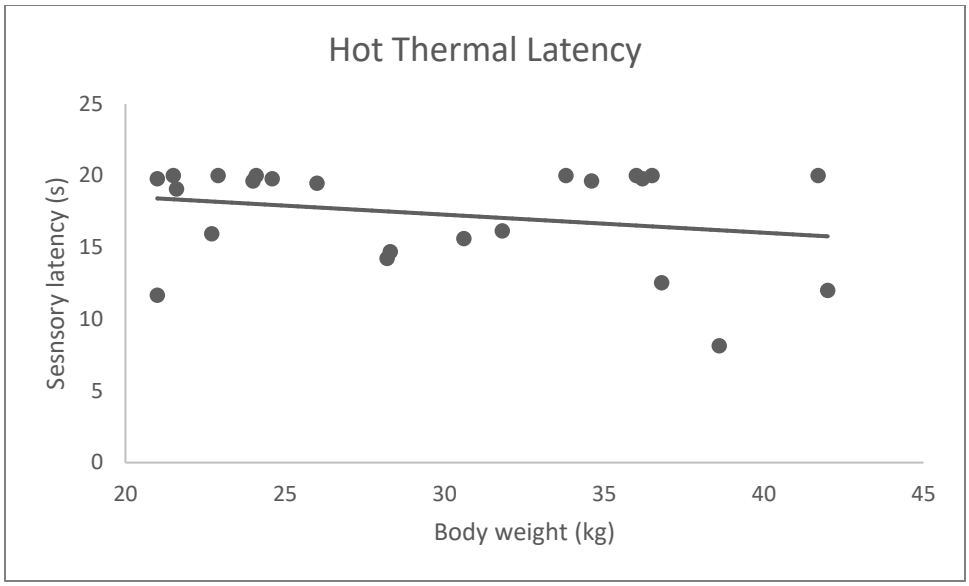


Figure 6. Hot Thermal Latency

Click here to access/download;Figure;Fig. 6 Hot Thermal Latency.pdf



Feasibility score

0 - No problem

1 - Mild difficulty

2 - Moderate difficulty

3 - Significant difficulty

4 - Extreme difficulty

5 - Impossible

Description

Minimum restraint needed; excellent cooperation; clear reaction to stimuli
Mild restraint needed; good cooperation; clear reaction to stimuli
Moderate restraint needed; good cooperation > 50% of the time;
mild sensitivity of feet being touched; mild variation in reaction to stimuli
Significant restraint needed and resisted lateral recumbency; good
cooperation < 25% of the time; moderate sensitivity to feet being touched; moderate variation in reaction to stimuli
Constant restraint required; not cooperative; unclear reaction to stimuli,
not confident in data collected
Could not collect data due to the dog's disposition and/or lack of
confidence in the reactions seen being due to the stimulus

Table 2

Parameters	Input
Method	Ramp and hold
Sequence	1
Baseline	39
Time Before Sequence (s)	0
Trigger	Auto
Destination Temperature (°C)	49
Deatinaytion Rate	8
Destination criterion	Temperature
Duration time (s)	30
Return option	Baseline
Return Rate	1
Number of Trials	1

Table 3

	Average ± SD	**Range**
Electronic von Frey (g)	521.1 ± 216.8	230.2 – 957.1
Pressure algometer (g)	1338.0 ± 308.6	758.9 – 1894.0
Hot thermal probe (s)	17.31 ± 3.55	8.13 – 20

Editorial comments:

62841_R1

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

>Reply: All remaining spelling and grammar issues have been corrected. All abbreviations are defined at first use.

2. Please provide an email address for each author.

>Reply: Email addresses have been added for each author.

3. Please revise the text, especially in the protocol, to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

>Reply: The text does not contain any personal pronouns.

4. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

>Reply: The protocol section has been edited so that all text is in the imperative tense and all instances of “could be”, “should be”, and “would be” have been replaced.

5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.
For example, Medoc TSA II NeuroSensory Analyzer etc

>Reply: All commercial language has been removed from the manuscript and replaced with generic terms.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

>Reply: The protocol contains all steps that are to be included in the video and is described in sufficient detail to allow replication of the protocol.

7. After including a one line space between each protocol step, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.

>Reply: All protocol text that should be included in the protocol section of the video has been highlighted.

8. Please add limitations of this method to your discussion.
>Reply: Limitations of the method are included in the discussion in lines 461-473.

9. Please sort the Materials Table alphabetically by the name of the material.
>Reply: The Materials Table has been sorted alphabetically by the name of the equipment.

Reviewers' comments:
Reviewer #1:

Line 61 - The term "sensory pathways" is quite generalized and non-descriptive related to the testing methods used. Specific mention of nociceptive, pressure and thermal receptors and pathways is needed.
>Reply: Thank you for pointing out the need for more detail here. A description of the receptors and afferent nerve fibers for the sensations tested in this protocol has been added (lines 66-71).

Line 64 - The described "loss or gain of function" is not accurate. Better descriptors are inhibition and facilitation of the nociceptive receptors and pathways.
>Reply: Thank you, this has been corrected.

Line 69 - One would argue that peripheral sensitization is also included here.
>Reply: Thank you, this has been added as suggested.

Line 74 - Not all studies have shown good feasibility and repeatability, especially as it relates to breed differences. These shortcomings need to also be included here.
>Reply: Thank you for pointing this out. Discussion of poor feasibility and repeatability of cold thermal QST and occasionally von Frey has been added to the introduction (lines 85-86) and is also included in the discussion (lines 485-490). The authors were unable to find any studies correlating poor feasibility and repeatability of QST to breed.

Line 102 - Need to add justification on why these two testing sites were selected and are representative locations for assessing nociception, especially as it relates to axial skeleton pain and the mentioned spinal cord injuries, crucial disease and osteoarthritis.
>Reply: Thank you, rationale for testing site selection has been added to the discussion (lines 447-452).

Line 105 - Missing manufacturer information and figures of testing device and application. Unclear why this section is highlighted?
>Reply: Thank you, figures of the testing device have been added (Figure 1). The authors are currently in the process of arranging to obtain professional photos of the equipment and application to include in the manuscript if it is accepted to move to the next stage of revisions. As per the Instructions for Authors, commercial language including manufacturer information cannot be included in the body of JoVE manuscripts. This information is cited in the Table of Materials. This section is highlighted to indicate the

portion of the protocol text to be included in the protocol section of the video, as per the Instructions for Authors.

Line 107 - Need to include the stiffness and diameter of the von Frey fiber used.
>Reply: Thank you, the diameter of the von Frey tip has been added. Unlike the von Frey filaments, the rigid tips for the electronic von Frey do not bend or deform, so the stiffness of the tip is irrelevant for this device.

Line 125 - Unclear what is meant by "stable backing". Seems to imply manual support of the limb.
>Reply: Thank you for pointing out that this is unclear. This does imply manual support of the limb, so the phrase "manual support" was added to provide clarity.

Line 128 - Unclear what "twitching" refers to. Is this a mild withdrawal reflex?
>Reply: Thank you for pointing out that this is unclear. Some dogs will twitch their paw without withdrawing it when the probe makes contact with their skin (which often dislodges the probe) while some dogs exhibit withdrawal of the limb just from the sensation of the probe making contact with their skin. These are two distinct actions and this has been clarified in the text.

Line 132 - Does the device display the rate of force application?
>Reply: Neither the electronic von Frey nor the pressure algometer display the rate of force application in g/sec. Both devices display the current force in g, so the QST handler must determine the approximate rate of force application by watching the change in the force displayed.

Line 142 - Need a better descriptor of feasibility and the factors that contribute to this score. Is this the clear endpoint to the applied stimulus or is it the ability for the dog to lay quietly in lateral recumbency for 10 continuous minutes, which seems unrealistic, especially for purpose-bred research dogs.
>Reply: Thank you for pointing out that this is unclear. The rubric for assigning feasibility scores (previously Table 2, now Table 1) is now referenced at this point in the protocol (line 168) to direct readers to the descriptions of the different feasibility scores and the factors that determine these scores. Feasibility scores are inherently subjective, as all of the factors that determine them are up for interpretation by the QST handler. The rubric provided was used for the authors' current work that this protocol is based on and this rubric has been used in several previous publications. Feasibility scores and the factors that determine them are discussed in greater detail in the last paragraph in the Representative Results section. Dogs are not required to remain in lateral recumbency for the entire testing procedure for each modality (now mentioned in step 2.2.7.1 of the protocol in lines 160-164) and whether or not they remain in lateral recumbency does not change the feasibility score unless it changes their cooperation with the procedure or the amount of restraint needed for each measurement. In the authors' experience, a considerable amount of the dogs actually do remain in lateral recumbency for the duration of one or more of the testing modalities, though these dogs and most of the dogs that have participated in published QST research are client owned pets. Indeed, purpose-bred research dogs may not behave the same.

Line 145 - Missing manufacturer information and figures of testing device and application.
>Reply: Thank you, figures of the testing device and application have been added (Figure 2). The authors are currently in the process of arranging to obtain professional photos of the equipment and application to include in the manuscript if it is accepted to move to the next stage of revisions. As per the Instructions for Authors, commercial language including manufacturer information cannot be included in the body of JoVE manuscripts. This information is cited in the Table of Materials.

Line 152 - I do not see the need to restate methods that have been reported in the above section. Only report the differences.
>Reply: Thank you for your input. Since JoVE is a methodology journal and the purpose of the articles are to help other scientists be able to properly use the equipment and techniques in a step-by-step protocol, the authors have decided to include each step of each modality, including those that are common to all modalities. The authors will defer to the editor for the final decision of whether or not restated methods should be included and would be happy to take them out if the editor deems them unnecessary.

Line 186 - Missing software manufacturer information and figures of testing device and application.
>Reply: Thank you, figures of the testing device and application have been added (Figure 3). The authors are currently in the process of arranging to obtain professional photos of the equipment and application to include in the manuscript if it is accepted to move to the next stage of revisions. As per the Instructions for Authors, commercial language including software manufacturer information cannot be included in the body of JoVE manuscripts. This information is cited in the Table of Materials.

Line 223 - If the thermode is a "one size fits all" for dogs, then why the mention of ensuring thermode selection based on subject sizes?
>Reply: Thank you for pointing this out. The discussion of thermode selection was included to provide the rationale for the use of the 16 x 16 mm thermode as opposed to the 30 x 30 mm thermode. Since the 30 x 30 mm thermode is not a reasonable option for the testing sites described even for large dogs, the authors see that this comment is confusing and does not contribute meaningfully to the protocol, so it was deleted. Thermode size selection has been added to step 4.1.1, as this is a more appropriate place in the protocol.

Line 271 - Need to include description of the different breeds.
>Reply: Thank you, this has been added as suggested.

Line 286 - Need to define where these distant secondary sites are located - in the pelvic limbs or axial skeleton?
>Reply: Thank you, this has been added as suggested and the wording in this sentence has been reordered for clarity.

Liner 288 - Under medical history need to include chronicity of the pain and concurrent medications or analgesics.
>Reply: Thank you, this has been added as suggested.

Line 328 - I would argue that anxiety can also cause a false decrease in nociceptive thresholds.
>Reply: Thank you for pointing this out. A false decrease in nociceptive thresholds due to stress has not been well documented like stress induced analgesia has been. The authors have seen some anxious dogs exhibit stress induced analgesia in which the dogs did not respond to any of the stimuli before the safety cut-off values were reached. The authors have also seen some anxious dogs become overly reactive. In these cases, the dogs become more reactive to the presence of the stimulus or to the testing procedure (this has been added to the text, lines 420-423). This usually has an impact on the feasibility score and decreases the QST operator's confidence in the data collected, as it is usually clear that any apparent decrease in nociceptive threshold is due to the dog reacting to something other than noxious sensation from the stimulus.

Line 332 - Rubbing the testing area would seem to alter the nociceptive thresholds as light touch or massage can affect these results.
>Reply: Thank you for pointing this out. Any touching or rubbing of the skin of the testing site prior to contact with the probe is intended to make the dog less reactive to the probe simply making contact with the skin. Any touch or rubbing should be light enough and brief enough that adaptation of the sensory receptors of interest does not occur (this has been clarified in the text, lines 429-431).

Line 343 - Need to include these behavioral responses in the application protocols as withdrawal reflexes are not the only parameter used to assess nociceptive thresholds.
>Reply: Thank you, this has been added where applicable in the protocol section (lines 149, 199, and 291).

Figure 1 - Remove the gridlines and add a linear regression line to the figure. It appears that larger sized dogs have higher nociceptive thresholds. Need to add this to the discussion. Also need to add 'nociceptive' in the vertical axis and 'body' to the horizontal axis labels.
>Reply: Thank you, the figure has been reformatted. The word 'sensory' has been used in the vertical axis label because it is uncertain if thresholds in dogs represent nociception, therefore, 'sensory thresholds' is a more accurate description. A brief summary of the relevant statistical analysis detailed in the original article the data set is from has been added to the representative results section. This is now Figure 4.

Figure 2 - Same comments as Figure 1.
>Reply: Thank you, the figure has been reformatted. This is now Figure 5.

Figure 3 - Same comments as Figure 1.
>Reply: Thank you, the figure has been reformatted. This is now Figure 6.

Table 1 - Needs significant reformatting. Remove column labels. Is the data normally distributed? If not, report nonparametric parameters. It seems like the coefficient of variation is quite high. Remove non-clinically relevant significant digits (3.548) and make them all consistent (521 ± 216).
>Reply: Thank you, the column labels have been removed. The significant digits were not changed because the digits included in the table are formatted to correspond to the digits displayed on the respective recording devices and follow the conventions of how

these numbers are most commonly reported in prospective QST studies. Formatting the numbers this way will give the reader a better sense of the numbers they will see on the recording devices while performing the protocol. This is now Table 2.

Table 2 - Reformate so that text fits on a single page. Remove column 1-2 labels.
>Reply: Thank you, the table has been reformatted as suggested. This is now Table 1.

Reviewer #2:

Line 41 - "distraction free" should be replaced with distraction-free
>Reply: Thank you, this has been corrected.

Line 46 - "end point" should be replaced with endpoint
>Reply: Thank you, this has been corrected.

Line 189 - "start up menu" should be replaced with startup menu
>Reply: Thank you, this has been corrected.

Lines 192 and 199 - "double clicking" should be replaced with double-clicking
>Reply: Thank you, this has been corrected.

Line 328 - "stress induced" should be replaced with stress-induced
>Reply: Thank you, this has been corrected.

Lines 342, 345, and 362 - "end point" should be replaced with endpoint
>Reply: Thank you, this has been corrected.

Line 398 - "effect" should be replaced with affect
>Reply: Thank you, this has been corrected.

References: (the suggested change is mentioned)
Line 422 (ref. no. 4) - ...197 (2), 216-219 (2013).
Line 425 (ref. no. 5) - ...193 (2), 545-550 (2012).
Line 436 (ref. no. 9) - ...73 (3), 461-471 (1997).
Line 439 (ref. no. 10) - ...69 (2), 147-152 (2000).
Line 441 (ref. no. 11) - "bupivicaine" should be replaced with bupivacaine
Line 442 (ref. no. 11) - ... 25 (1), 59-69 (1996).
Line 446 (ref. no. 12) - ... 43 (5), 542-548 (2014).
Line 449 (ref. no. 13) - ... 199 (2), 245-250 (2014).
Line 452 (ref. no. 14) - ... 44 (3), 615-624 (2017).
Line 454 (ref. no. 15) - ... 154 (9), 1807-1819 (2013).
Line 456 (ref. no. 16) - ... 51 (3), 535-543 (2012).
>Reply: Thank you for completing this bibliographic information, these references have been revised as suggested.

Reviewer #3:

Major Concerns:
I understand that Jove do not republish data or results without the express permission of the original publisher, however the figures /tables presented are not interpreted or discussed fully in the results and discussion sections e.g. what is the % feasibility scores for the 23 dogs, correlate feasibility score to baseline QST sensory thresholds, correlate weight, age, sex, or breed of dog to QST sensory thresholds. Is there an effect of age, gender, breed on QST? Was there a difference in QST sensory thresholds between trials? No correlation statistics has been performed (e.g. Spearman's rank) for figures 1-3. Which of the 3 tests is most reliable, accurate and less variable- interpret the descriptive statistics e.g. SD? Were additional dog's behavioural responses to the QST stimuli observed and closely monitored? E.g. pet owner scores?
>Reply: Thank you for pointing this out. A brief summary of the relevant statistical analysis detailed in the original article the data set is from has been added to the representative results section. Since JoVE is primarily a methodology journal and the purpose of the results section is to give the reader a general sense of representative data obtained from the protocol and in the interest of not simply reprinting statistical analysis that has already been published, the authors will defer to the editor to determine if more in-depth statistical analysis and discussion is appropriate for this type of article. The % feasibility score is not typically reported in QST studies. The most reliable modality is included in the discussion (line 478-479). The sensory thresholds and feasibility scores were the only data collected for each modality. The dogs' behavioral responses to the QST stimuli help determine the endpoint of testing and factor into the feasibility score. Owners were not present during testing for the data set included.

Minor Concerns:
Methods: the time interval between the 3 different QST sensory threshold tests is not described. I read that each dog is exposed to each of the 3 tests for one test period consisting of 5 trials 1-minute apart.
>Reply: Thank you for pointing this out, the interval between the testing modalities has been added.

Line 67: The introduction needs to cite the use of QST to assess sensory thresholds to measure neuropathic pain in dogs. e.g. authors need to consider Sanchis-Mora's work in Chiari-like malformation and syringomyelia (CM/SM) associated with Neuropathic pain in dogs. Further this group have developed an initial validation of a sensory threshold examination protocol (STEP) for phenotyping canine pain syndromes. DOI: 10.1016/j.vaa.2016.09.004
>Reply: Thank you for directing the authors' attention to these studies. They are now discussed and cited in the introduction and discussion.