Awareness of chronic pain in animals has increased because the perception of companion animals has changed as they are considered family members. Therefore, the behavioral and physiological consequences of chronic pain on the dog can be considered a concern for the owner because it can affect the level of well-being and, if not addressed, can lead to the phenomena of central and peripheral sensitization.\(^1,2\) For this reason, phenotyping possible functional changes in the peripheral and central nervous system for tailoring appropriate therapy and monitoring the success of therapeutic approaches is important, but still challenging.\(^3\)

In human medicine, quantitative sensory testing (QST) is used to detect loss of sensation and changes in somatosensory function like allodynia or hyperalgesia as an expression of peripheral or central sensitization.\(^4,5\) The so-called “QST batterie,” a combination of different, validated tests, can be performed within 1 hour in a human patient.\(^6\) It combines 7 different quantitative sensory tests to measure thermal detection threshold (cold, warm, and paradoxical heat sensation), thermal pain threshold (cold and heat pain), mechanical detection threshold (touch and vibration), and mechanical pain thresholds for pinprick or blunt pressure. Furthermore, the summation...
of pain in response to repeated pinprick stimulation, as well as a stimulus–response function for pinprick sensitivity and dynamic mechanical allodynia can be tested with this battery of test protocols.\(^6\)

Creating a comparable QST battery for companion animals could be a goal worth pursuing. Several QST protocols testing mechanical and heat thresholds have already been described,\(^7\)–\(^12\) but only a few studies are using cold stimulation in dogs so far.\(^13\)–\(^14\)

It is known from studies in other species,\(^15\)–\(^16\) that increases in cold sensitivity or cold pain threshold are associated with nerve injury, which underlines the potential role of cold stimulation during QST for diagnosing neuropathic pain. In rats, cold hyperalgesia could be generated experimentally by constriction of the sciatic nerve\(^15\) and it has been reported in human patients with neuropathic pain states like fibromyalgia or whiplash injury.\(^16\)–\(^17\) These patients experience a cold stimulus as painful at a higher temperature than healthy individuals, making the detection of somatosensory changes quantifiable.

Different techniques of cold stimulation have been used in humans, most commonly latency measurements with a constant low temperature using a thermal stimulation device or ice\(^18\) or automated testing with ramped-down cold stimuli. Here, the starting temperature of the thermal stimulation probe is close to normal skin temperature (eg, 32 °C) and decreases with a constant rate, until the patient reacts or a minimal cut-off temperature is reached.\(^6\)

In dogs, cold stimulation has not been widely used. Studies using a latency measurement protocol reported inconsistent results with an overall low response rate in healthy dogs: One study using a constant temperature of 0 °C reported a response rate between 13% and 19% of dogs, depending on the tested location,\(^15\) while another study demonstrated a response rate of 62% to a 5 °C latency measurement.\(^19\) A ramped-down cooling protocol from 25 to 0 °C with a cooling rate >1 °C/sec resulted in response rates of 21% to 26%.\(^20\)

To be able to experimentally test and clinically use different cold stimulation protocols like described above, a device is needed, that is easy to use and tolerated well by the animals. At the same time, the device needs to deliver precise and adjustable temperature output, so it can be used for a variety of studies or patients with different clinical pathologies. A cold QST device that can generate different heat or cold stimulation protocols is the Somedic MSA thermal stimulator (Somedic SenseLab MSA). The computer-controlled device can generate different cooling rates for ramped-down cold stimulation with a minimum temperature of 10 °C or a constant low temperature for latency measurements (minimally 11 °C). Although this device has been used for different QST studies in humans\(^21\)–\(^23\) it has not yet been evaluated in dogs.

For this reason, our study aimed to evaluate the applicability and repeatability of cold stimulation with this commercially available thermal stimulator in healthy dogs. Furthermore, we aimed to define aversive threshold values for different cooling protocols to determine a cooling rate that provides reliable results and could potentially be used in clinical patients with suspected chronic or neuropathic pain.

Our hypothesis was, that faster cooling rates would lead to a higher response rate.

**Methods**

**Animals**

Since, to our knowledge, this was the first study planning to evaluate different cooling rates in dogs, no robust data for a sample size calculation was available. Therefore, a sample size estimation as described by Walter et al\(^24\) was performed. Using \(\alpha = 0.05, \beta = 0.2\), and \(n = 3\) different cooling rates. Ten university-owned beagle dogs were chosen depending on their availability at the time of the study. Only dogs were considered, that were not currently used in other studies, that were healthy based on clinical examination, and not pregnant or lactating. Six female and 4 male, university-owned Beagle dogs from 2 litters with an age of 3 or 6 years with a median body weight of 13 kg (12 to 17 kg) were included in the study. Standard protocols for deworming and vaccination were followed and dogs were housed in groups of 4 to 6 dogs with daily access to an enriched dog run. The dogs were fed a commercial dog diet.

The study was reviewed and ethically approved by the Lower Saxony State Office for Consumer Protection and Food Safety (33.14-42502-04-14/1547) according \$8 German animal health and safety law.

**Thermal stimulation device**

The thermal stimulation device used in this study incorporates a Peltier element, the thermal probe (thermode), a computer unit, and a handheld stop button. Via a computer unit, the different cooling protocols could be chosen, and the stimulation started manually. During the measurements, temperature changes were displayed on the computer screen and the temperature, at which the handheld stop button was pressed, was automatically recorded. The thermode was connected to the stimulator and had a contact surface area of 20 X 20 mm.

**Study design**

The study was designed as a prospective, experimental, randomized, partially blinded trial conducted between January 10, 2022, and February 7, 2022.

The dogs were divided into 2 subgroups of 3 and 1 subgroup of 4 animals. During the experiment, dogs of a subgroup would be together in the experimental room, but only 1 dog at a time was tested. To ensure, that the dog that was tested still had visual and acoustical contact with the other animals of the subgroup, these were located in a children’s playpen with blankets and ad libitum access to water on the other side of the room. The order in which the dogs of the subgroups were measured and which...
Cooling protocols

During the study, 4 different cooling protocols were tested on 4 body regions (neck, lumbosacral, elbow, and knee).

One protocol consisted of a latency measurement (PL) with a constant probe temperature of 11 °C and a maximal stimulation time of 60 seconds. At the same time, the other protocols represented 3 different cooling rates. During these protocols the thermode would automatically cool down from 32 to 10 °C with rates of 0.5 (P0.5), 1 (P1), and 5 (P5) °C second⁻¹.

The experiment was performed 3 times per animal (Exp1 to 3) with 7 days between Exp1 and Exp2 and a period of 21 days between Exp2 and Exp3. The observer was blinded to the cooling rate during the allocation and conduction of the experiment, during the latency measurement blinding could not be maintained, since the set-up of the protocol was too different and easily distinguishable from the other 3 protocols.

Measurements

Before the first experiment, the fur over the measuring locations was clipped and baseline skin temperature was measured using a commercial contactless surface thermometer.

The cold threshold and latency measurements were performed in triplicate at the neck, lumbosacral area, elbow, and knee. In between measurements, skin temperature was checked, and the next measurement only started if the skin temperature was back to baseline temperature ± 1 °C.

To perform the measurements, 1 observer manually placed the contact surface of the thermode on the skin using minimal but constant pressure. Then, another researcher manually started the cooling protocol via the control unit at the computer. As soon as avoidance reactions were detected, the stimulation was terminated and the thermode was removed. Avoidance reactions were defined as follows: Withdrawing from the thermode (moving away from the thermode entirely or trying to reduce contact with the thermode by the movement of single body parts), vocalizing (yelping, whining, or howling), and startling or turning the head towards the thermode. During the measurements, the animals were unrestrained and able to choose a comfortable body position on their own. If the stimulation location could not be accessed in this position, the dog was gently moved, and the next measurement was only performed after the dog had regained a calm and relaxed state. If a dog would not calm down after 5 minutes light restraint was used by placing 1 observer’s hands on the sternum of the dog to prevent forward movement.

For P0.5, P1, and P5, the blinded observer terminated the stimulation via a handheld stop button as soon as avoidance reactions were noticed. The temperature at which the stimulation was stopped was saved by the computer unit of the device and recorded as thermal threshold (TT) in °C. During each experiment 1 to 3 sham measurements were performed; therefore, the probe was placed on the skin, but the operator did not start the computer unit.

During PL measurement the time to reaction in seconds was determined using a stopwatch. If no aversive reaction occurred, the measurement was terminated after 60 seconds.

A score (0-5) described by Williams et al²⁰ and slightly modified was used to evaluate the feasibility of each measurement. A score of 0 represented the best feasibility with no restraint needed and excellent cooperation while a score of 1 represented a measurement necessitating mild restraint while overall, the dog showed good cooperation. Further, a score of 2 was associated with moderate restraint and good cooperation for more than 50% of the time, while a score of 3 represented a measurement that could only be performed with significant restraint and the dog cooperated well for less than 25% of the time. If constant restraint was necessary and the dog was not cooperative a score of 4 was allocated and a score of 5 represented a measurement that could not be properly performed. Measurements that received a score of 0 and 1 were considered easy to perform and to be clinically reliable.

Data analysis

The statistical analyses were conducted with R version 4.2.0.²⁵ A mixed logistic model was fitted using the lme4 package²⁶ to compare the proportion of animals reacting to the cold stimulus depending on the rate of cooling, day of the experiment, and localization of the stimulus. A random intercept was added for each repetition nested within day and animal. Model residuals were inspected using the DHARMA package²⁷ relying on simulation to detect overdispersion, outliers, and deviation from the expected distribution. Statistical significance was set at P < .05; P-values for the fixed effects were obtained by likelihood ratio tests. Pairwise comparisons within the significant main effects were performed based on expected marginal means using the emmeans package;²⁸ P-values were corrected for multiple comparisons using the Tukey method.

Descriptive statistic was performed using standard software (Graphpad Prism version 9.3.1, Microsoft Excel version 16.64). Thermal thresholds and time to reaction are presented as mean and SD. For the 3 different cooling rates response rates are additionally reported as pooled response rates (responses to all 3 cooling rates together).

Results

All included dogs completed the study without complications. In 85% (1,234/1,440) of measurements (PL, L0.5, P1, and P5) a feasibility score of
A total of 1,413 out of 1,440 measurements were scored either 0 or 1 (95%). The lowest score given was a 3 and it was assigned in 0.5% (9/1440) of measurements.

A reaction to the cold stimulation could be detected in 12% (130/1080) of pooled measurements from the 3 cooling rate protocols over all experiments and in 13.6% (49/360), 11.1% (40/360), and 11.3% (41/360) for P0.5, P1, and P5, respectively.

With regards to the different experimental days response rate was 15% (54/360), 10% (36/360), and 11% (40/360) on Exp1, on Exp2, and on Exp3, respectively.

Pooled over all experimental days thermal thresholds (mean ± SD) were 17.7 ± 4 °C, 16.3 ± 4.6 °C and 13 ± 2.6 °C for P0.5, P1, and P5, respectively.

Logistic regression analysis revealed no significant influence of the cooling rate on the response rate (Figure 1) while the day of the experiment had a statistically significant effect on the number of responses to stimulation ($\chi^2(3) = 30.494, P < .001$).

The response rate was significantly higher at the elbow compared to the neck (log odds ratio = −1.309, SE = 0.299, z ratio = −4.374, $P = .0001$) or lumbosacral area (log odds ratio = −1.309, SE = 0.299, z ratio = −4.374, $P = .0001$). The same was true for measurements at the knee in comparison to the neck (log odds ratio = −1.008, SE = 0.306, z ratio = −3.299, $P = .0054$) and lumbosacral area (log odds ratio = −0.887, SE = 0.297, z ratio = −2.991, $P = .0148$). There was no statistically significant difference on response rate when knee and elbow were compared as measuring localizations.

Furthermore, the day of the experiment had a statistically significant effect on the response rate ($\chi^2(2) = 15.649, P < .001$). Significantly more reactions were observed during Exp1 compared to Exp2 (log odds ratio = −0.730, SE = 0.261, z ratio = −2.796, $P = .0054$) and during Exp1 when compared with Exp3 (log odds ratio = −1.242, SE = 0.280, z ratio = −4.432, $P < .001$) but no significant difference could be detected between Exp2 and Exp3 (log odds ratio = −0.512, SE = 0.297, z ratio = −1.723, $P = .1964$). In 37% (134/360) of latency measurements dogs reacted to the stimulation. The mean time-to-reaction was 13 ± 11 seconds.

Figure 1—Results of logistic regression analysis comparing the effect of the 3 cooling rates (0.5, 1, and 5 °C/s), the experiment number (Exp1, Exp2, and Exp3) and the location where measurements were performed (elbow, knee, and neck) on the proportion of healthy university owned Beagle dogs (n = 10) that reacted to cuneate cold stimulation (proportion that reacted to stimulation shown in light blue, proportion that did not react shown in dark blue). Day of the experiment and measuring location had a statistically significant influence on the proportion of dogs reacting to the stimulation. Different superscript letters represent statistically significant ($P < .05$) differences.
Discussion

Our study aimed to evaluate the applicability and repeatability of cold stimulation with a commercially available thermal stimulator in healthy dogs and define aversive threshold values for different cooling protocols. The hypothesis, that faster cooling rates would lead to a higher response rate could not be confirmed and the overall aversiveness of the different cooling protocols was low. Although studies in humans showed that faster cooling rates resulted in a more intense cold sensation, we were not able to detect this effect in our experimental dogs. A possible explanation could be, that the effects of the different rates on intensity of sensation were minimal in our dogs. Also, it is possible that the stimulus was not strong (cold) enough to produce a reliable response in the animal, especially since only gross, aversive reactions were defined as positive responses in our study. This is in contrast to humans, who are able to verbalize their sensations and the change in quality way before aversive reactions would occur and makes comparisons with these studies difficult. Using a stronger stimulus, like lower minimal temperatures, could have led more reliably to aversive responses in the dogs and maybe reveal differences between the cooling rates. Further, the dogs overall inconsistent response to the stimulation could have masked small differences between the rates.

Mean threshold values for the fastest cooling rate were lower than the other rates, which could potentially be the result of a delay in the response time of the dogs since the stimulus was faster/shorter or a delay in pressing the stop button by the observer. Especially with regards to the fact, that the dogs were not able to quickly verbalize changes in sensation, but a visible aversive reaction was necessary to be detected. Therefore, it is possible that for this chain of action to occur and to lead to the visible reaction took relatively longer in the cases where a shorter stimulus was used. Due to the inconsistancy of responses to the stimulation among dogs, the high variability of threshold temperatures, and the overall low response rate a statistical comparison of thermal threshold values was not attempted. This is in accordance with the result of another study using a ramped-down cooling protocol in dogs. Studies in humans that investigated different cooling protocols could not find significant differences between the rates of threshold values.

In human studies, the test subjects can discriminate cold sensation from cold pain and verbally express this. A decrease in skin temperature by less than 1 °C already leads to a cool sensation in humans, while decreasing skin temperature below 16 °C might be perceived as painful. In our study, it was not possible to differentiate between cold sensation and cold nociception since the outcome measure was an aversive reaction visible to the observer. This method of detecting a reaction to the cold stimulus did not allow for a graduation of the response, contrary to humans who can describe the quality of their sensations or use scales to describe it. Therefore, it is not known which endpoint really was measured, but seeing the mild and sparse reactions of the dogs it is easy to assume that it was rather cold sensation than cold nociception.

Potentially, aiming to achieve actual cold nociception by using a lower minimal temperature could have increased the response rate in our study and led to more robust data. It has been shown, that humans and rodents with neuropathic pain can develop cold allodynia and cold hyperalgesia and be more sensitive to cold stimulation at relatively high temperatures (>10 °C). Hence, even with the minimal temperatures we used in our experiments, it is possible that in dogs with neuropathic pain, a more pronounced reaction could be achieved and still make the tested stimulation device a useful tool to detect neuropathic pain in dogs. Therefore, further studies in clinical patients with possibly altered pain sensations are necessary.

The repeatability of quantitative sensory testing methods has been evaluated in humans and dogs with differing results. In humans, it is reported that the repeatability of cold pain detection was insufficient. In dogs, on the other hand, no difference could be detected between cold stimulation sessions although mean latencies were very close to cut-off time during both sessions. Briley et al faced the same problem during their investigation and abandoned the repetition of cold stimulation due to a lack of response to stimulation during their first experimental session. So far, the reason for this variable results is unknown, although differences in study design and protocol might have played a role.

In our study, response rates for the different cooling protocols were significantly lower during the last experiment. This could partly be the result of habituation to the stimulation protocol. Since the used minimal temperature presumably does not induce noxious cold pain, it is possible that the dogs would simply tolerate the stimulation without reacting to it anymore, especially since the dogs were experienced study animals, that had been used for other QST studies before, although other QST modalities were used and a time period of at least 2 months had passed since the last study. Nevertheless, this might have led to a more pronounced habituation effect in our study dogs than with client-owned dogs or clinical patients. The method showed good applicability with 95% of measurements receiving a FS of 0 or 1 and were considered as “easy measurements” and clinically trustworthy. It is possible that the cold stimulation was so well tolerated by the dogs because they were not restrained, and we allowed the animals to choose and stay in a comfortable body position. The same, very good feasibility was reported by another study using cold stimulation in dogs that were able to choose the position themselves. Contrary to another experiment where dogs were genteelly restrained in lateral recumbency during stimulation and 21% of measurements were categorized as “difficult” with a score of 3 to 5.

In accordance with both of these studies using cold stimulation in healthy dogs, the absolute
number of reactions to cold stimulation in the present study was low.

So far, cold stimulation in dogs has been mainly performed at different regions of the front and hind limbs. To the author’s knowledge, there is only 1 study comparing measurements at the limbs with stimulation performed at the neck, thoracolumbar area, and abdomen in dogs. In this study, significantly fewer responses to the stimulation of neck, thoracolumbar, and abdominal areas were reported. This is in accordance with the results of the present study and can be possibly explained by differences in skin thickness, with the skin being thicker in the neck and abdominal regions, and receptor density.

One limitation of our study was, that measurements could not be performed without touching the dogs with the manually held thermode. This process could have influenced the dogs during measurements. Since our university-owned dogs were used to getting clinically examined it is possible, that they would hold still, especially when touched with objects (like being auscultated with a stethoscope). This could have lowered the rate of response to the stimulation. A way to avoid this would have been to use client-owned dogs, which on the other hand might have resulted in a more heterogenic group of study subjects.

Furthermore, due to the design of the thermode and the need to apply it manually to the skin, the pressure with which it was pressed to the skin likely will not have been completely constant and potentially affected the stimulation of receptors. A thermode that could be fixed to the dogs and be operated from a distance or remotely could have overcome these limitations, but at present is not commercially available. Nevertheless, since the dogs were very cooperative and most measurements easily feasible, we presumed that contact pressure changes would be minimal.

Since only beagle dogs from 2 litters were used, we cannot rule out an influence of breed or genetics on the results of our study. On the other hand, this was the first study using this device for cold stimulation in dogs and the homogeneity of our study population allowed us to us a smaller sample size and reduced the number of animals taking part in our experiment.

In future studies, testing the different cooling protocols in dogs suffering from chronic pain and neuropathic conditions is of great interest to determine, if cold sensitivity is increased. Comparing threshold values and reaction rates to healthy dogs could help determine an optimal testing protocol for cold stimulation in a clinical setting. Further modification of the tested protocols, like using lower minimal temperatures, could be helpful to establish protocols with more consistent response rates.

The method was easy to apply and well tolerated, but habituation to the stimulus could not be excluded. Overall, the aversiveness of cold stimulation in the healthy dogs was limited and it is currently not possible to recommend a specific protocol. It needs to be determined if aversiveness is increased in diseased dogs.

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