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REVIEW PAPER

Use of nociceptive threshold testing in cats in experimental and clinical settings: a qualitative review

Chiara Adami[a] **and Claudia Spadavecchia**[b]

[a]Department of Clinical Sciences and Services, Royal Veterinary College, University of London, Hatfield, UK

[b]Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Berne, Langassstrasse 124, CH- 3027, Bern, Switzerland

Correspondence: Chiara Adami, Department of Clinical Science and Services, Royal Veterinary College, Hawkshead Lane, AL9 7TA, Hatfield, UK

Email: cadami@rvc.ac.uk

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Claudia Spadavecchia: Contribution to the design and critical revision of the manuscript

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Abstract

Objectives The objective of this work was to review the scientific articles on the use of nociceptive threshold testing (NTT) in cats, and to summarise the clinical and experimental applications in this species.

Databases used Pertinent literature was searched with PubMed, Scopus, Web of Science, Universitätsbibliothek Basel (swissbib Basel Bern) and Google Scholar. The search was then refined manually based first on article titles and abstracts, and subsequently on full texts.

Conclusions Of the four classical acute nociceptive models used for NTT, thermal and mechanical are most commonly used in cats. Thermal stimulation is applicable in experimental settings and has been used in pharmacodynamics studies assessing feline antinociception. Although mechanical stimulation is currently less used in cats, in the future it might play a role in the evaluation of clinical feline pain. However, the low response-reliability after stimulus repetition within a narrow time interval represents a major limitation for the clinical use of mechanical thresholds (MT) in this species.

Challenges remain when thermal thresholds (TT) are used to investigate analgesics that have the potential to affect skin temperature, such as opioids and alpha 2-adrenergic agonists, and when a model of inflammatory pain is reproduced in experimental cats with the purpose of evaluating NSAIDs as analgesics.

Keywords feline, mechanical and thermal thresholds, nociceptive model, nociceptive threshold testing, pain

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Introduction

Nociceptive threshold testing (NTT) makes use of a broad range of stimulation methods to assess and quantify nociceptive function and response. Most protocols described in cats have been developed for cutaneous application of mechanical and thermal stimulation. However, mechanical NTT has also been successfully applied to hollow viscera (Briggs et al. 1998).

Regardless of the type of stimulation used in the experimental setting, a realistic reproduction of clinical pain is probably impossible to achieve. Although characterised by a number of different features, a common denominator of clinical pain is its complexity and the diversity of the nociceptors involved where mechanical, thermal and chemical stimuli may all contribute to the activation of afferent pathways during postoperative surgical pain.

Nociception and pain are considered distinct processes. Nociception begins with the detection of injurious stimuli by a class of specialised receptors, with transmission of that information to the spinal cord and on up to the brain. This may result in a defensive, immediate reflex response (Sneddon, 2017). All reflexes, including those associated with nociception, are organised by centres at the lower hierarchy of the central nervous system; they can be elicited in decerebrated animals and are characterised by either autonomic or basic motor responses, including increased heart rate, withdrawal and muscular contractions (Woodworth & Sherrington 1904; Sneddon 2017). Complex behaviours, in response to noxious stimuli, can also include conditioned motor responses, usually as a result of learning (Le Bars et al. 2001). Pain is a negative affective and psychological response and is often accompanied by more complex or prolonged behavioural alterations indicative of discomfort, such as distrust of objects associated

with painful experiences and/or modification of social behaviour. Nociception may not result in pain, because of the ability of the central nervous system to modify nociceptive signals and prevent conscious perception of noxious stimuli, and pain can occur without nociception in the presence of central sensitisation. However, pain resulting from injury cannot occur without nociception (Sneddon 2017).

There is a huge body of literature on NTT in rodents, however these animals are often genetically very similar, leading to minimal variance in the detected thresholds. Cats used in research are, by comparison, much more genetically diverse and so it is expected that there will be greater variance in any tested population. There are many reports focusing on the application of NTT to cats. Older reports were aimed at investigating particular aspects of the afferent nociceptive organisation, or at establishing patterns to relate the neurophysiological activity of the sensory system to behavioural responses indicative of nociception (Beck et al. 1974; Casey & Morrow 1983). In contrast, more recent work has primarily focused on the pharmacodynamics of analgesic drugs in cats, with the purpose of identifying useful doses, routes of administration, onset times and duration of the effects (Millette et al. 2008; Pypendop et al. 2009; Ambros & Duke 2013).

When performing NTT testing it is important for the stimulus to be applied at a rate that will allow for conduction and interpretation of the stimulus. If the increase in stimulus strength is very rapid it might reach an excessively high level before the animal has had a chance to respond. It is also important that the approach can detect hyperalgesia as well as analgesia. In tests that use latency to the response, a very short latency will not likely allow for the detection of hyperalgesia.

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The objective of this work is to review the use and the clinical and experimental applications of NTT in cats, with particular focus on acute nociceptive models, and on the literature of the past 20 years.

Databases used and literature search

A literature search was conducted using PubMed, Scopus, Web of Science, Universitätsbibliothek Basel (swissbib Basel Bern) and Google Scholar. The keywords sets used for the initial screening were the following: 'nociceptive threshold testing + cats/feline', 'quantitative sensory testing/QST + cats/feline', 'mechanical/thermal/electrical thresholds + cats/feline', 'mechanical/thermal/electrical nociceptive model/antinociception + cats/feline', 'antinociceptive/analgesic effects/efficacy + cats/feline', and 'antinociceptive/analgesic pharmacodynamics + cats/feline'.

The search was refined based first on article titles and abstracts, and subsequently on full texts of the selected scientific reports. The reference list of each retrieved scientific paper was then scrutinised to identify further pieces of literature pertaining to the topic. All the identified scientific peer-review articles written in the English language and pertaining to the topic were included in the study. Related anaesthesia and neurophysiology textbooks were also reviewed.

The refined search identified 51 articles published between 1983 and 2019. Of these, nine were on the use of mechanical thresholds (MT) (of which six were experimental and three were clinical studies), 34 experimental studies were on the use of TTs, and eight on the use of both mechanical and TTs (of which one was a clinical report and the remaining seven were experimental studies).

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Mechanical stimulation

Mechanical stimulation has been used in cats to elicit mostly somatic, but also visceral nociception, in both experimental and clinical settings. Visceral nociception has been experimentally induced by inflating balloon catheters inserted into the rectum (Sawyer & Rech 1987; Briggs et al. 1998). Somatic nociception is induced and assessed by applying a force to a given area of the body. Force is defined as the push or pull on an object that causes it to change velocity; pressure is a measure of force per unit area. Therefore, for the outcome values to be comparable between devices that measure different variables, the surface area of the probe must be known, and recognised as part of the applied stimulus. A nociceptive threshold is defined, depending on the device used, as either the pressure (expressed in mmHg) or the force (expressed in g or Newtons) reached when the stimulus is intense enough to elicit a behavioural, conscious response in the cat, which is subjectively determined by the operator. Ideally, the stimulating probe should be applied perpendicular to the test site in order to ensure that the measured force has been wholly applied to the area of interest. There should be minimal distensible tissue so that the mechanical stimulus is not spread over a large surface area or that the stretching tissue attenuates the force applied. Mechanical stimuli are usually applied progressively and incrementally until a cut-off value is reached; the speed of increased force is variable and, for manual algometers, is operator dependent. Both sharp-ended pins and flat-ended/blunt probes have been used in animals (Moens et al. 2003; Haussler & Erb 2006; Machin et al. 2019).

It is commonly accepted that the elicited behaviours represent supraspinal responses to activation of nociceptors located in the skin, muscles and periosteum (Le Bars et al. 2001). Both myelinated Aδ fibres, with intermediate conduction speeds, and small, unmyelinated, slow-conducting C fibres are expected to be activated primarily,

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but conventional noxious mechanical stimuli do not produce selective activation of these nerve types (Le Bars et al. 2001). In some subjects/patients, activation of the Aß fibres, in response to touch and pressure, may be sufficient to evoke behavioural responses that could easily be misinterpreted as signs of nociception. This is more likely in the case of algometers that are applied intermittently than those that maintain contact with the skin where the Aß stimulation would be ongoing. As a result, one disadvantage of intermittently applied mechanical stimulation is a potential lack of specificity.

Several instruments have been specifically developed to perform mechanical stimulation in cats (Table 1). Slingsby and colleagues designed a finger-mounted algometer (Slingsby et al. 2001). The probe was made of a Force-Sensing Resistor (FSR), a thick polymer film, which exhibits a progressive decrease in resistance with increasing force applied to its surface. The 15 mm diameter-probe was soldered to a ribbon cable, connected to a battery powered measuring unit, calibrated with an accurate load beam and mounted on the index finger of the operator. The outcome force resulting from the application of the probe on the skin of the cat was expressed in Newtons. This device was subsequently used to evaluate the analgesic effect of post-operative meperidine in male cats undergoing castration, and in another clinical study investigating NSAID associated analgesia in 40 female cats undergoing ovariohysterectomy (OVH) (Slingsby & Waterman-Pearson 2000; Slingsby et al. 2001). Changes in MTs measured at the scrotum before and after surgery differentiated between meperidine and the negative control group in male cats. In female cats, thresholds measured at the surgical wound following OVH were lower than those measured before surgery. The authors did not state how quickly the force was applied in either study and did not describe how or where the probe was applied in the OVH study (Slingsby & Waterman-Pearson 2000; Slingsby et al. 2001).

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Another pressure testing device for use in cats was designed in 2007 by Dixon and colleagues (Dixon et al. 2007) and subsequently manufactured by Topcat Metrology Ltd (UK), marketed under the trade name "ProD-Plus". The device was composed of a 5 g plastic bracelet, inside which the authors mounted a blood pressure bladder and three brass pins, each tipped with a 2.4 mm diameter ball bearing, distributed 10 mm apart in a triangular pattern to apply a perpendicular pressure to the limb. For this device, the outcome was bladder pressure expressed in mmHg. This could not be translated into the force acting on the skin because neither the true contact area nor the actual pressure applied to each pin was known. The bracelet was applied on one forearm and bladder pressure was increased incrementally and measured with a strain gauge pressure transducer; the threshold pressure was recorded by pressing the hold button on the voltmeter when the cat reacted to the stimulus (the voltage was directly proportional to the pressure). The authors found that, whilst the thresholds varied a lot between different cats (68 to 202 mmHg in six cats), thresholds within each cat were consistent. This pressure algometer was used by the same authors to evaluate the analgesic efficacy of subcutaneous butorphanol (0.4 mg kg^{-1}) and carprofen (4 mg kg^{-1}). The NSAID was tested in a second phase of the trial, after kaolin was injected intradermally in the forearm to produce a model of inflammatory pain. In that study, the comparison between MTs measured before and after the administration of the opioid detected butorphanol antinociception. Excessively variable thresholds were obtained with the inflammatory model making this approach ineffective. The authors concluded that the device was light and easy to use and allowed the cats to remain unrestrained during testing. The repeatability of the thresholds was considered acceptable by the authors, who concluded that the algometer could be used for analgesic pharmacologic studies in cats (Dixon et al. 2007). However, the same authors developed and tested the device,

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which may have resulted in a certain degree of bias, although the device has been further modified and used by others who have confirmed its utility (Steagall et al. 2007; Millette et al. 2008; Slingsby et al. 2012).

Mechanical thresholds have also been used in cats to study experimentally induced visceral nociception (Sawyer & Rech 1987; Sawyer et al. 1993). A subsequent study (Briggs et al. 1998) investigated the analgesic effect of oxymorphone, butorphanol and acepromazine, alone and in combination, using this model. A silastic balloon catheter, inserted into the rectum and connected via a rubber tube to a plastic jug was pressurised to selected incremental values for 30-second periods. A positive result was considered when an undefined behavioural response was evoked. The authors interspersed lower pressures to prevent conditioning to the increasing pressures used. Although the model has been successfully used in horses (Muir & Robertson 1985) and found robust and reliable in rodents (Jones et al. 2004; Arvidsson et al. 2006; Nissen et al. 2018), its validity for investigating colorectal noxious distension was questioned by a more recent study in horses (Sanchez et al. 2005). The physical properties of the balloon are relevant and materials with linear compliance, such as mylar, should be selected over latex in order to ensure proper pressure application to the colorectal wall (Sanchez et al. 2005). Another drawback of this model is that it may fail to differentiate behavioural responses caused by nociception from those caused by an urge to defecate.

More recent studies used precision pressure algometers, such as the Electronic von Frey Anaesthesiometer (EVF) and the Small Animal ALGOmeter (SMALGO). The former represents the electronic version of the von Frey filaments and has been used most recently to assess acute and chronic pain in dogs and cats (Adami et al. 2018; Addison & Clements 2017). The EVF uses a sensory probe equipped with a rigid tip applying a force varying from 0 to 1000 g, which is measured, displayed and stored by

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the control unit. The SMALGO has been specifically developed for laboratory rodents and shares the same working principle as the EVF. However, it has a finger-mounted sensor probe whose applicable force ranges from 0 to 1500 g. With both devices, a progressively increasing force is applied by the operator over the targeted area, until an end-point behavioural response is observed. Although the stimulus is generally applied over an undefined time-period, most authors set a cut-off pressure/force value to avoid iatrogenic injury.

Addison and Clements (2017) found that use of both the von Frey filaments and the EVF, applied to the metacarpal/metatarsal pad to assess paw withdrawal thresholds, resulted in differentiation between healthy cats and those with osteoarthritis (Addison and Clements 2017). Another recent study evaluated the inter-rater and inter-device reliability of TTs measured with both the EVF and the SMALGO in non-painful cats (Adami et al. 2018). The authors found that the reliability of the measurements decreased after repetition within time-intervals shorter than one hour, indicating that the level of cooperation of feline patients may decrease after repeated testing or, alternatively, that the cats may anticipate the stimulus in order to end it. Similarly, learning and stimulus anticipation, resulting in decreased TTs have been described in dogs using algometry (Coleman et al. 2014). In another study, the SMALGO, applied to the skin of the upper lip, dorsal to the end of the canine root, was evaluated in cats with chronic gingivostomatitis, as compared to a healthy control group (Machin et al. 2019b). Although the authors found a low inter-observer and intra-observer variability, the study failed to differentiate between healthy and diseased cats. Moreover, there was no correlation between the scores of the chronic gingivostomatitis scale, used by the authors to score the severity of the oral lesions, and the thresholds measured in diseased

cats. Overall, these findings suggested that mechanical sensory testing with the SMALGO is not a reliable method to evaluate chronic oral pain in cats.

A final study compared the use of the EVF and von Frey filaments at different anatomical sites of non-painful cats (Machin et al. 2019a). The authors found a moderate agreement between the two devices, as suggested by the intra-class correlation coefficient of 0.49 (CI=0.13-0.70); however, the willingness of the cats to cooperate decreased with the repetition of the measurements after 24 hours. This drawback may limit the applicability of mechanical NTT in the clinical setting, where repeated testing may be needed to adjust the analgesic therapy to meet the patient's requirement. Despite its limitations, it is worth considering that one potential advantage of the mechanical NTT over thermal, electrical and chemical techniques may be that the use of pressure thresholds is often perceived, by both clinicians and cat owners, as less invasive and harmful than other types of stimuli. This aspect may allow and encourage the development of protocols to increase the clinical application of TT testing, for routine assessment of pain in cats.

At the date of writing, although inter-observer variability appears to be minimal for non-repeated TT measurements, there is still no evidence that, in cats, the thresholds consistently correlate with the severity of the clinical condition that causes pain, or with the intensity of pain itself. More prospective studies on the use of TTs in cats with clinical pain are needed to draw more solid conclusions with respect to the clinical usefulness of modern pressure algometers in this species. Even when used for testing analgesics in an experimental setting the documented thresholds have been highly variable making it difficult to record an antinociceptive effect.

Thermal stimulation

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A number of studies investigated the applicability of TT testing in various feline nociceptive models (Table 2). Thermal stimulation relies on the activation of two subtypes of nociceptors: mechano-heat units activated by noxious mechanical and hot thermal stimuli, and mechano-cold units activated by noxious mechanical and cold thermal stimuli (Djouhri & Lawson 2004). Two different outcome variables are often used during thermal stimulation: latency (the time elapsed between the start of application of a constant temperature and the observation of the target behavioural response) and threshold temperature (the measured temperature at which the response occurs with the application of an increasing temperature) (Casey & Morrow 1983; Slingsby & Taylor 2007; Addison & Clements 2017). Hot thermal testing is most commonly described, although cold (7°C temperature-controlled pressure-plate system) has also been used in cats and found more useful than kinetic gait analysis to differentiate between healthy limbs and those with osteoarthritis (Addison & Clements 2017).

There is a general concern that noxious thermal stimulation may activate mostly C but not Aδ fibres and therefore result in incomplete activation of nociceptive pathways, making the thermal model less likely than others to resemble the complexity of clinical pain (Mao 2012). Selectivity of receptor activation is greatly dependent on the mode of delivery of the thermal stimulus and on the steepness of the heating slope. In a murine model, stimuli capable of heating the cutaneous surface as rapidly as 6.5°C $second^{-1}$, such as laser radiation, activated Aδ units with a response latency of 2 seconds after the onset of the stimulus (Yeomans & Proudfit 1996). In contrast, thermal conduction, by means of rates of skin heating as slow as 0.9 °C $second^{-1}$, with relatively long latency of 5-6 seconds, evoked action potentials selectively in C nociceptors (Yeomans & Proudfit 1996). In cats, as well as in primates, thermal stimuli above 45 ºC are capable of

activating both the Aδ and C fibres, which respond with increasing discharge as the temperature is increased (Beck et al. 1974; Casey & Morrow 1983). Both laser and radiant heat stimulation responses are measured as latencies, whereas temperature thresholds are measured with contact thermodes. Contact thermodes unavoidably apply a pressure on the skin surface, which may also activate low-threshold non-nociceptive Aß fibres (Nathan et al. 1986; Svensson et al. 1997) but this is less likely to cause confusion if the thermode is continuously in contact with the skin vs an intermittent application.

Despite these limitations, thermal nociception has been used extensively over the last two decades and its use in cats has been more repeatable and reliable than both mechanical and electrical models. The use of cats to investigate and quantify afferent activity in response to thermal stimulation dates back to the 1960s (Kenshalo et al. 1967; Beck et al. 1974). One study used rapid onset thermal pulses ranging from 43 to 60ºC and concluded that the probability of evoking a nocifensive response in cats increased for cutaneous thermal stimuli between 50 and 55ºC (Casey & Morrow 1983).

In 2002, Dixon and colleagues developed a TT device, subsequently produced by Top Cat Metrology Ltd, which has been used for evaluation of various analgesics in cats (Dixon et al. 2002). A probe equipped with a heater element and a temperature sensor was held against a clipped area of the thorax using an elastic band and a pressure bladder, inflated to 100 mmHg to ensure even contact between the skin and the probe. The probe was heated at 0.6ºC second^{-1} until either a pre-defined behavioural response was elicited, or a cut-off value of 60ºC was reached. The measurement of the TTs with this device was repeatable and well tolerated by the cats but resulted in minor skin lesions. A further crossover trial carried out by the same authors in non-painful cats

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found the device useful to differentiate between pethidine antinociception and a placebo treatment (Dixon et al. 2002).

This device was used in various subsequent studies with a standardised approach, characterised by a cut-off temperature decreased to 55-55.5°C, to prevent skin lesions, the same application mode of the thermal stimulus and similar areas of the body tested. Many of these reports investigated the usefulness of TTs alone to evaluate the pharmacodynamics of various analgesics (Lascelles & Robertson 2004b; Robertson et al. 2005; Johnson et al. 2007; Wegner & Robertson 2007; Slingsby & Taylor 2008; Robertson et al. 2009; Slingsby et al. 2009; Slingsby et al. 2010) (Table 2), whereas some others compared mechanical and TT testing for the same purpose (Steagall et al. 2006; Steagall et al. 2007; Millette et al. 2008; Slingsby et al. 2012; Ambros and Duke 2013; Addison & Clements 2017) (Table 3).

In the context of pharmacological studies, TT testing has been commonly used to describe the analgesic effects of various opioids in cats, including “opioid-like” agents such as tramadol and tapentadol (Lascelles & Robertson 2004a; Johnson et al. 2007; Wenger & Robertson 2007; Pypendop et al. 2009; Steagall et al. 2015; Doodnaught et al. 2017). Overall, the results of these studies suggest that the thermal nociceptive model consistently detects opioid-antinociception, despite some contradictory findings; whilst buccal buprenorphine was found by some authors to significantly increase TTs (Robertson et al. 2005a; Doodnaught et al. 2018), it resulted in inconsistent thermal antinociception in another study (Steagall et al. 2015). A possible reason for these conflicting results is the variable bioavailability of buprenorphine after oral transmucosal administration, which was found to range between 16 and 60% (Pypendop et al. 2014).

An important drawback of using TTs to detect opioid antinociception in cats is that opioids increase body temperature (Niedfeldt & Robertson 2006; Posner et al. 2010), an effect that may act as a confounding variable and potentially affect the outcome because the raised baseline temperature may not be comparable to an untreated control.

The thermal nociceptive model has also been used to investigate the analgesic pharmacodynamics of α_2-adrenoceptor agonists in cats, with conflicting results. Slingsby & Taylor (2008) found that, among five different intramuscular dexmedetomidine doses tested, only the highest one (40 μg kg^{-1}) caused an increase in thresholds, which was less significant than with buprenorphine, used as positive control treatment. Another study failed to detect any difference using TT between intramuscular and oral transmucosal dexmedetomidine at the same dose (Slingsby et al. 2009).

In a subsequent report the same authors detected an additive antinociceptive effect with buprenorphine and dexmedetomidine combined in non-painful cats (Slingsby et al. 2010). In the light of these inconsistent findings, the authors concluded that the α_2-adrenoceptor agonists-induced vasoconstriction may alter the response to thermal stimulation by decreasing blood flow in the skin, which makes the thermal model suboptimal when this class of analgesics is investigated (Slingsby et al. 2009). The decrease in skin temperature appears to be dose dependent with doses >5 μg/kg causing some decrease (Pypendop personal communication 2020).

Besides the contact thermal algometer developed by Dixon (Dixon et al. 2002), another device using remote carbon dioxide laser stimulation has been validated for cats more recently (Farnworth et al. 2013a, Farnworth et al. 2013b). A visible, non-thermal, helium laser was used to guide and aim the thermal carbon dioxide laser beam over a target area of the cats' shaved thorax. The wavelength of the thermal laser was 10.6 μm,

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with a maximum power output of 10 W. The exhibition of either a body shift (e.g. rising) or the panniculus reflex were considered a positive response. The laser device was evaluated with respect to intra-individual and inter-individual variability. The authors found that, although individual responses were repeatable over a three-day period, the repeatability decreased after the third day of testing (Farnworth et al. 2013a). Moreover, heavier cats had increased latencies, suggesting that fat deposition in the sub-cutaneous layers, where the skin nociceptors occur, may act as a buffer and attenuate the response (Farnworth et al. 2013b). This laser device was used to investigate the analgesic effects of opioids, NSAIDs and α_2-adrenoceptor agonists (Farnworth et al. 2015). Although the results of this one study were inconclusive and did not allow differentiation between treatment groups, it is worth considering that using a CO_2 laser thermal stimulator may offer some advantages over other types of thermal probes. The monochromatic, long wavelength results in complete absorption regardless of the degree of pigmentation of the skin, which may be an issue with radiant heat methods (Le Bars et al. 2001). Moreover, the heating slope is steeper than with contact thermodes as the target temperature is reached within milliseconds, and the lack of cutaneous contact ensures avoidance of inadvertent activation of non-nociceptive nerve fibres (Treede et al. 1984; Le Bars et al. 2001). However, one potential disadvantage is the lack of skin temperature measurement before application of the stimulus, as well as the risk for blistering that was observed in some study cats.

Overall, many studies suggest that thermal nociception is well tolerated in cats and the results are reproducible (Lascelles & Robertson 2004a; Robertson et al. 2005a; Robertson et al. 2005b; Steagall et al. 2006; Pypendop et al. 2009). A limitation of TTs may be their applicability to pharmacodynamic studies focusing on analgesics that have the potential to alter the skin temperature in cats, such as opioids (Niedfeldt &

Robertson 2006; Posner et al. 2007) and α_2-adrenoceptor agonists at high doses - an effect that may affect the thresholds or the comparison with control animals and act as a confounding variable. The thermode method is unlikely to be very useful for investigating pain in clinical patients because it is time consuming, requires a skin area to be shaved for its placement and the repeated application of noxious heat may be regarded as upsetting by some animal caregivers. Methods that are more 'portable', such as a laser, that can be focused on an area of interest and used intermittently, may be more useful in the clinical setting but may still be unacceptable due to the repeated testing (Farnworth et al. 2015).

Electrical stimulation

The potential advantage of electrical stimulation is that it is reproducible, measurable and quantifiable. Single electrical stimuli of short duration, usually between 10 and 20 ms, are often applied in a sudden, abrupt fashion to measure latencies. Alternatively, electrical stimulation of gradually increasing intensity in the form of trains of stimuli, usually lasting some hundreds of ms, have been used in rodents to evaluate different responses organised on a hierarchical basis, namely reflex movements of the tail followed by more complex behavioural responses, such as attempts to escape (Le Bars et al. 2001).

A number of studies from the 1970s described the use of electrical stimulation in cats (Anderson & Pearl 1974; Berkley & Parmer 1974; Anderson & Pearl 1975; Lisney 1978). However, the majority of these neurophysiological studies were conducted in cats under general anaesthesia and did not use behavioural evaluation, which is an intrinsic component of NTT.

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More recently, Millette and colleagues evaluated the use of electrical threshold testing to characterise the antinociceptive effects of meperidine in pain-free cats (Millette et al. 2008). The authors used a current generator to deliver repeated stimuli with a duration of 1 ms and 1 ms delay between pulses, through two electrodes held against a clipped area of the mid-thorax. The current was increased at 1mA second $^{-1}$, and the cut-off was set at 5 mA. The main finding of Millette's study was that the electrical stimulus failed to detect meperidine antinociception, whereas the thermal and the TTs, also used by the authors, were both found useful for this purpose.

The application of electrical stimulation for nociceptive testing in cats has failed to earn popularity. A reason for this may be the many limitations of the electrical stimulus, which differs from every natural type of stimulus that an animal may encounter in its physiological environment.

Although studies conducted in both human volunteers and dogs demonstrated that electrical stimuli with frequencies of 2000, 250 and 5 Hz can selectively stimulate the Aß, the Aδ and the C fibres, respectively (Finkel et al. 2002; Sakai et al. 2004; Watabiki et al. 2010), to the best of these authors' knowledge, there are no published experiments in cats to verify these findings. As a result, nonselectivity of activation is another potential drawback of electrical stimulation, which can result in activation of Aδ, C as well as larger diameter fibres not directly implicated in nociception (Le Bars et al. 2001). Finally, there are technical considerations that may limit the applicability of electrical nociception. Based on its thickness and hydration, the skin offers variable impedance to electrical stimulation, which can considerably affect the response. This variability can be minimised by using a constant current and measuring impedance prior to stimulation (Le Bars et al. 2001).

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The very limited evidence, together with the small number of reports in this species, does not allow any conclusive statement with respect to the usefulness of the electrical nociceptive model in cats.

Chemical stimulation

Chemical stimuli differ from any other type of nociceptive stimulation, as they are slow, progressive and of longer duration. As a result, the chemical nociceptive model mostly produces complex behavioural responses rather than simple reflexes (Le Bars et al. 2001). Algogenic or irritant agents, such as capsaicin, formalin and kaolin, are either applied on the intact skin or injected subcutaneously or intradermally, to produce hyperalgesia and inflammation, and therefore evoke pain. A local cutaneous injury may produce primary hyperalgesia within the injured area, as well as secondary, neurogenic hyperalgesia, caused by central sensitisation, in the normal surrounding skin. (Baumann et al. 1991).

The duration of inflammation – and therefore of hyperalgesia – varies between chemical agents, routes of administration and, possibly, animal models. In rodents, both formalin and capsaicin reproduce an inflammatory model characterised by two well-identified phases, of which the acute one occurs within minutes from intradermal injection and lasts a few minutes, followed by secondary hyperalgesia starting around 10 minutes (Wheeler-Aceto & Cowan 1991; La et al. 2017). In cats, subcutaneous injection of kaolin in the paw results in well-defined and reproducible inflammation that lasted up to five days (Giraudel et al. 2005; Giraudel et al. 2009).

The failure to quantify NSAID associated analgesia and successfully differentiate between different agents within the same pharmaceutical class seems to be a common denominator of the studies that used the mechanical and thermal nociceptive models.

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Conceptually, since these models do not have an inflammatory component to the nociceptive stimulus it is not surprising that they have not succeeded. The addition of an inflammatory chemical has been used to test the anti-inflammatory antinociceptive effect of these drugs.

A study from the late 1970s used formalin, injected subcutaneously into the forepaw, to induce inflammation and then quantify the analgesic effects of morphine and meperidine with thermal latency testing in cats (Dubuisson & Dennis 1977). Similarly, kaolin was used in various studies to investigate NSAID associated antinociception in cats (Giraudel et al. 2005; Dixon et al. 2007; Taylor et al. 2007; Giraudel et al. 2009). Taylor and colleagues combined kaolin injection and TT testing to investigate the analgesic efficacy of ketoprofen in seven cats, and found that the combination of the two techniques did not detect antinociception. Ketoprofen increased the TT outside the 95% confidence interval but the study was probably underpowered (Taylor et al. 2007).

The intradermal or subcutaneous injection of chemicals has been used only in experimental cats with the greatest utility for testing the analgesic effect of drugs that have an anti-inflammatory component. There are no potential applications in the clinical setting.

Mixed nociceptive models comparing thermal and mechanical thresholds

A number of studies conducted in cats reported the simultaneous use of several threshold testing methods to investigate the pharmacodynamics of various analgesic agents, most of which were opioids (Steagall et al. 2006; Steagall et al. 2007; Millette et al. 2008; Steagall et al. 2008; Slingsby et al. 2012; Table 3).

The studies using different types of nociceptive stimulation seem to further confirm that the TTs, and to some extent the mechanical ones, consistently detect the antinociceptive effect of opioids (Millette et al. 2008; Slingsby et al. 2012), whilst less convincing findings were obtained when the two models were used to investigate ketamine (Ambros & Duke 2013). The authors concluded that, because ketamine seems to be more effective in pathological pain states characterised by central facilitation (Ghorpade and Advokat 1994), a model of acute nociception may not be the most appropriate one to detect ketamine analgesia (Ambros & Duke 2013).

Tramadol was found to have a limited effect on both TT and TTs (Steagall et al. 2008), although the increase in thresholds was more pronounced when tramadol was combined with 0.1 mg kg^{-1} acepromazine. However, the sedative effect, detected in all cats that received acepromazine (Steagall et al. 2008), could represent a confounding variable, by decreasing the behavioural responsiveness of the cats to nociceptive stimulation.

Conclusions

Mechanical and TT testing are the NTT methods that were found more reliable for use in cats within the last two decades, with TTs being the most widely applied in pharmacological studies. As indicated above TT testing may be influenced by changes in skin temperature associated with particular drugs. With a thermode technique the baseline temperature is measured but this needs to be accounted for with other methods where the skin temperature is not recorded automatically.

Whilst TT testing is mostly applicable to the experimental setting, there is a promising, increasing tendency to test the usefulness of MTs in cats with clinical pain. Therefore, mechanical nociception may, in the future, become part of the routine

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evaluation of feline patients suffering from various pain syndromes. The low repeatability of mechanical NTT within short time-intervals, as well as the lack of data in patients with acute and chronic pain, represent major limitations to its clinical application. Some studies showed that both MT and TT testing did not detect NSAID associated analgesia, suggesting that, in order to investigate the efficacy of drugs whose analgesic effect is mostly based on their anti-inflammatory properties, inflammation must be produced first.

Conflict of interest statement

The authors declare no conflict of interest.

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References

Adami C, Lardone L and Monticelli P (2018) Inter-rater and inter-device reliability of nociceptive threshold testing performed with the Electronic von Frey Anaesthesiometer and the SMall Animal ALGOmeter in healthy cats. J Feline Med Surg; article in press. DOI: 10.1177/1098612X18813426.

Addison ES, Clements DN (2017) Repeatability of nociceptive threshold testing in healthy cats in a clinical setting with comparison to cats with osteoarthritis. J Feline Med Surg 19, 1274-1282.

Ambros B (2016) Effect of pretreatment with hydromorphone or buprenorphine on thermal antinociception induced by fentanyl in awake cats. J Feline Med Surg 18, 818-25.

Journal Pre-proof

544 Ambros B, Duke T (2013) Effect of low dose rate ketamine infusions on thermal and
545 mechanical thresholds in conscious cats. Vet Anaesth Analg 40, 76-82.
546 Ambros B, Steagall PV, Mantovani F, et al. (2009) Antinociceptive effects of epidural
547 administration of hydromorphone in conscious cats. Am J Vet Res 70, 1187-1192.
548 Anderson KV, Pearl GS (1975) C-Fiber activity in feline tooth pulp afferents. Exp
549 Neurol 47, 357-359.
550 Arvidsson S, Larsson M, Larsson H, et al. (2006) Assessment of visceral pain-related
551 pseudo-affective responses to colorectal distension in mice by intracolonic
552 manometric recordings. J Pain 7, 108-118.
553 Beck PW, Handwerker HO, Zimmermann M (1974) Nervous outflow from the cat's
554 foot during noxious radiant heat stimulation. Brain Res 67, 373-386.
555 Berkley KJ, Parmer R (1974) Somatosensory cortical involvement in responses to
556 noxious stimulation in the cat. Exp Brain Res 20, 363-374.
557 Briggs SL, Sneed K, Sawyer DC (1998) Antinociceptive effects of oxymorphone-
558 butorphanol-acepromazine combination in cats. Vet Surg 27, 466-472.
559 Carrozzo MV, Alcorn J, Ambros B (2018) Effects of two fentanyl constant rate
560 infusions on thermal thresholds and plasma fentanyl concentrations in awake cats.
561 Vet Anaesth Analg 45, 831-838.
562 Casey KL, Morrow TJ (1983) Nocifensive responses to cutaneous thermal stimuli in the
563 cat: stimulus-response profiles, latencies, and afferent activity. J Neurophysiol 50,
564 1497-1515.
565 Coleman KD, Schmiedt CW, Kirkby KA, et al. (2014) Learning confounds algometric
566 assessment of mechanical thresholds in normal dogs. Vet Surg 43, 361-267.
567 Dixon MJ, Robertson, Taylor PM (2002) A thermal threshold testing device for
568 evaluation of analgesics in cats. Res Vet Sci 75, 205-210.

Journal Pre-proof

569 Dixon MJ, Taylor PM, Steagall PVM, et al. (2007) Development of a pressure
570 nociceptive threshold testing device for evaluation of analgesics in cats. Res Vet
571 Sci 82, 85-89.
572 Djouhri L, Lawson SN (2004) A beta-fiber nociceptive primary afferent neurons: a
573 review of incidence and properties in relation to other afferent A-fiber neurons in
574 mammals. Brain Res Brain Res Rev 46, 131-145.
575 Doodnaught GM, Evangelista MC and Steagall PVM (2017) Thermal antinociception
576 following oral administration of tapentadol in conscious cats. Vet Anaesth Analg
577 44, 364-369.
578 Doodnaught GM, Monteiro B, Edge D, et al. (2018) Thermal antinociception after
579 buccal administration of a high-concentration formulation of buprenorphine
580 (Simbadol) at 0.24 mg kg^{-1} in conscious cats. Vet Anaesth Analg 45, 714-716.
581 Dubuisson D, Dennis SG (1977) The formalin test: a quantitative study of the analgesic
582 effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain,
583 4: 161-174.
584 Farnworth MJ, Beausoleil NJ, Adams NJ, et al. (2013a) Validating the use of a carbon
585 dioxide laser for assessing nociceptive thresholds in adult domestic cats (*Felis*
586 *catus*). Appl Anim Behav Sci 143, 104-109.
587 Farnworth MJ, Barrett LA, Adams NJ, et al. (2013b) Body weight affects behavioural
588 indication of thermal nociceptive thresholds in adult domestic cats (*Felis catus*).
589 Appl Anim Behav Sci 149, 30-35.
590 Farnworth MJ, Barrett LA, Adams NJ, et al. (2015) Assessment of a carbon dioxide
591 laser for the measurement of thermal nociceptive thresholds following
592 intramuscular administration of analgesic drugs in pain-free female cats. Vet
593 Anaesth Analg 42, 638-647.

Ferreira TH, Rezende ML, Mama KR, et al. (2011) Plasma concentrations and behavioral, antinociceptive, and physiologic effects of methadone after intravenous and oral transmucosal administration in cats. Am J Vet Res 72, 764-771.

Finkel JC, Yang CI, Yarvitz JL, et al. (2002) Neuroselective sensory electrodiagnostic evaluation of 4% liposomal topical lidocaine. Anesth Analg 94, 1259-1262.

Ghoparde A, Advokat C (1994) Evidence of a role for N-methyl-D-aspartate (NMDA) receptors in the facilitation of tail withdrawal after spinal transection. Pharmacol Biochem Behav 48, 175-181.

Giraudel JM, Diquelou A, Lees P (2005) Development and validation of a new model of inflammation in the cat and selection of surrogate endpoints for testing anti-inflammatory drugs. J Vet Pharmacol Ther 28, 275-285.

Giraudel JM, King JN, Jeunesse EC, et al. (2009) Use of a pharmacokinetic/pharmacodynamic approach in the cat to determine a dosage regimen for the COX-2 selective drug robenacoxib. J Vet Pharmacol Ther 32, 18-30.

Haussler KK, Erb HN (2006) Mechanical nociceptive thresholds in the axial skeleton of horses. Equine Vet J 38, 70-75.

Hedges AR, Pypendop BH, Shilo-Benjamini Y, et al. (2014) Pharmacokinetics of buprenorphine following intravenous and buccal administration in cats, and effects on thermal threshold. J Vet Pharmacol Ther 37, 252-259.

Hung CY, Tan CH (2018) TRP Channels in Nociception and Pathological Pain. Adv Exp Med Biol 1099, 13-27.

Journal Pre-proof

Journal Pre-proof

617 Johnson JA, Robertson SA, Pypendop BH (2007) Antinociceptive effects of
618 butorphanol, buprenorphine, or both, administered intramuscularly in cats. Am J
619 Vet Res 68, 699-703.
620 Jones RC, Gebhart GF (2004) Models of visceral pain: colorectal distension (CRD).
621 Curr Protoc Pharmaco 5: Unit 5.36.
622 Kenshalo DR, Duncan DG, Weymark C (1967) Thresholds for thermal stimulation of
623 the inner thigh, footpad, and face of cats. J Comp Physiol Psychol 63, 133-138.
624 Lascelles BD, Robertson SA (2004a) Use of thermal threshold response to evaluate the
625 antinociceptive effects of butorphanol in cats. Am J Vet Res 65, 1085-1089.
626 Lascelles BD, Robertson SA (2004b) Antinociceptive effects of hydromorphone,
627 butorphanol, or the combination in cats. J Vet Intern Med 18, 190-195.
628 Leander JD (1983) Evidence that nalorphine, butorphanol and oxilorphan are partial
629 agonists at a kappa-opioid receptor. Eur J Pharmacol 86, 467-470.
630 Le Bars D, Gozariu M, Cadden SW (2001) Animal models of nociception. Pharmacol
631 Rev 53, 597-652.
632 Lisney SJ (1978) Some anatomical and electrophysiological properties of tooth-pulp
633 afferents in the cat. J Physiol 284, 19-36.
634 Machin H, Kato E, Adami C (2019a). Nociceptive threshold testing with Electronic von
635 Frey Anaesthesiometer and von Frey filaments in nonpainful cats: a pilot study.
636 Vet Anaesth Analg 46, 251-254.
637 Machin H, Pevere S, Adami C (2019b) Inter-observer and intra-observer reliability of
638 nociceptive threshold testing performed with the SMALGO (SMall animal
639 ALGOmeter) to detect and quantify pain associated to feline chronic gingivo-
640 stomatitis. J Feline Med Surg; article in press. DOI:
641 org/10.1177/1098612X19837343

Journal Pre-proof

642 Mao J (2012) Current challenges in translational pain research. Trends Pharmacol Sci,
643 33: 568-573.
644 Millette VM, Steagall PVM, Duke-Novakovski T, et al (2008) Effects of meperidine or
645 saline on thermal, mechanical and electrical nociceptive thresholds in cats. Vet
646 Anaesth Analg 35, 543-547.
647 Moens Y, Lanz F, Doherr MG, et al (2003). A comparison of the antinociceptive effects
648 of xylazine, detomidine and romifidine on experimental pain in horses. Vet
649 Anaesth Analg 30, 183-190.
650 Muir JT, Robertson S (1985) Visceral analgesia: Effects of xylazine, butorphanol,
651 meperidine, and pentazocine in horses. Am J Vet Res 46, 2081-2084.
652 Nathan PW, Smith MC, Cook AW (1986) Sensory effects in man of lesions of the
653 posterior columns and of some other afferent pathways. Brain 109, 1003-1041.
654 Niedfeldt RL, Robertson SA (2006) Postanesthetic hyperthermia in cats: a retrospective
655 comparison between hydromorphone and buprenorphine. Vet Anaesth Analg 33,
656 381-389.
657 Nissen TD, Brock C, Lykkesfeldt J, et al (2018). Pharmacological modulation of
658 colorectal distension evoked potentials in conscious rats. Neuropharmacology 15,
659 193-200.
660 Picker MJ, Negus SS, Craft RM (1990) Butorphanol's efficacy at mu and kappa opioid
661 receptors: inferences based on the schedule-controlled behavior of nontolerant and
662 morphine-tolerant rats and on the responding of rats under a drug discrimination
663 procedure. Pharmacol Biochem Behav 36, 563-368.
664 Porters N, Bosmans T, Debille M et al. (2014) Sedative and antinociceptive effects of
665 dexmedetomidine and buprenorphine after oral transmucosal or intramuscular
666 administration in cats. Vet Anaesth Analg 41, 90-96.

Journal Pre-proof

667 Posner LP, Gleed RD, Erb HN, et al. (2007) Post-anesthetic hyperthermia in cats. Vet
668 Anaesth Analg 34: 40-47.
669 Posner LP, Pavuk AA, Rokshar JL, et al. (2010) Effects of opioids and anesthetic drugs
670 on body temperature in cats. Vet Anaesth Analg 37, 35-43.
671 Pypendop BH, Ilkiw JE, Robertson SA (2006) Effects of intravenous administration of
672 lidocaine on the thermal threshold in cats. Am J Vet Res 67, 16-20.
673 Pypendop BH, Siao KT, Ilkiw JE, et al. (2009) Effects of tramadol hydrochloride on the
674 thermal threshold in cats. Am J Vet Res 70, 1465-1470.
675 Pypendop BH, Siao KT, Ilkiw JE (2010) Thermal antinociceptive effect of orally
676 administered gabapentin in healthy cats. Am J Vet Res 71, 1027-1032.
677 Pypendop BH, Shilo-Benjamini Y, Ilkiw JE (2016) Effect of morphine, methadone,
678 hydromorphone or oxymorphone on the thermal threshold, following intravenous
679 or buccal administration to cats. Vet Anaesth Analg 43, 635-642.
680 Robertson SA, Taylor PM, Lascelles BD, et al. (2003) Changes in thermal threshold
681 response in eight cats after administration of buprenorphine, butorphanol and
682 morphine. Vet Rec 153, 462-465.
683 Robertson SA, Lascelles BD, Taylor PM, et al. (2005a) PK-PD modeling of
684 buprenorphine in cats: intravenous and oral transmucosal administration. J Vet
685 Pharmacol Ther 28, 453-460.
686 Robertson SA, Taylor PM, Sear JW (2005b). Relationship between plasma
687 concentrations and analgesia after intravenous fentanyl and disposition after other
688 routes of administration in cats. J Vet Pharmacol Ther 28, 87-93.
689 Robertson SA, Wegner K, Lascelles BD (2009) Antinociceptive and side-effects of
690 hydromorphone after subcutaneous administration in cats. J Feline Med Surg 11,
691 76-81.

Journal Pre-proof

692 Sawyer DC, Rech RH (1987) Analgesia and behavioral effects of butorphanol,
693 nalbuphine, and pentazocine in the cat. J Am Anim Hosp Assoc 23, 438-446.
694 Sawyer DC, Rech RH, Durham RA (1993) Does ketamine provide adequate visceral
695 analgesia when used alone or in combination with acepromazine, diazepam, or
696 butorphanol in cats? J Am Anim Hosp Assoc 29, 257-263.
697 Sakai T, Tomiyasu S, Yamada H, et al. (2004) Quantitative and selective evaluation of
698 differential sensory nerve block after transdermal lidocaine. Anesth Analg 98,
699 248-251.
700 Sanchez LC, Merritt AM (2005) Colorectal distention in the horse: visceral sensitivity,
701 rectal compliance and effect of i.v. xylazine or intrarectal lidocaine. Equine Vet J
702 37(1): 70-74
703 Sawyer DC, Rech RH (1987) Analgesia and behavioral effects of butorphanol,
704 nalbuphine, and pentazocine in the cat. J Am Animal Hosp Assn 23, 438-446.
705 Scallan EM, Lizarraga I, Coursey CD, et al. (2019) Thermal antinociceptive, sedative
706 and cardiovascular effects of Governing Vessel 1 dexmedetomidine
707 pharmacopuncture in healthy cats. Vet Anaesth Analg 46, 529-537.
708 Siao KT, Pypendop BH, Escobar A, et al. (2012) Effect of amantadine on
709 oxymorphone-induced thermal antinociception in cats. J Vet Pharmacol Ther 35,
710 169-174.
711 Simon BT, Steagall PV, Monteiro BP, et al. (2016) Antinociceptive effects of
712 intravenous administration of hydromorphone hydrochloride alone or followed by
713 buprenorphine hydrochloride or butorphanol tartrate to healthy conscious cats.
714 Am J Vet Res 77, 245-251.
715 Simon BT, Scallan EM, Baetge CL, et al (2019) The antinociceptive effects of
716 intravenous administration of three doses of butorphanol tartrate or naloxone

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717 hydrochloride following hydromorphone hydrochloride to healthy conscious cats.
718 Vet Anaesth Analg 46, 538-547.
719 Simon BT, Scallan EM, Monteiro BP, et al. (2019b) The effects of aging on
720 hydromorphone-induced thermal antinociception in healthy female cats. Pain Rep
721 4, e722.
722 Slingsby LS, Jones A, Waterman-Pearson AE (2001) Use of a new finger-mounted
723 device to compare mechanical nociceptive thresholds in cats given pethidine or no
724 medication after castration. Res Vet Sci 70, 243-246.
725 Slingsby S, Murrell JC, Taylor PM (2010) Combination of dexmedetomidine with
726 buprenorphine enhances the antinociceptive effect to a thermal stimulus in the cat
727 compared with either agent alone. Vet Anaesth Analg 37, 162-170.
728 Slingsby LS, Murrell JC, Taylor PM (2012) Buprenorphine in combination with
729 naloxone at a ratio of 15:1 does not enhance antinociception from buprenorphine
730 in healthy cats. Vet J 192, 523-524.
731 Slingsby LS, Taylor PM (2008) Thermal antinociception after dexmedetomidine
732 administration in cats: a dose-finding study. J Vet Pharmacol Ther 31, 135-142.
733 Slingsby LS, Taylor PM, Monroe T (2009) Thermal antinociception after
734 dexmedetomidine administration in cats: a comparison between intramuscular and
735 oral transmucosal administration. J Feline Med Surg 11, 829-34.
736 Slingsby LS, Waterman-Pearson AE (2000) Postoperative analgesia in the cat after
737 ovariohysterectomy by use of carprofen, ketoprofen, meloxicam or tolfenamic
738 acid. J Small Anim Pract 41, 447-450.
739 Sneddon LU (2017) Comparative physiology of nociception and pain. Physiol 33, 63-
740 73.

Journal Pre-proof

741 Steagall PVM, Carnicelli P, Taylor PM, et al. (2006) Effects of subcutaneous
742 methadone, morphine, buprenorphine or saline on thermal and pressure thresholds
743 in cats. J Vet Pharmacol Ther 29, 531-537.
744 Steagall PV, Taylor PM, Brondani JT, et al. (2007) Effects of buprenorphine, carprofen
745 and saline on thermal and mechanical nociceptive thresholds in cats. Vet Anaesth
746 Analg 34, 344-350.
747 Steagall PV, Taylor PM, Brondani JT, et al. (2008) Antinociceptive effects of tramadol
748 and acepromazine in cats. J Feline Med Surg 10, 24-31.
749 Steagall PVM, Pelligand L, Giordano T, et al. (2013) Pharmacokinetic and
750 pharmacodynamic modelling of intravenous, intramuscular and subcutaneous
751 buprenorphine in conscious cats. Vet Anaesth Analg 40, 83-95.
752 Steagall PV, Monteiro BP, Lavoie AM, et al. (2015) A preliminary investigation of the
753 thermal antinociceptive effects of codeine in cats. J Feline Med Surg 17, 1061-
754 1064.
755 Steagall PVM, Taylor PM, Brondani JT (2007) Effects of buprenorphine, carprofen and
756 saline on thermal and mechanical nociceptive thresholds in cats. Vet Anaesth
757 Analg 34, 344-350.
758 Steagall PV, Taylor PM, Brondani JT, et al. (2008) Antinociceptive effects of tramadol
759 and acepromazine in cats. J Feline Med Surg 10, 24-31.
760 Svensson P, Rosenberg B, Beydoun A, et al. (1997) Comparative psychophysical
761 characteristics of cutaneous CO2 laser and contact heat stimulation. Somatosens
762 Mot Res 14, 113-118.
763 Taylor PM, Luangdilok CH, Sear JW (2016) Pharmacokinetic and pharmacodynamic
764 evaluation of high doses of buprenorphine delivered via high-concentration
765 formulations in cats. J Feline Med Surg 18, 290-302.

Journal Pre-proof

766 Taylor PM, Robertson SA, Dixon MJ (2007) Evaluation of the use of thermal thresholds
767 to investigate NSAID analgesia in a model of inflammatory pain in cats. J Feline
768 Med Surg 9, 313-318.
769 Treede RD, Jahnke MT, Bromm B (1984). Functional properties of CO_2 laser activated
770 nociceptive fibers in intact human skin nerve. In: Pain Measurement in men;
771 Neurophysiological correlates of pain (Bromm B Ed), pp 65-78, Elsevier,
772 Amsterdam.
773 Voets T, Droogmans G, Wissenbach U, et al. (2004). The principle of temperature-
774 dependent gating in cold- and heat-sensitive TRP channels. Nature 430, 748–754.
775 Wegner K, Robertson SA (2007) Dose-related thermal antinociceptive effects of
776 intravenous hydromorphone in cats. Vet Anaesth Analg 34, 132-138.
777 Wegner K, Robertson SA, Kollias-Baker C, et al. (2004) Pharmacokinetic and
778 pharmacodynamic evaluation of intravenous hydromorphone in cats. J Vet
779 Pharmacol Ther 27, 329-336.
780 Watabiki T, Nagakura Y, Wegner K (2010) Assessment of canine sensory function by
781 using sine-wave electrical stimuli paradigm. Physiol Behav 101, 327-330.
782 Woodworth RS, Sherrington CS (1904) A pseudoaffective reflex and its spinal path. J
783 Physiol (Lond) 31, 234-243.
784 Yeomans DC, Proudfit HK (1996) Nociceptive responses to high and low rates of
785 noxious cutaneous heating are mediated by different nociceptors in the rat:
786 electrophysiological evidence. Pain 68, 141-150.
787

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Table 1. Studies investigating the use of mechanical thresholds in both experimental and clinical cats (in chronological order)

Study (authors, year and publication type)	Number of cats	Type of algometer	Sensitive probe characteristics	Pressure/ force reached (range/ cut off value)	Area tested	Analgesics tested	End-point behavioural response as described by the authors	Baseline thresholds (site and time of measurement)
Briggs et al. 1998 (research paper)	8	Silastic balloon catheter inserted per rectum and connected to a pressurised plastic jug	NA	Approximately 0-50 mmHg (no cut-off)	Rectal mucosa	IV butorphanol, oxymorphone and ACP alone and in combination (saline as negative control group)	Stretching of the hind limbs, abdominal muscular contraction, back arching, changes in breathing pattern	Control thresholds measured before any drug administration
Slingsby & Waterman-Pearson 2000 (research paper)	40	Pressure FSR finger-mounted algometer	15 mm diameter flat surface	0.75-0.95 N (no cut-off)	Surgical wound (OVH)	SC Carprofen, Ketoprofen, Meloxicam and Tolfenamic acid	Flinch away from pressure	At the left flank, before premedication
Slingsby et al. 2001 (research paper)	40	Pressure FSR finger-mounted algometer	15 mm diameter flat surface	0-4 N (no cut-off)	Scrotum	IM meperidine (versus no-pethidine as negative control)	Cat pulling away	At the scrotum, before surgery
Dixon et al. 2007 (research paper)	11	ProD Plus pressure algometer	Three pins, each tipped with a 2.4 mm diameter ball – bearing pin	600 mmHg (cut-off)	Forearm	SC Butorphanol and Carprofen	Picking up the leg and shaking it, turning the head towards bracelet, licking/biting bracelet, vocalisation	Forearm, before kaolin injection
Ferreira et al. 2011 (research paper)	8	Two different devices: a C clamp and a mechanical algometer	1-cm^2 circular tip	5 and 20 kg cm^2 (cut-off) for the C clamp and the algometer, respectively	Metacarpus and antebrachium	IV and OTM methadone	Cat turning its head toward the stimulus, moving away from the stimulus, vocalising, or attempting to bite	Control thresholds measured before methadone administration
Porters et al. 2014 (research paper)	6	ProD Plus pressure algometer	4 mm diameter probe	20 N (cut off)	Pectoral muscle (shoulder joint)	Combination of dexmedetomidine and buprenorphine either IM or OTM	Jumping, limb withdrawal, head turning, vocalisation	Control thresholds measured before any drug administration

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Adami et al. 2018 (research paper)	13	EVF and SMALGO	0.8 mm diameter rigid tip (EVF) and 3 mm diameter pointed tip (SMALGO)	0-1000 g (EVF) and 0-1500 g (SMALGO)	Lumbosacral joint and medial aspect of the stifle	NA (reliability/repeatability study)	Attempts to escape, tail wiggling, hissing, attempts to bite, aggression, ears back and flat against the head, head turning towards the stimulation site, back muscle contraction and limb withdrawal	NA
Machin et al. 2019a (short communication)	15	EVF and VFF	Probe equipped with 0.8 mm diameter rigid tip (EVF)	0-1000 g (EVF) and 0.008-300 g (VFF)	Upper lip and medial aspect of the stifle	NA (reliability/ validation study)	Limb/head withdrawal, head turning, watching the application site, vocalisation, hissing, attempts to bite/scratch	NA
Machin et al. 2019b (research paper)	30 (15 healthy cats and 15 cats with CGS)	SMALGO	3 mm diameter pointed tip	0-1500 g	Upper lip above the canine root	NA (reliability /repeatability study)	Limb/head withdrawal, head turning, watching the application site, vocalisation, hissing, attempts to bite/scratch	NA

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Table legend: NA: not applicable; IV: intravenous; ACP: Acepromazine; MT: mechanical thresholds; OVH: Ovariohysterectomy; FSR: Force-Sensing Resistor; IM: intramuscular; SC: subcutaneous; NSAIDs: Non-Steroidal Anti-inflammatory Drugs; EVF: Electronic von Frey Anaesthesiometer; OTM: oral transmucosal; VFF: von Frey filaments; SMALGO: Small Animal Algometer; CGS: Chronic Gingivo Stomatitis.

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Table 2. Studies investigating the use of thermal thresholds in experimental cats (in chronological order)

Study (authors, year and publication type)	Number of cats	Type of algometer	Probe characteristics	Temperature reached (range/ cut off value)	Area tested	Analgesics tested	End-point behavioural response as described by the authors	Baseline thresholds (site and time of measurement)
Casey & Morrow 1983 (research paper)	29	Thermal algometer	Spring-loaded, water-cooled contact thermodes	43-60°C	Shaved outer thighs	NA	Vocalisation, movement of the stimulated limb, interruption of eating/approaching food	NA
Dixon et al. 2002 (research paper)	14	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	60ºC (cut-off)	Shaved skin of the dorso-lateral thorax	IM meperidine (n=6 out of 14 cats)	Visible (non-defined) reaction of the cat to the application of the stimulus	Shaved thorax before meperidine
Robertson et al. 2003 (research paper)	8	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the thorax	IM saline, morphine, buprenorphine or butorphanol (n=6 cats per group)	Flinching, turning or jumping	Before any treatment
Wegner et al. 2004 (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the thorax	IV hydromorphone	Flinching, turning or jumping	Before the analgesic treatment
Lascelles & Robertson 2004a (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the thorax	IV butorphanol (four different doses)	Flinching, turning or jumping	Shaved thorax before the analgesic treatment
Lascelles & Robertson 2004b (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the thorax	IM hydromorphone, butorphanol and combination of both	Flinching or twitching of the skin, jumping forward, turning to bite the probe	Shaved thorax before any analgesic treatment
Robertson et al. 2005a	6	Top Cat Metrology	As described by Dixon et al.	55ºC (cut-off)	Shaved area of	IV and OTM buprenorphi	Flinching, turning or	Shaved thorax

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(research paper)		thermal algometer	2002		the thorax	ne	jumping	before any analgesic treatment
Robertson et al. 2005b (research paper)	10	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV (n=10), TC (n=4), PO (n=2) and IN (n=2) fentanyl	Flinching, turning or jumping	Shaved thorax before any analgesic treatment
Pypendop et al. 2006 (short communicati on)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV lidocaine (saline as negative control group)	Jumping, flinching, turning towards the probe, licking or biting the probe area	Shaved thorax before any treatment
Johnson et al. 2007 (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the thorax	IM butorphanol, buprenorphi ne and combination of both	Turning to bite the probe, jumping away from the probe, jumping up from a recumbent position	Shaved thorax before any analgesic treatment
Wegner & Robertson 2007 (research paper)	7	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved skin of the lateral thorax	IV hydromorph one	Jumping, flinching or turning toward the probe	Shaved thorax before any analgesic treatment
Slingsby & Taylor 2008 (research paper)	12	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Various shaved areas of the thorax	Buprenorphi ne (n=12, dexmedetom idine at four different doses (n=10 each) and control saline (n=12)	Skin twitch, jumping or turning head towards the stimulus	Shaved thorax before any treatment
Pypendop et al. 2009 (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	PO tramadol (versus placebo)	Jumping, turning the head toward the probe, licking or biting the probe area or cable	Shaved thorax before the analgesic treatment
Robertson et al. 2009 (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	SC hydromorph one	Flinching, jumping or turning to look at the probe	Shaved thorax before the analgesic treatment

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Slingsby et al. 2009 (research paper)	12	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IM and OTM dexmedetomidine	Skin twitch, jumping or turning head towards the stimulus	Shaved thorax before the analgesic treatment
Pypendop et al. 2010 (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	PO gabapentin (versus placebo)	Jumping, turning the head toward the probe, licking or biting the probe area or cable	Shaved thorax before the analgesic treatment
Slingsby et al. 2010 (research paper)	12	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IM buprenorphine (2 different doses), dexmedetomidine (two different doses) and their association (the lowest dose of each)	Skin twitch, jumping or turning the head towards the stimulus	Shaved thorax before drugs administration
Siao et al. 2012 (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV oxymorphone and amantadine (oxymorphone and saline as control group)	Jumping, turning the head towards the probe, licking or biting the probe or cable	Shaved thorax before any treatment
Steagall et al. 2013 (research paper)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV, IM and SC bupernorphine	Flinching, jumping or turning to look at the probe	Shaved lateral thorax before analgesic treatment
Farnworth et al. 2013 a (research paper)	16	Remote carbon dioxide laser	5 mm diameter carbon dioxide beam guided by visible helium laser	Power output 165 mW for all cats	Two shaved areas of the lateral thorax, 4 cm^2 each	NA (validation study)	Moving away from the stimulus or exhibition of the panniculus reflex	NA
Farnworth et al. 2013b (research paper)	113	Remote laser device (Model 48-1, Synrad, Mulkiteo, USA)	3.5 mm diameter carbon dioxide beam guided by visible helium laser	500 mW was used; maximum power output of the device	Two shaved areas of the lateral thorax, 4	NA	Significant shifting (i.e. rising to its feet) or panniculus reflex	NA

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				set at 10 W	cm^2 each			
Ambros 2015 (research paper)		Top Cat Metrology thermal algometer (wireless)	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV hydromorphone or buprenorphine followed by a bolus of IV fentanyl (saline as negative control group)	Jumping, flinching, turning towards the probe, licking or biting the probe area	Shaved thorax before any treatment
Farnworth et al. 2015 (research paper)	60	Remote carbon dioxide laser	5 mm diameter carbon dioxide beam guided by visible helium laser	Maximum power output 10 W (cut-off)	Skin of both sides of the thorax	IM morphine, buprenorphine, medetomidine, tramadol, ketoprofen and saline (control)	Significant shifting (i.e. rising to its feet) or panniculus reflex	Skin of both sides of thorax before any treatment
Steagall et al. 2015 (short communication)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	PO codeine and OTM buprenorphine	Jumping, flinching, vocalisation, turning towards the probe	Shaved thorax before any treatment
Simon et al. 2016 (research paper)	8	Top Cat Metrology thermal algometer (wireless)	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV hydromorphone, alone and combined with either butorphanol or buprenorphine (saline as negative control group)	Flinching, vocalisation, rolling, jumping, turning the head towards the probe	Shaved thorax before any treatment
Pypendop et al. 2016	8	Top Cat Metrology thermal algometer (wireless)	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	Morphine, methadone or oxymorphone, administered either IV or OTM	Jumping, turning the head towards the probe, licking or biting the probe or cable	Shaved thorax before any treatment
Taylor et al. 2016 (research paper)	12	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the thorax	SC buprenorphine (at three different doses and different formulation)	Skin flick, jumping forward, turning to bite the band, vocalisation	Shaved thorax before any treatment

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Doodnaught et al. 2017	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the thorax	PO tapentadol (two different doses) (versus IM buprenorphine as positive control and placebo as negative control)	Vocalisation, rolling, jumping	Shaved thorax after 30 min acclimatisation, before the analgesic treatment
Doodnaught et al. 2018 (Letter to the Editor)	6	Top Cat Metrology thermal algometer	As described by Dixon et al. 2002	Non-specified	Non-specified	OTM buprenorphine	Non-specified	Before the analgesic treatment
Carrozzo et al. 2018 (research paper)	6	Top Cat Metrology thermal algometer (wireless)	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV fentanyl at two rates of infusion (5 and 3 μg kg hour^{-1})	Flinching, jumping, turning the head towards the probe, licking or biting the probe area, changing body position	Shaved thorax before the analgesic treatment
Scallan et al. 2019 (research paper)	8	Top Cat Metrology thermal algometer (wireless)	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	Dexmedetomidine either IM or at acupuncture point GV1 (same dose)	Flinching, skin twitch, further dilation of pupils, acute changes in facial conformation, intentional look or motion toward the probe, vocalisation	Shaved thorax before the analgesic treatment
Simon et al. 2019 (research paper)	8	Top Cat Metrology thermal algometer (wireless)	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV hydromorphone, alone and combined with either butorphanol or naloxone (saline as negative control group)	Flinching, vocalisation, rolling, jumping, turning the head towards the probe	Shaved thorax before any treatment
Simon et al. 2019b (research paper)	10 (all treated at 6,9 and 12 months of age)	Top Cat Metrology thermal algometer (wireless)	As described by Dixon et al. 2002	55ºC (cut-off)	Shaved area of the lateral thorax	IV hydromorphone (saline as negative control group)	Flinching, vocalisation, rolling, jumping, turning the head towards the probe	Shaved thorax before any treatment

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Table legend: NA: not applicable; GV1: Governing Vessel 1; IV: intravenous; IM: intramuscular; SC: subcutaneous; TT: thermal thresholds; SL: sublingual; TC: transcutaneous (compounded in pluronic lecithin organogel PLO); OTM: oral transmucosal; PO: oral administration; IN: intranasal.

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1 **Table 3. Studies investigating (and comparing) different thresholds in experimental cats (in chronological order)**

Study (authors, year and publication type)	Number of cats	Type of algometer	Sensitive probe characteristics	Pressure/ force /Temperature reached (range/ cut off value)	Area tested	Analgesics tested	End-point behavioural response as described by the authors	Baseline thresholds (site and time of measurement)	Application mode	Main results
Steagall et al. 2006	8	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	55 ºC (cut-off)	Shaved area of the thorax	SC buprenorphine, morphine, methadone or saline as negative control	Skin flicking, jumping forward, turning to bite the band, vocalisation	Shaved thorax, prior to any treatment administration	Manual, 0.6ºC sec $^{-1}$ (until end-point response or cut-off reached)	Morphine was the most effective treatment in increasing both TT and MT, as compared to both control group and baseline thresholds
		ProD Plus pressure algometer	Three pins, each tipped with a 2.4 mm diameter ball – bearing pin	650 mmHg (cut-off)	One forearm		Leg shake, head turn and/or vocalisation	Same forearm, prior to any treatment administration	Manual, no time limit (until end-point	
Steagall et al. 2007 (research paper)	8	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	55 ºC (cut-off)	Shaved area of the thorax	SC buprenorphine, carprofen or saline as negative control	Skin flicking, jumping forward, turning to bite the band, vocalisation	Shaved thorax, prior to any treatment administration	Manual, 0.6ºC sec $^{-1}$ (until end-point response or cut-off reached)	Both nociceptive models were effective in detecting buprenorphine analgesia (although the thermal model was superior to the mechanical one), but failed to detect carprofen analgesia
		ProD Plus pressure	Three pins, each tipped	650 mmHg (cut-off)	Craniolateral		Leg shake, head turn	Antebrachium, prior	Manual, no time limit	

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		algometer	with a 2.4 mm diameter ball – bearing pin		surface of one antebrachium		and/or vocalisation	to any treatment administration	(until end-point	
Steagall et al. 2008 (research paper)	8 (crossover)	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	55 ºC (cut-off)	Shaved area of the thorax	SC tramadol, ACP, and their combination (saline as negative control)	Skin flicking, jumping forward, turning to bite the cable	Shaved thorax, prior to any treatment administration	Manual, 0.6ºC sec^{-1} (until end-point response or cut-off reached)	SC tramadol had limited effect on both TT and MT, whereas the combination ACP + tramadol increased both TT and MT
		ProD Plus pressure algometer	Three pins, each tipped with a 2.4 mm diameter ball – bearing pin	650 mmHg (cut-off)	One forearm		Leg shake, head turn, biting at the probe, vocalisation	Same forearm, prior to any treatment administration	Manual, no time limit (until end-point	
Millette et al. 2008 (short communication)	8	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	55 ºC (cut-off)	Shaved area of the thorax	IM meperidine (saline as negative control)	Skin flicking, jumping forward, turning to bite the band, vocalisation	Shaved thorax, prior to any treatment administration	Manual, 0.6ºC sec^{-1} (until end-point response or cut-off reached)	Electrical nociception failed to detect meperidine analgesia, whereas both TT and MT were found useful for this purpose.
		ProD Plus pressure algometer	Three pins, each tipped with a 2.4 mm diameter ball – bearing pin	850 mmHg (cut-off)	Shaved thoracic limb		Picking up and shaking the leg, turning the head towards the bracelet, licking or biting the bracelet, vocalisation	Shaved thoracic limb, prior to any treatment administration	Manual, no time limit (until end-point	
		(Model	Constant Unit	5 mA (cut-	Clipped		Attempts to	Clipped	Continuous	

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		CCU1 Constant Current Unit; Astro-Med Inc	Generator equipped with a Grass stimulator, delivering stimuli via two skin electrodes	off)	area of the mid-thorax		look at, lick or bite the electrodes	area of the mid-thorax, prior to any treatment administration	deliver of stimuli at a rate of 1 mA sec^{-1} (duration of 1 ms with 1 ms delay between pulses)	
Ambros et al. 2009 (short communication)	7	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	As described by Dixon et al. 2002	As described by Dixon et al. 2002	Epidural hydromorphone (epidural saline as negative control)	As described by Dixon et al. 2002	Pre-treatment thresholds measured	As described by Dixon et al. 2002	Epidural administration of hydromorphone increased both MT and TT values (compared to both saline and baseline values)
		ProD Plus pressure algometer	Three pins, each tipped with a 2.4 mm diameter ball – bearing pin	As described by Dixon et al. 2007	As described by Dixon et al. 2007		As described by Dixon et al. 2007	Pre-treatment thresholds measured	As described by Dixon et al. 2007	
Slingsby et al. 2012 (short communication)	12	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	As described by Dixon et al. 2002	As described by Dixon et al. 2002	IM buprenorphine, naloxone and their combination	As described by Dixon et al. 2002	Pre-treatment thresholds measured	As described by Dixon et al. 2002	MT were not affected by buprenorphine treatment, whereas TT increased after buprenorphine administration compared to baseline; naloxone antagonised the thermal antinociceptive effect of buprenorphine
		ProD Plus pressure algometer	Three pins, each tipped with a 2.4 mm	As described by Dixon et	As described by Dixon		As described by Dixon et al. 2007	Pre-treatment thresholds	As described by Dixon et al. 2007	

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			diameter ball – bearing pin	al. 2007	et al. 2007			measured		
Ambros & Duke 2013 (research paper)	8	Top Cat Metrology thermal algometer	10 mm long, 10 mm wide and 5 mm deep probe containing a heater element	55 ºC (cut-off)	Shaved area of the thorax	IV ketamine CRI, delivered for two hours after loading dose, at two different rates	Jumping, flinching, turning towards the probe or licking/biting the probe area	Shaved thorax prior to any treatment administration	Manual, 0.6ºC sec^{-1} (until end-point response or cut-off reached)	Only the low dose of ketamine minimally affected both TT and MT. The results were inconclusive and ketamine analgesia could not be demonstrated with these nociceptive models
		ProD Plus pressure algometer	Three pins, each tipped with a 2.4 mm diameter ball – bearing pin	20 N (cut-off)	Anterolateral aspect of the antebrachium		Withdrawing, raising or shaking the limb, jumping forwards/turned forwards, trying to bite the actuator	Same forearm prior to any treatment administration	Manual, applying force increasing at 0.8 N sec^{-1}	
Addison & Clements 2017 (research paper)	21 (n=14 healthy cats and n=7 cats with OA)	Temperature-controlled thermal aluminium platform	NA	7ºC (cold plate) and 40ºC (hot plate)	Paws	NA (comparison between healthy cats and cats with OA)	Walking off the plate and number of times and duration that each paw was lifted off the plate	NA	Behavioural observation after 10 second habituation period	MT, measured with both EVF and VFF, were lower in cats with OA than in the healthy ones. Regarding TT, only the cold ones allowed differentiation between healthy and diseased limbs
		EVF and VFF	Probe equipped with 0.8 mm diameter rigid tip (EVF)	Up to 400 g (cut-off EVF) and 0.008-300 g (VFF)	Plantar or palmar aspects of the metacarpal or		Paw withdrawal (prior to filament buckling for VFF)		Manual, no time limit (until end-point response)	

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		metatarsal pad, respectively		

Table legend: SC: subcutaneous; IM: intramuscular; TT: thermal thresholds; MT: mechanical thresholds; ACP: Acepromazine; OA: Osteoarthritis; EVF: Electronic von Frey Anaesthesiometer; VFF: Von Frey Filaments

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