

## RESEARCH PAPER

**Assessment of a carbon dioxide laser for the measurement of thermal nociceptive thresholds following intramuscular administration of analgesic drugs in pain-free female cats**

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**Abstract**

**Objective** To assess the potential of a thermal carbon dioxide (CO<sub>2</sub>) laser to explore antinociception in pain-free cats.

**Study design** Experimental, prospective, blinded, randomized study.

**Animals** Sixty healthy adult female cats with a (mean ± standard deviation) weight of 3.3 ± 0.6 kg.

**Methods** Cats were systematically allocated to one of six treatments: saline 0.2 mL per cat; morphine 0.5 mg kg<sup>-1</sup>; buprenorphine 20 µg kg<sup>-1</sup>; medetomidine 2 µg kg<sup>-1</sup>; tramadol 2 mg kg<sup>-1</sup>, and ketoprofen 2 mg kg<sup>-1</sup>. Latency to respond to thermal stimulation was assessed at baseline and at intervals of 15–30, 30–45, 45–60, 60–75, 90–105 and 120–135 minutes. Thermal thresholds were assessed using time to respond behaviourally to stimulation with a 500 mW CO<sub>2</sub> laser. Within-treatment differences in response latency were assessed using Friedman's test. Differences amongst treatments were assessed using independent Kruskal–Wallis tests. Where significant effects were identified, pair-

wise comparisons were conducted to elucidate the direction of the effect.

**Results** Cats treated with morphine ( $\chi^2 = 12.90$ ,  $df = 6$ ,  $p = 0.045$ ) and tramadol ( $\chi^2 = 20.28$ ,  $df = 6$ ,  $p = 0.002$ ) showed significant increases in latency to respond. However, subsequent pairwise comparisons indicated that differences in latencies at specific time-points were significant ( $p < 0.05$ ) only for tramadol at 60–75 and 90–105 minutes after administration (21.9 and 43.6 seconds, respectively) in comparison with baseline (11.0 seconds). No significant pairwise comparisons were found within the morphine treatment. Injections of saline, ketoprofen, medetomidine or buprenorphine showed no significant effect on latency to respond.

**Conclusions and clinical relevance** The CO<sub>2</sub> laser technique may have utility in the assessment of thermal nociceptive thresholds in pain-free cats after analgesic administration and may provide a simpler alternative to existing systems. Further exploration is required to examine its sensitivity and comparative utility.

**Keywords** analgesia, behaviour, CO<sub>2</sub> lasers, domestic cat, nociception tests.

## Introduction

The domestic cat (*Felis catus*) was previously considered to have been underexplored in terms of its responses to pain and analgesia, but significant advances have since been made (Robertson 2008). Evidence suggests that the cat, as a species, displays substantial variation in its responses to different classes of analgesic compounds (Taylor et al. 2001; Robertson & Taylor 2004). Likewise, there appears to be substantial inter-individual variation in the effects and pharmacodynamics of specific analgesics, particularly for opioids (Lascelles & Robertson 2004; Johnson et al. 2007; Giordano et al. 2010; Steagall et al. 2013). These differences, as well as variations in injuries and clinical procedures, make the extrapolation of effects from other species, or even between individuals of the same species, difficult (Steagall & Monteiro-Steagall 2013). Research into techniques that allow pain and analgesic effects in cats to be objectively assessed is therefore prudent.

Thermal assessment techniques have been validated for use in cats. These include both methods using contact devices (Dixon et al. 2002) and those using remote carbon dioxide (CO<sub>2</sub>) laser stimulation (Farnworth et al. 2013a). Although methods using contact devices have been extensively explored and applied (Robertson et al. 2003; Steagall et al. 2007; Taylor et al. 2007a), the latter technique has been validated only in terms of its intra-individual repeatability (Farnworth et al. 2013a) and inter-individual variability (Farnworth et al. 2013b). It has not yet been used to explore the effects of pharmacological manipulation of nociceptive thresholds. Research in other species suggests that the CO<sub>2</sub> laser may be a valid tool for the assessment of nociception (Herskin et al. 2003; Guesgen et al. 2011; Di Giminiani et al. 2013), although its ability to measure variations in pain experienced post-castration are inconclusive (Ting et al. 2010). The fact that the laser technique can be potentially used with only moderate alteration of management routines and without the substantial need for habituation required by other techniques (Slingsby & Taylor 2008; Slingsby et al. 2010) suggests it may represent a useful tool if validated further.

This research sought to explore the effectiveness of a CO<sub>2</sub> thermal laser for the assessment of nociceptive thresholds in pain-free cats under analgesia. If this technique is to be considered useful for the assessment of analgesia, latency to display a behavioural response should show distinctions among cats

treated with any one of the five compounds known to have analgesic effects (morphine, buprenorphine, tramadol, ketoprofen and medetomidine) and a saline-treated control group. We hypothesized that latency to respond to thermal stimulation would differ within the morphine, buprenorphine, tramadol and medetomidine treatment groups over the duration of the test period, but not in cats treated with saline or ketoprofen, which has peripheral anti-inflammatory effects. Inflammation was likely to be absent in these test subjects.

## Materials and methods

### Cats and housing conditions

All procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 12/109). A total of 60 adult female domestic cats were used, including 32 entire and 28 spayed animals, with a mean  $\pm$  standard deviation (SD) weight of  $3.3 \pm 0.6$  kg and age of  $6.1 \pm 3.1$  years. The cats were permanently housed in a nutritional research facility in stable colonies of 10 individuals. Each colony was housed in an outdoor pen (2.4 m in height, 1.4 m in width, 4.4 m in depth), approximately half the volume of which was under cover. Records for the cats included (which were updated weekly) showed no longterm medical conditions, abnormal gait or substantial fluctuations in weight. The cats were therefore considered to be healthy and pain-free, although no blood analyses were performed to categorically confirm this. As treatment allocation was determined only shortly before the commencement of the experiment, food was not withheld in the colony housing and all subjects were fed a standard wet cat food diet *ad libitum* throughout the trial. Adverse side effects of treatment, such as excessive salivation or vomiting, were recorded during the experimental phase.

During testing, cats were individually held in eight metabolism cages (0.8 m in height, 0.8 m in width, 1.1 m in depth) in a non-climate-controlled room adjacent to, but separate from, the colony housing area (see Hendriks et al. 1999). These cages were regularly used for nutritional trials during which the cats were isolated and allowed to feed. The cats were therefore familiar with the cages and single housing, and thus there was no need to acclimatize the subjects. Prior to the cat being introduced to the cage, the depth of each cage was reduced to 0.55 m using a cardboard wall to ensure the cat did not have

access to a shelf at the rear of the cage and to prevent the reflection of the laser from the plastic rear wall. The metal cage door was replaced with a plasticated square mesh with 25 × 25 mm openings to prevent reflection of the laser and subsequent injury to the subjects or operators. For the cats' comfort, and to encourage sternal recumbency, each cage was furnished with a small wooden box, blanket and litter tray. Food and water were not provided in the individual cages during the test phase.

#### Laser device

Thermal nociceptive thresholds were measured using a remote laser device (Model 48-1; Synrad, Inc., WA, USA), which was mounted on a tripod to allow movement through vertical and horizontal planes. The CO<sub>2</sub> laser produced a beam measuring 3.5 mm in diameter, which was aimed using a non-thermal visible helium laser (JG-4A Class IIIA, wavelength 532 nm) attached to the external casing. The wavelength of the thermal laser was 10.6 μm (far infra-red) and the maximum power output was 10 W. For the purposes of this experiment, a 5% output was used (500 mW). As the non-visible component of the laser was potentially hazardous, safety goggles were employed by the experimenters at all times.

The visible (non-thermal) helium laser used to guide the thermal CO<sub>2</sub> laser has previously been demonstrated to have no discernible effect on the behavioural response latency of cats (Farnworth et al. 2013a) and hence was not used as a control in this experiment. In a previous study using cats, all responses to 500 mW thermal stimulation occurred in <60 seconds (Farnworth et al. 2013b) and therefore 60 seconds was set as the maximum duration for exposure to the thermal stimulus.

#### Thermal threshold testing procedure

The study was conducted over 5 days in February 2013. Approximately 24 hours prior to the commencement of testing, each cat's fur was clipped to skin level on both sides of the thorax as per the technique outlined in Farnworth et al. (2013b). The cats were not removed from their colony cages during this procedure. For all cats, data on age, current body weight and whether or not the cat had been spayed were taken from their records. Each cat was systematically allocated to one of six treatment groups by ordering their names alphabetically and

sequentially allocating them to groups 1–6; the primary researcher (MJF) was blinded to this systematic approach. Likewise, individuals were systematically allocated to a test day so that treatments were distributed across all test days rather than any single treatment being conducted on any single day. All tests were conducted between 09.00 and 17.00 hours. The total test period for each group was approximately 150–165 minutes.

For testing, eight cats from across the treatment groups were transferred to the experimental cages and were not returned until all nociceptive tests had been conducted on all subjects. On introduction to the test cages, cats were allowed 15 minutes to settle. The experimenters and equipment remained in the room during this time to habituate the cats to their presence. On commencement of the test sequence, the majority of the cats were quiet and in sternal recumbency.

Each cat was exposed seven times to a CO<sub>2</sub> thermal laser device during the test period. Cats were not returned to the colony cages between tests. The laser was directed onto the exposed area of skin from a distance of 2 m until the cat responded either by shifting significantly (i.e. rising to its feet or significantly easing its body) or exhibiting the panniculus reflex, or until the pre-determined cut-off time of 60 seconds was reached (Farnworth et al. 2013b). The laser was turned off immediately following either of these behavioural responses. The deactivation of the laser device and the timing of latency to respond were both performed manually (i.e. the timing device was not intrinsically connected to the laser on/off switch). As this introduced a margin of error based on the researcher's reaction time, the subject's latency to respond (time) was noted to the nearest 0.1 second. The researchers attempted to avoid stimulation of the same area of skin during subsequent tests on any given subject. To minimize variations in the distance of the laser from the cat, a line of tape was placed on the floor 2 m from the front of the cage and the front leg of the tripod on which the laser was mounted was placed on this line each time the laser device was moved. In the event that a cat was disturbed during testing (e.g. by the actions of an adjacent cat or staff activity) or moved incidentally (e.g. began to groom or urinate), the test was terminated and restarted as soon as possible (i.e. once the cat had resettled). Following an appropriate response the thermal laser was not re-applied until a minimum of 15 minutes had elapsed. The exact time between each test varied

depending upon the activity pattern of the individual (i.e. time to sternal recumbence).

The first thermal test in each cat was conducted prior to drug administration to establish a baseline response. The primary researcher (MJF) then exited the room to ensure he was blind to treatment and the appropriate drug was then injected by a qualified veterinarian (LAB). Latency to respond to thermal stimulation was measured during the time intervals 15–30, 30–45, 45–60, 60–75, 90–105 and 120–135 minutes. Intervals, rather than exact time-points, were used as the cats were unrestrained and laser line-of-sight could not be guaranteed at any precise time. Where a reading could not be made within a 15 minute interval, the data point was recorded as absent.

#### Drug treatments

Cats were randomly allocated to one of six treatments by the administering veterinarian, resulting in 10 cats per treatment group. A sample size of 10 was selected as it concurred with other similar thermal threshold testing protocols in the literature. The six treatment groups were: 1) saline (0.2 mL per cat; 0.9% NaCl; Baxter Healthcare Pty Ltd, New Zealand); 2) morphine (0.5 mg kg<sup>-1</sup>; morphine sulphate 10 mg mL<sup>-1</sup>; Hospira Pty Ltd, Australia); 3) buprenorphine (20 µg kg<sup>-1</sup>; Temgesic 0.3 mg mL<sup>-1</sup>; Reckitt Benckiser Ltd, New Zealand); 4) medetomidine (2 µg kg<sup>-1</sup>; Domitor 1 mg mL<sup>-1</sup>; Pfizer Global Pharmaceuticals, New Zealand); 5) tramadol (2 mg kg<sup>-1</sup>; Tramal 50 mg mL<sup>-1</sup>; CSL Biotherapies NZ Ltd, New Zealand); and 6) ketoprofen (3 mg kg<sup>-1</sup>; Ketofen 10%; Merial New Zealand, New Zealand). In treatment group 4, a 1:10 dilution ratio (medetomidine:saline) was used to ensure injectable volume equivalence among treatments. All cats received an intramuscular (IM) injection into the epaxial muscles between the iliac crest and the last rib. Injection was made using a 22 gauge, 3/4 inch needle from a 1 mL syringe.

#### Statistical analyses

We used IBM SPSS Statistics for Windows Version 22.0 (IBM Corp., NY, USA) to conduct our analysis. Our data were mostly nonparametric and our measures of central tendency and variation are expressed as the median (range). We tested for differences in weight and age among treatment groups using a one-way ANOVA procedure. Prior to

testing, we confirmed that data were normally distributed using the Kolmogorov–Smirnov test. After testing, we checked for homogeneity of variance using Levene's test.

The distribution of latencies to respond to thermal stimulation were not normal and thus a nonparametric Friedman's test was used to explore differences in response times across the duration of the monitoring period (135 minutes) for each of the treatments separately. When a subject's latency to respond exceeded the 60 second cut-off time, it was recorded as >60 seconds.

The effect of treatment on latency to respond at a particular time period (e.g. 15–30 minutes) was analysed by comparing response latencies amongst groups for each of the seven time periods using an independent Kruskal–Wallis test. When a significant effect was detected across a treatment group, pairwise Mann–Whitney tests were conducted to identify where inter-treatment differences occurred. Given the large number of potential comparisons, we restricted these to the period 60–75 minutes after the injection of the drug or saline. Data for each treatment group were also compared with data for the control group at each time-point. We adjusted the *p*-values using Bonferroni correction [critical value for significance (0.05)/number of comparisons] to reduce the likelihood of Type 1 errors.

## Results

#### Weight and age

Weight (Levene's test,  $F_{(5,53)} = 2.292$ ,  $p = 0.06$ ) and age (Levene's test,  $F_{(5,53)} = 0.485$ ,  $p = 0.786$ ) were homogeneous and normally distributed (weight: Kolmogorov–Smirnov test,  $p > 0.2$  for each treatment group; age: Kolmogorov–Smirnov test,  $p > 0.074$  for each treatment group). No differences in body weight ( $F_{(5,53)} = 1.176$ ,  $p = 0.33$ ) or age ( $F_{(5,53)} = 0.278$ ,  $p = 0.923$ ) were detected among the treatment groups. Weight and age differences were disregarded as potential explanations for different responses among treatments.

#### Effect of treatments on latency to respond to thermal stimulation

Readings were unavailable for 15 of 420 data points. Of these, six data points were absent in the saline group, four in the ketoprofen group, two in the medetomidine group, two in the buprenorphine

group and one in the morphine group. Response times of cats to thermal stimulation were highly variable across all six drug treatments (Fig. 1). However, the median and total range of pre-treatment response times for cats that received either an analgesic drug or saline solution were always <60 seconds (Table 1). No significant effects of treatment with regard to the total test period were found for the following: saline ( $\chi^2 = 3.922$ ,  $df = 6$ ,  $p = 0.687$ ); medetomidine ( $\chi^2 = 3.077$ ,  $df = 6$ ,  $p = 0.799$ ), and ketoprofen ( $\chi^2 = 5.816$ ,  $df = 6$ ,  $p = 0.444$ ). Although treatment with buprenorphine had no significant effect, there was a suggestion that latency to respond increased during the test phase ( $\chi^2 = 10.929$ ,  $df = 6$ ,  $p = 0.091$ ). By contrast, median response times in cats injected with morphine and buprenorphine exceeded 60 seconds on at least one of the post-treatment time intervals. Treatment with morphine ( $\chi^2 = 12.90$ ,  $df = 6$ ,  $p = 0.045$ ) and tramadol ( $\chi^2 = 20.28$ ,  $df = 6$ ,  $p = 0.002$ ) had significant effects on latency to respond over the course of the monitoring period.

Table 2 shows the numbers of tests in which the 60 second cut-off point was reached.

For those analgesics that showed a significant effect on latency to respond across the duration of the monitoring period, we conducted a series of pairwise comparisons. These were used to determine whether the difference occurred at 30–45 minutes, double this time (60–75 minutes) or double this time again (120–135 minutes) in comparison with the baseline response time. This represented three pairwise comparisons and the threshold value for significance was adjusted to  $p = 0.017$ .

For tramadol, significant differences in latency were recorded between the pre-treatment test and those at 60–75 minutes ( $Z = -2.803$ ,  $p = 0.005$ ) and 120–135 minutes after treatment ( $Z = -2.803$ ,  $p = 0.005$ ). Similarly, we recorded significant differences in the morphine treatment group between pre-treatment values and those at 60–75 minutes ( $Z = -2.701$ ,  $p = 0.007$ ) and 120–135 minutes ( $Z = -2.599$ ,  $p = 0.009$ ). We also determined the magnitude of the effect (effect size  $r$ ) for these two-

**Figure 1** Latency (seconds) of cats to respond to thermal stimulation generated by a carbon dioxide laser across six treatments. Quartiles are represented by the box and median latency by the horizontal bar. Whiskers represent the value at 1.5 times the size of the quartile box unless the maximum and minimum values fall within these values. For both tramadol and morphine, \*\*denotes a statistically significant effect across the entire test period on latency to respond ( $p < 0.05$ ). For buprenorphine, \*denotes a statistical trend ( $p < 0.1$ ).

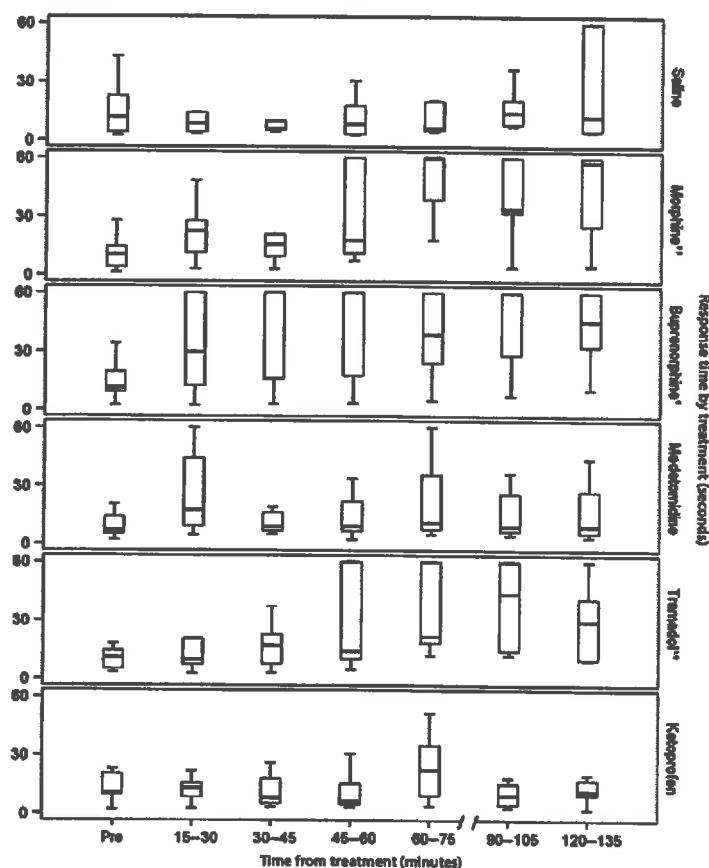


Table 1. Median (range) time to display the panniculus reflex following stimulation with a 500 mW thermal carbon dioxide laser in six treatment groups of 10 cats per group. Testing occurred across a monitoring period extending to a maximum of 135 minutes after an intramuscular injection of one of six treatment compounds. A cut-off time of 60 seconds was applied; data for subjects in which the cut-off time was reached are represented as >60

Treatment	Median (range) time to display panniculus reflex, seconds						
	Pre-test	15–30 minutes	30–45 minutes	45–60 minutes	60–75 minutes	90–105 minutes	120–135 minutes
Saline	11.8 (2.69–43.0)	8.5 (3.4–17.3)	6.1 (4.3–12.9)	8.3 (2.9–30.5)	6.2 (4.8–20.4)	14.2 (7.5–36.6)	12.0 (4.8 to >60)
Morphine	10.2 (1.3–7.8)	22.6 (3.1 to >60)	15.4 (3.1 to >60)	17.7 (7.4 to >60)	>60 (17.9 to >60)	34.0 (4.0 to >60)	58 (4.9 to >60)
Buprenorphine	11.2 (2.4–34.0)	29.6 (2.3 to >60)	>60 (3.0 to >60)	>60 (3.1 to >60)	38.6 (4.8 to >60)	>60 (7.1 to >60)	45.5 (10.3 to >60)
Medetomidine	6.8 (2.2–27.7)	17.3 (4.6 to >60)	8.9 (5.1–37.3)	9.0 (2.3 to >60)	11 (4.9 to >60)	9.1 (4.5 to >60)	9.2 (3.7 to >60)
Tramadol	11.0 (3.6–8.1)	9.9 (2.8 to >60)	17.1 (3.1 to >60)	14.1 (4.9 to >60)	21.9 (12.2 to >60)	43.6 (12.0 to >60)	29.7 (9.5 to >60)
Ketoprofen	10.6 (2.1–23.0)	12.9 (2.6–21.8)	8.2 (3.43 to >60)	6.4 (3.2–30.7)	22.3 (3.8–51.7)	9.5 (3.1 to >60)	11.6 (2.3 to >60)

way comparisons (Field 2009). Effect sizes for both tramadol and morphine were medium to large for both the pre-test *versus* 60–75 minutes and pre-test *versus* 120–135 minutes comparisons (Table 3). Effect sizes for buprenorphine fell within the range of those for tramadol and morphine.

The comparison of latencies to respond amongst groups at specific time-points indicated there was no significant effect of treatment on latency to respond to thermal stimulation during the pre-treatment ( $\chi^2 = 1.54$ ,  $df = 5$ ,  $p = 0.909$ ), 15–30 minutes ( $\chi^2 = 4.68$ ,  $df = 5$ ,  $p = 0.456$ ) and 30–45 minutes ( $\chi^2 = 6.669$ ,  $df = 5$ ,  $p = 0.246$ ) intervals. However, significant effects were detected amongst treatments at 45–60 minutes ( $\chi^2 = 12.254$ ,  $df = 5$ ,  $p = 0.030$ ), 60–75 minutes ( $\chi^2 = 21.02$ ,  $df = 5$ ,  $p = 0.001$ ), 90–105 minutes ( $\chi^2 = 18.38$ ,  $df = 5$ ,  $p = 0.003$ ) and 120–135 minutes ( $\chi^2 = 11.72$ ,  $df = 5$ ,  $p = 0.039$ ). We followed up on the effect of treatment at the halfway period of our trials (60–75 minutes) using Mann–Whitney tests in a series of pairwise comparisons. Bonferroni correction resulted in the reporting of our effects at a significance level of 0.01. There were no significant differences in latency to respond between cats injected with saline and those injected with buprenorphine ( $U = 20.0$ ,  $p = 0.04$ ), medetomidine ( $U = 40.0$ ,  $p = 0.965$ ), tramadol ( $U = 16.0$ ,  $p = 0.017$ ) and ketoprofen ( $U = 37.0$ ,  $p = 0.514$ ). However, the difference in latency to response was significant when data for cats in the saline treatment were compared with those for cats in the morphine treatment ( $U = 5.0$ ,  $p = 0.001$ ). Given that the Bonferroni correction provides a conservative indication of significance, determination of an effect size of drug treatment on latency to respond indicated a medium effect of buprenorphine (–0.47) and tramadol (–0.54) despite the nonsignificant Mann–Whitney tests. The effect size for ketoprofen was small (–0.149) and that for medetomidine was negligible (–0.01). Morphine showed a medium to large effect (–0.767) on latency to respond in comparison with saline.

#### Side effects of treatment and procedure

Side effects associated with the administration of drugs and the application of the thermal stimulus were observed and subsequently reported to, and noted by, the ethics committee concerned with the approval of these protocols. Firstly, 24 hours after the experiment, during routine checks, 24 of the 60 cats were found to show signs of mild blistering at sites at which the laser had been applied. Of the 24

**Table 2** Number of tests (numerator) within a given time period in which subjects (cats) reached the 60 second cut-off time

Treatment	Time phase, minutes						
	Pre-test	15–30	30–45	45–60	60–75	90–105	120–135
Saline (0.2 mL per cat)	0/10	0/8	0/10	0/7	0/9	0/10	2/10
Morphine (0.5 mg kg <sup>-1</sup> )	0/10	1/9	2/10	4/10	7/10	4/10	4/10
Buprenorphine (20 µg kg <sup>-1</sup> )	0/10	4/9	6/10	5/10	4/10	5/9	3/10
Tramadol (2 mg kg <sup>-1</sup> )	0/10	2/10	1/10	3/10	4/10	5/10	2/10
Ketoprofen (2 mg kg <sup>-1</sup> )	0/10	0/9	0/9	0/9	0/10	1/10	1/9
Medetomidine (2 µg kg <sup>-1</sup> )	0/10	1/9	0/10	1/9	1/10	1/10	2/10

The denominator is the total number of tests obtained for that time period. For group details, see Table 1.

**Table 3** Effect sizes for significant pairwise comparisons

Treatment	Pre-test versus 60–75 minutes Effect size <i>r</i>	Pre-test versus 120–135 minutes Effect size <i>r</i>
Morphine	-0.604	-0.572
Tramadol	-0.627	-0.627
Buprenorphine	-0.537	-0.604

Values for buprenorphine are included as normal hypotheses testing indicated significance remained below 0.1. Effect sizes of  $\approx 0.2$  (+ or -) are considered small, those of  $\approx 0.5$  (+ or -) as medium, and those of  $\approx 0.8$  (+ or -) as large. For group details, see Table 1.

cats with blistering, 18 had reached the maximum exposure time of 60 seconds on one or more occasion during testing. Blistering was dispersed across all treatment groups, but was most prevalent in the morphine, buprenorphine and tramadol groups (five of 10 individuals). Secondly, there was evidence of nausea shortly after the administration of morphine. Eight of the 10 cats in this group showed signs of excessive salivation or retching.

## Discussion

Significant changes in latency to respond to thermal nociceptive stimulation in the morphine and tramadol treatment groups support the proposal that this technique using a CO<sub>2</sub> laser may be useful for assessing nociceptive thresholds in cats provided with these analgesic drugs.

The morphine dose used here was comparatively high. However, as in other studies, such as that by Steagall et al. (2006), in which 0.2 mg kg<sup>-1</sup> was administered subcutaneously (SC), a significant

change in threshold response was observed at around 60 minutes. A previous study with IM injection [0.2 mg kg<sup>-1</sup> (Robertson et al. 2003)] showed no significant changes in thermal threshold until 4–6 hours following injection. Epidural administration [0.1 mg kg<sup>-1</sup> (Castro et al. 2009)] also resulted in a significant reduction in nociceptive response to a tail clamp at 1–12 hours.

Tramadol has been shown to significantly increase thermal thresholds 45 minutes after SC administration at 1 mg kg<sup>-1</sup>, but to have otherwise limited effect (Steagall et al. 2008). Significant increases in thermal threshold, measured using an attached device with a heating element, have been observed to persist for 45–90 minutes following IM injection of tramadol at a dosage of 2 mg kg<sup>-1</sup> (Jiwlawat & Durongphongtorn 2011), a finding that compares well with the results obtained in this experiment (Table 1). Further studies comparing different thermal techniques would be beneficial.

Buprenorphine did not demonstrate a clear significant effect on thermal nociceptive thresholds. Studies using intravenous (IV) (Steagall et al. 2009a) and SC (Steagall et al. 2006) administrations of buprenorphine at the dose used in this study demonstrated a clear effect on thermal threshold using the thermal device developed by Dixon et al. (2002) within 15 minutes and 45 minutes, respectively, of administration. The former was effective for up to 4 hours. Loss of significance across the sample may result from higher inter-individual variation in latency to respond to a low output thermal laser (Fig. 1). Our data suggest that the responses of individual cats at the same dose may also be highly variable as some individuals rapidly reached our cut-off time, whereas others demonstrated relatively little change across the testing period.

As expected, no significant effects were found in groups administered saline or ketoprofen. However, like other non-steroidal anti-inflammatory drugs (NSAIDs) [e.g. carprofen (Taylor et al. 2007b)], ketoprofen is an effective analgesic when administered postoperatively (Tobias et al. 2006) but is not generally expected to have an analgesic effect that can be elucidated through thermal stimulation in pain-free cats. This is because NSAID analgesics act by reducing inflammation and, therefore, nociceptor activation (Le Bars et al. 2001). This lack of response to both saline and an NSAID has been used to validate other emerging nociception assessment techniques in pain-free cats (Steagall et al. 2007). Assessment of absolute pain-free status in the present subjects did not include specific examinations or blood analyses. Conditions such as degenerative joint and renal diseases are more prevalent in older cats, but may be sub-clinically present in cats of a range of ages (Marino et al. 2014). Such conditions may be difficult to diagnose without thorough blood analyses. It is therefore not possible to rule out the presence of chronic low-level pain associated with such diagnoses in some subjects. However, a lack of nociceptive change in cats administered ketoprofen suggests a lack of inflammatory pain, at least in that treatment group. Two subjects in the saline group reached the 60 second cut-off time in the last test phase (see Table 2). Although aberrant results often occur, it is possible that the protracted length of the experimental period in this study in comparison with those in earlier studies (Farnworth et al. 2013a,b) may have increased the tendency for non-response.

A significant positive correlation between body weight and latency to exhibit a behavioural response has previously been demonstrated using thermal stimulation (Farnworth et al. 2013b). In addition, age-related changes in nociceptive sensitivity have been demonstrated in rodents (Chan & Lai 1982; Jourdan et al. 2000). Our results indicated that these factors did not differ significantly between treatment groups and therefore any variation amongst subjects attributable to age or weight is unlikely to impact upon overall latency to respond at the group level. The test periods used represent a further limitation in that they were not long enough to allow for evaluation of the overall effect of the analgesics. Further exploration of the CO<sub>2</sub> laser for such purposes is required.

In general, our data showed substantial over-dispersion (see Table 1 and Fig. 1). There were clear

differences in latencies to respond amongst cats within the same treatment group at a given time-point. Opioids are known to elicit substantial inter-individual variability in cats (Taylor et al. 2007c); this variability has recently been discussed relative to buprenorphine (Steagall et al. 2014). It is likely that the over-dispersion of response times explains why findings in the buprenorphine group did not achieve statistical significance overall and why the effects of morphine were not established statistically through corrected *posthoc* analysis. However, analysis of effect size did identify that the changes in response time seen in the tramadol, morphine and buprenorphine groups were similar. This suggests that the lack of significance is likely to have been caused by small sample sizes rather than a lack of effect. Smaller cohort studies of thermal nociceptive thresholds commonly use a crossover design, which functions to minimize inter-individual variability. It may be judicious to use such a design with a thermal CO<sub>2</sub> laser. This study appears to have been adequately powered to establish differences between control treatments and analgesic treatments, but it may not have been sufficiently powered to detect differences between opioids or to account for a large degree of inter-individual variation.

Medetomidine showed no significant effect on thermal thresholds; however, the amount used in this study was well below that used in other studies (e.g. Ansah et al. 2002). In part, this was to avoid excessive levels of sedation, which are known to impact upon animals' ability to demonstrate nociceptive response (Hunt et al. 2013). The IM administration of medetomidine at  $\geq 50 \mu\text{g kg}^{-1}$  has been shown to result in peak sedation scores (Ansah et al. 1998) and is often utilized as an adjunctive sedative during anaesthesia (Wiese & Muir 2007). In cats, analgesia is achieved with dosages of both  $15 \mu\text{g kg}^{-1}$  and  $10 \mu\text{g kg}^{-1}$  (Ansah et al. 2002; Steagall et al. 2009b). Medetomidine was included at a substantially lower dosage here ( $2 \mu\text{g kg}^{-1}$ ) in an attempt to assess the sensitivity of the CO<sub>2</sub> laser protocol. This result suggests that either medetomidine had no analgesic or sedative effect at this dose or that this thermal technique is not able to elucidate small changes in nociception. Retrospectively, it appears that a validated dose rate of  $10 \mu\text{g kg}^{-1}$  (Cullen 1996) would have been appropriate.

Although our results appear promising, there are areas which require further exploration and some findings indicate potential drawbacks. This technique lacks the direct contact of attached thermal



devices and hence, although normal behavioural patterns are not disrupted, it is difficult to take measurements at exact time-points because of the subject's movement patterns. We were also unable to ascertain the effect of skin temperature variations on latency to respond to a remote thermal stimulus. This is of particular interest because opioids such as morphine and buprenorphine cause significant increases in body temperature (Posner et al. 2010), and other drugs such as dexmedetomidine have been shown to impact upon thermoregulatory processes (Talke et al. 1997).

It is important to note there was some evidence of blistering in cats exposed for the full 60 seconds, possibly as a result of reduced reactivity brought about by the analgesic and/or sedative effects of treatment. This effect was not previously observed in other similar experiments (Farnworth et al. 2013b), but it suggests a need to establish the time-point at which damage occurs and to reduce the exposure time accordingly. However, the use of an earlier cut-off point is likely to require a statistical technique that can account for higher numbers of right-censored data points (those reaching the cut-off point) from cats provided with analgesics. Although we attempted to minimize the likelihood that a single point of stimulation would be reused, our inability to definitively ensure this may have resulted in some sensitization to the thermal stimulus. Future exploration may include marking the site of each test to be undertaken on the subject's skin with ink. Targeting of the mark with the visible laser would preclude the unintentional overlap of stimulation sites.

Future studies using this technique should attempt to measure sedation and perhaps address a narrower array of analgesics using a broader set of dose rates. They may also wish to address how this technique applies to analgesia following surgical interventions and in animals already experiencing pain. It would also be useful to develop this technique in conjunction with thermographic imaging to quantify any effects of changes in skin temperature resulting from external temperature fluctuations or physiological changes as a result of drug administration. It is reasonable to conclude that the research hypotheses were supported by our findings and that a CO<sub>2</sub> laser is able to determine changes in antinociceptive thresholds in cats tested following the administration of opioids. The utility of this technique warrants further exploration.

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