Assessment of a carbon dioxide laser for the measurement of thermal nociceptive thresholds following intra-muscular administration of analgesic drugs to pain-free female cats.

Abstract

Objective: To assess the potential for using a thermal carbon dioxide (CO_2) laser to assess anti-nociception in pain-free cats.

Animals: Sixty healthy adult female cats with a mean weight (± SD) of 3.3 kg (± 0.6 kg).

Methods: This is a prospective, blinded and randomised study. Cats were systematically allocated to one of six treatments 1) saline 0.2 ml/cat; 2) morphine 0.5 mg/kg; 3) buprenorphine 20 µg/kg; 4) medetomidine 2 µg/kg; 5) tramadol 2mg/kg; 6) ketoprofen 2 mg/kg. Latency to respond to thermal stimulation was assessed prior to intramuscular injection and at 6 time periods following injection (15-30; 30-45; 45-60; 60-75; 90-105; 120-135 min). Thermal thresholds were assessed using time to respond behaviourally to stimulation with a 500 mW CO_2 laser with maximum latency to respond set at 60 seconds. Differences in response latency for each treatment across the duration of the experiment were assessed using a Friedman's test. Differences between treatments at any given time were assessed using an independent Kruskal-Wallis test. Where significant effects were identified, pair-wise comparisons

were conducted at 30-45, 60-75 and 120-135 min to further explain the direction of the effect.

Results: Cats treated with morphine (χ^2 = 12.90; df = 6; P = 0.045) and tramadol (χ^2 = 20.28; df = 6; P = 0.002) showed significant increases in latency to respond over the duration of the test period. However, subsequent pairwise comparisons indicated that latencies at specific time points were only significantly different (P < 0.05) for tramadol at 60-75 and 90-105 min after administration. No significant pairwise comparisons were found within the morphine treatment group. Injection of saline, ketoprofen, medetomidine or buprenorphine showed no significant effect on latency to respond.

Conclusions: This project further validates the CO_2 laser technique for use in cats. It can be used for assessment of thermal nociceptive thresholds in pain-free cats after analgesic administration and shows some promise in differentiating amongst analgesic treatments. It may provide a simpler alternative to existing systems although further exploration is required both in terms of its sensitivity and comparative utility (i.e. relative to other thermal threshold systems). Future experiments should seek to quantify the effects of skin temperature and sedation on latency to respond. Given that this technique was found to cause minor skin blistering in individuals that reached the 60 s exposure limit, a cut off time of <45 s is recommended.

Keywords: Analgesia, Behaviour, Cat, CO_2 laser, NSAID, Opioid, Pain assessment

Introduction

Domestic cats (*Felis catus*) have previously been identified as underexplored in terms of their responses to pain and analgesia but significant advances have been made (Robertson 2008). Evidence suggests that cats, as a species, display substantial variation in their response to different classes of analgesic compounds (Taylor et al. 2001; Robertson & Taylor 2004). Likewise there appears to be a large degree of inter-individual variation around specific analgesic effects and pharmacodynamics, particularly with 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 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 contact devices (Dixon et al. 2002) and remote CO_2 laser stimulation (Farnworth et al. 2013b). Although the contact devices have been extensively explored and applied (Robertson et al. 2003; Steagall et al. 2007; Taylor et al. 2007a), the latter technique has only been validated in terms of its intra-individual repeatability (Farnworth et al. 2013b) and inter-individual variability (Farnworth et al. 2013a). It has not yet been used to explore the effects of pharmacological manipulation of nociceptive thresholds. Research in other species suggests that the CO_2 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 potential to use

the laser technique with only moderate alteration of management routines and without substantial need for habituation required by other techniques (Slingsby & Taylor 2008; Slingsby et al. 2010), suggest it could be a useful tool if validated further.

Analgesics that act primarily upon the dorsal horn of the spinal cord are considered to have central effect (Robertson & Taylor 2004). This central action has been shown to result in thermal hypoalgesia (Dixon et al. 2002). Effectiveness was established relative to two confirmed centrally-acting analgesics, morphine (pure mu-agonist) and buprenorphine (a partial opioid mu-agonist and antagonist of kappa-receptors) which have previously been evaluated in cats using thermal thresholds (Robertson et al. 2003; Steagall et al. 2006; Pypendop et al. 2008). Medetomidine, an alpha-two agonist with both sedative and analgesic effects (Cullen 1996; Steagall et al. 2009b) was also used. Previous thermal threshold studies have been successfully conducted with respect to its active isomer dexmedetomidine (Slingsby & Taylor 2008). In addition two other compounds with analgesic activity were evaluated, all of which have received some attention in the literature. Tramadol has been validated using a thermal stimulus (Pypendop et al. 2009) and is a centrally acting synthetic analogue of codeine (Cagnardi et al. 2011). Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) and an effective analgesic following ovariohysterectomy in cats (Slingsby & Waterman-Pearson 1998). NSAID do not have a central action, but rather act to inhibit prostaglandin synthesis and therefore inflammatory response (Robertson & Taylor 2004).

This research sought to explore the effectiveness of a CO_2 thermal laser for the assessment of nociceptive thresholds in pain-free cats under analgesia. If this

technique is to be considered useful for assessment of analgesia, latency to display a behavioural response should allow distinctions to be made between cats treated with one of the five compounds known to have analgesic effects (morphine, buprenorphine, tramadol, ketoprofen, or medetomidine) as compared to a saline control group. We hypothesised that latencies to respond to thermal stimulation will differ within the morphine, buprenorphine, tramadol and medetomidine treatment groups over the duration of the test period but not for saline or ketoprofen which has peripheral anti-inflammatory effects which are likely 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, 32 entire and 28 spayed, with a mean weight (± SD) of 3.3 (± 0.6) kg and a mean age (± SD) of 6.1 (± 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 (2.4m height x 1.4m width x 4.4m depth) with approximately half the volume of each pen under cover. Cats included had no long-term medical conditions identified in their records (which were updated weekly) nor abnormal gait or substantial fluctuations in weight. They 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 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 height x 0.8 m width x 1.1 m 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, avoiding the need to acclimate 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 reflection of the laser from the plastic rear wall. The metal cage door was replaced with a plasticated square mesh with openings measuring 25 x 25 mm 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, Mulkiteo, Washington, USA) which was mounted on a tripod to allow movement through vertical and horizontal planes. The CO_2 laser produced a 3.5mm diameter beam which was aimed using a non-thermal visible helium laser (JG-4A Class IIIA, wavelength 532nm) attached to the external casing. The wavelength of the

thermal laser was 10.60 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). Given that 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_2 laser has previously been demonstrated to have no discernable effect on the behavioural response latency of cats (Farnworth et al. 2013b) therefore it was not used as a control in this experiment. In a previous study using cats, all responses to 500mW thermal stimulation occurred in less than 60 s (Farnworth et al. 2013a), therefore 60 s was set as the maximum duration for exposure to the thermal stimulus.

Thermal threshold testing procedure

The study was conducted over five days in February 2013. Approximately 24 h 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. (2013a). The cats were not removed from their colony cages during this procedure. For each cat, age, current body weight and whether they 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 group 1 through 6, the primary researcher (MF) was blinded to this systematic approach. Likewise individuals were systematically allocated to a test day meaning treatments were distributed across all test days as opposed to any single treatment being conducted on

any single day. All tests were conducted between 0900 h and 1700 h. The total test period for each group was approximately 150-165 min.

For testing, each group of eight cats was transferred to the experimental cages and was only returned after all nociceptive tests had been conducted on all group members. On introduction to the test cage cats were allowed 15 min 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_2 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 significant easing of the body) or exhibiting the panniculus reflex, or until the pre-determined cut-off time of 60 s was reached (Farnworth et al. 2013a). Following either of these behavioural responses the laser was turned off. Deactivation of the laser device was manual. 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 s. The researchers attempted to avoid stimulation of the same area of skin during subsequent tests on any given subject. To minimise 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, 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 min 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 was conducted for each cat prior to drug administration to establish a baseline response. The primary researcher (MF) then exited the room to ensure they were blind to treatment and the appropriate drug was then injected by a qualified veterinarian (LB). Latency to respond to thermal stimulation was measured during the following time intervals: 15-30; 30-45; 45-60; 60-75; 90-105; 120-135 min. 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 min interval the datum point was recorded as absent.

Drug treatments

Cats were randomly allocated to one of 6 treatments by the administering veterinarian, resulting in 10 cats per treatment group. The six treatments groups were 1) saline (0.2 ml/cat; 0.9% NaCl; Baxter Healthcare Pty Ltd, Auckland, New Zealand); 2) morphine (0.5 mg/kg; morphine sulphate 10 mg/ml; Hospira, Mulgrave, Victoria, Australia); 3) buprenorphine (20µg/kg; Temgesic 0.3 mg/ml; Reckitt Benckiser, Auckland, New Zealand); 4) medetomidine (2 µg/kg; Domitor 1mg/ml; Pfizer, Auckland, New Zealand); 5) tramadol (2mg/kg; Tramal 50mg/ml; CSL Biotherapies, Auckland, New Zealand); 6) ketoprofen (3 mg/kg; Ketofen 10%; Merial, Auckland, New Zealand). For treatment group 4, a 1:10 dilution ratio

(medetomidine:saline) was used to ensure injectable volume equivalence among treatments. All cats received an intramuscular injection into the epaxial muscles between the iliac crest and the last rib. Injection was made using a 22-gauge ¾ inch needle from a 1 ml syringe.

Statistical analyses

We used SPSS 22 (IBM inc., Chicago, Illinois, USA) to conduct our analysis. Our data was mostly nonparametric and our measures of central tendency and variation are expressed as median (range). We tested for differences in weight and age among treatment groups using a one-way ANOVA procedure. Prior to testing we first confirmed that data was normally distributed using the Kolmogov-Smirnov test and after testing for homogeneity of variance using the Levene's test.

Distribution of latencies to respond to thermal stimulation were not normal and so a non-parametric Friedman's test was used to explore differences in response times across the duration of the monitoring period (135 min) for each of the treatments separately. For median calculations values exceeding 60 s were recorded as >60 s.

The effect of treatment on latency to respond at a particular time period (e.g. 15-30 min) was analysed by comparing response latencies between treatment groups at each of the seven time periods. This was done using an independent Kruskal-Wallis test. When a significant treatment effect was detected, pair-wise comparisons based on a Mann Whitney test were conducted to identify where specifically inter-treatment differences occurred. Given the large number of potential comparisons we restricted

these to the period 60 – 75 min after injection of the drug or control and between the saline control and each of the drug treatments only (5 pair wise comparisons).We adjusted the p values using the Bonferroni correction (Critical value for significance (0.05)/number of comparisons) to reduce the likelihood of Type 1 errors.

Results

Weight and age

We confirmed the variances in 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 homogenous and the distribution of data was normal for weight (Kolmogorov-Smirnvo test, $P > 0.2$ for each treatment group) and age (Kolmogorov-Smirnvo test, $P > 0.074$ for each treatment group) among treatment groups. We could detect no differences in the body weights ($F_{(5,53)} = 1.176$, $P = 0.33$) or ages ($F_{(5,53)} = 0.278$, $P = 0.923$) of cats among the treatment groups This suggested we could disregard weight and age differences as potential explanations of different responses among treatments.

Effect of treatments on latency to respond to thermal stimulation

Readings were unable to be taken for 15/420 datum points. Of these, six datum points were absent in the saline group, four for ketoprofen, two for medetomidine, two for buprenorphine and one for morphine. Response times of cats to thermal stimulation were very variable across all six drug treatments (Fig 1). However median and total range of pre-treatment response times for cats that received either an analgesic drug or

saline solution were always below 60 s (see Table 1). No significant effects of treatment with regards to the total test period, were found for the following treatments: 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 did increase during the test phase ($\chi 2 = 10.929$; df = 6; P = 0.091). In contrast median response times of cats injected with morphine and buprenorphine were greater than 60 s on at least one of the post-treatment time intervals. Treatment with morphine ($\chi 2 = 12.90$; df = 6; P = 0.045) and tramadol had a significant effect on latency to respond ($\chi 2 = 20.28$; df = 6; P = 0.002) over the course of the monitoring period. The number of tests which reached the 60 s cut-off point are shown in table 2.

_____

Tables 1 and 2 here

_____

_____

Figure 1 here

_____

For those analgesics for which we demonstrated a significant effect of latency to respond across the duration of the monitoring period we conducted a series of pair wise comparisons to determine whether the difference occurred at 30-45 min, double this time (60-75 min) and double this time again (120-135 min) when compared to the response time immediately prior to injection of the analgesic drug. This

represented three pairwise comparisons and we adjusted our threshold value for significance to P = 0.0167.

For tramadol, significant differences were recorded between the pre-treatment [median(range) = 11.0 s (3.6-18.1)] and 60-75 min after treatment [21.9 s (12.2- >60 s)] (Z = -2.803, P = 0.005) and 120-135 min after treatment[29.7 s (9.5 – >60 s)] (Z = -2.803, P = 0.005). Similarly we recorded significant differences for morphine treatment at the same time intervals namely pre-treatment [median(range) = 8.7 s (1.3-27.8)] and 60-75 min ([median(range) = > 60 s (17.9 - >60)] Z = -2.701, P = 0.007) and pre-treatment and 120 -135 min [median(range) = 48.1 s (4.9->60)] (Z = -2.599, P = 0.009 (Table 3,4). We also determined the magnitude of the effect (effect size r) for these two way comparisons (Field 2009). Effect sizes for both tramadol and morphine were medium to large for both the pre-test vs. 60-75 min and pre-test vs. 120-135 comparisons (Table 3). Similarly effect sizes for Buprenorphine fell within the range for tramadol and morphine.

_____

Table 3 here

_____

There was no significant effect of treatment on latency to respond to thermal stimulation during the pre-treatment interval ($\chi 2 = 1.54$; df = 5; P = 0.909), 15-30 min ($\chi 2 = 4.68$; df = 5; P = 0.456), and 30-45 min ($\chi 2 = 6.669$; df = 5; P = 0.246) after injection However, a significant effect of treatment was detected at 45-60 min ($\chi 2 = 12.254$, df = 5, P = 0.030), 60-75 min ($\chi 2 = 21.02$, df = 5, P = 0.001), 90-105 min ($\chi 2 = 18.38$, df = 5, P = 0.003) and 120-135 min ($\chi 2 = 11.72$, df = 5, P = 0.039) after

injection (Table 6). We followed up on the effect of treatment at the half way period of our trials (60-75 min) by using Mann-Whitney tests in a series of pair wise comparisons. The Bonferroni correction resulted in our effects being reported at a 0.01 level of significance. There were no significant differences in latency to respond between cats injected with saline where compared to 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 latency to response was significant when saline treatment was compared to morphine treatment (U = 5.0, P = 0.001). Reflecting the fact 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) in spite of the non-significant Mann-Whitney tests. The effect of ketoprofen was small (-0.149) and negligible for medetomidine (-0.01). Morphine showed a medium-large effect (-0.767) on latency to respond when compared with saline.

Side effects of treatment and procedure

Side effects associated with drug administration and 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 h after the experiment, during routine checks, it was identified that 24/60 cats showed signs of mild blistering where the laser had been applied. Of the 24 cats with blistering 18 had reached the maximum exposure time of 60s 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 (5/10 individuals). Secondly there was evidence of nausea shortly after the administration of morphine. Eight of the ten cats in this group showed signs of excessive salivation or retching.

Discussion

A significant positive correlation between body weight and latency to exhibit a behavioural response has previously been demonstrated when using thermal stimulation (Farnworth et al. 2013a). In addition age-related changes in nociceptive sensitivity have been demonstrated in rodents (Chan et al. 1982, Jourdan et al. 2000). Our results indicated that these factors were not significantly different between treatment groups and therefore the likelihood that these factors substantially impacted upon the results is minimal.

This study provides some evidence that a CO_2 laser may be used to explore analgesic efficacy and can be used to distinguish between treatments that are known to have an analgesic effect and those that are not. In particular increased latency to respond to thermal stimulation was noted for morphine and tramadol. It is reassuring to note that no statistical difference was identified between baseline measurements for any treatment, although more than a single baseline measurement for each cat may have allowed clearer comparisons within treatments.

A significant positive correlation between body weight and latency to exhibit a behavioural response has previously been demonstrated when using thermal

stimulation (Farnworth et al. 2013a). In addition age-related changes in nociceptive sensitivity have been demonstrated in rodents (Chan et al. 1982, Jourdan et al. 2000). Our results indicated that these factors were not significantly different between treatment groups and therefore the likelihood that these factors substantially impacted upon the results is minimal.

As expected no significant effects were found for groups administered saline or ketoprofen. Although, as for other NSAIDs (e.g. carprofen: Taylor et al. 2007c), ketoprofen is an effective analgesic when administered post-operatively (Tobias et al. 2006), it is generally not expected to have analgesic effect which 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 non-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).

The morphine dose used here was high relative to that used in other studies. However, as for other studies (0.2 mg/kg, subcutaneously: Steagall et al. 2006) a significant change in threshold response was observed at around 60 min. A previous study with intramuscular injection at lower doses (0.2 mg/kg: Robertson et al. 2003) showed no significant changes in thermal threshold until 4-6 h following injection. Epidural administration (0.1 mg/kg: Castro et al. 2009) also resulted in significant reduction in nociceptive response to a tail clamp between 1-12h.

Tramadol has been shown to significantly increase thermal thresholds 45 min after subcutaneous administration at 1 mg/kg, but with 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 between 45-90 min following intramuscular injection of tramadol at a dosage of 2 mg/kg (Jiwlawat & Durongphongtorn 2011) which compares well with the results obtained in this experiment (Fig. 2). Further studies comparing the different thermal techniques would be beneficial.

Buprenorphine did not demonstrate a clear significant effect on thermal nociceptive thresholds. Studies using intravenous (Steagall et al. 2009a) and subcutaneous (Steagall et al. 2006) administration of buprenorphine at the same dose as this study demonstrate a clear effect on thermal threshold when using the thermal device developed by Dixon et al. (2002) within 15 min and 45 min of administration respectively. The former was effective for up to 4 h. 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 response of individual cats may also be highly variable at the same dose with some individuals rapidly reaching out cut-off time whilst others demonstrated relatively little change across the testing period. It is also worthy of note that cats reached the 60 s cut-off point during the final test within the saline treatment group. Although a definitive reason cannot be provided for this it is likely that the extended testing period resulted in increased stress for some cats. Habituation to this length of study period may be required for these cats.

In general our data showed substantial over-dispersion (see Table 1; Fig. 1). There were clear differences in latencies to respond amongst cats within the same treatment

at a given time point. Opioids in general are known to elicit substantial inter-individual variability in cats (Taylor et al. 2007b), this variability has recently been discussed relative to buprenorphine (Steagall et al. 2014). The over-dispersion of response times likely explains why buprenorphine did not achieve statistical significance overall and why the effects of morphine were unable to be statistically established through corrected post-hoc analysis. However, analysis of effect size did identify that the changes in response time seen for tramadol, morphine and buprenorphine were similar. This suggests that the lack of significance is likely caused by sample sizes being too small rather than providing evidence of a lack of effect. Smaller cohort studies of thermal nociceptive thresholds commonly use cross-over studies which function to minimise the inter-individual variability. It may be judicious to use such a design with a thermal carbon dioxide laser.

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). Intramuscular administration of medetomidine at 50 µg/kg or over has been shown to result in peak sedation scores (Ansah et al. 1998) and it is often utilised as an adjunctive sedative during anaesthesia (Wiese & Muir 2007). In cats, analgesia is achieved at both 15 and 10 µg/kg (Ansah et al. 2002; Steagall et al. 2009b). Medetomidine was included at a substantially lower dosage here (2 µg/kg) in an attempt to assess the sensitivity of the CO2 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 a validated dose rate of 10 µg/kg (Cullen 1996) would have been appropriate.

Although preliminary results appear promising, there are a number of areas which require further exploration and some findings indicate potential drawbacks. This technique lacks the direct contact of attached thermal devices which means that, whilst it does not disrupt normal behavioural patterns, it is difficult to take measurements at exact time points dependent upon 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 given that 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).

This study used a similar number of subjects per treatment when compared to other thermal threshold studies. It may be judicious to increase sample size in future protocols, especially given the variability of response. This study appears adequately powered to establish differences between control treatments and analgesic treatments but may not be sufficiently powered to detect differences between opioids, or to account for a large degree of inter-individual variation. When multiple comparisons were made, significant effects were often lost when p-values were corrected. However comparisons between this and other studies make a strong case that a CO_2 laser is a valid experimental tool for assessing pharmacological effect.

It is important to note there was some evidence of blistering in cats exposed for the full 60 s, 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. 2013a) but suggests a need to establish at what time point damage occurs and to reduce the exposure time accordingly. However, the use of an earlier cut-off point will likely require the use of 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 attempts were made to minimise the likelihood that a single point of stimulation would be reused The inability to definitively ensure such may have resulted in some sensitisation to the thermal stimulus. Future exploration may include placing one ink mark on the subjects skin for each test to be undertaken. Targeting of the mark with the visible laser would preclude unintentional overlap of stimulation sites.

The 15 min intervals used may have had some effect on the median response times, although all attempts were made to minimise this. 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 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 carbon dioxide laser is able to determine changes

in anti-nociceptive thresholds of cats tested following administration of opioids. The utility of this technique requires, and warrants, further exploration

Acknowledgements

The authors would like to thank the staff and students at the Massey University Feline Unit for their kind assistance with this work.

References

Ansah OB, Raekallio M, Vainio O (1998) Comparison of three doses of dexmedetomidine with medetomidine in cats following intramuscular administration. J Vet Pharmacol Ther 21**,** 380-387.

Ansah OB, Vainio O, Hellsten C et al. (2002) Postoperative pain control in cats: Clinical trials with medetomidine and butorphanol. Vet Surg 31**,** 99-103.

Cagnardi P, Villa R, Zonca A et al. (2011) Pharmacokinetics, intraoperative effect and postoperative analgesia of tramadol in cats. Res Vet Sci 90**,** 503-509.

Castro DS, Silva MFA, Shih AC et al. (2009) Comparison between the analgesic effects of morphine and tramadol delivered epidurally in cats receiving a standardized noxious stimulation. J Feline Med Surg 11**,** 948-953.

Chan SH, Lai YY (1982) Effects of aging on pain responses and analgesic efficacy of morphine and clonidine in rats. Exp Neurol 75, 112-119.

Cullen LK (1996) Medetomidine sedation in dogs and cats: A review of its pharmacology, antagonism and dose. Brit Vet J 152**,** 519-535.

Di Giminiani P, Petersen LJ, Herskin MS (2013) Nociceptive responses to thermal and mechanical stimulations in awake pigs. Eur J Pain 17**,** 638-648.

Dixon MJ, Robertson SA, Taylor PM (2002) A thermal threshold testing device for evaluation of analgesics in cats. Res Vet Sci 72**,** 205-210.

Farnworth MJ, Barrett LA, Adams NJ et al. (2013a) Body weight affects behavioural indication of thermal nociceptive threshold in adult domestic cats (Felis catus). Appl Anim Behav Sci 149, 30-35.

Farnworth MJ, Beausoleil NJ, Adams NJ et al. (2013b) Validating the use of a carbon dioxide laser for assessing nociceptive thresholds in adult domestic cats (Felis catus). Appl Anim Behav Sci 143**,** 104-109.

Field A (2009). Discovering statistics using SPSS. Sage publications Washington DC, United States of America.

Giordano T, Steagall PVM, Ferreira TH et al. (2010) Postoperative analgesic effects of intravenous, intramuscular, subcutaneous or oral transmucosal buprenorphine administered to cats undergoing ovariohysterectomy. Vet Anaesth Analg 37**,** 357-366.

Guesgen MJ, Beausoleil NJ, Minot EO et al. (2011) The effects of age and sex on pain sensitivity in young lambs. Appl Anim Behav Sci 135**,** 51-56.

538 Hendriks WH, Wamberg S, Tartellin MF (1999). A metabolism cage for quantitative
539 urine collection and accurate measurement of water balance in adult cats
540 (*Felis catus*). J Anim Physiol A Anim Nutr 82, 94-105.
541 Herskin MS, Müller R, Schrader L et al. (2003) A laser-based method to measure
542 thermal nociception in dairy cows: Short-term repeatability and effects of
543 power output and skin condition. J Anim Sci 81**,** 945-954.
544 Hunt JR, Grint NJ, Taylor PM et al. (2013) Sedative and analgesic effects of
545 buprenorphine, combined with either acepromazine or dexmedetomidine, for
546 premedication prior to elective surgery in cats and dogs. Vet Anaesth Analg
547 40**,** 297-307.
548 Jiwlawat S, Durongphongtorn S (2011) The antinociceptive effects of tramadol on the
549 thermal threshold response in cats. Thai J Vet Med 41**,** 171-177.
550 Johnson JA, Roberston SA, Pypendop BH (2007) Antinociceptive effects of
551 butorphanol, buprenorphine, or both, administered intramuscularly in cats. Am
552 J Vet Res 68**,** 699-703.
553 Jourdan D, Boghossian S, Alloui A, Veyrat-Durebex C, Coudore MA, Eschalier A,
554 Alliot J (2000). Age-related changes in nociception and effect of morphine in
555 the Lou rat. Eur J Pain 4, 291-300.
556 Lascelles BDX, Robertson SA (2004) Use of thermal threshold response to evaluate
557 the antinociceptive effects of butorphanol in cats. Am J Vet Res 65**,** 1085-
558 1089.
559 Le Bars D, Gozariu M, Cadden SW (2001) Animal models of nociception. Pharmacol
560 Rev 53**,** 597-652.
561 Posner LP, Pavuk AA, Rokshar JL et al. (2010) Effects of opioids and anesthetic
562 drugs on body temperature in cats. Vet Anaesth Analg 37**,** 35-43.
563 Pypendop BH, Siao KT, Ilkiw JE (2009) Effects of tramadol hydrochloride on the
564 thermal threshold in cats. Am J Vet Res 70**,** 1465-1470.
565 Pypendop BH, Siao KT, Pascoe PJ et al. (2008) Effects of epidurally administered
566 morphine or buprenorphine on the thermal threshold in cats. Am J Vet Res 69**,**
567 983-987.
568 Robertson SA (2008) Managing Pain in Feline Patients. Vet Clin N Am - Small 38**,**
569 1267-1290.
570 Robertson SA, Taylor PM (2004) Pain management in cats - Past, present and future.
571 Part 2. Treatment of pain - Clinical pharmacology. Journal of Feline Medicine
572 and Surgery 6**,** 321-333.
573 Robertson SA, Taylor PM, Lascelles BDX et al. (2003) Changes in thermal threshold
574 response in eight cats after administration of buprenorphine, butorphanol and
575 morphine. Vet Rec 153**,** 462-465.
576 Slingsby LS, Murrell JC, Taylor PM (2010) Combination of dexmedetomidine with
577 buprenorphine enhances the antinociceptive effect to a thermal stimulus in the
578 cat compared with either agent alone. Vet Anaesth Analg 37**,** 162-170.
579 Slingsby LS, Taylor PM (2008) Thermal antinociception after dexmedetomidine
580 administration in cats: A dose-finding study. J Vet Pharm Ther 31**,** 135-142.
581 Slingsby LS, Waterman-Pearson AE (1998) Comparison of pethidine, buprenorphine
582 and ketoprofen for postoperative analgesia after ovariohysterectomy in the cat.
583 Vet Rec 143**,** 185-189.
584 Steagall PV, Pelligand L, Giordano T et al. (2013) Pharmacokinetic and
585 pharmacodynamic modelling of intravenous, intramuscular and subcutaneous
586 buprenorphine in conscious cats. Vet Anaesth Analg 40**,** 83-95.

Steagall PV, Taylor PM, Brondani JT et al. (2007) Effects of buprenorphine, carprofen and saline on thermal and mechanical nociceptive thresholds in cats. Vet Anaesth Analg 34**,** 344-350.

Steagall PVM, Carnicelli P, Taylor PM et al. (2006) Effects of subcutaneous methadone, morphine, buprenorphine or saline on thermal and pressure thresholds in cats. J Vet Pharm Ther 29**,** 531-537.

Steagall PVM, Mantovani FB, Taylor PM et al. (2009a) Dose-related antinociceptive effects of intravenous buprenorphine in cats. Vet J 182**,** 203-209.

Steagall PVM, Millette V, Mantovani FB et al. (2009b) Antinociceptive effects of epidural buprenorphine or medetomidine, or the combination, in conscious cats. J Vet Pharm Ther 32**,** 477-484.

Steagall PVM, Monteiro-Steagall BP (2013) Multimodal analgesia for perioperative pain in three cats. J Feline Med Surg 15, 737-743.

Stegall PVM, Monteiro-Steagall BP, Taylor PM (2014) A review of the studies using buprenorphine in cats. J Vet Intern Med 28, 762-770.

Steagall PVM, Taylor PM, Brondani JT et al. (2008) Antinociceptive effects of tramadol and acepromazine in cats. J Feline Med Surg 10**,** 24-31.

Talke P, Tayefeh F, Sessler DI et al. (1997) Dexmedetomidine does not alter the sweating threshold, but comparably and linearly decreases the vasoconstriction and shivering thresholds. Anesthesiology 87**,** 835-841.

Taylor PM, Robertson SA, Dixon MJ (2007a) Evaluation of the use of thermal thresholds to investigate NSAID analgesia in a model of inflammatory pain in cats. J Feline Med Surg 9**,** 313-318.

Taylor PM, Robertson SA, Dixon MJ et al. (2001) Morphine, pethidine and buprenorphine disposition in the cat. J Vet Pharm Ther 24**,** 391-398.

Taylor PM, Slingsby LS, Pypendop BH et al. (2007b) Variable response to opioid analgesia in cats. In: Proceedings of the Spring Conference of the Association of Veterinary Anaesthetists Conference, Paris, France 34, 6-7.

Taylor PM, Steagall PVM, Dixon MJ et al. (2007c) Carprofen and buprenorphine prevent hyperalgesia in a model of inflammatory pain in cats. Res Vet Sci 83**,** 369-375.

Ting STL, Earley B, Veissier I et al. (2010) Effects of Burdizzo castration on CO2 laser induced thermal nociception of Holstein-Friesian calves of different ages. Appl Anim Behav Sci 126**,** 12-18.

Tobias KM, Harvey RC, Byarlay JM (2006) A comparison of four methods of analgesia in cats following ovariohysterectomy. Vet Anaesth Analg 33**,** 390-398.

Wiese AJ, Muir WW (2007) Anaesthetic and cardiopulmonary effects of intramuscular morphine, medetomidine and ketamine administered to telemetered cats. J Feline Med Surg 9**,** 150-156.

631 Table 1. Median and range for behavioural latency of cats to respond to a carbon
632 dioxide laser. Values are median (minimum to maximum) in seconds for cats across
633 monitoring period that extended to a maximum of 135 min.
634

Treatment	**Pre-Test** sec	**15-30 min** sec	**30-45 min** sec	**45-60 min** sec	**60-75 min** sec	**90-105 min** sec	**120-135 min** sec
Saline	*11.8 (2.6-43)*	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->60)
Morphine	*10.2 (1.3-27.8)*	22.6 (3.1->60)	15.4 (3.1->60)	17.7 (7.4->60)	>60 (17.9->60)	34.0 (4.0->60)	58 (4.9->60)
Buprenorphine	*11.2 (2.4-34)*	29.6 (2.3->60)	>60 (3.0->60)	>60 (3.1->60)	38.6 (4.8->60)	>60 (7.1->60)	45.5 (10.3->60)
Medetomidine	*6.8 (2.2-27.7)*	17.3 (4.6->60)	8.9 (5.1-37.3)	9.0 (2.3->60)	11 (4.9->60)	9.1 (4.5->60)	9.2 (3.7->60)
Tramadol	*11.0 (3.6-18.1)*	9.9 (2.8->60)	17.1 (3.1->60)	14.1 (4.9->60)	21.9 (12.2->60)	43.6 (12 - >60)	29.7 (9.5->60)
Ketoprofen	*10.6 (2.1-23)*	12.9 (2.6-21.8)	8.2 (3.43->60)	6.4 (3.2-30.7)	22.3 (3.8-51.7)	9.5 (3.1->60)	11.6 (2.3->60)

635
636

Table 2: Number of tests (numerator) for a given time period where subjects (cats) reached the 60s cut-off time. Testing occurred after an intramuscular injection of one of six treatment compounds and was executed using a 500 mW thermal carbon dioxide laser. The denominator is the total number of tests obtained for that time period.

Treatment	Time Phase (min)						
	Pre	15-30	30-45	45-60	60-75	90-105	120-135
Saline (0.2ml/cat)	0/10	0/8	0/10	0/7	0/9	0/10	2/10
Morphine (0.5mg/kg)	0/10	1/9	2/10	4/10	7/10	4/10	4/10
Buprenorphine (20µg/kg)	0/10	4/9	6/10	5/10	4/10	5/9	3/10
Tramadol (2mg/kg)	0/10	2/10	1/10	3/10	4/10	5/10	2/10
Ketoprofen (2mg/kg)	0/10	0/9	0/9	0/9	0/10	1/10	1/9
Medetomidine (2µg/kg)	0/10	1/9	0/10	1/9	1/10	1/10	2/10

Table 3. Effect sizes for pair size comparisons presented in Table 3. Figures for Bupremorphine are also included as normal hypotheses testing indicated significance remained below 0.1. Effects sizes = 0.2 are considered small, = 0.5 medium and = 0.8 large.

Treatment	Pre-test vs 30-45 min Effect size r	Pre-test vs 60-75 min Effect size r	Pre-test vs 120-135 min Effect size r
Morphine	-0.148	-0.604	-0.572
Tremadol	-0.307	-0.627	-0.627
Buprenorphine	-0.399	-0.537	-0.604

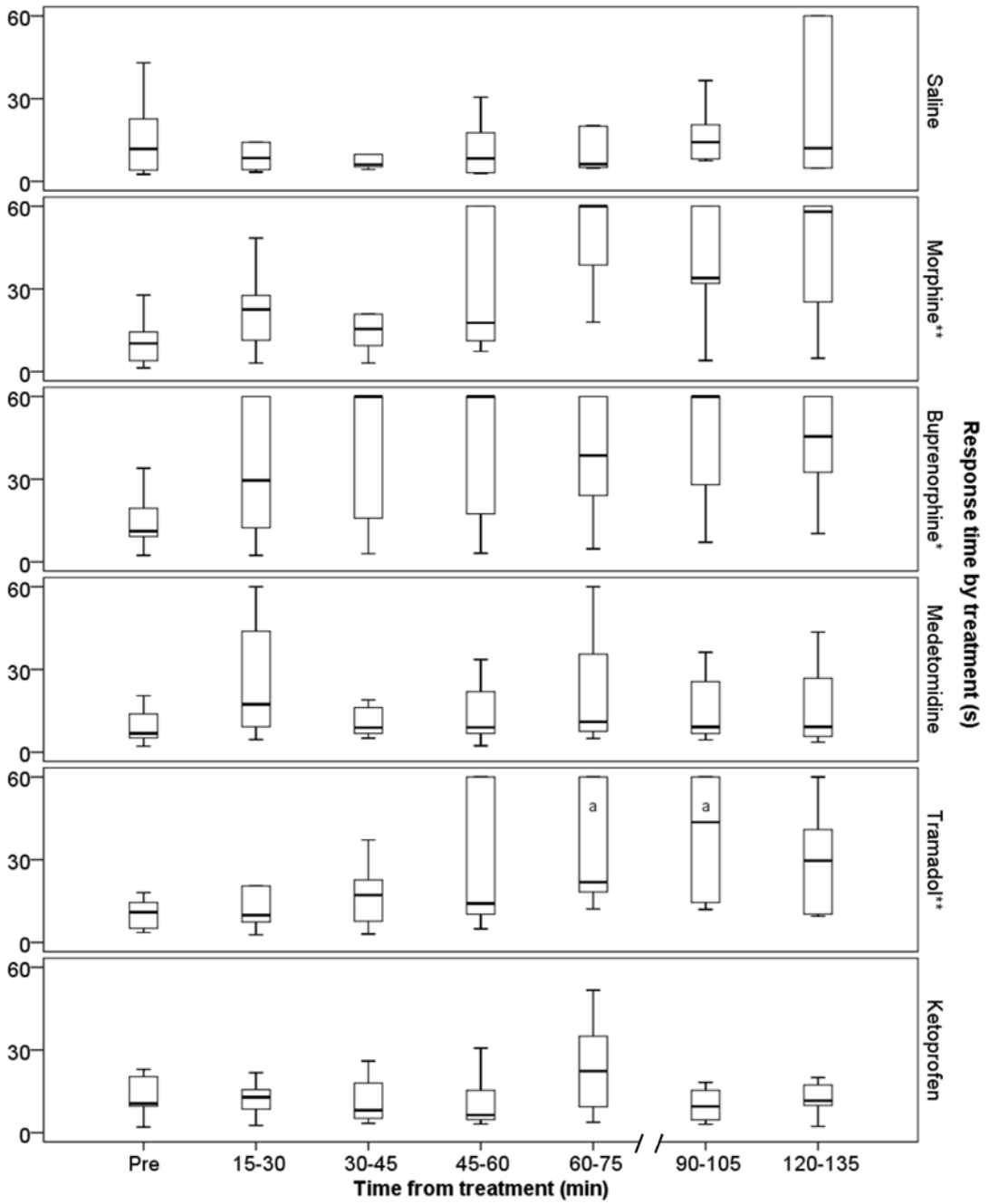

Figure 1: Quartiles (box) and Median latency (horizontal bar) of cats to respond to thermal stimulation using a carbon dioxide laser across six treatments. For both tramadol and morphine ** denotes a statistically significant effect across the entire test period on latency to respond ($P < 0.05$). For buprenorphine * denotes a statistical trend ($P < 0.1$). Within treatments the letter (a) denotes statistical significance ($P<0.05$) between the response at the relevant time period and the pre-treatment response.