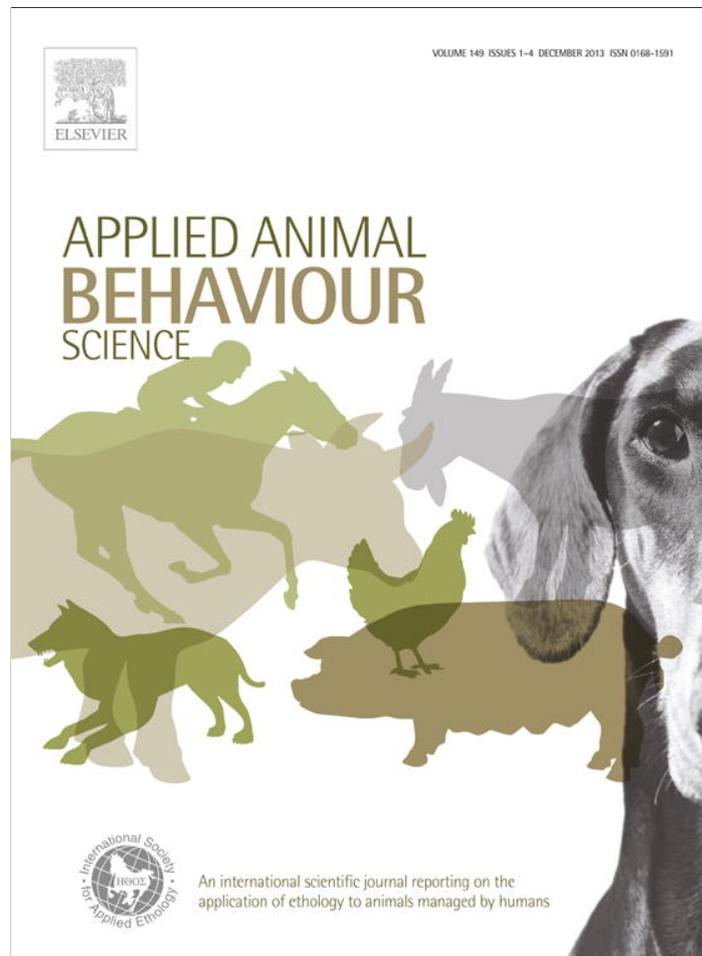


Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



**This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.**

**Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.**

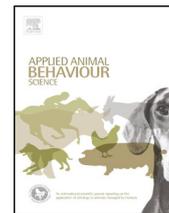
**In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:**

**<http://www.elsevier.com/authorsrights>**



Contents lists available at ScienceDirect

## Applied Animal Behaviour Science

journal homepage: [www.elsevier.com/locate/applanim](http://www.elsevier.com/locate/applanim)

## Body weight affects behavioural indication of thermal nociceptive threshold in adult domestic cats (*Felis catus*)



Mark J. Farnworth<sup>a,\*</sup>, Lorelle A. Barrett<sup>b</sup>, Nigel J. Adams<sup>a</sup>, Ngaio J. Beausoleil<sup>b</sup>, Margreet Hekman<sup>c</sup>, David G. Thomas<sup>c</sup>, Natalie K. Waran<sup>d</sup>, Kevin J. Stafford<sup>b</sup>

<sup>a</sup> Animal Welfare and Biodiversity Research Group, Department of Natural Sciences, Unitec Institute of Technology, Private Bag 92025, Auckland 1025, New Zealand

<sup>b</sup> Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand

<sup>c</sup> Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand

<sup>d</sup> Jeanne Marchig International Centre for Animal Welfare Education, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG, United Kingdom

### ARTICLE INFO

#### Article history:

Accepted 25 September 2013

Available online 4 October 2013

#### Keywords:

Body condition

Cat

CO<sub>2</sub> laser

Nociception

Pain assessment

Thermal threshold

### ABSTRACT

Carbon dioxide (CO<sub>2</sub>) thermal lasers have previously been validated for the assessment of nociception in cats. This experiment sought to assess the potential impact of factors associated with age, sex, body weight and sterilisation upon nociceptive threshold as measured by latency to display a behavioural response. Cats ( $N = 113$ ) were exposed to a CO<sub>2</sub> thermal laser three times during a 45–60 min test period depending upon the interval between tests. A minimum of 15 min elapsed between consecutive tests on any one individual. Time to display either a skin twitch or withdrawal was measured. Intra-class correlations showed the three measurements to be repeatable across tests for any given cat ( $ICC = 0.482$ ;  $P < 0.001$ ). Males had a significantly longer mean latency to respond than females (14.83 s and 12.59 s respectively;  $P = 0.028$ ). Analyses of co-variance established that the body weight of females significantly affected response threshold ( $P = 0.013$ ) but for males this effect was marginal ( $P = 0.058$ ). All other factors included in the analyses were non-significant. A post hoc  $t$ -test for males and females with overlapping body weights found no significant differences between the sexes ( $P = 0.721$ ). The precise reason for the effect of body weight on latency to respond is unknown and further exploration is needed particularly as it relates to subcutaneous fat deposition and skin temperature. It is concluded that, for cats, the body weight of the subject should be standardised or included in any analyses for assessment of nociception. Inclusion of body weight data in analyses may also prove useful when using a CO<sub>2</sub> laser protocol in other species.

© 2013 Elsevier B.V. All rights reserved.

### 1. Introduction

The assessment of nociceptive thresholds using a reflex withdrawal from thermal stimulation has been

commonplace in rodent based research for many years (e.g. the tail-flick test: D'Amour and Smith, 1941). Systems that use thermal stimulation to measure nociception are of particular interest given that heat is a naturally occurring stimulus and heat mediated withdrawal is a fundamental nociceptive response in mammals (Herskin et al., 2009).

Carbon dioxide (CO<sub>2</sub>) lasers have been implemented to elicit behavioural responses to thermal nociceptive thresholds in various species including rats (Kao and Jaw, 2012), sheep (Guesgen et al., 2011), pigs (Di Giminiani et al.,

\* Corresponding author at: Department of Natural Sciences, Unitec Institute of Technology, Private Bag 92025, Auckland 1025, New Zealand. Tel.: +64 9815 4321x7071.

E-mail address: [mfarnworth@unitec.ac.nz](mailto:mfarnworth@unitec.ac.nz) (M.J. Farnworth).

2013; Herskin et al., 2009) and cattle (Ting et al., 2010; Veissier et al., 2000). More recently the CO<sub>2</sub> laser technique was validated as a tool for measuring thermal nociceptive thresholds in domestic cats (*Felis catus*) (Farnworth et al., 2013). The use of a CO<sub>2</sub> laser is considered ideal as it is non-invasive and can be used at a purely nociceptive level of stimulation (Kramer et al., 2012).

When a CO<sub>2</sub> laser is used on cats at low power settings the primary behavioural response, taken to indicate thermal nociceptive threshold, is a skin twitch known as the panniculus reflex (Farnworth et al., 2013). Use of thermal stimuli often results in habituation or sensitisation following successive exposures which are considered to be drawbacks of thermal techniques (Bölskei et al., 2010). However, similar to other devices used for measuring thermal thresholds in cats (Dixon et al., 2002) the behaviours elicited by the low power CO<sub>2</sub> laser technique show no evidence of habituation or sensitisation over a period of 1 h (Farnworth et al., 2013). Panniculus reflex, being the predominant and relatively invariant behavioural response, also minimises the likelihood that errors associated with subjective interpretation will be made. Reflex responses have been validated in other species for assessment of pain thresholds (e.g. cattle: Veissier et al., 2000) and may be particularly useful as they are able to predict subsequent behaviours. Measurement of reflex responses may therefore avoid the need to assess more overt behavioural responses, such as jumping or biting at the device, used in other thermal assessments of nociception and pain in cats (Slingsby et al., 2010; Steagall et al., 2007, 2008).

The use of a remotely applied CO<sub>2</sub> laser for measuring nociceptive thresholds in cats allows testing of greater numbers of individuals than has previously been possible using thermal (Taylor et al., 2007; Steagall et al., 2008), electrical (Millette et al., 2008) and pressure-based (Dixon et al., 2007) contact devices. This is because there is no requirement to attach devices to the animals used. Additionally, this means there is no need to habituate animals to the devices or exclude those that do not habituate to its presence (e.g. Dixon et al., 2007). Laser-based assessment has also been shown to require little manipulation or restriction of the test subjects or interference with normal management processes (Herskin et al., 2009; Veissier et al., 2000). These factors make it an ideal tool for the exploration of inter-individual differences in thermal nociceptive responses using a larger cohort.

Nociceptive response can be considered to be mechanistically heterogeneous. It has been found to differ based upon general characteristics such as age (rats: Gagliese and Melzack, 2000) and sex (Greenspan et al., 2007). Complex interactions between two or more factors are also evident such as sex and age (sheep: Guesgen et al., 2011) or age and body weight (piglets: Janczak et al., 2012). In addition to body weight, obesity may also have impacts upon nociceptive mechanical and thermal thresholds (rats: Iannitti et al., 2012). Finally specific individual experiences and conditions, such as previous experience of injurious events (rats: Ren et al., 2004) and positive maternal affiliation (lambs: Hild et al., 2010) have also been shown to affect nociceptive thresholds.

Little is known about how basic variables may affect thermal nociceptive thresholds in cats. With this in mind we undertook to explore individual variation in cats and its effect on thermal nociceptive response as characterised by latency to a behavioural response. We hypothesised that age, sex, body weight and neutered status of the individual would have an effect on nociceptive threshold in the domestic cat, as measured by latency to respond following thermal stimulation with a CO<sub>2</sub> laser.

## 2. Materials and methods

### 2.1. Subjects and housing conditions

All procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 11/101). A total of 113 domestic cats were used (60 male; 53 female) (Table 1). All cats were adult and over 1 year of age. The cats were permanently housed in a nutritional research facility and were fed a standard wet cat food diet ad libitum throughout the trial. Cats were housed in stable colonies of 10 individuals in outdoor pens (2.4 height × 1.4 width × 4.4 depth m); with approximately half the volume of each pen under cover.

During testing, cats were held in eight individual metabolism cages (0.8 height × 0.8 width × 1.1 depth m) in a 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 mm × 25 mm to prevent reflection of the laser and subsequent injury to the subjects or operators. For the cats' comfort, and to encourage sternal recumbence, each cage was furnished with a small wooden box and blanket. Food and water were not provided during the test phase.

### 2.2. Experimental protocol

#### 2.2.1. Thermal threshold testing procedure

The study was conducted over 5 days in February 2012. Approximately 24 h prior to the commencement of testing each cat's fur was clipped to skin level on both sides of the animal as per the technique outlined in Farnworth et al. (2013). The cats were not removed from their colony cages during this procedure. For each cat the sex, age, current body weight and neutered status (Table 1) were taken from their records. Cats were weighed on a weekly basis at the facility and the most recent weight was included in analyses. Each cat was randomly allocated to a group of eight, and the sequence in which groups were tested was randomised across the 5-day experimental period. All tests were conducted between 09:00 h and 17:00 h. The test period for each group was between 45 and 60 min.

**Table 1**

Basic cohort characteristics for male ( $N=60$ ) and female ( $N=53$ ) domestic cats included in an experiment to explore thermal nociceptive threshold as measured by latency to respond to a thermal CO<sub>2</sub> laser stimulus.

Sex	Number neutered	Mean ( $\pm$ SE) age (years)	Mean ( $\pm$ SE) body weight (g)	Median (range) condition score (1–9) <sup>a</sup>	Mean latency ( $\pm$ SE) to respond (s)
Male	59/60	7.1 ( $\pm$ 0.4)	4367.1 ( $\pm$ 84.6)	5 (4–8)	14.83 ( $\pm$ 0.85)
Female	31/53	5.9 ( $\pm$ 0.5)	3290.8 ( $\pm$ 67.2)	5 (4–7)	12.59 ( $\pm$ 0.94)

<sup>a</sup> Measured using the Purina® 9-point scale (Nestlé Purina PetCare Company, MO, USA). Data available for 105/113 subjects (male = 54/60; female = 51/53).

Immediately prior to tests of thermal nociception, each group was transferred from their normal housing to the experimental cages and were only returned after all three nociceptive tests had been conducted. 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. The test sequence began after the habituation period, when the majority of the cats were quiet and in sternal recumbency.

Each cat was exposed three times to a CO<sub>2</sub> thermal laser device during the test period (see Section 2.2.2). 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 safety cut-off time was reached (see Section 2.2.2). Following either of these behavioural responses, the laser was turned off and the latency to respond (time) noted to the nearest 0.1 s. In the event that the 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 after 5 min. Following an appropriate response the thermal laser was not re-applied until a minimum of 15 min had elapsed, well beyond the time required for heat decay at the site of stimulation in humans (Leandri et al., 2006) and the time interval required to prevent sensitisation in pigs (Di Giminiani et al., 2013). The exact time between each test varied dependent upon the activity pattern of the individual (i.e. time to sternal recumbency).

### 2.2.2. Laser device

Thermal nociceptive thresholds were measured using a remote laser device (Model 48-1, Synrad, Mulkey, Washington, USA). The CO<sub>2</sub> laser produced a 3.5 mm diameter beam 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 10.60  $\mu$ m (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<sub>2</sub> laser has previously been demonstrated to have no discernable effect on the behavioural response latency of cats (Farnworth et al., 2013), therefore it was not used as a control in this experiment. As the power setting was greater than the 165 mW used in Farnworth et al. (2013) settings were first tested on two cats not used in the study. At 500 mW all responses occurred in less than 60 s

with no evidence of reddening or skin damage. Therefore, 60 s was set as the maximum duration for exposure to the thermal stimulus. If no response was seen within this time the test was terminated.

### 2.3. Additional data collection

Provisional statistical analysis of the data indicated that body weight was a significant variable in determining latency to respond. Therefore it was considered of value to collect data on the body condition scores of the individuals used. Body condition was scored using the Purina® 9-point scale (Nestlé Purina PetCare Company, MO, USA) where a score of 1–4 indicates 'too thin', 5 is 'ideal' and 6–9 'too heavy'. These data were collected 5 months after the original latency to respond data and were then incorporated into re-analysis of the original data set. Only 105/113 cats were still available for body condition scoring. Analysis and discussion of the impact of condition score were made with caution given the uncertainty around variation in body condition scores for individuals during the interim 5 months.

### 2.4. Statistical analyses

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 19.0 for Windows (IBM Inc., Chicago IL, USA). The data were log<sub>10</sub> transformed to achieve a normalised distribution with homogeneity of variances. To reduce analytical complexity, and because older cats are more likely to have underlying conditions (e.g. degenerative joint disease) which may impact upon nociceptive response (e.g. Lascelles et al., 2012), cats were assigned to one of two age categories 'younger adults' (1–6.99 years;  $N=57$ ) and 'older adults' (7 years or over  $N=56$ ).

Repeatability of the latency to respond for the cats was tested across the three exposures using a single measures intra-class correlation (ICC). A Cronbach's alpha test was used to assess the reliability of the data. Differences and correlations were considered significant at  $P < 0.05$ . Following a robust result from the ICC the log<sub>10</sub> transformed mean value for the three exposures was used for subsequent analyses. Suitability of the mean transformed data for use with an Analysis of Co-Variance (ANCOVA) was assessed using a Spearman's Rank Correlation.

These provisional analyses suggested that inclusion of body weight as a co-variant would result in a more powerful  $F$ -test when examining the possible influence of other factors on latency to respond. The use of ANCOVA was therefore appropriate. However, a requirement of the ANCOVA protocol is that the co-variant (i.e. body weight)

should be uncorrelated with other treatment factors. Factors of interest in this study were the sex and age of the animal and, for females, whether or not they were sterilised. An independent samples test indicated that sex of the cat was not independent of its body weight which confounded interpretation of the ANCOVA analysis. We had no evidence of confounding factors between our other treatment factors (age and sterilisation status) and body weight.

We explored differences in mean response times between males and females using a simple *t*-test. Given that males were also significantly heavier than females it was not clear whether this difference in response time was driven by body weight, as suggested by the significant correlation between body weight and  $\log_{10}$  mean response time, or by sex. Consequently we explored the effect of the age, sterilisation and body weight on mean response time using ANCOVA for males and females separately. To explore the underlying effect of sex and body weight a post hoc *t*-test was conducted on male and female cats with body weights that overlapped. Note that all males, with the exception of one individual were sterilised (Table 1) so we did not include this factor in further analysis of the male data.

### 3. Results

#### 3.1. Provisional analyses

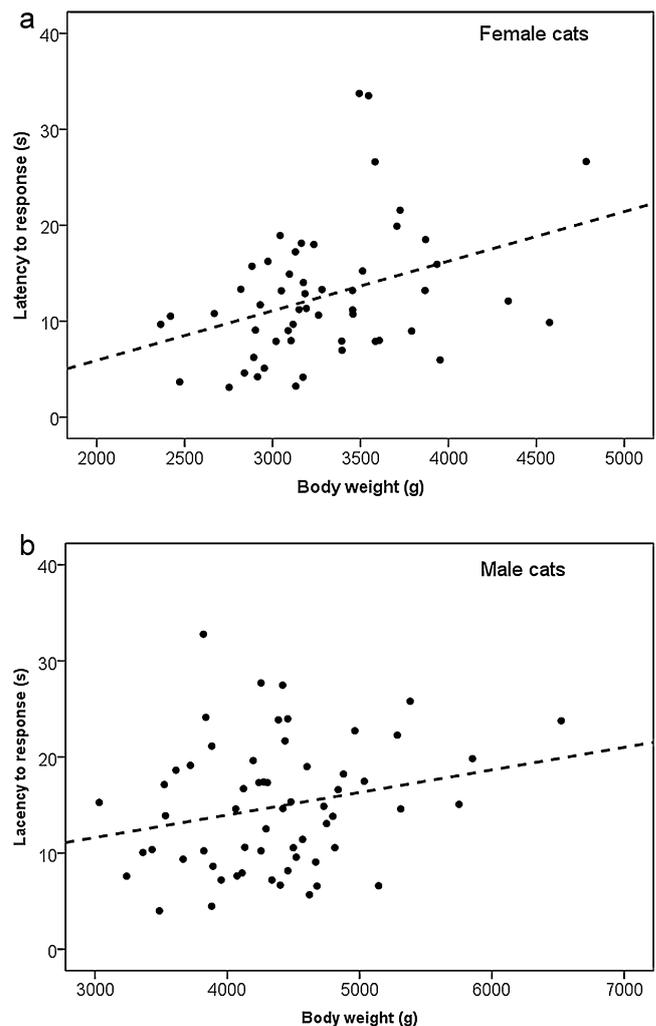
The mean body weight of males was significantly greater than that of females (Table 1;  $t=9.757$ ;  $DF=111$ ;  $P<0.0001$ ). Males also had a longer mean  $\log_{10}$  response time ( $\pm SE$ ) than females ( $1.098 \pm 0.27$  s and  $1.005 \pm 0.32$  s respectively). Untransformed mean latency to respond across all tests was 14.83 s for males (range: 4.0–32.8 s) and 12.59 s for females (range: 3.1–33.7 s). These differences were significant ( $t=2.223$ ;  $DF=111$ ;  $P=0.028$ ).

#### 3.2. Latency to respond and repeatability

The ICC demonstrated significant repeatability for individuals across tests (ICC=0.482; 95% confidence limits: 0.371–0.587;  $F_{(112,224)}=3.787$ ;  $P<0.001$ ). A Cronbach's alpha of 0.736 suggested that the source data were reliable. Following the ICC, mean  $\log_{10}$  values were used for further analyses.

#### 3.3. Effects of individual variables on response latency

Preliminary analysis indicated a significant correlation between body weight and mean  $\log_{10}$  response time (Spearman Rank Correlation (SRC)  $r=0.327$ ;  $P<0.0001$ ) which supported the use of ANCOVA to control for the effects of body weight and generate a more powerful test to assess the influence of other factors on latency to respond (Section 2.4). There was no significant correlation between condition score and mean  $\log_{10}$  response time (SRC  $r=0.122$ ;  $P=0.215$ ) in our sample of cats. However there was a significant positive correlation between body weight and condition score for both males (SRC  $r=0.539$ ;  $P<0.0001$ ) and females (SRC  $r=0.45$ ;  $P<0.001$ ) within the sample.



**Fig. 1.** (a/b) Association between body weight (g) and mean latency to respond (s) to thermal stimulation using a  $\text{CO}_2$  laser for both female ( $N=53$ ) and male ( $N=60$ ) cats. Following Analysis of Co-Variance the association is significant for females ( $P=0.013$ ) and marginal for males ( $P=0.058$ ). Latency to respond is presented as a back-transformed mean for ease of interpretation.

For females, ANCOVA confirmed a significant effect of body weight on mean  $\log_{10}$  response time (Fig. 1a;  $F=6.727$ ;  $DF=1$ ;  $P=0.013$ ), but there was no effect of sterilisation or age ( $F=1.399$ ;  $DF=1$ ;  $P=0.243$  and  $F=0.001$ ;  $DF=1$ ;  $P=0.98$  respectively). Unlike females, males exhibited no significant effect of body weight on mean  $\log_{10}$  response time, although non-significance was marginal (Fig. 1b;  $F=3.758$ ;  $DF=1$ ;  $P=0.058$ ). As for females, there was no significant effect of age on latency to respond in males ( $F=0.325$ ;  $DF=1$ ;  $P=0.571$ ). A post hoc *t*-test for those individuals with similar body weight was conducted. This analysis included 46 males and 38 females that had overlapping measurements of body weight (range 3032–4783 g). There was no significant difference in mean  $\log_{10}$  response times between males and females in this group ( $t=-0.370$ ;  $DF=83$ ;  $P=0.721$ ).

### 4. Discussion

Based upon the literature one may expect a range of inter-individual variables to impact upon the nociceptive

thresholds of domestic cats. However, these findings indicate that this technique is only significantly influenced by the body weight of the test subject, and then definitively only in females. The non-significant result obtained when comparing males and females of similar body weight indicates that the assertion of [Farnworth et al. \(2013\)](#), that variation in nociceptive thresholds may be associated with the sex of the cat per se, is not supported.

Body weight has not previously been cited as impacting upon nociceptive threshold in cats and there appears little information around this parameter for other species. [Di Giminiani et al. \(2013\)](#) have demonstrated that smaller pigs (~30 kg) respond more quickly to CO<sub>2</sub> laser stimulation than larger pigs (~60 kg). However, as for other studies using juveniles, the effect of body weight has not been analysed independently of age ([Ting et al., 2010](#); [Guesgen et al., 2011](#)) and it is not possible to establish whether or not it was a contributing variable in any differences observed. It is possible that both behavioural differences in younger animals and cutaneous neural density will impact upon speed of response ([Di Giminiani et al., 2013](#)).

Body weight does not have a quantifiable effect on thermal pain thresholds in humans with normal body mass indices (e.g. [Neziri et al., 1996](#)). In contrast, there is evidence that obesity affects latency to respond to thermal stimulation either as a result of obesity mediated inflammatory responses or thermal sensitivity. Some studies demonstrate a reduced latency of obese rats to respond to thermal stimulation following simulated inflammation ([Iannitti et al., 2012](#)), whilst other studies demonstrate increased latency of obese humans to respond following thermal stimulation of peripheral areas of skin ([Miscio et al., 2005](#)). The 9-point condition score system used in this study is a common measure of obesity in companion animals. Condition scores were significantly correlated with body weight despite the time elapsed between the two data collection phases. However condition score failed to elucidate the underlying impact on latency to respond. It is important to note that the post hoc collection of these data may have substantially impacted upon their comparative value. Condition scores were unable to account for seasonal and individual fluctuations during the interim 5 months. However, it could also be the case that condition score is not an accurate enough measure to explain the underlying effects of body weight on behavioural expression of nociceptive threshold. It is suggested therefore that other physical parameters should be explored which give a more accurate understanding of body weight. For example a comparison between increased body weight as a result of increased musculature versus a similar gain caused by subcutaneous fat.

Body weight may have a more complex impact upon latency to respond which includes interactions with other unquantified variables. For example [Ting et al. \(2010\)](#) note that initial skin temperature directly affects the speed of the response in calves. This is one drawback of a remote method. Whereas the thermal contact device developed by [Dixon et al. \(2002\)](#) accounts for this variation by measuring initial skin temperature and temperature at point of response, the CO<sub>2</sub> laser does not. The cats were also not housed in a temperature controlled room and daily

fluctuations may have impacted upon measurements. For humans the level of subcutaneous fat deposition is found to significantly decrease skin temperature around the abdomen ([Savastano et al., 2009](#)). This may translate into a longer duration for heavier individuals to respond to thermal stimulation as observed by [Miscio et al. \(2005\)](#) and here in cats. The body weight of our subjects and the range of condition scores may mean that skin temperature did vary significantly between individuals. Future tests using the laser should attempt to control for this by using a temperature controlled room and, if possible, by gauging skin temperature, possibly through use of a thermal imaging camera.

Previous research has indicated a sex difference in pain perception for some species and gonadal hormones are known to have an effect on pain responses ([Greenspan et al., 2007](#)). No such effect of sex per se (i.e. independent of body weight) was found for cats in this study. Our post hoc analysis of a sub-set of cats of similar body weight suggests that there was no significant difference between nociceptive responses of castrated males and spayed or unspayed females. It is important to note that such a comparison is investigative only given that it creates an artificial grouping which may include animals that are not directly comparable (i.e. the lightest males compared with heaviest females). The lack of entire males in the study and inability to assess circulating levels of oestrogens in cycling females mean this finding should be considered with caution. Female cats in New Zealand have been shown to have two reproductive peaks per year ([Aguilar and Farnworth, 2012](#)), therefore, circulating levels of oestrogens may have varied substantially between individuals.

It is apparent that for adult cats, age has no significant effect on nociceptive threshold as measured using a CO<sub>2</sub> laser, at least when 'young' cats (1–7 years) were compared with older cats (>7 years). Sheep have been noted as having divergent responses to CO<sub>2</sub> laser stimulation during early development of males and females ([Guesgen et al., 2011](#)). Assessment of sub-adult cats may prove valuable in understanding development of nociceptive responses for this species.

This is the first such procedure to explore any potential confounding factors that may impact upon behavioural expression of nociceptive thresholds in cats. Other techniques have not been validated against inter-individual variation in response thresholds. It may be particularly difficult to assess such variations in small cohort studies but the effects of inter-individual variability could, to some extent, be reduced if comparisons between treatments are made using the same test subjects (e.g. [Robertson et al., 2003](#)). Otherwise this work suggests that future studies involving thermal nociception should attempt to standardise the body weight of cats used or include weight as a covariate in any analyses.

This research confirms the conclusion of [Farnworth et al. \(2013\)](#). Behavioural response latency following thermal stimulation using a CO<sub>2</sub> laser is individually repeatable and therefore a valid method for exploration of nociception in cats. Increases in power output have previously been shown to reduce behavioural latency to respond ([Herskin et al., 2009](#); [Veissier et al., 2000](#)). The use of a 500 mW laser

output, as compared to 165 mW in Farnworth et al. (2013) saw a decrease in mean latency to respond (~14 s vs. 28.6 s) without a concomitant loss of repeatability. An increased power output of 500 mW is therefore recommended.

Further research is required to explore thermal stimulation using a CO<sub>2</sub> laser and its applications beyond its validity as a simple tool. Firstly, the underlying effect of body weight on nociception in cats requires more exploration. Moving beyond, it remains to be seen as to whether latency to a behavioural response, elicited at low power thermal stimulation, has any value as a measure of analgesic effect. Similarly it needs to be determined if this nociceptive response is an effective measurement for assessment of pain states following actual tissue damage or surgery.

## 5. Conclusions

The use of CO<sub>2</sub> lasers in the assessment of thermal nociceptive thresholds has been further validated as a technique in domestic cats. However, this large cohort study reveals that inter-individual variations, particularly the body weight of the subject, may impact upon the response to a previously validated technique. Further research is required to explore this effect and its underlying mechanisms beyond simple measures of body weight and condition. This research also supports the need for nociceptive threshold assessments to be investigated relative to inter-individual variations, both on a technique and a species basis. The variable that has been indicated as having an impact upon nociceptive thresholds in domestic cats may not be the same in other species.

## Acknowledgements

The authors would like to thank the staff and students at the Massey University Feline Unit for their generous provision of time and assistance.

## References

- Aguilar, G.D., Farnworth, M.J., 2012. Stray cats in Auckland, New Zealand: discovering geographic information for exploratory spatial analysis. *Appl. Geogr.* 34, 230–238.
- Bölcseki, K., Pethő, G., Szolcsányi, J., 2010. Noxious heat threshold measured with slowly increasing temperatures: novel rat thermal hyperalgesia models. In: Szallasi, A. (Ed.), *Analgesia: Methods and Protocols*, vol. 617. Springer Science and Business Media, Rueil-Malmaison, France, pp. 57–66, *Methods Mol. Biol.*
- D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.* 72, 74–79.
- Di Giminiani, P., Petersen, L.J., Harskin, M.S., 2013. Nociceptive responses to thermal and mechanical stimulations in awake pigs. *Eur. J. Pain* 17, 638–648.
- Dixon, M.J., Robertson, S.A., Taylor, P.M., 2002. A thermal threshold testing device for evaluation of analgesics in cats. *Res. Vet. Sci.* 72, 205–210.
- Dixon, M.J., Taylor, P.M., Steagall, P.V.M., Brondani, J.T., Luna, S.P.L., 2007. Development of a pressure nociceptive threshold testing device for evaluation of analgesics in cats. *Res. Vet. Sci.* 82, 85–92.
- Farnworth, M.J., Beausoleil, N.J., Adams, N.J., Barrett, L.A., Stevenson, M., Thomas, D.G., Waterland, M.R., Waran, N.K., Stafford, K.J., 2013. 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.
- Gagliese, L., Melzack, R., 2000. Age differences in nociception and pain behaviours in the rat. *Neurosci. Biobehav. Rev.* 24, 843–854.
- Greenspan, J.D., Craft, R.M., LeResche, L., Arendt-Nielsen, L., Berkley, K.J., Fillingim, R.B., Gold, M.S., Holdcroft, A., Lautenbacher, S., Mayer, E.A., Mogil, J.S., Murphy, A.Z., Traub, R.J., 2007. Studying sex and gender differences in pain and analgesia: a consensus report. *Pain* 132, S26–S45.
- Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., Jones, G., Stafford, K.J., 2011. The effects of age and sex on pain sensitivity in young lambs. *Appl. Anim. Behav. Sci.* 135, 51–56.
- Hendriks, W.H., Wamberg, S., Tartellin, M.F., 1999. A metabolism cage for quantitative urine collection and accurate measurement of water balance in adult cats (*Felis catus*). *J. Anim. Physiol. A Anim. Nutr.* 82, 94–105.
- Herskin, M.S., Ladewig, J., Arendt-Nielsen, L., 2009. Measuring cutaneous thermal nociception in group-housed pigs using laser technique—effects of laser power output. *Appl. Anim. Behav. Sci.* 118, 144–151.
- Hild, S., Andersen, I.L., Zanella, A.J., 2010. The relationship between thermal nociceptive threshold in lambs and ewe–lamb interactions. *Small Ruminant Res.* 90, 142–145.
- Iannitti, T., Graham, A., Dolan, S., 2012. Increased central and peripheral inflammation and inflammatory hyperalgesia in Zucker rat model of leptin receptor deficiency and genetic obesity. *Exp. Physiol.* 97, 1236–1245.
- Janczak, A.M., Ranheim, B., Fosse, T.K., Hild, S., Nordgreen, J., Moe, R.O., Zanella, A.J., 2012. Factors affecting mechanical (nociceptive) thresholds in piglets. *Vet. Anaesth. Analg.* 2012, 628–635.
- Kao, Y.-C., Jaw, F.-S., 2012. Laser-induced withdrawal test for electrophysiological recordings of nociception. *J. Comp. Physiol. A* 198, 79–83.
- Kramer, J.L.K., Haefeli, J., Jutzeler, C.R., 2012. An objective measure of stimulus-evoked pain. *J. Neurosci.* 32, 12981–12982.
- Lascelles, B.D.X., Dong, Y.-H., Marcellin-Little, D.J., Thomson, A., Wheeler, S., Correa, M., 2012. Relationship of orthopedic examination, gonio-metric measurements, and radiographic signs of degenerative joint disease in cats. *BMC Vet. Res.* 8 (10), 1–8.
- Leandri, M., Saturno, M., Spadavecchia, L., Iannetti, G.D., Cruccu, G., Truini, A., 2006. Measurement of skin temperature after infrared laser stimulation. *Neurophysiol. Clin.* 36, 207–218.
- Millette, V.M., Steagall, P.V.M., Duke-Novakovski, T., Livingston, A.J., 2008. Effects of meperidine or saline on thermal, mechanical and electrical nociceptive thresholds in cats. *Vet. Anaesth. Analg.* 35, 543–547.
- Miscio, G., Guastamacchia, G., Brunani, A., Priano, L., Baudo, S., Mauro, A., 2005. Obesity and peripheral neuropathy risk: a dangerous liaison. *J. Peripher. Nerv. Syst.* 10, 354–358.
- Neziri, A.Y., Scaramozzino, P., Andersen, O.K., Dickenson, A.H., Arendt-Nielsen, L., Curatolo, M., 1996. Reference values of mechanical and thermal pain tests in a pain-free population. *Eur. J. Pain* 15, 376–383.
- Ren, K., Anseloni, V., Zou, S.-P., Wade, E.B., Novikova, S.I., Ennis, M., Traub, R.J., Gold, M.S., Dubner, R., Lidow, M.S., 2004. Characterization of basal and re-inflammation-associated long-term alteration in pain sensitivity following short-lasting neonatal local inflammatory insult. *Pain* 110, 588–596.
- Robertson, S.A., Taylor, P.M., Lascelles, B.D.X., Dixon, M.J., 2003. Changes in thermal threshold response in eight cats after administration of buprenorphine, butorphanol and morphine. *Vet. Rec.* 153, 462–465.
- Savastano, D.M., Grobach, A.M., Eden, H.S., Brady, S.M., Reynolds, J.C., Yanovski, J.A., 2009. Adiposity and human regional body temperature. *Am. J. Clin. Nutr.* 90, 1124–1131.
- Slingsby, L.S., Murrell, J.C., Taylor, P.M., 2010. Combination of dexmedetomidine with buprenorphine enhances the antinociceptive effect to a thermal stimulus in the cat compared with either agent alone. *Vet. Anaesth. Analg.* 37, 162–170.
- Steagall, P.V.M., Taylor, P.M., Brondani, J.T., Luna, S.P.L., Dixon, M.J., Ferreira, T.H., 2007. Effects of buprenorphine carprofen and saline on thermal and mechanical nociceptive thresholds in cats. *Vet. Anaesth. Analg.* 34, 344–350.
- Steagall, P.V.M., Taylor, P.M., Brondani, J.T., Luna, S.P.L., Dixon, M.J., 2008. Antinociceptive effects of tramadol and acepromazine in cats. *J. Feline Med. Surg.* 10, 24–31.
- Taylor, P.M., Robertson, S.A., Dixon, M.J., 2007. 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.
- Ting, S.T.L., Earley, B., Veissier, I., Gupta, S., Crowe, M.A., 2010. Effects of burdizzo castration on CO<sub>2</sub> laser induced thermal nociception of Holstein–Friesian calves of different ages. *Appl. Anim. Behav. Sci.* 126, 12–18.
- Veissier, I., Rushen, J., Colwell, D., de Passillé, A.M., 2000. A laser-based method for measuring thermal nociception of cattle. *Appl. Anim. Behav. Sci.* 66, 289–304.