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Pain assessment and alleviation in the domestic cat (*Felis catus*)

A thesis presented in fulfilment of the requirements for the degree of

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in

Veterinary Science

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This thesis is dedicated to my parents and my Grandma without whom I may never have realised the value of education and found my love of biology.
Abstract

This thesis begins by exploring current knowledge around the domestic cat population and gonadectomy as well as pain assessment tools and pain alleviation for cats. It identifies a number of areas where knowledge is either absent or in need of updating and limitations in tools for the assessment of pain in cats. It therefore proposes the undertaking of the projects which can be found in the subsequent chapters.

Chapters two and three compared the attitudes and practices of veterinarians in New Zealand, Australia and the United Kingdom (UK) around pre-pubertal gonadectomy and provision of analgesia for cats. This used an electronic questionnaire which received 717 responses. Most respondents believed pre-pubertal gonadectomy was either entirely or ‘sometimes’ desirable (556/621). Age of patient at gonadectomy was significantly affected by country surveyed and respondents’ provision or non-provision of services for pounds. Post hoc Tukey HSD analysis indicated the mean age of both spaying and castration (both 4.3 months) in the UK was significantly different from both Australia (spaying: 3.4 months, castration: 3.2 months) and New Zealand (spaying: 3.4 months, castration: 3.2 months) (all p < 0.001). Mean ages at spaying and castration were also significantly different (p=0.008; p=0.019 respectively) for non-providers (spaying: 3.9 months, castration: 3.8 months) of services to pounds when compared to providers (spaying and castration: both 3.6 months).

With respect to the use of analgesics there has been a substantial increase in provision of analgesia to cats undergoing gonadectomy when compared to the early literature. There were significant differences in prevalence of analgesia provision prior to and
following spaying and castration (both $p < 0.001$). There were also significant post-
operative and post-discharge differences in provision of analgesia for castration, as
compared to spaying (both $p < 0.001$), and a similar effect was seen pre/intra-
operatively ($p = 0.002$).

Significant effects amongst countries and between genders relative to the desirability
of pre-pubertal gonadectomy were identified. Respondents from the UK were more
likely to answer ‘no’ ($p=0.004$) or ‘sometimes’ ($p=0.05$) as compared to those from
New Zealand or Australia. Females were more likely to respond with ‘sometimes’ as
opposed to ‘yes’ than males. Reasons for considering pre-pubertal gonadectomy
desirable or sometimes desirable focussed on reducing unwanted pregnancies and
improving population control, as well as improving rates of adoption, owner
compliance and cat behaviour and health. Post-operative provision of analgesia
following both castration ($p < 0.001$) and spaying ($p < 0.001$) also differed amongst
countries of practice. Veterinarians in Australia and New Zealand were more likely to
provide post-operative analgesia than those from the UK. Veterinarians from the UK
more commonly used non-steroidal anti-inflammatory drugs (NSAID) in the pre/intra-
operative phase ($P < 0.001$) than veterinarians from either New Zealand or Australia.

Differences in attitudes towards pre-pubertal gonadectomy amongst countries may
relate to the specific Veterinary Association’s guidelines or possibly differences in
social discourse which affect perception of cats. There is substantial overlap between
the reported minimum age of gonadectomy and the age at which cats can enter early
puberty, allowing a window for unintentional pregnancy when pre-pubertal
gonadectomy does not occur. The differences in use of analgesics amongst the UK,
Australia and New Zealand may reflect differing professional considerations of the
risks associated with the use of NSAID. In the interests of animal welfare, pain relief should perhaps be provided or offered more frequently for owner administration.

Chapters four through six explored the value of a thermal carbon dioxide (CO$_2$) laser for the assessment of nociceptive thresholds in cats. To begin repeatabilities were established based on individual responses to three thermal tests on the same day and across 4 consecutive days. A total of 12 thermal tests were conducted on 16 adult cats (50% male). A non-thermal helium aiming laser was used as a control to ensure the animals were responding to the thermal component of the device. All thermal tests elicited a behavioural response 97% of which were a skin twitch known as the panniculus reflex. No control tests resulted in this reflex behaviour. There was no evidence that cats became sensitised or habituated to the low power thermal stimulus on any given day (p=0.426) or across days (p=0.115). There was also no difference in latency to respond between males and females (p=0.094), although there was a significant day of testing and sex interaction (p=0.042). Significant intra-class correlations (ICC) demonstrated that individual responses were repeatable over days 1 to 3 (all p<0.05) but not over day 4 (p=0.096). A significant intra-class correlation was also evident across all days when data were combined (p<0.0001).

Significant repeatabilities in the first laser-based experiment were low ranging from 0.241 to 0.414 therefore a larger sample was used (n=113) to establish any other factors, including age or sex effects, that impacted upon thermal sensitivity. In this next phase cats were exposed to a more powerful (500mW) CO$_2$ thermal laser three times during a 45-60 min test period with a minimum of 15 min elapsed between consecutive tests on any one individual. Again time to display a behavioural response was repeatable across tests for any given cat (ICC=0.482; p<0.001). 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.

Finally the CO₂ laser’s ability to assess analgesia in pain-free cats was explored. Sixty healthy adult female cats were used and randomly allocated to one of six treatments 1) saline 0.2 ml/cat; 2) morphine 0.5 mg/kg; 3) buprenorphine 20µg/kg; 4) medetomidine 2 µg/kg; 5) tramadol 2mg/kg; 6) ketoprofen 2mg/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 again assessed using time to respond behaviourally to stimulation with a 500mW CO₂ laser. Maximum latency to respond was set at 60 sec but given that this technique was found to cause minor skin blistering in individuals that reached the 60s exposure limit, a cut off time of <45s is recommended.

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 to further explain the direction of the effect. Cats treated with morphine (p=0.045) and tramadol (p=0.002) showed significant increases in latency to respond over the duration of the test period. Treatment with buprenorphine also resulted in increases in latency to respond although only at the level of a statistical trend (p=0.091). Injection of saline, ketoprofen or medetomidine showed no significant effect on latency to respond.
longest latency to respond after injection of morphine was achieved at 60-75 min whilst that of buprenorphine occurred at 90-105 min.

These projects validated the CO₂ laser technique for use in cats and demonstrate that it can be used for assessment of analgesia and may be useful for differentiating amongst analgesic treatments. This technique 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 possible experiments using this technique are to be found in the discussion chapter.

Keywords: Analgesia; Cat; CO₂ laser; Gonadectomy; Pain assessment; Pre-pubertal; Veterinary attitudes
# Contents

Abstract .......................................................................................................................... 5

Contents ....................................................................................................................... 11

List of tables and figures .............................................................................................. 18

Acknowledgements ...................................................................................................... 19

Preface .......................................................................................................................... 21

1. Literature Review: ............................................................................................... 23

1.1 The domestic cat population ......................................................................... 25

1.1.1 Problems associated with the cat population in New Zealand ............... 25

1.2 Reasons for Gonadectomy ............................................................................ 27

1.3 Nociception and pain ..................................................................................... 28

1.3.1 Defining nociception and pain .................................................................... 28

1.3.2 Acute pain .............................................................................................. 30

1.3.3 Inflammatory pain .................................................................................. 31

1.3.4 Chronic pain ........................................................................................... 31

1.3.5 Pain severity ........................................................................................... 32

1.3.6 Pain duration .......................................................................................... 33

1.4 Impact of painful experiences ....................................................................... 34
1.4.1 Central sensitization and the development of chronic/maladaptive pain
35

1.5 Gonadectomy of domestic cats .................................................................35

1.5.1 Timing of gonadectomy ....................................................................36

1.5.2 Impact of gonadectomy on cats .......................................................37

1.6 Current use of analgesia for pain in cats ..............................................38

1.7 Managing the pain associated with gonadectomy in cats .................39

1.7.1 Peri-operative pain management ......................................................40

1.7.2 Post-operative and recovery pain management ..............................42

1.8 Current limitations to effective pain management in cats .................44

1.9 Assessing pain .....................................................................................45

1.9.1 Behavioural assessment of pain .......................................................45

1.9.2 Limitations of behavioural assessment of pain ...............................47

1.9.3 Behavioural measurement of pain in cats .......................................49

1.9.4 Pain scales .....................................................................................50

1.9.5 Quantitative sensory testing ..........................................................53

1.10 Objectives of this study ......................................................................55

1.11 References .........................................................................................56

Survey Paper One .....................................................................................67
2. Veterinary attitudes towards pre-pubertal gonadectomy of cats: A comparison of samples from New Zealand, Australia and the United Kingdom. ......................... 69

2.1 Abstract ......................................................................................................... 69

2.2 Introduction ................................................................................................... 71

2.3 Materials and methods ................................................................................ 74

2.3.1 Statistical analyses ................................................................................. 75

2.4 Results ......................................................................................................... 77

2.4.1 Basic demographics ............................................................................... 77

2.5 Discussion ..................................................................................................... 86

2.6 References ..................................................................................................... 92

Survey Paper Two ........................................................................................................ 95

3. Veterinary provision of analgesia for domestic cats (*Felis catus*) undergoing gonadectomy: A comparison of samples from New Zealand, Australia and the United Kingdom. ..................................................................................................................... 97

3.1 Abstract ......................................................................................................... 97

3.2 Introduction ................................................................................................... 98

3.3 Materials and methods ................................................................................ 102

3.4 Statistical analyses ..................................................................................... 103

3.5 Results ......................................................................................................... 105
4. Validating the use of a carbon dioxide laser for assessing nociceptive thresholds in adult domestic cats (Felis catus).

4.1 Abstract

4.2 Introduction

4.3 Materials and methods

4.3.1 Subjects and housing conditions

4.3.2 Experimental protocol

4.4 Statistical analyses

4.5 Results

4.5.1 Latency to respond

4.5.2 Repeatability
5. Body weight affects behavioural indication of thermal nociceptive threshold in adult female domestic cats (*Felis catus*). ................................................................. 145

5.1 Abstract ....................................................................................................... 145

5.2 Introduction ................................................................................................. 146

5.3 Materials and methods ................................................................................ 148

5.3.1 Subjects and housing conditions .......................................................... 148

5.3.2 Experimental protocol .......................................................................... 149

5.3.3 Additional data collection .................................................................... 151

5.4 Statistical analyses....................................................................................... 152

5.5 Results ......................................................................................................... 154

5.5.1 Provisional analyses................................................................................ 154

5.5.2 Effects of individual variables on response latency............................. 155

5.6 Discussion ................................................................................................... 157

5.7 Conclusions ................................................................................................. 161

5.8 References ................................................................................................... 162
6. Assessment of a carbon dioxide laser for the measurement of thermal nociceptive thresholds following intra-muscular administration of morphine, buprenorphine, tramadol, ketoprofen and medetomidine to pain-free female cats.......................... 167

6.1 Abstract ........................................................................................................ 167

6.2 Introduction ................................................................................................. 168

6.3 Materials and methods ............................................................................... 171

6.3.1 Cats and housing conditions ................................................................. 171

6.3.2 Laser device ........................................................................................... 172

6.3.3 Thermal threshold testing procedure ..................................................... 173

6.3.4 Drug treatments ...................................................................................... 175

6.3.5 Statistical analyses .................................................................................. 175

6.4 Results ....................................................................................................... 176

6.4.1 Weight .................................................................................................... 176

6.4.2 Effect of treatments on latency to respond to thermal stimulation ...... 177

6.4.3 Side effects of treatment and procedure.............................................. 181

6.5 Discussion .................................................................................................. 182

6.6 References ................................................................................................. 185

7. General discussion. ...................................................................................... 189
7.1 Gonadectomy and pain in cats ................................................................. 189

7.2 Value of these studies ........................................................................ 191

7.3 Summary of key findings ..................................................................... 192

7.4 Implications of findings for veterinary practice and pain research in cats.. 195

7.5 Limitations of the research ................................................................. 198

7.5.1 Survey studies ................................................................................ 198

7.5.2 Laser-based studies .......................................................................... 199

7.6 Future enquiry .................................................................................... 202

7.7 References .......................................................................................... 206

8. Appendix 1 ................................................................................................ 209

9. Appendix 2: DRC 16s Statements of contribution to doctoral thesis containing publications for chapters 2-6 inclusive. .............................................. 213
List of tables and figures

Table 2-1: Respondent variables ................................................................. 78
Table 2-2: Minimum age of gonadectomy ................................................. 79
Table 2-3: Minimum age of spay relative to service provision ............... 80
Table 2-4: Is pre-pubertal gonadectomy desirable? Response by gender .......... 82
Table 2-5: Responses to open ended questions about pre-pubertal gonadectomy ................................................................................................. 85
Table 3-1: Analgesia use for cats undergoing gonadectomy ..................... 106
Table 3-2: Provision of analgesia relative to country of practice .......... 109
Table 3-3: Analgesics used during and after gonadectomy relative to country of practice .................................................................................... 111
Table 4-1: Intra-class correlations and repeatability of laser protocol ........ 134
Table 5-1: Cat characteristics .................................................................... 155
Table 6-1: Number of cats reaching 60 s cut-off by treatment ............. 178

Figure 5-1a/b: Body weight and response latency .................................... 156
Figure 6-1: Individual variation in latency to respond to buprenorphine .... 179
Figure 6-2: Latency to respond relative to treatment and across the testing period. ........................................................................................................ 180
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Cats, like most animals for which humans have a burden of care, undergo elective surgeries regularly. Not least of these is gonadectomy and, as a result, it is commonly used as a treatment within most studies of cat pain. There is an apparent gap within the scientific literature around gonadectomy. Provision of analgesia to cats before and following gonadectomy has not been explored for a number of years and information may now be out-dated given the rapid advances being made in veterinary medicine. For New Zealand and Australia the timing of gonadectomy in terms of the typical patient’s age is not known. Furthermore how exactly cats experience pain is not fully understood and, although gonadectomy is considered by practitioners to be less painful than other surgeries, how responses are mediated by pain severity is not easily assessed. Current techniques for assessing pain in cats use rating scales which may be influenced by the raters’ experiences and subjective perceptions. Pharmacological studies use attached thermal, mechanical or electrical devices to elicit a nociceptive response and assess efficacious pain management. These devices require substantial habituation and limit sample sizes. It is therefore judicious to explore techniques which may reduce subjectivity whilst being unconstrained by a need for habituation. Carbon dioxide lasers have been used in a range of species to explore nociceptive thresholds and can be utilised with minimal impact upon management routines. To date this technique has not been applied to cats.

This thesis comprises a literature review which expands upon the concepts of gonadectomy, pain and pain management in cats and is followed by five experimental chapters. Chapters two through five have all been published in peer-reviewed journals.
and chapter six is currently in submission. The full manuscript titles, authorships and journals in which they were published can be found on the first page of each chapter. The main body of each chapter is presented as it can be found in the peer-reviewed literature.

In the interests of expediting the reading process all readers should be aware that chapters two and three used the same survey and therefore have similar methodologies. Likewise chapters four, five, and six used the same population of cats and testing facility and therefore also share significant components of their methodologies.
1. Literature Review:

Gonadectomy, pain and current methods used to assess pain in domestic cats (*Felis catus*)

This chapter is written in the style of the New Zealand Veterinary Journal
1.1 The domestic cat population

Globally the total domestic cat (*Felis catus*) population is estimated to be 600 million (Peterson *et al.* 2012) and is increasing as the world’s human population becomes more urbanised (Dabritz and Conrad 2010). In New Zealand the cat population has been estimated to be 1.4 million with approximately 48% of households owning at least one cat (MacKay 2011). Although the largest proportion of the cat population is identified as owned (Bradshaw *et al.* 1999), a significant number of cats lie outside traditional ownership models and are either unowned or semi-owned (Toukhsati *et al.* 2007). For example, in the United States of America (USA) unowned and semi-owned cats are thought, in combination, to comprise somewhere between 14 and 63% (Mahlow and Slater 1996) over and above the owned population, however, a definitive number remains elusive.

1.1.1 Problems associated with the cat population in New Zealand

Owned cats are considered an integral component of their owners’ lives and provide significant emotional and social support (Zasloff and Kidd 1994; Sable 1995). However, in countries such as New Zealand, where the cat is both a companion animal and a legally recognised pest (Littin 2010), public perception of cats can be considered ambivalent (Farnworth *et al.* 2011b). The cat population therefore presents several distinct social problems both in terms of its management and mitigation of its impact.
Perhaps the most commonly recognised problem in New Zealand is the impact of cats upon native fauna including birds (van Heezik et al. 2010), but also reptiles and insects (Gillies and Clout 2003; Flux 2007). The majority of cats in New Zealand are free-roaming (Farnworth et al. 2011a) and this provides opportunity for them to be recognised as a public nuisance (Gunther and Terkel 2002).

A persistent cat population requires that many individuals are reproductively viable, especially as a substantial number of unowned cats are removed from urban areas every year by charitable organisations (Aguilar and Farnworth 2012). Given that New Zealanders report a high rate of sterilisation for owned cats (McKay et al. 2009), it is probable that population persistence is based on semi-owned or unowned individuals in urban environments, most of which are unsterilized. Such a dynamic is evident in Australia (Marston and Bennett 2009). This allows rapid population growth as urban cats are often found in relatively high densities compared to those outside urban environments. For example, in New Zealand, the density of cats within Dunedin city was reported as 223 cats/km² (van Heezik et al. 2010) compared to 2-3 cats/km² in rural Hawke’s Bay (Langham 1990). Anthropogenic food sources also mean that unowned cats in urban environments are less likely than those in rural or uninhabited areas to be nutritionally limited and fecundity may therefore be high (Schmidt et al. 2007).
1.2 Reasons for Gonadectomy

The aim of this review is not to address cat control methods and it should be noted that there is unlikely to be a single solution which can be applied to curb cat population growth. Within the scientific literature the three most common approaches mentioned are: curtailing the movement of owned cats (Toukhsati et al. 2012) or restricting ownership (Metsers et al. 2010); trapping and removal of unowned or semi-owned individuals for gonadectomy and adoption or euthanasia (Schmidt et al. 2009; Lohr et al. 2013); or trapping, neutering and return of unowned or semi-owned cats to the site of capture (TNR) (Natoli et al. 2006; Longcore et al. 2009).

Within New Zealand, given the risk that free-roaming cats pose to wildlife, public support for TNR appears to be equivocal (Farnworth et al. 2011b). However lethal control of the cat population may also be undesirable for groups that are particularly concerned with animal protection (Farnworth et al. 2013). Putting public debate to one side, it is clear that a strong component of any strategy to control cat population must be centred on reducing reproductive viability of the extant owned and semi-owned populations, namely via gonadectomy. In general, the younger this can be done the smaller the likelihood of any one individual cat contributing further to unwanted population growth. Gonadectomy is also considered to be a necessary surgery, not only to control population growth, but also to prevent undesirable behaviours such as roaming, urine spraying and aggression, particularly in males (Kustritz 2002).

How and when veterinarians choose to gonadectomise cats may have a substantial impact upon population management. In turn these decisions may also be, in part,
dictated by the social discourse around cats. Although early-age sterilisation is considered by some to be contentious (Spain et al. 2004) veterinary perception of those risks may be mediated by the manner in which veterinary professionals contextualise the wider risks posed by cats within their country of practice. As yet this effect remains to be quantified but recent literature from the UK suggests that the practice of early sterilisation, anecdotally widely used in New Zealand, appears to require greater support (Joyce and Yates 2011).

1.3 Nociception and pain

1.3.1 Defining nociception and pain

Nociception is a physiological process which involves the stimulation of afferent A and C nerve fibres by thermal, mechanical or chemical stimuli (Snider and McMahon 1998). The free endings of the myelinated Aδ and unmyelinated C nerve fibres respond to chemical, thermal and mechanical insults by generating an action potential which is processed in the superficial laminae of the dorsal horn of the spinal cord (Iannetti et al. 2004). This processing results in a reflex response to the potentially noxious event. The term noxious is defined as any stimulus which is damaging or potentially damaging (Millan 1999). Nociception is distinguished from pain as, in the absence of the stimulus becoming noxious; it is not consciously processed by the central nervous system. In humans both thermal and mechanical stimulation may result in a reflex response below the level required to be registered as painful (Snider and McMahon 1998). In essence nociception functions to allow avoidance of
potentially harmful stimuli before they result in actual tissue damage (Millan 1999).

Pain, like nociception, also results from activation of peripheral sensory neurones. During processing the neurotransmitter glutamate, released from the Aδ and C fibres, activates the neurones of the dorsal horn which relay the noxious event to the cortex (Iannetti et al. 2004). It is this secondary process which results in pain perception and therefore its negative emotional and physical impacts.

Pain is defined in humans as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP, 1994). For animals Molony (1997) defined pain as “..an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues.. [which]... changes the animal’s physiology to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery..”. Pain is considered a major contributor to reduced animal welfare, to the extent that absence or management of pain is identified explicitly in all discourses concerning improvement of welfare. The earliest formalized system for welfare evaluation that highlights the importance of pain to animal welfare is the five freedoms where it is explicitly identified in “freedom from pain, injury and disease” (Webster 2001). Pain continues to be recognised as an integral component of animal suffering in more recent constructs which seek to promote positive welfare (Mellor 2012).

Pain is evolutionarily functional in that it allows for the formation of memories of noxious stimulation and future avoidance of such events, with greater severity eliciting more active avoidance (Woolf and Salter 2000). However, Molony (1997) identified that pain becomes non-functional, and therefore is most likely to compromise welfare, when “..the intensity or duration of the experience is not
appropriate for the damage sustained and when physiological and behavioural responses are unsuccessful in alleviating it”. As a result pain can be identified as a complex experience that includes both the physical impacts of its intensity, duration and location but also emotional impacts associated with its aversive nature and the animal’s ability to manage such sensations. In the event that the level of pain exceeds normal intensity or duration, or, in animals, is perceived likely to do so by human caregivers, additional steps (e.g. provision of analgesia) should be taken to safeguard animal welfare.

The manner in which pain is experienced probably depends upon the species and its neuroanatomy. However, recent studies clearly identify that behavioural responses to painful or nociceptive events and those nerve fibres which trigger them, are highly conserved and exhibited by both vertebrates (Jirkof et al. 2013; Kluivers-Poodt et al. 2013; Li et al. 2013; Pratt et al. 2013; Yarnell et al. 2013) and invertebrates (Elwood 2012). Generally, as pain is considered to be consciously perceived in the cerebral cortex of the brain, it is questioned whether those animals which lack a cortex can experience pain (e.g. fish: Rose 2007).

1.3.2 Acute pain

Acute pain is the initial response to tissue damage, or potential damage, at a level which exceeds, or has the potential to exceed, the normal physiological range of the nociceptors (Millan 1999). Acute pain activates behavioural responses that have evolved to allow both current and future avoidance of the stimulus (Le Bars et al.
Physiologically it also results in a complex feedback cascade which promotes recovery from any injury caused, this feedback mechanism functions, in the acute phase, to trigger responses which are proportional to the insult (Millan 1999).

### 1.3.3 Inflammatory pain

Following injury associated with acute phase pain, arachidonic acid is converted to prostaglandin via the action of cyclooxygenase (Vane et al. 1998). Prostaglandins function to alter blood flow to the area affected and promote immune responses through chemotaxis, all of which further activate local nociceptors (Funk 2001). This results in hyperalgesia, a process by which the localised region becomes more sensitive to mechanical stimulation and during which the nociceptive threshold is reduced (Millan 1999). However, functional inflammatory responses are localised and short-lived with prostaglandins being rapidly degraded (Funk 2001). Therefore, once the stimulus has been removed, inflammation and the associated pain, rapidly decline.

### 1.3.4 Chronic pain

Unlike acute and inflammatory pain, chronic pain can be described as maladaptive in that it no longer functions to allow withdrawal from the stimulus or to promote recovery (Millan 1999). Most commonly it is associated with (and treated during) neurological or oncological disease progression (Bergh and Budsberg 2005) or
degenerative disorders (Lascelles 2010; Lascelles and Robertson 2010). However it may also result from neuropathy following acute trauma (Hsu 2011). Therefore efficient management of acute pain may reduce the likelihood that chronic pain conditions will develop. Chronic pain, being mal-adaptive, is a significant contributor to both reduced welfare (Robertson and Lascelles 2010) and long-term psychological disorders such as depression (Apkarian et al. 2009).

1.3.5 Pain severity

In human medicine “cutpoints” are used to characterise the severity of painful experiences using a system of self-reporting. Such cutpoints, characterised as ‘mild’ ‘moderate’ and ‘severe’ are affected by a number of factors associated with both the biology and the life history of an individual (Hirschfeld and Zernikow 2013). Determining the severity of any pain experience is important for understanding the likely impact of that pain on the individual. It would also allow the best course of action in terms of treatment protocols and urgency to be established. It therefore follows that, to best mitigate welfare compromises for animals that are experiencing pain, there needs to be some mechanism by which the severity of the experience can be gauged.

Surveys of veterinary professionals indicate that there is significant support for the notion that animals experience a level of pain which increases relative to the amount of tissue damage associated with surgery (Lascelles et al. 1999; Williams et al. 2005). Current severity scales developed for rodents use facial characteristics to assess
severity which are compiled by exposing animals to increasing levels of insult (Langford et al. 2010). Measurement of pain severity using facial expressions has since been validated in rabbits and mice using pain resulting from routine management protocols for example tattooing of rabbits (Keating et al. 2012) and vasectomy of mice (Leach et al. 2012).

1.3.6 Pain duration

Severity is only one aspect of painful experiences that has the potential to impact upon an animal’s welfare. The duration of a painful event and the degree of damage caused may also result in greater degrees of sensitisation and a greater likelihood of ‘hyperalgesia’ (Costigan et al. 2009). Painful experiences, if prolonged and performed without analgesia can cause persistent behavioural and physiological changes indicative of hyperalgesia (Eicher et al. 2006). Therefore veterinary surgeries such as gonadectomy which result in acute pain could result in longer term pain conditions if not effectively treated.

Chronic pain can develop in response to long-term medical conditions and results in behavioural changes (Van Loo et al. 1997). Robertson and Lascelles (2010) suggest that defining pain as chronic or acute based on duration fails to address the underlying causal factors. They therefore suggest that pain be re-categorised into ‘adaptive’ and ‘maladaptive’ pain. Chronic pain is maladaptive as it has the potential to cause long-term emotional suffering and withdrawal from normal interactions and positive experiences. Such responses may be particularly problematic in companion animals as
they may change the fundamental relationship between companion animal and owner. This phenomenon has been explored in horses (Fureix et al. 2010) and dogs (Wiseman et al. 2001), but is yet to be investigated in cats. As a result chronic pain, and especially that associated with chronic conditions, presents a significant issue within veterinary care and is of particular relevance in geriatric veterinary medicine (Robertson and Lascelles 2010).

1.4 Impact of painful experiences

Pain not only has immediate implications for welfare in terms of acute experience, i.e. immediate unpleasantness and other negative emotions (Flaherty 2012), it may also alter the behaviour of the sufferer in the mid-term, for example reduced feed intake (Scott et al. 2003) and activity (Farnworth et al. 2011c). Pain also has the potential to cause longer-term changes in pain responsiveness (Anand et al. 1999), social behaviours and ability to cope with environmental stressors (Walker et al. 2003) if it is persistent or uncontrolled. Acutely painful events may be of particular importance in early life, especially if unmanaged. Pain pathways are highly adaptable and neural plasticity (the ability of a neurone to change its function or structure) results in myriad ways for pain to be experienced and expressed (Woolf and Salter 2000). Therefore injurious events have the potential to change pain sensitivity across the individual’s lifetime, especially during early-life when the peripheral nervous system is continuing to develop (Torsney and Fitsgerald 2003). Early-life inflammatory events have also been linked to the development of localised hypersensitivity in the adult (Randich et al. 2006). Such effects are particularly important within veterinary science, especially
when animals undergo significant surgical procedures at an early age, as is the case for gonadectomy. Research in human infants indicates that subsequent surgeries in the same anatomical region result in higher post-operative pain scores and a greater need for analgesia (Peters et al. 2005).

1.4.1 Central sensitization and the development of chronic or maladaptive pain

Conscious perception of acute pain associated with surgically-induced tissue damage is not the only potential welfare issue associated with gonadectomy. It is also possible that failure to effectively manage neurological transmission of noxious inputs to the dorsal horn during surgery results in central sensitisation (Reichert et al. 2001). Although the pain is not consciously perceived because the animal is unconscious due to general anaesthetic, the individual’s body still reacts physiologically to the insult as the signal is modulated. Therefore, if nociception is uncontrolled during surgery there may be longer-term implications than those associated with the post-operative recovery phase (Gurney 2012).

1.5 Gonadectomy of domestic cats

Gonadectomy is a routine elective surgery for cats and involves either the removal of the ovaries and uterus (ovariohysterectomy or spaying) or the testes (neutering or
castration). Ovariohysterectomy can be performed via a ventral mid-line or a lateral (flank) incision into both the skin and underlying fascia. The uterine suspensory ligament is broken down (separated from the uterus), the ovarian pedicles are severed and the uterus crushed and severed above the cervix (de Tora and McCarthy 2011). Subsequently the cervix and ovarian blood vessels are ligated and the skin and fascia sutured. Castration for cats generally involves two incisions into the scrotum and removal of the testes by severing the spermatic cord and blood vessels. These are then typically tied off and the wound is left without sutures (Looney et al. 2008). Both procedures are performed under anaesthesia following pre-medication with a sedative and recommended in conjunction with an analgesic (Robertson 2008). However the nature of these procedures means that they also result in pain once the cat regains consciousness. As spaying is more invasive than castration and causes more extensive tissue damage it is therefore cited as being more painful (Williams et al. 2005). Given the substantial proportion (~90%) of owned cats that are gonadectomised in New Zealand (McKay et al. 2009) these procedures, by virtue of their frequency, may have the potential to significantly affect cat welfare if post-operative pain is not managed effectively.

1.5.1 Timing of gonadectomy

The age at which puberty occurs in cats varies between individuals. Comparison between the sexes identifies it as occurring between 5 and 9 months for queens and 8 to 10 months for toms. However, it may occur as early as 3.5 months (Little 2011a,b). Owned Cats are typically gonadectomised at around 6 months of age (Howe 1997)
but this age may differ depending upon the origin of the cat. For example, cats in welfare shelters and pounds are more likely to be gonadectomised earlier (Spain et al. 2004) often at between 8-16 weeks. In part this is to ensure that adopted cats are not reproductively viable but also results from shelters not requiring owner consent for the procedure. Early gonadectomy is encouraged in the literature as a primary mechanism by which cat population growth can be effectively managed (Bushby and Griffin 2011). Early-age neutering is contentious in some countries due to a perception that it may have long-lasting behavioural and physiological impacts on cat development (USA: Spain et al. 2002; UK: Murray et al. 2008). Little is known about how early gonadectomy is utilised and understood in other countries. It may be of particular interest to explore attitudes to such practices in countries like New Zealand where the cat is both a companion animal and a legally recognised pest.

1.5.2 Impact of gonadectomy on cats

In 2000 Pascoe asserted that veterinary medicine still remained guilty of ignoring pain following surgery, particularly surgeries which could be considered routine such as gonadectomy. Gonadectomy results in a substantial level of pain in the short-term post-operative phase (Slingsby and Waterman-Pearson 1998). It is therefore likely that any major welfare problems for both individuals and the population will be experienced in the short to medium-term during the post-surgical period. The degree of pain caused will be relative to the amount and type of tissue damage inflicted (Gregory 2010). In addition it is possible that other biological factors such as sex and age may influence pain sensitivity and, therefore, the post-operative experience of
cats. Sex and age effects have been demonstrated in other species (e.g. sheep: Guesgen et al. 2011; pigs: Di Giminiani et al. 2013) but are, as yet unexplored in cats. In addition, external factors such as the expertise of the surgeon have also been demonstrated to significantly affect the post-surgical recovery of dogs, either as a result of greater tissue damage or longer anaesthesia (Michelsen et al. 2012). How pain is pre-emptively managed prior to the procedure and also managed thereafter has clear implications for post-surgical experience and recovery (Pascoe 2000; Robertson 2005; Dyson 2008; Robertson 2008; Gurney 2012). Although pre-pubertal gonadectomy prevents the development of physical and behavioural sexual characteristics (Root Kustritz 2007) there is little evidence that it has any substantial long-term impacts upon the patient (Spain et al. 2004).

1.6 Current use of analgesia for pain in cats

The existing literature suggests that cats may be more likely to experience unmanaged post-surgical pain than dogs. This is because cats are currently considered to receive less adequate analgesia (Robertson 2005) when compared to dogs (Williams et al. 2005) which results from three main concerns. Firstly, that there is a poorer understanding of pain responses in cats, secondly practitioner wariness around the use of analgesics in cats and thirdly the cat’s unique physiology results in non-standard metabolism of some analgesics and hence increased toxicity or reduced effect (Robertson and Taylor 2004; Taylor and Robertson 2004). In older literature under-provision of analgesia is evident even when the pain experienced by cats is considered equivalent to that of dogs (Lascelles et al. 1999). There are also discrepancies in
analgesic provision among different categories of cats. For example, males receive analgesia less often than females during and following gonadectomy. In Britain, Lascelles et al. (1999) identified that only 26% and 16% of practitioners provided cats with pre-operative pain relief for ovariohysterectomies and castrations, respectively. Similarly, post-operative provision of analgesia for cats in Canada was 16.6% following ovariohysterectomy and 9.3% after castration (Dohoo and Dohoo 1996) and 6% following ovariohysterectomy of dogs and cats combined in Australia (Watson et al. 1996). The use of analgesics for cats undergoing ovariohysterectomy and castration were 36.3% and 17.2% in France (Hugonnard et al. 2004); 64% and 50% in New Zealand (Williams et al. 2005) and 62% and 38% in Finland, although the Finnish study also included use of pre-operative local anaesthetics (Raekallio et al. 2003). Much of this literature is relatively old given the likelihood that veterinary medicine and veterinary practices are in perpetual change. An assessment of current analgesia provision for cats is warranted.

1.7 Managing the pain associated with gonadectomy in cats

Based on behavioural indicators, the pain associated with gonadectomy may persist for up to 55 h (Waran et al. 2007). The timing of analgesia is therefore important to best address that pain. In general pain management during and following surgery can be considered to fall into one of three time phases: 1) the pre- and intra-operative phase (also known as the peri-operative phase); 2) the post-operative phase; 3) the recovery phase.
1.7.1 Peri-operative pain management

In healthy pain-free animals, surgical pain may be managed in the first instance through the use of analgesic drugs administered prior to surgery (Dyson 2008). Premedication with analgesics has two major purposes. Firstly to provide analgesic coverage for the early recovery phase and secondly to pre-emptively block nociceptor response to the surgical process (Pascoe 2000). The latter may prevent central sensitisation and reduce post-operative need for analgesia (Kelly et al. 2001). However, for effective pre-emptive analgesia, the drug must be administered well enough in advance for it to reach peak concentration at the point of surgery (Dyson 2008).

Pre-medications administered prior to gonadectomy often contain both analgesics and sedatives (Hunt et al. 2013) as well as anaesthetics which directly block noxious inputs during surgery. Traditional pre-operative analgesics may include non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, given either separately or in combination (Gurney 2012). Opioids generally function to inhibit the transmission of painful stimuli to the brain. Opioid agonists bind to receptors found predominantly in the spinal cord, but also in the brain and gastro-intestinal tract. Binding of opioids to these receptors results in either full or partial expression of a physiological cascade which alters perception of pain. Three types of opioid receptor have been identified those being mu, kappa and delta, with mu being considered the most potent (Stein 1995). However, each receptor type contains several sub-types dependent upon their anatomical location and function. As a consequence opioid agonists have myriad functions dependent upon their receptor specificity and affinity (Gurney 2012).
analgesic action of NSAIDs differs to that of opioids. NSAIDs prevent prostaglandin synthesis by inhibiting production of the enzymes cyclooxygenase (COX) 1 and 2 (Lascelles et al. 2007). NSAIDs therefore reduce the inflammatory and pyretic responses as well as the associated pain that develops after tissue injury (Robertson and Taylor 2004). COX-2 inhibitors are considered to be superior to COX-1 for use in cats given that they are less active in the gastro-intestinal tract and are therefore less likely to result in renal or gastro-intestinal side-effects (Staffieri et al. 2013).

There is some evidence that pre-emptive NSAID analgesia reduced self-administration of opioids post-operatively in human patients following hysterectomy (Karaman et al. 2006). In addition, evidence exists that the use of pre-operative analgesia reduces analgesic requirements post-operatively in dogs (Lascelles et al. 1997; Lascelles et al. 1998). Lascelles and Waterman (1997) suggest that administration of pre-operative analgesics to cats may be an effective route to minimise welfare compromise caused by post-surgical pain, however there is a paucity of corresponding data. A single study suggests that pre-emptive NSAID (meloxicam) may be better than an opioid at reducing post-surgical pain (Gassel et al. 2005). Multimodal pain relief has also been shown to synergistically reduce post-operative pain in humans following hysterectomy (Gilron et al. 2005). Similar protocols are supported for cats undergoing ovariohysterectomy in that they reduce pain arising from multiple sources (Gurney 2012). For example, some sedatives such as medetomidine, an alpha-two agonist, also have analgesic effects and are used to provide multi-modal pain relief during surgery (Robertson 2008). However the post-operative value of such drugs is limited as reversal of sedation may eliminate any analgesic action (Cullen 1996).
Whether opioids or NSAIDs or a combination is used is often dependent upon the practitioners’ confidence in using them. The use of both opioids and NSAIDs has historically raised concerns for practitioners, with the former being unjustifiably associated with mania (Robertson 2005) and the latter for their toxic effects on renal function and integrity (Robertson and Taylor 2004; Lascelles et al. 2007). The degree of concern associated with each appears to differ between countries. For example, in NZ, 93% of vets indicated concern about the side-effects associated with NSAID use (Williams et al. 2005) compared to only 75% in the UK some 6 years prior (Lascelles et al. 1999).

**1.7.2 Post-operative and recovery pain management**

Historically practitioners have been reluctant to use post-operative pain relief for cats given the possibility of undesirable side-effects and frustration associated with excessive paperwork for controlled substances (Tobias et al. 2006). Post-operative pain relief is not routinely used within all veterinary practices (Ansah et al. 2002), despite the now common acceptance within the literature that gonadectomy results in post-surgical pain (Polson et al. 2012). To some extent post-operative pain can be managed by judicious use of long-acting analgesics in the pre-medication, as described above. For example NSAIDs such as ketoprofen and meloxicam have been shown to have a discernible effect on pain responses for up to 24 hours (Dyson 2008). Likewise the opioid buprenorphine, a partial mu-agonist, demonstrates a slow dissociation from the mu receptor, resulting in long-lasting analgesia (Giordano et al. 2010). Testing of buprenorphine has resulted in it being identified as one of the most
effective analgesics for use in cats (Robertson et al. 2005). Pre-emptive use of analgesics may prevent activation of pain pathways reducing subsequent need for additional post-operative analgesia. Comparisons of pre-operative NSAIDs such as carprofen and ketoprofen with the opioid buprenorphine suggest they are all able to manage gonadectomy related post-operative pain and are better than a local anaesthetic agent (bupivacaine) alone (Tobias et al. 2006).

Given that some analgesics are cleared more slowly by cats than other species, they therefore have a longer action (Taylor and Robertson 2004) and the provision of additional pain relief must consider the effective duration of any pre-medication. Any overlap in the effects of drugs given before, during and after surgery may function to provide more effective analgesia but also introduces concerns around over-medication. In general the requirement for post-operative pain management is assessed once the patient has recovered from surgery. It therefore hinges upon careful monitoring of the patient’s response to interaction and demeanour. Most recent literature has validated the efficacy of both NSAIDs and opioids for managing post-operative pain in cats as a component of their inclusion in pre-medications (Al-Gizawiy and Rudé 2004; Tobias et al. 2006; Giordano et al. 2010; Cagnardi et al. 2011; Polson et al. 2012; Staffieri et al. 2013). It is the older literature which has focussed on post-operative analgesia as a discrete process (Ansah et al. 2002) and these authors themselves indicate that there is a significant move towards pre-emptive pain management. How exactly these changes in the process of managing surgical pain are implemented in practice has not been quantified.

Within the literature there is little evidence of consideration of ‘at home’ analgesia beyond its provision for chronically painful conditions (Gunew et al. 2008; Sparkes et al. 2010). Currently there are NSAIDs available for owner administration (Robertson
There is certainly evidence that owners expect pain management for their animals and that they perceive gonadectomy to result in pain for a significant period after discharge from veterinary care (Demetriou et al. 2009). However, there is a dearth of literature surrounding provision of analgesia following discharge after elective procedures that cause acute and short-term pain and how and when practitioners offer these options.

### 1.8 Current limitations to effective pain management in cats

Exactly when and how pain should be managed to minimise negative impacts on patient welfare is unclear, including whether pre-emptive analgesia or post-operative analgesia is most effective (Gurney 2012). Currently, understanding the provision of effective pain management for cats undergoing gonadectomy is limited both by our incomplete understanding of analgesia in the cat (Court 2013) and by the methods that are available for pain assessment in cats (see below). Currently the routes by which pain can be managed in cats are many. This includes a wide range of analgesics with differing modes of action which can be used solely or in combination and numerous routes by which administration can occur (Viñuela-Fernández et al. 2007). In this regard there is a need to understand not only pharmacology but also when and how these analgesics are administered.

As post-operative analgesia is often provided “as needed” it is first necessary to have objective measures that allow pain to be assessed. Current ‘objective’ tools tend to be limited in value outside the research context given their cost and complexity (Dixon et al.
Furthermore their labour or time-intensive nature, constrain the numbers of subjects that can be reasonably assessed. Conversely, tools for use in the clinic, while more practical to use, are beset with issues relating to their validity and subjective interpretation (Holton et al. 1998).

1.9 Assessing pain

The assessment of pain, broadly speaking, uses two core sets of indicators. The first is behavioural assessment and the second assessment using changes in physiological parameters (see: Weary et al. 2006). It is the first of these that is addressed further in this review. It is, however, also acknowledged that this distinction is somewhat arbitrary and that many assessment protocols may use a combination of both behavioural and physiological parameters.

1.9.1 Behavioural assessment of pain

Although apparently ubiquitous, pain is also a private experience. This means that it requires unambiguous cues in order to be recognised by others. Behaviour is therefore cited as one of the most effective and non-invasive routes by which pain can be assessed (Rutherford 2002). Behavioural cues of pain, for example facial expression, are well documented within humans (Bieri et al. 1990). Terstegen et al. (2003) and Koh et al. (2004) identify that, for humans unable to self-report using language,
management of pain is compromised by an inability to correctly interpret behavioural observations. Likewise the use of pain scales that are not validated for the patient being assessed (e.g. those validated for use in normally developed children) may reduce prescribed pain relief. This information is of comparative interest. In the absence of language, behavioural indicators of pain in humans are shown to be highly conserved, irrespective of assumed cognitive abilities. These include vocalisations, postures and avoidance responses (Terstegen et al. 2003) all of which have been similarly demonstrated in other mammals (e.g. piglets, Marx et al. 2003). However even between people there are discrepancies in interpretation based on observer experience (Boerner et al. 2013), patient age (Sheu et al. 2011) and observer nationality (Torres et al. 2013). Facial cues have been explored in humans and other species (e.g. mice, Langford et al. 2010) as have inter-individual abilities to recognise conspecific pain states. These suggest that individual animals are able to recognise, and respond to, pain in others (e.g. sheep, Hild et al. 2011).

Research into communication of pain in animals builds upon, and to some extent mirrors, research into pain recognition in profoundly mentally impaired adults and children. Scales intended to be filled out by the patient are subsequently altered and validated for use by a third party, much as is seen in veterinary practice (Holton et al. 1998). The persistent question that remains is whether or not behavioural self-reporting of pain can be unambiguously assessed by observers of non-human animals. Behavioural assessment using observation is the most commonly used method by which pain is assessed, both clinically and in pain research. The use of combinations of pain behaviours allow greater accuracy to be gained, although often single behaviours may be suitable for simple presence/absence observations (Prunier et al. 2013).
Indicator behaviours are those which may be taken as reliable expressions of pain because they are performed by animals only during or shortly after injurious procedures. A repertoire of core pain behaviours can be accumulated for a given species (Prunier et al. 2013) and may form the basis of any pain scale (Cloutier et al. 2005). For most species these behaviours are those that occur or increase in frequency after injury, but they may also include behaviours which are reduced or extinguished following an injurious event (Farnworth et al. 2011c).

1.9.2 Limitations of behavioural assessment of pain

The ability of humans to reliably interpret the pain experienced by non-human animals is one of the major obstacles in identifying and treating non-human pain. The ability to recognise such indicators may depend on familiarity with a species and an understanding of its behaviour (Robertson 2003). Once such knowledge is gained it is still not possible to establish whether this experience has the same qualities and intensities across species (Paul-Murphy et al. 2004). Behavioural assessment of pain is often considered to be relatively subjective and to contain inherent observer bias when there is a lack of objective criteria against which it is judged (Roughan and Flecknell 2003). This may also include over-emphasizing the value of more obvious signs like vocalisations (Conzemius et al. 1997; Viñuela-Fernández et al. 2007), use of incorrect components of the behaviour, or focussing on the wrong body parts in formulating a diagnosis (Leach et al. 2011). Therefore recent research has focussed on ways in which behavioural assessment can either be linked to physiological indicators or objectively validated (Brondani et al. 2011). Another difficulty with the use of
behaviour to objectively assess pain is that the observer cannot be easily blinded to the surgical procedure used (Grint et al. 2006) and therefore cannot be prevented from making some subjective assessment around the perceived pain severity.

Pain assessments based on observations are potentially problematic, especially in a research environment, as they do not always provide objective criteria for assessment. Despite both construct and diagnostic validation of such tools, it is difficult to ensure intra- and inter-observer reliability as assessment depends upon the observer’s perception and experience of the species in question (Fox et al. 2000). The most easily validated behavioural indicators are those that are found to be expressed in animals that have undergone a procedure versus those that have not and which can be extinguished or reduced in animals receiving analgesia versus those that have not (Weary et al. 2006). The latter of these (i.e. non-provision of analgesia to animals in pain) is ethically problematic and many experiments now avoid such measures. This being said assessment of pain may also include subjective rating scales (Roughan and Flecknell 2003). However, earlier exploration of the reliability of rating-scales indicated that there was significant variability in pain scoring for dogs when more than one observer was asked to use the same scale (Holton et al. 1998). This continues to be a problem identified in contemporary literature using validated scales (Brondani et al. 2011). Reliability and validity are further confounded by the meaning ascribed to different words by the implementer (Bath 1998), especially if translated between languages (Brondani et al. 2013).
1.9.3 Behavioural measurement of pain in cats

The development of behavioural indicators of pain in the cat, as in other species, involves detailed and comparative analyses of behaviour both prior to and following a surgical procedure or painful experience. Such analyses, which do (Waran et al. 2007) or do not (Rütgen et al. 2011) include post-operative analgesia have resulted in identification of a number of indicator behaviours. In the case of the preceding experiments both identified an abnormal postural change characterised by the limbs being drawn tightly to the chest. Indicator behaviours are however affected by the surgery undertaken. In the above examples abdominal surgery resulted in tucked postures similar to those in other species undergoing abdominal surgeries (Farnworth et al. 2011c). Post-surgical pain in cats following onychectomy or tenectomy was predominantly identified by paw-shakes (Cloutier et al. 2005). Generally changes to normal behavioural functioning are those most commonly used to identify pain in cats, which may persist well beyond time in clinic. Owner reports of behavioural change following ovariohysterectomy included general reductions in activity, something also identified in the above protocols. However observations can also include reductions in play, reduction in affiliative behaviours and expression of an abnormal gait (Väisänen et al. 2007), behaviours which are unlikely to be expressed until the animal is returned to its normal environment. Such changes are difficult to observe in the clinic given the restricted environment provided. As a result clinical observations also include degree of restlessness/activity (Ansah et al. 2002) and response to interaction which will include the position of the animal within the cage but also their response to interaction with the wound site (Grint et al. 2006).
Caution is required in interpreting individual indicator behaviours in isolation. For example, although attention to wound site has been identified as an indicator of pain for numerous species (Sutherland and Tucker 2011), at least one study of cats has shown that this behaviour increased following administration of butorphanol when a decrease was expected (Rütgen et al. 2011). This suggests that attention to the wound site could also be mediated by a reduction in pain making interaction less aversive. It is possible then that typical pain behaviours may be expressed even when pain is being managed. It is also difficult to delineate between behavioural changes that result from pain and those that result from other stressors such as a novel environment (Stella et al. 2013). In this regard the majority of behavioural assessment tools used for cats use multiple measurements of behavioural response.

### 1.9.4 Pain scales

As the name suggests, Simple Descriptive Scales (SDS) rely on fairly broad categories of behaviour and on inference from the observer (Slingsby and Waterman-Pearson 1998). The patient’s pain experience is ascribed a descriptor based on its perceived severity. As such, how behaviour is interpreted may depend upon individual relationships with the particular animal under observation and awareness of its unique behavioural characteristics. SDS are similar to basic Visual Analogue Scales (Benito-De-La-Vibora et al. 2008) which allow a subjective rating to be made for a given animal on a scale of 0 (no pain) to 100 (worst pain imaginable).
Descriptive pain scales are routinely used as a component of pain studies in cats. They rely on the scoring of a basic behavioural repertoire following observation of, and/or interaction with, the patient. A subjective score is then provided (usually 0-4) to estimate the degree of pain being experienced (Taylor et al. 2010; Staffieri et al. 2013). Such scales are reported to produce results comparable to mechanical nociceptive threshold testing (Polson et al. 2012). In a research context, these sorts of scales may not be sufficiently subtle to reveal the efficacy of an analgesic or variations in pain as a result of the method of analgesic administration (Giordano et al. 2010), but may be used at clinic to make decisions about the need for additional (or some) post-operative provision of analgesia.

Composite pain scales (CPS) are used to assess pre- and post-surgical behaviour for changes related to pain (Scott et al. 2003). As well as physiological assessments of heart rate, respiration and appetite, they contain a range of behavioural components which are known from the literature to alter following surgery (Reid et al. 2007; van Loon et al. 2010) as well as a range of statements with which the observer can agree or disagree. These components, or the composite scales themselves, have been primarily validated in dogs (Reid et al. 2007) but also in cats (Brondani et al. 2011) and have been used to explore the pain associated with various procedures and different analgesic regimes e.g. (Kongara et al. 2013). A composite pain scale validated for cats uses behavioural cut-points to score the expression of the patient from 0 (the behaviour is absent or normal) upwards towards overt expression or abnormal expression. The upper bound can be 2, 3 or 4 depending on the possible range of observed changes (Brondani et al. 2011). Summing these cut-point scores provides a composite score which, in itself, provides a threshold above which analgesia should be provided or increased (Reid et al. 2007). Historically simple CPS
have been shown to correlate relatively poorly with pressure based pain threshold (Conzemius et al. 1997), leading researchers to increase the categorical complexity of the scales and introduce interactive components.

The cumulative nature of CPS provides a route by which variations in the pain experience may be more easily elucidated. However, this is dependent on the weighting provided to each component. In one case the CPS was not sufficiently sensitive to detect differences between analgesic regimes, whereas an individual component of the CPS was (Al-Gizawiy and Rudé 2004). In this case, only the palpation test demonstrated increased post-surgical analgesia from the use of carprofen when compared with butorphanol. Response to palpation is frequently used in behavioural assessments of pain in cats and its incorporation into CPS leads them to be known as Interactive Visual Analogue Scales (Cloutier et al. 2005) or Dynamic Interactive Visual Analogue Scales (Polson et al. 2012). However the use of palpation could be considered contentious as it causes sufficient pain to the animal through interaction with damaged tissues to elicit avoidance or an attack (Bley et al. 2004). In essence this is causing pain to measure pain.

Pain assessment based on subjective observational scales may be problematic. Validation of a cat-specific CPS by (Brondani et al. 2011) has demonstrated that it has both construct validity (ability to measure the effect being investigated) and internal reliability (highly related scores indicate assessment of a single dimension (i.e. pain)). However the authors also indicate that further validation is required to establish inter- and intra-observer reliability. It is reasonable to assert that the more behavioural components there are to be assessed within a given scale, and the greater the number of people expected to make that assessment, the harder it is to ensure reliability of
measurements. This is a conundrum whereby more complex scales show improved validity but SDS show greater utility in a clinical setting.

1.9.5 Quantitative sensory testing

Some methods of pain assessment in cats use remotely measurable criteria (e.g. time to response; skin temperature at response). The use of such methods aims to overcome difficulties associated with observer bias by providing a discrete measurable response indicative of the detection of a noxious stimulus. They are relatively commonly used in rodents to model pain development and to test the efficacy of analgesic (e.g. Pitcher et al. 1999) and are becoming more commonly used in a wide range of mammalian species, including cats. Devices are used to apply a stimulus to the subject. The threshold for testing is the point at which stimulation becomes noxious is noted by a behavioural change in the animal indicative of pain (Stubsjøen et al. 2010), the threshold noted and the stimulus removed or deactivated (Dixon et al. 2002).

Quantitative sensory testing (QST) assesses sensory nerve function using a range of stimuli. These include pressure (Dixon et al. 2007; Steagall et al. 2007), temperature (Dixon et al. 2002; Robertson et al. 2003) and electrical stimulation (Millette et al. 2008). These current methods, used primarily for the assessment of nociceptive sensitivity and validation of analgesics in cats, are relatively complex. They require direct manipulation of the subject and application of the stimulus to the skin using an attached device in order to measure cutaneous temperature, applied force or electrical current. These processes require the attachment of such devices to the animal itself, as
such they require significant time for habituation to wearing the device (Steagall et al. 2006). In cats active behavioural responses to noxious stimuli are taken as indicators of pain threshold. These include shaking of the limb, biting at the device, attempts to avoid the stimulus by moving, vocalisation and focus of attention upon the site of stimulation (Millette et al. 2008).

Quantitative Sensory Testing in other species has implemented carbon dioxide (CO$_2$) lasers (e.g. sheep: Guesgen et al., 2011; cows: Veissier et al., 2000; pigs: Herskin et al., 2009) to reliably assess thermal nociceptive thresholds. The benefits of CO$_2$ laser stimulation include remote application, consistent stimulus application and immediate cessation of stimulation following the response. Unlike other QST methods (Dixon et al. 2002) there is no requirement for a period of habituation prior to commencing the experiment.

For the CO$_2$ laser in particular, there is complete absorbance of thermal energy by the skin, regardless of pigmentation and the beam penetrates the skin to the depth at which most cutaneous thermosensitive receptors are found, approximately 100nm (Gülsoy et al. 2001; Le Bars et al. 2001). In addition, laser stimulation protocols are relatively simple which increases their utility. Such protocols can also be widely adapted and applied to unrestrained animals, this allows them to be used in a wider range of environments (e.g. on-farm) without a need for specialised handling or monitoring equipment (Ting et al. 2010) with the caveat that protective equipment should be employed. In those QST that have been validated thermal stimuli appear more useful than both electrical and pressure based systems for elucidating changes in nociceptive threshold (Steagall et al. 2007; Millette et al. 2008). However, measurement of thermal pain sensitivity using a CO$_2$ laser has not yet been attempted nor validated in cats.
1.10 Objectives of this study

The following studies are undertaken in an attempt to understand how and when veterinary professionals gonadectomise cats and, given the age of the literature, to establish contemporary use of analgesia for gonadectomy within practice. This is particularly relevant given the range of approved analgesics for cats and their differing modalities. Exploring these dimensions will allow for a greater understanding of exactly when pain may compromise the welfare of cats undergoing gonadectomy. This is important in terms of the pain caused by surgery but also relative to the age and developmental stage of the patient.

In addition it is intended that we will explore and validate a novel method for more objectively assessing nociception and pain in cats that allows for greater utility. Any new method should attempt to limit subjective interpretation of results if it is to be useful for evaluating and developing efficacious pain management techniques. In this regard the following thesis will explore the use of thermal stimulation using a carbon dioxide laser starting with validation of the tool for use in cats. Large cohort studies will then be undertaken to explore the underlying mechanisms and inter-individual factors that impact upon thermal nociceptive thresholds in cats. The technique will also be applied to healthy cats under the influence of traditional analgesics to establish if it presents a potentially viable alternative to current QSTs in clinical research.
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Survey Paper One

This chapter is formatted in the style of the New Zealand Veterinary Journal and has been published as:

2. Veterinary attitudes towards pre-pubertal gonadectomy of cats: A comparison of samples from New Zealand, Australia and the United Kingdom.

2.1 Abstract

AIM: To compare the attitudes and practices of a sample of veterinarians in New Zealand, Australia and the United Kingdom (UK) towards pre-pubertal gonadectomy of cats.

METHODS: Respondents’ demographics were gathered using an electronic questionnaire distributed via professional veterinary associations in the target countries, as were minimum age at gonadectomy and typical age of puberty. Desirability of pre-pubertal gonadectomy was gauged and justified. Two-way Analyses of Variance (ANOVA) followed by post hoc Tukey HSD tests were used to test whether there were differences in minimum ages for gonadectomy among and between countries and between providers and non-providers of services to pounds (or animal welfare centres). Views on the desirability of pre-pubertal gonadectomy relative to demographics were explored using a Likelihood Ratios Test.

RESULTS: The survey received 717 responses. Most respondents believed pre-pubertal gonadectomy was either entirely or ‘sometimes’ desirable (556/621), few thought it was undesirable (65/621). Minimum age at gonadectomy was significantly affected by country surveyed and provision or non-provision of services for pounds. Post hoc Tukey HSD analysis indicated the mean age of both spaying and castration (both 4.3 months) in the UK was significantly different from both Australia (spaying:
3.4 months, castration: 3.2 months) and New Zealand (spaying: 3.4 months, castration: 3.2 months)) (all p < 0.001). Mean ages at spaying and castration were also significantly different (p=0.008; p=0.019 respectively) for non-providers (spaying: 3.9 months, castration: 3.8 months) of services to pounds when compared to providers (spaying and castration: both 3.6 months).

Likelihood Ratio Tests indicated significant effects amongst countries and between genders relative to the desirability of pre-pubertal gonadectomy. Respondents from the UK were more likely to answer ‘no’ (p=0.004) or ‘sometimes’ (p=0.05) as compared to those from New Zealand or Australia. Females were more likely to respond with ‘sometimes’ as opposed to ‘yes’ than males. Reasons for considering pre-pubertal gonadectomy desirable or sometimes desirable focussed on reducing unwanted pregnancies and improving population control, as well as improving rates of adoption, owner compliance and cat behaviour and health.

CONCLUSIONS AND CLINICAL RELEVANCE: In general pre-pubertal gonadectomy is considered a desirable procedure by those practitioners that responded to the survey. However age at which any such procedure occurs differs depending upon a number of factors. Differences among countries may relate to the specific Veterinary Association’s guidelines or possibly differences in social discourse which affect perception of cats. There is substantial overlap between the reported minimum age of gonadectomy and the age at which cats can enter early puberty, allowing a window for unintentional pregnancy when pre-pubertal gonadectomy does not occur.
2.2 Introduction

Pre-pubertal gonadectomy in cats is defined as ‘neutering well before the onset of puberty’ (Howe et al. 2000) and is any age prior to 23 weeks, the traditional age for gonadectomy being 6 months (Howe 1997) with a normal range of five to nine months (Preston Stubbs et al. 1996; Root Kustritz 2007). It is a broad category which encompasses and exceeds ‘early age neutering’ which, in New Zealand, is considered to occur between the ages of 1.5-4 months (Walsh and Worth 2008) and generally between the ages of 1.5-3.5 months in the United States of America (USA) (Root Kustritz 2002). The latter age is considered appropriate by proponents of early age neutering in the UK (Joyce and Yates 2011). Both pre-pubertal gonadectomy and early age neutering allow early sale or adoption of cats without the associated risk of breeding (Spain et al. 2004).

The age at which puberty occurs in cats varies substantially; however it is generally considered to occur between five and nine months for queens and eight to ten months for toms but can occur as early as 3.5 months (Little 2011a/b). This indicates that the risks of accidental pregnancy or insemination are greater if pre-pubertal gonadectomies are performed closer to the traditional age of puberty, a risk that is seemingly mitigated through the use of early age neutering. Studies in the United Kingdom (UK) and New York State in the USA indicate that 51% and 39% of practitioners respectively recommended gonadectomy no earlier than six months of age for client owned cats (UK: Murray et al. 2008; US: Spain et al. 2002). The appropriateness of gonadectomy at the traditional age is being debated (Joyce and Yates 2011). A contemporary study in the US indicates that, at 6 months or younger,
43% of cats were spayed or neutered compared to 77% at between 6 months and 12 months of age and 87% between 12 months and 4 years (Trevejo et al. 2011). This suggests that, in the USA at least, later gonadectomy continues to be a common practice.

Pre-pubertal gonadectomy is considered desirable for cats being re-homed from shelters (Spain et al. 2004) as it prevents unintended pregnancies whilst allowing animals to be re-homed early enough for effective socialisation to occur (Root Kustritz 2002). It is also recommended for tom cats to prevent potentially dangerous post-pubescent aggressive behaviour (Root Kustritz 2007). In addition to reducing the number of cats being relinquished at shelters, pre-pubertal gonadectomy may also reduce the number of adult cats entering shelters as those relinquished are significantly more likely to be entire than spayed or neutered (Patronek et al. 1996).

Anecdotally, practitioners may only encounter a cat once prior to pubescence, therefore encouraging and practicing pre-pubertal gonadectomy prior to sale or adoption may function to reduce the number of cats reaching sexual maturity. This may be confounded as post-adoption/post-purchase compliance with spay and neuter programmes, even if the surgery costs are pre-paid, is low (Bushby and Griffin 2011). In general, sexually entire cats younger than 10 months of age are less likely to be registered with a veterinarian and vaccinated annually (Murray et al. 2009) suggesting that some owners of cats may wait until after pubescence to engage veterinary services. Cat owner education about the benefits of early veterinary care, and in particular pre-pubertal gonadectomy, is therefore important.

Both surgical (Aronsohn and Faggella 1993a), anaesthetic (Aronsohn and Faggella 1993b) and analgesic (Mathews 2008) practices have been deemed safe for use in cats between the ages of 1.5 and 3.5 months. The immediate impact of surgery on pre-
pubescent cats (e.g. in terms of pain experienced) during a critical developmental phase, as opposed to older more developed cats, remains relatively unexplored. There are few short-term (Howe 1997) or long-term side effects following pre-pubertal gonadectomy (Preston Stubbs et al. 1996; Howe et al. 2000; Spain et al. 2004) and there is potential for behavioural benefits for early-age neutered toms (Spain et al. 2004). Reviews of the topic continue to suggest that the benefits outweigh the costs, with the caveat that a practitioner must consider the anaesthetic risks to the patient (Bushby and Griffin 2011; Joyce and Yates 2011), consider its individual health parameters and consult with the owner (Reichler 2009). How contentious pre-pubertal gonadectomy (before 6 months of age) is has not been fully ascertained, however early age gonadectomy (before 4 months of age) remains contentious due, in part, to the historical perception that it may carry long-term risks (Bushby and Griffin 2011). A recent study of 875 veterinarians in the UK showed that only 28% believed gonadectomy between 3 and 4 months of age was appropriate, less than 5% thought it appropriate below 3 months (Murray et al. 2008).

The attitudes of veterinarians, positive or negative, towards pre-pubertal gonadectomy will clearly impact upon its use and therefore its effectiveness in controlling the cat population. Much of the exploration around veterinary attitudes towards pre-pubertal gonadectomy has occurred in the USA (e.g. Spain et al. 2002) and the UK (e.g. Murray et al. 2008) and focussed on early age gonadectomy. However ‘traditional’ veterinary attitudes and practices concerning pre-pubertal gonadectomy of cats may vary between countries, especially in countries such as New Zealand and Australia where the cat is both a popular pet and a ‘pest’ species subject to stringent controls driven, in part, by public concern for native wildlife (e.g. New Zealand: Farnworth et al. 2011; Australia: Toukhsati et al. 2012).
This research sought to establish the general attitudes of veterinary practitioners towards pre-pubertal gonadectomy (minimum age at surgery and perceived costs or benefits to the patient) in three different countries, namely New Zealand, the UK and Australia. It is hypothesised that there will be a wide age range given for pre-pubertal gonadectomy of cats. Although the majority of cats will be pre-pubescent in this age range, cats which reach puberty early remain at risk of unwanted pregnancy and sexual activity. In addition this research explored whether professional association with welfare centres impacted upon the age at gonadectomy and the perceived desirability of pre-pubertal gonadectomy. We hypothesise that the desirability of pre-pubertal gonadectomy and willingness to perform the procedure earlier will vary relative to country of practice. We also hypothesise that provision of services to welfare centres and pounds will significantly impact upon perception and use of the procedure.

2.3 Materials and methods

This study targeted companion animal veterinarians in New Zealand, Australia and the UK. A questionnaire containing 34 questions, taking approximately 10-15 minutes to complete (Appendix 1) was disseminated on-line (www.surveymonkey.com) through a direct link promoted by the New Zealand Veterinary Association’s Companion Animal Society (NZVA-CAS), the British Veterinary Association (BVA) and British Small Animal Veterinary Association (BSAVA) and the Australian Small Animal Veterinary Association (ASAVA). To improve response rates the associations...
and people responsible for promoting the survey were prompted to remind their members on two occasions during data collection.

Only responses to 16 of the 34 questions are considered in this paper. This included, basic information about the respondent including gender, year of qualification and country of current residence. In addition, respondents were asked whether they currently performed services for pounds, shelters or animal welfare centres and, if so, what proportion of their caseload comprised such activities.

In the final section respondents were asked the age at which they considered a cat to be adult for the purposes of analgesia and anaesthesia, the age at which they considered puberty to occur in cats, the minimum age at which they would perform a spay or castration, and whether or not they considered pre-pubertal gonadectomy to be desirable. In the case of questions concerning the minimum age that is it appropriate/acceptable for cats to undergo gonadectomy, response options were in two week increments. An open-ended discretionary question allowed respondents to clarify why they considered pre-pubertal gonadectomy to be desirable or undesirable. Respondents were also asked about their use of analgesics and anaesthetics during routine desexing, responses to those questions are not considered further in this paper.

### 2.3.1 Statistical analyses

The data were described in terms of percentage of respondents providing a given answer as well as providing mean or median values and ranges where appropriate. Statistical analyses were conducted using the Statistical Package for the Social
Sciences (SPSS) version 19.0 for Windows (IBMInc, Chicago IL, USA). We used a multiple logistic regression approach to examine the relationship among country of practice, time since graduation, gender of respondent and whether or not the respondent performed services for shelters, pounds or welfare centres on veterinary attitudes towards the desirability of pre-pubertal gonadectomy. This approach followed an assessment of the suitability of the data to this approach using Pearson Chi-square Test.

Differences in minimum age at which spaying and castration were performed were assessed among the countries of practice and between providers and non-providers of services to welfare centres and pounds using a two-way ANOVA. Differences among countries relative to estimations of when puberty occurred were established using a one-way ANOVA. For ‘minimum age at time of spay’ a single outlier was removed (12 months, next highest category reported 6.5 months) and age categories were aggregated (1.5 and 2 months became ‘2’; 2.5 and 3 months became ‘3’ etc.). These manipulations ensured that the assumption of homogeneity of variance, using Levene’s test, were not violated (p>0.05). Post hoc Tukey HSD analysis was then used to establish where the significant difference occurred among countries of practice.

For open-ended responses to whether or not pre-pubertal gonadectomy was considered desirable, a descriptive analysis of common themes was conducted to allow comparisons among countries and to ascertain whether or not responses were consistent with the literature. Responses were categorised by using key words to limit the sample to possible cases (e.g. ‘pregnancy’) and then each case was read and subjectively assigned to a theme by the researcher. For example a respondent indicating ‘sometimes’ with the rationale ‘to prevent unwanted pregnancy’ would
enter the theme ‘if unwanted pregnancy, unnecessary breeding or early oestrus are probable’, if the rationale were ‘owners often fail to spay females and pregnancy can occur’ it was assigned to the theme ‘if owner or purchaser is unlikely to sterilise at a later date/ prior to mating’.

2.4 Results

2.4.1 Basic demographics

There were 717 responses to the survey. Of these 249 (34.7%) were from New Zealand, 269 (37.5%) were from the UK and 199 (27.8%) from Australia. For New Zealand this response rate represents 41.6% of the total NZVA-CAS membership (S Blaikie,\(^1\) pers. Comm.). The UK and Australian samples groups were less easily contacted and the percentage response rates for the UK (269/4500; 6%; T Sainty\(^2\), pers. comm.) and Australia (199/1460; 13.6%; M Cole\(^3\), pers. comm.) are substantially lower than those for New Zealand. A greater percentage of females responded than males when compared to the veterinary association’s statistics (ASAVA\(^3\): \(♀=49.2\%\) \(♂=50.8\%\); BVA\(^2\): \(♀=57.9\%\) \(♂=42.2\%\); NZVA-CAS\(^1\): \(♀=49\%\) \(♂=51\%\)) (Table 2-1). The age at which puberty was considered to occur was not significantly different between the countries surveyed (5.7-5.9 months; \(F_{(2,616)}=1.534; p=0.217\) )

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3 M Cole, Administration Officer, ASAVA, Unit 40, 6 Herbert Street, St. Leonards, New South Wales, Australia
Table 2-1: Respondent variables

Table 2-1: Responses (n=717) to questions from a survey concerning pre-pubertal gonadectomy of cats. Responses are shown by the country in which the respondent currently practices, New Zealand (n=249), United Kingdom (n=269) or Australia (n=199). Data are represented as total number (percentage), median (total range) or mean (total range) for a given response.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Response</th>
<th>New Zealand</th>
<th>United Kingdom</th>
<th>Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n=717)</td>
<td>Male</td>
<td>104 (41.8)</td>
<td>91 (33.8)</td>
<td>50 (25.1)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>145 (58.2)</td>
<td>178 (66.2)</td>
<td>149 (74.9)</td>
</tr>
<tr>
<td>Do you provide services for pounds or welfare centres? (n=715)</td>
<td>Yes</td>
<td>119 (52.2)</td>
<td>144 (53.7)</td>
<td>67 (33.5)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>130 (47.8)</td>
<td>124 (46.3)</td>
<td>131 (65.5)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>125 (59.5)</td>
<td>108 (45.6)</td>
<td>110 (63.2)</td>
</tr>
<tr>
<td>Is pre-pubertal gonadectomy desirable? (n=621)*</td>
<td>No</td>
<td>16 (7.6)</td>
<td>36 (15.2)</td>
<td>13 (7.5)</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>69 (32.9)</td>
<td>93 (39.2)</td>
<td>51 (29.3)</td>
</tr>
<tr>
<td>Median years since graduation</td>
<td>20 (1-53)</td>
<td>18 (1-58)</td>
<td>14 (1-56)</td>
<td></td>
</tr>
<tr>
<td>Median percentage of workload comprising gonadectomy of cats</td>
<td>6-10% (0-85)</td>
<td>6-10% (0-80)</td>
<td>11-15% (0-80)</td>
<td></td>
</tr>
<tr>
<td>Median age (months) a cat is considered ‘adult’ for the purposes of analgesia and anaesthesia</td>
<td>5.5 (1-12)</td>
<td>5.5 (1-12)</td>
<td>5.5 (1-12)</td>
<td></td>
</tr>
<tr>
<td>Spay: Mean minimum age (months)**</td>
<td>3.4 (&lt;1-8)</td>
<td>4.3 (&lt;1-6.5)</td>
<td>3.4(1-5-6)</td>
<td></td>
</tr>
<tr>
<td>Castration: Mean minimum age (months)**</td>
<td>3.4 (1.5-6)</td>
<td>4.3 (&lt;1-8)</td>
<td>3.2(1-6)</td>
<td></td>
</tr>
<tr>
<td>Mean age at puberty (months)</td>
<td>5.9 (1.5-10)</td>
<td>5.6 (&lt;1-10)</td>
<td>5.8(2.5-9)</td>
<td></td>
</tr>
</tbody>
</table>

*UK respondents were more likely to respond ‘no’: W=8.342; df=1; p=0.004 or ‘sometimes’: W=3.848; df=1; p=0.05 as compared to ‘yes’ than those from either New Zealand or Australia. No significant differences were found between New Zealand and Australia.

**Following post hoc Tukey HSD analysis UK respondents’ minimum ages for spay and castration were significantly different (both p<0.001) to those for New Zealand and Australia. New Zealand and Australia did not significantly differ for either procedure.

The minimum age which was considered appropriate/acceptable for either spaying or castration was highly variable (<1.5–6.5+ months). A single respondent from New Zealand mentioned a 0 month minimum age for spaying and a 1 month minimum age for castration.
Zealand indicated a minimum age of 12 months for both procedures and these data were excluded from analysis as outliers. Most respondents considered pre-pubertal (≤ 5.5 months) spaying or castration to be appropriate (579/609 and 583/617 respectively), although a substantial number of these did not consider early age spaying or castration (4.5, 5 or 5.5 months) to be appropriate (192/579 and 177/583 respectively) (table 2-2).

**Table 2-2: Minimum age of gonadectomy**

Table 2-2: Minimum age (months) at which respondents to a survey on pre-pubertal gonadectomy would spay (n=609) or castrate (n=617) a cat.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>minimum age (months)</th>
<th>Pre-pubertal / Early-age</th>
<th>Pre-pubertal only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1.5</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Spay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>16</td>
<td>83</td>
</tr>
<tr>
<td>Total (%)</td>
<td>0.5</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Castration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>27</td>
<td>91</td>
</tr>
<tr>
<td>Total (%)</td>
<td>1</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>
Table 2-3: Minimum age of spay relative to service provision

Table 2-3: Mean minimum age (response range) in months at which respondents to a survey on pre-pubertal gonadectomy (n=632) that did or did not provide services for pounds or welfare centres performed spaying or castration of cats.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Provision of services to pounds or welfare centres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=297)</td>
</tr>
<tr>
<td></td>
<td>No (n=335)</td>
</tr>
<tr>
<td>Spay*</td>
<td>3.6 (&lt;1 - 6.5)</td>
</tr>
<tr>
<td>Castration**</td>
<td>3.6 (&lt;1 – 8)</td>
</tr>
<tr>
<td></td>
<td>3.9 (&lt;1 - 6.5)</td>
</tr>
<tr>
<td></td>
<td>3.8 (&lt;1 – 8)</td>
</tr>
</tbody>
</table>

Minimum age at which a given procedure would be performed was significantly different between practitioners that do and do not provide services for pounds or welfare centres. *(F(1,265)=76.657; p=0.008); ** (F(1,263)=37.932; p=0.019).

2.4.1.1 Differences between veterinary practitioners

Our data proved suitable for use in a multinomial logistic regression ($\chi^2=4.145; \text{df}=4; p=0.387$). Likelihood ratio tests were used to assess the effect of each parameter in the model. To ensure the model was as parsimonious as possible aspects of the multinomial logistic regression which lay well beyond the parameters for significance (>0.5), namely time since graduation ($\chi^2=0.854; \text{df}=2; p=0.652$) and whether or not the respondent performed services for shelters, pounds or welfare centres ($\chi^2=0.696; \text{df}=2; p=0.704$), were excluded and a reduced model was then executed using the parameters which were either significant or close to significance (p<0.1). These were country of practice ($\chi^2=17.424; \text{df}=4; p=0.002$) and gender of respondent ($\chi^2=5.707; \text{df}=2; p=0.058$). Significant results were then obtained for country of practice ($\chi^2=18.836; \text{df}=4; p=0.001$) and gender of respondent ($\chi^2=8.496; \text{df}=2; p=0.014$) in the simplified model. These were retained and further analysed using parameter estimates to establish the magnitude of any effect.
Respondents from the UK were significantly more likely than respondents from New Zealand or Australia to report ‘no’ or ‘sometimes’ as opposed to ‘yes’ when asked if pre-pubertal gonadectomy was desirable (‘no’: W=8.342; df=1; p=0.004; ‘sometimes’: W=3.848; df=1; p=0.05). The likelihood ratio parameter estimates indicated UK respondents were 2.6 times more likely to say ‘no’ and 1.5 times more likely to say ‘sometimes’. Non-significant differences were identified between the likelihood ratio parameter estimates that respondents from Australia or New Zealand would say ‘no’ or ‘sometimes’ as compared to ‘yes’ (‘no’: W=0.067; df=1; p=0.796; ‘sometimes’: W=1.437; df=1; p=0.231) (Table 2-1).

Levene’s test indicated the response variable (minimum age at gonadectomy) showed similar variances across the two factors: country of origin and provision of charity services. Q-Q plots conducted for the response variable (age) for each of the levels of these two factors all plotted close to a straight line indicating little departure from normality. In addition, ANOVA procedures may be reasonably robust to departure from normality if samples sizes are not dramatically different and are large. Our data fulfil both criteria. Given our survey design we feel confident that our responses from veterinarians are independent of each other. Accordingly we used the two-way ANOVA protocol based on Type III sum of squares and estimated marginal means to perform the tests. This approach accounted for potential problems associated with uneven samples sizes among groups.

There were significant differences in the minimum age at which gonadectomy would occur for both spaying ($F_{(2,265)}=275.286; p=0.004$) and castration ($F_{(2,263)}=191.495; p=0.005$) among countries. Similarly, whether or not individuals provided services to pounds or animal welfare centres had a significant effect on the minimum age at which spaying ($F_{(1,265)}=76.657; p=0.008$) and castration ($F_{(1,263)}=37.932; p=0.019$)
were performed (Table 2-3). Post hoc Tukey HSD analysis indicated that, for country of practice, the difference lay between the UK as compared to New Zealand and Australia for both procedures where the minimum age was significantly greater (spay and castration p<0.001; see Table 2-1). There were no significant differences between New Zealand and Australia for either procedure (spay: p=0.998; castration: p=0.434) (Table 2-1). There was no significant interaction between country of practice and provision of services to shelters and pounds on minimum age at either spaying or castration ($F_{(2,625)}=0.240; p=0.787$ and $F_{(2,623)}=0.329; p=0.720$, respectively).

The gender of the respondent had a significant effect upon the responses given to the question ‘do you consider pre-pubertal gonadectomy to be desirable’ (Table 2-4). Likelihood Ratio Tests indicated that men were significantly less likely to respond with ‘sometimes’ as opposed to ‘yes’ when compared to women ($W=8.146; df=1; p=0.004$). There was no significant difference in the likelihood that women or men would say ‘no’ as opposed to ‘yes’ ($W=0.166; df=1; p=0.683$).

**Table 2-4: Is pre-pubertal gonadectomy desirable? Response by gender**

<table>
<thead>
<tr>
<th>Is pre-pubertal gonadectomy desirable?</th>
<th>Respondent gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Male 128 (61.8)</td>
</tr>
<tr>
<td></td>
<td>No 23 (11.1)</td>
</tr>
<tr>
<td></td>
<td>Sometimes* 56 (27.1)</td>
</tr>
</tbody>
</table>

* Male veterinarians were significantly less likely to say ‘sometimes’ as opposed to ‘yes’ when compared with female veterinarians ($W=8.146; df=1; p=0.004$). There was no difference in the likelihood that either male or female veterinarians said ‘no’ as opposed to ‘yes’ ($W=0.166; df=1; p=0.683$).
2.4.1.2 Open ended responses

Reasons for indicating that pre-pubertal gonadectomy was not desirable (Table 2-5) were similar across the three countries. They tended to focus upon negative consequences for the patient including perceptions that unspecified aspects of the procedure meant it was too risky or respondents indicated they believed it to be unnecessary.

Those respondents that indicated it was ‘sometimes’ desirable considered it more desirable for cats that were likely to breed unintentionally either through early oestrus or being in an uncontrolled environment (e.g. mixed-sex households). Support was also strong for the pre-pubertal gonadectomy of animals from shelters, pet shops and pounds rather than those kept as companions by private clients. Respondents from New Zealand and Australia appeared more concerned with owner compliance around gonadectomy than respondents from the UK, indicating that if animals were unlikely to be seen regularly pre-pubertal gonadectomy was preferable. Respondents from the UK showed strong support for the pre-pubertal gonadectomy of feral and stray cats, especially those that were to be returned to their point of pick-up, rather than be rehomed. A small number (5-11%) considered it to be desirable only if the potential benefits, such as improved prospects for re-homing, outweighed specific concerns, for example complications resulting from non-maturation of the reproductive tract in males. A substantial minority of those who responded with either ‘yes’ (18-25%) or ‘sometimes’ (6-22%) also cited that the cat population required controlling due to over-population and or public and ecological nuisance.
For veterinarians that indicated pre-pubertal gonadectomy was ‘sometimes’ desirable those from the UK were 13 times more likely to use it as part of a Trap-Neuter-Release (TNR) programme than either vets from New Zealand or Australia (26% vs. 2%). Conversely, veterinarians from Australia and New Zealand were nearly four times more likely to respond that there was a cat over-population problem associated with a public or ecological nuisance (22% vs. 6%; table 2-4).

In contrast to those indicating ‘sometimes’, respondents stating ‘yes’ believed that pre-pubertal gonadectomy improved the health and welfare of their patients by reducing the risk of mammary tumours and disease transmission (e.g. Feline Immunodeficiency Virus). They also felt that it resulted in a faster recovery following a procedure that was less traumatic. Many (26-49%) considered that the procedure made patients better pets by improving behaviour through prevention of sexual behaviours such as spraying and roaming in males and behaviours associated with oestrus in females. By far the greatest reason for undertaking the procedure, reported by 55-57% of those that believed pre-pubertal gonadectomy to be desirable, was to prevent unwanted litters and pregnancies.
Table 2-5: Responses to open ended questions about pre-pubertal gonadectomy

Table 2-5: Major themes arising from descriptive analysis of respondents’ reasoning behind answers to the question ‘Do you consider pre-pubertal gonadectomy to be desirable?’ when responding to a survey about pre-pubertal gonadectomy in cats. Data are represented as percentage of total providing a specific response. Total number of written responses 553. Due to their open-ended nature single responses may contain multiple themes.

<table>
<thead>
<tr>
<th>Response</th>
<th>Major Themes</th>
<th>New Zealand</th>
<th>Australia</th>
<th>UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (n=51)</td>
<td>Unnecessary/ costs outweigh benefits</td>
<td>6/14 (43%)</td>
<td>4/11 (37%)</td>
<td>7/26 (28%)</td>
</tr>
<tr>
<td></td>
<td>Affects growth and hormonal profile</td>
<td>0/14 (0%)</td>
<td>2/11 (18%)</td>
<td>7/26 (28%)</td>
</tr>
<tr>
<td></td>
<td>Causes health problems</td>
<td>3/14 (21%)</td>
<td>1/11 (9%)</td>
<td>4/26 (16%)</td>
</tr>
<tr>
<td></td>
<td>Procedure (e.g. anaesthesia) carries excessive risk</td>
<td>2/14 (14%)</td>
<td>2/11 (18%)</td>
<td>4/26 (16%)</td>
</tr>
<tr>
<td></td>
<td>Personal objection</td>
<td>3/14 (21%)</td>
<td>2/11 (18%)</td>
<td>4/26 (16%)</td>
</tr>
<tr>
<td>Sometimes</td>
<td>If unwanted pregnancy, unnecessary breeding or early oestrus are probable.</td>
<td>18/60 (30%)</td>
<td>12/46 (26%)</td>
<td>28/84 (33%)</td>
</tr>
<tr>
<td>(n=190)</td>
<td>If the animal is from a shelter, petshop or to be re-homed early.</td>
<td>16/60 (27%)</td>
<td>17/46 (37%)</td>
<td>17/84 (20%)</td>
</tr>
<tr>
<td></td>
<td>If owner or purchaser is unlikely to sterilise at a later date/ prior to mating.</td>
<td>17/60 (28%)</td>
<td>15/46 (33%)</td>
<td>5/84 (6%)</td>
</tr>
<tr>
<td></td>
<td>To prevent the cat over-population problem/ nuisance/ ecological damage.</td>
<td>13/60 (22%)</td>
<td>10/46 (22%)</td>
<td>5/84 (6%)</td>
</tr>
<tr>
<td></td>
<td>If stray or feral or a colony cat (TNR)*.</td>
<td>1/60 (2%)</td>
<td>1/46 (2%)</td>
<td>22/84 (26%)</td>
</tr>
<tr>
<td></td>
<td>If the risks to health are outweighed by benefits (e.g. able to be re-homed).</td>
<td>3/60 (5%)</td>
<td>4/46 (9%)</td>
<td>9/84 (11%)</td>
</tr>
<tr>
<td></td>
<td>Can prevent problem behaviours (e.g. roaming/spraying).</td>
<td>7/60 (12%)</td>
<td>5/46 (11%)</td>
<td>5/84 (6%)</td>
</tr>
<tr>
<td></td>
<td>If requested by owner or breeder.</td>
<td>4/60 (7%)</td>
<td>2/46 (4%)</td>
<td>2/84 (2%)</td>
</tr>
<tr>
<td></td>
<td>Depends on individual cat (size, maturity).</td>
<td>3/60 (5%)</td>
<td>3/46 (7%)</td>
<td>3/84 (4%)</td>
</tr>
<tr>
<td></td>
<td>For females only.</td>
<td>3/60 (5%)</td>
<td>3/46 (7%)</td>
<td>2/84 (2%)</td>
</tr>
<tr>
<td></td>
<td>Depending on time of year/season.</td>
<td>3/60 (5%)</td>
<td>2/46 (4%)</td>
<td>1/84 (1%)</td>
</tr>
<tr>
<td></td>
<td>If it minimises disease risk (e.g. FIV).</td>
<td>2/60 (3%)</td>
<td>0/46 (0%)</td>
<td>1/84 (1%)</td>
</tr>
<tr>
<td>Yes (n=195)</td>
<td>Prevents unwanted litters/pregnancies</td>
<td>71/126 (56%)</td>
<td>48/88 (55%)</td>
<td>51/90 (57%)</td>
</tr>
<tr>
<td></td>
<td>Improves behaviour</td>
<td>49/126 (39%)</td>
<td>43/88 (49%)</td>
<td>23/90 (26%)</td>
</tr>
<tr>
<td></td>
<td>Better way to control population/prevent nuisance or ecological damage</td>
<td>31/126 (25%)</td>
<td>16/88 (18%)</td>
<td>17/90 (19%)</td>
</tr>
<tr>
<td></td>
<td>Improves health and welfare outcomes / faster recovery</td>
<td>19/126 (15%)</td>
<td>16/88 (18%)</td>
<td>8/90 (9%)</td>
</tr>
<tr>
<td></td>
<td>Easier, safer or less traumatic for patient</td>
<td>16/126 (13%)</td>
<td>11/79 (13%)</td>
<td>7/90 (8%)</td>
</tr>
<tr>
<td></td>
<td>Ensures owner compliance</td>
<td>9/126 (7%)</td>
<td>9/88 (10%)</td>
<td>5/90 (6%)</td>
</tr>
<tr>
<td></td>
<td>No reason not to/no evidence of risk</td>
<td>3/126 (2%)</td>
<td>2/88 (2%)</td>
<td>1/90 (1%)</td>
</tr>
</tbody>
</table>

*T Trap Neuter Release: the process by which unowned cats are spayed or neutered and returned to the area they were captured but are not adopted or housed.
2.5 Discussion

This paper presents the first data on attitudes towards pre-pubertal gonadectomy in Australia and New Zealand and is the first to use a single survey to explore the relative attitudes of veterinarians among these countries and the UK. There are no significant differences in the age at which puberty was typically considered to occur (5.7-5.9 months). Therefore differences in the reported minimum ages for spaying and castration are not simply artefacts of an overall difference in age at which cats are considered to enter puberty among the three countries.

It is important to note that the subject of this survey, pre-pubertal gonadectomy, encompasses early-age neutering, therefore confounding of the results is inevitable. Most veterinarians in the sample did not oppose pre-pubertal gonadectomy and substantially fewer opposed this than is found for early age neutering in the UK (Murray et al. 2008). However, pre-pubertal gonadectomy can encompass ages that are considered ‘conventional’ (e.g. 5 months: Preston Stubbs et al. 1996). As such, reduced opposition when compared to early age neutering is not unlikely. Most respondents would spay or castrate cats at an age considered to be pre-pubertal, however, a substantial minority (35%) would not perform these procedures at ages congruent with ‘early age neutering’. As puberty varies widely, and can occur as early as 3.5 months of age (Little 2011a), cats may enter puberty and become pregnant, or sire young, between these two time frames. If gonadectomy is to be used to maximum effect for limiting the unwanted cat population then it is reasonable that practitioners should consider implementing spaying and castration at ages between 3.5 months of age (Root Kustritz 2002) and 4 months of age (Walsh and Worth 2008). Our data
indicates that veterinarians in New Zealand and Australia are significantly more likely to spay or neuter cats at or below these ages compared to veterinarians from the UK. Accordingly, many New Zealand and Australian veterinarians are already meeting the age targets for gonadectomy currently being encouraged in the literature (≤ 3.5 months: Joyce and Yates 2011).

The difference in minimum age for spaying and castration among countries has at least three possible explanations. The first is that the difference between New Zealand and the UK may lie in the practices endorsed by the veterinary associations of each country. The NZVA policy states that it ‘supports pre-pubertal desexing of dogs and cats from 8 weeks of age’ (NZVA 2009) whilst the BVA and BSAVA follow the guidelines of The Cat Group who argue ‘a case for ‘earlier’ neutering at around four months’ (Anonymous n.d.). However, the ASAVA guidelines state that ‘Veterinarians should exercise their professional judgement of the appropriate age for desexing individual cats’. The fact that Australian practitioners show agreement with those in New Zealand indicates that other aspects may be affecting minimum age at gonadectomy beyond explicit veterinary association guidelines.

The introduction of cats, and their resulting impact on naïve native fauna, means they are considered a pest species in New Zealand (Farnworth et al. 2010) and Australia (Bengsen et al. 2011). An informal search of the scientific literature did not reveal similar discussions within the UK. Neutering at the earliest possible age is cited as a major route by which cat population, and therefore impact, can be curbed (Bushby and Griffin 2011) without compromising cat welfare. For veterinarians that indicated pre-pubertal gonadectomy was ‘sometimes’ desirable the foci of the open-ended responses differed between countries. In the UK pre-pubertal gonadectomy was more often reported as desirable as part of a Trap-Neuter-Release (TNR) as compared with
New Zealand or Australia. Conversely, veterinarians from Australia and New Zealand were more likely to report a perception that there was a cat over-population problem. Although both responses are associated with a perceived need to manage the cat population anecdotally TNR is not commonly used in New Zealand or Australia as it does not mitigate the immediate effects of stray or feral cats. Farnworth *et al.* (2011) have previously shown that TNR in New Zealand is only marginally more popular with the general public than lethal trapping. The second possibility is that veterinarians in Australia and New Zealand, undertake gonadectomy sooner as a result of the propensity in those countries for stray and feral cats to be controlled using lethal methods. Further exploration of veterinary attitudes towards stray and feral cats in these countries may be of value.

Finally, a third possibility may result from the sampling method. The UK convenience sample included both the BVA and BSAVA, the memberships of which overlap. Samples from New Zealand and Australia only included the associations that concern small animal practitioners. It is possible that the general membership opinions of the BVA and BSAVA differ. The BSAVA members may or may not have more knowledge of current practices as they relate specifically to cats, and shelter medicine, than the more general BVA membership. Unfortunately this research cannot determine this as it cannot distinguish between responses from these two UK groups.

Although provision of services to welfare centres or pounds was not found to affect the desirability of pre-pubertal gonadectomy it did have a significant effect on the minimum age at which gonadectomy occurred. Providers of such services were more likely to use early age neutering than non-providers. Given that shelters often require gonadectomy of animals before adoption and early adoption is preferred to allow effective socialisation (Root Kustritz 2002), it is perhaps unsurprising that they spay
and castrate earlier. Shelters may be more able to implement earlier gonadectomy as there are no requirements for owner consent for relinquished cats. Veterinarians who provide such services may also be more likely to be exposed to cat overpopulation problems and euthanasia of unwanted cats than non-providers. Similarly, the literature indicates that early age neutering rather than pre-pubertal gonadectomy is considered useful for shelter cat management (Spain et al. 2004; Trevejo et al. 2011). This may be positively encouraged from as young as 1.5 months when re-homing cats (Bushby and Griffin 2011; Joyce and Yates 2011) mainly for the improvement of animal welfare and population management reasons. Consistent with this, respondents that indicated pre-pubertal gonadectomy was ‘sometimes’ desirable primarily focussed on its ability to benefit cats from a shelter (see Root Kustritz 2007). They also indicated it was useful to ensure owner compliance with recommendations for gonadectomy, which has been shown to be low (Bushby and Griffin 2011). Given that many cats are free-roaming before the traditional neutering age of 6-8 months focussing only on cats from shelters, or those of recalcitrant owners, may not be sufficient to curb population growth. A study in the USA indicated that there was a clear difference in veterinary perception of the need for pre-pubertal gonadectomy dependent on whether the patient originated from a shelter or a private client (Spain et al. 2002). This may be problematic as evidence from the UK suggests that for cats, the majority of unplanned pregnancies occur between 4-9 months of age (Murray et al. 2009). Therefore the longer an owned cat remains entire the greater the risk of accidental pregnancy.

Our study indicated differences in the desirability of pre-pubertal gonadectomy among countries based upon country of practice and the gender of the respondent. However, in contrast to Murray et al. (2008), we found no relationship between the acceptability of neutering between 3-4 months and time since graduation.
Respondents from the UK were significantly more likely than those from New Zealand or Australia to indicate that pre-pubertal gonadectomy was undesirable or only sometimes. This may reflect the current discussion of early-age neutering which continues to be considered controversial in the UK (Murray et al. 2008; Joyce and Yates 2011; Sparkes 2011). This may not be the case for New Zealand and Australia, however further research should consider, and make explicit, the differences between the terms ‘early-age neutering’ and ‘pre-pubertal gonadectomy’. Overall, male practitioners were more likely to unequivocally answer in the affirmative when compared to females.

Veterinarians who stated it was undesirable to perform pre-pubertal gonadectomy often focussed on the perceived risks, including long-term health problems. These have been cited as potential issues in early literature (e.g. Romatowski 1993) however all subsequent explorations of the effects of patient age on post-surgical complications or developmental problems conclude that there is little or no foundation to these concerns (Howe et al. 2000; Reichler 2009; Bushby and Griffin 2011). We suggest that rejection of pre-pubertal gonadectomy may fail to consider the wider implications of cat overpopulation, including high euthanasia rates for unwanted cats (Spain et al. 2004). Based upon the euthanasia statistics alone it has been suggested that veterinarians should perhaps receive greater education around the procedures and benefits of early-age neutering (Root Kustritz 2007). Practitioners that indicated pre-pubertal gonadectomy was desirable identified a range positive benefits including behavioural benefits (see Spain et al. 2004); the potential to improve population management (see Reichler 2009; Joyce and Yates 2011). They also cited that there were fewer surgical complications during pre-pubertal gonadectomy (Howe 1997).
Our New Zealand sample is a more substantial proportion of NZVA-CAS members than is the case for equivalent professional organisations in the UK and Australia. Consequently our conclusions should be considered with some degree of caution.

In conclusion, it is evident that pre-pubertal gonadectomy is contentious for a small minority, but less so than early age neutering. Veterinarians who provide services to pounds and welfare centres spay and castrate animals earlier than those who do not, possibly due, in part, to a greater recognition of the social and environmental effects of cat overpopulation. Continued education about the benefits of pre-pubertal gonadectomy and the lack of empirical evidence supporting health concerns may continue to improve its acceptance. This is of particular relevance to the substantial proportion of veterinarians that consider pre-pubertal gonadectomy relevant only to certain groups of cats (e.g. those from shelters). As time of implementation for any veterinary procedure is also dependent upon owners’ attitudes towards them we propose this would be a suitable topic for future research. Given our results indicate that veterinary attitudes may differ between countries it is therefore important to explore them both nationally and comparatively rather than rely on inference from studies in other nations.
2.6 References


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Survey Paper Two

This chapter is formatted in the style of the New Zealand Veterinary Journal and has been published as:


As both chapters two and three used the same survey to collect data, the methodologies provided are similar in nature.

3.1 Abstract

AIM: To compare the use and provision of analgesia to cats undergoing gonadectomy by a sample of veterinarians in New Zealand, Australia and the United Kingdom.

METHODS: Small animal veterinarians’ views and practices on provision of analgesia to cats at three different time phases (pre/intra-operatively, post-operatively and post-discharge) were gathered using an electronic questionnaire. Respondents were also asked to state the pharmacological agent(s) used and the dosage rate(s). Differences in provision of analgesia were assessed relative to the respondent using binary logistic regression. The effects of sex of the patient and time of provision were explored using McNewar’s Test and Cochran’s Q respectively. Differences between drug types used amongst countries was tested using a cross-tabulation.

RESULTS: The survey received 717 valid responses. There has been a substantial increase in provision of analgesia to cats undergoing gonadectomy when compared to the early literature. There were significant differences in prevalence of analgesia provision at the three different time phases for spaying and castration (both p < 0.001). Likewise, there were significant post-operative and post-discharge differences in provision of analgesia for castration, as compared to spaying (both p < 0.001), and a similar effect was seen pre/intra-operatively (p = 0.002).
Post-operative provision of analgesia following both castration (p < 0.001) and spaying (p < 0.001) differed amongst countries of practice. Veterinarians in Australia and New Zealand were more likely to provide post-operative analgesia than those from the United Kingdom. Respondents from New Zealand and Australia were significantly more likely to provide post-operative analgesia compared to those from the United Kingdom. Veterinarians from the UK more commonly used non-steroidal anti-inflammatory drugs (NSAID) in the pre/intra-operative phase (P < 0.001) than veterinarians from either New Zealand or Australia.

CONCLUSIONS AND CLINICAL RELEVANCE: Contemporary use of analgesics for cats appears focused on provision at clinic and may not address the effects of surgery beyond the first 24 hours. The UK, Australia and New Zealand clearly differ in the types of analgesia administered, possibly reflecting differing professional considerations of the risks associated with the use of NSAID. In the interests of animal welfare, pain relief should perhaps be provided or offered more frequently for owner administration.

3.2 Introduction

As the most common elective surgery for cats, gonadectomy is of legitimate interest both in terms of animal welfare and effective companion animal population management. Most owners that present cats at clinic do so for elective surgeries, meaning that the cats are free from pain prior to provision of veterinary care (Dyson 2008). As such, pain in general and, more specifically, the pain associated with
gonadectomy, require careful management. Failure to do so for such a common procedure may impose substantial welfare costs on the animals concerned.

The efficacy of pain management for cats is often elucidated through research which uses ovariohysterectomy as the surgical model (e.g. Al-Gizawy et al. 2004; Giordano et al. 2010; Cagnardi et al. 2011). Following ovariohysterectomy behavioural changes indicative of pain persist for up to 55 hours even if pre-operative buprenorphine is given (Waran et al. 2007) and owners reported persistent behavioural changes in their cats for several days following gonadectomy (Väisänen et al. 2007). Pain behaviours following gonadectomy have been found to diminish following provision of post-operative butorphanol (Rütgen et al. 2011). Despite this, analgesia for owner administration following discharge from the clinic has received little attention in the literature. It should be noted that Meloxicam is available as an oral analgesic and has been reported as commonly used “off-label” for treatment of pain in cats (Robertson, 2005). Studies on carprofen given to healthy cats subcutaneously for 6 days (day 1: 4mg/kg; day 2 and 3: 2mg/kg; day 4 and 6 1mg/kg; Steagall et al. 2009) found no gastro-intestinal side-effects or haematological changes. These data indicate that prolonged post-operative pain management for cats by their owners may be possible as long as there are no complicating factors (e.g. reduced renal function) and owners do not exceed the stated dose.

In general analgesia provision is affected by the gender, time since graduation (Lascelles et al. 1999) and practice size (Raekallio et al. 2003) of the practitioner. Historically, research into provision of analgesia for cats during or following gonadectomy demonstrates it to be low, especially when compared to dogs (e.g. Williams et al. 2005). It is then, perhaps, unsurprising that analgesia for cats has been described as under-provisioned (Robertson, 2005). In general, analgesia provision for
Castration is less common compared to ovariohysterectomy (Dohoo and Dohoo, 1996; Lascelles et al. 1999; Wright 2002; Huggonard et al. 2003; Raekallio et al. 2003). In part this is due to the perceived differences in pain caused by the two procedures (Williams et al. 2005). This sex difference is also expressed through owner reports where male cats received a significantly lower pain severity score than female cats (Väisänen et al. 2007). However, experimentally, this difference may become non-significant after 1.5 hours (Cagnardi et al. 2011).

Overall, the literature suggests that under-provision of analgesia to cats arises from their unique physiology, a lack of approved NSAID for use in cats (Lascelles et al. 2007) and a general wariness amongst veterinary practitioners when using certain drug types. For example, the perception that opioids induce mania in cats persists (Robertson 2005), despite the fact that this only resulted from doses of 20mg/kg (Dhasmana et al. 1972). Similarly it is suggested that concern around the impact of non-steroidal anti-inflammatory drugs (NSAID) on renal function and integrity is evident amongst veterinarians, despite there being suggestion that this is mitigated if they are used correctly (Robertson and Taylor, 2004). The pre-operative use of NSAID such as meloxicam and carprofen is reported as common practice in the UK and, in healthy cats, renal side effects appear to be rare (Lascelles et al. 2007). However, others suggest it should only be used post-operatively and after recovery from anaesthesia (Wright, 2002).

The timing of administration of analgesia is also important as are the combinations of analgesic agents used. Effective timing and combinations of analgesics may act to prevent central sensitisation by pre-emptively blocking nociceptors before they are activated in humans (Husain et al. 2009) although little evidence of this can be found for cats. Similarly, it has been cited in other species that pre-emptive analgesia may
attenuate the post-operative pain response. For example, Lascelles et al. (1997) identified that pre-emptive analgesia using pethidine reduced post-surgical hyperalgesia in dogs following ovariohysterectomy. In turn it is argued that this reduction in nociceptive input may function to reduce the requirements for analgesia post-operatively (Pascoe 2000; Wright 2002). Little research has been conducted that addresses pre-emptive analgesia provision and efficacy for cats specifically.

Elective surgeries also result in pain with a number of root causes (e.g. inflammation and acute tissue injury). Processing of these different insults occurs through a variety of complex mechanisms. In terms of cats, there is little explicit evidence of the value and efficacy of multimodal analgesia in the literature (Lascelles et al. 2007) although it is anecdotally reported as commonly used, and efficacious, in a clinical setting (Robertson, 2005). In general, post-operative NSAID provide long periods of analgesia, for example meloxicam may provide up to 24 hours of pain relief for cats (Robertson, 2005) whereas the opioid hydromorphone may only be effective for up to 5 hours (Wegner and Robertson, 2003). Post-operative pain assessment following ovariohysterectomy using carprofen, ketoprofen or meloxicam was found to provide appropriate analgesia for the majority of cats for up to 18 hours (Slingsby and Waterman-Pearson, 2000). As such NSAID may be able to significantly reduce pain for the patient even after discharge from the clinic. The length of post-operative cover will depend on whether they are provided as part of the pre-medication, immediately following the operation or sometime after regaining consciousness.

This paper sought to explore the current provision of analgesia to both male and female cats during and following gonadectomy. It is considered that provision of analgesia will be contingent upon the sex of the patient and will be less likely as time since the operation increases. In addition, provision of analgesics is considered
relative to the practitioner’s gender, time since graduation and country of practice, to establish any effects. We also hypothesise that analgesia provision may be affected by the characteristics of the veterinarian. Finally descriptions of the analgesics used are provided and discussed.

3.3 Materials and methods

This study targeted companion animal veterinarians in New Zealand, Australia and the UK. A questionnaire containing 34 questions, taking approximately 10-15 minutes to complete (Appendix 1) was disseminated on-line (www.surveymonkey.com) through a direct link promoted by the New Zealand Veterinary Association’s Companion Animal Society (NZVA-CAS), the British Veterinary Association (BVA) and British Small Animal Veterinary Association (BSAVA) and the Australian Small Animal Veterinary Association (ASAVA). To improve response rates the associations and people responsible for promoting the survey were prompted to remind their members on two occasions during data collection.

Only responses to 17 of the 34 questions are considered in this paper as the other questions related to practices and attitudes concerning implementation of pre-pubertal gonadectomy (Farnworth et al. 2013). Information gathered included, basic information about the respondent including gender, year of qualification and country of current practice. In addition respondents were asked about their provision of analgesia, including drugs and dosages used, for male and female cats during gonadectomy. Respondents were also asked about analgesia provision during three
specific phases, these being: the intra/pre-operative phase, the post-operative phase and post-discharge phase.

3.4 Statistical analyses

The data were described in terms of percentage of respondents providing a given answer as well as providing mean or median values and ranges where appropriate. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 19.0 for Windows (IBM Inc, Chicago IL, USA). For the purposes of analysis graduation time was condensed into the categories 0-10, 11-20 and 21+ years. Statistical significance was established at p < 0.05.

McNemar’s test was used to explore whether there were differences in the likelihood (yes or no) that analgesics were provided to male and female cats at three different time stages during gonadectomy, namely pre/intra-operatively, post-operatively and after discharge. We then used Cochran’s Q test to test for differences in the likelihood (yes or no) of pain relief provision among the three time phases for both males (castration) and females (spaying). We adjusted the significance levels, to account for the multiple tests, using a Bonferroni correction.

Binary logistic regression was used to determine the possible impact of the main effects of gender of veterinarian, country of practice and graduation time category (0-10 years, 11-20 years, and ≥20 years) on analgesia provision (yes or no) to cats undergoing gonadectomy. The possible effects of the two-way interaction between gender and graduation time and between gender and country of practice and the effect
of the three-way interaction of gender, graduation time and country of practice were also examined. We investigated this relationship separately for each combination of time phase and procedure (castration or spay). This avoided issues around independence of error within a single analysis due to repeat measure caused by multiple non-responses from each veterinarian to accommodate the use of repeated tests on the same data set we adjusted our threshold significance level using the Bonferroni Correction. The adjusted significant level was calculated at \( P = 0.008 \).

Finally data concerning the drugs used were tabulated to identify the number of practitioners using multimodal therapies. For the purposes of analysis drugs reported by respondents using trademarked names (e.g. Rimadyl) were re-categorised based on their generic active ingredient (e.g. carprofen). We examined the association between the use of NSAID only, opioid only and opioid/NSAID combination drug regimens and country of practice using a cross tabulation procedure. Other combinations are presented but were too infrequent to allow meaningful statistical analysis. In the analysis we controlled for procedure (castration or spaying). As for the previous analyses the association was tested separately for the two time phases namely the pre-/intra-operative and immediately post-operative periods. Significance levels were also subject to a Bonferroni adjustment with the new threshold level set at 0.025.
3.5 Results

3.5.1 Basic demographics

There were 717 responses to the survey. Of these 249 (34.7%) were from New Zealand, 269 (37.5%) were from the UK and 199 (27.8%) from Australia. For New Zealand this response rate represents 41.6% of the total NZVA-CAS membership (S Blaikie, pers. Comm.). The UK and Australian samples groups were less easily contacted and the percentage response rates for the UK (269/4500; 6%; T Sainty, pers. Comm.) and Australia (199/1460; 13.6%; M Cole, pers. Comm.) are substantially lower than those for New Zealand. A greater percentage of females (F) responded (Australia 75%; New Zealand 58%; UK 66%) than males (M) when compared to the veterinary associations statistics which are as follows: ASAVA: F=49.2% M=50.8%; BVA: F=57.9% M=42.2%; NZVA-CAS F=49% M=51%.

3.5.2 Differences in analgesia use relative to procedure and phase

The likelihood that pain relief would be provided differed between male and female cats at each of the pre/intra-operative, post-operative and after discharge phases (p = 0.002; p < 0.001; p <0.001 respectively: Table 3-1). Similarly the likelihood of pain relief provision showed a significant decline across the pre, post- and after discharge

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5 T Sainty, Head of Membership, BVA, 7 Mansfield Street. London, UK
6 M Cole, Administration Officer, ASAVA, Unit 40, 6 Herbert Street, St. Leonards, New South Wales, Australia

105
periods for both male (Cochran’s $Q = 803.55; df = 2; p < 0.001$) and female
(Cochran’s $Q = 730.331; df = 2; p < 0.001$) cats.

Table 3-1: Analgesia use for cats undergoing gonadectomy

Table 3-1. Overall number (and percentage) of respondents from New Zealand, Australia and the UK (total n=717) reporting provision of analgesia at three different time phases (pre/intra-operative, post-operative and post-discharge) to cats undergoing gonadectomy. Variation in total number is due to missing responses.

<table>
<thead>
<tr>
<th>Analgesia provided</th>
<th>Pre/intra-operative$^a$</th>
<th>post-operative$^a$</th>
<th>post-discharge “at home”$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Castration</td>
<td>Spay</td>
<td>Castration$^b$</td>
</tr>
<tr>
<td>Yes</td>
<td>650/705 (92.2%)</td>
<td>624/657 (95%)</td>
<td>327/646 (50.6%)</td>
</tr>
</tbody>
</table>

$^a$ Percentage of practitioners providing analgesia amongst the three time phases is statistically significantly different for both castration ($\chi^2 = 1045.24; df = 2; p < 0.001$) and spaying ($\chi^2 = 853.21; df = 2; p < 0.001$).

$^b$ Percentage of practitioners providing analgesia for males is statistically significantly different as compared to females during the post-operative ($\chi^2 = 30.955; df = 1; p < 0.001$) and the post-discharge ($\chi^2 = 54.034; df = 1; p < 0.001$) phases.

3.5.3 Differences in analgesia provision amongst respondents

3.5.3.1 Pre-operative analgesia

A large majority of veterinarians ($\geq 79\%$) in all combinations of categories (i.e. country, gender and graduation time) provide pain relief during the pre-operative stage. Accordingly the number of responses to the category of “no provision of analgesia” was small or zero. Because of this incomplete information from predictors
the statistical procedures associated with the logistic regression are inappropriate. We elected not to conduct the analysis at this time phase.

### 3.5.3.2 Post-operative analgesia for castration

A test of the full model against a constant only model was statistically significant, indicting the inclusion of the independent variables of gender, country of practice and graduation time significantly improved the chance of predicting category membership (i.e. whether or not pain provision was provided) \( \chi^2 = 84.482; \ df = 15; \ p < 0.001 \). Examination of the main effects of gender, country of practice and graduation time individually revealed that only the main effect of country of practice was significant (Wald statistic = 13.907; \( \df = 2; \ p = 0.001 \)) (see Table 3-2). The proportion of the variance accounted for by the regression model was relatively small at around 16% (Nagelkerke R\(^2\) = 0.167).

Expressed as an odds ratios, New Zealand veterinarians are 2.382 (95% CI: 1.372-4.138) times more likely to provide post-operative analgesia after castration than UK veterinarians. The equivalent odds ratio for the Australia-United Kingdom comparison is 2.885 (95% CI: 1.510-5.512)
3.5.3.3 Post-operative analgesia for spaying

A test of the full model against a constant only model was statistically significant, indicating the inclusion of the independent variables of gender, country of practice and graduation time significantly improves on chance in predicting category membership (i.e. whether or not pain provision was provided) ($\chi^2=113.24; \text{df} = 15; p < 0.001$). Individual examination of the main effects of gender, country of practice and graduation time revealed that only the main effect of country of practice was significant (Wald statistic = 23.819; df = 2; p < 0.001) (see Table 3-2). The proportion of the variance accounted for by the regression model was relatively small at around 22% (Nagelkerke $R^2 = 0.223$).

Expressed as odds ratio New Zealand veterinarians are 4.038 (95% CI: 2.199-7.412) times more likely to provide post-operative analgesia than UK veterinarians. The equivalents odds ratio for the Australia-United Kingdom comparison is 3.193 (95% CI: 1.604-6.357)
Table 3-2: Provision of analgesia relative to country of practice

Table 3-2: Number (percentage of valid responses) of veterinarians that provide post-operative analgesia to cats undergoing gonadectomy. Data are presented relative to the respondent’s country of practice. Differences among the total number of responses and the number of responses in any category are due to missing datum points for specific questions.

<table>
<thead>
<tr>
<th>Country of practice</th>
<th>Respondents providing post-operative analgesia for castration</th>
<th>Respondents providing post-operative analgesia for spaying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>New Zealand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 249)</td>
<td>137</td>
<td>62.6%</td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 199)</td>
<td>115</td>
<td>65.3%</td>
</tr>
<tr>
<td>United Kingdom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 269)</td>
<td>73</td>
<td>31.0%</td>
</tr>
</tbody>
</table>

3.5.4 Provision of analgesia for owner administration

A test of the full model against a constant only model was not statistically significant, indicting the inclusion of the independent variables of gender, country of practice and graduation time did not improve on chance in predicting category membership (i.e. whether or not pain provision was provided) for castration ($\chi^2=12.507; \text{ df } = 15; \text{ p } = 0.640$) or spaying ($\chi^2=18.139; \text{ df } = 15; \text{ p } = 0.244$).
3.5.5 Analgesics used relative to country of practice

When controlling for procedure (castration or spay), we demonstrated a significant association between country of practice and the drug regime (opioids only, NSAID only, combination of opioids and NSAID) for the pre/intra-operative time phase (Castration: $\chi^2 = 171.521; \text{df} = 4; \ p < 0.001$, Spay: $\chi^2 = 191.853; \text{df} = 4; \ p < 0.001$). The significant difference was driven by the higher likelihood that veterinarians in the United Kingdom would use NSAID only or in combination with opioids when compared to veterinarians from New Zealand and Australia (Table 3-3). The differences in drug regime were less marked for the immediate post-operative period (Table 3-3). However there remained a similar significant association between country of practice and drug regime for castration ($\chi^2 = 13.026; \text{df} = 4; \ p < 0.011$) but not for spaying ($\chi^2 = 8.161; \text{df} = 4; \ p = 0.086$). The significant effect of country of practice on drug regime appeared largely driven by the relatively higher use of opioids for analgesia provision in New Zealand. Due to wide variations in the manner in which respondents reported the dosage used for each analgesic and a lack of information about how these were administered these data were excluded from further analysis.
Table 3-3: Analgesics used during and after gonadectomy relative to country of practice

Table 3-3. Response of veterinarians (n = 717) to a survey about pain relief for cats undergoing gonadectomy. Data include those that provide one or more analgesics, based on the reported class(es) used. Data represent the analgesic classes used either pre/intra-operatively and post-operatively by veterinarians from the UK, New Zealand or Australia. Numbers are presented as reported use/number of respondents using analgesia. Variation between numbers is due to non-report of drugs used.

<table>
<thead>
<tr>
<th>Time Phase</th>
<th>Analgesia</th>
<th>New Zealand</th>
<th>Australia</th>
<th>United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spay</td>
<td>Castration</td>
<td>Spay</td>
</tr>
<tr>
<td>Pre/intra-operative(^a)</td>
<td>Opioid(^c) only</td>
<td>171/213</td>
<td>162/227</td>
<td>98/164</td>
</tr>
<tr>
<td></td>
<td>NSAID(^d) only</td>
<td>13/213</td>
<td>13/227</td>
<td>29/164</td>
</tr>
<tr>
<td></td>
<td>Opioid/NSAID combination</td>
<td>5/213</td>
<td>5/227</td>
<td>19/164</td>
</tr>
<tr>
<td></td>
<td>Other(^e) only</td>
<td>5/213</td>
<td>8/227</td>
<td>5/164</td>
</tr>
<tr>
<td></td>
<td>Opioid/opioid combination</td>
<td>7/213</td>
<td>8/213</td>
<td>4/164</td>
</tr>
<tr>
<td></td>
<td>NSAID/NSAID combination</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Opioid/other combination</td>
<td>6/213</td>
<td>5/227</td>
<td>5/164</td>
</tr>
<tr>
<td></td>
<td>NSAID/other combination</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Other combination</td>
<td>-</td>
<td>2/227</td>
<td>1/164</td>
</tr>
</tbody>
</table>

| Post-operative\(^b\) | Opioid only | 34/188 | 27/137 | 11/137 | 6/115 | 12/109 | 8/73 |
|                      | NSAID only | 127/188 | 98/137 | 103/137 | 97/115 | 87/109 | 56/109 |
|                      | Opioid/Opioid combination | 1/188 | 1/137 | - | - | - | - |

\(^a\) Use of drug categories ‘opioid only’ NSAID only’ and ‘opioid/NSAID combination’ differ significantly amongst countries for castration and spaying respectively (\(\chi^2 = 171.521; \text{df} = 4; p < 0.001; \chi^2 = 191.853; \text{df} = 4; p < 0.001\)).

\(^b\) Use of drug categories ‘opioid only’ NSAID only’ and ‘opioid/NSAID combination’ differ significantly amongst countries for castration (\(\chi^2 = 13.026; \text{df} = 4; p < 0.011\)) but not for spaying (\(\chi^2 = 8.161; \text{df} = 4; p = 0.086\)).

\(^c\) Opioids in categories include use of: Morphine; buprenorphine; butorphanol; methadone; pethidine

\(^d\) Non-steroidal anti-inflammatory drugs (NSAID) in categories include reported use of: Meloxicam; carprofen; ketoprofen; tolfenamic acid

\(^e\) ‘Other’ in categories includes reported use of: Medetomidine; Ketamine; acepromazine and other miscellaneous agents reported fewer than 4 times by the respondents which are neither opioid nor NSAID
3.6 Discussion

In this sample veterinary provision of analgesia for cats undergoing gonadectomy is substantial in the pre/intra-operative phase. It appears from comparison with earlier literature from Australia, New Zealand and the UK that routine provision of analgesia has increased in both the pre/intra-operative and post-operative phases (Watson et al. 1996; Lascelles et al. 1999; Williams et al 2005). Some caution should be taken in interpretation of these findings given the different survey vehicles used and populations sampled. For example, Williams et al. (2005) specifically asked about peri-operative use of analgesia which could include some overlap between the pre/intra- and post-operative distinction used in this research. It also surveyed a larger sample of veterinarians and not just those that work in companion animal practice. Watson et al. (1996) were also not specifically companion animal focused and the use of analgesics for ovariohysterectomy of dogs and cats was reported as a combined percentage of 6% (Watson et al. 1996). It should also be noted that, as with any survey, the likelihood for non-response bias is substantial as those choosing not to answer the survey may represent a specific group (e.g. veterinarians that are not interested in analgesia). Even allowing for these distinctions, the authors are confident in their assertion that veterinarians are far more likely to use analgesia for cats in contemporary practice.

In the pre/intra-operative phase the difference for provision of analgesia to male and female cats is significant. However provision for both procedures exceeds 90%. This suggests not only a change in the number of practitioners using analgesia but also greater equity of analgesia provision between the two procedures. Previous reporting
in the literature suggests that male veterinarians and those in practice for longer were less likely to provide analgesia (Lascelles et al. 1999). Our analyses indicate that although the category ‘time since graduation’ may explain some of the likelihood that analgesia is provided, the effects are trivial, country of practice explains the majority of any effect. Likewise no evidence for differences between male and female practitioners were found. These changes suggest an overall recognition of the importance of managing pain, both in practice and likely in veterinary education. The under-provisioning of analgesia for cats (Robertson, 2005), in these three countries at least, appears to be waning.

Post-operative analgesia provision is also prevalent in the three countries surveyed indicating that the duration of pain management for cats undergoing gonadectomy is, for many, able to address issues of pain in the clinic. However, post-operative administration of drugs is not as common as pre/intra-operative management, particularly in the UK (Table 3-2). Given the reduced percentage of practitioners using post-operative analgesia improvements may still be able to be made. However it is important to note that those practitioners that provide pre/intra-operative NSAID should exercise caution. Post-operative overdosing of meloxicam (oral provision after previous parenteral dosing) has resulted in renal insufficiency in eight cats in the UK (Dyer et al. 2010).

Also of note is the inequity between male and female cats, possibly reflecting the continued perception, rightly or otherwise, that castration is less painful that ovariohysterectomy (Wright, 2002). Anecdotally some respondents to the survey indicated that they would like to provide post-operative pain relief but that clients did not expect to pay extra for the medication and veterinary practices could not be expected to carry the financial burden. This raises an interesting dilemma for
veterinary practices in terms of meeting the needs of the patient and the client (owner) as well as establishing exactly how much, and what period, of analgesia is appropriate for gonadectomy. Further research should investigate owner willingness to pay for analgesia and veterinary perception of obligation to provide analgesia following surgery.

It was found that there is a general paucity of post-discharge pain relief. Practitioners were not asked why they chose to provide, or not provide, analgesia. Possible reasons for non-provision of post-discharge analgesia may include concerns about longer term use of NSAID (Robertson, 2008; Dyer et al. 2010), a lack of owner willingness to pay and a lack of awareness of practitioners as to the potential duration of pain caused by gonadectomy. A recent survey of pet owners’ expectations in Great Britain suggests that 61% of owners would expect their pet to be sent home with pain relief following surgery (Demetriou et al. 2009). The findings in this research suggest this expectation is not being met. Further research on pain management following discharge, extending beyond gonadectomy of cats into a range of surgeries and species, may be useful.

In the UK sample pre/intra-operative use of NSAID is substantially more commonplace than in New Zealand and Australia. Likewise, although the use of a combined pre/intra-operative therapy is relatively uncommon, it is behavior more frequently by practitioners in the UK. In part this may be associated with the length of time that NSAID have been commonly used for pre/intra-operative use, although exactly how is hard to clarify. It is possible that earlier approvals of early generation NSAID with a smaller body of research around their use increased the likelihood that practitioners would experience adverse side-effects. In the UK NSAID such as meloxicam and carprofen have been reported as widely used for peri-operative
analgesia (Robertson and Taylor, 2004) with carprofen approved in the late 1990s (Taylor, 1999) and meloxicam in the early 2000s (Robertson and Taylor, 2004). In New Zealand rimadyl was approved for use with cats in 1994. As previously cited there is still some controversy around the use of NSAID in general. Some authors argue that risk is minimal if the dose and anaesthetic regime are appropriate (e.g. Gurney, 2012) whilst others argue more strenuously for caution based on the potential risks (e.g. Wright, 2002). An article from the popular veterinary media in New Zealand (Robson, 2007), states that the risks of pre/intra-operative NSAID use in cats outweigh the potential benefits. A comparison of Williams et al. (2005) with Lascelles et al. (1999) indicates that in New Zealand 93% of vets indicated concern about the side-effects associated with NSAID use, compared with only 75% in the UK some 6 years prior. It is also possible that countries with fewer veterinary schools (e.g. New Zealand only has a single veterinary school) show reduced diversity in veterinary practices based on the dominant perspective of lecturers. A survey of the attitudes of teaching staff at veterinary schools towards analgesia use in cats may be able to ascertain whether or not this influence is evident.

It is not within the scope of this paper to argue for or against pre/intra-operative NSAID use or to dispute what is ‘reasonable caution’. However, as asserted by Lascelles et al. (2007), there is a clear need for more research which specifically addresses the value of single-mode and multi-modal NSAID therapies and multi-modal therapies in general (Robertson, 2008) in the treatment and amelioration of pain in cats. An epidemiological study of NSAID linked mortality or lasting harm in otherwise problem-free cats may also be useful.

Wider pre/intra-operative use of NSAID in the UK appears to reduce the likelihood that practitioners will provide further post-operative analgesia. Conversely
practitioners in Australia and New Zealand show reduced usage of NSAID in the pre/intra-operative phase but more use of NSAID post-operatively. The use of NSAID is often promoted as, in general, it provides a far longer period of analgesia than commonly used opioids (Robertson, 2008). Therefore, later administration, or a post-operative boost, may serve to extend the period of pain relief. Many authors identify that pre-emptive pre/intra-operative use of analgesics, including NSAID, could subsequently reduce the post-operative pain response, and hence the need for analgesia (e.g. Kelly et al. 2001). Additionally pre/intra-operative NSAID may also reduce post-operative inflammatory pain. It is reasonable then to pose the question: If limited NSAID analgesia is to be used is it better to provide it post-operatively to extend the period of analgesia (as in New Zealand and Australia), or earlier to avoid subsequent sensitisation and need for increased post-operative analgesia (as in the UK). There is some research demonstrating the effect of central sensitisation in dogs (Lascelles et al. 1997, 1998; Welsh et al. 1997), however currently there is none for cats. The authors recommend more research be undertaken in order to explore this question.

It should be noted that respondents were not asked about the mode or timing of delivery for the analgesics given, or the reasons for selection or avoidance of some compounds. This information would be valuable as it would allow elucidation of various aspects of analgesia use in cats. This would include the potential to identify if animals would be under full or partial pain management at various stages of the surgical process.

In conclusion, there is clear evidence of significant improvements in analgesia provision for cats across the countries surveyed, although more attention to the pain management of discharged patients may be warranted. The authors identify that much
research is still required to understand the value of pre/intra-operative and multi-modal analgesia use in cats, especially as it relates to central sensitisation.

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119
Laser validation paper one

This chapter is formatted in the style of Applied Animal Behaviour Science and has been published as:

4. Validating the use of a carbon dioxide laser for assessing nociceptive thresholds in adult domestic cats (Felis catus).

4.1 Abstract

Thermal sensitivity in cats has historically been assessed using complex devices which require direct application to the patient and can therefore, in themselves, affect the measurement. This study aimed to validate the use of a remote low power (165 mW) carbon dioxide laser device for the assessment of thermal nociceptive thresholds in cats. Repeatabilities were established based on individual responses to three thermal tests on the same day and across 4 consecutive days. A total of 12 thermal tests were conducted on each of eight male and eight female de-sexed adult cats. As a control a non-thermal helium aiming laser was used to ensure the animals were responding to the thermal component of the device. All thermal tests elicited a behavioural response with the large majority being a skin twitch known as the panniculus reflex (97%). None of the non-thermal tests resulted in this reflex behaviour. There was no evidence that cats became sensitised or habituated to the low power thermal stimulus on any given day (P = 0.426) or across days (P = 0.115), or that there was any interaction between the two time factors (P = 0.084). There was also no difference in latency to respond between males and females (P = 0.094), although there was a significant interaction between the day of testing and the sex of the subject (P = 0.042). Significant intra-class correlations demonstrated that individual responses were repeatable over days 1 to 3 (all P < 0.05) but not over day 4 (P = 0.096). A significant intra-class correlation was also evident across all days when data were combined (P < 0.0001). This technique shows some promise in assessing individual nociceptive
thresholds and as a tool to establish associated individual differences. It could, with more exploration, also provide an alternative thermal mechanism for testing the efficacy of analgesics in cats. The significant repeatabilities were low ranging from 0.241 to 0.414, this suggests that a number of extraneous factors may have influenced responses to CO₂ laser stimulation at low power levels (165 mW). Further exploration of this technique on a larger sample than used here may allow elucidation of any other factors, including age or sex effects, that impact upon thermal sensitivity in the domestic cat.

4.2 Introduction

Cats are a commonly owned companion animal (Bernstein, 2005) and may be subjected to a number of surgical procedures to accommodate them in an anthropocentric environment. A range of surgical procedures are conducted to reduce reproductive rates (e.g. ovario-hysterectomy; Grint et al., 2006; Rütgen et al., 2011; Waran et al., 2007) or damage to the owner’s property (e.g. onychectomy; Cloutier et al., 2005). All involve tissue damage, and therefore result in pain. The provision of analgesia to cats prior to, during or following surgery, although improving, is not consistent (Robertson, 2005) and is underutilised in cats compared to dogs (Williams et al., 2005). It is therefore important to further investigate feline-specific pain and nociception, allowing improvements in understanding individual variation to be made, which may ultimately improve diagnoses and treatments.
Pain sensitivity is considered to be the point at which an animal is able to sense, and respond to, a noxious stimulus (Allen, 2004). Noxious stimuli have an actual (or perceived) ability to damage tissues (Woolf and Ma, 2007). Nociceptive tests that use behavioural responses as indicators have been identified as useful for the assessment of pain sensitivity in animals (Herskin et al., 2009) as the two are intrinsically linked. Sensitivity to noxious stimuli may vary between individual animals relative to their age (Gagliese and Melzack, 1999; Ting et al., 2010), sex (Chesterton et al., 2003; Fillingim and Gear, 2004; Greenspan et al., 2007) or prior experience, or may result from a complex interaction between multiple developmental factors (Guesgen et al., 2011). Increased pain sensitivity in cats may also result from central sensitisation during and following surgeries if effective peri-operative analgesia is not employed (Lascelles and Waterman, 1997). Therefore, reliable measurement of the sensitivity of cats to noxious stimuli is important and may ultimately improve the understanding of pain and analgesic effect.

Current methods for the assessment of nociceptive sensitivity and validation of analgesics in cats are relatively complex. They require direct manipulation of the subject and application of the stimulus to the skin using an attached device (e.g. pressure devices: Dixon et al., 2007; thermal devices: Dixon et al., 2002) as well as cutaneous measurement of temperature or applied force. These processes may affect the variables being measured and require significant time for habituation. In addition, directly applied thermal devices have been reported, in some cases, to be insufficiently reliable to evaluate analgesia in cats (Taylor et al., 2007).

Measurement of thermal thresholds has been used to assess nociception and to evaluate analgesic effects in rats (Malmberg and Yaksh, 1992) and mice (Pinardi et al., 2003). In comparison, carbon dioxide (CO₂) lasers have been used to reliably
assess thermal nociceptive thresholds in a range of species (e.g. sheep: Guesgen et al., 2011; pigs: Herskin et al., 2009; cows: Veissier et al., 2000). The benefits of CO$_2$ laser stimulation include remote application, consistent stimulus application and immediate cessation of stimulation following the response. There is also complete absorbance of thermal energy by the skin, regardless of pigmentation (Gülsoy et al., 2001; Le Bars et al., 2001). In addition, laser stimulation followed by a clear behavioural response should require relatively little behavioural monitoring experience, particularly if the response is a stereotypic reflex, such as the panniculus reflex (a skin twitch), that has been associated with normal neural functioning (Van Soens et al., 2009).

To date, thermal devices used for threshold testing in cats have produced active behavioural responses (e.g. jumping or looking at the site of stimulation) (Slingsby et al., 2010) indicative of conscious pain perception. These thermal contact devices cannot be instantaneously removed from the subject to allow the site of application to cool rapidly. Previous thermal laser stimulation experiments in livestock have often used relatively high power outputs resulting in physical withdrawal of the target area (e.g. 2.25-4.5 W: Veissier et al., 2000). Where a range of power outputs have been used in pigs, increasing power has concomitantly reduced response latency as well as changed the type and frequency of the behaviour displayed (Herskin et al., 2009). It has also been suggested that low power output (2.5 W) does not produce reliable measures of response in cattle (Veissier et al., 2000). It has not currently been established as to whether, in cats, low level stimulation resulting in a nociceptive response (panniculus), might be repeatable and could therefore be explored as a tool for measurement of sensitivity to pain.
This study investigates the repeatability of a thermal laser device for use in testing the thermal nociceptive thresholds of cats. We hypothesise that the usefulness of such an approach depends on the repeatable nature of the response.

4.3 Materials and methods

4.3.1 Subjects and housing conditions

All procedures were approved by the Massey University Animal Ethics Committee (Massey University, Palmerston North, New Zealand, MUAEC protocol 11/49). The experiment used eight male and eight female, adult and de-sexed, domestic cats (Felis catus) with a mean age of 4.2 years (s.d. 1.5 years). The cats were permanently housed in a nutritional facility and were fed a standard wet cat food diet ad libitum during the trial. Cats were housed in stable colonies of 10 individuals in outdoor pens (2.4 m H x 1.4 m W x 4.4 m L); with approximately half the volume of each pen under cover.

During the experimental phase, six individual metabolism cages (0.8 m H x 0.8 m W x 1.1 m L) were used 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 acclimatise the subjects. Although the cages housed a single cat, cats were, at all times, in visual contact with other individuals. 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 similarly replaced with a plastic coated 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 by providing a slightly raised vantage point, each cage was furnished with a small wooden box and blanket. Food and water were not provided during the 1-1.5 h test phase.

4.3.2 Experimental protocol

4.3.2.1 General procedure

Approximately 24 h prior to the commencement of the study each cat was removed from the colony housing and a patch of fur was clipped to skin level from each side of the animal before they were returned to their colony cages. The area exposed measured approximately 4 cm² on each side of the cat, starting at the third rib 1 cm sternally from the corresponding vertebra. Optimum positioning of the exposed area of skin (i.e. able to be accessed by the laser operator when the cat was in full sternal recumbency) was ascertained using two cats that did not participate in the subsequent study.

Each individual cat was tested every day for 4 consecutive days. Since all cats could not be housed in the test cages simultaneously, they were allocated to three groups of mixed sex, two of six individuals and one of four. The sequence in which these groups were tested was randomised across the 4 days to reduce any potential circadian effects. Testing began at 10:00 h and ended at 16:00 h, with each group being in the
test room for between 1 and 1.5 h. Cats were returned to the colony cages between days but not between tests. On introduction to the test cage, each cat was 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 when the majority of cats were quiet and in sternal recumbency.

4.3.2.2 Laser device

Thermal nociceptive thresholds were measured using a purpose-built remote laser device (M.P.B. Technologies Inc., Dorval, Canada). The CO$_2$ laser produced a 5 mm diameter beam which was aimed using a non-thermal visible helium laser housed within the casing of the laser device. The wavelength of the thermal laser was 10.60 µm (far infra-red) and the power output used was 165 mW. Given that the non-visible component of the laser was potentially hazardous, personal protective equipment was employed at all times, including safety goggles. Cages were lined with non-reflective materials to eliminate risk of injury by reflection of the laser.

4.3.2.3 Testing procedure

Each cat was exposed three times on a given day to a CO$_2$ thermal laser device for 4 consecutive days. As a control, each cat was also exposed to the visible (non-thermal) helium laser three times per day across 4 consecutive days. The helium laser was manufactured as an integrated component of the thermal laser device and used for
guidance. This resulted in a total of 24 exposures for each cat, 12 to the thermal and 12 to the non-thermal laser.

After the habituation period (15 min), three threshold tests were conducted on each cat. Testing did not commence until the cat was in the appropriate position (sternal recumbency). The laser was directed onto the exposed area of skin from a distance of 2 m until the cat responded either by moving away from the stimulus or exhibiting the panniculus reflex, or until the safety cut-off time was reached. Following either of these behavioural responses, the laser was turned off and the time to respond (latency) noted to the nearest 0.1 s. For each cat 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). The exact time between each test varied dependent upon the activity pattern of the individual (i.e. time to sternal recumbency).

To ensure that the response observed was related to the thermal laser and not to the visible helium laser used for sighting, each cat was also exposed to the visible (non-thermal) helium laser only (i.e. the red helium laser was directed onto the exposed skin but the thermal laser was not activated) on three separate occasions randomly during each of the 4 trial days. Each helium laser test ceased when the cat responded in either of the ways identified as endpoints for thermal laser testing or when the time exceeded the safety cut-off time. Non-thermal laser tests were applied opportunistically throughout the 1-1.5 h thermal laser testing period and therefore did not necessarily have a 15 min interval between them.

To establish whether the device would elicit behavioural responses within an appropriate time its settings were first tested on two cats not used in the study. At 165
mW all responses occurred in less than 90 s with no evidence of reddening or skin damage. Therefore, these parameters were considered appropriate maxima for thermal laser testing of cats. For other species increased laser power has been shown to be associated with a shorter latency to respond (Herskin et al., 2009; Veissier et al., 2000). For this reason a constant low power was used to maximise the probability that any changes in latency would be identified (i.e. longer latencies would better allow variation between individuals to be identified) and to minimise the assumed negative effects of thermal stimulation at higher power settings. As skin temperature is expected to increase at a constant rate in response to CO\(_2\) laser stimulation (Veissier et al., 2000), latency to respond behaviourally to thermal stimulation was measured rather than skin temperature *per se*.

### 4.4 Statistical analyses

Data were analysed and transformed using the Statistical Package for the Social Sciences (SPSS) version 19.0 for Windows (IBMInc, Chicago IL, USA). The data were not normally distributed and were therefore \(\log_{10}\) transformed and re-tested and shown to be normal with homogeneity of variances. A mixed design analysis of variance (ANOVA) was used to assess whether the latency to respond varied significantly amongst tests on a given day (intra-day), amongst test days (inter-day) or between the sexes or whether there was an interaction amongst any combination of the three factors. One of the core assumptions of this analysis is that of sphericity. To test sphericity we used Mauchly’s Test which tests for the equivalence of the hypothesised and observed variance/co-variance patterns. Data are presented in this
paper as both raw mean values and transformed mean values where required and are identified appropriately.

Repeatability of the latency to respond for the cats was tested using a single measures intra-class correlation (ICC). Repeatability was assessed for the subjects on each day (intra-day repeatability) and across the 4 days using all 12 latencies (inter-day repeatability). Inter-day repeatability was also assessed using ICC for male and female cats separately to establish whether there were possible sex effects on repeatability. A Cronbach’s alpha test was used to assess the reliability of the data. Differences and correlations were considered significant at $P < 0.05$.

As 98% (188/192) of the non-thermal tests exceeded the 90 s cut-off time these were not included for analysis. They were taken as indicative that latencies recorded in response to the CO$_2$ laser were a direct result of thermal stimulation.

### 4.5 Results

All thermal laser exposures elicited a behavioural response within the 90 s allowed. Of the 192 non-thermal tests only four (2%) resulted in a behavioural response within the 90 s timeframe. All four responses were associated with movement of the cat away from the light and, therefore, none were associated with the panniculus reflex. By contrast, of the 192 thermal tests 186 (97%) were terminated as a result of the subject exhibiting the panniculus reflex.
4.5.1 Latency to respond

For all analyses, Mauchly’s Test of Sphericity was not significant (all P > 0.4). In general, latency to respond showed substantial individual variation, with a mean response time ± SE across the 192 thermal tests of 28.6 ± 1.05 s (total range: 7.6 – 76.5 s). The log-transformed mean was 1.41 ± 0.15. Intra-day latency to respond did not significantly vary (ANOVA intra-day effect; $F_{2,28} = 1.213; P = 0.313$) nor was there any evidence of a change in latency over 4 consecutive days (Inter-day effect; $F_{3,42} = 1.918; P = 0.141$). Similarly there was no significant interaction between intra-day and inter-day response latencies ($F_{6,84} = 1.298; P = 0.287$).

There was no significant effect of sex on latency to respond ($F_{1,14} = 3.238; P = 0.094$) however there was a significant interaction between sex and inter day latency ($F_{3,42} = 2.987; P = 0.042$). Exploration using estimated marginal means indicated that females had lower response time latencies than males on days 2 (24.6 s and 31.9 s respectively), 3 (23.5 s and 34 s respectively) and 4 (21.3 s and 28.6 s respectively) but not on day 1 (32.1 s and 31.4 s respectively). Means as expressed are untransformed.

4.5.2 Repeatability

Intra-class correlations demonstrated significant intra-day repeatability on the first 3 days of testing but not on the 4th (Table 4-1). Similarly, inter-day repeatability was
statistically significant for the 16 cats (Table 4-1). Further analysis indicated that inter-day repeatability was statistically significant for both males (ICC = 0.178; $F_{7,77} = 3.599; P = 0.002$) and females (ICC = 0.283; $F_{7,77} = 5.725; P < 0.0001$). With the exception of day 3, the Cronbach’s alpha score exceeded 0.7 for all ICC indicating that the source data were reliable (day 3 = 0.6; reliability marginal).

Table 4-1: Intra-class correlations and repeatability of laser protocol

Table 4-1: Results of intra-class correlations for latency to respond to a thermal laser by 16 cats. Correlations reflect repeatability over three tests on any 1 day of testing (intra-day reliability) and overall repeatability for all tests on all days (across 4 consecutive days)

<table>
<thead>
<tr>
<th>Day</th>
<th>Intra-class correlation</th>
<th>95% confidence interval</th>
<th>DF*</th>
<th>F Test Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.414</td>
<td>0.105</td>
<td>0.707</td>
<td>15 (30)</td>
<td>3.118</td>
</tr>
<tr>
<td>2</td>
<td>0.255</td>
<td>-0.042</td>
<td>0.592</td>
<td>15 (30)</td>
<td>2.026</td>
</tr>
<tr>
<td>3</td>
<td>0.334</td>
<td>0.028</td>
<td>0.652</td>
<td>15 (30)</td>
<td>2.505</td>
</tr>
<tr>
<td>4</td>
<td>0.197</td>
<td>-0.09</td>
<td>0.545</td>
<td>15 (30)</td>
<td>1.737</td>
</tr>
<tr>
<td>All days</td>
<td>0.241</td>
<td>0.112</td>
<td>0.473</td>
<td>15 (165)</td>
<td>4.817</td>
</tr>
</tbody>
</table>

* total degrees of freedom for analyses ($DF_2$) shown in parentheses

4.6 Discussion

This research supports the use of a CO$_2$ lasers to measure thermal nociceptive thresholds in cats. As demonstrated in other species, cats consistently exhibit a relatively simple behavioural response to thermal laser stimulation. In this regard, the panniculus reflex should be considered a useful and obvious behaviour for the testing of nociceptive thresholds in cats. The behavioural responses in this study were less
marked than those reported in other species with only a minority of individuals actively withdrawing from the stimulus. Similar laser-based studies in other species have reported more active avoidance of the stimulus (e.g. kicking of the leg; Guesgen et al., 2011; Herskin et al., 2009; Veissier et al., 2000). This suggests that low power stimulation can be effective and repeatable in some species. It may also minimise the negative aspects of thermal stimulation in domestic cats.

There were no effects of test number or of test day on the latency to respond. This indicates there was no evidence of habituation or sensitisation to the stimulus, likely also related to the low power outage used. Previous studies using higher power devices have demonstrated that animals respond relatively quickly (94% response in < 25 s; Herskin et al., 2009), with a tendency towards a decreased latency in subsequent tests (Veissier et al., 2000). This study supports the notion that low power thermal stimulation results in longer latencies to respond, although higher outputs need to be explored further in domestic cats. It is unclear whether the subsequent lack of habituation resulted from a response at a lower skin temperature than required for other species (e.g. 45-55°C in cattle; Veissier et al., 2000) or whether it results from a more gradual increase in skin temperature which stimulates different nociceptors (C as opposed to Aδ fibres) as seen in the rat (Yeomans and Proudfit, 1996). To establish this, future assessments could include thermographic images of the affected skin area. As the experimental room was not climate controlled it is possible that fluctuations in ambient temperature may also have had a small effect on rates of heating and cooling of the skin and, therefore, latency to respond.

The statistically significant repeatability demonstrated for this technique shows it to be a promising tool in assessing nociception in the cat and does not support the assertion by Veissier et al., (2000) that high power settings are absolutely required to
ensure valid and reliable measures. It indicates that, although responses may be variable between individuals, the position of the individual within the cohort is predictable. In this regard single exposures (and responses) may be sufficient for any future nociceptive threshold assessments. The long latencies at lower powers may be of benefit to studies where small numbers of animals are used. For horses, it has been noted that high power thermal sources require a short cut-off time to avoid tissue damage, during which some individuals may not have responded (Love et al., 2011). This can limit the value of the data collected as slow responses are not quantified. A similar issue was noted for sheep during a laser-based trial using a high power output (Guesgen et al., 2011). In addition, where multiple factors may affect nociceptive response (e.g. age, sex or level of subcutaneous body fat), a longer latency to respond may be useful in differentiating between them.

However, low power outputs and long latencies to respond do appear to have drawbacks. A potential drawback of using a low power laser device with longer latency times is that other factors may strongly influence the response. Of particular interest is the loss of repeatability on day 4. This test day coincided with inclement weather (a winter storm) which may explain why the cats’ responses were not repeatable. A fear response to the storm may have masked the response to a less aversive event (i.e. low level thermal stimulation) for some of the cats affecting repeatability on day 4. Storms and unpredictable noises have been identified as common fear inducing events for dogs (Overall et al., 2001), and cats have also been noted as having noise related fear responses (Bowen and Heath, 2004). In humans fear has been shown to induce hypoalgesia and increase the latency of a reflex withdrawal response following low intensity thermal stimulation (Rhudy et al., 2004). Similarly a conditioned fear response is known to increase latency to respond to a
thermal tail flick test in rats (Seo et al., 2008). This suggests that external disturbances, particularly those which may cause stress or fear, should be controlled, wherever possible, when using a low power CO$_2$ laser technique.

Use of this technique to establish the effectiveness of analgesia may be limited due to the wide variation in latency to respond. Current thermal techniques use the skin temperature at which a behavioural response occurs rather than the time to respond, which appears to have significantly less variation ($40.8 \pm 2.2$ ºC: Lascelles and Robertson, 2004; $44.4 \pm 2.5$ ºC: Siao et al., 2011). Long response latencies, which may be useful for establishing variation between individuals, may result in the need to have larger sample populations for testing the effects of analgesia. Further exploration of underlying nociceptive mechanisms in cats and an investigation into the responses of individuals to low output thermal devices following surgery and administration of analgesia are required. There is also a need to explore the use of higher power laser outputs to establish if a decrease in response latency may further improve the utility of this methodology for cats.

There are numerous extraneous factors which could explain the relatively low ICC coefficients, however these are hard to effectively explore in small cohort studies. This study showed a non-significant difference in latency to respond between males and females, but arguably suggested a trend ($P < 0.1$) that females responded more quickly than males. There was a significant interaction between sex and daily response with females responding more quickly than males on days 2, 3 and 4 but not on day 1. Therefore the influence of sex on nociceptive thresholds of cats requires further exploration. Sex has been shown to have a significant and complex interaction with age in a similar study of pain threshold in lambs (Guesgen et al., 2011). Genotype has been identified as the basis for variations in pain perception (Mogil,
and phenotypic expression is also known to influence pain perception in humans (e.g. hair colour: Liem et al. 2005). Whether this effect extends to coat colour in non-human animals is currently unknown. However, we suggest that this study should be performed on a larger cohort to explore these factors further.

4.7 Conclusions

Although there are a number of potential factors that influence the response of the individual cat to thermal laser stimulation, this study demonstrated that use of a CO$_2$ laser on a low power setting generated a repeatable response for a given individual both within and between days. However there was a significant degree of inter-individual variation and an indication that the sex of the cat may influence the response. Therefore this technique warrants further exploration using a larger cohort, specifically to address factors that may influence the response latency.

4.8 References


This chapter is formatted in the style of Applied Animal Behaviour Science and has been published as:


As chapters four, five and six utilise the same thermal technique and the same housed population of cats some of the methodological processes have been repeated.
5. Body weight affects behavioural indication of thermal nociceptive threshold in adult female domestic cats (*Felis catus*).

5.1 Abstract

Carbon dioxide (CO\textsubscript{2}) 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\textsubscript{2} 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; \( P < 0.001 \)). 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\textsubscript{2} laser protocol in other species.
5.2 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$_2$) 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., 2013; Herskin et al., 2009) and cattle (Ting et al., 2010; Veissier et al., 2000). More recently the CO$_2$ 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$_2$ 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$_2$ 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 (Bölcskei 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$_2$ laser technique show no evidence of habituation or sensitisation over a period of one hour (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$_2$ 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\textsubscript{2} laser.

### 5.3 Materials and methods

#### 5.3.1 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 5-1). 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 h x 1.4 w x 4.4 d m); with approximately half the volume of each pen under cover.

During testing, cats were held in eight individual metabolism cages (0.8 height x 0.8 width x 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 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 recumbence, each cage was furnished with a small wooden box and blanket. Food and water were not provided during the test phase.

5.3.2 Experimental protocol

5.3.2.1 Thermal threshold testing procedure

The study was conducted over five 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 5-1) 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 five 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.

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$_2$ 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 safety cut-off time was reached. 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 recumbence).
5.3.2.2 Laser device

Thermal nociceptive thresholds were measured using a remote laser device (Model 48-1, Synrad, Mulkiteo, Washington, USA). The CO\textsubscript{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 \textmu 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\textsubscript{2} 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.

5.3.3 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, Missouri, 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.

5.4 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\textsubscript{10} transformed to achieve a normalised distribution with homogeneity of variances. To reduce analytical complexity cats were assigned to one of two age categories ‘younger adults’ (1-6.99 years; \(N = 57\)) and ‘older adults’ (7 years or over \(N = 56\)). All cats were adult and over 1 year of age.

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\textsubscript{10} 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 \textit{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 5-1) so we did not include this factor in further analysis of the male data.
5.5 Results

5.5.1 Provisional analyses

The mean body weight of males was significantly greater than that of females (Table 5-1. \( t = 9.757; \ DF = 111; \ P < 0.0001 \)). Males also had a longer mean log_{10} response time (± SE) than females (1.098 ± 0.27 s and 1.005 ± 0.32 s respectively). These differences were significant \( t = 2.223; \ DF = 111; \ P = 0.028 \).

5.5.1.1 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 values were used for further analyses. Untransformed mean latency to respond (± SE) across all tests was 14.83 ± 0.85 s for males (range: 4.0 – 32.8 s) and 12.59 ± 0.94 s for females (range: 3.1 – 33.7 s).
Table 5-1: Cat characteristics

Table 5-1: 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$_2$ laser stimulus.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Neutered</th>
<th>Mean age (years)</th>
<th>Mean body weight (g)</th>
<th>Median condition score (1-9)*</th>
<th>Mean latency (± SE) to respond (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>59/60</td>
<td>7.1 (± 0.4)</td>
<td>4367.1 (± 84.6)</td>
<td>5 (4-8)</td>
<td>14.83 (± 0.85)</td>
</tr>
<tr>
<td>Female</td>
<td>31/53</td>
<td>5.9 (± 0.5)</td>
<td>3290.8 (± 67.2)</td>
<td>5 (4-7)</td>
<td>12.59 (± 0.94)</td>
</tr>
</tbody>
</table>

*Measured using the Purina® 9-point scale (Nestlé Purina PetCare Company, Missouri, USA). Data available for 105/114 subjects (male = 54/60; female = 51/53).

5.5.2 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.

For females, ANCOVA confirmed a significant effect of body weight on mean log$_{10}$ response time (Fig. 5-1a; $F = 6.727; DF = 1; P = 0.013$), 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. 5-1b; $F = 3.758; DF = 1; P = 0.058$). 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 ($t = -0.370; DF = 83; P = 0.721$).

Figure 5-1a/b: Body weight and response latency

Figure 5-1a/1b: Association between body weight (g) and mean latency to respond (s) to thermal stimulation using a CO2 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.
5.6 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 (~30kg) respond more
quickly to CO₂ laser stimulation than larger pigs (~60kg). 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). Studies in humans with normal body weight indices, which have been incorporated into the analyses, do not report a quantifiable effect (e.g. Neziri et al., 1996). For 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$_2$ 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 males). 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$_2$ laser, at least when ‘young’ cats (1-7) were compared with older cats (>7). Sheep have been noted as having divergent responses to CO$_2$ laser stimulation during early development of males and females (Guesgen at 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$_2$ 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$_2$ 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.

5.7 Conclusions

The use of CO$_2$ 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.

5.8 References


163


Laser Validation Paper Three

This chapter is formatted in the style of Veterinary Anaesthesia and Analgesia and has been submitted as:


165
6. Assessment of a carbon dioxide laser for the measurement of thermal nociceptive thresholds following intramuscular administration of morphine, buprenorphine, tramadol, ketoprofen and medetomidine to pain-free female cats.

6.1 Abstract

**Objective:** To explore the potential for using a thermal carbon dioxide (CO$_2$) laser to assess analgesia in pain-free cats.

**Animals:** Sixty healthy adult female cats with a mean weight (± SD) of 3314.4 g (± 636 g).

**Methods:** Cats were randomly allocated to one of six treatments 1) saline 0.2 ml/cat; 2) morphine 0.5 mg/kg; 3) buprenorphine 20µg/kg; 4) medetomidine 2 µg/kg; 5) tramadol 2mg/kg; 6) ketoprofen 2mg/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 500mW CO$_2$ laser with maximum latency to respond set at 60 sec. 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 to further explain the direction of the effect.

**Results:** Cats treated with morphine ($\chi^2 = 12.90; \text{df} = 6; P = 0.045$) and tramadol ($\chi^2 = 20.28; \text{df} = 6; P = 0.002$) showed significant increases in latency to respond over the duration of the test period. Treatment with buprenorphine also resulted in increases in
latency to respond although only at the level of a statistical trend ($\chi^2 = 10.93; \text{df} = 6; P = 0.091$). Injection of saline, ketoprofen or medetomidine showed no significant effect on latency to respond. The longest latency to respond after injection of morphine was achieved at 60-75 min whilst that of buprenorphine occurred at 90-105 min.

**Conclusions:** This project further validates the CO$_2$ laser technique for use in cats. It can be used for assessment of analgesia 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 60s exposure limit, a cut off time of <45s is recommended.

**6.2 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 analgesics 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 such as buprenorphine (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 nociceptive threshold assessment techniques have been validated for use in cats. These include both contact devices (Dixon et al. 2002) and remote CO₂ laser stimulation (Farnworth et al. 2013b). Although the former technique has 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 CO₂ 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 ability to use the laser technique without interference with management routines or the 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.

This research sought to explore the effectiveness of a CO₂ thermal laser for the assessment of nociceptive thresholds in pain-free cats under analgesia. 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 and buprenorphine (a partial agonist at mu-opioid receptors) which have previously been evaluated in cats using thermal thresholds (Robertson et al. 2003; Steagall et al. 2006; Pypendop et al. 2008). In addition three other compounds with analgesic activity were used, 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). NSAIDs do not have a central action, but rather act to inhibit prostaglandin synthesis and therefore inflammatory response (Robertson & Taylor 2004). The final treatment used medetomidine, an alpha-two agonist with both sedative and analgesic effects (Cullen 1996; Steagall et al. 2009b). 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) to assess the sensitivity of the CO₂ laser protocol. Saline was included as a control.

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 with 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 and inflammation is absent in these test subjects.

6.3 Materials and methods

6.3.1 Cats and housing conditions

All procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 12/109). A total of 60 female domestic cats were used, 32 entire and 28 spayed, with a mean weight (±SD) of 3.314 kg ± 0.636 kg and a mean age (±SD) of 6.1 years ± 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); they were therefore considered to be healthy and pain-free. 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

171
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.

### 6.3.2 Laser device

Thermal nociceptive thresholds were measured using a remote laser device (Model 48-1, Synrad, Mulkiteo, Washington, USA). 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 $\mu$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$_2$ 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 non-treated cats, all responses to 500mW thermal stimulation occurred in less than 60 s with no evidence of reddening or skin damage (Farnworth et al. 2013a). 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.

6.3.3 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 randomly allocated to one of six treatment groups, and likewise randomly allocated to a test 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 was transferred to the experimental cages and were only returned after all 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. 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 CO₂ 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 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 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, well beyond the time interval required to prevent sensitisation in pigs (Di Giminiani et al. 2013). 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. Readings were unable to be taken for 15/420 data 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.
6.3.4 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; Pfizer, Auckland, New Zealand); 5) tramadol (2mg/kg; Tramal; 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.

6.3.5 Statistical analyses

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). Accordingly we tested for differences in weight among treatment groups using a one-way ANOVA procedure after testing for homogeneity of variance.
The experimental protocol restricted the maximum time cats were exposed to the laser to 60 s at which point the response time was recorded as greater than 60 sec. The resulting distribution of response time was 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.

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 were conducted to identify where specifically inter-treatment differences occurred. Given that multiple pair-wise comparisons were performed and that adjustments to the p-values are often highly conservative the significant unadjusted values were also retained (Fig.6-1) although with the caveat that they cannot be considered to be reliable. Significance was considered at \( P \leq 0.05 \).

6.4 Results

6.4.1 Weight

After confirming the variances of weight among treatment groups were homogenous (Levene’s test, \( F_{(5,53)} = 2.292, \ P = 0.06 \)), we could detect no differences in the body weights of cats among the treatment groups (\( F_{(5,53)} = 1.176, \ P = 0.33 \)). This suggested we could disregard weight differences as a potential explanation of different responses among treatments.
6.4.2 Effect of treatments on latency to respond to thermal stimulation

Response times of cats to thermal stimulation were very variable across all six drug treatments (Fig 1). However baseline (median pre-treatment) response times for cats that received either an analgesic drug or saline solution were always below 60 s (range: 6.3-12.2 s and 7.25 -11.53 s respectively). No significant effects of time were found for groups treated with medetomidine ($\chi^2 = 3.077; \text{df} = 6; P = 0.799$), ketoprofen ($\chi^2 = 5.816; \text{df} = 6; P = 0.444$) or saline ($\chi^2 = 3.922; \text{df} = 6; P = 0.687$). In contrast median response times of cats injected with morphine and buprenorphine reached 60 s on at least one of the post-treatment time intervals. The number of tests which reached the 60s cut-off point are shown in table 6-1. When we tested for an effect of time since injection, treatment with morphine ($\chi^2 = 12.90; \text{df} = 6; P = 0.045$) and tramadol had a significant effect on response time ($\chi^2 = 20.28; \text{df} = 6; P = 0.002$) over the course of the observation period. Treatment with buprenorphine demonstrated a statistical trend ($\chi^2 = 10.929; \text{df} = 6; P = 0.091$).
Table 6-1: Number of cats reaching 60 s cut-off by treatment

Table 6-1: 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre</th>
<th>15-30</th>
<th>30-45</th>
<th>45-60</th>
<th>60-75</th>
<th>90-105</th>
<th>120-135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0.2ml/cat)</td>
<td>0/10</td>
<td>0/8</td>
<td>0/10</td>
<td>0/7</td>
<td>0/9</td>
<td>0/10</td>
<td>2/10</td>
</tr>
<tr>
<td>Morphine (0.5mg/kg)</td>
<td>0/10</td>
<td>1/9</td>
<td>2/10</td>
<td>4/10</td>
<td>7/10</td>
<td>4/10</td>
<td>4/10</td>
</tr>
<tr>
<td>Buprenorphine (20µg/kg)</td>
<td>0/10</td>
<td>4/9</td>
<td>6/10</td>
<td>5/10</td>
<td>4/10</td>
<td>5/9</td>
<td>3/10</td>
</tr>
<tr>
<td>Tramadol (2mg/kg)</td>
<td>0/10</td>
<td>2/10</td>
<td>1/10</td>
<td>3/10</td>
<td>4/10</td>
<td>5/10</td>
<td>2/10</td>
</tr>
<tr>
<td>Ketoprofen (2mg/kg)</td>
<td>0/10</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/10</td>
<td>1/10</td>
<td>1/9</td>
</tr>
<tr>
<td>Medetomidine (2µg/kg)</td>
<td>0/10</td>
<td>1/9</td>
<td>0/10</td>
<td>1/9</td>
<td>1/10</td>
<td>1/10</td>
<td>2/10</td>
</tr>
</tbody>
</table>

In general the data showed substantial over-dispersion. There were clear differences in latencies to respond amongst cats within the same treatment. An example of this is provided using five of ten cats from the buprenorphine treatment group (fig. 6-1.).
Figure 6-1: Individual variation in latency to respond to buprenorphine

Figure 6-1: Chart shows latency to respond to thermal stimulation with a carbon dioxide laser for five of ten cats. ‘Pre’ is the baseline measurement with each subsequent measurement occurring after intramuscular administration of buprenorphine (20 µg/kg).

For tramadol, adjusted p-values indicated significant differences between the pre-treatment and 60-75 min after treatment (P = 0.016) and 90-105 min after treatment (P = 0.033). This reflected an increase in the median response time from 11.35 s before injection to 37.97 s at 60-75 min and 60 s at 90-105 min afterwards. However for tramadol unadjusted significance values (all P < 0.05) suggested increased latencies to respond from 45-60 min to 120-135 min inclusive. For morphine an increased latency to respond occurred only at 60-75 min (unadjusted P = 0.003), Likewise unadjusted significant differences (all P < 0.05) in latency to respond were found between 30-45 min and 120-135 min inclusive for buprenorphine.
Figure 6-2: Latency to respond relative to treatment and across the testing period.

Figure 6-2: 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 of treatment on latency to respond (P < 0.05). For buprenorphine * denotes a statistical trend (P < 0.1). Within treatments the letter (a) is used to indicate where an unadjusted statistical difference (P < 0.05) is found relative to pre-treatment latency, (b) denotes statistical significance retained when the p value is adjusted for multiple comparisons.
There was no significant effect of treatment on latency to respond to thermal stimulation during the pre-treatment interval ($\chi^2 = 1.54; \text{df} = 5; P = 0.909$). However, a significant effect of treatment was found at 60-75 min ($\chi^2 = 21.02, \text{df} = 5, P = 0.001$), 90-105 min ($\chi^2 = 18.38, \text{df} = 5, P = 0.003$) and 120-135 min ($\chi^2 = 11.72, \text{df} = 5, P = 0.039$) after injection. At 60-75 min median response time ranged from 7.96 s for cats injected with medetomidine to 60 s for cats provided morphine. At 90-105 min after injection response time ranged from 10.5 s for cats given saline to 60 s for cats injected with buprenorphine. At 120-135 min response time ranged from 10.12 s for cats provided medetomidine to 48.14 s for cats given buprenorphine. Whilst the overall conclusion of both a time and treatment effect on the response time of cats to thermal stimulation is considered to be robust some caution should be exercised in interpretation of specific differences. Multiple comparisons on the same set of data may increase the likelihood of Type 1 errors.

6.4.3 Side effects of treatment and procedure

Animals were closely monitored throughout this protocol, however there were some side effects. 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. There was no evidence of this having occurred during a previous experiment (Farnworth et al. 2013a). These adverse effects were reported to the ethics committee concerned with approval of this project. Secondly
there was evidence of nausea associated with the administration of morphine. Eight of the ten cats showed signs of excessive salivation or retching.

6.5 Discussion

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 and, to a lesser extent, buprenorphine.

As expected no significant effects were found for groups administered saline or ketoprofen. Although, as for other NSAIDs (e.g. carprofen: Taylor et al. 2007b), 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. Studies 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. 6-2). 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. The unadjusted significance values within this study (Fig. 6-2) suggest a similar effect. Loss of significance across the sample may result from higher inter-individual variation in latency to respond to a low output thermal laser (Fig. 6-1.). Buprenorphine has previously been identified as having short-term analgesic effect which requires additional and regular dosing to maintain effect (Robertson & Taylor 2004). 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. This variability likely explains why this treatment did not achieve statistical significance overall.
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). 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 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 skin temperature (Posner et al. 2010) and dexamethasone impacts 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 CO₂ 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 reduce the exposure time to 45 s. 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 analgesia-treated cats.

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. 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 the analgesic treatment.

6.6 References


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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.


7. General discussion.

7.1 Gonadectomy and pain in cats

Gonadectomy is the most common elective surgery for cats in the developed world. It is widely accepted that surgical procedures such as gonadectomy result in pain (Robertson 2008) but are essential for effective cat population management (Spain et al. 2004). Population management may be best achieved by performing gonadectomy at as young an age as is practicable, however pre-pubertal gonadectomy of cats appears to be contentious, especially for non-shelter based practitioners (Joyce and Yates 2011). The attitudes and practices of New Zealand veterinarians with regard to pre-pubertal gonadectomy, and thus, how contentious this practice is in New Zealand in comparison to other countries, have not been previously established.

Alongside concern about the increase in the global cat population (Peterson et al. 2012) which provides a continued impetus for gonadectomy of cats, the management of pain is now recognised as an important component of good veterinary practice (Stafford and Mellor 2007). However, post-surgical pain management in cats/domestic animals is still reported to be influenced by a range of external factors. These include the graduation date and gender of the practitioner, as well as the species and sex of the patient (Williams et al. 2005). The most commonly cited literature concerning analgesia use in cats and dogs is approximately 15 years old (Dohoo and Dohoo 1998; Lascelles et al. 1999), and it is likely that veterinary practices and attitudes have changed significantly since such time. There is also a dearth of studies exploring differences in veterinary practice amongst countries using the same survey
vehicle at the same time. To date, geographical and temporal differences in post-surgical analgesia provision for cats have only been inferred from separate studies in the literature.

These studies are to better understand firstly when cats may experience pain during their development and secondly how that experience is managed by veterinarians. This is especially pertinent as early exposure to unregulated pain may have long-term implications for the patient. Clinical management of post-surgical pain is likely to be driven by changes within veterinary education and analgesic drug development which are, in turn, research-driven and informed. Such research is predicated on methods of pain assessment which can be effectively validated and consistently applied.

In clinical situations, rating scales based on the evaluation of a range of behavioural and physiological indicators are most commonly used to evaluate post-surgical pain and the need or otherwise for analgesia provision. Although such scales have been demonstrated to have construct validity (Brondani et al. 2011) and can be applied to large cohorts of animals they are time consuming. Despite their usefulness within the clinic, they are also difficult to compare across studies given the potential for subjective variations in interpretation by different implementers.

Conversely thermal threshold devices such as the one developed by Dixon et al. (2002) have commonly been used to assess analgesic effectiveness in cats. Such devices elicit relatively simple behavioural indicators which minimises subjective interpretation of nociceptive threshold, i.e. response to a painful stimulus; however, their requirement for attachment of the device limits the number of animals which can be tested. The use of a carbon dioxide thermal laser may provide a useful alternative for measuring thermal pain sensitivity in cats as it can be applied to large numbers of
subjects and also elicits a relatively simple behavioural indicator of thermal pain threshold. It may also avoid some of effects of wearing device on variable measured.

7.2 Value of these studies

Cat ownership and population management are contentious topics within the public sphere. Veterinarians have a major role to play providing both public education and the best possible care for cats. The survey studies outlined in Chapters two and three aimed to update the literature relating to pre-pubertal gonadectomy and analgesia use in cats. In particular, these studies attempt to provide information to fill some of those gaps including understanding of the perceived positive and negative impacts of pre-pubertal gonadectomy as well as reasons for not performing the procedure. The investigations of differences among countries in the minimum age of gonadectomy and provision of peri- and post-surgical pain relief will help reveal factors that influence these veterinary practices. In turn it also helps identify areas of research that may guide best practice and which may make meaningful improvements in cat welfare. This is particularly valuable in an era when practitioners regularly find employment in countries other than that in which they were trained.

Following on from this information I also sought to develop the use of an objective tool for the assessment of pain in cats. Such a tool may be especially pertinent as it would allow future studies to assess the impacts of pre-pubertal gonadectomy and analgesia provision in cats. In the three experimental studies, I have returned to first principles and taken steps to validate a thermal carbon dioxide laser which may prove
useful. In addition I performed one of the largest single studies on thermal nociception in cats, identifying a potentially confounding variable (i.e. weight) to the assessment of thermal pain using a laser. This information may benefit any future studies employing laser devices to measure pain sensitivity. Finally my research has also demonstrated the potential for the panniculus reflex to be used to assess nociceptive thresholds in pain-free cats with and without pharmacological intervention.

7.3 Summary of key findings

From this series of studies there have been a number of significant findings. First, it is evident that the attitudes of veterinary practitioners differ relative to their country of practice. General attitudes concerning the appropriate age for gonadectomy of cats and also the minimum age at which pre-pubertal gonadectomy occur suggest that practitioners in the United Kingdom gonadectomise later and have more concerns around pre-pubertal gonadectomy. These differences also extend to how and when post-gonadectomy analgesia is provided, with practitioner in the UK more likely to use NSAIDs in pre-medications and less likely to provide post-operative analgesia. This is the first project to explore the attitudes of veterinarians towards cats and gonadectomy amongst different countries. It also suggests that veterinary perception of cats may be, to some extent, governed by social discourses around cat population management.
The second survey study provided detailed information on the provision of analgesia before, during and after gonadectomy of cats in NZ, Australia and the UK. It is encouraging to see that the use of analgesics has substantially increased in practice when compared to earlier studies (Watson et al. 1996; Lascelles et al. 1999; Williams et al. 2005). A lack of significant differences in provision associated with practitioner gender or age may suggest a fundamental change in the use of analgesics within the profession, at least in these countries.

Of significant interest are the apparent differences in practices towards the use of analgesics amongst practitioners from the three countries surveyed, including differing times of administration. Practitioners from the UK were found to provide post-operative analgesia less often than did those from NZ or Australia. However, UK vets provided NSAIDs, as opposed to opioids, more commonly in the pre/intra-operative phase. What exactly might explain this difference requires further investigation but could be associated with differences in education, drug approvals and drug legislation amongst the countries surveyed.

Although analgesia provision has improved, my research does identify some potential gaps and continued shortfalls. The pain associated with castration continues to be less likely to be managed than that associated with ovariohysterectomy, both following surgery and after discharge from veterinary care. Although castration may cause less pain than ovariohysterectomy pain management should differ by type, not by likelihood. In addition, how pain is experienced by the patient, and subsequently develops in the post-operative recovery phase, appears to be managed in substantially different ways. Although the per/intra-operative period shows substantial levels of management to avoid post-operative development of sensitisation of hyperalgesia resulting from intra-operative nociception, post-operative pain management appears
variable. The greatest potential for poor management of pain appears to occur after
the patient has been discharged, with relatively few practitioners providing analgesia
for owner administration. This is despite evidence that ovariohysterectomy pain
persists for a period likely to extend beyond typical time at clinic (Waran et al. 2007),
as well as the majority of owners reporting being able to recognise behavioural
changes associated with pain post-gonadectomy (Väisänen et al. 2007), and
expecting analgesia to be provided if necessary (Demetriou et al. 2009).

The latter experimental components of this thesis explored the validity of a low output
thermal CO$_2$ laser for determining nociceptive thresholds (i.e. pain sensitivity) in cats.
Validation of this method would be useful for pain research, especially given that
previous investigations of thermal pain sensitivity in dogs and cats have used either
complicated and expensive testing equipment (Dixon et al. 2002) or lasers with
relatively high outputs on large domestic species (Herskin et al. 2003) which, simply
based on epidermal thickness, may not be suitable for small mammals.

The first study in this suite demonstrated that the measures of thermal pain thresholds
achieved using the CO$_2$ laser were indeed repeatable for a given cat, both on any
given day and across all days combined. However there were some indications that
repeatability may have been influenced by both external conditions and inter-
individual factors. It also identified that, for the almost all tests, the stereotypical
‘panniculus reflex’ was sufficient to elucidate thermal nociceptive threshold.

Subsequent testing of a much larger population of cats of varying age, sex and
reproductive status revealed that body weight showed a positive correlation with
latency to respond to thermal stimulation. In other words, heavier cats took longer to
respond to the thermal stimulus and were thus less sensitive to thermal pain. This
effect was particularly strong in female cats and was not simply an artefact of weight differences between the sexes. However, the exact reason for the effect of body weight on thermal sensitivity requires further investigation.

Finally, as most thermal threshold testing protocols are used to investigate analgesic effects in cats (Steagall et al. 2007), it was important to further validate the technique using a range of pharmaceuticals already shown to be effective using other thermal devices. The laser technique allowed some differentiation between those analgesics expected to have an effect (i.e. centrally-acting opioids) and those not expected to have an effect on pain sensitivity in pain/inflammation-free cats (i.e. NSAIDs). It was also reassuring to note that the saline control did not significantly alter latency to respond.

7.4 Implications of findings for veterinary practice and pain research in cats

These findings have substantial potential to alter veterinary practice, or at the very least galvanise discussion around gonadectomy and analgesia use in feline veterinary practice. Cat population management is currently a much discussed topic, particularly in New Zealand and Australia where cats cause significant damage to native wildlife. Early gonadectomy has been identified as a significant factor in achieving effective population control (Bushby and Griffin 2011). The fact that practitioners from different countries display diverse practices and attitudes suggests that there is still a need to further discuss how, when and why cats are gonadectomised at different ages.
and whether it is important. Results from New Zealand and Australia are encouraging from a population management perspective in that they suggest early gonadectomy is normal practice for many veterinarians. If this is the case, there is still a need to discuss why the unowned cat population continues to show signs of growth (Aguilar and Farnworth 2013) and what this may mean for alternative cat management strategies.

Pain management is seen as one of the major foci of effective veterinary care yet, historically, a lack of up-to-date knowledge about effective pain management practices has affected analgesia provision in cats (Williams et al. 2005). Although this knowledge has clearly improved there is still a need to clarify how, when and why veterinarians provide analgesia for surgical pain in cats. There is also an obvious need for a wider discussion of NSAID use within New Zealand and Australia and whether, or not, current use is appropriate.

My research may also bring attention to the, as yet, unexplored value (in terms of business practice, but also animal welfare) of providing greater pain management outside the clinic. On a wider scale, and in an international setting, there is a need for discussion around expectations for analgesia provision, especially as veterinary professionals now, more than ever, are likely to experience practice outside the country in which they gained their qualifications. If anything, this research has revealed a paucity of studies which attempt to draw a wider understanding of variation amongst veterinary practices.

There is substantial variation in pain management provided for cats during gonadectomy, as well as in the range of ages at which it occurs. This suggests a clear need to better understand the development of post-surgical pain in cats as well as how
this is impacted upon by the age (and developmental stage) of the patient. There is also a need to explore some areas where pain management varies. For example whether castration is being under-managed or whether the administration of analgesia is disproportionate to the pain caused.

The laser technique shows significant promise as a research tool for investigating pain in cats. Firstly it has been shown to have practical utility in that it minimises the need to habituate the animals to wearing a device which has been necessary in other studies (Robertson et al. 2003). It can also be used on larger groups of animals with minimal disruption to management protocols and doesn’t require development of any other specialist equipment. Although the technique has some way to go before it can be considered fully validated, especially with regard to cats that are not pain-free, it has certainly demonstrated some parity with other thermal nociceptive techniques currently used in cat pain studies. Additionally, it may be a valuable technique, if developed further (and substantially so), for use in a clinical setting where, although most practitioners state pain assessment techniques are important, relatively few use them (Reid et al. 2007; Robertson 2008).

The ability to use the technique on large cohorts is particularly valuable as it may allow greater exploration of the underlying developmental and genetic mechanisms of pain sensitivity in cats. By way of demonstration, the second laser study in this thesis, which used 113 cats, was able to identify that weight affected nociceptive response, a heretofore unexplored component of nociceptive response in cats and one which requires more attention. Understanding the effects of such factors on measures of pain sensitivity provides an opportunity for future research.
7.5 Limitations of the research

7.5.1 Survey studies

The survey used (Chapters two and three) was targeted specifically at vets registered with national companion animal societies. This means that the results potentially represent the views of veterinarians that are most involved with gonadectomy of cats. One may therefore expect these findings to reflect a more contemporary view of analgesia and not represent the views of veterinarians for whom companion animals are a minor component of daily practice. The results should therefore be interpreted cautiously and may over-represent the current state-of-play as regards analgesia use for cats. This is further confounded by non-response bias, as with all surveys there is a strong potential for non-response bias, for example those practitioners not concerned with analgesia use may be less likely to respond. Mens’ opinions are typically under-represented. This may also result in an over-estimation of the level of change that has occurred. In the online protocol used in the current research, there was no mechanism by which the non-responders could be identified, and so it was not possible to ascertain their reasons for not engaging with the survey. However the response rate for New Zealand was good when compared with similar surveys (Williams et al. 2005; Keown et al. 2011).

Once the analgesia survey responses were returned it became apparent that some of the content/questions may not have been as useful for the intended purpose as expected. For example, although questions regarding analgesic dose rate were included, enquiry about the route of administration was not. There is substantial
evidence that the route of delivery has a significant impact upon the timing and depth of analgesia (Pypendop et al. 2008; Steagall et al. 2013). Also, dose rates were not always provided in a manner which could be compared among respondents. For example, a common reply to this question was “as recommended”, and dose recommendations likely vary among countries and products. In addition to mode of delivery, it was not possible to establish when analgesia was provided, either before or after surgery, beyond the broadly descriptive time phases used in the survey (pre/intra-operative and post-operative). Again this limited the value of the information gathered. Finally, although this survey asked about which analgesics were and were not used, it did not explore reasons for not using certain classes of drugs which resulted in some level of conjecture as to why differences occurred amongst countries.

It is often difficult to balance the need for respondents to complete the survey and the number of questions provided. This survey compared favourably with other similar surveys (Lascelles et al. 1999; Williams et al. 2005) in that it only addressed one species and one procedure. Despite this there were still a number of respondents that either did not complete the survey or failed to answer some questions.

7.5.2 Laser-based studies

Validation of a remote technique for assessing nociceptive thresholds is difficult. In the present studies, a number of processes could not be integrated based upon the budget and equipment available. For example, unlike other thermal devices (Dixon et al. 2002), the laser was not designed to allow measurement of skin temperature.
meant that I was unable to account for fluctuations in initial skin temperature associated with ambient temperature changes or changes associated with drug treatment (Posner et al. 2010) that may have affected the time required for the skin, and therefore thermal nociceptors, to reach response threshold. However, room temperature did not vary greatly (~16-21°C) and not sufficiently that it was likely to cause thermal stress. Unlike other experiments (e.g. Bortolami et al. 2013; Staffieri et al. 2013) involving pharmacological manipulation of the subjects, sedation scores were not allocated during this study. It is therefore difficult to elucidate between delays in nociceptive response caused by anti-nociception and those resulting from sedation.

Although a maximum cut-off time for application of the thermal stimulus was imposed, it was not possible to ascertain if skin temperature had risen to the point that damage was likely to occur until after the fact (i.e. blister formation). In chapters 4 and 5 no blistering was recorded in cats that approached the 60 s cut off. However, in chapter 6, following administration of analgesics, cats that exceeded the cut-off (approximately 50% of subjects) showed signs of mild blistering 24 hours after the experiment. Whether this resulted from changes in skin temperature, altered blood flow or animal responsiveness to stimulation could not be ascertained.

In addition to being unable to measure skin surface temperature, the location of thermal stimulation could not be closely controlled as is possible with attached thermal probes. Although all tests were conducted on the same patch of shaved skin it is possible that neurone density, fat depth and skin thickness may have been variable across that small area. In addition, small movements of the cats could have altered the location of thermal stimulation over the course of a particular test, potentially increasing the time required to activate thermal nociceptors and thus, the latency to 200
respond. Without effective automation of targeting, possibly by marking the skin to guide the visible laser, it is unlikely that this could have been accounted for and may serve to explain some of the intra-individual variation observed.

Although I was able to demonstrate an effect of body weight on latency to respond and to eliminate adult age and sex as factors, it remains to be elucidated as to exactly why this effect occurs. A measurement of body condition using a standard 9-point Purina scale was made, and although this correlated with body weight, it was not significantly associated with latency to respond. This suggests that there may be other underlying aspects associated with body weight (e.g. muscle density) which remain to be explored.

It would have been pertinent to explore the nociceptive thresholds of pre-pubertal cats (those under six months) to better understand the development of nociceptive thresholds. However, at the time of the study the two most recent cohorts of cats in the colony were one year of age and kittens < 2 weeks of age. The latter cohort was very small (<10 kittens). This meant the opportunity to assess nociceptive thresholds in developing cats, and likewise in cats undergoing gonadectomy at a range of ages was impractical. This would prove a very useful avenue of research for the future.

All of the cats used in this protocol were familiar with both the room and the holding cages in which testing occurred. However, they were not given an opportunity to familiarise themselves with the aspects of this particular protocol (i.e. extended time in the cages without access to food). Some of the experimental phases required cats be held for up to 90 minutes in the morning and this may have disrupted normal feeding and toileting processes. As a result extended periods of isolation without access to
food may have resulted in some animals experiencing more stress than others and
stress may have some effect on physiological response (Stella et al. 2013).

Finally, the relationships between thermal nociceptive thresholds and pain associated
with actual tissue damage (thermal or otherwise) are complex. It is the latter
component which is most relevant to post-surgical experience. In these experiments
once the nociceptive response was observed the thermal stimulation ceased and thus,
in most cases, no actual tissue damage was caused. Although other experiments
indicate a reflex response is a pre-cursor to a pain response (Veissier et al. 2000), the
relevance of measures of baseline thermal pain sensitivity in cats to post-surgical pain
needs further exploration. It would also have been useful to film the study allowing
behavioural changes prior to, and just after, the reflex response to be further explored.

7.6 Future enquiry

Firstly, although there appear to be a number of differences in how veterinarians treat
cats amongst countries, there are many more questions to be asked. What exactly
drives these differences? How do practitioners provide information about
gonadectomy to clients? Can veterinarians play a significant role, beyond surgical
processes, in improving cat management and owner engagement with their cats?
Would this in turn reduce the unowned or abandoned cat populations?

In order to explore these questions a further survey of practitioners is needed. Firstly
to explore veterinary attitudes towards cats and their social value and secondly to
understand the underlying motivations that relate to the use pre-pubertal
gonadectomy. The second component could also consider the attitudes of cat owners to pre-pubertal gonadectomy and how these opinions may, or may not, be affected by veterinary opinions.

Research into the understanding of veterinary attitudes towards cats should include a wider range of veterinarians and not just companion animal veterinarians, for example little is known about how wildlife and large animal practitioners view cats. The latter two groups could also be surveyed to explore if self-identified companion animal practitioners represent the wider veterinary field in terms of analgesia use for companion animals.

To further explore differences amongst countries it would be helpful to administer a single survey across countries that included more explicit questions around reasons for non-provision of analgesia, or certain classes of analgesics. Such a survey could use contacts at various veterinary associations and would allow for wider analysis of the veterinary profession’s uses of analgesia. Direct comparisons rather than inference may provide valuable information for both developed and developing veterinary associations as to obligations and expectations, both cultural and professional, around pain management of veterinary patients.

With respect to the use of the carbon dioxide laser as a thermal stimulus to measure nociception in cats, validation is simply the start of any process. Of course this technique should be considered as only one of a suite of available methods. As such, further validation should occur relative to those other techniques. For example how do the results from latency to respond experiments correlate with assessment using attached thermal devices that measure skin temperature (Dixon et al. 2002) or mechanical stimulation (Steagall et al. 2007)? Similarly how does this technique
relate to other more clinically applied behavioural scales and dynamic interactive visual analogue scales (Polson et al. 2012)? There is a substantial need to explore how cats respond to this technique post-surgery. Currently the technique has only been validated using behavioural responses to stimulation of peripheral thermal nociceptors in pain-free cats. The effects of thermal stimulation on response thresholds when applied peripherally (away from the surgical site on undamaged skin) following surgery, or applied close to the wound site would be of interest. Is there an overall hypersensitivity to thermal stimulation or only around the wound site?

Once wider validation of the technique has occurred, the next phase should begin to consider whether or not this protocol can be used to assess pain management techniques following surgery as is the case for other nociceptive devices (Robertson et al. 2003; Taylor et al. 2007; Pypendop et al. 2009). Currently the technique has only explored the effects of biological factors (e.g. weight, adult age) and of pharmaceuticals in pain/inflammation-free cats and it would be judicious to now begin to apply it to cats that have undergone gonadectomy and for whom pain is managed using a range of analgesic interventions. It would also be of interest to explore whether pain response to gonadectomy differs relative to the age of the patient. This could be applied to the range of ages identified as being used in practice in chapter two; it could also explore the effects of gonadectomy in older animals, especially those gonadectomised as mid to late age adults (6-10 years).

As for sheep (Guesgen et al. 2011), the laser technique may be useful for exploring the development of the nociceptive pathway in cats during development. These experiments used only adult cats of one year or older. Assessing kittens may allow for a better understanding of pain in cats and would also allow for the exploration of post-surgical pain in cats gonadectomised at different ages. To date studies on the impact
of pain following gonadectomy have not explored how early-age sterilisation may impact younger cats differently from older cats. This is especially pertinent as pre-pubertal gonadectomy may become more common worldwide as a method for controlling cat populations.

This technique could also be used to explore varying degrees of post-surgical pain including assessment of response latencies after different elective surgeries which are considered to inflict greater or lesser amounts of pain. In essence allowing an objective assessment of how variation in surgical injury impacts upon subsequent peripheral hypo- or hypersensitivity. It could also be used to explore how cats with chronically painful disorders, for example degenerative joint disease (Lascelles and Robertson 2010), respond to thermal nociceptive stimulation to understand how chronic inflammation can influence more global pain sensitivity. This could possibly include assessment as to how thresholds develop and change as chronic diseases progress. There is currently a substantial need for research which addresses the assessment and management of pain in geriatric animals (Lascelles and Robertson 2010).

As noted above, one current limitation of the thermal laser technique is the inability to directly measure skin temperature during stimulation. It may be possible for the laser to be developed to allow for assessment of skin temperature by incorporating a thermographic imaging camera. With substantial investment the question also begs to be asked as to whether a small low power thermal laser could ever be developed for assessment of cats in the clinic. This is especially interesting given the general global trend for technology to become smaller and more affordable over time. If further research demonstrated that this technique could be used to assess post-surgical pain, pain management and a patient’s need for further analgesia, it may be possible to use
it to develop a more objective, remote and rapid diagnostic tool for use by carers. Such an implement would also minimise the need to interact with animals during the post-surgical recovery phase.

7.7 References

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8. Appendix 1

This survey was developed by Mark Farnworth (mfarnworth@unitec.ac.nz), Senior Lecturer in Animal Behaviour and Welfare at Unitec New Zealand (an Institute of Technology) in collaboration with Professor Kevin Stafford and Professor Natalie Waran. It should take no longer than 10-15 minutes of your time. If you have any concerns with the content you can contact us via the e-mail address above.

The recognition, evaluation and alleviation of animal pain associated with surgery, injury or disease is a fundamental objective of veterinary medicine. Understanding attitudes towards the management of pain following routine surgeries, as well as identifying issues associated with the process of such surgeries, is an important component of advancing best practice.

The results of the survey will establish current practice in regards to sterilization of cats. Such information will be useful for those working in this area as well as in understanding comparisons between practices in different countries. The results may also help educational facilities to recognize areas that should be targeted in terms of continuing education.

Please note that questions are repeated for SPAY and NEUTER surgeries as these may be different. Attempt to fully answer all questions. By completing this survey you are giving informed consent for the information submitted to be used for the purposes of research. All returns will be anonymous and unidentifiable. Responses will be kept in a password protected file and accessible only to the primary researchers and their associates.

*1. Are you male/female?
*2. In what year did you qualify as a veterinarian?

*3. From which institution did you gain your first veterinary qualification?

*4. In which country are you currently practising as a veterinarian?

*5. In which state do you currently practise?

6. Do you provide veterinary services to shelters, pounds or animal welfare centres?

7. Approximately how much of your total caseload (%) is performed for shelters, pounds or animal welfare centres?

8. Approximately what percentage of your total workload comprises gonadectomy of cats?

9. At what age (months) do you consider a typical cat to be "adult" for the purposes of anaesthesia and analgesia?

*10. Do you provide pre/intra-operative analgesia for ADULT cats undergoing CASTRATION?

11. In approximately which year did you begin to provide pre/intra-operative analgesia?

*12. Which analgesic; what dosage (mg/kg)?

*13. Do you provide pre/intra-operative analgesia for ADULT cats being SPAYED?

14. In approximately which year did you begin to provide pre/intra-operative analgesia?

*15. Which analgesic; what dosage (mg/kg)?
16. Do you provide post-operative analgesia to ADULT cats after SPAYING?

17. In approximately which year did you begin to provide post-operative analgesia?

18. Which analgesic; what dosage (mg/kg)?

19. Do you provide post-operative analgesia to ADULT cats after CASTRATION?

20. In approximately which year did you begin to provide post-operative analgesia?

21. Which analgesic; what dosage (mg/kg)?

22. Do you provide analgesia for owner administration following patient discharge for a SPAY?

23. In approximately which year did you begin to provide analgesia for discharged patients?

24. Which analgesic; what dosage (mg/kg)?

25. Do you provide analgesia for owner administration following patient discharge for a SPAY?

26. In approximately which year did you begin to provide analgesia for discharged patients?

27. Which analgesic; what dosage (mg/kg)?

28. When SPAYING a healthy cat what is the MINIMUM age (months) at which you will perform surgery?

29. Why would you not perform a spay below your stated minimum age?
30. When CASTRATING a healthy cat what is the MINIMUM age (months) at which you will perform surgery?

31. Why would you not perform a spay below your stated minimum age?

32. At what age (months) do you consider puberty to typically occur in cats?

33. Do you consider pre-pubertal gonadectomy to be desirable?

34. Please provide an explanation of your response to question 33.
9. **Appendix 2: DRC 16s Statements of contribution to doctoral thesis containing publications for chapters 2-6 inclusive.**