Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
The Use of Thermal Nociceptive Threshold Testing to Assess the Effect of Analgesic Drugs on the Pain Response of Dairy Cattle

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

Master of Veterinary Science

in

Veterinary Clinical Sciences

at Massey University, Palmerston North,
New Zealand.

Lorelle Anne Barrett

2012
Painful procedures are routinely performed on cattle and the use of analgesia can abate this pain. Thermal nociceptive threshold (TNT) testing is used to assess pain sensitivity and the effect that painful conditions and analgesia have on this. However, little work has used TNT testing in cattle for these purposes. This research was carried out to determine if TNT testing could be used to assess the effects of analgesic drugs in both pain-free cattle and those that had undergone liver biopsy.

A carbon dioxide laser was used as the noxious thermal stimulus. In the first experiment, the effects of an alpha2-adrenoreceptor agonist (medetomidine) and a non-steroidal anti-inflammatory drug (ketoprofen) were compared with the effect of saline on TNTs of pain-free cattle. TNTs were measured 20 minutes before treatments were administered, then again at 20, 40 and 60 minutes after treatment. Medetomidine significantly increased the cows’ TNT at 60 minutes post-treatment. This increased TNT may be due to the central analgesic properties of the drug. Ketoprofen had no effect on TNTs.

In the second experiment, TNTs were measured to determine if different analgesic protocols moderated central sensitisation that may have occurred after liver biopsy. Behavioural observations were also used to assess pain in the post-biopsy period. Cows were assigned into one of four groups: control (local anaesthetic (LA) + sham-biopsy); LA + biopsy; LA + ketoprofen + biopsy; LA + meloxicam + biopsy. TNTs were measured 1 day before liver biopsy was performed, and once daily on the 3 days post-biopsy. Behavioural observations were made in the 4 hours after biopsy and on the 3 days post-biopsy. TNTs of biopsied cows did not differ from sham-biopsy cows. This may be because liver biopsy did not induce central sensitisation, or because the TNT method used did not reflect localised hyperalgesia. Behaviour also did not differ between treatment groups. These findings suggest that liver biopsy as it was performed here does not induce significant pain in cattle.

It is concluded that TNT testing may be useful to investigate the effects of some analgesics on the acute pain response of pain-free cattle, but it has not been useful in demonstrating central sensitisation after liver biopsy. Further development and refinement of the methodology is required in order for this technique to be of future use for similar research in cattle.
Thanks and appreciation firstly to my supervisors, Professor Kevin Stafford, Dr Ngaio Beausoleil and Dr Jackie Benschop. Your encouragement, constructive criticism and general affability has allowed me to pursue this ambition with confidence, and has given me a solid approach to research and scientific writing that I hope to take forward.

A huge thank-you to Robin Whitson, Liz Gillespie and Odine Johnstone of VLATU. Your help and cooperation made this research possible and is greatly appreciated.

Thanks to Dr Stefan Smith for your professional time involved with the liver biopsies.

To my lovely assistants, Nicole Andrews, Rachel Munn and James Bowden, many thanks for helping to make this research run smoothly (well, most of the time!) and for the company in the cow shed/paddock.

Thanks are also due to Dr Geoff Jones for providing helpful advice on the data analysis.

Thanks to Dr Mark Waterland and Peter Lewis (electronics workshop) for their assistance with the lasers. In particular, thanks to Peter for accommodating numerous short-notice visits for fine-tuning!

Finally, lots of love and thanks to Mum and Dad. My regression from full-time professional back to full-time student would not have been possible without your willing support, thank-you for helping me achieve this goal.
## Contents

Abstract ........................................................................................................................................... i  
Acknowledgements ........................................................................................................................ ii  
Contents ........................................................................................................................................ iii  
Abbreviations .................................................................................................................................... vii  
List of Tables ...................................................................................................................................... viii  
List of Figures .................................................................................................................................... ix  
1 Introduction ................................................................................................................................... 1  
2 Literature Review ........................................................................................................................... 4  
  2.1 Introduction ............................................................................................................................. 4  
  2.2 Pain ......................................................................................................................................... 6  
    2.2.1 Neurophysiology of Pain .................................................................................................. 6  
      2.2.1.1 Nociception .............................................................................................................. 6  
      2.2.1.2 Pain Perception ........................................................................................................ 9  
      2.2.1.3 Descending Modulation of Pain ............................................................................ 9  
    2.2.2 Pathophysiological Pain States ....................................................................................... 10  
      2.2.2.1 Peripheral Sensitisation ....................................................................................... 10  
      2.2.2.2 Central Sensitisation ............................................................................................ 10  
      2.2.2.3 Hyperalgesia – The Effect of Sensitisation ............................................................ 11  
    2.2.3 The Dorsal Horn & Neuronal Plasticity ........................................................................ 13  
  2.3 Assessing Pain in Cattle ........................................................................................................... 13  
    2.3.1 Nociceptive Threshold Testing (Analgesiometry or Quantitative Sensory Testing) 14  
      2.3.1.1 Thermal NTT ........................................................................................................... 15  
      2.3.1.1.1 Radiant Heat Source .................................................................................... 15  
      2.3.1.1.2 Contact Thermodes .......................................................................................... 15  
      2.3.1.1.3 CO₂ Laser ........................................................................................................ 16  
      2.3.1.2 Mechanical NTT ..................................................................................................... 17  
      2.3.1.3 Electrical NTT ....................................................................................................... 18  
      2.3.1.4 Research Opportunities Using NTT ................................................................ 19  
    2.3.2 Quantifying Pain-Related Behaviour ............................................................................. 19
2.3.2.1 Behaviour as an Indicator of Pain in Cattle .......................................................... 20

2.3.2.2 Research Opportunities Using Quantified Behavioural Observation ................. 22

2.3.3 Physiological Parameters ...................................................................................... 22

2.4 The Effect of Analgesia on the Acute Pain Response in Cattle: .................................. 24

2.4.1 Local Anaesthetic .................................................................................................. 24

2.4.1.1 The Effect of LA on the Acute Pain Response of Cattle ..................................... 25

2.4.2 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) ............................................. 26

2.4.2.1 The Effect of NSAIDs on the Acute Pain Response of Cattle ............................ 26

2.4.3 Local Anaesthetic plus NSAIDs: .......................................................................... 27

2.4.4 Alpha2-Adrenoceptor Agonists ........................................................................... 28

2.4.5 Opioids ............................................................................................................... 29

2.5 Conclusions ............................................................................................................. 30

3 The Effect of Ketoprofen and Medetomidine on the Thermal Nociceptive Threshold of Dairy Cattle: A Pilot Study ................................................................. 31

3.1 Introduction ............................................................................................................. 32

3.2 Materials and Methods ......................................................................................... 34

3.2.1 Animals .............................................................................................................. 34

3.2.2 Experimental Design .......................................................................................... 34

3.2.3 Experimental Procedure ..................................................................................... 36

3.2.4 Laser Equipment and TNT Testing ................................................................... 37

3.2.5 Statistical Analysis .............................................................................................. 39

3.2.5.1 Exploratory Data Analysis ......................................................................... 39

3.2.5.2 Construction of Model ............................................................................... 40

3.3 Results .................................................................................................................... 40

3.3.1 Exploratory Data Analysis .................................................................................. 40

3.3.2 Construction of Model ....................................................................................... 42

3.3.3 Model Output .................................................................................................... 42

3.3.4 Behavioural Responses ...................................................................................... 44

3.3.5 Sedation Scores ................................................................................................. 44

3.3.6 Thermal Burn Injury .......................................................................................... 45

3.4 Discussion .............................................................................................................. 45

3.4.1 Ketoprofen & Medetomidine Do Not Act Centrally in Cattle .............................. 46
3.4.2 Are TNTs Suitable for Assessing Central Analgesia? ............................................. 47
3.4.3 Current Methodology May Have Affected the Experimental Outcome ............. 48
  3.4.3.1 Location of Stimulus Application ..................................................................... 48
  3.4.3.2 Stimulus Intensity May Affect Results ............................................................. 49
  3.4.3.3 Onset of Analgesia and Post-Treatment Assessment Period ......................... 49
    3.4.3.3.1 Medetomidine ............................................................................................. 49
    3.4.3.3.2 Ketoprofen .................................................................................................. 50
  3.4.3.4 Effect of Ambient Temperature ...................................................................... 51
  3.4.3.5 Effect of Study Day .......................................................................................... 51
3.5 Conclusions .................................................................................................................. 52

4 Assessment of NSAID Analgesia on the Pain Response of Dairy Cattle after Liver Biopsy 53
  4.1 Introduction ............................................................................................................... 54
  4.2 Materials and Methods ............................................................................................. 55
    4.2.1 Animals .................................................................................................................... 56
    4.2.2 Experimental Design and Procedure ................................................................. 56
    4.2.3 Treatment Groups ............................................................................................... 57
    4.2.4 Liver and Sham Biopsy ........................................................................................... 58
    4.2.5 Laser and Nociceptive Threshold Testing ............................................................ 58
    4.2.6 Behavioural Observations ................................................................................... 59
    4.2.7 Statistical Analysis ............................................................................................... 61
      4.2.7.1 TNT Testing ..................................................................................................... 61
        4.2.7.1.1 Exploratory Data Analysis ...................................................................... 61
        4.2.7.1.2 Construction of Model ............................................................................ 61
      4.2.7.2 Behavioural Observations .............................................................................. 62
        4.2.7.2.1 Exploratory Data Analysis ...................................................................... 62
        4.2.7.2.2 Analysis of Variance (ANOVA) ............................................................... 62
  4.3 Results ........................................................................................................................ 63
    4.3.1 TNTs ....................................................................................................................... 63
      4.3.1.1 Exploratory Data Analysis .......................................................................... 63
      4.3.1.2 Construction of Model ............................................................................... 63
      4.3.1.3 Model Output ............................................................................................... 63
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>Action potential</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>DH</td>
<td>Dorsal horn</td>
</tr>
<tr>
<td>DHN</td>
<td>Dorsal horn neurons</td>
</tr>
<tr>
<td>DoA</td>
<td>Duration of action</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscular; intramuscularly</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal; intraperitoneally</td>
</tr>
<tr>
<td>IT</td>
<td>Intrathecal; intrathecally</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous; intravenously</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LA</td>
<td>Local anaesthetic</td>
</tr>
<tr>
<td>MNT</td>
<td>Mechanical nociceptive threshold</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>NT</td>
<td>Nociceptive threshold</td>
</tr>
<tr>
<td>NTT</td>
<td>Nociceptive threshold test/testing</td>
</tr>
<tr>
<td>PAF</td>
<td>Primary afferent fibre</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>PO</td>
<td>Per os (oral administration)</td>
</tr>
<tr>
<td>SIH</td>
<td>Stress-induced hypoalgesia</td>
</tr>
<tr>
<td>SRT</td>
<td>Spinoreticular tract</td>
</tr>
<tr>
<td>STT</td>
<td>Spinothalamic tract</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time to maximum plasma concentration (of drugs)</td>
</tr>
<tr>
<td>TNT</td>
<td>Thermal nociceptive threshold</td>
</tr>
</tbody>
</table>
List of Tables

**Table 2.1:** Properties of C- & A-fibres (derived from Millan, 1999 and Raja et al., 1999) ............ 8

**Table 3.1:** Order of treatments for 6 cows within each of 3 mobs (total n=18). All 3 mobs followed the same order as that outlined here. KET = ketoprofen, MED = medetomidine, SAL = saline ................................................................................................................................. 36

**Table 3.2:** Subjective Assessment of Level of Sedation Post-Treatment ....................................... 37

**Table 3.3:** Ethogram of end-point response behaviours performed by cows exposed to a laser thermal stimulus (derived from Herskin et al., 2003) ........................................................................................................... 39

**Table 3.4:** Point estimates of regression coefficients with standard errors (SE), log transformed estimates and their 95% confidence intervals from mixed effects model of thermal nociceptive thresholds of 18 cows. Values in bold-type are significantly different at p < 0.05. ........................................................................................................ 43

**Table 4.1** Schedule for assessing pain-response to liver biopsy in dairy cows. ...................... 57

**Table 4.2:** Ethogram of cow behaviour measured in the paddock after actual or sham liver biopsy (derived from Beausoleil and Stafford, 2012). .................................................................................................................. 60

**Table 4.3:** Point estimates of regression coefficients with standard errors, log transformed estimates and their 95% confidence intervals from an accelerated failure time model of thermal nociceptive thresholds of 24 cows. Values in bold-type are significantly different at p < 0.05. LA = local anaesthetic; LAK = LA + ketoprofen; LAM = LA + meloxicam ......................................................... 65

**Table 4.4:** Results of statistical analysis of transformed cow behaviour data for the total 4 hour observation period and hourly aggregates on day 1. Values in bold-type are significantly different at p < 0.05 .......................................................................................................................................... 68

**Table 4.5:** Results of statistical analysis of transformed cow behaviours data for the daily 2 hour observation periods on days 2-4. Values in bold-type are significantly different at p < 0.05..... 68
List of Figures

Figure 3.1: Plot of the raw mean TNT (expressed as latency to respond) as a function of time after treatment. Vertical lines represent standard error of the mean. n=18 for all data points except medetomidine at 20, 40 & 60 minutes, where n=14. ....................................................... 41

Figure 3.2: Plot of the raw mean TNT (expressed as latency to respond) as a function of time and study day. Vertical lines represent standard error of the mean. n=18 for all data points except medetomidine at 20, 40 & 60 minutes, where n=14. ....................................................... 41

Figure 3.3: Total proportion (+/- 95% confidence interval) of behavioural response types shown by cows to TNT testing across all three study days. Lift = lifts leg, Kick = kicks leg, Step = step, Tail = tail flick, WtShift = weight shift, NR = no response. .......................................................... 44

Figure 4.1: Histogram of the mean TNT (expressed as latency to respond) of treatment groups across study days. Vertical lines are the standard error of the mean. C = control group, LA = LA + biopsy, LAK = LA + ketoprofen + biopsy, LAM = LA + meloxicam + biopsy............................ 64

Figure 4.2: Boxplots of raw TNTs (expressed as latency to respond) as a function of study day, stratified by treatment. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam................................................................. 64

Figure 4.3: Total proportion (+/- 95% confidence interval) of types of behavioural response shown by cows to laser stimulus across all days. WS = weight shift, NR = no response to laser. .................................................................................................................................................... 66

Figure A.1: Box-plots of TNTs (expressed as latency to respond) as a function of time from treatment, stratified by treatment. Dom = medetomidine, Ket = ketoprofen, Sal = saline. ........ 91

Figure A.2: Box-plots of TNTs (expressed as latency to respond) as a function of time from treatment, stratified by study day (A, B, C). ................................................................................ 91

Figure B.1: Box plots of number of tail-flicks performed by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. .............................................................................................................................. 92

Figure B.2: Box plot of number of looks at biopsy site performed by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. .............................................................................................................................. 93

Figure B.3: Box plot of numbers of transitions performed by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. .............................................................................................................................. 94

Figure B.4: Box plot of proportion of time spent feeding by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly aggregates from day 1. B is the daily totals
for days 2-4 C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. ................................................................. 95

**Figure B.5:** Box plot of proportion of time spent ruminating by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. ................................................................. 96

**Figure B.6:** Box plot of proportion of time without feeding or rumination occurring in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. ................................................................. 97

**Figure B.7:** Box plot of proportion of time spent standing by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. ................................................................. 98

**Figure B.8:** Box plot of proportion of time spent lying by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. ................................................................. 99

**Figure B.9:** Box plot of proportion of time spent walking by cattle in the post-biopsy observation period, stratified by treatment group. A is the hourly total from day 1. B is the daily total for days 2-4. C= control, LA= local anaesthetic (LA), LAK= LA + ketoprofen, LAM = LA + meloxicam. ................................................................. 100