Electroencephalographic and cardiovascular responses to castration in *Bos indicus* bull calves and the mitigating effects of lidocaine or meloxicam administration

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This thesis is presented for the degree of
Master of Research (with Training) of Murdoch University 2017

ACKNOWLEDGEMENTS

Where to begin? I have so many acknowledgements that I'm sure to miss someone, but some specific thank yous are definitely in order. For unfailing patience and support, my fabulous supervisors Gabby Musk, Ellie Drynan and Mike Laurence. To the collaborators of this project Drs Musk, Laurence, Theresa Collins, Karina Gleerup, Craig Johnson, Tim Hyndman and Jonno Tuke for their guidance, support and input throughout the project and putting up with my neuroses regarding 'the boys' and, dare I say, Taffy. The production animal department staff, including Johnno and Stace for the fabulous help and camaraderie through the project and beyond. Meat and Livestock Australia deserve unqualified recognition as this project is simply beyond the typical realm for a residency research student, however the funding they provided made this possible.

Some appreciation goes to the KrispyKreme of Myaree – we really did add to your bottom line during the project, and I'm still uncomfortably dealing with the calorific consequences.

To 'the boys', the cattle involved in the project, heartfelt gratitude for unknowingly being involved and making the time both fun and fascinating. Finally to my family and Griffin, for just always being there. I love you all.

DECLARATION

I declare that this thesis is my own account of my research and contains work which has not previously been submitted for a degree at any tertiary education institution.

Heidi Lehmann

ABSTRACT

Australian *Bos indicus* cattle are legally able to be castrated without anaesthesia or analgesia up to the age of 12 months. Castration surgery is known to cause pain to cattle, though a reliable and consistent assessment of pain must be first optimised before analgesic therapies can be tested for this procedure. Studies in conscious cattle have demonstrated the difficulty in isolating pain responses from the stress of handling and human contact.

This study aimed to investigate electroencephalographic and cardiovascular responses indicative of nociception in *Bos indicus* bull calves undergoing surgical castration whilst under general anaesthesia. Further, the mitigating effects of administration of local anaesthetic or systemic meloxicam on these electroencephalographic and cardiovascular responses were investigated.

A total of 36 six-to-eight month old *Bos indicus* bull calves were included in this prospective, randomised, experimental study. Animals were randomly allocated to three groups of twelve (groups L – 260 mg of 2% lidocaine subcutaneously and intratesticularly five minutes prior to castration, M - 0.5 mg kg^{-1} of meloxicam subcutaneously 30 minutes prior to castration and C – no preoperative analgesia administered). Anaesthesia was induced and maintained with halothane (0.9-1.1%) in oxygen. Electroencephalogram, heart rate (HR) and mean blood pressure (MAP) were recorded for 300 seconds prior to (baseline, B) and from the start of surgery (first testicle incision, T1). HR and MAP were compared at ten-second intervals for 90 seconds from the start of T1. Median frequency (F_{50}), spectral edge frequency (F_{95}) and total power of the electroencephalograph (P_{tot}) were analysed using area-under-the-curve comparing T1 to B.

All electroencephalographic variables were significantly different between B and T1. No differences in F_{50} were found between groups during T1. F_{95} and P_{tot} were significantly different between group L and groups C and M during T1. There were transient significant changes in HR and MAP in groups L and M compared to group C during the 20-50 second periods.

This study is the first description of electroencephalographic and cardiovascular responses to castration in *Bos indicus* cattle, and the effect of two different analgesic strategies in reducing these responses. Administration of lidocaine prior to castration significantly attenuated the acute post-operative nociceptive response. In addition, the preoperative administration of meloxicam attenuated the cardiovascular, but not the electroencephalographic, responses to castration in the peracute period. These findings provide support for the preoperative administration of lidocaine and give impetus for further research into the peracute anti-nociceptive effects of meloxicam for castration in *Bos indicus* bull calves.

TABLE OF CONTENTS

Acknowledgements i
Declaration i
Abstract ii
Table of Contents iii
Table Index vi
Figure Index vi
Abbreviation List vii

1 LITERATURE REVIEW 1

1.1 Australian Cattle Industry 1
1.1.1 Current Australian Pastoral Cattle Practices 1
1.1.2 Routine Husbandry Procedures 1
1.1.3 Current Western Australian Welfare Regulations 2
1.1.4 Why should these Practices be examined? 2
1.2 Pain & Nociception 3
1.2.1 Introduction 3
1.2.2 Pain & Nociception Background 3
1.2.3 General Pain Assessment 5
1.2.4 Animal Pain Assessment 5
1.2.5 Cattle Pain Assessment 6
1.2.6 Cattle Analgesia Therapy 7
1.2.7 Species-specific Pain Assessment 7
1.3 Electroencephalography 8
1.3.1 Electroencephalography Overview 8
1.3.2 Electroencephalography uses in Human and Animal Healthcare 9
1.3.3 Animal Studies Using Electroencephalography 9
1.3.4 Minimal Anaesthesia Model 16
1.3.5 Electroencephalography Output and Data Analysis 16
1.3.6 Electroencephalography Practical Components 18
1.4 Summary 19
1.5 Project Study Aims 19

2 MATERIALS AND METHODS 21

2.1 Animals 21
2.1.1 Animal Ethics Approval 21
2.1.2 Signalment 21
2.1.3 Farm Induction Protocol 21
2.2 Herd Management 22

2.2.1 Feeding *22*
2.2.2 Environment *22*
2.2.3 Enrichment *23*
2.2.4 Sentinel animals *24*
2.3 Castration and Electroencephalography **24**
2.3.1 Management on procedure day *24*
2.3.2 Study Groups *25*
2.3.3 Anaesthetic Management *25*
2.3.4 Instrumentation *26*
2.3.5 Monitoring *28*
2.3.6 Electroencephalography data acquisition *28*
2.3.7 Cardiovascular data acquisition *29*
2.3.8 Castration *29*
2.3.9 Anaesthetic recovery *30*
2.4 Weights and blood sample collection **32**
2.4.1 Weighing *32*
2.4.2 Blood collection *32*
2.5 Data acquisition and statistical analysis **32**
2.5.1 Electroencephalographic data sourcing *32*
2.5.2 Cardiovascular data sourcing *32*
2.5.3 Statistical analysis *33*
2.6 Funding **34**
2.6.1 Project Funding *34*
3 RESULTS **35**
3.1 Animals and Anaesthesia **35**
3.1.1 Animals *35*
3.1.2 Anaesthesia and Recovery *35*
3.1.3 Monitoring *36*
3.2 Data **36**
3.2.1 Electroencephalography Data *36*
3.2.2 Cardiovascular data *41*
4 DISCUSSION **43**
4.1 Aims and Study Overview **43**
4.1.1 General Aims *43*
4.1.2 Study Motivation *44*
4.2 Electroencephalographic Findings **44**
4.2.1 Electroencephalographic response in control treatment group *44*
4.2.2 Lidocaine and electroencephalographic response modification *45*
4.2.3 Meloxicam and EEG Response Modification *46*

4.2.4 Minimal Anaesthesia Model and EEG recording 46
4.3 Cardiovascular Responses 48
4.3.1 Introduction 48
4.3.2 Cardiovascular responses with lidocaine 49
4.3.3 Cardiovascular responses with meloxicam 49
4.4 Study Limitations 50
4.4.1 General limitation overview 50
4.4.2 Sample size 50
4.4.3 Data collection and analyses 50
4.4.4 Control group ethics 51
4.4.5 Noxious stimuli assessment 51
4.4.6 Pharmacokinetic assessment 51
4.5 Conclusions and Practical Implications 53
4.5.1 EEG findings 53
4.5.2 Cardiovascular findings 53
4.5.3 Practical Implications and Future Development 53
5 APPENDIX 54
6 REFERENCES 56

TABLE INDEX

Table 1-1 *Advantages and disadvantages of EEG and behavioural analysis used in pain studies of animals. Adapted from (Johnson 2007).* 9

Table 1-2 *EEG studies published utilising equid species (horses, ponies, mules, donkeys).* 11

Table 1-3 *EEG studies published utilising companion animal species (dogs, cats).* 12

Table 1-4 *EEG studies published utilising laboratory animal species (rabbits, rats).* 13

Table 1-5 *EEG studies published utilising ruminant species (sheep, cattle, goats, deer).* 14

Table 1-6 *EEG studies published utilising other species (pigs, wallabies).* 15

Table 3-1 *Animal weight and times (intubation, general anaesthesia and time to remove first testicle)* 35

Table A 1 *The predicted median frequency (F_{50}) area-under-the-curve (AUC) from the mixed effects model for the three groups.* 54

Table A 2 *The model interactions (a) and predicted spectral edge frequency (F_{95}) area-under-the-curve (AUC) (b) from the mixed effects model for the three groups.* 54

Table A 3 *The model interactions (a) and predicted total power (Ptot) area-under-the-curve (AUC) (b) from the mixed effects model for the three groups.* 55

FIGURE INDEX

Figure 1-1 *Nociceptive pathways in animals including transduction, transmission, modulation, projection and perception. From Anderson and Muir (2005).* 4

Figure 1-2 *The schematic representation of the three frequencies (F_{50}, F_{95} and P_{tot}) used in EEG spectrum analysis. Adapted from Murrell and Johnson.* 17

Figure 2-1 *Calf number 23 seen following castration illustrating the pedometer above the right metatarsal-phalangeal joint and the tag number marked on the right rump for afar identification purposes.* 22

Figure 2-2 *Satellite image of paddock with the external fence highlighted (dashed line). The 'x' marks the location of the self-feeder. Image extracted from Google Maps (google.com/maps).* 23

Figure 2-3 *Example placement of the base-apex ECG needles.* 27

Figure 2-4 *Example placement of the EEG dermal electrodes, as viewed from a right-sided oblique dorsoventral aspect.* 27

Figure 2-5 *Flow diagram of study for EEG assessment.* 31

Figure 3-1 *Median frequency (F_{50}) of halothane-anaesthetised six-to-eight month old Bos indicus bull calves in three treatment groups.* 38

Figure 3-2 *Spectral edge frequency (F_{95}) of halothane-anaesthetised six-to-eight month old* Bos *indicus bull calves in three treatment groups* 39

Figure 3-3 *Total power (P_{tot}) of halothane-anaesthetised six-to-eight month old* Bos *indicus bull calves in three treatment groups.* 40

Figure 3-4 *Percentage change in heart rate (HR) from the baseline in each of the ten second epochs (T_{10} to T_{90}) following castration.* 41

Figure 3-5 *Percentage change of mean arterial blood pressure (MAP) from the baseline in each of the ten second epochs (T_{10} to T_{90}) following castration.* *42*

ABBREVIATION LIST

AUC	area-under-the-curve
BIS	Bispectral
BS	burst suppression
BVDV	bovine viral diarrhoeal virus
CMPS-SF	composite multivariate pain score – short form
CO_2	carbon dioxide
CVS	cardiovascular
DAP	diastolic arterial pressure
ECG	electrocardiograph/y
EEG	electroencephalograph/y
F_{50}	median EEG frequency
F_{95}	spectral edge EEG frequency
Fe'CO_2	end-tidal carbon dioxide
Fe'Halo	expired halothane concentration
FFT	fast fourier transformation
Fi'Halo	inspired halothane concentration
FiO_2	inspired oxygen concentration
f_R	respiratory rate
Group C	control group
Group L	lidocaine-treated group
Group M	meloxicam-treated group
h	hours
ha	hectare
HR	heart rate
IM	intramuscular
IV	intravenous
L	litres
m	meters
MAC	minimum alveolar concentration
MAP	mean arterial pressure
min	minute
MLA	Meat & Livestock Australia Limited
N_2O	nitrous oxide
$PaCO_2$	arterial partial pressure of carbon dioxide
PEEP	positive end-expiratory pressure
PIP	peak inspiratory pressure
P_{tot}	total EEG power
QBA	qualitative behavioural analysis
SAP	systolic arterial pressure
SC	subcutaneous
SEP	somatosensory evoked potentials
spO_2	peripheral oxygen haemoglobin saturation
T	temperature
VPC	ventricular premature contraction
V_T	tidal volume

1 LITERATURE REVIEW

1.1 Australian Cattle Industry

1.1.1 Current Australian Pastoral Cattle Practices

The Australian cattle industry contributes significantly to the Australian agricultural economy. In 2014 the Australian beef industry comprised nearly 30 million head of cattle across 80,000 properties. This accounted for 55% of the country's combined agricultural activity that incorporates livestock and cropping enterprises (MLA 2015). The off-farm meat value of this industry is nearly $AU13b per annum. The Northern cattle industry of Australia is based on broad-acre properties located in northern Western Australia, the Northern Territory and Queensland. The cattle type is predominantly the *Bos indicus* species, principally the Brahman breed, due to their inherent parasite and heat resistance (Frisch et al. 1984). *Bos indicus* cattle are generally considered to be a more difficult breed to handle, tend to have a much larger flight distance and be more unpredictable in their behaviour compared to *Bos Taurus* cattle such as Angus or Friesian (Fordyce et al. 1988; Zavy et al. 1992).

1.1.2 Routine Husbandry Procedures

The cattle in the northern beef industry of Australia are typically mustered once a year for routine husbandry and marking procedures, including castration, dehorning, branding, ear-tagging and prophylactic disease control. Numerous documents are published by Meat and Livestock Australia Limited (MLA) pertaining to the practice of husbandry procedures, including guidelines for best practice with reference to applicable legal obligations (Newman et al. 2007). The MLA is the producer-owned body responsible for marketing, research and development for the livestock and red meat industries of Australia. The guidelines outline the husbandry procedures in detail, including the recommendation for using a scalpel blade rather than a sharpened pocket-knife for castration, and ensuring the

procedures are not done in dusty or wet conditions to reduce the chance of infection.

1.1.3 Current Western Australian Welfare Regulations

Western Australia, as with all states of Australia, has individual animal welfare legislation that dictates the Australian Animal Welfare Standards and Guidelines (AHA 2014) and the Model Code of Practice for cattle (PISC 2004) to varying degrees. Currently the standards stipulate that animals over the age of six months cannot be surgically castrated without the appropriate use of analgesia. Though this applies to all cattle in Western Australia, an ambiguous caveat exists allowing older, previously un-mustered bulls to be castrated without analgesia, though "preferably" to be completed by a veterinarian. Consequently, most, if not all, northern cattle are castrated without the use of anaesthesia or analgesia up to the age of 12 months given the yearly muster and geographical remoteness of northern cattle properties. Putting this reality into perspective, these animals may be up to 300-400kg by that age. With no chemical restraint utilised it is only physical restraint combining human and techniques employing, for instance, ropes and crushes, that provide the requisite immobility for the procedure to be carried out. The restraint techniques have their own human and animal welfare implications given the known reactivity and potentially violent response of larger cattle undergoing painful husbandry procedures with no analgesia (Stafford 2013).

1.1.4 Why should these Practices be examined?

The pain and distress caused by castration without the use of anaesthesia or analgesia has been long known, with the capacity to impact the industry's market success. Veterinarians noted that castration caused a reduction in well-being over sixty years ago (Fenton et al. 1958), while pressure from both the modern-day consumer and non-consumer sectors to uphold livestock welfare considerations can impact market strength (Weary et al. 2004). Producers and veterinarians acknowledge that the castration process has a significant cost to an animal, but also that it is necessary for management and productivity reasons. If a means to reduce the welfare

impact of cattle castration that is simple to utilise and cost-effective, it be may be readily adopted. This approach to refinement has already occurred in the merino sheep industry of Australia where the use of the local anaesthetic agent Trisolfen ® has greatly increased the welfare of merino lambs undergoing the mulesing procedure (Lomax et al. 2013). Further assessment into the pain caused by castration and its potential alleviation is required to improve both producer and consumer confidence in the Australian beef industry. Further to this, the concept of pain and nociception will be evaluated, along with possible paths of research.

1.2 Pain & Nociception

1.2.1 Introduction

Whilst the debate may still be ongoing regarding the level to which animals 'feel' pain, it is incontrovertible that the pain pathways and consequences of their stimulation are very similar, if not identical, in all mammals. The husbandry procedures that occur on a daily basis in various livestock industries such as castration, dehorning and tail docking are widely accepted to cause pain (Weary et al. 2004). To understand the definition and concept of pain in these livestock, some of the basic concepts must be understood.

1.2.2 Pain & Nociception Background

The description of pain widely accepted in scientific literature is that given by the International Association of Pain (IASP) in 1979 as "*an unpleasant sensory and emotional experience*, *associated with actual or potential tissue damage*, *or described in terms of such damage*" (Bonica 1979). In 2001 an additional stipulation was added so that "*the inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment*". This second component of the pain definition was included initially to cover non-verbal or pre-verbal humans and infants however it can be applied to animals.

Pain is a normal defense mechanism of a living being and is essential for survival because of the behavioral changes elicited by the person or animal when pain is detected. A lack of pain has been shown to shorten life expectancy considerably (Miranda et al. 2002). The basic physiology of pain and the underlying processes of nociception are discussed before looking further into pain assessment in animals.

Nociception is the physiological process of pain detection by a pain receptor or nociceptor and the transmission of that signal to the brain (Shilo et al. 2013). The conscious perception of nociception is known as pain, though it is recognised to be a very complex experience. While we cannot currently, perhaps ever, understand the thoughts of a non-verbal animal, as indicated above this inability to articulate or express themselves does not preclude their ability to feel pain.

The nociceptive pathway incorporates the detection of the noxious stimuli at the periphery, be it mechanical, chemical or thermal, that is known as transduction, whereby the signal is changed from the physical action to the electrical signal. After transduction, the signal is transmitted to the spinal cord where modulation occurs, prior to projection, and finally perception in the cerebral cortex (Figure 1-1).

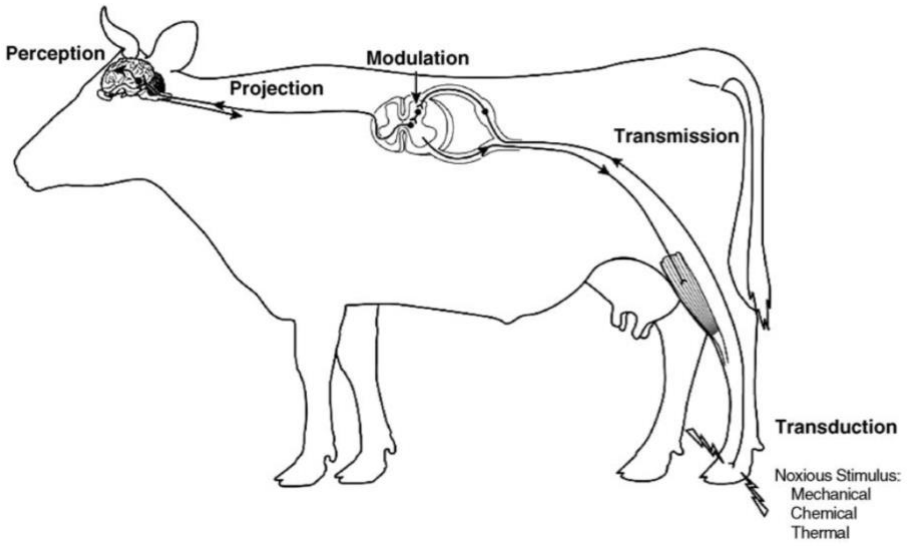

Figure 1-1 *Nociceptive pathways in animals including transduction, transmission, modulation, projection and perception. From Anderson and Muir (2005).*

1.2.3 General Pain Assessment

The knowledge that procedures like castration cause pain in animals is not sufficient for the basis of analgesia application. Demonstration of this theory in a scientific manner allows quantification of pain or nociception, and in turn, the ability of an analgesic treatment to diminish it. As may be appreciated, literate verbal humans are able to self-report the level of pain being perceived through a variety of measuring aids from simple descriptive scales to complex reporting algorithms. The ability to self-report is the gold standard for pain assessment, and in humans this method highlights that pain is an explicitly individual experience. Anecdotally this variability in responses to the same noxious stimuli is described in veterinary medicine amongst different breeds and types of animals, some being more stoic than others.

1.2.4 Animal Pain Assessment

To ensure the successful treatment of pain in animals, first the accurate recognition of pain must happen. Many pain assessment strategies for animals are adapted from techniques used for humans. As the recognition of pain is a fundamental pre-requisite to its treatment, there have been many methods of both subjective and objective assessment investigated, though none are yet to be defined as 'gold-standard' (Murrell et al. 2006). The various pain behaviours and consistent objective measures in many species are currently unknown (Price et al. 2003; Petherick et al. 2014). It is in the hospital or other confined setting where veterinarians and owners of animals are often assessing pain and deciding on treatment. Importantly, normal pain behaviour is altered by both these settings and the act of being observed in many animals (Paul-Murphy et al. 2004). Consequently, the understanding of specific behavioural signs of pain in animals is complex and not readily utilised in day-to-day practice.

Confounders to the objectivity of pain assessment in animals include the use of analgesic and anaesthetic agents (Johnson et al. 1997; Murrell et al. 2006). Many agents used in clinical anaesthetic practice have either analgesic properties or a depressant effect on the spinal transmission of nociception. A variety of pain-type assessment has been used in animals, though they can be

broadly categorised into either predominantly subjective or objective means. Subjective methods include qualitative behavioural assessment and visual pen scoring, while more objective means include heart rate variability, infrared thermography and activity monitoring. Animals in pain and distress have elevated levels of the hormone cortisol, though it is a non-specific indicator of stress (Lester et al. 1991; Choi et al. 2012). Plasma cortisol levels may be used in carefully designed experimental settings where the base levels of stress from handling and human interaction can be evaluated (Stafford et al. 2002; Bergamasco et al. 2011).

1.2.5 Cattle Pain Assessment

Recognition and assessment of pain in cattle undergoing husbandry procedures is a continuing field of research. Whilst it is known that animals in pain may be more dangerous to handle, less productive and less fertile, there is no consistent method of pain assessment available. It has been postulated that as a prey animal that does not get assistance from others in its herd, displaying pain-behaviour may only attract predator attention, a distinctly non-survival oriented activity (Stafford 2013).

Many subjective pain assessment tools including visual pen scoring, videography, vocalisation and chute exit speed have been trialled and reviewed (Coetzee 2013). Notably behavioural assessments tend to have a common flaw associated with large inherent variability due to personnel previous experience and bias affecting the result (Johnson 2007). The focus to obtain a validated objective measure for cattle pain following presumed painful procedures is ongoing. Some advances in objective pain assessment have been forthcoming in the last decade, including the development of a composite pain scale (de Oliveira et al. 2014) and a pain expression scale (Gleerup et al. 2015) which may be used in specified clinical situations. Work using other objective measures that are applicable only in the research setting include the use of infrared thermography, heart-rate variability, electroencephalography, nociceptive threshold assessment and pedometry (Coetzee 2013; Musk et al. 2016). It must be acknowledged that only a minority of these mentioned pain assessments have been completed in *Bos indicus* species (de Oliveira et al. 2014; Musk et al. 2016), with the majority of

studies in *Bos taurus*, such as Angus or Friesian, typical meat and milking breeds.

1.2.6 Cattle Analgesia Therapy

As cattle are a part of the food chain through milk or meat production strict controls exist mandating the use of analgesics in the cattle industry world-wide. In the developed world there is a marked difference between continental or country divisions regarding the products that are licensed for use in cattle, resulting in a stymied ability to treat pain in these animals. Interestingly the administration of local anaesthesia prior to castration and dehorning is legal requirement in some European countries (DEFRA 2003), recommended and available in Australia (PISC 2004) but no analgesics are licensed for the treatment of pain in livestock in the USA (Bayley 2010). In the Australian farm setting, only local anaesthetics and non-steroidal anti-inflammatory agents have been licensed for use in cattle, though in the research setting other drugs may also be used.

1.2.7 Species-specific Pain Assessment

Between cattle species there are marked differences in temperament. Older references suggest that *Bos indicus* cattle such as Brahmans have a flightier nature, and are more reactive to handling (Fordyce et al. 1988; Zavy et al. 1992). However, more recent reports indicate that with low-stress handling techniques and acclimation to the handling environment these animals can be treated like *Bos taurus* species, translating to increased productivity (Cooke 2014). A previous study using behavioural and objective measures of pain in *Bos indicus* bull calves following castration indicated differences in Qualitative Behavioural Assessment (QBA), pedometry and weight gain, however these measures did not consistently demonstrate a difference between study groups (Musk et al. 2016).

The only validated pain assessment tool available for use in cattle is a composite pain scale developed by de Oliveira et al. (2014) at the University of Estadual Paulista, Brazil. This scale, known as the UNESP-Botucatu pain scale, is particularly relevant to the current study of Brahman cattle from the

Northern Australian beef industry, having been developed using Nellore cattle, a breed of *Bos indicus* cattle commonly found in Brazil. Further pain assessment tools comprise behavioural scales including a facial pain score developed in dairy cattle (Gleerup et al. 2015) and QBA in Angus steers during handling prior to slaughter (Stockman et al. 2012), however these are in *Bos taurus* species. Further research into objective pain assessment in cattle, specifically *Bos indicus* species, is therefore warranted.

A number of objective measurements of pain in cattle have been investigated, including a number of neurophysiological techniques. One technique, electroencephalographic response to likely noxious (painful) stimulus, allows investigation of this while the animal is anaesthetised providing an ethical model (Murrell et al. 2006). Electroencephalography techniques have been investigated in *Bos taurus* cattle for a number of noxious stimuli, however no *Bos indicus* EEG studies have been completed. As an area with a paucity of data, the use of EEG techniques assessing responses to noxious stimuli in *Bos indicus* cattle merits investigation.

1.3 Electroencephalography

1.3.1 Electroencephalography Overview

Neurophysiological techniques in both man and animals have been increasingly used to try to establish the specific features of the nociceptive and pain pathways indicated earlier. As mentioned in the previous section EEG can be used as an objective measure of pain in both animals and humans. Murrell and Johnson (Murrell et al. 2006) have reviewed the use of EEG for pain assessment in animals in great detail. The basis of EEG is the recording of the electrical activity of the brain at various locations on the scalp or head, with consequent analysis allowing identification of nociception.

Electroencephalographic techniques include raw data analysis, spectral analysis and somatosensory evoked potentials (SEP). Some basic features of these techniques were identified by Johnson (2007) (Table 1-1).

Table 1-1 *Advantages and disadvantages of EEG and behavioural analysis used in pain studies of animals. Adapted from (Johnson 2007).*

COMPONENT	EEG ANALYSIS	BEHAVIOURAL ANALYSIS
Restraint required	Animals must be anaesthetised due to movement limitation required	Animals must be conscious
Sample numbers required	Small number can produce statistical differences	Larger numbers required for statistical differences
Analysis type used	Mathematical concepts used are complex	No complex mathematical concepts involved
Analysis timeframe	Rapid analysis (computer based)	Laborious data analysis involved time and personnel input
Pain type studied	Suited to acute pain stimulus	Suited to more prolonged perception of pain
Cross-species application	Consistent responses in wide variety of mammalian species	Behaviour specific to species and even type
Pain type differentiation	Differentiation of visceral and somatic pain	Behaviour specific to noxious stimuli
Pain required	Pain research without the conscious perception of pain in research animals	Research animals must suffer pain in order for the pain-related behaviours to be measured

1.3.2 Electroencephalography uses in Human and Animal Healthcare

There has been many studies in humans utilising EEG data for the assessment of anaesthetic depth, the principal clinical use in human medicine (Whyte et al. 2003). In the research setting the magnitude and nature of the EEG response to a noxious stimulus is tightly linked to the intensity of the stimulus in humans (Chen et al. 1989). In the veterinary medicine sphere there is no clinically utilised EEG technologies. Limitations of applying EEG analysis to the clinical setting in veterinary anaesthesia are principally linked to reliability, validity and logistics. Furthermore, in human anaesthesia, one of the principal factors associated with professional liability is the occurrence of awareness and recall from anaesthesia and surgical procedures, a factor at this point not, and perhaps never to be, encountered in the veterinary realm. Bispectral (BIS) analysis allows a semi-temporal readout providing a useful single value linked directly to the depth of anaesthesia and as such, awareness.

1.3.3 Animal Studies Using Electroencephalography

Over the last 20 years there has been a number of reports of using EEG analysis, principally for analgesic therapy assessment. These studies are summarised in respect to authors and year of publication, species investigated, number and nature of population assessed, anaesthesia protocol

employed, noxious stimuli employed and outcome of electroencephalographic assessment (Tables 1-2 – 1-6). Due to the unpredictable and changeable plane of anaesthesia during equine procedures, it is this species where the most trials of clinical application of EEG have occurred (Johnson et al. 1997; Grint, Johnson, Clutton, et al. 2014; Grint, Johnson, De Sa Lorena, et al. 2014). Despite these studies, no reliable clinical application of EEG during equine anaesthesia has been established. Much of the variance may be associated with the confounding components found to be commonly used in general anaesthesia in the 21st century. For example, ketamine is a nearly ubiquitous agent used during equine anaesthesia (Taylor 2015), and has been shown to have profound effects on the EEG response of horses, specifically generating a high-voltage slow waveform response (Purohit et al. 1981).

Studies carried out in animal models in both clinical and experimental settings include the assessment of spontaneous EEG changes during castration in both ponies and donkeys (Grint, Johnson, Clutton, et al. 2014), changes in EEG responses due to the effects of tramadol or morphine in dogs undergoing castration (Kongara et al. 2013) and EEG changes attributed to local anaesthesia techniques during antler removal in red deer (Johnson, Wilson, et al. 2005). In such settings there is often the need for the use of an injectable anaesthetic for induction of anaesthesia prior to maintenance of anaesthesia with an inhaled drug, due to safety concerns of both the animals and personnel involved.

Table 1-2 *EEG studies published utilising equid species (horses, ponies, mules, donkeys).*

Author	Year	Population	Nature of Stimuli	Anaesthesia	Outcome
Grint, Johnson, De Sa Lorena et al.	2014	11 horses 11 mules 4 ponies	Castration	ACP, thiopentone then halothane	A response to castration observed in 73%/82%/0% horses, 36%/36%/0% of mules, 75%/100%/25% of ponies for F_{50}, *P*tot and F_{95} respectively. Acepromazine IV vs IM administration caused reduced EEG response.
Grint, Johnson, Clutton et al.	2014	6 ponies 6 donkeys	Castration	ACP, thiopentone then halothane	EEG responses to noxious stimuli noted in both donkeys and ponies. Donkeys have a greater change in *P*tot in response to castration than ponies.
Murrell et al.	2003	9 ponies 4 horses	Castration	ACP, GGE and thiopentone then halothane	Desynchronisation of EEG during castration, increased F_{50} may be specific nociceptive response in horses.
Johnson, Bloomfield and Taylor	1999	8 ponies	Binaural broad band click	Thiopentone then ketamine CRI and halothane	Ketamine reduced F_{95}, F_{50} and midlatency of auditory evoked potentials. F_{95} might be indicative of general CNS depression, F_{50} of antinociception.
Johnson and Taylor	1998	8 ponies	None applied	Thiopentone then halothane, methoxyflurane or isoflurane	MAC multiples of 1, 1.25 and 1.5x, isoflurane depressed all EEG values recorded more than the 1x halothane MAC. All methoxyflurane MAC levels resulted in increased EEG values than the lowest than the 1x halothane MAC.
Johnson, Young and Taylor	1994	9 ponies	None applied	ACP, thiopentone then halothane	The F_{95} had the best correlation with end-tidal halothane levels.
Ekström, Short and Geier	1993	8 horses	Bilateral stifle arthroscopy	Detomidine, ketamine then halothane or isoflurane	EEG frequency shift changes observed suggest that isoflurane provided better analgesia than halothane for this group of horses.
Mayhew and Washbourne	1992	27 ponies Horses not reported	Brainstem auditory evoked potential	Etomidate sedation	Positive relationship between waveform I-V interpeak latency and inter-aural distance was confirmed in ponies and horses.
Otto and Short	1991	18 horses	None applied	Conscious Xylazine, ketamine then halothane	Conscious – electrical activity distributed mainly in δ and β frequency bands, minor activity in the θ and α frequency ranges. Anaesthesia - Increasing depth accompanied by a pronounced shift from β to θ and δ bands.
Mayhew and Washbourne	1990	Not reported	Brainstem auditory evoked potential	Etomidate sedation	EEG waveform description.

Table 1-3 *EEG studies published utilising companion animal species (dogs, cats).*

Author	Year	Population	Nature of Stimuli	Anaesthesia	Outcome
Kongara et al.	2013	16 dogs	Castration	ACP, atropine with tramadol or morphine, thiopentone then halothane	During testicle 1 ligation the tramadol group had higher F_{50} and lower *P*tot than the morphine group. No F_{95} differences. No post-operative CMPS-SF differences.
Kongara, Chambers and Johnson	2012	24 dogs	Ovariohysterectomy	ACP with atropine and morphine, tramadol or morphine and tramadol, thiopentone or halothane	No differences between F_{50} and *P*tot in any group; F_{95} lower in morphine c.f. low-dose morphine and tramadol group; lower CMPS-SF in low-dose and tramadol group c.f. tramadol or morphine alone.
Kongara, Chambers and Johnson	2010	8 dogs (crossover)	Supramaximal electrical stimuli	ACP w either tramadol, parecoxib, morphine or saline, propofol then halothane	F_{50} increased in tramadol/parecoxib and saline group c.f. morphine; abolished F_{50} response in morphine group.
Bergamusco et al.	2003	20 dogs (10 analysed)	None applied	Propofol CRI	Prevalence of slow rhythms (δ and θ) with fast rhythms (α and β) poorly represented.
Taylor and Vierk	2003	3 cats	5mm stainless steel probe	Conscious, ketamine IM only or with ketamine CRI	No changes in EEG following IM ketamine; ketamine infusion (10.0-22.2 mg/kg/h), total and low-frequency EEG power and autonomic responses to nociceptive stimulation were eliminated.

Table 1-4 *EEG studies published utilising laboratory animal species (rabbits, rats).*

Author	Year	Population	Nature of Stimuli	Anaesthesia	Outcome
Murrell et al.	2010	60 rats	Ovariohysterectomy	Induction and maintenance w halothane; 4 groups of CRIs: saline, thiopental, ketamine or fentanyl	Stable F_{50} during surgery so unsuitable noxious stimuli indicator, maybe due to length or surgery or predominantly visceral component; control group similar changes to other minimal anaesthesia model studies.
Murrell, Waters and Johnson	2008	40 rats	None applied	Halothane, isoflurane, desflurane and sevoflurane	Used 1.25, 1.5 and 1.75x MAC of each agent, burst suppression (BS) almost complete at all levels of isoflurane; no BS at any levels of halothane; BS evident in all levels of desflurane and sevoflurane w increasing burst suppression ration (BSR) with increasing levels.
Murrell et al.	2007	46 rats	Mechanical, thermal and electrical noxious stimuli on tail	Halothane	EEG changes caused by the stimuli are quantitatively different from each other; F_{50} increased with electrical stimuli, some channels in thermal stimuli with none during mechanical; predominantly noxious stimuli (mechanical and thermal) may demonstrate cortical stimulation versus non-specific electrical stimuli.
Antunes et al.	2003	12 rats	Pedal withdrawal reflex	Isoflurane or halothane	EEG and auditory evoked potentials were suppressed more by isoflurane than. halothane.
Rampil and Laster	1992	23 rats	Alligator clip on tail	Isoflurane	Increasing isoflurane MAC multiples, increased burst-suppression; burst-suppression evidence did not predict lack of somatic response.
Kaieda et al.	1989	24 rabbits	Nil	Induction w halothane or isoflurane then thiopentone w fentanyl CRI; ventilated w 70% N_2 in O2 or 70% N_2O.	Greatest change in EEG in halothane and N_2O group

Table 1-5 *EEG studies published utilising ruminant species (sheep, cattle, goats, deer).*

Author	Year	Population	Nature of Stimuli	Anaesthesia Protocol	Outcome
Lehmann et al.	2017	36 calves	Surgical castration	Halothane	Increased F_{50} and F_{95} with decreased P_{tot} in response to castration ameliorated by local anaesthesia; intermittent decreased heart rate and mean arterial pressure ameliorated by both local anaesthesia and systemic nonsteroidal antinflammatory therapy.
Bergamasco et al.	2011	12 Holstein calves	Castration	Conscious	No treatment effect was noted between groups (IV sodium salicylate vs control) for cortisol and EEG measurements.
Johnson et al.	2009	55 lambs	Castration w rubber rings	Halothane	F_{50} and F_{95} demonstrated an increasing sensitivity to the noxious stimulation of castration with increasing age.
Gibson et al.	2009	17 Friesian calves	Neck tissue and blood-vessel transection	Ketamine and propofol then halothane	The EEG responses seen following neck-tissue and blood-vessel transection were qualitatively distinct, and suggested that cutting neck tissues caused greater noxious sensory input than transection of only the major blood vessels of the neck.
Gibson et al.	2007	20 Friesian calves	Scoop dehorning	Ketamine and propofol then halothane	Increase in the F_{50} and F_{95} and a decrease in *P*tot following dehorning and no change in the group that had a local block.
Johnson, Stafford et al.	2005	41 lambs	Castration w rubber rings	Halothane	Increase in the F_{50} in the younger lambs and an increase in *P*tot in both groups, which was of greater magnitude in the older lambs; no significant changes in the F_{95}.
Johnson, Wilson et al.	2005	29 male red deer	Dehorning	Ketamine and propofol then halothane	Lidocaine ring block of the antler pedicle provides adequate analgesia for velvet antler removal. The use of antler pedicle compression bands represents a noxious stimulus in its own right.
Jongman et al.	2000	98 lambs	Castration, mulesing, formalin, docking, ear-tagging.	Conscious	Mulesing, docking and castration compared to handling, shearing and ear tagging suggest that mulesing at both the time of treatment and during 15 min after treatment results in a response that is similar to that of induced lameness.
Antognini and Carstens	1999	10 goats	Clamp on dew claw	Isoflurane	Isoflurane blunted EEG and midbrain reticular formation–thalamus activation response to noxious stimulation at 1.1 MAC and higher.
Ong et al.	1997	8 sheep	Electrical stimulus	Conscious	Following stimulus, an overall increase in the EEG power spectrum occurred in the first four seconds, then rapidly returned to normal.

Table 1-6 *EEG studies published utilising other species (pigs, wallabies).*

Author	Year	Population size	Nature of Stimuli	Anaesthesia	Outcome
Diesch et al.	2010	20 joeys	Haemostat clamp on toe	Halothane	Joeys aged < 127 days had little or no EEG activity; periods of spontaneous EEG activity were present by 142 days. EEG responses to a noxious stimulus were non-existent in joeys aged 94–127 days, showed a minimal decrease in the F_{50} in ages 142-181 days and a greater decrease 187-261 day old joeys.
Haga and Ranheim	2005	47 male piglets	Castration	Halothane	Injecting lidocaine into the funiculus spermaticus or testes is effective in reducing signs of nociception caused by castration. Lidocaine injection is less noxious than castration without local anaesthetic.

1.3.4 Minimal Anaesthesia Model

As indicated previously there are confounding effects of various anaesthetic agents on the EEG, including choices of induction, maintenance and analgesic agents. The minimal anaesthesia model was first developed and applied to horses anaesthetised with halothane by Murrell et al. (2003) where the anaesthetic depth was kept at as 'light' a level as possible to maintain unconsciousness but allow EEG changes evoked by noxious stimuli to be demonstrated (Murrell et al. 2006). This model is still used to allow researchers to ethically examine the effects of a non-analgesic control group, a continuing source of controversy in the current veterinary research realm (Slingsby 2010).

As indicated above the volatile agent halothane is used as the inhalant of choice in the minimal anaesthesia model. Investigations into the various other volatile agents available including isoflurane, sevoflurane and desflurane (Murrell et al. 2008) have elucidated the effects of these agents on the spontaneous EEG activity of the brain of rats at differing levels of minimal alveolar concentration (MAC). It was discovered that the newer agents of isoflurane, sevoflurane and desflurane cause marked burst-suppression effects on the EEG activity at low levels of inhaled agent, from 1.25 x MAC and above. Burst-suppression is the phenomenon of alternating periods of slow high amplitude waves (the burst) followed by periods of so-called flat EEG (the suppression) (Amzica 2009). Given the considerable reduction of use in halothane in the human medical setting, commercial producers are currently only producing the drug for use in equine practice and research establishments.

1.3.5 Electroencephalography Output and Data Analysis

The EEG can be recorded intra-cranially (near-field), or most commonly from electrodes placed on the surface of the scalp (far-field) (Murrell et al. 2006). In humans, custom-made scalp caps with a multitude of electrodes are manufactured. In animals a number of different arrangements and types of electrodes have been trialled, though currently the most popular,

due to the ease of use, are the sub-dermal needle-type electrodes. In humans there are often up to 32 channels of EEG activity recorded, allowing location of activity to specific anatomical regions. In animals, however, the anatomical differences and relatively smaller cranium size mean that at most animal EEG recordings are usually only from one to two channels.

In humans a multitude of EEG frequencies have been categorised, including delta (0-4Hz), theta (4-8Hz), alpha (8-12Hz) and beta (>12Hz). The level of each frequency is reported and has been linked to functional aspects including cognitive memory and performance (Klimesch 1999). Whilst some studies have tried to assign similar categories to animals, the power spectrum following Fast Fourier Transformation (FFT) is the most applicable analysis to animals currently available (Kongara et al. 2013). Three derived values of the transformed data, the median frequency or F_{50} below which 50% of the total power of the EEG is contained, the spectral edge frequency or F_{95} below which 95% of the total EEG power is located and the total power or P_{tot} which contains the whole spectrum of EEG recorded are described (Figure 1-2) (Murrell et al. 2006).

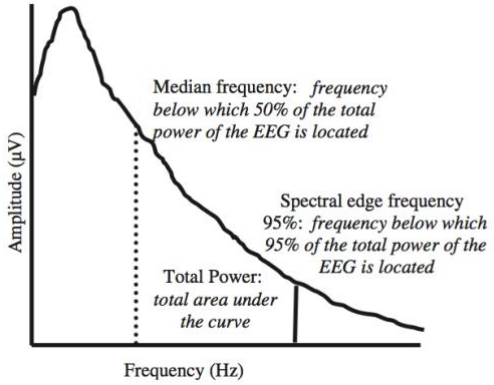

Figure 1-2 *The schematic representation of the three frequencies (F_{50}, F_{95} and P_{tot}) used in EEG spectrum analysis. Adapted from Murrell and Johnson.*

Following temporally-applied acute noxious stimuli in animals these three frequency descriptors have been reported to undergo specific changes. Generally a shift of the EEG towards higher frequency and lower amplitude occurs during the classic arousal pattern and tends to result in predictable changes in the EEG (Grint, Johnson, De Sa Lorena, et al. 2014). The median frequency and spectral edge frequency have been shown consistently to increase during nociception, and the total power of the EEG to fall (Murrell et

al. 2006). This pattern of change has been demonstrated across a range of species and noxious stimuli including equids during castration (Murrell et al. 2003), red deer during antler removal (Johnson, Wilson, et al. 2005), calves during dehorning (Gibson et al. 2007) and dogs during castration (Kongara et al. 2013). These studies also show that the use of local anaesthesia can obtund these responses to a high degree. Within the three frequency descriptors both a decrease in the total power of the EEG and an increase in the median frequency have been associated with noxious stimuli. There is growing evidence that the spectral edge frequency is more closely related to the depth of anaesthesia than the noxious stimuli *per se* (Kongara et al. 2013). Furthermore, the total power descriptor has shown preference for indicating the occurrence of somatic versus visceral pain (Johnson, Wilson, et al. 2005).

1.3.6 Electroencephalography Practical Components

There have been investigations into the impact of various anaesthetic agents on the changes in EEG activity, including the use of agents with known analgesic properties and those without. Anaesthesia induction agents such as propofol and ketamine that have a short duration of action and therefore minimal or short-lived effects on EEG activity are preferred for EEG studies (Johnson et al. 1999). In some animals the induction of anaesthesia with an inhaled drug is simple but for larger animals such as cattle and horses this approach is not commonly performed and injectable agents are often required. Whilst there is debate about the effect of the induction agent on EEG activity, most reports adequately account for this potential issue by including a control group that is anaesthetised with the same protocol as the treatment group(s) (Gibson et al. 2007). As EEG activity has inherent individual variation, comparisons within each animal between the pre-noxious stimulus baseline and the time following noxious stimulus are commonly used.

As indicated previously EEG is useful for describing the response to acute noxious stimuli only. This property is demonstrated in Gibson et al.'s dehorning study (2007) where baseline EEG activity returned within 90 seconds of the noxious stimuli application. Local anaesthetics are useful to assess the response, or lack thereof, in EEG activity during noxious stimuli application due to the complete abolition of the nociceptive signal

transduction. In Australia, the only local anaesthetic that is licensed for use in beef cattle is lidocaine (lignocaine), that has no withholding period for both milk and meat.

Given that the EEG changes with acute noxious stimuli changes, there is a theory that if an inflammatory reaction can be generated per-acutely, (ie. within minutes), then the use of anti-inflammatories and their effect on the generation of this reaction may be investigated. In rats it has been shown that the inflammatory response after the intradermal injection of formalin creates an acute inflammatory reaction that is measurable within minutes (Fischer et al. 2014). This theory provides an avenue for assessing if pre-treatment with non-steroidal anti-inflammatories, such as meloxicam which is readily available, attenuates the pain response to soft tissue injury (e.g. castration) evident in cattle.

1.4 Summary

Research into the assessment of pain and pain mechanisms in cattle needs ongoing refinement to allow use in practical settings, thus the drive for continuing it. To further elucidate this area, specifically in the important Northern Australian beef industry, further research must be completed. Given the previous complications associated with measuring the pain response in conscious *Bos indicus* cattle, a role for the use of EEG and the minimal anaesthesia model is clear. Some obstacles do exist, as highlighted above, principally associated with the perceived temperament issues of *Bos indicus* cattle and the suitability of this species for induction of anaesthesia with an inhalant drug.

1.5 Project Study Aims

The aims of the project were:

1) to characterise the electroencephalographic and cardiovascular responses to castration in *Bos indicus* bull calves in response to nociception during halothane anaesthesia.
2) Assess the specific attenuation of these responses by the use of intra-testicular lidocaine or subcutaneous meloxicam.

The hypothesis of the project was that the local anaesthesia will markedly reduce the nociceptive response to castration, however the meloxicam will not. The data produced from investigating these aims will add to the current knowledge and literature regarding cattle during castration. Specifically, it may allow further development of welfare guidelines for the Australian cattle industry.

2 MATERIALS AND METHODS

2.1 Animals

2.1.1 Animal Ethics Approval

This study was approved by the Murdoch University Animal Ethics Committee, #R2730 15 in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.1.2 Signalment

Thirty-six six to eight month-old healthy male entire Brahman (*Bos indicus*) calves sourced from a private supplier in the Kimberly region of Western Australia were used in the study. The animals had previously undergone dehorning and weaning. All animals had bilaterally descended testicles.

2.1.3 Farm Induction Protocol

The bulls were allowed to acclimatise to the University farm paddocks for one week. At this point a number of procedures were performed as part of the farm induction protocol and instrumentation for the study: collection of blood for Bovine Viral Diarrhoea Virus (BVDV) antibody testing (Department of Agriculture WA, Kensington, Australia); application of a pour-on insecticide treatment (Cydectin, Virbac, Australia); and the fitting of a pedometer (Afitag II, Afimilk, Israel) to the right distal metatarsus. Pedometry data was not part of this thesis so is not further reported. The bulls were identified with the use of a numbered (#1-48) ear tag put in the right pinnae, with the same number marked on the pedometer and sprayed (Spray & Mark, Dy-Mark, Australia) onto the left and right rump.

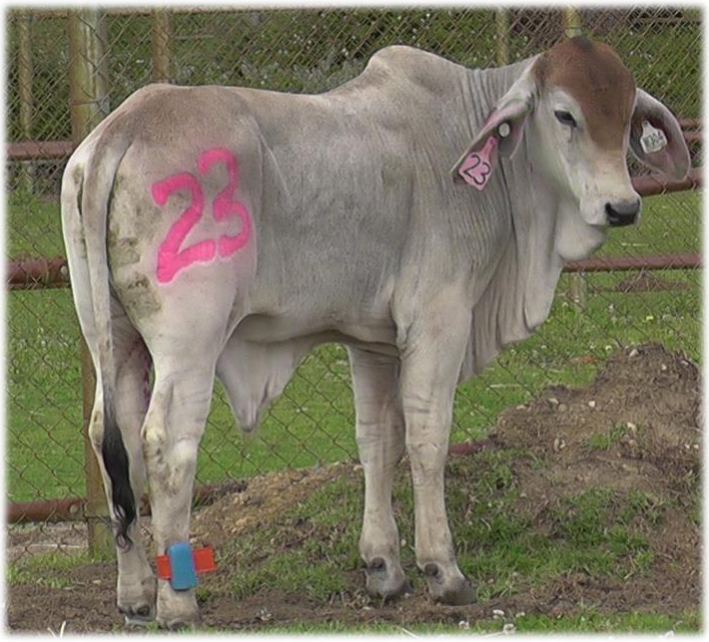

Figure 2-1 *Calf number 23 seen following castration illustrating the pedometer above the right metatarsal-phalangeal joint and the tag number marked on the right rump for afar identification purposes.*

2.2 Herd Management

2.2.1 Feeding

The bulls were held in a 1.1 ha paddock with irrigated kikuyu pasture available for free-grazing and oaten hay bales intermittently fed out. A beef cattle specific balanced mixed pelleted food was available on a daily basis (EasyBeef®, Milne Feeds, Perth, Australia) and during the study period was fed only after the morning drafting had been completed. Once all anaesthetic procedures had been completed the pelleted food was available from a bulk feeder *ad lib*.

The bulls had access to one ground-level stock trough for water, and a fountain present in the paddock.

2.2.2 Environment

The main paddock had irrigated kikuyu pasture, a large piggery building inset into the paddock and multiple large trees around the boundary and in the paddock. No other structures were in the paddock. A rectangular livestock trough with continuous flow float system was accessible from all

sides at the south-west end of the paddock. The round self-feeder was placed near the piggery building at the north-east end.

Figure 2-2 *Satellite image of paddock with the external fence highlighted (dashed line). The 'x' marks the location of the self-feeder.*
Image extracted from Google Maps (google.com/maps).

2.2.3 Enrichment

No specific environmental enrichment was provided though a number of features associated with the paddock and its surrounds gave the bulls a high level of behavioural enrichment. Various bird-life, including ducks, galahs and black cockatoos came into the paddock on a regular basis. All four sides of the paddock had ring-lock wire fences and multiple species including horses, donkeys, sheep and other cattle within close visual or nuzzling distance. The

paddock was located immediately behind the university farm's teaching buildings and yards, where various activities occurred regularly. Additionally a laneway passed the paddock that regularly had farm vehicles travelling along it.

An unintended enrichment provision developed with the self-flushing cleaning system beneath the piggery building. A slow running fountain constantly ran to fill up a counter-weighted swinging bucket that would empty on reaching a threshold level to flush the space below the piggery grating. The bulls were often playing with both the fountain and the swing-buckets, and seemed to enjoy it.

2.2.4 Sentinel animals

Prior to the anaesthetic procedures commencing, a sentinel animal, an aged good-temperament halter-trained Illawarra cow, was introduced to the main paddock. During the anaesthetic procedures this cow lived in the recovery paddock. Additionally during the anaesthetic procedures a five year-old Angus bull receiving daily medical attention for an injured leg acted as a second sentinel animal within the hospital building.

2.3 Castration and Electroencephalography

2.3.1 Management on procedure day

Each morning all the bulls were herded via a laneway into the collection yards for daily assessments to be performed. Animals scheduled for anaesthesia and surgery were weighed and drafted into a small transport vehicle to travel to the University hospital. Other procedures performed at this time included blood sampling or post-operative assessment as required. If blood sampling was necessary, the bulls were restrained in a head bale (Leicht, Australia) and blood was collected from the jugular vein. The four bulls for experimentation each day were loaded onto a trailer and moved to the hospital holding yards. In pairs the bulls were directed into the in-hospital race prior to the anaesthetic procedure.

After restraint in a custom-made squeeze chute and head bale assembly, two halters were applied; a soft cotton restraint halter and a large heavy gauge nylon long lead halter. The heavy gauge halters lead was passed through the custom-made tilt-table assembly (Murdoch University, Australia) and fixed to the gate of this crush via a lever system to help manoeuvre the bull into position.

Once the bull was standing in the tilt-table crush assembly, the soft halter was tightened to the allocated point on the tilt-table. Three heavy nylon straps were passed around the bull to restrain the animal against the tilt table with a neck, a cranial thoracic strap and a caudal abdominal strap. Once these straps were tightened the tilt-table was activated and the bull was effectively restrained in left lateral recumbency. A blindfold was placed over the eyes and forehead and the fore and hind legs were secured together by soft cotton ropes and fastened to the corresponding points on the tilt-table.

2.3.2 Study Groups

The study had three equal groups: castration only (C, n=12); castration with meloxicam (M, n=12) and castration with lidocaine (L, n=12). The allocation was block randomised to ensure that the last animal to be castrated on a given day was equally represented across the three study groups.

2.3.3 Anaesthetic Management

Once in lateral recumbency a custom-made mask (H. Lehmann, Murdoch University, Australia) was fitted over the bull's muzzle to facilitate induction of anaesthesia. A Tafonius Junior large animal anaesthetic machine and ventilator (Vetronic, UK) with a large animal circle circuit was attached to the mask assembly and delivered halothane (Halothane BP, Pharmachem, Australia) in oxygen during spontaneous ventilation. The initial anaesthetic machine settings were 100% oxygen at 8 L/min and halothane at 1 %. The low initial concentration of halothane was delivered to allow the animal time to get used to the smell. After five minutes the halothane vaporiser setting was increased to 5 % and the mask was held firmly in place until the animal

was anaesthetised. It took approximately 30 minutes to reach a depth of anaesthesia adequate for endotracheal intubation.

Direct or tracheal intubation was achieved by digital palpation of the epiglottis and arytenoid cartilages of the larynx with two personnel retracting the bull's jaw and tongue to allow access. Intubation was accomplished with either an 18 mm, 20 mm or 22 mm internal diameter silicone large animal cuffed endotracheal tube (Surgivet, Australia). Once intubated, anaesthesia was maintained with halothane in oxygen with the aim of maintaining a pre-EEG baseline end-tidal halothane ($F_E'Halo$) of 0.9-1.1%. Positive-pressure ventilation was applied to maintain the end-tidal carbon dioxide ($F_E'CO_2$) between 40-50mmHg.

2.3.4 Instrumentation

During the induction period an electrocardiogram (ECG) was recorded in a base-apex manner using dermal needles. Following intubation comprehensive physiological monitoring equipment was applied to the animal. This monitoring equipment included a pulse-oximetry probe on the tongue, an oscillometric blood-pressure cuff on the proximal tail, a 20-gauge, 1.16 inch cannula (BD Insyte, Becton Dickinson Infusion Therapy, USA) in the auricular artery for invasive blood pressure measurement and arterial blood sampling, a thermistor temperature probe in the caudal nasal passages and a three-lead far-field EEG using dermal needles (Neuroline subdermal, Ambu, Malaysia). Figure 2.3 and 2.4 illustrate the ECG and EEG needle placement.

Figure 2-3 *Example placement of the base-apex ECG needles.*

Figure 2-4 *Example placement of the EEG dermal electrodes, as viewed from a right-sided oblique dorsoventral aspect.*

The electrode and colour codes are as follows: red = non-inverting, midway between medial canthi; blue = inverting, sitting superficially over right mastoid process; green = earth, just distal to midline poll.

2.3.5 Monitoring

Physiological variables and inspired and expired gas composition was continuously measured with a multi-parameter monitor (Carescape B650 Anaesthetic Monitor, GE Healthcare, Finland) and manually recorded every five minutes during anaesthesia. During the 30-minute induction phase of anaesthesia the following parameters were measured: inspired oxygen concentration (FiO_2 %), end-tidal oxygen concentration (FEO_2 %), inspired halothane concentration (FiHalo %), Fe'Halo (%), halothane-vaporiser setting (%), Fe'CO_2 (mmHg), respiratory rate (f_R) and heart rate (HR) via the ECG.

Once the trachea was intubated additional parameters were also recorded: peak-inspiratory pressure (PIP), positive-end expiratory pressure (PEEP), tidal volume (V_T), peripheral oxygen haemoglobin saturation (spO_2), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), intranasal temperature (T). The time of commencement of induction of anaesthesia, time to intubation, number of attempts at intubation, total anaesthesia time, time to extubation, time to standing and eye position was recorded. Analgesic drug, dose, route and time of administration were also recorded. A single arterial blood sample was collected into a pre-heparinised syringe (Pico50, Radiometer, Denmark) prior to removal of the arterial catheter and analysed with temperature correction for electrolytes and blood gas status (ABL 700 series, Radiometer, Denmark).

2.3.6 Electroencephalography data acquisition

A far-field EEG was obtained using dermal needles (Neuroline subdermal, Ambu, Malaysia) with the non-inverting electrode placed midline between the medial canthi, the inverting electrode over the right mastoid process and the earth electrode 2-4cm caudal to the poll as previously described (Murrell et al. 2006).

Following a ten-minute period of stable anaesthesia with FE'Halo at 0.9-1.1% and $FE'CO_2$ 40-55mmHg a five minute baseline EEG was obtained. The first procedure (first testicle castration or lidocaine injection) was then completed. Group M received 0.5 mg/kg of meloxicam (Ilium Meloxicam 20, Troy Laboratories, Australia) subcutaneously during the induction phase of

anaesthesia 30 minutes prior to castration, and Group L received 260 mg lidocaine (Ilium Lignocaine 20, Troy Laboratories, Australia) subcutaneously (into the distal scrotal skin surrounding the incision site) and intra-testicularly (into the body of each testicle) following the baseline data acquisition. In Group L a further five-minute period following the injection of lidocaine was recorded prior to castration. Each bull had the left testicle removed first. Five minutes later the right testicle was removed. Time points were recorded as timestamps on the EEG trace as baseline start, lidocaine injection, first testicle start, first testicle finish, second testicle start, second testicle finish and completion of the EEG trace (finish). The timeline of the anaesthesia and EEG collection is illustrated in Figure 2-3.

2.3.7 Cardiovascular data acquisition

Heart rate (derived from base-apex ECG electrodes, bpm), peripheral oxygen haemoglobin saturation (SpO_2 %) and invasive arterial blood pressure via the auricular arterial line were recorded every five minutes throughout anaesthesia. The heart rate and blood pressure data were digitised at a rate of 1 Hz (Powerlab 8/35, AD Instruments, Australia) and continuously recorded (LabChart Pro, AD Instruments, Australia) on a personal computer (Satellite C850, Toshiba Corporation, Japan). Baseline data of heart rate and blood pressure were defined as an averaged 300 seconds immediately prior to the start of surgery (groups C and M) or the injection of lidocaine (group L). Data extraction and analysis were completed off-line following the study.

2.3.8 Castration

Castration was completed on all bulls by a trained, experienced clinician. An open castration technique was used: the testicle was stabilised in the distal scrotum, a skin incision was made over the most distal point of the scrotum, the *tunica vaginalis* was incised and the testicle was extracted, and firm pressure was placed on the spermatic cord until it ruptured. Any remaining fibrous connections were severed by sharp dissection.

2.3.9 Anaesthetic recovery

Following the completion of the EEG recording, all instrumentation was removed from the bull, the anaesthetic was discontinued, the bull disconnected from the anaesthetic machine, pulled onto a carrying plate of a fork lift (I8, Nissan Forklift, Japan) and transferred to a recovery paddock. The recovery paddock contained the sentinel cow and any other bulls that had already been castrated on that day. The bulls remained in left lateral recumbency during recovery, had a blind fold placed, were extubated once rejecting the tube or swallowing then restrained in the same position with pressure on the neck, head and dependent forelimb for as long as possible.

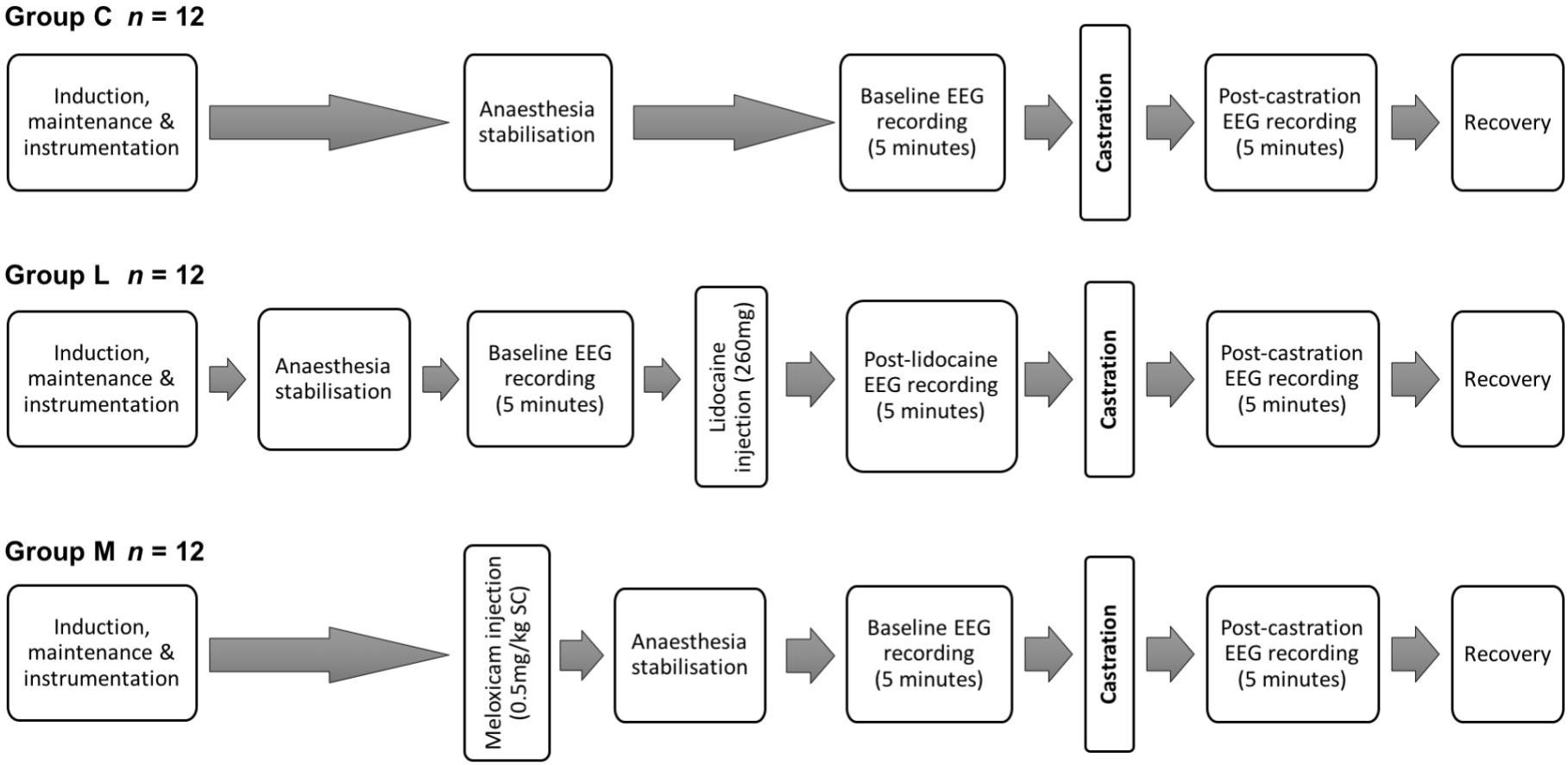

Figure 2-5 *Flow diagram of study for EEG assessment.*

2.4 Weights and blood sample collection

2.4.1 Weighing

The bulls were weighed on in-line scales in the race (Gallagher, Australia) on arrival, day -7 (7 days prior to anaesthesia), day 0 (day of anaesthesia), day 6, day 10 and on departure.

2.4.2 Blood collection

Blood was collected from the jugular vein during anaesthesia and on days 3 and 6 following anaesthesia.

2.5 Data acquisition and statistical analysis

2.5.1 Electroencephalographic data sourcing

The EEG data was directed through a custom-made break-out box (C. Johnson, Massey University, New Zealand) and the signal amplified through a bioamplifier (DAM 50 differential amplifier, World Precision Instruments, USA). The EEG was recorded with a gain of 1000x in alternating current mode, a low filter setting of 1Hz and a high filter setting of 100 Hz. The data was then digitised at a rate of 1 Hz (Powerlab 8/35, AD Instruments, Australia) and continuously recorded (LabChart Pro, AD Instruments, Australia) on a personal computer (Satellite C850, Toshiba Corporation, Japan). Data extraction and analysis was completed off-line following the completion of the procedures. Noise was noted in the EEG signal and earthing the tilt-table to the break-out box resolved the majority of this noise.

2.5.2 Cardiovascular data sourcing

The heart rate from the ECG and arterial blood pressure were recorded simultaneously to allow temporal association of events. An additional single channel bioamplifier (BioAmp ML132, AD Instruments, Australia) and bridge

amplifier (BridgeAmp ML110, AD Instruments, Australia) were utilised to retrieve the ECG and blood pressure measurements respectively, and the data then acquired by the PowerLab arrangement (see 2.7 a).

2.5.3 Statistical analysis

Continuous data was assessed for normality using Shapiro-Wilk analysis. Normally distributed data were compared between treatment groups with one-way ANOVA analysis and results displayed as mean ± 1 S.D. Non-parametric data were analysed with Mann-Whitney U analysis and displayed as median (range).

The raw EEG data was inspected for any noise artefacts such as electromyography signals. Fast fourier transformation (FFT) was completed using custom-written software (C. Johnson, Massey University, New Zealand). The median frequency (F50), spectral edge frequency (F95) and the total power (Ptot) of the EEG was then established using 1-Hz frequency bins on each timestamped period of data.

The spectral data was then smoothed, and summarised by the normalised area-under-the-curve (AUC) utilising the statistical software package R (The R Foundation for Statistical Computing, United States). This normalised AUC was then regressed against the timestamp and treatment. A mixed effect model was fitted with a random intercept term to account for the repeated measures.

The measurements of HR and arterial blood pressure taken over 300 seconds following the first incision into the scrotum (T1) were compared to the 300 seconds of baseline measurements. Within each 300 second epoch, averages were collected over each ten-second time period for the first 90 seconds following incision and were labelled T_{10} through to T_{90}. The values for each time-period are presented as a percentage change from the baseline. The mean arterial blood pressure (MAP) was assessed. Normality of all data was assessed with the Shapiro-Wilk test. Normally distributed data were compared with a one-way ANOVA. Gabriel's post-hoc analysis was performed if $p < 0.05$. SPSS software (Version 22.0.0.0, IBM, USA) was used to complete all analysis. Data are presented as mean ± SD unless otherwise stated.

2.6 Funding

2.6.1 Project Funding

The project was funded by the Australian Government and Meat and Livestock Australia (MLA) corporation (grant B.AWW.0242).

3 RESULTS

3.1 Animals and Anaesthesia

3.1.1 Animals

There were no significant differences in the weight of the animals between groups, the time from the start of delivery of halothane to intubation, total general anaesthesia time or time for removal of the first testicle (Table 3-1).

Table 3-1 *Animal weight and times (intubation, general anaesthesia and time to remove first testicle).*

Mean (±SD) of weight, time to intubation and total general anaesthesia time of the treatment groups with median (range) for the time for removal for the first testicle (start of incision to rupture of the spermatic cord).

	Treatment Group			
Variable	**C**	**L**	**M**	**P-value**
Weight (kg)	238 ± 17	233 ± 24	239 ± 16	0.689
Intubation time (minutes)	33.0 ± 6.5	36.1 ± 10.1	35.5 ± 7.5	0.624
General Anaesthesia time (minutes)	79.9 ± 8.6	82.2 ± 19.3	82.2 ± 9.9	0.956
Time to testicle removal (seconds)	34 (18-49)	40 (20-84)	33 (22-61)	0.467

3.1.2 Anaesthesia and Recovery

The Fe´CO_2 was maintained in the target range over the combined 600 seconds of baseline and T1 time periods [Group C, 6.5 ± 0.6 kPa (49 ± 5 mmHg); Group L, 6.5 ± 0.5 kPa (49 ± 4 mmHg); Group M, 6.7 ± 0.5 kPa (50 ± 4 mmHg)] with no differences between groups (p = 0.628). The $PaCO_2$ at the end of surgery was higher than the target value [Group C, 7.8 ± 1.0 kPa (59 ± 8 mmHg); Group L, 7.7 ± 0.8 kPa (58 ± 6 mmHg); Group M, 7.6 ± 0.8 kPa (57 ± 6 mmHg)] with no differences between groups (p = 0.756). All other cardiorespiratory parameters remained within the normal range throughout the study.

All bulls in each treatment group had an excellent recovery based on subjective assessment. Well-defined recovery plateaus became apparent with

the rejection of the endotracheal tube in all animals around 10 minutes after being disconnected from the anaesthetic machine. Following extubation, the earliest attempts to rise occurred 10 minutes later. Occasionally the bull remained recumbent, resting, and required visual and auditory stimulation to rise. All recoveries were calm and excitement-free.

No regurgitation was noted during anaesthesia or recovery in any of the bulls.

3.1.3 Monitoring

During the induction of anaesthesia, ECG monitoring showed two isolated ventricular premature contractions (VPC) in three animals. Three animals had visual evidence of mild rumenal bloating requiring an increase in tidal volume and therefore peak inspiratory pressure (PIP) to maintain the target $Fe´CO_2$. No adverse impacts on the cardiovascular parameters were noted following the increase in PIP. All animals recovered uneventfully from anaesthesia, and all were observed eating within 30 minutes of standing. Postoperative assessment of pain was performed via qualitative behavioural assessment and subjective veterinary assessment (data not shown). None of the animals required rescue analgesia.

3.2 Data

3.2.1 Electroencephalography Data

Data from all 36 bulls were included in the analysis. Somatic responses (swallowing, ear flicking or extremity movement) were observed and noted following incision in five animals: two animals in groups L and M, and one in group C.

The final model for F_{50} indicated that the only significant predictor of AUC was timestamp (F: 65.1668, $P - value$: < 0.0001). For F_{95} and P_{tot} the final model indicated that the main effects timestamp and treatment, as well as the two-way interaction, were all significant predictors of AUC (*Table A1 – A3* in Appendix).

In a comparison between the 300 seconds of baseline and the 300 seconds following T1, F_{50} was increased in all groups ($p < 0.0001$). No differences in the magnitude of change in F_{50} between groups ($p = 0.6491$) were observed (Fig. 3.1). F_{95} was also increased in all groups following T1, compared to baseline ($p = 0.0001$). An increase in F_{95} in groups C and M and a decrease in group L (Fig. 3.2) ($p = 0.0005$) were observed. P_{tot} after T1 was decreased in all groups compared to baseline ($p < 0.0001$). There were significant differences in the change of P_{tot} between all groups ($p = 0.0163$) (Fig. 3.3): L decreased by the least, C by the most, and M was intermediate to L and C. No difference in group L was seen for any variable following injection of lidocaine (F_{50}, p = 0.093; F_{95}, p = 0.998; P_{tot}, p = 0.225).

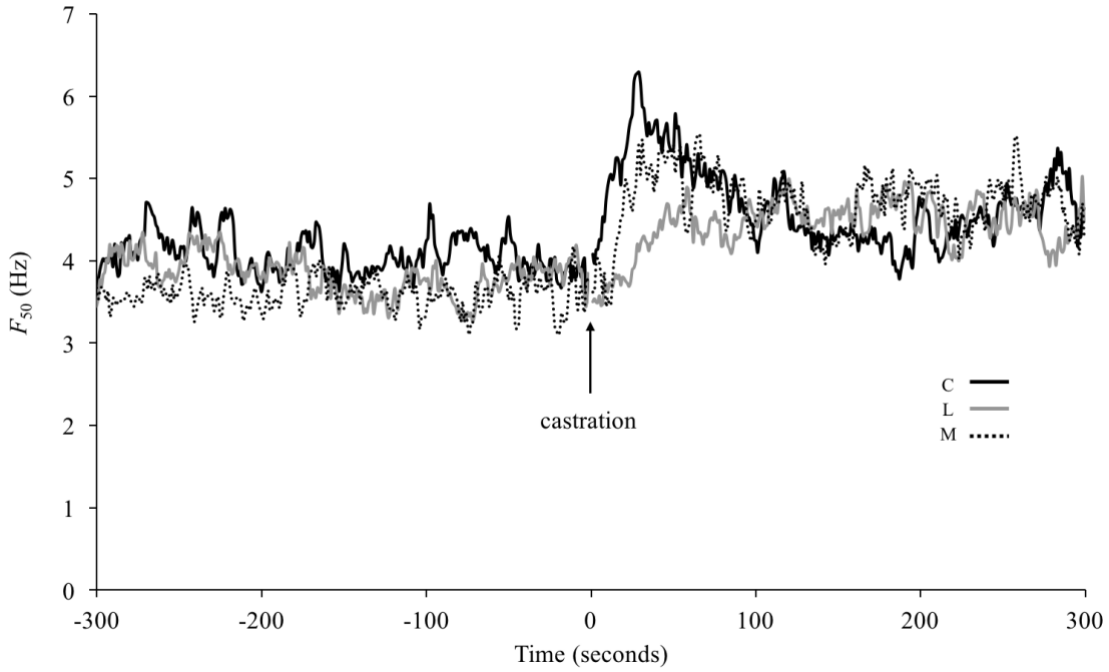

Figure 3-1 *Median frequency (F_{50}) of halothane-anaesthetised six-to-eight month old Bos indicus bull calves in three treatment groups.*

Group C - no preoperative analgesia, n = 12; group L – pre-operative lidocaine, n = 12 and group M – preoperative meloxicam, n = 12 are shown. Both baseline period and the 300 seconds following castration of the first testicle (T1) are shown on the x-axis. All treatment groups were different compared to baseline ($p < 0.0001$). There were no differences between groups during T1 ($p = 0.6491$). Castration occurred at 0 seconds. Median results are shown.

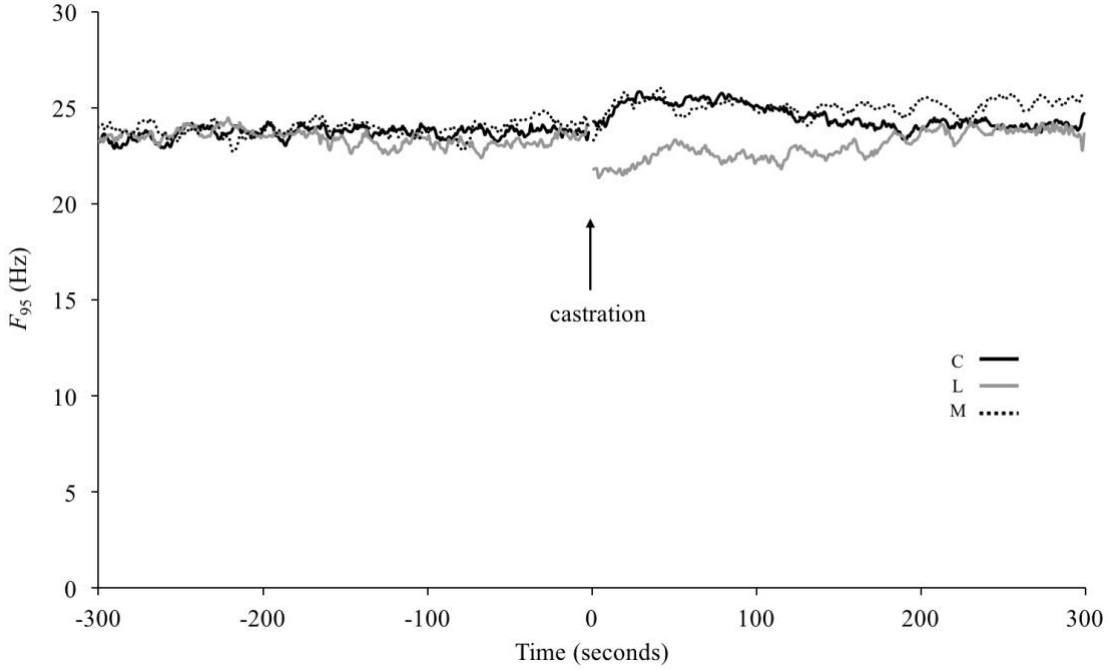

Figure 3-2 *Spectral edge frequency (*F_{95}*) of halothane-anaesthetised six-to-eight month old* Bos *indicus bull calves in three treatment groups.*

Group C - no preoperative analgesia, n = *12; group L – preoperative lidocaine,* n = *12 and group M – preoperative meloxicam,* n =*12 are shown. Both baseline period and the 300 seconds following castration of the first testicle (T1) are shown on the* x-*axis. All treatment groups were different compared to baseline (*p < *0.0001). All groups were different during T1 (*p = *0.0005). Castration occurred at 0 seconds. Median results are shown.*

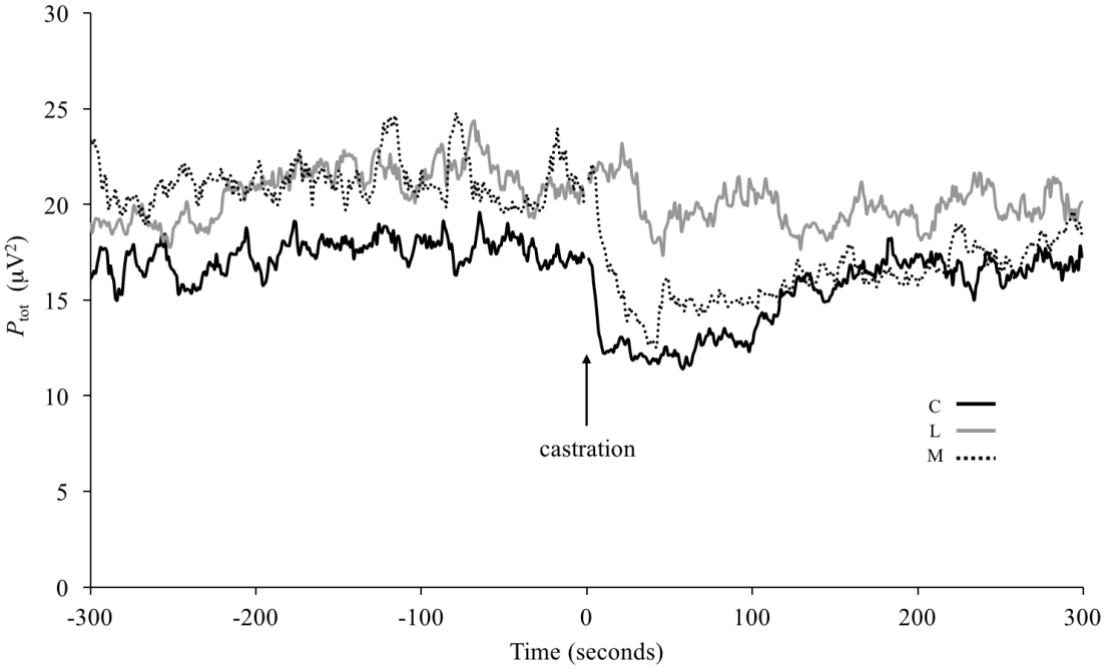

Figure 3-3 *Total power (*P_{tot}*) of halothane-anaesthetised six-to-eight month old* Bos *indicus bull calves in three treatment groups.*

Group C - no preoperative analgesia, n = *12; group L – preoperative lidocaine,* n = *12 and group M – preoperative meloxicam,* n = *12 are shown. Both baseline period and the 300 seconds following castration of the first testicle (T1) are shown on the* x-*axis. All treatment groups were different compared to baseline (*p < *0.0001). There were significant differences between groups during T1 (*p = *0.0163). Castration occurred at 0 seconds. Median results are shown.*

3.2.2 Cardiovascular data

Cardiovascular data from 23 animals was collected and included in these analyses (group C, n = 7; group L, n = 8; group M, n = 8). The remaining 13 animals did not have cardiovascular data recorded due to the appropriate equipment not being available over the first three days of experimentation. There were no differences in the baseline values of HR or MAP between the three groups. HR decreased from baseline and was different between groups C and L at T_{20} (p = 0.03), T_{30} (p = <0.001) and T_{40} (p = 0.009) and between groups L and M at T_{30} (p = 0.015) (Fig. 5a). MAP also decreased from baseline and was different between groups C and L at T_{20} (p = 0.003), T_{30} (p <0.001), T_{40} (p = <0.001), T_{50} (p = 0.018), T_{70} (p = 0.027) and T_{80} (p = 0.045); between groups C and M at T_{40} (p = 0.025) and T_{50} (p = 0.024); and between groups L and M at T_{20} (p = 0.013) and T_{30} (p = 0.002).

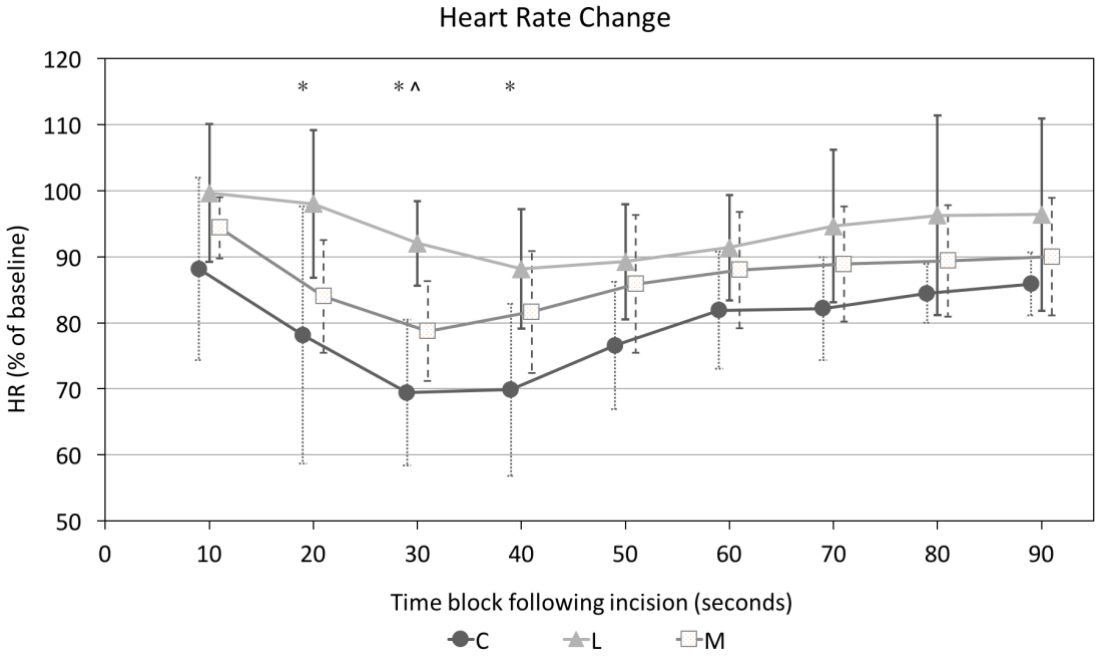

Figure 3-4 *Percentage change in heart rate (HR) from the baseline in each of the ten second epochs (T_{10} to T_{90}) following castration.*

Significant differences (p < *0.05) between the groups following Gabriel post-hoc analysis indicated by* * *(group C compared to L),* ^ *(group C compared to M), and + (group L compared to M). C = castration without preoperative analgesia, L = castration with preoperative lidocaine, M = castration with preoperative meloxicam.*

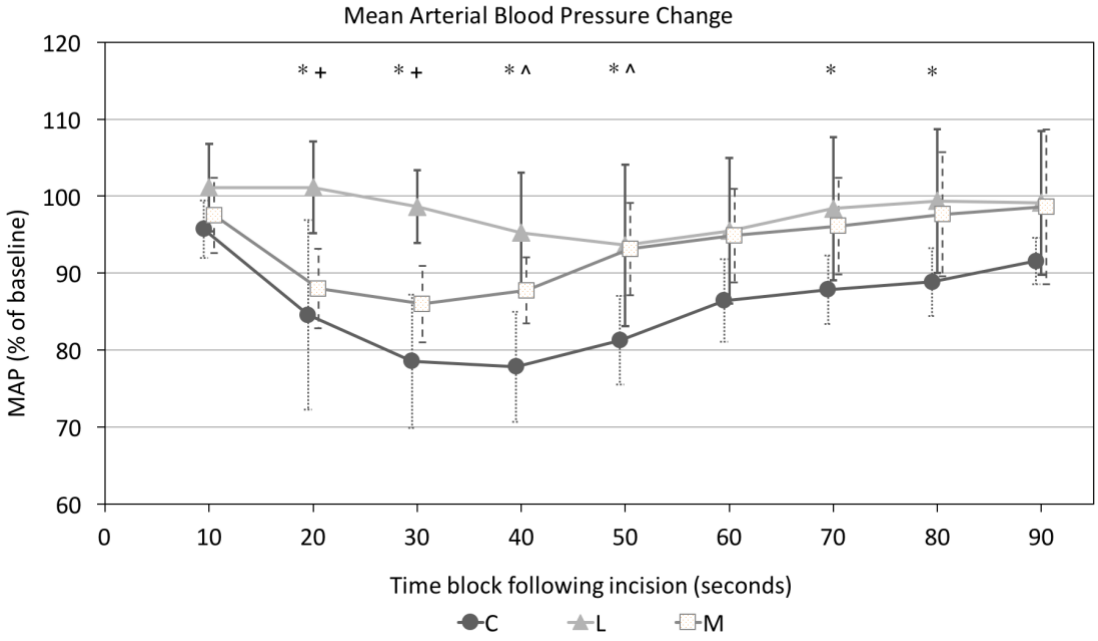

Figure 3-5 *Percentage change of mean arterial blood pressure (MAP) from the baseline in each of the ten second epochs (T_{10} to T_{90}) following castration.*

Significant differences ($p < 0.05$*) between the groups following Gabriel post-hoc analysis indicated by * (group C compared to L), ^ (group C compared to M), and + (group L compared to M). C = castration without preoperative analgesia, L = castration with preoperative lidocaine, M = castration with preoperative meloxicam.*

4 DISCUSSION

4.1 Aims and Study Overview

4.1.1 General Aims

The project aims of characterising the electroencephalographic and cardiovascular responses to surgical castration in *Bos indicus* bull calves indicative of nociception during halothane anaesthesia were successfully completed. Once elucidated, the mitigation of these responses by analgesic therapy of either intra-testicular lidocaine or subcutaneous meloxicam was also examined. The hypotheses were partially supported with marked mitigation of both EEG and cardiovascular response to castration following lidocaine treatment, though only cardiovascular response mitigation evident following meloxicam therapy.

The electroencephalographic findings were similar to previous mammalian studies assessing noxious stimuli with the characteristic nociceptive response occurring. F_{50} increased in all three experimental groups (C, M and L) compared to baseline levels without any significant difference between the groups. F_{95} increased in groups C and M but decreased in group L. P_{tot} decreased in all groups but the decrease was least in group L and greatest in group C.

Equally, the cardiovascular responses to surgical castration displayed similar attenuation of the nociceptive response shown in the EEG responses. Group L was associated with the greatest attenuation of cardiovascular responses following the noxious stimulus. The cardiovascular responses in group M were intermediate to groups L and C.

In brief, lidocaine attenuated, but did not abolish, the EEG and cardiovascular response to surgical castration whereas bull calves pretreated with meloxicam were only significantly different from the control group with respect to their cardiovascular responses, not their EEG descriptors.

4.1.2 Study Motivation

As outlined in the first chapter (section 1), the project had its motivational origins from the relative lack of welfare requirements in the legislation dictating animal welfare standards during castration of farmed cattle. Despite the well established concept of pain caused by castration the legislation in Australia is lacking in any practical applications of pain reduction in this context (AHA 2014). With ever-increasing pressure from consumer and non-consumer sectors to ensure livestock welfare considerations are maintained, the data generated in this study adds to the literature in support of the concept of pain occurring during castration and a method by which to assess potential analgesic therapies.

4.2 Electroencephalographic Findings

4.2.1 Electroencephalographic response in control treatment group

The electroencephalographic responses typically associated with nociception are increases in F_{50} and F_{95}, and a decrease in P_{tot} (Gibson et al. 2007; Grint, Johnson, Clutton, et al. 2014; Grint, Johnson, De Sa Lorena, et al. 2014) in the peracute period following noxious stimuli. These findings have been sustained in multiple ruminant studies examining the EEG responses to procedures including dehorning and castration (Johnson, Stafford, et al. 2005; Johnson, Wilson, et al. 2005; Gibson et al. 2007). The calves in the current project had analogous results in all three EEG frequencies examined following the start of T1 (Figures 3-1, 3-2 and 3-3). The comparisons between the baseline period and T1 were markedly significant in all three frequencies with $p < 0.0001$ in all three analyses. Previous establishment of the increase in F_{50} and F_{95} and the decrease of P_{tot} following the start of the noxious stimuli being across-species indicators of mammalian nociception gives merit to state that *Bos indicus* bull calves experience nociception during castration. The establishment of this control group response allowed the effect of provision of analgesia to be assessed.

4.2.2 Lidocaine and electroencephalographic response modification

Local anaesthetic techniques including testicular infiltration have been successfully used in a number of species and reduction in nociception during castration has been described (Haga et al. 2005; Thüer et al. 2007; Moldal et al. 2013). Gibson et al. (2007) demonstrated that a lidocaine ring block prevented any EEG response to dehorning in Holstein calves. The presence of a reduced, but not eliminated, response in the current study suggests that the local anaesthetic block was incomplete. Previous data supports this occurrence with the possibility of uneven tissue infiltration (Haga et al. 2005) or low local anaesthetic levels present in the cremaster muscle at the time of surgery (Ranheim et al. 2005). Haga and Ranheim (2005) found incomplete anaesthesia produced in piglets during castration following intra-testicular or intra-furnicular lidocaine, postulating that the scrotal ligament and intra-abdominal portion of the spermatic cord may be responsible for the continued nociceptive signal. The current data supports this premise and may reflect nociception originating from the spermatic cord. Analysis of the EEG response comparing the periods before and after injection of lidocaine revealed no significant changes in any of the EEG parameters. This absence of noxiousness associated with the process of injection is consistent with other studies in cattle and piglets (Haga et al. 2005; Gibson et al. 2007).

The use of local anaesthetic techniques might be expected to abolish nociception because of the signal transduction interruption as seen previously during dehorning of calves (Gibson et al. 2007). Certainly, targeted local anaesthesia techniques including nerve-stimulator guided blockade and intrathecal anaesthesia have a high rate of full sensory blockade (Campoy et al. 2012).

The electroencephalographic responses normally associated with nociception are increases in F_{50} and F_{95}, and a decrease in P_{tot} (Gibson et al. 2007; Grint, Johnson, Clutton, et al. 2014; Grint, Johnson, De Sa Lorena, et al. 2014). For F_{95}, an antinociceptive response will typically be characterised as no change from the baseline, and so the decrease in F_{95} seen in group L was seemingly paradoxical. This pattern was first described in a study assessing EEG responses to reticular stimulation in cats, termed “synchronisation”, and

is considered a modified form of EEG activation (Prince et al. 1966). It was referred to as "paradoxical arousal" in a study on isoflurane-anaesthetised sheep where its incidence was correlated with the intensity of stimulus (Otto et al. 2003). Such data provide a plausible explanation as to why the decreased F_{95} was seen in the current study in only group L, where the most significant anti-nociception effect was expected, and thus only the higher intensity stimulus at the point of testicle retraction elicited a response.

4.2.3 Meloxicam and EEG Response Modification

There were no EEG changes in response to castration associated with the preoperative administration of meloxicam. Many investigations into the effects of NSAIDs on nociception, specifically during surgery on animals, have found no differences in the variables considered. These studies report that preoperative administration of meloxicam does not affect the F_{50} in anaesthetised dogs (Kaka et al. 2015) and that the administration of carprofen does not alter minimum alveolar concentration (MAC) of isoflurane in dogs (Ko et al. 2009). However, a significant difference between the control and meloxicam-treated animals may have been expected given previous studies supporting a similar response in animal models of acute nociception (Díaz-Reval et al. 2004; Otto et al. 2005). A previous study by Dumka and Srivastava (2004) reported that therapeutic plasma concentrations of meloxicam were present 30 minutes after the subcutaneous administration of 0.5 mg kg^{-1} to cross-breed calves. It is feasible that anti-nociceptive plasma levels of meloxicam were not present by the start of surgery in the current study given the species, age and size differences in the study population compared to this study. Consequently, higher doses and/or drug administration more than 30 minutes before surgery may have produced different results.

4.2.4 Minimal Anaesthesia Model and EEG recording

Obtaining valid EEG measurements during anaesthesia necessitates minimal influence of anaesthetic and analgesic drugs, along with physiological variables that may be altered by anaesthesia. The stability of the $F_{E'}Hal$ and the physiological parameters, $F_{E'}CO_2$, temperature and oxygenation over the

duration of the study indicate that these parameters were not responsible for the EEG changes presented here. Partial pressures of CO_2 were greater than usually reported in other minimal anaesthesia studies (Murrell et al. 2010; Kongara et al. 2013). These results reflect the difficulty of maintaining normocapnia in cattle and are not unusually high for large ruminants (Klein et al. 1988). The values recorded are considerably less than those which would be expected to have a direct effect on the EEG (Paulson et al. 1974). Furthermore, during anaesthesia, mechanical ventilation was managed by interpreting the information provided by capnography. The discrepancies between the $F_E'CO_2$ and $PaCO_2$ in this study reflect the limitations of capnography, as opposed to the gold-standard temporaneous arterial blood gas analysis. Such discrepancies may be the result of high ventilation-perfusion mismatch resulting in an increase of alveolar dead-space.

Halothane was used in the current study as the sole agent for both induction and maintenance of anaesthesia. This anaesthetic protocol differs significantly from most other large animal studies assessing EEG when intravenous agents including thiopentone or ketamine have been used (Johnson, Wilson, et al. 2005; Gibson et al. 2007; Grint, Johnson, De Sa Lorena, et al. 2014). Induction of anaesthesia with an inhaled drug delivered by facemask in large animals has been reported previously: in trained horses (Pascoe et al. 1993) and small calves (Keegan et al. 2006). In older and thus larger cattle, the technique of induction of anaesthesia with a facemask for delivery of the drug is rarely reported (Thurmon et al. 1968). The facilities at the Murdoch University farm permitted this technique to be used without adverse incident occurring for either the animals or personnel involved. Halothane, along with other common volatile anaesthetic agents including isoflurane and sevoflurane, have been examined for their cerebral depressant effects at equipotent doses in rats during EEG recording (Murrell et al. 2008). Sevoflurane is the most popular choice amongst humans for mask induction due to the sweet odour, non-irritant nature of the agent and rapid onset and offset of action due to low blood solubility co-efficient. Unfortunately, 1xMAC or higher equivalent doses of sevoflurane cause burst suppression resulting in isoelectric periods of EEG, making it unsuitable for EEG assessment.

4.3 Cardiovascular Responses

4.3.1 Introduction

Comparable and transient decreases in HR and MAP were evident in all three experimental groups following the commencement of surgery, with group L having the least change compared to baseline values, and group M an intermediate change. These short-lived reductions in heart rate and blood pressure have previously been reported in anaesthetised ruminants during the application of noxious stimuli (Gibson et al. 2007; Johnson et al. 2009) but the current study is the first such description in cattle during castration. Previous descriptions indicating dominant sympathetic nervous system responses with an increase in heart rate and blood pressure to noxious stimuli frequently focus on a delayed change measured in minutes to hours following noxious stimuli (Peers et al. 2002; Coetzee 2013). The timing of recordings taken in previous studies compared to the peracute period recorded here may explain the disparate results. This theory is supported with the apparent return to near to baseline levels of both the HR and MAP by approximately 100 seconds following surgery. Studies with analogous results to the current study similarly used continuous computer-recorded data from the moment of the incision (Gibson et al. 2007; Johnson et al. 2009). This methodology allows interrogation of the interval immediately from the start of the incision. The mechanism of bradycardia and reduced blood pressure observed in this study is not clear. Given the short period in which changes occurred, a neural mechanism is the most likely explanation. The reduced HR and MAP in the current study may result from vasovagal response to noxious stimuli (van Lieshout et al. 1991). This response may subsequently be overridden by the stress response of surgery and anaesthesia, as could be occurring in the reports of animals when relatively delayed cardiovascular measurements were recorded (Grondahl-Nielsen et al. 1999; Peers et al. 2002). Further work to elucidate this mechanism, possibly using an anti-cholinergic treatment, is required.

The possibility that the cardiovascular responses reported here were vagally-mediated, might be investigated further by examining whether an

antimuscarinic drug (e.g. atropine) can attenuate this response. Advanced physiological data, for example, respiratory values including airway pressure and dynamic lung compliance, along with cardiac output and stroke volume, may also enable elucidation of the mechanism given the known impact of vagus nerve activity on these parameters (van Lieshout et al. 1991).

4.3.2 Cardiovascular responses with lidocaine

The pre-operative administration of 260 mg of lidocaine (group L) resulted in the greatest attenuation of cardiovascular responses following the noxious stimulus. Minimal reductions in HR and MAP were evident in these animals until $T_{30.}$ This time (T_{30}) coincides with when the maximal traction was placed on the spermatic cord just prior to rupture (see Table 3-1), indicating that visceral stimulation, and not the initial incision, may have caused the delayed response in this group. A comparable response has been reported in conscious calves being castrated with local anaesthesia where the skin incision and handling of the testicle provoked minimal behavioural reaction, however spermatic cord traction induced pain-related behaviours (Thüer et al. 2007). A more complex local anaesthetic technique such as epidural or intrathecal anaesthesia may result in complete analgesia (Stilwell et al. 2008). Using such an involved technique is seldom used for the process of castration in livestock, particularly in large-scale field settings.

4.3.3 Cardiovascular responses with meloxicam

Following 0.5 mg kg^{-1} meloxicam SC prior to castration (group M), the cardiovascular response to surgery was intermediate between that of groups L and animals that had not had any pre-operative analgesia (group C). This result is interpreted as a reduction in the nociceptive response following castration with meloxicam. This explanation conflicts with the traditional concept of inflammation occurring some time after the initial activation of nociception and pain perception. Anti-nociceptive actions of non-steroidal anti-inflammatory drugs, in addition to their anti-inflammatory actions, have previously been reported in sheep and cattle using ketoprofen and carprofen, and the mechanism of action is considered to be centrally mediated (Otto et

al. 2003; Lizarraga et al. 2006). The current data are enhancing the concept of inflammation occurring as a part of nociceptor transduction, rather than as a consequence of it. Further investigation of the meloxicam-induced reduction of acute nociception during husbandry procedures in cattle is undoubtedly warranted.

4.4 Study Limitations

4.4.1 General limitation overview

Some limitations exist in this study, and acknowledgement and understanding of these limitations with the impact on the validity of the data gained is crucial. A number of limitations are overt and were considered prior to the project commencing, including the number of animals used and how the data analysis was being completed. There are further limitations that may be considered in a purely theoretical realm, but require examining to display the full understanding of the project.

4.4.2 Sample size

A total number of 36 *Bos* indicus bull calves were used in this study. These numbers are generous in large animal research. Large animal studies often have small treatment group numbers, with the restriction originating from financial and logistic factors (de Vries et al. 2016). Certainly there are few anaesthesia-based research based projects using cattle of this size, with numbers often much smaller. Indeed other ruminant and equid projects have at most used 30 or fewer animals (Johnson, Wilson, et al. 2005; Grint, Johnson, De Sa Lorena, et al. 2014).

4.4.3 Data collection and analyses

The data analysis was performed by personnel present at the experimental phase who were not blinded to the treatment groups. As the data was recorded and extracted via computational methods the bias from is expected to be minimal.

4.4.4 Control group ethics

The ethical justification for including control group (group C) is an interesting component of the study. It must be made clear that this control group allowed evaluation of the nociceptive responses by the bull calves, and valid comparison with the treatment groups. As all animals being castrated in this study were anaesthetised the welfare of these study animals was markedly higher than their compatriots on Australian cattle farms where all husbandry procedures including castration, dehorning, branding and tagging are done without the benefit of either anaesthesia or analgesia. A recent review of the ethics of using such control groups in the pre-eminent veterinary anaesthesia and analgesia serial publication supported their use (Slingsby 2010). In additional support of the project, the Murdoch University Animal Ethics Committee approved the undertaking.

4.4.5 Noxious stimuli assessment

The noxious stimulus in the current study was an irreversible surgical procedure. A standardised repeatable stimulus, such as those used in minimum alveolar concentration (MAC) determination studies, may provide more information about the analgesic efficacy of various drugs. It was chosen to use the surgical procedure to allow a real-world demonstration of the nociception of castration in *Bos indicus* bull calves, along with the amelioration of this response with the analgesic treatments.

4.4.6 Pharmacokinetic assessment

The pharmacokinetics of neither lidocaine or meloxicam are known in *Bos indicus* cattle, with dosing and timing coming from studies in *Bos taurus* species cattle. There is the potential that unsatisfactory time or dosing of both drugs influenced the results achieved. From a practical aspect the pharmacokinetics of these agents would have to be applied to a field-setting to allow real-world welfare improvements. From the current study it may be inferred that a suitable plasma level of meloxicam occurred during the 30 minutes from subcutaneous injection to surgery start time. In a field-setting, it would be useful to ascertain is this level can be achieved more rapidly via an

intramuscular injection to encourage uptake of the practice. The lack of collection of pharmacokinetic data during this study is also a shortfall, however due to additional cost and logistical implications it was elected to not be included.

4.5 Conclusions and Practical Implications

4.5.1 EEG findings

As expected, the *Bos indicus* bull calves in this study demonstrated typical mammalian nociceptive responses to the noxious stimuli of surgical castration. Furthermore the data indicates the reduction of this response by the use of intratesticular lidocaine. Meloxicam had no appreciable effect on the EEG response to surgical castration.

4.5.2 Cardiovascular findings

A novel finding of the transient decrease in blood pressure occurring after the start of surgical castration in *Bos indicus* bull calves is reported herein. This result was additionally supported by the decreased heart rate during the same period. Surprisingly, the use of both lidocaine and meloxicam attenuated this cardiovascular response to surgical castration.

4.5.3 Practical Implications and Future Development

In conclusion, this study is the first description of EEG and cardiovascular responses to castration in *Bos indicus* cattle, and the effect of two different analgesic drugs in reducing these responses. Administration of lidocaine prior to castration significantly attenuated the acute post-operative nociceptive response in six-to-eight month old *Bos indicus* bull calves. In addition, the preoperative administration of meloxicam attenuated the cardiovascular, but not the EEG, responses to castration in the peracute period. These findings provide support for the preoperative administration of lidocaine and give impetus for further research into the peracute anti-nociceptive effects of meloxicam for castration in *Bos indicus* bull calves.

5 APPENDIX

Table A 1 *The predicted median frequency (F_{50}) area-under-the-curve (AUC) from the mixed effects model for the three groups.*

TS	fit	se	lower	upper
B	4.040895	0.207325	3.627398	4.454392
T1	4.758077	0.207325	4.344580	5.171573

Table A 2 *The model interactions (a) and predicted spectral edge frequency (F_{95}) area-under-the-curve (AUC) (b) from the mixed effects model for the three groups.*

a

	numDF	denDF	F-value	p-value
(Intercept)	1	66	12131.899366	0.0000000
TS	2	66	23.612333	0.0000000
Tx	2	33	3.474494	0.0427203
TS:Tx	4	66	7.316009	0.0000612

b

TS	Tx	fit	se	lower	upper
B	C	23.30691	0.4043657	22.50456	24.10926
T1	C	24.40731	0.4043657	23.60496	25.20966
B	L	23.36051	0.4043657	22.55816	24.16286
T1	L	23.08607	0.4043657	22.28372	23.88842
B	M	23.75512	0.4043657	22.95277	24.55747
T1	M	24.91579	0.4043657	24.11344	25.71814

Table A 3 *The model interactions (a) and predicted total power (Ptot) area-under-the-curve (AUC) (b) from the mixed effects model for the three groups.*

a

	numDF	denDF	F-value	p-value
(Intercept)	1	33	964.155543	0.0000000
TS	1	33	34.944006	0.0000013
Tx	2	33	2.684924	0.0831081
TS:Tx	2	33	4.676048	0.0162946

b

TS	Tx	fit	se	lower	upper
B	C	18.70859	1.12872	16.45502	20.96215
T1	C	15.64272	1.12872	13.38915	17.89628
B	L	20.93368	1.12872	18.68012	23.18724
T1	L	20.20549	1.12872	17.95193	22.45906
B	M	21.48978	1.12872	19.23622	23.74334
T1	M	17.69135	1.12872	15.43779	19.94492

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