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Electroencephalography during castration in six-month old Bos indicus cattle

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Executive Summary

Pain is a sensory and emotional experience and given the difficulties in assessing the emotional component of pain in animals this project aimed to focus on the sensory component (nociception) of the pain experience associated with castration in *Bos indicus* bull calves. To this end the electroencephalogram (EEG) and the changes in mean arterial blood pressure (MAP) and heart rate (HR) in the immediate period following castration were measured. In addition, post-operative pain assessment including liveweight changes, activity and behaviour were described over the 13 days after surgery.

Forty-two 6-8 month old *Bos indicus* bull calves were divided into four study groups: The study groups were as follows: anaesthesia without castration (NC) n=6; anaesthesia with castration (C) n=12; anaesthesia and castration with pre-operative lidocaine (CL) n=12; anaesthesia and castration with pre-operative meloxicam (CM) n=12. Animals were randomly allocated to each study group. An open castration technique was employed. In the CL group, 260 mg of lidocaine was injected into the testicles and subcutaneous tissue at the incision site 5 minutes prior to castration. In the CM group, 0.5 mg/kg of meloxicam was administered by subcutaneous injection 30 minutes prior to castration. All the animals were anaesthetised with halothane according to the minimal anaesthesia model (Murrell and Johnson, 2006).

The EEG response to castration indicated that *Bos indicus* bull calves elicit a nociceptive response to castration without analgesia. The pre-operative administration of lidocaine attenuated, but did not abolish, the EEG response to surgical castration. Moreover, bull calves pre-treated with meloxicam exhibited a diminished EEG response compared to the control group. However, this response was still increased compared to the lidocaine group. Overall these data demonstrate that both lidocaine and meloxicam reduce nociception following surgical castration, however lidocaine does so to a significantly greater extent.

Without analgesia the cardiovascular response to castration was a decrease in MAP and HR. The percentage decrease in MAP and HR in the CL and CM groups was less than in group C. Lidocaine prevented a decrease in MAP and HR associated with the first incision and meloxicam prevented a decrease in MAP in response to the first incision. These data support the EEG findings that both lidocaine and meloxicam interfere with the nociceptive response to castration.

Post-operative assessments were not able to consistently differentiate between study groups with regards to liveweight, activity and behaviour. There were only subtle differences in activity and behaviour in the animals that received meloxicam.

The effects of lidocaine on the EEG and cardiovascular response to castration in the period immediately after surgery were as expected. Lidocaine is a local anaesthetic of medium duration of action when given as single injection. In lambs the duration of action is reported to be 100 ± 38 minutes (Ghadirian et al., 2016). Consequently it is unlikely that this drug will provide analgesia in the days following surgery. Meloxicam is a non-steroidal anti-inflammatory that is likely to have analgesic actions beyond the acute phase to up to 48 hours. With an understanding of the mode of action, duration of effect and analgesic efficacy of lidocaine and meloxicam, the results of this study support the recommendation that the pre-operative administration of both lidocaine and meloxicam will improve the welfare of animals undergoing castration.

Pre-operative administration of local anaesthetic (e.g. lidocaine) and a non-steroidal anti-inflammatory (e.g. meloxicam) have an anti-nociceptive effect during castration of *Bos indicus* bull calves. It is likely that these effects on nociception will reduce the emotional component of the pain experience in the days following castration. The administration of these drugs prior to castration will increase the financial cost of castration but will improve animal welfare.

Table of Contents

1	Background.....	5
1.1	Background.....	5
1.2	Significance for Industry	6
1.3	Overarching aim.....	6
2	Project objectives	6
3	Methodology	6
3.1	Animals.....	6
3.2	Study groups.....	7
3.3	Anaesthesia.....	7
3.4	Acute responses to castration.....	8
3.4.1	Electroencephalography (EEG)	8
3.4.2	Cardiovascular responses.....	8
3.5	Recovery from anaesthesia	9
3.6	Post-operative assessments.....	9
3.6.1	Liveweight.....	9
3.6.2	Pedometry	9
3.6.3	Behavioural ethograms	9
3.6.4	Quantitative Behavioural Analysis (QBA)	11
3.7	Statistical analyses.....	11
3.7.1	EEG	11
3.7.2	Cardiovascular responses.....	11
3.7.3	Liveweight and pedometry	11
3.7.4	Behavioural ethograms	12
3.7.5	Quantitative Behavioural Analysis (QBA)	12
4	Results.....	13
4.1	Anaesthesia.....	13
4.2	Acute responses to castration.....	13
4.2.1	EEG	13
4.2.2	Cardiovascular responses.....	15
4.3	Post-operative assessments.....	17
4.3.1	Liveweight.....	17
4.3.2	Pedometry	18
4.3.3	Behavioural ethograms	20
4.3.3.1	Behaviour analysis Days -1 to 13 in paddock	20
4.3.3.2	Behaviour analysis Day 0 in yard.....	23
4.4	Quantitative Behavioural Analysis (QBA)	26
5	Discussion.....	26
5.1	Acute responses to castration.....	27
5.1.1	EEG	27
5.1.2	Cardiovascular responses.....	29
5.2	Post-operative assessments.....	29
5.2.1	Liveweight and pedometry	29

5.2.2	Behaviour	30
6	Conclusions/recommendations	31
7	Key messages	31
8	Bibliography	32
9	Appendix	34

1 Background

1.1 Background

There is growing societal concern over the treatment of livestock and in particular, performing routine surgical procedures such as castration without pain relief. The provision of pain relief for surgical castration of calves is likely to be required by some international standards and this may affect market access. Currently, pastoral cattle in Australia are surgically castrated, without anaesthesia or analgesia, up to 8 months of age. Pain assessment in cattle is difficult and there is no single validated pain assessment technique that can be reliably utilised to determine pain in cattle. As a result it is difficult to assess the efficacy of analgesic drugs used in this species. Industry requires a practical, best practice technique that may or may not include the use of local anaesthetic and/or systemic pain relief. Importantly, industry requires a management technique that can be used under field conditions and one that gains consumer acceptance.

Pain is defined by the International Association for the Study of Pain (IASP: www.iasp-pain.org) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. The sensory component of this definition is referred to as nociception while the emotional experience is more difficult to describe. A painful (or noxious) stimulus will send messages to the dorsal horn of the spinal cord, onto the thalamus and then the cerebral cortex in the brain. These pathways cause electrical activity in the brain which can be recorded with electroencephalographic (EEG) equipment. This approach to pain assessment is not always necessary as the emotional aspects of the pain experience can often be exhibited by body language and thus more easily described in some species. Unfortunately pain assessment in *Bos indicus* cattle is very difficult as they are a species which do not display overt behavioural changes during periods of pain or distress. This is supported by the conclusion reached in B.AWW.0223 Final Report. For this reason, studying the EEG of *Bos indicus* cattle is likely to elucidate whether or not nociceptive pathways are stimulated in the same way as they are in other species. This will enable a better understanding of the animals' response to painful procedures such as castration, and how it can be best managed with analgesic drugs.

Previous work by this group investigated the efficacy of analgesic strategies for castration of 6-month old *Bos indicus* cattle and the usefulness of a number of pain assessment tools in this species (B.AWW.0223 Final Report). While it was apparent that the administration of lignocaine by intra-testicular and subcutaneous (scrotal) injection 5 minutes prior to surgery and post operative meloxicam provided analgesia for these cattle it was not clear if this analgesic strategy was superior to the others that were tested. Nor was it possible to identify a single reliable and useful tool for pain assessment in these animals.

In light of the difficulties in assessing pain in 6-month old *Bos indicus* cattle, examining the EEG response to noxious stimuli is warranted. Data from neurophysiological experiments in animals and functional neuroimaging studies in humans have conclusively demonstrated the involvement of the cerebral cortex in pain processing (Schnitzler and Ploner, 2000). This approach includes the collection of EEG information which has been used in a range of species to measure the depth of anaesthesia and to assess pain (Murrell and Johnson, 2006). Dogs, rats, chickens, donkeys, horses, sheep and cattle are some of the species that have been studied previously (Diesch et al., 2009, Gibson et al., 2009, Grint et al., 2014a,

Haga and Dolvik, 2005, Kongara et al., 2011, McIlhone et al., 2014). The EEG is the electrical activity recorded from electrodes placed on the head of an animal. The electrodes produce a signal which requires amplification and recording so equipment capable of this is essential. It is standard for EEGs to be performed during anaesthesia to minimise the interference from movement of the animal. A minimal anaesthesia model is employed whereby general anaesthesia is administered to a stable plane such that the animals are unconscious, but still able to demonstrate EEG responses to noxious stimulation (Murrell and Johnson, 2006). Halothane is the anaesthetic agent of choice as it appears to be devoid of antinociceptive properties and causes less depression of the cerebral cortex compared to isoflurane, sevoflurane and desflurane (Murrell et al., 2008).

1.2 Significance for Industry

Evaluation of the EEG response to castration will provide strong and quantifiable data to demonstrate whether or not there is a neurophysiological response to castration which is likely to be associated with a pain experience in the post-operative period. This approach will provide industry with evidence on which to base their recommendations for the management of pain *Bos indicus* cattle undergoing castration.

1.3 Overarching aim

The overarching aim of this work is to produce sound recommendations to industry regarding the management of pain associated with routine husbandry procedures such as castration in *Bos indicus* bull calves. This aim will be met by measuring the acute EEG and cardiovascular responses to castration and by evaluating weight changes, activity levels and behavioural changes in the post-operative period.

2 Project objectives

There were two objectives for this work:

1. Describe the EEG activity during castration of 6-month old *Bos indicus* cattle.
2. Determine the efficacy of analgesic strategies for the management of pain associated with castration of 6-8 month old *Bos indicus* cattle using EEG.

3 Methodology

3.1 Animals

The study was approved by the Animal Ethics Committee of Murdoch University (R2730 15). Forty-two six to eight month old *Bos indicus* bull calves were sourced from a private supplier in the Kimberley region of Western Australia. The animals had previously been dehorned. The animals were transported to the Murdoch University farm two weeks prior to the study and were treated with an endectocide on arrival. Blood was also collected for testing for Bovine Viral Diarrhoea Virus and the animals were randomly allocated a study number for identification. The numbers 1-48 were marked on an ear tag and with spray paint on the rump.

The cattle were held in a 1.1 ha farm paddock with irrigated kikuyu pasture for free-grazing. Supplementary feed was provided daily: oaten hay bales and a beef cattle specific balanced

mixed pelleted food (EasyBeef®, Milne Feeds, Perth, Australia). There was a ground-level stock trough for water.

A halter-trained Illawarra cow was also held in this paddock during the acclimatisation period. This cow later accompanied the bull calves during recovery from anaesthesia

3.2 Study groups

The study groups were as follows:

1. Anaesthesia without castration (NC) n=6
2. Anaesthesia with castration (C) n=12
3. Anaesthesia and castration with pre-operative lidocaine (CL) n=12
4. Anaesthesia and castration with pre-operative meloxicam (CM) n=12

Animals were randomly allocated to a study group. In the NC group animals were anaesthetised and monitored for a period of time equivalent to that required for castration in the treatment groups (~70 minutes). In each of the treatment groups surgical castration was performed by an experienced veterinary surgeon. An open castration technique was employed. In the CL group, 260 mg of lidocaine was injected into the testicles and subcutaneous tissue at the incision site 5 minutes prior to castration. In the CM group, 0.5 mg/kg of meloxicam was administered by subcutaneous injection 30 minutes prior to castration.

3.3 Anaesthesia

Three or four animals were anaesthetised each day. On these days the animals were weighed and transported from the farm paddock to the surgery area (in the barn) in a trailer (~ 2 km). The animals were held in a small concrete yard prior to anaesthesia.

Immediately prior to anaesthesia, a single animal was drafted into the race and restrained with a head bail for placement of two halters. The animal was then moved into the section of race which was a tilt-table. Three straps were positioned (over the neck, behind the elbow and in front of the stifle) and the halter was secured. The table was then tilted to the horizontal position, the straps were tightened, leg ropes were positioned and tied in place and a blind fold was placed over the eyes.

Anaesthesia was induced with halothane in oxygen by a face mask attached to a large animal anaesthetic machine. The trachea was intubated when the depth of anaesthesia was adequate. Anaesthesia was maintained with halothane in oxygen in accordance with the 'minimal anaesthesia model' (Murrell and Johnson, 2006). This model uses halothane alone for induction and maintenance of anaesthesia and anaesthetic depth is kept at a level to maintain unconsciousness and immobility but to allow EEG changes evoked by noxious stimuli to be demonstrated. The model requires the control of the physiological consequences of anaesthesia to be kept within narrow limits for body temperature, blood pressure and the partial pressure of oxygen and carbon dioxide in arterial blood (Gibson et al., 2007b). To this end the expired halothane concentration (FE'HAL) was maintained between 0.9 and 1.1%, and body temperature, blood pressure and the blood gases (oxygen and carbon dioxide) were maintained within normal limits.

Following endotracheal intubation and instrumentation for monitoring (nasal temperature probe, arterial catheter for invasive blood pressure measurement, pulse oximeter and capnograph) a stable phase of anaesthesia was maintained for at least 10 minutes prior to recording of the electroencephalogram.

3.4 Acute responses to castration

The acute nociceptive response to castration was assessed by monitoring the EEG and the cardiovascular changes immediately following castration. These data were collected from the treatment groups only (C, CL and CM).

3.4.1 Electroencephalography (EEG)

A far-field EEG was obtained using dermal needles (Neurone subdermal, Ambu, Malaysia). The non-inverting electrode was placed midline between the medial canthi of the eyes, the inverting electrode over the right mastoid process and the earth electrode 2-4 cm caudal to the poll as previously described (Gibson et al., 2007b). Noise was noted in the EEG signal and earthing the tilt-table to the breakout box resolved the majority of this noise. Following a ten-minute period of stable anaesthesia with $\text{FE}'\text{HAL}$ at 0.9-1.1% and $\text{FE}'\text{CO}_2$ at 45-55 mmHg a five-minute baseline EEG was obtained for each animal. For the CL group the first stimulus was injection of lidocaine and for the C and CM groups the first stimulus was incision of the scrotum and castration. For the CL group a further five-minute baseline EEG was recorded following injection of lidocaine and prior to castration. Each bull had the left testicle removed first and after five minutes the right testicle was removed. Time points were recorded as time stamps on the EEG trace as baseline start (B), lidocaine injection, first testicle start (T1), first testicle finish, second testicle start, second testicle finish and completion of the EEG trace (finish). The EEG data was directed through a custom made breakout 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 1000 in alternating current mode, a low filter setting of 1 Hz and a high filter setting of 100 Hz. The data were 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.

The raw EEG data was inspected for any noise artefacts such as electromyography signals. These segments of record were excluded from analysis. Fast Fourier transformation (FFT) was completed using custom-written software (C. Johnson, Massey University, New Zealand). The median frequency (F_{50}), spectral edge frequency (F_{95}) and the total power (P_{tot}) of the EEG were then established using 1-Hz frequency bins on each time stamped period of data.

3.4.2 Cardiovascular responses

Mean arterial blood pressure (MAP) and heart rate (HR) changes following castration were recorded continuously by measuring invasive blood pressure and performing electrocardiography, respectively. For measurement of blood pressure a catheter was placed in the auricular artery after the induction of anaesthesia. The catheter was connected to a fluid filled non-distensible tube and transducer and in turn to the Powerlab data

acquisition system. Likewise the electrocardiography signal was read by Powerlab to measure the heart rate. Baseline data for these parameters was collected for 5 minutes prior to the first incision and for 5 minutes after removal of each testicle. The same time stamps as those used for the EEG were used for the MAP and HR.

3.5 Recovery from anaesthesia

For recovery from anaesthesia a small paddock adjacent to the surgery area was used. At the end of anaesthesia the animal was moved from the barn to the recovery paddock with a forklift. A blindfold was kept in place and the trachea was extubated once the animal could swallow and protect its airway. The recovery was unassisted. The halter-trained Illawarra cow resided in this paddock during the anaesthesia and castration phase of the study to accompany the bull calves during recovery from anaesthesia.

3.6 Post-operative assessments

Post-operative assessments were performed in all the study groups (NC, C, CL and CM). In group NC these assessments were done until Day 10. In groups C, CL and CM these assessments were done until Day 13.

3.6.1 Liveweight

The animals were weighed at various timepoints during the study period: on arrival at the University farm, seven days before anaesthesia and surgery (Day -7), on the day of surgery (Day 0) and six, ten and 13 days after surgery.

3.6.2 Pedometry

A pedometer (Afimilk, Kibbutz Afikim, Israel) was fitted to each animal on the same day as animal identification numbers were attributed. The pedometer was fixed to a hind limb, just above the fetlock, with a nylon strap. The pedometer data was downloaded wirelessly to a base unit mounted in the paddock. The pedometers had a range of 400 m, which covered the area of the paddock, and a recall period of 12 h, which allowed for retrospective retrieval of data when the animals left the paddock for anaesthesia and surgery, or in the event of wireless communication failure.

Pedometry data generated values for daily activity (steps/hour), rest (minutes/24 hours) and bout (average minutes spent resting) from seven days prior to anaesthesia and surgery (Day -7) to 13 days afterwards (Day 13).

3.6.3 Behavioural ethograms

An ethogram was developed by observing bulls in the paddock (pre and post-surgery days) and in the recovery yard (on the day of surgery). It contained 5 states and 24 events describing the behaviour that the bulls demonstrated. The duration of the states was presented as a proportion (%) of time for the 2-minute clip as they are mutually exclusive, while the events were actions that occurred for less than 5 seconds and were either counted every time one occurred within the 2-minute filming period and scored as either a 0 (absent) or 1 (present).

Two digital camcorders (Panasonic HC-V520M) mounted on portable tripods approximately 1.2 m above ground were used to film the cattle before and after castration. Video clips for the cattle in the paddock were analysed on days -1, 0, 1, 2, 3, 6 and 13-16. Filming on days 0 and 3 occurred at 06:30-08:00. On days -1, 2, 6 and 13-16, filming occurred at 17:00-18:00. And on day 1, filming occurred at 13:00-14:00. Clips for the cattle in the recovery yard were analysed at half an hour, 1 h, 2 h, and 3 h after castration.

Table 1. Behavioural categories for quantifying animal behaviour over 2 minutes when animals were either in paddock or yards.

States (proportion of time)	Description
Play ¹	Head butting*, jumping* running* and bunt (push in an active manner)*
Feed	Head lowered on floor visibly eating ²
Walking/Seeking	Moving around in pen, not standing stationary
Standing	Standing stationary on four legs
Lying	Lying on floor
Events (Counts)²	
Chew Paddock fixtures	Chewing paddock fixtures (wire, fences, trees, gates, feeder)
Defecation	Discharge of faeces
Ear-flick	Ear movement backwards and forwards
Headflick	Quick head movement to the hind limbs
Hind leg kick/stamp	Hind leg kick or stamp
Hindlimb stumble	Temporary loss of balance in one or both hind limbs
Hind quarter grooming	The grooming of the bulls' scrotum or hind legs
Licking nose	Licking of own nose
Lying, head flat ¹	Lateral lying* or lying with neck extended*
Mounting	Mounting another bull
Neck Curl	Neck curl while lying down
Pawing ground	Striking ground with forelegs
Stumbling	Temporary loss of balance
Tail swishing	The movement of the tail from side to side
Urination	Discharge of urine
Weight shifting	The shifting of the body from one leg to another

¹ Behaviour were auto correlated, thus only the listed term (e.g. play) was included in analysis

* Behaviour were auto correlated and terms (e.g. butting, jumping and running) were no longer used in analysis

² Each event was 5 sec or less

Events (counted as present or not)	Description
Alert	The bull is looking ahead with ears forward
Autogroom	The grooming of itself
Headshake	Shaking of whole head
Instigate grooming	The grooming of another bull
Receive grooming	Bull receives grooming from another
Low Head Position	The head is below shoulder level
Lying limb extended	Lateral lying with all four limbs extended
Lying nose to dirt	Sternum lying with nose in dirt
Saw-horse stance	Legs abducted and extended while standing
Social	Physically interacting with another bull
Restless	The bull continuously performed numerous different states
Ruminating	Chewing cud
Walking backwards	Stepping backwards

3.6.4 Quantitative Behavioural Analysis (QBA)

These analyses are ongoing and so these data are not included in this report.

3.7 Statistical analyses

3.7.1 EEG

The raw data was transformed to generate values for median frequency (F_{50}), spectral edge frequency (F_{95}) and the total power (P_{tot}). This 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 time-stamp and treatment. A mixed effect model was fitted with a random intercept term to account for the repeated measures. The same analysis was applied to the CL group to assess the effect of lidocaine injection.

3.7.2 Cardiovascular responses

MAP and HR changes were expressed as raw data and as a percentage change from baseline over averaged 10-second time blocks and were compared with one-way ANOVA with Gabriel's post-hoc analysis. Significance was $p \leq 0.05$.

3.7.3 Liveweight and pedometry

Four response variables were considered: weight, activity, bout, and rest. For each of these response variables, a mixed effect model was fitted with the predictors: treatment, day, and

pre-treatment (baseline) level. The treatment had four levels, C, CL, CM, and NC. In the case of weight, due to missing data, the treatment level NC could not be considered. The pre-treatment level was the mean of the response variable for each bull before any treatment was given. The day was treated as a categorical random variable. For each model, the random intercept for each bull fitted the data the best. Starting with day, treatment and pre-treatment level as the main effects a two-way interaction between treatment and day was performed. The best model was then selected using a likelihood ratio test with a cut-off of <0.05 .

The final models were (Appendix 1):

- Weight: the predictors in the final model were pre-treatment weight and day.
- Activity: the predictors in the final model were pre-treatment activity and day.
- Bout: the predictors in the final model were pre-treatment bout, day and treatment.
- Rest: the predictors in the final model were pre-treatment bout, day, treatment and a interaction between day and treatment.

3.7.4 Behavioural ethograms

To understand the correlation between all the behaviours and states, a correlation matrix was performed. Behavioural categories that had a correlation score of more than 0.7 were combined to allow for the data to remain singular. Behaviours that were demonstrated by less than three individuals throughout the study were also excluded. A principle components analysis (PCA) produced dimensions with the main 4 representing the percentage of total variation seen. The factor scores for the first four Eigenvalues were generated and only factors that had a loading of more than 75% of the largest loading were considered to be significant to the principle components. Within each dimension, factor scores representing a specific behaviour were then sorted in ascending order to classify similar behavioural categories as a group.

The PCA scores for each day of castration was used as the dependent variable in a repeated measures ANOVA, with the categorical factor being treatment, and the within effect being day of castration in order to analyse differences between treatment groups over time or day. A few data points were missed unavoidably if timing of clip collection of different bulls was required simultaneously in different locations. Thus, if data for a bull in the paddock were missed then an average of all bulls was used. If data for a bull in the yard were missed, then a treatment group average for that day was used. The data were further subjected to a post hoc Tukeys Test to detect differences between treatment groups.

3.7.5 Quantitative Behavioural Analysis (QBA)

These analyses are ongoing and these data are not included in this report.

4 Results

4.1 Anaesthesia

Anaesthesia was induced successfully in each animal with halothane delivered by mask. Time from placement of the facemask to oral endotracheal intubation was 34.9 (± 8.1) minutes, and the duration of anaesthesia (from the initial placement of the facemask to turning off the vapouriser at the end) was 81.4 (± 13.1) minutes. Intubation was successful on the first attempt in 29 animals, on the second attempt in one animal and on the third attempt in six animals. Multiple attempts (>1) at intubation occurred twice in group C and three times each in groups CL and CM. The time (median [range]) for removal of testicle one (T1) was 34.6 [17.8-83.9] seconds. There were no significant differences between the weight, time to intubation, total general anaesthesia time or time for removal of the first testicle between the treatment groups.

One animal in the NC group sustained a soft tissue injury during recovery from anaesthesia so analgesia was administered. The data from this animal were excluded if they were significantly different from others in the NC group at the same time point.

4.2 Acute responses to castration

4.2.1 EEG

Data from the 36 bulls in groups C, CL and CM was suitable for analysis. Somatic responses such as movement of a leg or the head, tail or ear flicking, chewing or tongue movement were noted following incision in two animals in groups L and M groups and one in group C. Visual inspection of the EEG data permitted the removal of the electric noise evident on these traces as a result of these movements.

The AUC analysis of the F_{50} revealed a significant difference at T1 compared to the baseline in all groups ($p < 0.0001$) but no difference between the treatment groups ($p = 0.6491$). For the F_{95} there was a significant difference between the T1 time point and baseline ($p = 0.0001$) and this difference was significant between treatment groups ($p = 0.0005$) with an increased AUC for groups C and CM, and a decrease in group CL, compared to baseline. The P_{tot} was significantly different between baseline and T1 ($p < 0.0001$) and this difference was significant between treatment groups ($p = 0.0163$) with the decrease of AUC in the C and CM groups being significantly more than that in the L group (Figure 1). There was no difference between the baseline and lidocaine injection EEG response for F_{50} ($p = 0.093$), F_{95} ($p = 0.998$) or P_{tot} ($p = 0.225$).

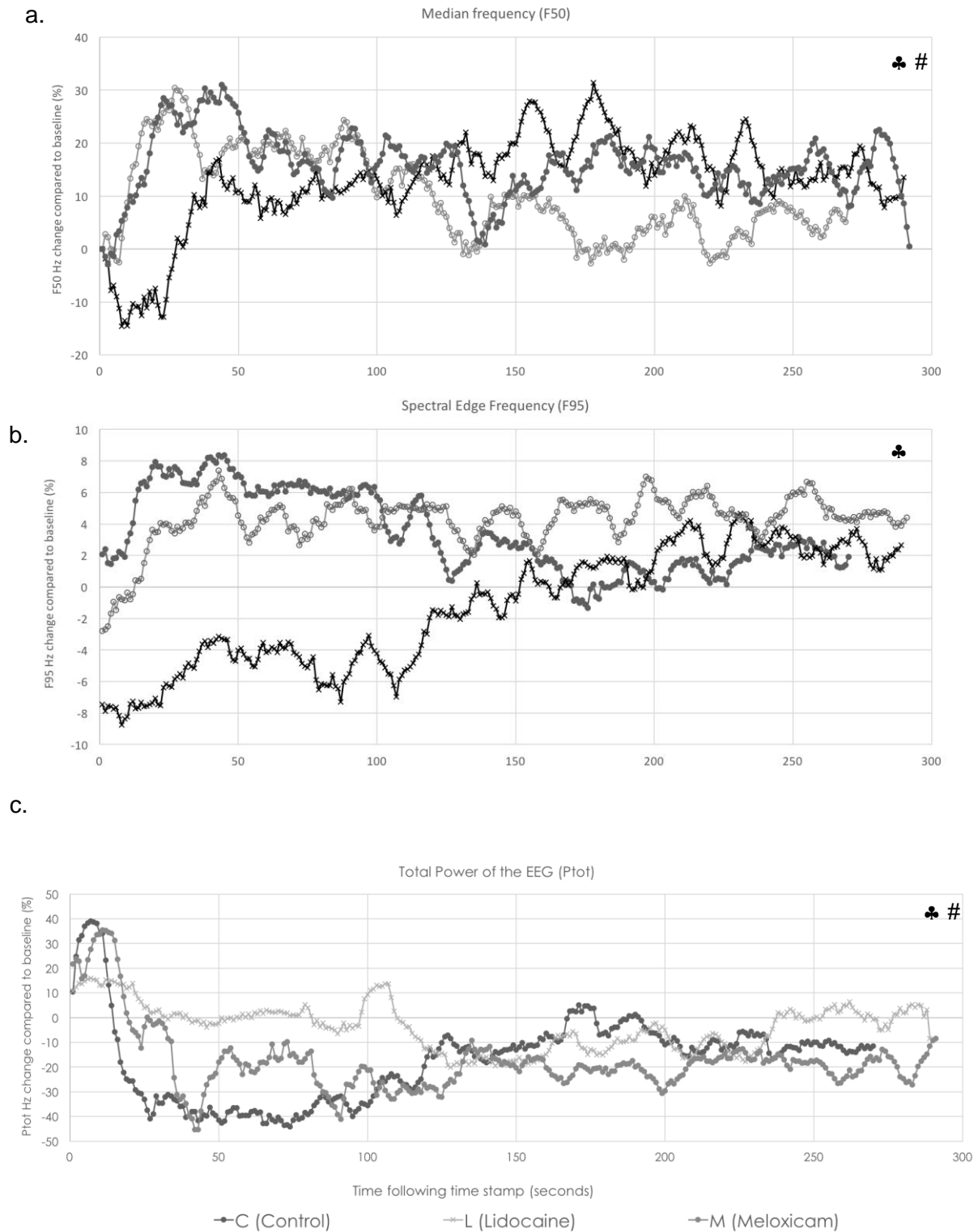


Figure 1. a. Median frequency (F_{50}), b. spectral edge frequency (F_{95}) and c. total EEG power (P_{tot}) change compared to baseline (%) in the 300 seconds following surgical removal of the first testicle (T1). ♣ significant difference between baseline and the 300 seconds following incision; # significant difference between the treatment groups over the 300 seconds following incision. Data points are the mean of each group. Incision at 0 seconds.

The EEG data will be submitted for publication in the journal 'Veterinary Anaesthesia and Analgesia'. The manuscript is in the advanced stages of preparation: "Electroencephalographic responses to surgical castration in *Bos indicus* bull calves indicate anti-nociceptive effects of lidocaine or meloxicam". These data were also presented at the Association of Veterinary Anaesthetist's meeting in Lyon, France in April 2016 (oral abstract).

4.2.2 Cardiovascular responses

Data for 23 animals were collected (C: n=7, CL: n=8, CM: n=8). Without analgesia the cardiovascular response to castration was a decrease in MAP and HR. The percentage decrease in MAP and HR in the CL and CM groups was less than in group C in all time-blocks up to 100 seconds after incision (Figures 2 and 3). Lidocaine prevented a decrease in MAP and HR associated with the first incision ($p < 0.001$) in the 20-30 s time block (Tables 2 and 3). Meloxicam prevented a decrease in MAP in response to the first incision ($p = 0.025$) between 30 and 50 seconds (Table 3).

Table 2: Mean arterial blood pressure

Time Block	Group C		Group CL		Group CM		p values		
	Mean	SD	Mean	SD	Mean	SD	CvCL	CvCM	CLvCM
0-10 s	95.7	3.7	101.13	5.7	97.5	4.9	0.123	0.857	0.380
10-20 s	84.6	12.3	101.13	6	88	5.2	0.003	0.802	0.013
20-30 s	78.6	8.6	98.6	4.7	86	5	<0.001	0.091	0.002
30-40 s	77.9	7.2	95.2	7.8	87.8	4.3	<0.001	0.025	0.095
40-50 s	81.3	5.8	93.6	10.5	93.1	6	0.018	0.024	0.999
50-60 s	86.4	5.3	95.5	9.4	94.9	6.1	0.072	0.101	0.997
60-70 s	87.9	4.4	98.4	9.3	96.1	6.3	0.027	0.099	0.892
70-80 s	88.9	4.4	99.4	9.3	97.6	8.1	0.045	0.111	0.956
80-90 s	91.6	3	99.1	9.3	98.6	10	0.246	0.298	0.999

Table 3: Heart rate

Time Block	Group C		Group CL		Group CM		p values		
	Mean	SD	Mean	SD	Mean	SD	CvCL	CvCM	CLvCM
0-10 s	88.1	13.8	99.6	10.4	94.4	4.6	0.113	0.562	0.662
10-20 s	78.4	19.5	98	11.1	84	8.5	0.030	0.787	0.143
20-30 s	69.4	11	92	6.4	78.8	7.6	<0.001	0.123	0.015
30-40 s	69.9	13	88.1	9	81.6	9.2	0.009	0.117	0.528
40-50 s	76.6	9.6	89.2	8.7	85.9	10.5	0.056	0.207	0.861
50-60 s	81.9	8.8	91.4	8	88	8.8	0.122	0.436	0.814
60-70 s	82.1	7.8	94.6	11.5	88.9	8.7	0.058	0.453	0.553
70-80 s	84.4	4.4	96.2	15.1	89.4	8.4	0.118	0.744	0.488
80-90 s	85.9	4.8	96.4	14.6	90	8.9	0.178	0.826	0.540

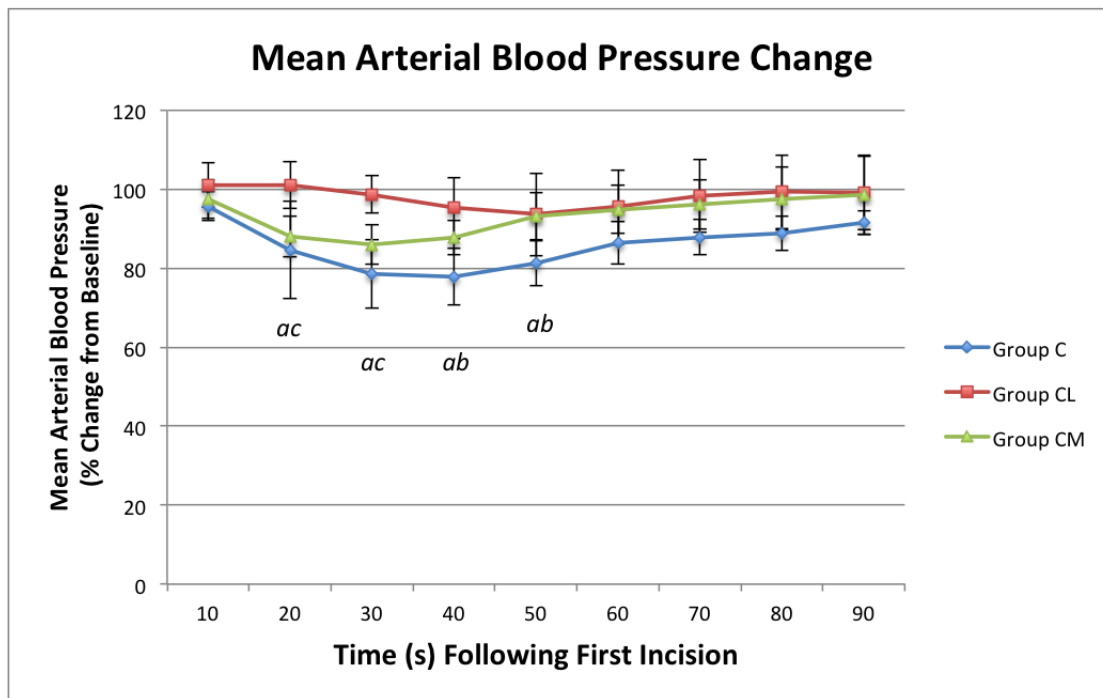


Figure 2: Mean (SD) of the average mean arterial blood pressure (mmHg) as a percent of the individual baseline values at 10-second time blocks (averaged) following the start of castration. a = C vs CL $p < 0.05$, b = C vs CM $p < 0.05$, c = CL vs CM $p < 0.05$.

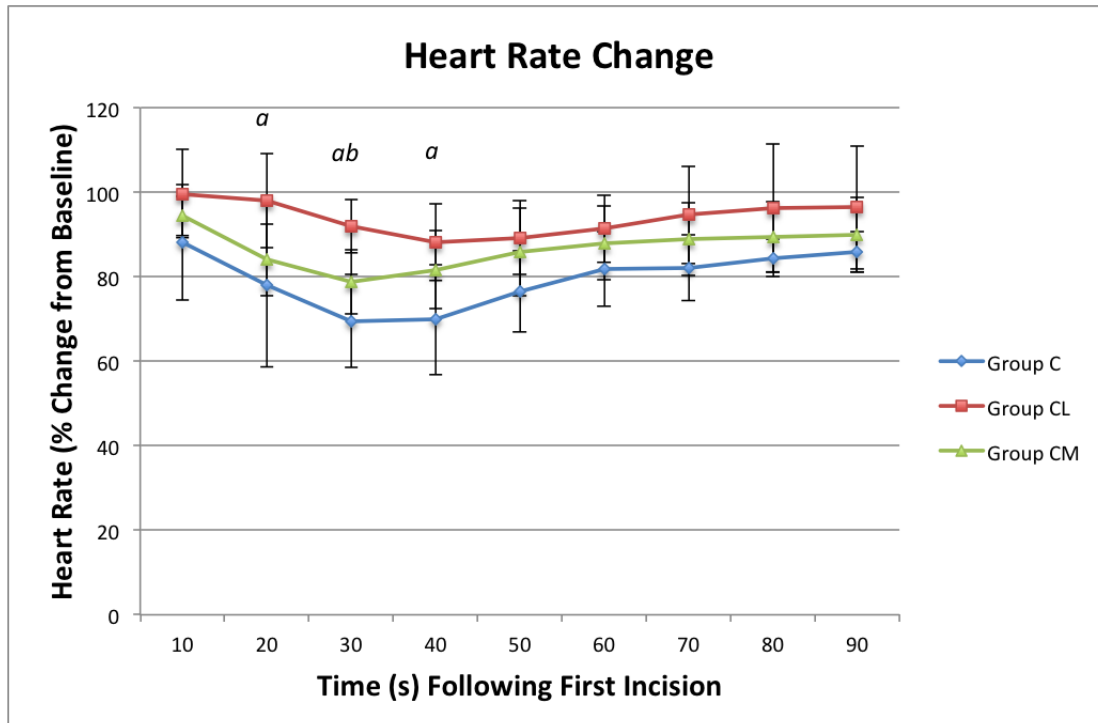


Figure 3: Mean (SD) of the mean heart rate (beats/minute) as a percent of the individual baseline values at 10-second time blocks (averaged) following the start of castration. a = C vs CL $p < 0.05$, b = C vs CM $p < 0.05$.

The cardiovascular data will be submitted for publication in the journal 'Veterinary Anaesthesia and Analgesia'. The manuscript is in the early stages of preparation: "Lidocaine and meloxicam analgesia decrease nociception as indicated by cardiovascular responses of halothane-anaesthetised *Bos indicus* bull calves during surgical castration". These data will be presented as an abstract at the International Veterinary Emergency and Critical Care Society Meeting in Texas, USA in September 2016 (oral abstract).

4.3 Post-operative assessments

4.3.1 Liveweight

All the cattle gained weight during the study. There was no difference in the weight change over time between groups. There was no effect of treatment or day on weight (Appendix 1).

Table 4: Mean (SD) weight over the course of the study.

Study group	Arrival at the University Farm	Day -7	Day 0	Day 6	Day 10	Day 13	Departure from the University Farm
No castration (NC)	237.5 (±15.9)	234.8 (±14.4)	228.3 (±17.4)	243.3 (±21.7)	255.3 (±20.7)		253.3 (±21.6)
Castration without analgesia (C)	225.3 (±17.1)	226.4 (±17.8)	236.1 (±18.1)	236.6 (±17.8)	241.3 (±17.6)	249.6 (±19.6)	253.7 (±21.7)
Castration with lidocaine (CL)	225.1 (±16.9)	226.6 (±18.2)	236.7 (±18.8)	236.6 (±17.8)	249.5 (±19.4)	253.2 (±21.0)	253.2 (±21.0)
Castration with meloxicam (CM)	225.9 (±16.9)	229.3 (±16.0)	237.1 (±18.7)	237.1 (±17.6)	241.3 (±17.2)	249.6 (±19.2)	252.7 (±21.4)

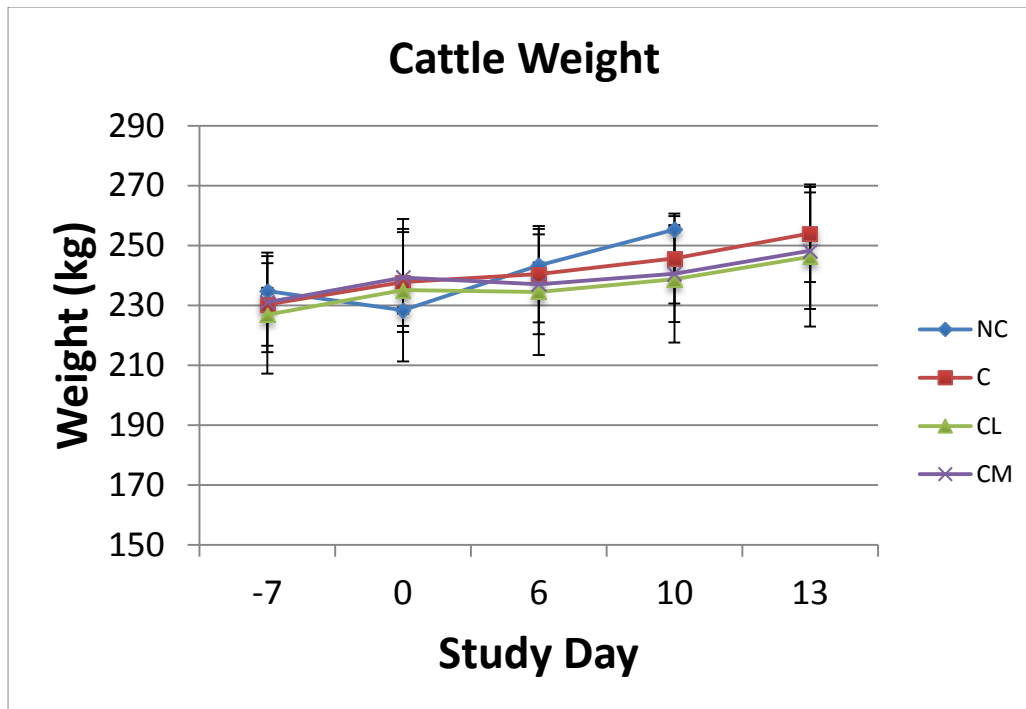


Figure 4: Mean (SD) weight over the course of the study.

4.3.2 Pedometry

The pedometry data generated three parameters: activity (steps/hour), rest (minutes/24 h) and bout (averages minutes spent resting). There was no treatment effect for activity. For rest there was a treatment and day interaction term whereby the duration of rest over the study was greatest in the CM group and lowest in the C group. Furthermore the duration of rest decreased in all groups over the study period. For bout there was a treatment effect in that the duration of bouts were longest in the CM group and shortest in the C group. Finally, those animals that had longer bouts prior to anaesthesia and surgery maintained the habit of taking longer bouts after anaesthesia and surgery (Appendix 1).

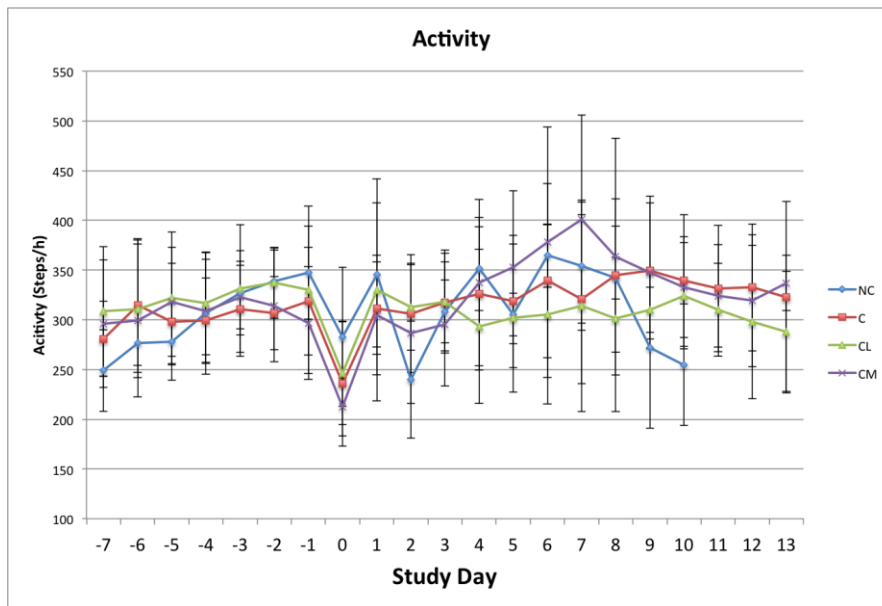


Figure 5: Activity (steps/h). Values are mean (SD).

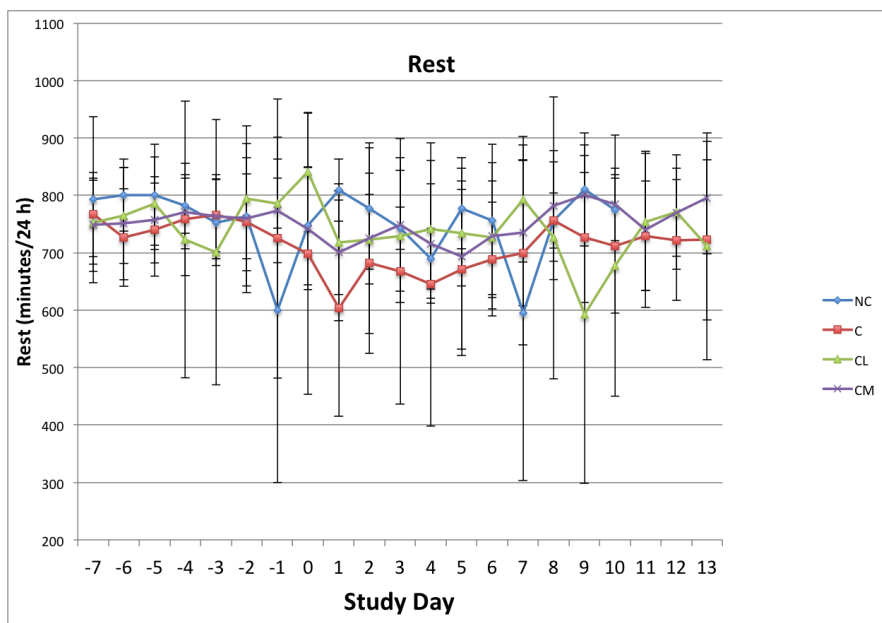


Figure 6: Rest (minutes/24 hours). Values are mean (SD).

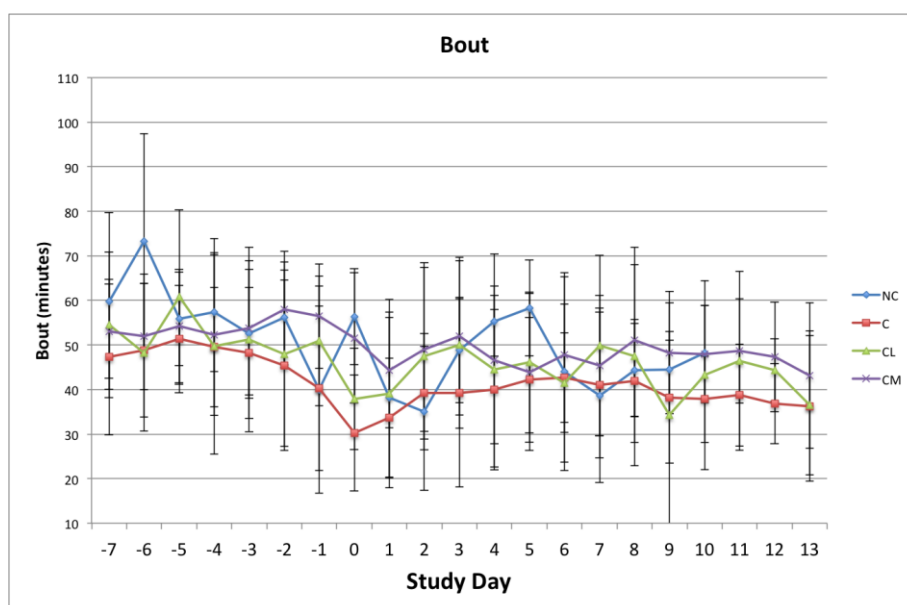


Figure 7: Bout (average duration of rest in minutes). Values are mean (SD).

4.3.3 Behavioural ethograms

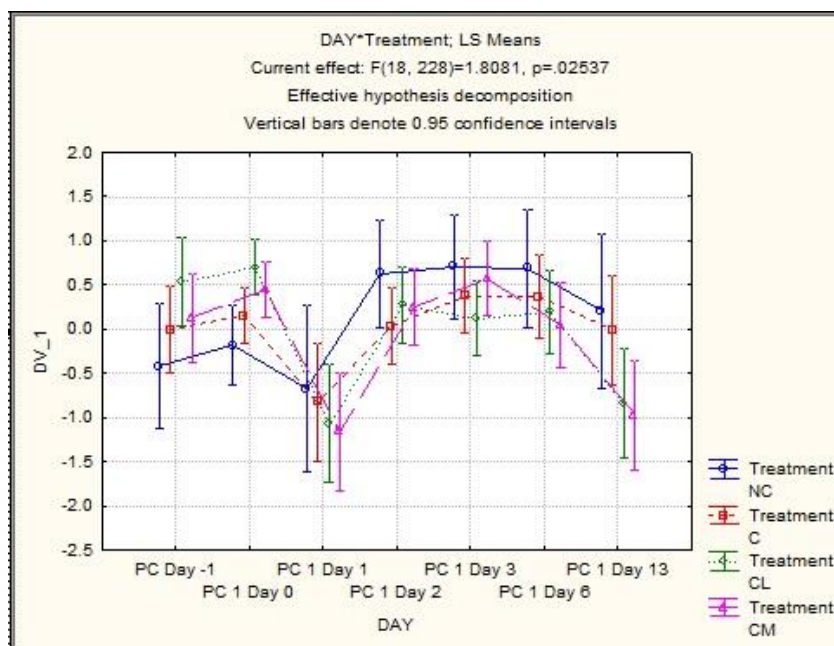
4.3.3.1 Behaviour analysis Days -1 to 13 in paddock

No correlation between any behaviour were found, however certain behaviours including, mounting, stumbling, urination, defecation, neck curl and lying head flat were removed from analysis due to the low frequency of occurrence. The principle components analysis (PCA) generated four main factors with Eigenvalues > 1.0 (Table 5). Terms that are strongly loaded (>75% of the highest absolute correlation coefficient value) on either axis of each dimension are described. However, on principle component (PC) 1 and PC2, terms only loaded strongly on the low end only of each dimension. Terms loaded on PCA1 were hind-quarter groom (-0.76), autogroom (-0.68), head flick (-0.68), tail swish (-0.63) and restlessness (-0.63) and terms loaded on PC2 were feed (-0.95), walk/seek (-0.94) and stand (-0.94). Terms loaded with PC3 were social (-0.71) and bunt (-0.6) on the low end and lying (0.55) on the high end, respectively. Terms loaded with PC4 were alert (-0.6) on the low end, and ruminating (0.58) on the high end.

Table 5: Results of PCA and repeated measures ANOVA for bulls in the paddock. * $p < 0.05$.

Value Number	PC1	PC2	PC3	PC4
Eigenvalue	3.757	2.923	2.273	1.888
% Total Variance	14.451	11.240	8.743	7.263
Treatment F3, 102	0.52	1.073	0.253	0.053
Treatment P-value	0.672	0.372	0.859	0.984
Day F3, 102	13.81	1.812	2.114	2.644
Day P-value	0.00*	0.098	0.053	0.017*
Day x Treatment	1.81	0.694	1.394	1.132
Day x Treatment P-value	0.025*	0.815	0.135	0.322

The PC1 repeated measures ANOVA (Figure 8a and 8b) showed a day by treatment effect ($p < 0.05$) for all groups. On Day +1, bulls in all treatment groups performed significantly more 'restless' behaviour, including *hind quarter grooming*, *grooming*, *head flick* and *tail swishing* than on other days, and a trend towards less *lying* and *ruminating* behaviour.

**Figure 8a:** Repeated Measures ANOVA for the four treatment groups for PCA1 in the paddock.

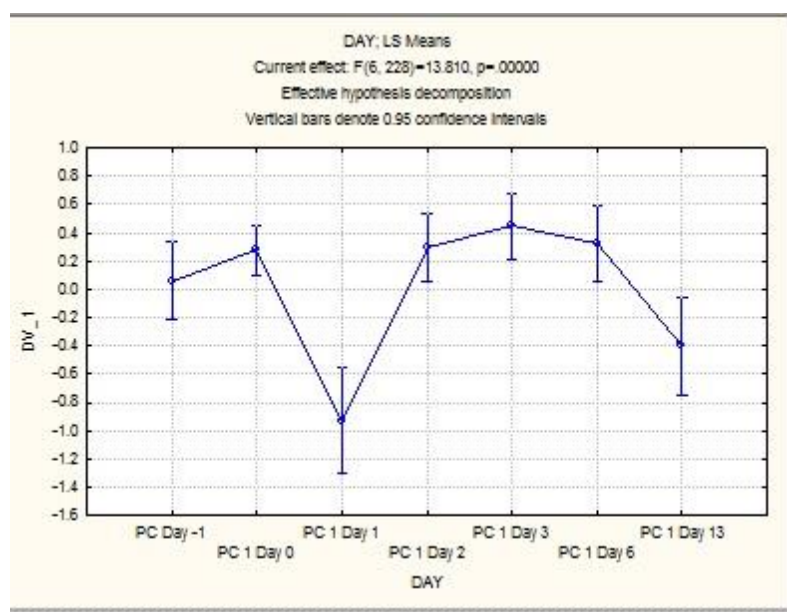


Figure 8b: Repeated Measures ANOVA for the combined groups over day for PCA1 in the paddock.

A post hoc analysis for PC1 day effects found that the following significant differences: day 1 with day -1 ($P<0.01$), with day 0 ($P<0.01$), day 2 ($P<0.01$), day 3 ($P<0.01$) and day 6 ($P<0.01$). PC1 values on day 13 were different to day -1 ($P<0.05$), day 0 ($P<0.001$), day 2 ($P<0.01$), day 3 ($P<0.01$) and day 6 ($P<0.01$).

There were no significant treatment effects in PC2 and PC3. There was a significant day effect for PC4 where all treatment groups performed more *alert* behaviour and less *ruminating* on day 2 compared to the other days ($P<0.05$) (Figure 9).

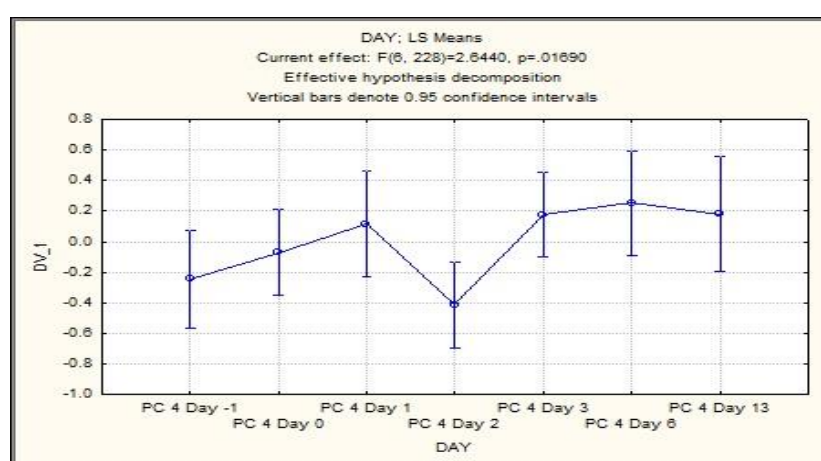


Figure 9: Repeated Measures ANOVA for the treatment groups over day for PCA4 in the paddock.

4.3.3.2 Behaviour analysis Day 0 in yard

Bull #2 (NC) was removed from the study as the bull sustained an injury upon recovery and was immediately treated with an anti-inflammatory. The footage of Bulls #19 (C), #20 (CL) and #31 (C) post-surgery was inadequate and therefore not used in this study.

The correlation matrix found that the behaviour for social and play (0.8), social and instigate/receive grooming (0.9), bunt and play (0.9), instigate/receive grooming and autogroom (0.7), bunt and social (0.8), and lying with neck extended and lying head flat (0.8) were highly correlated. As they were so similar, the behavioural counts for social, instigate/receive grooming, bunt and lying with neck extended were taken out of the analysis to allow the data to remain singular. Behaviours, including mounting, urination, defecation, hind limb stumbles, pawing and lying nose to dirt, were removed from further analysis due to the low frequency of occurrence.

The PCA generated four main factors with Eigenvalues > 1.0 that are listed in Table 6. Terms that are strongly loaded (>75% of the highest absolute correlation coefficient value) on either axis of each dimension are described, however on PC2, only terms associated with the low end were identified. Terms strongly loaded for PC1 were *lying* (-0.78), *lying head flat* (-0.59) on the low end, and *low head position* (0.64), and *alert* (0.74) on the high end of the dimension axis, respectively. Terms strongly loaded for PC2 were *licking nose* (0.60), *hind-quarter grooming* (0.75) and *autogroom* (0.78). Terms strongly loaded for PC3 were *standing* (-0.69) at the low end and *feeding* (0.53) on the high end. Terms strongly loaded for PC4 were *restless* (-0.47), *play* (-0.44), *walk/seek* (-0.44) on the negative axis and *saw-horse* (0.31) on the positive axis.

Table 6: Yard results. * $p < 0.05$.

Value Number	PC1	PC2	PC3	PC4
Eigenvalue	3.25	2.67	1.91	1.63
% Total Variance	12.98	10.75	7.65	6.52
Treatment F3, 102	1.573	3.083	1.233	2.226
Treatment P-value	0.214	0.04*	0.313	0.103
Time F3, 102	1.09	6.576	1.807	0.896
Time P-value	0.357	0.00*	0.151	0.446
Time x Treatment	2.548	0.867	2.222	1.002
Time x Treatment P-value	0.011*	0.558	0.026*	0.444

The results showed a time by treatment effect ($P < 0.05$) for PCA 1, with the post hoc test showing a significant difference between CM and C at 3 hours ($P < 0.01$) (Figure 10).

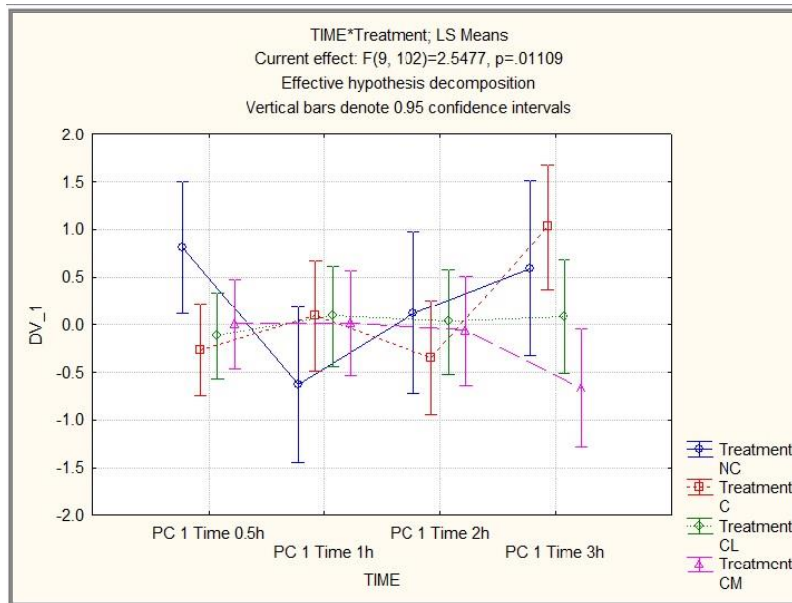


Figure 10: Repeated Measures ANOVA for the time x treatment groups for PCA1 in the yard.

PC2 showed a time effect where all treatment groups increased *nose licking*, *hind quarter grooming* and *autogrooming* from 0.5h until 3h ($P<0.001$) (Figures 11a and 11b).

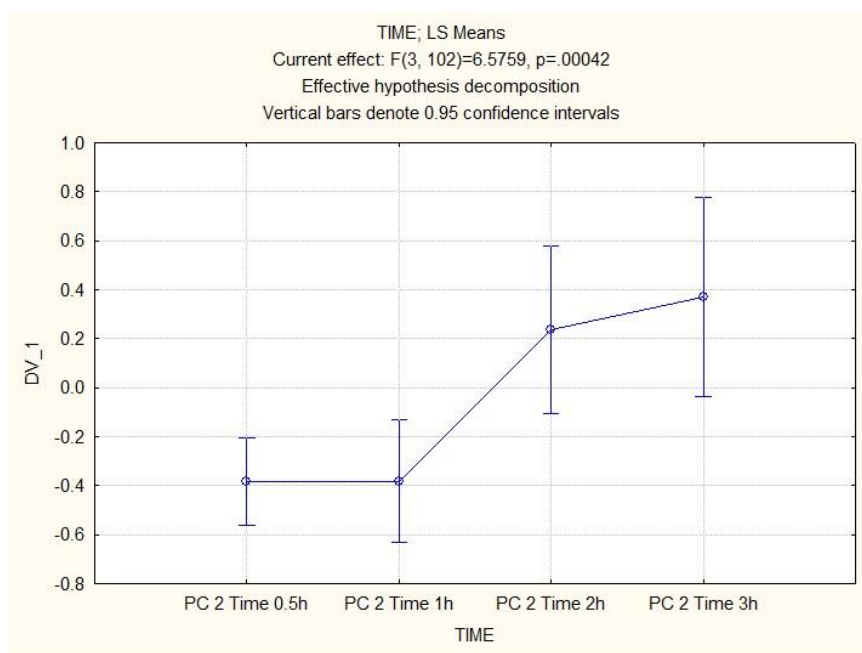


Figure 11a: Repeated Measures ANOVA over time for groups for PCA2 in the yard.

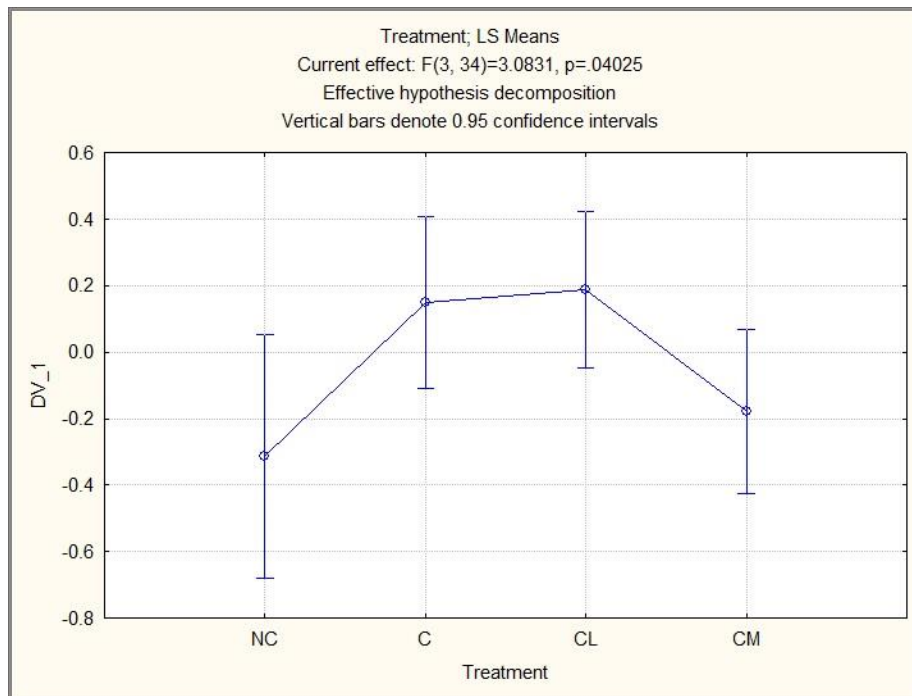


Figure 11b: Repeated Measures ANOVA for treatment groups for PCA2 in the yard.

A post hoc for PC2 day effects found the following significant differences: 3 h with 0.5 h ($P>0.05$) and with 1 h ($P<0.001$), and 2 h with 1 h ($P<0.05$). In addition, there was difference between C at 3 h and CM at 0.5 h ($P<0.05$) and 1 h ($P<0.01$). There was a significant difference between C at 3 h and the NC at 0.5 h ($P<0.01$).

PC3 also showed a time by treatment effect where NC cattle were feeding more and standing less at 0.5 h compared to 3 h ($P<0.05$) (Figure 12).

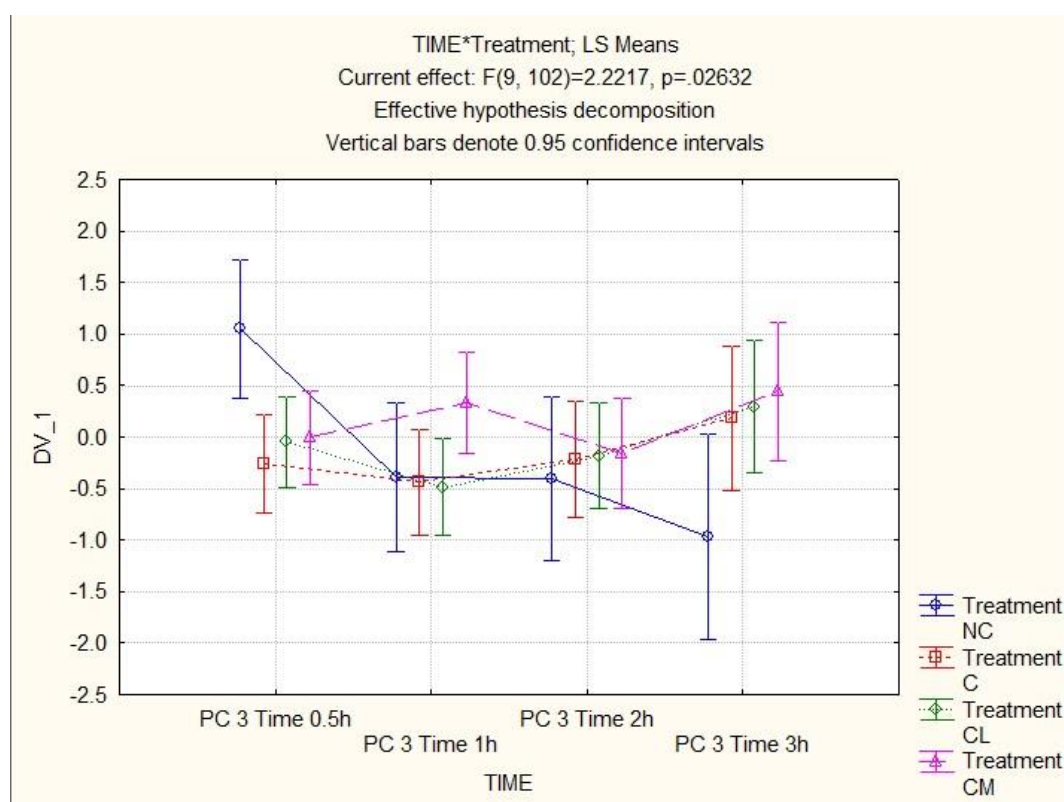


Figure 12: Repeated Measures ANOVA for PC3 for time x treatment groups in yard.

4.4 Quantitative Behavioural Analysis (QBA)

These analyses are ongoing and these data are not included in this report.

5 Discussion

The primary aim of the current study was to describe the EEG changes associated with castration in *Bos indicus* bull calves utilising the minimal anaesthesia model. The effect of pre-operative administration of lidocaine and meloxicam on this EEG response was also evaluated. In addition, the acute cardiovascular response to castration was assessed as well as a range of post-operative pain measures. The focus on the acute response associated with nociception was deemed important as there is little information in the literature about pain assessment in *Bos indicus* animals and previous work by this group of researchers was unable to identify a reliable and consistent method to differentiate animals that had been or had not been castrated with or without analgesia (Final Report B.AWW.0223). The most useful assessments in the previous study were weight changes and pedometry variables.

5.1 Acute responses to castration

5.1.1 EEG

The EEG response to castration was recorded and indicates that *Bos indicus* bull calves mount a nociceptive response to castration without analgesia. The pre-operative administration of lidocaine attenuated, but did not abolish, the EEG response to surgical castration. Moreover, bull calves pre-treated with meloxicam exhibited a diminished EEG response compared to the control group. However, this response was still increased compared to the lidocaine group. These results were not as expected: the lidocaine group was expected to show a complete abolition of an EEG response to castration, and the meloxicam group was expected to show no mitigation. Overall these data demonstrate that both lidocaine and meloxicam reduce nociception following surgical castration, however lidocaine does so to a significantly greater extent.

Previous studies have described the EEG responses seen in different species during noxious stimuli and concurrent analgesic administration (Gibson et al., 2007a, Johnson et al., 2005b, Murrell et al., 2010, Johnson et al., 1999). This study is the first description of such EEG responses and changes accorded to analgesic therapy in *Bos indicus* cattle. In animal studies three different EEG derived frequencies are principally investigated in both anaesthetised and conscious animals (F_{50} , F_{95} P_{tot}). An increase in F_{50} in deer, horses, donkeys and ponies is indicative of nociception with the minimal anaesthesia model (Johnson et al., 2005b, Grint et al., 2014c, Murrell et al., 2003). Additionally studies in conscious sheep have further supported the link between an increase in F_{50} and pain-related behaviour (Ong et al., 1997). The current study reflected this change with an increase in the F_{50} in animals that had not received analgesia, however this result did not reach significance. Additionally, lidocaine administration was associated with attenuation of the increase in F_{50} , indicative of anti-nociception, a finding similarly described during the castration of piglets (Haga and Ranheim, 2005).

In the current study, the F_{95} was significantly greater after castration, compared to baseline, in all the groups and between all three treatment groups with a reduced magnitude of response in group CL compared to groups C and CM. During castration of lambs and donkeys (Grint et al., 2014b, Johnson et al., 2009) and the removal of antler in deer (Johnson et al., 2005b) the F_{95} increased in response to noxious stimuli, whereas during castration of ponies (Murrell et al., 2003) and during ovariohysterectomy of rats (Murrell et al., 2010) no change in this parameter was observed. This diversion of response in the face of noxious stimuli may be associated with the stimulus itself, or a species specific response. An increased F_{95} linked to nociception in cattle was found following scoop-dehorning of Friesian calves under halothane anaesthesia without analgesia (Gibson et al., 2007a).

The P_{tot} decreased significantly in each study group immediately following the first incision, a finding consistent with other studies of castration using the minimal anaesthesia model (Grint et al., 2014b, Haga and Ranheim, 2005, Murrell et al., 2003). Further support for changes in the spectral frequencies presented here being associated with nociception, is the maintenance of similar anaesthetic depth with the physiological parameters of $FE'HAL$, $FE'CO_2$, temperature and oxygenation held within tight limits for all animals used in the study as dictated by the minimal anaesthesia model (Murrell et al., 2003). These results

subsequently support the premise that surgical castration of *Bos indicus* bull calves results in nociception, circumventing the difficulty of behavioural assessment in this species.

To optimise the quality of EEG data collected in studies such as this it is important to consider the influence of any anaesthetic or analgesic drug administered to the animal and to maintain physiological parameters within a normal range. Different anaesthetic agents cause different levels of cortical depression and thus alter the EEG. Halothane, the agent shown to have least cortical depression and no analgesic properties at a light plane of anaesthesia was used in this study (Murrell et al., 2008). Additionally, anaesthesia was induced with halothane delivered by a facemask so no other drugs were administered, in accordance with the minimal anaesthesia model (Murrell and Johnson, 2006). Previous studies have used short-acting injectable anaesthetic induction agents to ensure safety of the animals and personnel in large animals used in similar studies (Johnson et al., 2005b, Johnson and Taylor, 1997). Using tilt-table restraint and mask placement, no operator or bull calf was injured during anaesthetic induction.

The premise that lidocaine would prevent any change to the EEG was not confirmed in the current study. Nevertheless, the EEG response following the first incision was significantly reduced in magnitude in group CL and this result indicates an anti-nociceptive action of lidocaine. Additionally, towards the end of the castration procedure, between 20 and 40 seconds following the start of surgery, a delayed nociceptive response is seen in group CL. This delayed response suggests that the distribution of lidocaine in this study did not completely desensitise the surgical site.

Previous studies demonstrate that the use of intra-testicular, subcutaneous or intra-funicular lidocaine prior to castration under general anaesthesia results in reduced, but not complete ablation of, nociception. This reduced nociceptive response is demonstrated by a decrease in the HR and an increase in the MAP in dogs (Huuskonen et al., 2013), a reduced rise in the pulse rate (PR) and MAP, and heart rate variability (HRV) in cats (Moldal et al., 2013), and EEG alterations in piglets (Haga and Ranheim, 2005). The incomplete blockade of the nociceptive response in the current study is likely associated with insufficient intra-abdominal effects of lidocaine-mediated nociceptors during traction on the spermatic cord. Similar separate reactions have been shown during castration in cattle treated with local anaesthesia where the initial skin incision and handling of the testicle provoked little behavioural reaction, however spermatic cord traction did induce pain-related behaviours (Thüer et al., 2007). This reaction is also supported by the reappearance of nociceptive EEG responses in group CL towards the end of removal of the testicle during traction on the spermatic cord. Given the castration method used currently, which is completed with the removal of the testicle via a stretching and ultimately tearing action, only more complex local anaesthetic techniques such as epidural or intrathecal anaesthesia would be able to fully block the surgical site.

The EEG responses in group CM in the current study highlights some interesting components of the mechanism of action of non-steroidal anti-inflammatory drugs as well as their possible clinical applications. As discussed above the significantly different responses seen in both the F_{95} and P_{tot} between each study group indicates a nociceptive response in the control group and the amelioration of this reaction in both analgesic treatment groups. However, the nociceptive response was greater when meloxicam had been administered, compared to when lidocaine had been administered. Previous studies have suggested a

centrally-mediated mode of action for non-steroidal anti-inflammatory drugs, principally associated with a down-regulation of nociceptive spinal pathways (Díaz-Reval et al., 2004). The results of this study lend support to the theory that meloxicam has a central effect which attenuates, to some extent, the nociceptive response to castration.

There are some limitations to this study. A sham injection to assess the effect of injecting lidocaine as a noxious stimulus in itself was not used in this study. However, the analysis comparing the baseline to the EEG response following lidocaine injection indicated no significant changes in any of the EEG parameters. This finding is consistent with other studies in cattle and piglets (Gibson et al., 2007a, Haga and Ranheim, 2005). Furthermore, it must be emphasised that EEG analysis following surgical stimuli is useful for the assessment of peracute nociception and further pain assessment requires additional post-operative, longitudinal assessment including behavioural and physiological factors.

To summarise the EEG findings: the administration of lidocaine prior to castration was most effective at preventing the acute post-operative nociceptive response as assessed by the EEG following castration in 6-8 month old bull calves. Pre-operative meloxicam also attenuated the EEG response to castration although further research into the mechanism of this effect is warranted. These findings provide support for the pre-operative administration of both lidocaine and meloxicam for castration in *Bos indicus* bull-calves.

5.1.2 Cardiovascular responses

The cardiovascular responses to castration were recorded simultaneously with the EEG. The alteration in the MAP and HR were similar insofar as a decrease in the HR was associated with a decrease in the MAP. This simultaneous decrease occurs as the HR is a major determinant of blood pressure according to the equation: arterial blood pressure = heart rate x stroke volume x systemic vascular resistance.

In deer it has previously been shown that nociception is associated with an acute and temporary decrease in HR, but not blood pressure (Woodbury et al., 2005). Similarly in piglets there was a decrease in HR following castration but MAP increased (Haga and Ranheim, 2005). Following castration in lambs the HR has also been reported to decrease transiently (Johnson et al., 2005a). In cattle a transient bradycardia following dehorning is reported (Gibson et al., 2007b). This acute decrease in HR is associated with a noxious stimulus and supports the data in the current study that a nociceptive response is apparent in cattle that were castrated without analgesia. Lidocaine and, to a lesser extent, meloxicam attenuated this response.

To summarise the cardiovascular responses to castration: the administration of lidocaine and meloxicam provides analgesia for castration of *Bos indicus* bull calves during anaesthesia with halothane.

5.2 Post-operative assessments

5.2.1 Liveweight and pedometry

There were no differences between the groups with regards to changes in weight over the course of the study. This parameter has previously been incorporated into pain assessment strategies in cattle in other studies (Coetzee, 2013), including our previous study (Final

Report B.AWW.0223), as a useful tool to differentiate animals that have well controlled pain to those that do not. In the current study the change in weight was not different between treatment groups. All the animals gained weight during the study. It is possible that despite having animals in this study that were not castrated that the impact of general anaesthesia is great enough to mask any subtle alterations in feed intake and weight gain.

Pedometry proved a useful tool for pain assessment in the previous study (Final Report B.AWW.0223) where we showed that animals that had received pre-operative meloxicam were more active in the post-operative period. With regards to rest, the previous study demonstrated that all animals that were castrated, with or without analgesia, rested for less time than animals that had not been castrated. This result suggests that animals that have not had a painful experience are more restful. Furthermore, our previous study showed that animals that had received post-operative meloxicam had fewer rest bouts than those that did not receive post-operative meloxicam. This difference was not evident after the first post-operative day (Day 1). Overall these results are perplexing. On one hand animals that are less painful, or not painful at all, rest for longer and more often and are therefore less active. On the other hand animals that have had post-operative pain relief rested less, at least on the first day after surgery.

In the current study, the animals that had received meloxicam rested for longer and more often than those that did not receive meloxicam. In turn, the animals that rested the least were those that had not received any analgesia. These results did not translate to a difference in activity between the groups.

5.2.2 Behaviour

In general, the analysis of behaviour in both the home paddock over 13 days, and recovery yard on the day of surgery, indicated there were subtle differences between bulls that were expected to be in pain and those that were not. Some behavioural patterns were common across all treatment groups over the 2-week study, yet some subtle changes between treatment groups occurred after recovery on day 0. The analysis in the home paddock indicated the main dimension (PC1) depicted a level of comfort, where all animals on Day +1 demonstrated more *restless* behaviour than on any other day prior to day 13. All bulls exhibited more *hindquarter grooming*, *autogrooming*, *head flicking* and *tail swishing* behaviour regardless of their treatment on this day. This suggests that the restless behaviour may be in response to the activity of the previous day, including the effects of handling and general anaesthesia, and was not restricted to the castrated only animals.

If continuous filming of these bulls was used to count behaviour over 24 hour periods perhaps subtler pain behaviour would be evident, however this was not practical for this study given the desire to allow the bulls to remain in an extensive home paddock. It is likely that the daily time period for recording of observations was insufficient to determine whether the bulls were in pain or not. Given they are rangeland cattle not well habituated to people, more post-operative confinement to allow better video surveillance may equally have prevented the expression of behaviour subtleties.

On the day of surgery, the main dimension (PC1) showed a time x treatment effect between CM and C at 3 h. Cattle castrated with meloxicam showed more *lying* and *lying with their head flat on their ground* compared to the cattle that were castrated only. This suggests that

the pre-operative meloxicam may have provided sufficient pain relief to enable more resting behaviour than those that did not receive any analgesia. The C bulls showed more *low head position* and *alert* behaviour than CM bulls which might also be reflective of a pain or fear response. Interestingly, we did not see differences between lying behaviour between C and NC animals in this dimension, but given NC bulls were pain free their desire to lie down at 3 h may have been non-existent. There appears to be an absence of an effect on resting behaviour in the CL group.

A time effect on PC2 ($P < 0.001$) where all treatment groups increased grooming behaviour such as nose *licking*, *hind quarter grooming* and *autogrooming* from 0.5 h until 3 h. Bulls in Group C performed more of such grooming at 3 h compared to those in Group CM at 0.5 and 1 h, and to Group NC at 0.5 h. Hence, such grooming might indicate normal activity within 3 h following handling and recovery from anesthesia, but may be slightly more pronounced in bulls without pain relief.

A time by treatment effect on PC3 showed NC cattle were *feeding* more and *standing* less at 0.5 h compared to 3 h ($P < 0.05$). This suggests completely pain free cattle such as NC bulls return to feeding earlier compared to cattle that had just undergone castration. Thus, bulls given some analgesia took a longer time to return to feeding than those pain free. Hence early return to feeding may be an indicator of the absence of pain, but it is confounded by the effect of the anaesthesia.

6 Conclusions/recommendations

The results of this study provide strong evidence that the administration of pre-operative lidocaine and meloxicam decreases the acute nociceptive response to castration in *Bos indicus* bull calves. The acute response, as evidenced by the EEG and the cardiovascular responses is transient, and it is essential to ensure that pain assessment beyond the acute phase is addressed. Our efforts at post-operative pain assessment were somewhat unrewarding in identifying a method to reliably differentiate the treatment groups.

In conclusion, pain assessment in cattle is difficult and despite multiple approaches to this problem in this cohort of animals we were unable to readily identify a reliable method of clearly differentiating the treatment groups in the post-operative period. Nevertheless, in the acute phase following castration changes, both the EEG and in cardiovascular performance (MAP and HR) support the premise that lidocaine, and to a lesser extent meloxicam mitigate the nociceptive response to castration in *Bos indicus* bull calves. In the days following castration animals that had received meloxicam were more restful.

7 Key messages

Pre-operative administration of local anaesthetic (e.g. lidocaine) and a non-steroidal anti-inflammatory (e.g. meloxicam) have an anti-nociceptive effect during castration of *Bos indicus* bull calves. The administration of these drugs prior to castration will increase the financial cost of surgery but will improve animal welfare.

8 Bibliography

- COETZEE, J. F. 2013. Assessment and management of pain associated with castration in cattle. *Vet Clin North Am Food Anim Pract*, 29, 75-101.
- DÍAZ-REVAL, M. A. I., VENTURA-MARTÍNEZ, R., DÉCIGA-CAMPOS, M., TERRÓN, J. A., CABRÉ, F. & LÓPEZ-MUÑOZ, F. J. 2004. Evidence for a central mechanism of action of S-(+)-ketoprofen. *European Journal of Pharmacology*, 483, 241-248.
- DIESCH, T. J., MELLOR, D. J., JOHNSON, C. B. & LENTLE, R. G. 2009. Electroencephalographic responses to tail clamping in anaesthetized rat pups. *Laboratory Animals*, 43, 224-231.
- GHADIRIAN, S., VESAL, N., MAGHSOUDI, B. & AKHLAGH, S. H. 2016. Comparison of lidocaine, lidocaine–morphine, lidocaine–tramadol or bupivacaine for neural blockade of the brachial plexus in fat-tailed lambs. *Veterinary Anaesthesia and Analgesia*, 43, 109-116.
- GIBSON, T., JOHNSON, C., STAFFORD, K., MITCHINSON, S. & MELLOR, D. 2007a. Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal*, 55, 152-157.
- GIBSON, T. J., JOHNSON, C. B., MURRELL, J. C., HULLS, C. M., MITCHINSON, S. L., STAFFORD, K. J., JOHNSTONE, A. C. & MELLOR, D. J. 2009. Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning. *New Zealand Veterinary Journal*, 57, 77-83.
- GIBSON, T. J., JOHNSON, C. B., STAFFORD, K. J., MITCHINSON, S. L. & MELLOR, D. J. 2007b. Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal*, 55, 152-157.
- GRINT, N. J., JOHNSON, C. B., CLUTTON, R. E., WHAY, H. R. & MURRELL, J. C. 2014a. Spontaneous electroencephalographic changes in a castration model as an indicator of nociception: A comparison between donkeys and ponies. *Equine Veterinary Journal*, n/a-n/a.
- GRINT, N. J., JOHNSON, C. B., CLUTTON, R. E., WHAY, H. R. & MURRELL, J. C. 2014b. Spontaneous electroencephalographic changes in a castration model as an indicator of nociception: A comparison between donkeys and ponies: Electroencephalographic response to nociception in donkeys and ponies. *Equine Veterinary Journal*, n/a-n/a.
- GRINT, N. J., JOHNSON, C. B., DE SA LORENA, S., LUNA, S., HUSSNI, C. A., WHAY, H. R. & MURRELL, J. C. 2014c. Electroencephalographic Responses to a Noxious Surgical Stimulus in Mules, Horses, and Ponies. *Journal of Equine Veterinary Science*.
- HAGA, H. A. & DOLVIK, N. I. 2005. Electroencephalographic and cardiovascular variables as nociceptive indicators in isoflurane-anaesthetized horses. *Veterinary Anaesthesia and Analgesia*, 32, 128-135.
- HAGA, H. A. & RANHEIM, B. 2005. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary anaesthesia and analgesia*, 32, 1-9.
- HUUSKONEN, V., HUGHES, J. M. L., ESTACA BAÑON, E. & WEST, E. 2013. Intratesticular lidocaine reduces the response to surgical castration in dogs: Intratesticular lidocaine decreases response to surgery. *Veterinary Anaesthesia and Analgesia*, 40, 74-82.
- JOHNSON, C. B., BLOOMFIELD, M. & TAYLOR, P. M. 1999. Effects of ketamine on the equine electroencephalogram during anesthesia with halothane in oxygen. *Vet Surg*, 28, 380-5.
- JOHNSON, C. B., STAFFORD, K. J., SYLVESTER, S. P., WARD, R. N., MITCHINSON, S. L. & MELLOR, D. J. 2005a. Effects of age on the electroencephalographic response

- to castration in lambs anaesthetised using halothane in oxygen. *N Z Vet J*, 53, 433-437.
- JOHNSON, C. B., SYLVESTER, S. P., STAFFORD, K. J., MITCHINSON, S. L., WARD, R. N. & MELLOR, D. J. 2009. Effects of age on the electroencephalographic response to castration in lambs anaesthetized with halothane in oxygen from birth to 6 weeks old. *Vet Anaesth Analg*, 36, 273-9.
- JOHNSON, C. B. & TAYLOR, P. M. 1997. Effects of alfentanil on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Research in Veterinary Science*, 62, 159-163.
- JOHNSON, C. B., WILSON, P. R., WOODBURY, M. R. & CAULKETT, N. A. 2005b. Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Veterinary anaesthesia and analgesia*, 32, 61-71.
- KONGARA, K., CHAMBERS, J. P. & JOHNSON, C. B. 2011. Effects of tramadol, morphine or their combination in dogs undergoing ovariohysterectomy on peri-operative electroencephalographic responses and post-operative pain. *New Zealand Veterinary Journal*, 60, 129-135.
- MCILHONE, A. E., BEAUSOLEIL, N. J., JOHNSON, C. B. & MELLOR, D. J. 2014. Effects of isoflurane, sevoflurane and methoxyflurane on the electroencephalogram of the chicken. *Veterinary Anaesthesia and Analgesia*, n/a-n/a.
- MOLDAL, E. R., ERIKSEN, T., KIRPENSTEIJN, J., NODTVEDT, A., KRISTENSEN, A. T., SPARTA, F. M. & HAGA, H. A. 2013. Intratesticular and subcutaneous lidocaine alters the intraoperative haemodynamic responses and heart rate variability in male cats undergoing castration. *Vet Anaesth Analg*, 40, 63-73.
- MURRELL, J. C. & JOHNSON, C. B. 2006. Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics*, 29, 325-335.
- MURRELL, J. C., JOHNSON, C. B., WHITE, K. L., TAYLOR, P. M., HABERHAM, Z. L. & WATERMAN-PEARSON, A. E. 2003. Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia*, 30, 138-146.
- MURRELL, J. C., MITCHINSON, S. L., LESPERANCE, L., SIVAKUMARAN, S. & JOHNSON, C. B. 2010. Electroencephalography during ovariohysterectomy in rats anaesthetized with halothane: EEG changes during OVH in rats. *Veterinary Anaesthesia and Analgesia*, 37, 14-24.
- MURRELL, J. C., WATERS, D. & JOHNSON, C. B. 2008. Comparative effects of halothane, isoflurane, sevoflurane and desflurane on the electroencephalogram of the rat. *Laboratory animals*, 42, 161-170.
- ONG, R. M., MORRIS, J. P., O'DWYER, J. K., BARNETT, J. L., HEMSWORTH, P. H. & CLARKE, I. J. 1997. Behavioural and EEG changes in sheep in response to painful acute electrical stimuli. *Aust Vet J*, 75, 189-93.
- SCHNITZLER, A. & PLONER, M. 2000. Neurophysiology and Functional Neuroanatomy of Pain Perception. *Journal of Clinical Neurophysiology*, 17, 592-603.
- THÜER, S., MELLEMA, S., DOHERR, M. G., WECHSLER, B., NUSS, K. & STEINER, A. 2007. Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods in calves. *The Veterinary Journal*, 173, 333-342.
- WOODBURY, M. R., CAULKETT, N. A., JOHNSON, C. B. & WILSON, P. R. 2005. Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): cardiovascular and somatic responses. *Veterinary Anaesthesia and Analgesia*, 32, 72-82.

9 Appendix

See attachment: Appendix 1.