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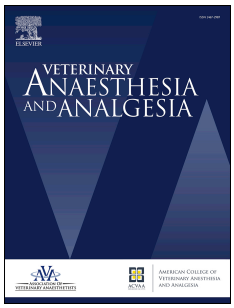

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RESEARCH PAPER

Mitigation of electroencephalographic and cardiovascular responses to castration in *Bos indicus* bulls following the administration of either lidocaine or meloxicam

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Authors' contributions

H.L. – Design, experimental procedure, data collection, statistical analysis, management and interpretation, preparation and revision of manuscript.

G.M. – Design, experimental procedure, data collection, preparation and revision of manuscript.

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T.H. – Design, experimental procedure, preparation and revision of manuscript.

J.T. – Statistical analysis.

T.C. – Design, experimental procedure.

K.B.G. – Design, experimental procedure.

C.B.J. – Design, experimental procedure, data collection, management and interpretation, preparation of manuscript.

Abstract

Objective To investigate the mitigating effects of administration of local anaesthetic or systemic meloxicam on the electroencephalographic (EEG) and cardiovascular responses during surgical castration of *Bos indicus* bull calves.

Study Design Prospective, randomized, experimental study.

Animals 36 six-to-eight month old *Bos indicus* bull calves, mean ± SD weight of 237 ± 19kg.

Methods 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 EEG (P_{tot}) were analysed using area-under-the-curve comparing T1 to B.

Results All EEG variables were significantly different between B and T1 ($p \leq 0.0001$). No differences in F_{50} were found between groups during T1 ($p = 0.6491$). F_{95} and P_{tot} were significantly different between group L and groups C and M during T1 ($p = 0.0005$ and 0.0163 respectively). There were transient significant changes in HR and MAP in groups L and M compared to group C during the 20-50 second periods.

Conclusions The EEG changes indicate nociceptive responses in all three groups during surgical castration, greater in group L compared to groups C and M. Both analgesics attenuated the peracute cardiovascular response. Lidocaine and meloxicam administered prior to castration attenuated these responses in *Bos indicus* bull calves.

Clinical Relevance These findings provide support for the pre-operative administration of lidocaine and potentially meloxicam for castration in *Bos indicus* bull calves.

Keywords analgesia, *Bos indicus*, castration, cardiovascular, electroencephalography.

Introduction

Surgical castration of young cattle is a common husbandry procedure and in various parts of the world, including the United States and Australia, the procedure is performed without the use of anaesthesia or analgesia (Bayley 2010; Coetzee 2013; AHA 2014). Many studies have demonstrated that castration without analgesia is a cause of significant pain in cattle (Coetzee 2013). A wide range of experimental techniques including behavioural (de Oliveira et al. 2014), physiological and neuroendocrine assessments (Petherick et al. 2014a; Petherick et al. 2014b; Laurence et al. 2016; Musk et al. 2016) have been used to assess pain in cattle.

Societal expectations of production animal welfare are increasing. These values are having a positive impact on the drive for innovative animal welfare science and practical approaches to mitigate pain in livestock are a research focus (Weary & Fraser 2004). The World Organisation for Animal Health (2015), states that ‘where painful

procedures cannot be avoided, the resulting pain should be managed to the extent that available methods allow'. Strategies to alleviate surgical pain have been investigated in a number of farmed species including the use of topical analgesics following mulesing in merino lambs (Lomax et al. 2008) and local anaesthetic ring blocks for velvet antler removal in deer (Johnson et al. 2005). There are only a small number of studies investigating the use of analgesia in *Bos indicus* cattle following painful husbandry procedures (Petherick et al. 2014b; Petherick et al. 2014a; Laurence et al. 2016; Musk et al. 2016).

The use of electroencephalography (EEG) for assessing nociception in various animal species has been reported (Murrell & Johnson 2006), but assessments in cattle have been limited to *Bos taurus* (Gibson et al. 2007; Bergamasco et al. 2011). Electroencephalography assesses the sensory component of pain, as opposed to the emotional and behavioural response, and therefore produces objective data. In humans, the magnitude and type of EEG response to a noxious stimuli is tightly linked to the intensity of the noxious stimuli (Chen et al. 1989).

For maintenance of anaesthesia, halothane causes less cortical depression than isoflurane, sevoflurane and desflurane (Murrell et al. 2008). By using halothane alone for the induction and maintenance of anaesthesia, anaesthetic depth can be maintained at a level that maintains unconsciousness and immobility but allows EEG changes that are evoked by noxious stimuli to be demonstrated (Murrell & Johnson 2006). This method of anaesthesia has been referred to as the 'minimal anaesthesia model' (Murrell & Johnson 2006; Johnson et al. 2012).

Previous studies have demonstrated attenuation of EEG response to noxious stimuli by various analgesic medications. Local anaesthetic infiltration completely blocked nociception during dehorning in cattle (Gibson et al. 2007) and markedly decreased responses have been seen during the castration of piglets (Haga & Ranheim 2005).

The aim of the current study was to record the EEG and cardiovascular responses to assess the degree to which local anaesthesia with lidocaine, or systemic meloxicam, ameliorated the noxious effects of surgical castration in halothane-anaesthetised *Bos indicus* bull calves.

Materials and Methods

Approval for this study was granted by the Animal Ethics Committee of Murdoch University (Permit number R2730/15) following the guidelines of the National Health and Medical Research Council of Australia's Code of Practice for the Care and Use of Animals for Scientific Purposes (2013).

Thirty-six *Bos indicus* bull calves at six-to-eight months of age were sourced from an extensive cattle station in the north-west of Australia. The animals were transported to the Murdoch University farm (Murdoch, WA, Australia) two weeks before the study commenced. The cattle had not been handled by the farmer beyond routine husbandry procedures and were not accustomed to contact with humans. Access to kikuyu pasture, oaten hay and water was allowed *ad lib* and a complete pelleted ration was fed daily (EasyBeef pellets, Milne AgriGroup Pty Ltd, WA, Australia) at approximately 3% of bodyweight. On the morning of castration, the study animals were weighed on in-race scales (Gallagher Animal Management, Australia). All animals were in normal body condition and were clinically healthy with normal appetite, drinking, defecation and urination patterns.

The cattle were assigned to three experimental groups (n = 12). Group allocation was via block randomisation to ensure that the last animal to be castrated on a given day was equally represented across the three study groups. Four animals were castrated each experimental day. The size of each study group reflected a previous study in cattle assessing EEG changes following noxious stimuli (Gibson et al. 2007) where treatment groups of ten were assessed. In one group, analgesia was provided by lidocaine (group

L). In the second group, analgesia was provided by meloxicam (group M). In the third group, preoperative analgesia was not administered (group C).

Anaesthesia

Pelleted food was withheld starting from the day before induction of anaesthesia. Each bull was directed into a custom-made tilt-table (Murdoch University Production Animal Department, WA, Australia) and restrained in left lateral recumbency with a blind-fold placed over the eyes.

Anaesthesia was induced using 5% halothane (Halothane BP, Pharmachem, Australia) in oxygen delivered via facemask from a large animal circle system connected to an anaesthetic machine with a ventilator (Tafonius Junior; Vetronic, UK) and precision vapourizer (Ohmeda Fluotec 4, UK). Once jaw tone was sufficiently relaxed, orotracheal intubation with an 18 mm, 20 mm or 22 mm internal diameter endotracheal tube (Surgivet, USA) was performed by digital palpation of the larynx. After tracheal intubation, mechanical ventilation was initiated and anaesthesia was maintained with halothane in oxygen. Following the completion of the EEG recording, all instrumentation was removed from the animal and the anaesthetic was discontinued. Following transfer to a recovery paddock, the animals remained in left lateral recumbency and had a blindfold placed over the eyes. The trachea was extubated when signs of light anaesthesia (swallowing and reacting to the presence of the tube) were apparent. Once the animals were in sternal recumbency, they were left to stand without assistance.

Instrumentation and Monitoring

Respiratory gases were sampled at the y-piece and monitored using a multiparameter monitor (Carescape B650 Anaesthetic Monitor, GE Healthcare, Finland). Inspired oxygen percentage (FIO_2) and $F_E´Hal$ were maintained at greater than 85% and between 0.9–1.1%, respectively, by adjustment of fresh gas flow and vapouriser settings. Initial ventilator settings were a tidal volume of 10 mL kg^{-1} and a respiratory rate of 10 breaths $minute^{-1}$. These settings were adjusted as required to maintain an end-tidal carbon dioxide ($F_E´CO_2$) of 6.0-7.3 kPa (45-55 mmHg).

Heart rate (HR) (derived from the ECG), peripheral arterial oxygen haemoglobin saturation (SpO_2), invasive mean arterial blood pressure (MAP) and nasopharyngeal temperature (T) were recorded every five minutes throughout anaesthesia. Invasive blood pressure was measured via a 20 gauge catheter placed in an auricular artery and a disposable pressure transducer (TruWave 3cc; Edwards Lifesciences, CA, USA) zeroed at the level of the right atrium connected via non-distensible tubing to the multiparameter monitor. ECG was recorded from Lead II with subdermal electrodes (Neurone subdermal; Ambu, Malaysia) placed in a base-apex configuration. Time to intubation and total anaesthesia time 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 at completion of anaesthesia. This blood was used for electrolyte and blood gas analysis (ABL 700 series; Radiometer, Denmark).

Electroencephalography data acquisition

151 An EEG was recorded using dermal needles (Neurone subdermal; Ambu, Malaysia).
152 The non-inverting electrode was placed midline between the medial canthi of the eyes,
153 the inverting electrode over the right mastoid process and the ground electrode 2-4 cm
154 caudal to the poll as previously described (Mayhew & Washbourne 1990). Electrodes
155 were connected to a signal amplifier (DAM 50 differential amplifier, World Precision
156 Instruments, FL, United States) via a custom-made breakout box (C. Johnson, Massey
157 University, New Zealand). The EEG was recorded with an amplifier gain ratio of
158 1000:1 in alternating current mode, a high-pass filter setting of 1 Hz and a low-pass
159 filter setting of 100 Hz. The data were digitised at a rate of 1 Hz (Powerlab 8/35, AD
160 Instruments, NSW, Australia) and continuously recorded (LabChart Pro, AD
161 Instruments, NSW, Australia) on a personal computer (Satellite C850, Toshiba
162 Corporation, Japan). Assessors, aware of treatment allocation, completed data extraction
163 and analysis off-line following the study.
164
165 *Group treatments*
166 In group L, 260 mg of lidocaine hydrochloride (1.1 ± 0.1 mg kg^{-1}) (Ilium Lignocaine
167 20, 2%, Troy Laboratories, NSW, Australia) was injected five minutes prior to the start
168 of surgery. Each injection was divided so approximately 6 mL of drug was injected into
169 each testicle and the remaining 2 mL was injected subcutaneously into the scrotal skin.
170 All lidocaine injections were carried out in the same manner by the clinician who also
171 performed the subsequent surgery. In group M, 0.5 mg kg^{-1} of meloxicam (Ilium
172 Meloxicam 20, 2%, Troy Laboratories, Australia) was injected subcutaneously in the
173 right lateral neck at least 30 minutes prior to castration. In group C, analgesia was not
174 administered prior to castration. Details of the relative timing of data recording and

175 treatments for each group are given in Fig. 1. All animals were monitored for 14 days
176 following surgery.
177
178 *Surgery*
179 Once baseline data had been recorded, castration of the left testicle was completed by a
180 single experienced clinician using an open technique as described by Newman (2007).
181 Briefly, the skin and tunica vaginalis were incised, the connective tissue surrounding the
182 testicle was dissected bluntly, after which continuous gentle traction was placed on the
183 spermatic cord until rupture occurred. The surgical wound was then left open. Any
184 somatic responses at the time of castration were noted. Data during T1 was recorded for
185 300 seconds from the start of incision. Following T1 elapsing, the right testicle was then
186 castrated in the same manner. Data from the right testicle was not included in analysis
187 due to contamination of data from the commenced surgery.
188
189 *Data analysis*
190 The raw EEG data were manually inspected and any noise artefacts excluded from
191 further analysis. Fast Fourier transformation (FFT) was carried out using custom-
192 written software (C. Johnson, Massey University, New Zealand). The median frequency
193 (F_{50}), spectral edge frequency (F_{95}) and total power (P_{tot}) of the EEG were calculated
194 for each one-second epoch. The following periods were extracted from the data for
195 statistical comparison: baseline (B) defined as 300 seconds immediately prior to the
196 start of surgery or injection of lidocaine; lidocaine injection (L) defined as 300 seconds
197 immediately following injection of lidocaine; castration of left testicle defined as 300
198 seconds immediately following skin incision of scrotum of the left testicle (T1).

199

200 Data were smoothed, and summarised by the normalised area-under-the-curve (AUC)
201 utilising the statistical software package R (Version 3.2.2 [2014-08-14], The R
202 Foundation for Statistical Computing, United States). For each time stamp (B and T1)
203 and treatment group (C, L and M) combination, the normalised AUC was calculated for
204 F_{50}, F_{95} and P_{tot}. A mixed effect model was fitted using the nlme package
205 (http://CRAN.R-project.org/package=nlme). The response variable was the normalised
206 AUC (F_{50}, F_{95} and P_{tot}), and the fixed effects were treatment and timestamp as main
207 effects with a two-way interaction term between the main effects. Primary residual plots
208 indicated the necessity of random intercept for each subject. This was confirmed by
209 Akaine's Information Criterion, Bayesian Information Criterion and a likelihood ratio
210 test. Model selection of the fixed effects was performed using an F-test using a cutoff of
211 $p < 0.05$ while maintaining the principal of marginality. Residual analysis was used to
212 check the final model assumptions.

213

214 The measurements of HR and MAP taken over 300 seconds following the first incision
215 into the scrotum (T1) were compared to the 300 seconds of baseline measurements
216 collected immediately prior to the first incision. Within each 300 second epoch,
217 averages were collected over ten-second time periods for the first 90 seconds following
218 incision and were labelled T_{10} through to T_{90}. The values for each time period are
219 presented as a percentage change from the baseline. Normality of all data was assessed
220 with the Shapiro-Wilk test. Normally distributed data were compared with a one-way
221 ANOVA. Gabriel's post-hoc analysis was performed if $p < 0.05$. SPSS software
222 (Version 22.0.0.0, IBM, USA) was used to complete these analyses.

Several continuous variables were analysed using SPSS software (Version 22.0.0.0, IBM, USA) following assessment of normality: weight; time from mask application to intubation; time from incision commencement to the rupture of the first spermatic cord (described as 'removal of testicle'); time from mask application to extubation (described as 'total general anaesthesia time'); $F_E{}'CO_2$ and arterial carbon dioxide partial pressure ($PaCO_2$). Normally distributed data were compared with a one-way ANOVA. Tukey's post-hoc analysis was performed if $p < 0.05$. Non-parametric data were compared by independent *T*-test. Parametric data are presented as mean ± standard deviation and non-parametric data are expressed as median (range).

Results

Animals and anaesthesia

There were no significant differences in the weight of the animals amongst 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 1). The $F_{E}'CO_2$ 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. During the induction of anaesthesia, ECG monitoring showed two isolated ventricular premature contractions (VPC) in three animals. Three animals had visual evidence of mild ruminal bloating requiring an increase in tidal volume (and therefore peak inspiratory pressure (PIP) to maintain the target $F_{E}'CO_2$. No adverse impacts on the cardiovascular parameters were noted following the increase in PIP. Regurgitation was not observed. All animals recovered uneventfully from anaesthesia, and all were observed eating within 30 minutes of standing. Postoperative assessment of pain was performed (data not shown). None of the animals required rescue analgesia.

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 time stamp (*F*: 65.1668, *P – value*: < 0.0001). For F_{95} and P_{tot} the final model indicated that the main effects of timestamp and treatment, as well as the two-way interaction, were significant predictors of AUC (*Table 2-4* in Appendices).

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. 2). 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) ($p = 0.0005$) was 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. 4): 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).

Cardiovascular data

Data from 23 animals were 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. No differences in baseline values of HR or MAP between the three

279 groups was apparent. HR decreased from baseline and was different between groups C
280 and L at T_{20} (p = 0.030), T_{30} (p = <0.001) and T_{40} (p = 0.009) and between groups L and
281 M at T_{30} (p = 0.015) (Fig. 5a). MAP also decreased from baseline and was different
282 between groups C and L at T_{20} (p = 0.003), T_{30} (p <0.001), T_{40} (p = <0.001), T_{50} (p =
283 0.018), T_{70} (p = 0.027) and T_{80} (p = 0.045); between groups C and M at T_{40} (p = 0.025)
284 and T_{50} (p = 0.024); and between groups L and M at T_{20} (p = 0.013) and T_{30} (p = 0.002)
285 (Fig. 5b).

Discussion

The aim of the current study was to assess the degree to which preoperative local anaesthesia with lidocaine or systemic meloxicam ameliorated the noxious effects of surgical castration on the EEG and cardiovascular responses in *Bos indicus* bull calves. In the current study, F_{50} increased in all three experimental groups (C, M and L) without there being 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. 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 short, lidocaine attenuated, but did not abolish, the EEG and cardiovascular response to surgical castration whereas bull calves pre-treated with meloxicam were only significantly different from the control group with respect to their cardiovascular responses, not their EEG descriptors.

Gibson and others (2007) found that a lidocaine ring block prevented any EEG response to dehorning in Holstein calves. The presence of a reduced response in the current study suggests that the local anaesthetic block was incomplete and may reflect nociception originating from the spermatic cord. Analysis of the EEG response comparing the periods before and after injection of lidocaine (but still before surgical castration) 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 & Ranheim 2005; Gibson et al. 2007).

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 et al. 2014a; Grint et al. 2014b). For F_{95}, an antinociceptive response will typically be seen as neither an increase nor a decrease from 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 & Shanzer 1966). It was referred to as "paradoxical arousal" in a study on isoflurane-anaesthetised sheep where its

incidence was correlated to the intensity of stimulus (Otto & Mally 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.

There were no EEG changes in response to castration associated with the preoperative administration of meloxicam. 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 in the current study may have been expected given previous studies, supporting a similar response in animal models of acute nociception (Díaz-Reval et al. 2004; Otto & Adams 2005). Although a previous study 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 (Dumka & Srivastava 2004), it is feasible that anti-nociceptive plasma concentrations of meloxicam were not present by the start of surgery in the current study. Consequently, higher doses and/or drug administration more than 30 minutes before surgery may have produced different results.

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 & Fisher 1988). The values recorded are considerably less than those which would be

expected to have a direct effect on the EEG (Paulson & Sharbrough 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 et al. 2005; Gibson et al. 2007; Grint et al. 2014b). 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.

For the analyses of the cardiovascular responses to surgical stimuli, comparable and transient decreases in HR and MAP were evident in all three experimental groups. These brief 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 have frequently focussed on delayed changes measured in minutes to hours following noxious stimuli, rather than the peracute period reported here (Peers et al. 2002; Coetzee 2013). The timing of recordings may explain the disparate results compared to the current study. Studies with analogous results to the current study used continuous computer-recorded data from the moment of the incision (Gibson et al. 2007; Johnson et al. 2009). This methodology is able to

interrogate the interval immediately following the start of the first 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 a 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).

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, 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.

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. Anti-nociceptive actions of non-steroidal anti-inflammatory drugs, further to their anti-inflammatory actions, have previously been reported in sheep and cattle following the administration of ketoprofen and carprofen (Otto & Mally 2003; Lizarraga & Chambers 2006). Further investigation of the meloxicam-induced reduction of acute nociception during husbandry procedures in cattle is undoubtedly warranted.

There were some limitations to this study. The number of animals in each group was small, and an *a priori* power study was not completed. Large animal studies often have treatment group numbers restricted due to financial and logistic limitations (de Vries et al. 2016). 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. 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 this was expected to be minimal.

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.

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Figure Legends

Figure 1 Flow diagram of the experimental protocol for each of the three groups (group C - no pre-operative analgesia, *n* = 12; group L – preoperative lidocaine, *n* = 12; group M – preoperative meloxicam, *n* = 12). All animals were six-to-eight month old *Bos* indicus bull calves. Arrow size is not indicative of time between components.

Table 1 Mean (± SD) of weight, time to intubation and total general anaesthesia time 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; group M – preoperative meloxicam, n=12). The median (range) is shown for the time for removal for the first testicle (start of incision to rupture of the spermatic cord). *P-values < 0.05.

Figure 2 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; 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 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; group M – preoperative meloxicam, n =1 2) 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 4 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; 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.

Figure 5 Percentage change in heart rate (HR) (a) and mean arterial blood pressure (MAP) (b) 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 =

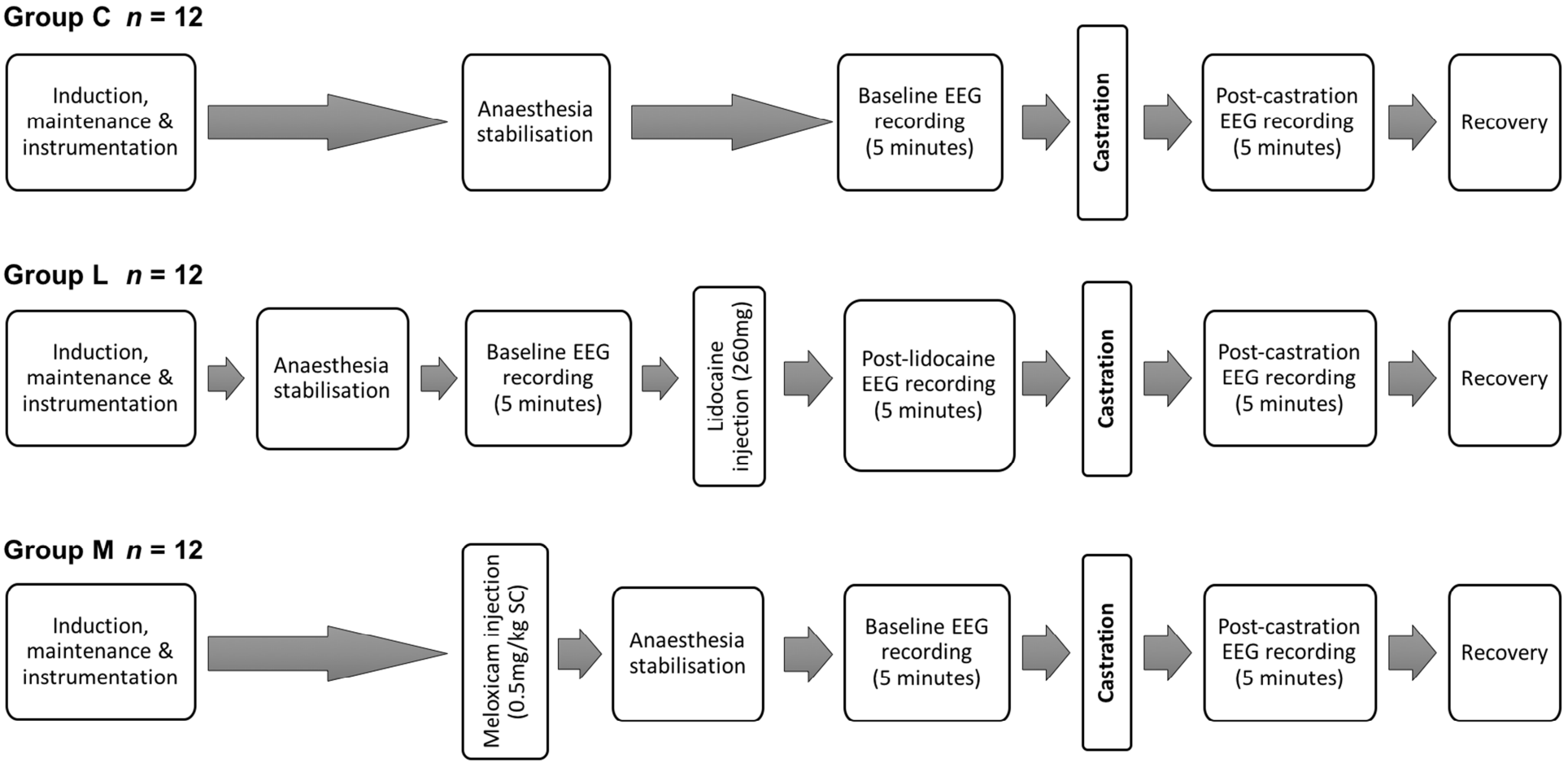
52 castration with preoperative lidocaine, M = castration with preoperative

53 meloxicam.

54 *Table 1.*

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

55 *Figure 1*



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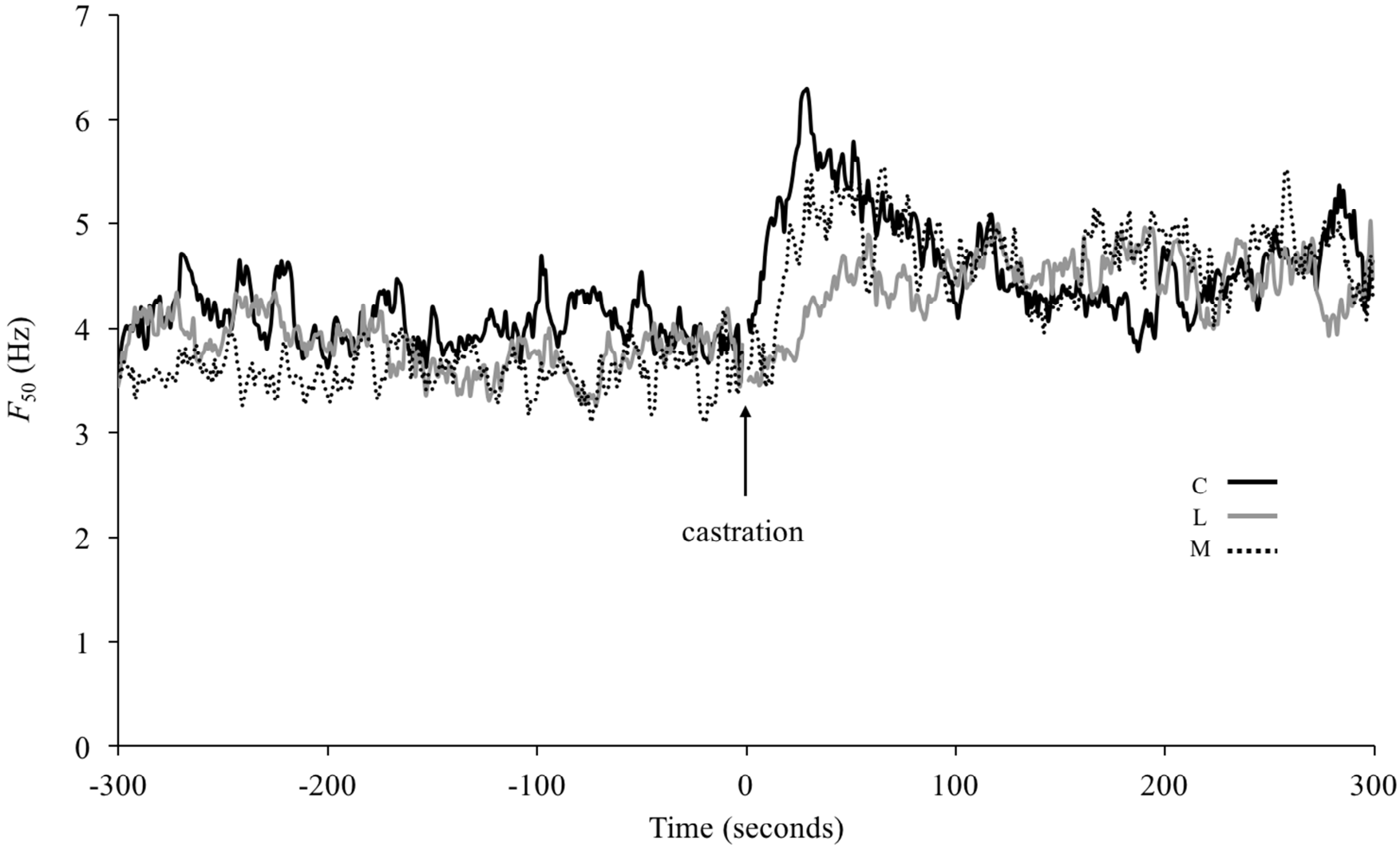
57 *Figure 2*



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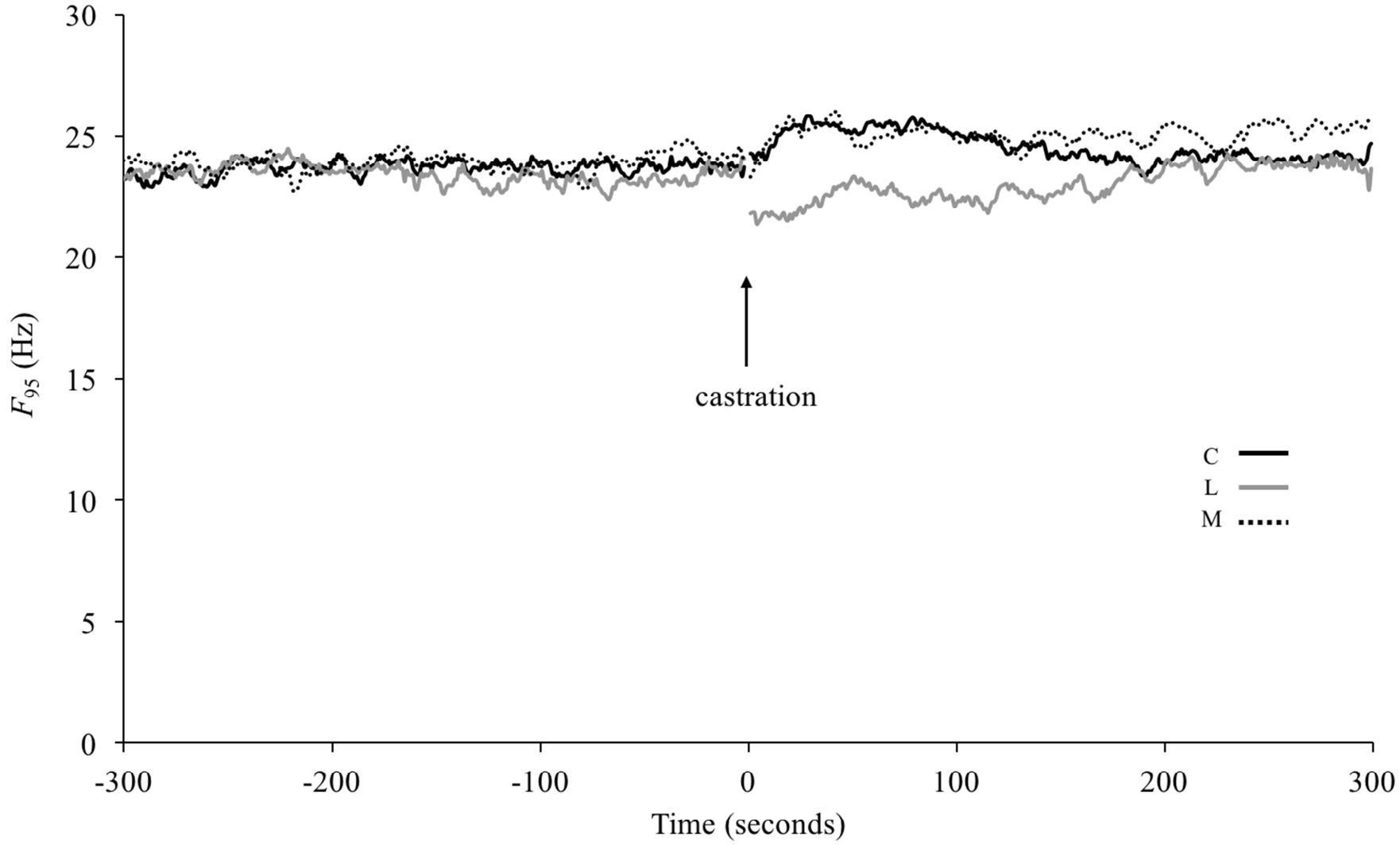
59 *Figure 3*



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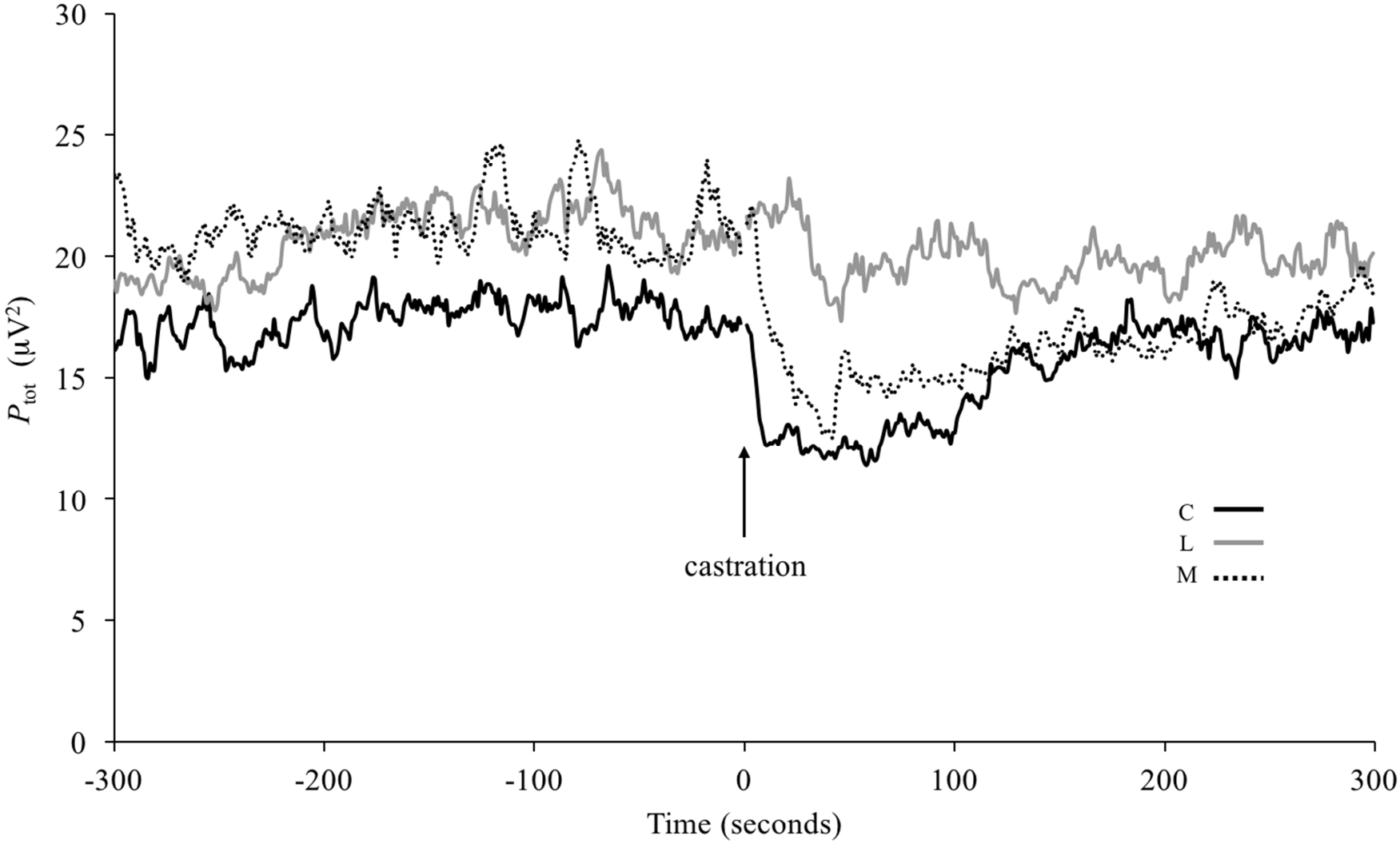
61 *Figure 4*



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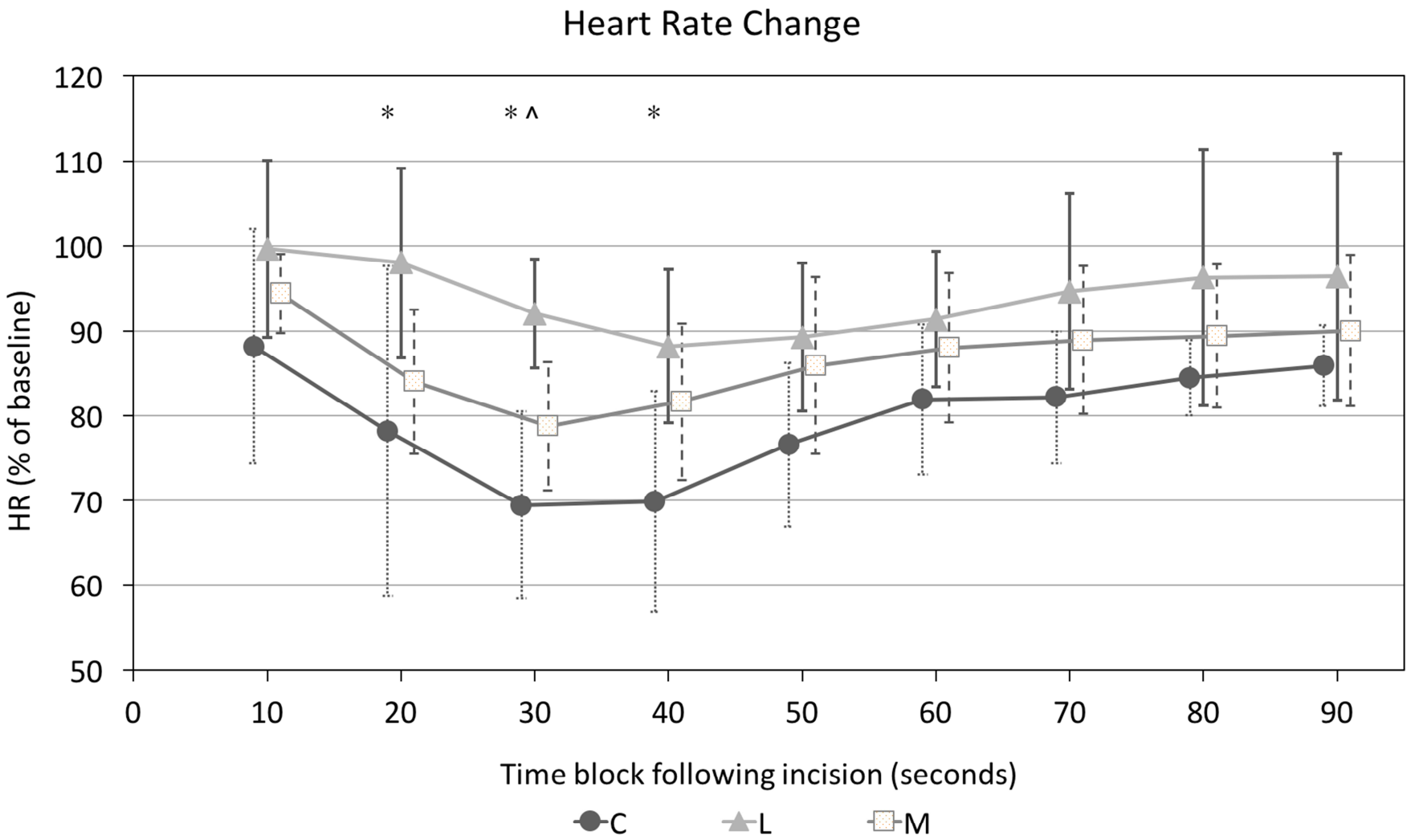
63 *Figure 5a*



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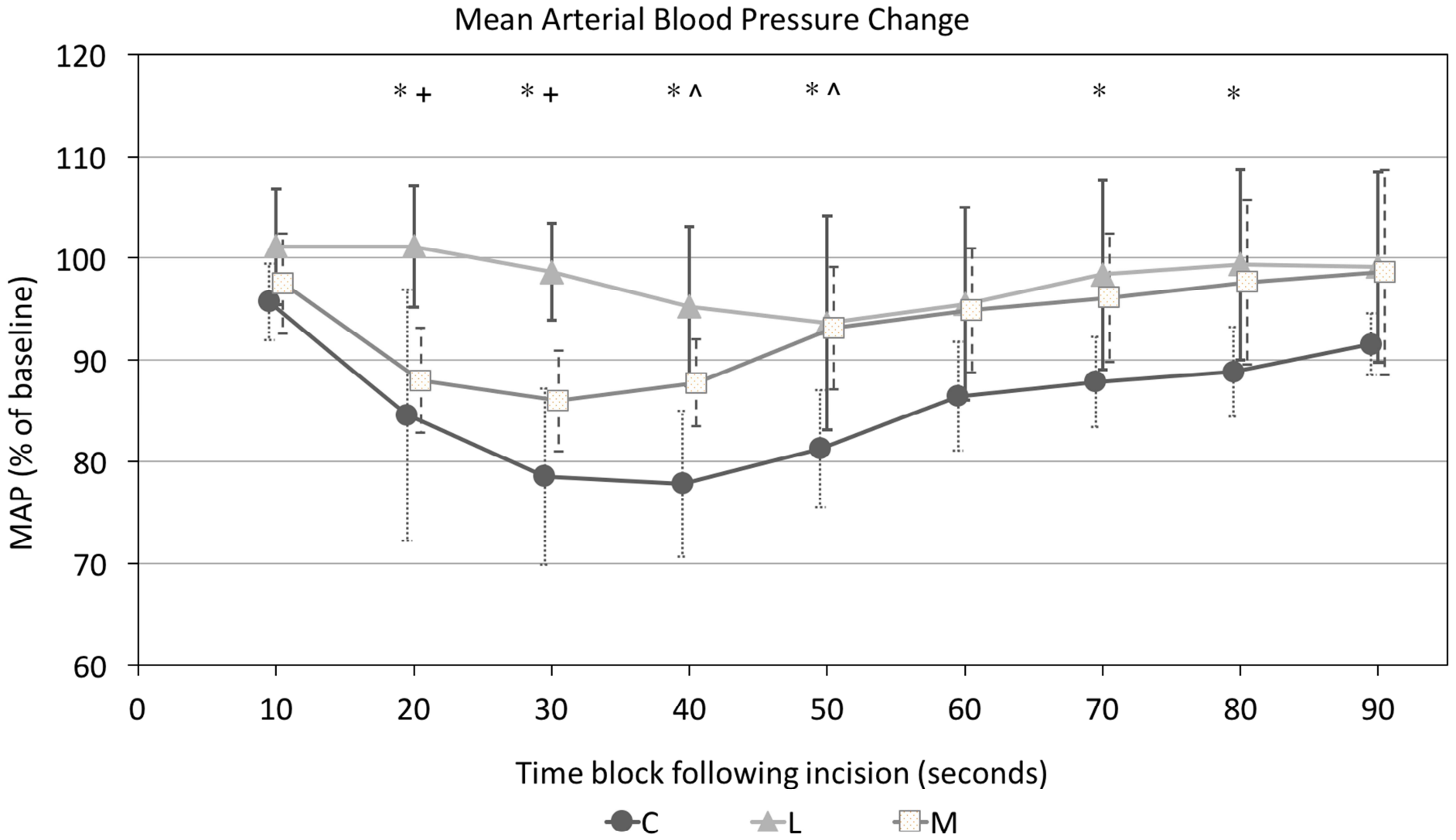
66 *Figure 5b*



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