

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

The Effect of Early Post-Natal Castration on Subsequent Electroencephalogram Response to Tail Docking in Lambs

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

Master of Veterinary Science

at Massey University, Palmerston North,
New Zealand.

Steven Impey

2015

Abstract

The objective of this study was to investigate the effect of early age painful stimulation on the cortical response to subsequent painful stimulation in lambs. Using the electroencephalogram (EEG), the current study measured the effect of early age castration at one day of age on the cortical pain response to tail docking at 23 days of age in lambs.

Lambs were randomly assigned to rubber ring castration (n=12) or handling (n=12) at one day of age. At 23 days of age lambs were tail docked under a minimal plane of anaesthesia maintained using halothane in oxygen ($P_{E\text{Hal}} = 1\%$). EEG data was recorded for two minutes pre-docking, and for eight minutes following tail docking. EEG median frequency, spectral edge frequency and total power were derived using fast Fourier transform. Data were analysed for group (castrated versus handled), time and group by time effects using mixed model analysis, as well as for the effect of group on pre-docking EEG.

Castrated lambs showed an increased cortical response to pain, demonstrated by a greater increase in EEG median frequency (Mixed model analysis; $F = 5.45$, $P = 0.03$) and greater reduction in total power ($F = 5.15$, $P = 0.03$) in response to subsequent tail docking.

These findings indicate that early age noxious stimulation results in an increased cortical response to subsequent noxious stimulation at approximately three weeks of age in lambs. The greater cortical response in the castrated lambs would likely correspond to an increased perception of pain, and therefore the potential for a greater degree of suffering and welfare compromise in response to subsequent painful injuries, for example lambing, injury and footrot.

There was also a tendency toward a higher pre-docking total power of the EEG in the castrated lambs when compared with handled lambs (Satterthwaite's t-test; $T = 1.86$, $P =$

0.08). The higher pre-docking total power may indicate a greater background activity in the nociceptive centres of the castrated lambs. However, the significance of this finding is not clear at this stage, and further work is necessary to better define the basis and clinical importance of this observation.

Key Words: Pain, electroencephalogram, sheep, lamb, castration, hyperalgesia

Acknowledgements

This study was approved by the Massey University Animal Ethics Committee (MUAEC Protocol 11/46).

Without the ongoing encouragement and supervision of Dr Ngaio Beausoleil this thesis would likely not have been completed. Thank you, Ngaio, for your efforts and for your thorough and valuable contributions toward improving the quality of my thinking and consequently my work.

Many thanks also to Professor Craig Johnson, for leading the research, for his supervision, for teaching me some of his impressive technical expertise, and for his faith in my abilities. To Professor Kevin Stafford, who initially got me involved in research at Massey, and provided continued infectious enthusiasm. And to Debbie Hill, for her patience with my questions throughout, and her help in finalising and submitting the thesis.

I extend my gratitude to the staff at the Massey University Large Animal Teaching Unit, for their assistance in managing the lambs, setting up equipment, and carrying out the procedures. Particular thanks to Robin Whitson, who helped me catch and castrate many of the lambs early in the morning, and provided some great conversation.

On a more personal note, I wish to thank my friends, flatmates and family who have been greatly accommodating in reading and being read drafts, entertaining long 'discussions' about neurophysiology, and providing me much needed breaks from reading and writing.

Lastly, to my mother Claire, thank you for your help, advice, and for proof reading the final draft. But more importantly, thank you for your continued support and ongoing encouragement.

Table of Contents

Abstract.....	ii
Acknowledgements.....	iv
Table of Contents.....	v
List of Figures	vii
List of Tables	ix
List of Abbreviations	x
1 Introduction.....	1
2 Literature Review	5
2.1 The Study of Pain.....	5
2.1.1 Definition of Pain and Nociception.....	5
2.1.2 Castration and Tail Docking as Painful Husbandry Procedures.....	7
2.1.3 Types of Pain	11
2.1.4 Pain Pathways	11
2.2 Hyperalgesia and the Potential Longer Term Effect of Early Pain Exposure	16
2.2.1 Defining Hyperalgesia	16
2.2.2 General Mechanisms of Hyperalgesia	18
2.2.3 The Evidence for Hyperalgesia in Response to Early Age Noxious Stimulation	27
2.3 Evaluating Pain and the Electroencephalogram	31
2.3.1 Key Considerations for Evaluating Pain in Animals	31
2.3.2 Evaluating Pain through Behavioural and Physiological Measures.....	32
2.3.3 Introduction to the Electroencephalogram.....	34
2.3.4 Uses of the Electroencephalogram.....	36
2.3.5 The Electroencephalogram and Anaesthesia	37
2.3.6 Placement of Electrodes	39
2.3.7 Fast Fourier Transform and the Electroencephalogram Power Spectrum.....	41
2.3.8 Human and Animal Power Spectra	44
2.3.9 Potential Limitations of the Electroencephalogram.....	46
2.4 Summary and Aims of the Study.....	51
3 Materials and Methods	54
3.1 Animals and General Care.....	54
3.2 Study Design and First Treatment.....	54
3.3 Anaesthesia and Tail-Docking	55
3.4 Electroencephalogram Set-Up	56

3.5	Data Collection	58
3.6	Analysis of the Electroencephalogram.....	58
3.7	Statistical Analysis	60
4	Results.....	62
4.1	Mixed Model Analysis of Group, Time, and Group by Time Effects	62
4.2	Analysis of Baseline	67
5	Discussion	69
5.1	Review of the Aims of the Study	69
5.2	Principle Findings	70
5.2.1	Summary of Principle Findings.....	70
5.2.2	Physiological Basis for Hyperalgesia, and the Potential for Allodynia.....	71
5.2.3	Use of EEG variables to Evaluate Pain in Animals.....	72
5.2.4	Behavioural Evidence for the Effects of Early Painful Events on Later Pain Responses	78
5.3	Minor Findings.....	83
5.3.1	Higher Pre-docking EEG Total Power in previously castrated lambs	83
5.3.2	Duration of the EEG Response to Tail Docking.....	86
5.4	Potential Limits of Study Design and Recommendations for Future Studies	87
5.4.1	Potential Limitations of Summarising the EEG spectrum as F50, F95 and Ptot	87
5.4.2	Potential Effects of Anaesthetic Depth on the EEG.....	89
5.4.3	Potential Limitations of Sample Size.....	91
5.4.4	Limitation of Evaluating EEG Recording from Only the Right Cerebral Hemisphere	92
5.4.5	Future Studies and the Inclusion of an Analgesia Control Group	93
5.4.6	Testosterone as a Potential Confounder	95
5.5	Application of the Findings.....	96
5.5.1	Application of the Findings to the Current Animal Welfare Recommendations in New Zealand	96
5.5.2	Variation in Developmental Timeframes between Species	97
5.5.3	The Human Clinical Relevance of the Findings	98
5.6	Conclusions.....	100
6	Appendices	102
6.1	Flow Chart for Steps in Data Manipulation.....	102
6.2	Paper Abstract, World Congress of Veterinary Anaesthesiology	103
7	References	104

List of Figures

- Figure 2.1: Flow diagram showing the relationship between nociception, pain and suffering in animals..... 14
- Figure 2.2: Schematic diagram of pain pathway from stimulus to perception, and then to behavioural response in the lamb 15
- Figure 2.3: Diagram representing the distinct phenomena of hyperalgesia and allodynia; where hyperalgesia represents an increase pain sensation of painful stimuli, and allodynia represents a pain sensation in response to normally innocuous stimuli. Figure source: Cervero (2008)..... 17
- Figure 2.4: Diagram showing the relationship between electrode placement and electrocardiogram presentation of a normal ECG recording from the heart. Note that the waveforms show varied appearance depending on the axis of recording despite being a recording of the same net electrical activity of the heart. Figure source: <http://www.ivline.org/2010/05/quick-guide-to-ecg.html>, accessed 14 July 2015 39
- Figure 2.5: The international 10-20 electrode placement system for EEG recording, as standardised by the American Electroencephalographic Society, a total of 21 electrodes are used in the 10-20 EEG montage. Figure source: Sharbrough et al. (1991) 40
- Figure 2.6: Schematic representation of an EEG power spectrum. The dashed line represents median (50%) frequency, the solid line represents spectral edge (95%) frequency. Figure source: Murrell and Johnson (2006) 43
- Figure 2.7: Schematic representation of an EEG power spectrum. The dashed line represents median (50%) frequency, the solid line represents spectral edge (95%) frequency. Figure source: Murrell and Johnson (2006) 49
- Figure 3.1: Placement of EEG electrodes (solid lines) for the bilateral three electrode montage, with non-inverting (1), common inverting (2), and ground (3) leads; and the ECG electrodes (dashed lines), with inverting (4), non-inverting (5) and shared ground (6) leads 57
- Figure 4.1: Raw (non-transformed) average median frequency for castrated (solid line) and handled (broken line) lambs as a percentage of the average 110 second pre-docking period. Time 0 marks tail docking. Vertical bars indicate standard error. Pre-docking data is included for comparison, but was not statistically analysed. Standard error was calculated for post tail docking data, and therefore it has not been applied to the pre-docking data..... 63

- Figure 4.2: Raw (non-transformed) average median frequency (pooled castrated and handled data) response following tail docking, shown as a percentage of baseline. Time 0 marks tail docking. Vertical bars indicate standard error 63
- Figure 4.3: Raw (non-transformed) average spectral edge frequency for castrated (solid line) and handled (broken line) lambs as a percentage of the average 110 second pre-docking period. Time 0 marks tail docking. Vertical bars indicate standard error. Pre-docking data is included for comparison, but was not statistically analysed. Standard error was calculated for post tail docking data, and therefore it has not been applied to the pre-docking data..... 64
- Figure 4.4: Raw (non-transformed) average spectral edge frequency for castrated (solid line) and handled (broken line) lambs as a percentage of the average 110 second pre-docking period. Time 0 marks tail docking. Vertical bars indicate standard error. Pre-docking data is included for comparison, but was not statistically analysed. Standard error was calculated for post tail docking data, and therefore it has not been applied to the pre-docking data..... 65
- Figure 4.5: Raw (non-transformed) average total power for castrated (solid line) and handled (broken line) lambs as a percentage of the average 110 second pre-docking period. Time 0 marks tail docking. Vertical bars indicate standard error. Pre-docking data is included for comparison, but was not statistically analysed. Standard error was calculated for post tail docking data, and therefore it has not been applied to the pre-docking data..... 66
- Figure 4.6: Raw (non-transformed) average total power (pooled castrated and handled data) response following tail docking, shown as a percentage of baseline. Time 0 marks tail docking. Vertical bars indicate standard error. Filled circles (•) indicate time points that are significantly different from baseline 67
- Figure 4.7: Raw total power of castrated and handled lambs, recorded at 1 Hz. Time of tail docking is marked by the vertical black line (time = 0). Pre-docking standard error is 0.09 for castrated lambs, and 0.05 for handled lambs. EEG recording began 115 seconds before tail docking, and 5 seconds of data either side of tail docking was excluded from analysis, giving 110 seconds pre-docking recording. Post tail docking raw data are included on this graph for visual reference, but were not included in the statistical analyses 68

List of Tables

Table 4.1:	Results of the mixed model analysis for F50, F95 and Ptot following tail docking; values were calculated as a percentage of the baseline 62
Table 4.2:	Treatment group mean and standard error of baseline for F50, F95 and Ptot averaged over 110 second pre-docking period, and T-test results for group comparison 68

List of Abbreviations

CNS	Central nervous system
CT	Computed tomography
EEG	Electroencephalogram
F50	Median (50 th percentile) frequency of the electroencephalogram
F95	Spectral edge (95 th percentile) frequency of the electroencephalogram
FFT	Fast Fourier transform
fMRI	Functional magnetic resonance imaging
Hz	Hertz
IASP	International Association for the Study of Pain
KHz	Kilohertz
LTP	Long term potentiation
PHP	Painful husbandry procedure
Ptot	Total power of the electroencephalogram
SE	Standard error