

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.

Electroencephalographic responses of calves to the noxious sensory input of slaughter by ventral neck incision and its modulation with non-penetrative captive bolt stunning

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

Doctor of Philosophy

In

Physiology

Massey University

Palmerston North

New Zealand

Troy John Gibson

2009

11th May 2009

Declaration of regulatory compliance

This is to certify that the work performed in the Doctoral Thesis entitled "*Electroencephalographic responses of calves to the noxious sensory input of slaughter by ventral neck incision and its modulation with non-penetrative captive bolt stunning*" in the Institute of Veterinary, Animal and Biomedical Sciences at Massey University, Palmerston North, New Zealand:

- Is the original work of the candidate, except where cited in text, diagrams and/or in the acknowledgements.
- That the text, excluding appendices does not exceed 100,000 words.
- All the ethical requirements applicable to the study have been complied with as required by Massey University and relevant legislation.

Animal Ethical Authorisation codes: Protocol no. 04/86 and 06/61.

Candidate: Troy J. Gibson

Chief Supervisor: Dr Craig Johnson

Signature:



Signature:

Date: 11th May 2009

Date:

11th May 2009

Candidate's Declaration

This certifies that the research carried out for my Doctoral thesis entitled "*Electroencephalographic responses of calves to the noxious sensory input of slaughter by ventral neck incision and its modulation with non-penetrative captive bolt stunning*", in the Institute of Veterinary, Animal and Biomedical Sciences at Massey University, Palmerston North, New Zealand is my own work except where cited in text, diagrams and/or in the acknowledgements and that the thesis material has not been used in part or whole for any other qualification.

Candidate: Troy J. Gibson

Signature:



Date: 11th May 2009

11th May 2009

Supervisor's Declaration

This certifies that the research carried out for the Doctoral thesis entitled "*Electroencephalographic responses of calves to the noxious sensory input of slaughter by ventral neck incision and its modulation with non-penetrative captive bolt stunning*", was done by Troy J. Gibson in the Institute of Veterinary, Animal and Biomedical Sciences at Massey University, Palmerston North, New Zealand. The thesis material has not been used in part or whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

Chief Supervisor: Dr Craig Johnson

Signature:

Date:



Massey University

Declaration Confirming Content of Digital Version of Thesis

I confirm that the content of the digital version of this thesis

Title: *Electroencephalographic responses of calves to the noxious sensory input of slaughter by ventral neck incision and its modulation with non-penetrative captive bolt stunning*

is the final amended version following the examination process and is identical to this hard bound paper copy.

Student's Name: Troy J. Gibson

Student's Signature:

A handwritten signature in blue ink that reads "Troy Gibson".

Date: 11th May 2009

ABSTRACT

Slaughter by ventral neck incision (VNI) is performed on some animals without prior stunning in New Zealand and other countries. A single incision with a razor sharp blade is made in the ventral aspect of the neck, sectioning both carotid arteries and jugular veins, though, not the vertebral arteries. There are a number of potential welfare concerns surrounding slaughter by VNI including pain due to the incision, which may lead to distress during the time before loss of consciousness. The aims of this thesis were to identify cortical responses indicative of noxious stimulation due to slaughter by VNI using analysis of the electroencephalogram (EEG) power spectrum and to investigate the effects of non-penetrative captive bolt (NPCB) stunning on these cortical responses.

The studies utilised adaptations of a minimal anaesthesia model, which has been validated in a range of mammalian species. Surgical dehorning was used as a validation technique for this methodology in cattle and demonstrated a 'typical' EEG response to noxious stimulation. Cattle slaughtered by VNI without prior stunning produced specific responses in the EEG that strongly indicated responses to noxious stimulation. Causation was investigated in cattle where blood flow through the brain remained intact during neck tissue incision (NTI) or the major blood vessels of the neck were isolated and transected independently of other neck tissues (BVT). The response to neck incision in intact animals was principally due to the noxious sensory input due to incision of neck tissues and not mainly as a result of loss of blood flow through the brain. NPCB stunning produced states of cortical activity that were incompatible with the maintenance of sensibility and pain perception. Experimental examination of the time to onset of undoubted insensibility was attempted in cattle subsequent to a pilot study in sheep. The generation of somatosensory-evoked potentials was problematic in cattle.

The conclusions of this thesis are that incision of neck tissues during slaughter without prior stunning constitutes a substantial noxious stimulus. Were an animal conscious, this stimulus would be perceived as painful until the onset of hypoxia-induced insensibility. This would represent a significant compromise to animal welfare.

ACKNOWLEDGEMENTS

This thesis could not have been possible without the help, expertise, support and encouragement of my supervisors: Dr Craig Johnson, Professor David Mellor, Professor Kevin Stafford and Dr Geoff Barnes.

I am grateful to the C Alma Baker Trust for the award of C Alma Baker Trust Postgraduate Scholarship. The Ministry of Agriculture and Forestry of New Zealand and the Department of Environment, Food and Rural Affairs of the United Kingdom jointly funded the work presented in this thesis.

Special thanks goes to all the people that have assisted me during my experimental work, it was often cold, it was often wet, it was sometimes dark but because of the team it was always fun. Dr Jo Murrell for all your expert help with experimentation, dinner parties and neuro drinks. Sheryl Mitchinson and Corrin Hulls for all your hard work and friendship during the course of my studies. I would also like to thank the following for their help in experimental work and helpful discussion: Leanne McCracken, Kavitha Kongara, Nicki Grint, Zoe Matthews, Dr Paul Chambers and Des Waters. Thank you to my office mates: Amanda McIlhone, Megan McGregor and Laureline Maynier for all the helpful discussion, fun and for putting up with my untidy desk. Thank you Tamara Diesch for translating obscure German theses.

I am indebted to a number of people that have helped me during my studies: Neil Ward for his help with experimentation and computers, Mike Hogan for your advice and use of post-mortem room and equipment. Members of: the Massey University Large Animal Teaching Unit; the Small Animal Production Unit; the Deer Research Unit; and Dairy Unit 1. Especially their managers: Robin Whitson, Debbie Chesterfield and Martin Chesterfield. Dr Alistair Johnstone for your histological expertise, Alan Nutman for helpful discussion, and Allain Scott for your support during my studies. Many thanks to my stock agent Sam Jones, able to find suitable stock within a moments notice.

Thank you to my friends and family for all your love and support during the course of my studies. My parents John and Yvonne Gibson for your encouragement and support. I am very lucky to have parents like you, thanks. Finally my biggest thanks goes to my loving wife Willow for your love, encouragement, support and understanding.

TABLE OF CONTENTS

ABSTRACT	I
ACKNOWLEDGEMENTS	III
TABLE OF CONTENTS	V
LIST OF FIGURES	IX
LIST OF TABLES	XV
ABBREVIATIONS AND ACRONYMS.....	XVII
CHAPTER 1 SLAUGHTER WITHOUT STUNNING.....	1
1.1 INTRODUCTION	1
1.2 RESEARCH FOCUS	3
1.2.1 <i>Primary focus</i>	3
1.2.2 <i>Secondary foci</i>	3
1.3 STRUCTURE OF THE THESIS	4
1.4 REFERENCES	5
CHAPTER 2 BACKGROUND AND LITERATURE REVIEW	7
2.1 ANIMAL WELFARE.....	7
2.2 SOME REFLECTIONS ON THE ETHICS OF SLAUGHTER	9
2.2.1 <i>The Ethics of Slaughter Based Research</i>	10
2.3 JEWISH AND MUSLIM SLAUGHTER BACKGROUND.....	11
2.3.1 <i>Jewish Slaughter</i>	11
2.3.2 <i>Muslim Slaughter</i>	15
2.4 EMERGENCY SLAUGHTER.....	17
2.5 NEW ZEALAND LEGISLATION CONCERNING THE SLAUGHTER OF ANIMALS WITHOUT PRIOR STUNNING.....	18
2.6 PHYSIOLOGY OF PAIN	21
2.6.1 <i>Measurement of Pain in Livestock</i>	23
2.7 ELECTROENCEPHALOGRAM AND EVOKED POTENTIALS	24
2.7.1 <i>Electroencephalogram</i>	25
2.7.1.1 Recording of the EEG	26
2.7.1.2 Interpretation of Noxious Sensory Input with the EEG	29
2.7.1.3 Minimal Anaesthesia Model.....	34
2.7.2 <i>Evoked Potentials</i>	35
2.7.2.1 Interpretation of the Evoked Potential Waveform	37
2.8 TIME TO INSENSIBILITY	38
2.9 BLOOD SUPPLY TO THE BOVINE BRAIN	43
2.9.1 <i>Vertebral Arteries during Slaughter in Cattle</i>	46
2.10 PAIN DURING SLAUGHTER	48
2.11 REFERENCES	51
CHAPTER 3 VALIDATION OF THE ACUTE ELECTROENCEPHALOGRAPHIC RESPONSES OF CALVES TO NOXIOUS STIMULUS WITH SCOOP DEHORNING	65
3.1 ABSTRACT.....	66
3.2 INTRODUCTION	67
3.3 MATERIALS AND METHODS	69
3.3.1 <i>Animals</i>	69
3.3.2 <i>Anaesthesia</i>	69
3.3.3 <i>EEG and ECG Recording</i>	70
3.3.4 <i>Experimental Procedure</i>	71

3.3.5 EEG and ECG Analysis.....	72
3.3.6 Statistical Analysis.....	72
3.4 RESULTS.....	73
3.5 DISCUSSION.....	77
3.6 REFERENCES.....	80
CHAPTER 4 ELECTROENCEPHALOGRAPHIC RESPONSES OF CALVES TO SLAUGHTER BY VENTRAL NECK INCISION WITHOUT PRIOR STUNNING.....	85
4.1 ABSTRACT.....	86
4.2 INTRODUCTION.....	87
4.3 MATERIALS AND METHODS.....	89
4.3.1 Animals.....	89
4.3.2 Anaesthesia.....	89
4.3.3 EEG and ECG Recording.....	90
4.3.4 Experimental Procedure.....	90
4.3.5 EEG and ECG Analysis.....	92
4.3.6 Histopathology.....	93
4.3.7 Statistical Analysis.....	93
4.4 RESULTS.....	94
4.5 DISCUSSION.....	101
4.6 REFERENCES.....	105
CHAPTER 5 COMPONENTS OF ELECTROENCEPHALOGRAPHIC RESPONSES TO SLAUGHTER: EFFECTS OF CUTTING NECK TISSUES COMPARED TO MAJOR BLOOD VESSELS.....	109
5.1 ABSTRACT.....	110
5.2 INTRODUCTION.....	111
5.3 MATERIALS AND METHODS.....	112
5.3.1 Animals.....	112
5.3.2 Anaesthesia, EEG and ECG.....	112
5.3.3 Experimental Procedure.....	113
5.3.4 Data Analysis.....	115
5.3.5 Statistics.....	116
5.4 RESULTS.....	117
5.4.1 EEG Power Spectra Indices.....	117
5.4.2 Compressed Spectral Arrays in Individuals.....	120
5.4.3 Blood Pressure Responses.....	121
5.5 DISCUSSION.....	123
5.6 REFERENCES.....	127
CHAPTER 6 ELECTROENCEPHALOGRAPHIC RESPONSE TO CONCUSSIVE NON- PENETRATIVE CAPTIVE BOLT STUNNING IN CATTLE.....	131
6.1 ABSTRACT.....	132
6.2 INTRODUCTION.....	133
6.3 MATERIALS AND METHODS.....	135
6.3.1 Animals.....	135
6.3.2 Anaesthesia.....	135
6.3.3 EEG and ECG Recording.....	136
6.3.4 Experimental Procedure.....	136
6.3.5 Data and Statistical Analysis.....	137
6.4 RESULTS.....	139
6.5 DISCUSSION.....	144
6.6 REFERENCES.....	147
CHAPTER 7 AMELIORATION OF ELECTROENCEPHALOGRAPHIC RESPONSES TO SLAUGHTER BY NON-PENETRATIVE CAPTIVE BOLT STUNNING AFTER VENTRAL NECK INCISION IN HALOTHANE ANAESTHETISED CALVES.....	151
7.1 ABSTRACT.....	152
7.2 INTRODUCTION.....	153
7.3 MATERIALS AND METHODS.....	154

7.3.1 Animals.....	154
7.3.2 Anaesthesia and Instrumentation.....	154
7.3.3 Experimental Procedure.....	155
7.3.4 Data Analysis and Statistics.....	156
7.4 RESULTS.....	157
7.5 DISCUSSION.....	163
7.6 REFERENCES.....	166
CHAPTER 8 INHERENT VARIABILITY OF ACUTE ELECTROENCEPHALOGRAPHIC RESPONSES TO NOXIOUS SUPRAMAXIMAL ELECTRICAL STIMULI IN CALVES.....	169
8.1 ABSTRACT.....	170
8.2 INTRODUCTION.....	171
8.3 MATERIALS AND METHODS.....	172
8.3.1 Animals.....	172
8.3.2 Anaesthesia.....	173
8.3.3 EEG, ECG and Arterial Blood Pressure Recoding.....	173
8.3.4 Experimental Procedure.....	174
8.3.5 Data and Statistical Analysis.....	175
8.4 RESULTS.....	175
8.5 DISCUSSION.....	181
8.6 REFERENCES.....	183
CHAPTER 9 PILOT STUDY TO DEVELOP A METHOD OF GENERATING AND RECORDING SOMATOSENSORY EVOKED POTENTIALS WITHOUT SURGICALLY IMPLANTED CORTICAL ELECTRODES.....	185
9.1 ABSTRACT.....	186
9.2 INTRODUCTION.....	187
9.3 MATERIALS AND METHODS.....	188
9.3.1 Animals.....	188
9.3.2 Anaesthesia.....	188
9.3.3 EEG and SEP Recording.....	188
9.3.4 Experimental Procedure.....	189
9.4 RESULTS.....	190
9.5 DISCUSSION.....	193
9.6 REFERENCES.....	195
CHAPTER 10 INVESTIGATION OF CORTICAL FUNCTION WITH THE USE OF SOMATOSENSORY-EVOKED POTENTIALS IN RESPONSE TO VENTRAL NECK INCISION OR NON-PENETRATIVE CAPTIVE BOLT STUNNING IN CALVES.....	197
10.1 ABSTRACT.....	198
10.2 INTRODUCTION.....	199
10.3 MATERIALS AND METHODS.....	200
10.3.1 Animals.....	200
10.3.2 Anaesthesia and Blood Pressure.....	201
10.3.3 Superficial Peroneal Nerve Preparation.....	201
10.3.4 EEG and SEP.....	202
10.3.5 Experimental Procedure.....	203
10.3.6 Interruption of EEG and SEP Waveforms.....	203
10.3.7 Statistics.....	204
10.4 RESULTS.....	206
10.4.1 SEP.....	206
10.4.2 Spontaneous EEG.....	208
10.4.3 Blood Pressure.....	210
10.5 DISCUSSION.....	212
10.6 REFERENCES.....	214
CHAPTER 11 GENERAL DISCUSSIONS AND CONCLUSIONS.....	217
11.1 NOXIOUSNESS OF SLAUGHTER.....	218
11.2 MODULATION OF THE EEG RESPONSES TO VNI WITH NPCB STUNNING.....	219
11.3 CORTICAL FUNCTION AFTER NPCB STUNNING OR VNI SLAUGHTER.....	220

11.4 EXPERIMENTAL LIMITATIONS	221
11.5 FUTURE WORK.....	225
11.6 FINAL CONCLUSION.....	227
11.7 REFERENCES	228
APPENDIX 1 EEG SPECTRAL ANALYSIS PROGRAMME CODE	233
APPENDIX 2 STUNNING POSITION IN CATTLE	237
APPENDIX 3 PUBLISHED SCIENTIFIC PEER-REVIEWED PAPERS	239
APPENDIX 4 SCIENTIFIC CONFERENCE PRESENTATIONS.....	277

LIST OF FIGURES

FIGURE 2.1. FLOW CHART DETAILING THE IMPORTANT STEPS IN THE RECORDING AND ANALYSIS OF THE EEG, FROM A SUBJECT, INCLUDING AMPLIFICATION, ANALOGUE FILTERING, ANALOGUE-TO-DIGITAL CONVERSION AND COMPUTER-BASED ACQUISITION TO DIFFERENT FORMS OF EEG ANALYSIS.....	28
FIGURE 2.2. DIAGRAMMATIC REPRESENTATION OF THE EEG POWER SPECTRUM AND THE FOUR SINGLE DESCRIPTORS USED IN ANALYSIS. A: MEDIAN FREQUENCY (F50); B: 95% SPECTRAL EDGE FREQUENCY (F95); C: TOTAL EEG POWER (PTOT); AND D: FREQUENCY BAND ANALYSIS.....	31
FIGURE 2.3. COMPRESSED SPECTRAL ARRAY FROM THE EEG OF A ANAESTHETISED CALF, WITH TIME (SECONDS) ALONG THE X-AXIS, FREQUENCY (HZ) ALONG THE R-AXIS AND POWER (μV^2) ON THE Y-AXIS.	32
FIGURE 2.4. FLOW CHART DETAILING THE MAJOR STEPS IN THE RECORDING AND ANALYSIS OF THE SOMATOSENSORY EVOKED POTENTIALS, FROM SUBJECT, AMPLIFICATION, ANALOGUE FILTERING, ANALOGUE-TO-DIGITAL CONVERSION, TRIGGERING, WAVEFORM SUMMATION AND INTERPRETATION.....	36
FIGURE 2.5. EXAMPLE OF A SOMATOSENSORY EVOKED POTENTIAL (SEP) WAVEFORM AND THE MEASUREMENT OF LATENCY (MSEC) AND AMPLITUDE (μV). THIS EVOKED POTENTIAL REPRESENTS THE SUMMATION OF 32 REPETITIONS.	37
FIGURE 2.6. DIAGRAMMATIC ILLUSTRATION OF THE BLOOD SUPPLY TO THE BRAIN IN CATTLE AND SHEEP. ADAPTED FROM BALDWIN (1971); BLACKMORE AND DELANY (1988).....	45
FIGURE 3.1. DIAGRAM OF THE EXPERIMENTAL DESIGN FOR SCOOP DEHORNING ALONE (DH) OR SCOOP DEHORNING WITH LIDOCAINE RING BLOCK (DH+LA) GROUPS OVER TIME (MINUTES) FROM INDUCTION OF ANAESTHESIA (0 MINUTES) TO RECOVERY (45 MINUTES).	71
FIGURE 3.2. MEDIAN FREQUENCY (F50) (MEAN) OF THE ELECTROENCEPHALOGRAM (EEG) BEFORE AND AFTER DEHORNING IN CALVES DEHORND WITH (—) (DH+LA) OR WITHOUT (—) A LIDOCAINE RING BLOCK (DH) (A = VALUES SIGNIFICANTLY DIFFERENT BETWEEN TREATMENT GROUPS, $P<0.01$; B = VALUES SIGNIFICANTLY DIFFERENT FROM PRE-TREATMENT DH, $P<0.01$), 0 SECONDS = TIME POINT OF DEHORNING.	74
FIGURE 3.3. MEAN 95% SPECTRAL EDGE FREQUENCY (F95) OF THE ELECTROENCEPHALOGRAM (EEG) BEFORE AND AFTER DEHORNING IN CALVES DEHORND WITH (—) (DH+LA) OR WITHOUT (—) A LIDOCAINE RING BLOCK (DH) (A = VALUES SIGNIFICANTLY DIFFERENT BETWEEN TREATMENT GROUPS, $P<0.01$; B = VALUES SIGNIFICANTLY DIFFERENT FROM PRE-TREATMENT DH, $P<0.01$), 0 SECONDS = TIME POINT OF DEHORNING.	74
FIGURE 3.4. MEAN TOTAL POWER (PTOT) OF THE ELECTROENCEPHALOGRAM (EEG) BEFORE AND AFTER DEHORNING IN CALVES DEHORND WITH (—) (DH+LA) OR WITHOUT (—) A LIDOCAINE RING BLOCK (DH) (A = VALUES SIGNIFICANTLY DIFFERENT BETWEEN TREATMENT GROUPS, $P<0.01$; B = VALUES SIGNIFICANTLY DIFFERENT FROM PRE-TREATMENT DH, $P<0.01$), 0 SECONDS = TIME POINT OF DEHORNING.....	75
FIGURE 4.1. DIAGRAM OF THE EXPERIMENTAL DESIGN IN MINUTES FOR VENTRAL NECK INCISION (GROUP VNI) AND SHAM INCISION (GROUP SI). ANAESTHESIA OCCURRED AT 0 MINUTES AND VENTRAL NECK INCISION (VNI) AT 25 MINUTES.	91

FIGURE 4.2. MEAN 95% SPECTRAL EDGE FREQUENCY (F95) FOLLOWING VENTRAL NECK INCISION (VNI) AT 0 SECONDS. ELECTROENCEPHALOGRAM (EEG) FROM THE RIGHT (—) AND FROM THE LEFT (—) OF THE CRANIUM. A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT RIGHT (P<0.05), B = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT LEFT CEREBRAL HEMISPHERE (P<0.05).95

FIGURE 4.3. MEAN TOTAL POWER (PTOT) OF THE ELECTROENCEPHALOGRAM (EEG) FOLLOWING VENTRAL NECK INCISION (VNI) 0 SECONDS. EEG FROM THE RIGHT (—) AND FROM THE LEFT (—) OF THE CRANIUM. A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT RIGHT (P<0.05), B = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT LEFT CEREBRAL HEMISPHERE (P<0.05).95

FIGURE 4.4. MEDIAN FREQUENCY (F50) (MEAN) FOLLOWING VENTRAL NECK INCISION (VNI) AT 0 SECONDS. ELECTROENCEPHALOGRAM (EEG) FROM THE RIGHT (—) AND FROM THE LEFT (—) OF THE CRANIUM. A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT RIGHT (P<0.05), B = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT LEFT CEREBRAL HEMISPHERE (P<0.05).96

FIGURE 4.5. CHARACTERISTICS OF THE SPONTANEOUS ELECTROENCEPHALOGRAM (EEG) IN INDIVIDUAL ANIMALS OVER TIME (SECONDS) AFTER VENTRAL NECK INCISION (VNI). THE SPONTANEOUS TRACES WERE VISUALLY INSPECTED AND WERE EITHER CLASSIFIED AS: VNI ARTEFACT, ACTIVE EEG, TRANSITIONAL EEG, HALF EEG, AND ISOELECTRIC EEG. R = EEG RECORDED FROM THE RIGHT AND L = EEG RECORDED FROM THE LEFT HAND SIDE OF THE CRANIUM. THE TIME DURATION IN SECONDS ARE DISPLAYED ON THE INDIVIDUAL BARS; CLEAR GAPS WAS OUT OF RANGE.....96

FIGURE 4.6. THE COMPRESSED SPECTRAL ARRAY FROM CALF 4 LEFT CEREBRAL HEMISPHERE, REPRESENTING A TYPICAL ARRAY IN RESPONSE TO VENTRAL NECK INCISION (VNI) AT 0 SECONDS.97

FIGURE 4.7. MEAN PTOT FOLLOWING APPLICATION OF A NON-NOXIOUS SHAM INCISION (SI) AT 0 SECONDS. ELECTROENCEPHALOGRAM (EEG) FROM THE RIGHT (—) AND FROM THE LEFT (—) OF THE CRANIUM.97

FIGURE 4.8. MEAN \pm STANDARD ERROR OF THE MEAN (—), FEMORAL ARTERIAL BLOOD PRESSURE (MMHG) BEFORE AND AFTER VENTRAL NECK INCISION (VNI). A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES (P<0.05).98

FIGURE 5.1. DIAGRAM OF THE EXPERIMENTAL DESIGN IN MINUTES FOR NECK TISSUE INCISION (NTI) AND BLOOD VESSEL TRANSECTION (BVT). ANAESTHESIA INDUCTION OCCURRED AT 0 MINUTES, AND THE TIMES TO NECK INCISION AND VESSEL TRANSECTION ARE INDICATED. LIKEWISE THE TIMING OF HEPARINIZATION AND PENTOBARBITONE EUTHANASIA ARE INDICATED WITH VERTICAL GREY BLOCKS. 115

FIGURE 5.2. PERCENTAGE CHANGE IN MEDIAN FREQUENCY (F50) OF THE EEG FOLLOWING AND NECK TISSUE INCISION (NTI) (—) AND BLOOD VESSEL TRANSECTION (—) (BVT) AT 0 SECONDS IN INDIVIDUAL ANIMALS, WITH MEAN PERCENTAGE CHANGE OVERLAID FOR NTI (—) AND BVT (—). DATA ARE DISPLAYED AS 10-POINT MOVING AVERAGES. 118

FIGURE 5.3. PERCENTAGE CHANGE IN 95% SPECTRAL EDGE FREQUENCY (F95) OF THE EEG FOLLOWING NECK TISSUE INCISION (—) (NVI) AND BLOOD VESSEL TRANSECTION (—) (BVT) AT 0 SECONDS IN INDIVIDUAL ANIMALS, WITH MEAN PERCENTAGE CHANGE OVERLAID FOR NTI (—) AND BVT (—). DATA ARE DISPLAYED AS 10-POINT MOVING AVERAGES. 118

FIGURE 5.4. PERCENTAGE CHANGE IN TOTAL POWER (PTOT) OF THE EEG POWER SPECTRUM OF THE EEG FOLLOWING AND NECK TISSUE INCISION (—) (NTI) AND BLOOD VESSEL TRANSECTION (—) (BVT) AT 0 SECONDS IN INDIVIDUAL ANIMALS, WITH MEAN PERCENTAGE CHANGE OVERLAID FOR NTI (—) AND BVT (—). DATA ARE DISPLAYED AS 10-POINT MOVING AVERAGES. 119

FIGURE 5.5. AN EXAMPLE OF THE TYPICAL RESPONSE TO NECK TISSUE INCISION (NTI) ON THE COMPRESSED SPECTRAL ARRAY FROM CALF 3 LEFT CEREBRAL HEMISPHERE. NECK TISSUE INCISION OCCURRED AT 0 SECONDS..... 120

FIGURE 5.6. AN EXAMPLE OF THE TYPICAL RESPONSE TO THE TRANSECTION OF THE MAJOR BLOOD VESSELS (BVT) SUPPLYING THE BRAIN ON THE COMPRESSED SPECTRAL ARRAY FROM CALF 1 RIGHT CEREBRAL HEMISPHERE. TRANSECTION OF BLOOD VESSELS OCCURRED AT 0 SECONDS.	121
FIGURE 5.7. MEAN CHANGES IN BLOOD PRESSURE (MMHG) FOLLOWING NECK TISSUE INCISION (—) (NTI) AND TRANSECTION OF BLOOD VESSELS (—) (BVT) AT 0 SECONDS (— ± SEM). A = SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS, P<0.001.	122
FIGURE 6.1. DIAGRAM OF THE EXPERIMENTAL DESIGN WITH INDUCTION AT 0 MINUTES AND NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING AT 25 MINUTES (MARKED WITH A VERTICAL GREY BLOCK).	137
FIGURE 6.2. EEG TRACE FROM A CALVE SHOWING THE COURSE OF CHANGES FROM ACTIVE EEG (A), TO TRANSITIONAL EEG (B) AND TO AN ISOELECTRIC EEG (C).	138
FIGURE 6.3. MEAN CHANGES TOTAL POWER (PTOT) OF THE ELECTROENCEPHALOGRAM (EEG) BEFORE AND AFTER NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING ONLY; SIMPLE RESPONSE (N=4). RIGHT CEREBRAL HEMISPHERE (—), LEFT CEREBRAL HEMISPHERE (—). (A) = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES PTOT (P<0.05) RIGHT CEREBRAL HEMISPHERE.....	140
FIGURE 6.4. MEAN CHANGES TOTAL POWER (PTOT) OF THE ELECTROENCEPHALOGRAM (EEG) BEFORE AND AFTER NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING ONLY; BIPHASIC RESPONSE (N=5). RIGHT CEREBRAL HEMISPHERE (—), LEFT CEREBRAL HEMISPHERE (—). (A) = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES PTOT (P<0.05) RIGHT CEREBRAL HEMISPHERE.....	140
FIGURE 6.5. EXAMPLE OF THE COMPRESSED SPECTRAL ARRAY FROM A CALF WITH A SIMPLE RESPONSE AFTER NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING AT 0 SECONDS.	141
FIGURE 6.6. EXAMPLE OF THE COMPRESSED SPECTRAL ARRAY FROM A CALF WITH A BIPHASIC RESPONSE AFTER NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING AT 0 SECONDS.	141
FIGURE 6.7. CHARACTERISTICS OF THE SPONTANEOUS ELECTROENCEPHALOGRAM (EEG) IN INDIVIDUAL ANIMALS OVER TIME (SECONDS). VISUALLY EXAMINED AND CLASSED AS EITHER, ACTIVE, TRANSITIONAL, CYCLIC EEG AND ISOELECTRIC. R REPRESENTS EEG RECORDED FROM THE RIGHT AND L THE EEG RECORDED FROM THE LEFT SIDE OF THE CRANIUM. TIME DURATIONS IN SECONDS IS DISPLAYED ON THE INDIVIDUAL BARS; CLEAR GAPS REPRESENT PERIODS WHERE THE EEG WAS OUT OF RANGE.	142
FIGURE 6.8. CHANGES IN MEAN FEMORAL ARTERIAL BLOOD PRESSURE (MMHG) BEFORE AND AFTER NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING (— ±SEM). (A) = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES (P<0.05). THE ARROW DENOTES THE APPLICATION OF NPCB STUN.	142
FIGURE 7.1. DIAGRAM OF THE EXPERIMENTAL DESIGN. ANAESTHESIA WAS INDUCED AT 0 MINUTES, FOLLOWING WHICH ANAESTHESIA WAS STABILIZED. A 10-MINUTE PRE-TREATMENT RECORDED WAS TAKEN; THIS WAS IMMEDIATELY PRECEDED BY VENTRAL NECK INCISION WITH NON-PENETRATIVE CAPTIVE BOLT STUNNING (VNI+NPCB) 5 SECONDS AFTER (MARKED WITH A VERTICAL GREY BLOCK). THE ELECTROENCEPHALOGRAM (EEG) WAS RECORDED FOR A FURTHER 10 MINUTES FOLLOWING TREATMENT.	156
FIGURE 7.2. MEAN CHANGES IN TOTAL POWER (PTOT) OF THE ELECTROENCEPHALOGRAM (EEG) BEFORE AND AFTER VENTRAL NECK INCISION FOLLOWED 5 SECONDS LATER BY NON-PENETRATIVE CAPTIVE BOLT STUNNING (VNI+NPCB). RIGHT (—) AND LEFT (—) CEREBRAL HEMISPHERE. A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES, RIGHT CEREBRAL HEMISPHERE (P<0.05), B = SIGNIFICANT DIFFERENCE FROM PER-TREATMENT VALUES LEFT CEREBRAL HEMISPHERE (P<0.05).....	158
FIGURE 7.3. A TYPICAL EXAMPLE OF A COMPRESSED SPECTRAL ARRAY FROM AN INDIVIDUAL CALF BEFORE AND AFTER VENTRAL NECK INCISION FOLLOWED 5-SECONDS LATTER BY NON-PENETRATIVE CAPTIVE BOLT STUNNING (VNI+NPCB).	159

FIGURE 7.4. AN EXAMPLE OF THE SPONTANEOUS ELECTROENCEPHALOGRAM (EEG) OF AN INDIVIDUAL CALF BEFORE, DURING AND AFTER VENTRAL NECK INCISION (VNI) AND NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING, WITH INDIVIDUAL POWER SPECTRUMS FOR TWO-SECOND EPOCHS DURING (A) PRE-TREATMENT ELECTROENCEPHALOGRAM, (B) VNI ARTEFACT, AND (C) TRANSITIONAL ELECTROENCEPHALOGRAM AFTER NPCB STUNNING. ARROWS REPRESENT THE VNI AND NPCB..... 159

FIGURE 7.5. CHARACTERISTICS OF THE SPONTANEOUS ELECTROENCEPHALOGRAM (EEG) IN INDIVIDUAL ANIMALS OVER TIME (SECONDS) AFTER VENTRAL NECK INCISION FOLLOWED 5 SECONDS LATER BY NON-PENETRATIVE CAPTIVE BOLT STUNNING (VNI+NPCB). THE SPONTANEOUS TRACES WERE VISUALLY INSPECTED AND WERE EITHER CLASSIFIED AS: VNI ARTEFACT, ACTIVE EEG, TRANSITIONAL EEG, CYCLIC HALF, AND ISOELECTRIC WAVEFORM. ARROWS REPRESENT TIME POINT OF VNI AND NPCB. R REPRESENTS EEG RECORDED FROM THE RIGHT AND L THE EEG RECORDED FROM THE LEFT SIDE OF THE CRANIUM. TIME DURATIONS IN SECONDS ARE DISPLAYED ON THE INDIVIDUAL BARS; CLEAR GAPS REPRESENT PERIODS WHERE THE EEG RECORDING WAS OUT OF RANGE..... 161

FIGURE 7.6. MEAN \pm STANDARD ERROR OF THE MEAN (—) CHANGES IN FEMORAL ARTERIAL BLOOD PRESSURE (MMHG) BEFORE AND AFTER VENTRAL NECK INCISION FOLLOWED 5 SECONDS LATER WITH A NON-PENETRATIVE CAPTIVE BOLT STUN (VNI+NPCB). (A) = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES ($P<0.05$). SHORT DASHES REPRESENT VNI, LONG DASHES REPRESENT NPCB..... 161

FIGURE 8.1. EXPERIMENTAL DESIGN, AFTER INDUCTION OF ANAESTHESIA AT 0 MINUTES, SHOWING STABILISATION OF ANAESTHESIA, INSTRUMENTATION, PRE-TREATMENT RECORDING, THE 70-VOLT SUPRAMAXIMAL ELECTRICAL STIMULUS (GREY VERTICAL BOX) AND THE POST-TREATMENT RECORDING..... 174

FIGURE 8.2. INTERVAL PLOTS SHOWING THE MEDIANS AND 95% CONFIDENCE INTERVALS OF THE INITIAL EEG RESPONSE (RIGHT AND LEFT CEREBRAL HEMISPHERES): DATA SUMMED (Σ) FROM 30 SECONDS BEFORE AND AFTER APPLICATION OF A 70-VOLT SUPRAMAXIMAL ELECTRICAL STIMULUS TO THE LEFT SHOULDER OF CALVES. F50 (A), F95 (B) AND PTOT (C)..... 177

FIGURE 8.3. MEAN CHANGES IN MEDIAN FREQUENCY (F50) AFTER A 70-VOLT SUPRAMAXIMAL ELECTRICAL STIMULUS APPLIED AT 0 SECONDS TO THE LEFT SHOULDER OF CALVES. RIGHT (—) AND LEFT (—) CEREBRAL HEMISPHERE. A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES, RIGHT CEREBRAL HEMISPHERE ($P<0.05$), B = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES, LEFT CEREBRAL HEMISPHERE ($P<0.05$). 178

FIGURE 8.4. MEAN CHANGES IN 95% SPECTRAL EDGE FREQUENCY (F95) AFTER A 70-VOLT SUPRAMAXIMAL ELECTRICAL STIMULUS APPLIED AT 0 SECONDS TO THE LEFT SHOULDER OF CALVES. RIGHT (—) AND LEFT (—) CEREBRAL HEMISPHERE. A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES, RIGHT CEREBRAL HEMISPHERE ($P<0.05$), B = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES, LEFT CEREBRAL HEMISPHERE ($P<0.05$)..... 178

FIGURE 8.5. MEAN CHANGES IN TOTAL POWER (PTOT) AFTER A 70-VOLT SUPRAMAXIMAL ELECTRICAL STIMULUS APPLIED AT 0 MINUTES TO THE LEFT SHOULDER OF CALVES. RIGHT (—) AND LEFT (—) CEREBRAL HEMISPHERE. A = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES, RIGHT CEREBRAL HEMISPHERE ($P<0.05$), B = SIGNIFICANT DIFFERENCE FROM PRE-TREATMENT VALUES, LEFT CEREBRAL HEMISPHERE ($P<0.05$)..... 179

FIGURE 9.1. MEAN SOMATOSENSORY EVOKED POTENTIALS (—) \pm SEM (—), RECORDED IN RESPONSE TO A 10-VOLT ELECTRICAL STIMULUS TO THE EXPOSED SUPERFICIAL PERONEAL NERVE OF EWE 2, AT HALOTHANE VAPORISER SETTINGS OF 1% (CONNECTED AND DISCONNECTED), 1.7%, 2.1% AND 2.3%, AND AT 0.5% AFTER 15-MINUTE RECOVERY, AND FOLLOWING EUTHANASIA WITH PENTOBARBITONE. THE ELECTRICAL STIMULUS WAS DELIVERED AT 0 MILLISECONDS WITH THE STIMULUS ARTEFACT REMOVED FOR CLARITY. PEAKS ARE DESIGNATED EITHER P (POSITIVE DEFLECTION) OR N (NEGATIVE DEFLECTION), AND THE SUBSCRIPT NUMBER IS THE TIME IN MILLISECONDS AFTER STIMULATION..... 192

FIGURE 10.1. SUPERFICIAL PERONEAL NERVE PREPERATION. A: THE EXPOSED SUPERFICIAL PERONEAL NERVE LAYED OVER TWO STIMULATING ELECTRODES (POSITVE DISTAL AND NEGATIVE PROXIMAL). A SURGICAL SWAP, LOCATED PROXIMALLY, WAS SOAKED IN HARTMANN’S SOLUTION, WAS EARTHED AND ACTED AS THE NERVE BATH. B: THE FINISHED PREPERATION WITH MOISTEND SWAPS PREVENTING DEYDRATION..... 202

FIGURE 10.2. DIAGRAMMATIC REPRESENTATION OF THE EXPERIMENTAL DESIGN FOR BOTH VENTRAL NECK INCISION (VNI) AND NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING GROUPS. CALVES IN BOTH GROUPS WERE ANAESTHETIZED AT 0 MINUTES. TREATMENTS OCCURRED APPROXIMATELY 115 MINUTES FROM INDUCTION OF ANESTHESIA. 205

FIGURE 10.3. EXAMPLES OF SOMATOSENSORY EVOKED POTENTIALS (SEP) FROM TWO CALVES AFTER EITHER VENTRAL NECK INCISION SLAUGHTER (CALF 8 VNI GROUP) OR NON-PENETRATIVE CAPTIVE STUNNING (CALF 7 NPCB GROUP). THESE WERE THE ONLY DATA SETS WHERE PRE-TREATMENT SEPs WERE ABLE TO BE GENERATED. EACH WAVEFORM IS THE SUMMATION OF 32 TRACES. THERE WAS A STIMULUS DELAY OF 100 MS WITH THE STIMULUS BEING DELIVERED AT 0 MINUTES. 207

FIGURE 10.4. CHANGES IN THE CHARACTERISTICS OF SPONTANEOUS ELECTROENCEPHALOGRAM (EEG) IN INDIVIDUAL ANIMALS AFTER VENTRAL NECK INCISION (VNI). THE TRACES WERE VISUALLY INSPECTED AND WERE CLASSIFIED AS: VNI ARTEFACT, ACTIVE EEG, TRANSITIONAL EEG, CYCLIC HALF ACTIVITY, ISOELECTRIC WAVEFORM AND HIGH AMPLITUDE HIGH FREQUENCY HAHF. R REPRESENTS EEG RECORDED FROM THE RIGHT AND L THE EEG RECORDED FROM THE LEFT SIDE OF THE CRANIUM. THE TIME DURATIONS IN SECONDS ARE DISPLAYED ON THE INDIVIDUAL BARS; CLEAR GAPS REPRESENT PERIODS WHERE THE EEG RECORDING WAS OUT OF RANGE. 209

FIGURE 10.5. CHARACTERISTICS OF THE SPONTANEOUS ELECTROENCEPHALOGRAM (EEG) IN INDIVIDUAL ANIMALS AFTER NON-PENETRATIVE CAPTIVE BOLT STUNNING (NPCB). THE TRACES WERE VISUALLY INSPECTED AND WERE CLASSIFIED AS: ACTIVE EEG, TRANSITIONAL EEG, CYCLIC HALF ACTIVITY AND ISOELECTRIC WAVEFORMS. R REPRESENTS EEG RECORDED FROM THE RIGHT AND L THE EEG RECORDED FROM THE LEFT SIDE OF THE CRANIUM. THE TIME DURATIONS IN SECONDS ARE DISPLAYED ON THE INDIVIDUAL BARS; CLEAR GAPS REPRESENT PERIODS WHERE THE EEG RECORDING WAS OUT OF RANGE. 210

FIGURE 10.6. MEAN BLOOD PRESSURE AFTER VENTRAL NECK INCISION (VNI) (—) AND NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING ONLY (—). CALF 9 FROM THE VNI GROUP HAD ONE ONLY CARTIOD CUT (—). A = SIGNIFICANT DIFFERENCE FROM PRETREATMENT VALUES IN THE VNI GROUP. B = SIGNIFICANT DIFFERENCE FROM PRETREATMENT VALUES IN THE NPCB STUNNING GROUP..... 211

LIST OF TABLES

TABLE 2.1. STUDIES INTO THE EFFECTS OF EXPERIMENTAL PAIN IN HUMANS AND ANIMALS ON EEG FREQUENCY BAND POWER.	33
TABLE 2.2. MAJOR STUDIES INVESTIGATING THE TIME TO INSENSIBILITY AFTER VENTRAL NECK INCISION (VNI) SLAUGHTER WITHOUT PRIOR STUNNING IN VARIOUS SPECIES AND THE PARAMETER REPORTED IN PUBLICATIONS.	39
TABLE 2.3. STUDIES OF THE TIME TO INSENSIBILITY IN CATTLE WITH DIFFERENT STUNNING TECHNIQUES: PENETRATIVE CAPTIVE BOLT (PCB); NON-PENETRATIVE CAPTIVE BOLT (NPCB); ELECTRICAL STUNNING. THE PARAMETER USED AND TIME TO INSENSIBILITY ARE INDICATED.	41
TABLE 2.4. STUDIES OF THE TIME TO INSENSIBILITY IN SHEEP WITH DIFFERENT STUNNING TECHNIQUES: PENETRATIVE CAPTIVE BOLT (PCB); NON-PENETRATIVE CAPTIVE BOLT (NPCB); ELECTRICAL STUNNING. THE PARAMETER USED AND TIME TO INSENSIBILITY ARE INDICATED.	42
TABLE 2.5. STUDIES OF THE TIME TO INSENSIBILITY IN POULTRY WITH DIFFERENT STUNNING TECHNIQUES, PENETRATIVE CAPTIVE BOLT (PCB) AND NON-PENETRATIVE CAPTIVE BOLT (NPCB). THE PARAMETER USED AND TIME TO INSENSIBILITY ARE INDICATED.	42
TABLE 3.1. MEAN \pm STANDARD ERROR OF THE MEAN OF HEART RATE (BEATS PER MINUTE) AS A PERCENTAGE OF INDIVIDUAL PRE-TREATMENTS AT TIME POINTS AFTER THE START OF DEHORNING FOR SCOOP DEHORNING ALONE (DH) OR SCOOP DEHORNING WITH LIDOCAINE RING BLOCK (DH+LA) GROUPS.	76
TABLE 4.1. MEAN \pm STANDARD ERROR OF THE MEAN, CHANGES IN HEART (BEATS PER MINUTE) AT TIME POINTS AFTER VENTRAL NECK INCISION (VNI).	99
TABLE 4.2. HISTOLOGY RESULTS FOR CALVES AFTER VENTRAL NECK INCISION (VNI). SECTIONS TAKEN FROM OBEX, SPINAL CORD, PONS, CEREBELLUM, MIDBRAIN, THALAMUS, INSULA AND SOMATOSENSORY CORTEX. CHATTER = PROCESSING ARTEFACT.	100
TABLE 6.1. MEAN \pm STANDARD ERROR OF THE MEAN OF HEART RATE (BEATS PER MINUTE) AT INDIVIDUAL TIME POINTS AFTER NON-PENETRATIVE CAPTIVE BOLT (NPCB) STUNNING.	143
TABLE 7.1. MEAN \pm STANDARD ERROR OF THE MEAN, CHANGES IN HEART (BEATS PER MINUTE) AT TIME POINTS AFTER VENTRAL NECK INCISION FOLLOWED 5 SECONDS LATER BY NON-PENETRATIVE CAPTIVE BOLT STUNNING (VNI+NPCB) OF CALVES.	162
TABLE 8.1 MEAN \pm STANDARD ERROR OF THE MEAN (SEM), CHANGES IN HEART RATE (BEATS PER MINUTE (BPM)) AND BLOOD PRESSURE (MMHG) AT DIFFERENT TIME POINTS AFTER A 70-VOLT SUPRAMAXIMAL ELECTRICAL STIMULUS. A = PRE-TREATMENT VALUE, B = 70-VOLT SUPRAMAXIMAL ELECTRICAL STIMULUS.	180

ABBREVIATIONS AND ACRONYMS

AEP	Auditory evoked potentials
ANOVA	Analysis of variance
bpm	beats per minute
BSE	Bovine spongiform encephalopathy
BVT	Major neck blood vessel transection
CJD	Creutzfeldt-jakob disease
CNS	Central nervous system
CI	Confidence interval
DH	Dehorned only
DH+LA	Dehorned plus lidocaine ring block
ECG	Electrocardiogram/electrocardiographic
ECoG	Electrocorticogram
EEG	Electroencephalogram/electroencephalographic
EMG	Electromyogram / electromyographic
EP	Evoked potential
F50	Median frequency
F95	95% Spectral edge frequency
FFT	Fast Fourier Transformation
FE'HAL	End-tidal halothane partial pressure
fMRI	Functional magnetic resonance imaging
HALF	High amplitude low frequency
HAHF	High amplitude high frequency
H&E	Hematoxylin and Eosin
HPA	Hypothalamic-pituitary adrenocortical axis
I.M	Intramuscular
NPCB	Non-penetrative captive bolt
NTI	Neck tissue incision with intact blood circulation through the brain
PET	Positron emission tomography
Ptot	Total power of the electroencephalogram
SAM	Sympathetic adrenomedullary system

SD	Standard deviation
SEM	Standard error of the mean
SEP	Somatosensory evoked potentials
SI	Sham incision
TBI	Traumatic brain injury
VAS	Visual analogue scale
VEP	Visual evoked potentials
VNI	Ventral neck incision

CHAPTER 1

SLAUGHTER WITHOUT STUNNING

1.1 Introduction

The slaughter of livestock for human consumption, animal based products and emergency euthanasia, have a number of potential areas for significant welfare compromise, from the farm gate to slaughter room floor. Yarding, loading/unloading, transportation, fatigue, heat stress, disease and dehydration all have the potential to cause significant welfare compromise. Once at the slaughter works the animals are in an unknown environment, and they are often mixed with or in close proximity to unfamiliar animals, causing potential stress, agitation and injury. From the yards to the stun/kill box, the animals are further exposed to stresses such as unfamiliar sounds, sights, smells, reflections and contrasts in colours. The design of stun/kill boxes, lighting, restraints and conveyors can affect the welfare of the animals prior to stunning/slaughter. All these events can cause suffering. Any consideration of the welfare of livestock submitted for slaughter must therefore consider all the events involved during entire slaughter process not just the act of slaughter itself (FAWC 2003).

Significant improvements in animal welfare during slaughter have been made, particularly with: improved animal handling techniques through education (Grandin 1994a, 2005); improvements in slaughterhouse design (Grandin 1994b, 2003a) and management (Grandin 1994b 2005); and the development of improved stunning/slaughter restraint systems that reduce distress (Dun 1990; Grandin 1990, 1992; Grandin and Regenstein 1994; Grandin 1994ab, 2003ab). Although the slaughter process encompasses everything from the farm gate to the slaughter room floor, the act of killing has received much attention, especially with regard to the potential to experience pain from the neck cut, the time taken to become insensible and the methods for rendering the animals insensible to any pain and distress.

Slaughter without prior stunning is of interest to numerous individuals and groups including scientific and religious communities, government regulatory authorities, animal welfare groups, farmers, the livestock industry and the general public. In certain religions, slaughter of livestock for human consumption without prior stunning is an important and essential part of practicing and observing their religious faiths. Slaughter without prior stunning is sometimes also practiced for emergency slaughter of livestock on farms, during transport and at slaughterhouse yards, for several reasons including minimising further suffering caused by injury or sickness, or to maintain biosecurity.

Slaughter by ventral neck incision (VNI) without prior stunning is carried out on some animals in New Zealand and other countries. A single incision with a razor sharp blade is made in the ventral aspect of the neck, which transects carotid arteries and jugular veins, skin, muscle, trachea, oesophagus, sensory nerves and connective tissues, but not the vertebral arteries (Mellor and Littin 2004). There are a number of potential welfare concerns surrounding slaughter without prior stunning including possible pain caused by the incision itself, and distress during the time before loss of consciousness. Previous studies have demonstrated that the electrocorticogram (ECoG) remains grossly unchanged for 7.5 ± 2 seconds (Daly et al 1988) and onset of undoubted insensibility occurs between 5 to 60 seconds after neck incision (Levinger 1961; Nangeroni and Kennett 1964; Blackmore et al 1979; Groß 1979; Blackmore and Newhook 1981; Newhook and Blackmore 1982b; Blackmore et al 1983; Gregory and Wotton 1984c, Daly et al 1988; Bager et al 1992; Mellor and Littin 2004). There is insufficient understanding of conscious processes to be able to interpret these electrical events in terms of the precise progression towards loss of consciousness, but it seems likely from these data that there is a window between incision of the neck and loss of consciousness during which the animal could potentially experience pain and distress and suffer as a result.

1.2 Research Focus

1.2.1 Primary focus

It is to quantitatively investigate in cattle the potential noxiousness or otherwise of slaughter by ventral neck incision (VNI), with or without stunning, during the period before the onset of hypoxia-induced insensibility.

1.2.2 Secondary foci

- i. Prior to experimentation in which calves were to be slaughtered it was necessary to validate, in calves, the use of a minimal anaesthesia model and the electroencephalogram (EEG) for assessing responses to noxious stimulation (Murrell and Johnson 2006). This was to be done with the use of surgical or amputation dehorning (by scoop) as the noxious stimulus. Large amounts of data exist using surgical dehorning (Stafford and Mellor 2005), with which data generated using the minimal anaesthesia model were to be compared.
- ii. Electroencephalographic responses to slaughter by VNI without prior stunning in calves were then to be identified. The data generated were to be examined for any of the characteristic signs which had been previously associated with noxious stimulation in the calves during dehorning in experiment (i) and in other species in response to other invasive techniques (Murrell and Johnson 2006).
- iii. The causation of detected EEG response to slaughter by VNI without prior stunning in calves was then to be investigated. Any potential noxious stimulation seen in experiment (ii) was anticipated to be due to neck incision, to changes in blood flow to the brain or to a combination of the two. A study

was designed to compare the EEG responses to loss of carotid blood flow to the brain without tissue damage to the neck and transection of neck tissue similar to VNI but without disrupting the blood flow to and from the brain.

- iv. The impact of non-penetrative captive bolt (NPCB) stunning of calves was then to be investigated using its effects on the EEG to evaluate alterations in functional activity of the cerebral cortex in response to concussive stunning and how those alterations relate to insensibility.
- v. The modulation of detectable EEG responses to slaughter in calves by the application of a NPCB stun after the neck cut (VNI) was then to be investigated.
- vi. Finally, develop a model for the generation and recording of somatosensory-evoked potential (SEP) for use in slaughter research and identify SEP responses to slaughter and stunning was to be undertaken, and the impact of slaughter by VNI or NPCB stunning on the propagation of SEPs was to be investigated in two groups of calves.

All of this work was completed.

1.3 Structure of the Thesis

The thesis comprises 11 Chapters that commence with the literature review (Chapter 2). This is followed by experimental chapters (3 to 10 Chapters). Each experimental chapter has a separate abstract, introduction, materials and methods, results and discussion. All experimental chapters have either been accepted, submitted to or are prepared for submission to peer-reviewed scientific journals. The thesis concludes with the general discussions and conclusions (Chapter 11).

1.4 References

- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP.** Onset of insensibility at slaughter in calves - effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162-73, 1992
- Blackmore DK, Newhook JC, Petersen GV.** Electrical stunning and humane slaughter. *New Zealand Veterinary Journal* 27, 224-, 1979
- Blackmore DK, Newhook JC.** Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* 29, 219-22, 1981
- Blackmore DK, Newhook JC, Grandin T.** Time of onset of insensibility in 4-week-old to 6-week-old calves during slaughter. *Meat Science* 9, 145-9, 1983
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325-9, 1988
- Dun CS.** Stress reactions of cattle undergoing ritual slaughter using two methods of restraint. *Veterinary Record* 126, 522-5, 1990
- FAWC.** Report on the welfare of farmed animals at slaughter or killing. Part 1. red meat animals. Farm Animal Welfare Council, London, 2003
- Grandin T.** Humanitarian aspects of Shechita in the United States. *Judaism* 39, 436-46, 1990
- Grandin T.** Observations of cattle restraint devices for stunning and slaughtering. *Animal Welfare* 1, 85-90, 1992
- Grandin T.** Euthanasia and slaughter of livestock. *Journal of the American Veterinary Medical Association* 204, 1354-60, 1994a
- Grandin T.** Farm animal-welfare during handling, transport, and slaughter. *Journal of the American Veterinary Medical Association* 204, 372-7, 1994b
- Grandin T, Regenstein JM.** Religious slaughter and animal welfare: a discussion for meat scientists. *Meat Focus International*, 115-23, 1994
- Grandin T.** Slaughterhouse design and religious slaughter. In: DVD. 2003a
- Grandin T.** Transferring results of behavioural research to industry to improve animal welfare on the farm, ranch and the slaughter plant. *Applied Animal Behaviour Science* 81, 215-28, 2003b

- Grandin T.** Maintenance of good animal welfare standards in beef slaughter plants by use of auditing programs. *JAVMA-Journal of the American Veterinary Medical Association* 226, 370-3, 2005
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141-3, 1984c
- Groß R.** Elektroencephalographische und elektrokardiographische verlaufsuntersuchungen nach bolzenschußbetäubung und nach töten durch entbluten in der form des rituellen schlachtens. durch die tierärztliche hochschule, Hannover, 1979
- Levinger IM.** Untersuchungen zum schächtproblem. Dr thesis, University of Zürich, Zürich, 1961
- Mellor DJ, Littin KE.** Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13, S127-S32, 2004
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Nangeroni LL, Kennett PD.** Electroencephalographic studies of the effects of Shechita slaughter on cortical function in ruminants. Cornell University, New York, 1964
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295-300, 1982b
- Stafford KJ, Mellor DJ.** Dehorning and disbudding distress and its alleviation in calves. *The Veterinary Journal* 169, 337-49, 2005

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

This review examines the existing literature concerning the slaughter of animals without prior stunning, investigating: the ethics of slaughter and slaughter based research, slaughtering without stunning, the time to insensibility in different livestock species, the potential role of the vertebral arteries in maintaining sensibility in cattle and the possible pain of slaughter. The review also investigates the different techniques that have been used in the investigation of pain in animals and novel techniques that may improve the understanding of the potential noxious sensory input of slaughter without stunning.

2.1 Animal Welfare

Concern for the welfare of livestock from within the public domain has become an increasingly important driving force for changes in the management of livestock. This has occurred in parallel with changes in thinking about the way humans interact with animals, resulting in an increased awareness of the welfare of animals under the influence of humans. This has contributed to the development of the new scientific field of “Animal Welfare Science”. This field endeavours to qualify, quantify and improve welfare or minimise suffering, using best scientific practices and theory. The changing views of what the public feel that animals experience, coupled with advances in science and international export requirements have resulted in significant improvements in animal welfare with changes in government legislation reflecting the increased concern for and awareness about the welfare of animals under the influence of human actions both directly and indirectly.

The term ‘animal welfare’ did not arise in science to express a scientific concept, rather it came about to express the ethical concerns of people with regard to the treatment of animals (Duncan and Fraser 1997). There are many different

definitions of animal welfare (Swanson 1995; Spedding 2000). For instance: ‘welfare is normally defined as a state of well-being, in which at least basic needs are met and suffering is minimized’ (Spedding 2000). This is a simple definition, which deals mainly with the physical needs of the animal and implies that suffering can only be minimized. This definition does not take into account the idea that animals can be physically fit and mentally unwell (i.e. experiencing boredom, anxiety, fear, etc); also it fails to include positive states of welfare (e.g. happiness, excitement, etc). When people (scientists and the public alike) think of animal welfare they find it relatively easy to describe it in terms of the absence of negative states. Even the ‘Five Freedoms’, developed by the Farm Animal Welfare Council (FAWC 2003), focus on the absence of negative states of welfare. Duncan (2002) suggested that most people reach general agreement on what animal welfare is. They find it fairly easy to describe states that reduce welfare and a little more difficult to say what good welfare is. The question to be asked is whether a definition of animal welfare is absolutely necessary; can progress be made without definitions?

Although most people have similar concepts of what constitutes poor and good welfare, it is important to realise that the state of being of animals is rarely at one of these extremes. In the Massey University laboratory where the present thesis work was undertaken, it has been found to be useful to define the state of being of an animal over a range or continuum between very poor welfare and excellent welfare. In addition, its multifaceted character has been highlighted including as it does nutritional, environmental, health, behavioural and mental domains (Mellor and Stafford 2001). Anxiety, fear, pain, and distress are part of the mental experience of animals and, when severe enough, may lead to severe suffering or poor welfare. Where an animal is located on the continuum between negative and positive welfare depends on the interrelationship between these different domains in the particular circumstances of the animal. In the present case, the context of livestock slaughter.

2.2 Some Reflections on the Ethics of Slaughter

The commonly held concept of ethics is that it is the analysis of right or wrong. However ethics is more than cataloguing decisions about right or wrong thoughts and actions; rather it is the analysis of the different ways such decisions are made (Mellor and Littin 2004). Different decisions can be analysed according to a range of ethical principles, and individuals may employ several such principles and give them different weight in order to arrive at the particular decisions they make (Battye 1998; Mellor 1998; Mellor and Battye 2004). Ethics applies both to the choices of individuals and groups.

Human-animal interactions will always produce ethical and moral questions, especially with regard to modern livestock based agriculture and slaughter. The way humans interact with farmed species, particularly in highly industrialised and urbanised countries, has changed dramatically in the last hundred years. For instance, consumers are no longer required to raise, kill and butcher animals for meat, which is now supplied shrink-wrapped in styrofoam containers bearing no resemblance to the animal of origin. The distancing of consumers from the farm gate has resulted in the situation where some of them are unaware of processes involved in the farming and slaughter of animals. Attitudes can range from a primary preoccupation with the price on the supermarket shelf to an overwhelming concern about how animals are treated. Regarding the latter, a survey of meat eaters in the United Kingdom found that if confronted with the hypothetical prospect of having to kill animals for their own needs, the majority said they would cease eating meat altogether (Gregory 2004). There are many different stakeholders interested in the farming, treatment and slaughter of animals. These groups often have differing ethical and moral viewpoints on the use of animals in human society.

There will always be ethical and moral issues surrounding whether or not animals should be killed, whether it is for human food, welfare, euthanasia, human/animal disease control, production or economics. Death is inevitable for all living organisms, so regardless of the reason or justification the main ethical concern must be to minimise suffering during slaughter (Spedding 1998; FAWC 2003). However this can be complicated by human rights, such as the right to worship and practice beliefs, where those practices involving slaughter preclude the use of

stunning techniques and where there is debate concerning the potential for suffering during slaughter.

2.2.1 The Ethics of Slaughter Based Research

Animal based research generates a number of ethical and moral concerns, including whether or not animals should be used for such purposes at all, and if they are, whether or not any harm caused to them by such use can be justified by the anticipated benefits (Mellor 1998). A related issue is how well animals can model normal human body function and perturbed states such as injury and disease.

Conducting animal research to seek potential benefit for the same species avoids some issues related to modelling, unless a purpose of the work is to model another animal species, for instance sheep investigated for their own sake and to model cattle. However, the question of the expected benefits still arises, whether these relate to the individual animal, the species, to human interests (economics) or other matters. When conducting animal-based research, regardless of the reason or justification, the benefits of the research must outweigh the potential harms in accord with utilitarian ethical precepts, which are most commonly used to justify the use of animals in science (Mellor 1998). Indeed, the benefits must be maximised and the harms minimised so that the separation between them is the greatest that can practically be achieved (Battye 1998; Mellor 1998). This is especially true for Slaughter-based research, which is a particularly strong example of the importance of ensuring that the benefits of the research greatly outweigh the suffering of the individual animals involved. However, although it is true that any suffering in the tens of animals used to improve the humaneness of slaughter techniques will benefit millions of other animals over many years, and potentially worldwide, the animals involved in such slaughter research have no knowledge that their suffering will provide for some greater good. It is therefore essential for scientists involved in slaughter research to recognize the potential for suffering in the animals they use and to minimise that suffering. This can be done through the adherence to the Three Rs principles (Russell and Burch 1959): i.e. animal use should be avoided if possible (*replacement*); if that is not possible, the number used must be minimised (*reduction*);

and the animals used must be treated in ways that keep the negative impact on them as low as possible (*refinement*). A good example is a novel methodological refinement developed in the Massey University laboratory. It allows noxious sensory input to be investigated without the animals consciously experiencing pain and distress (Johnson et al 2008) and has obvious potential applications to slaughter research, as outlined below.

2.3 Jewish and Muslim Slaughter Background

Certain religions, including the Sikh, Jewish and Muslim religions, have strict rules concerning the treatment, slaughter and preparation of animals for human consumption. These rules in some cases forbid the stunning of animals prior to slaughter. Such religious requirements are important for the practice of these faiths and therefore discussion about them can become highly emotional and political. The slaughter practiced by the Sikh religion involves the decapitation of accepted species; this is referred to as Jhatka slaughter (Shimshony and Chaudry 2005). Slaughter for both Jewish and Muslim religions involves VNI of fully conscious animals that are free from injury and disease (Carding 1970). The incision involves the transection and severing of the skin, muscles, carotid arteries, jugular veins, trachea, oesophagus, sensory nerves and connective tissues. Some Muslim communities do allow reversible stunning (Anil and Sheard 1994). The banning of religious slaughter practices without significant evidence would certainly be viewed as a hostile act by adherents to these faiths (Grandin and Regenstein 1994). It is therefore important for researchers to remain objective, critically and cautiously interpreting data when evaluating these practices from an animal welfare viewpoint (Grandin and Regenstein 1994).

2.3.1 Jewish Slaughter

The Jewish religious method of animal slaughter is referred to as Shechita. The method of Shechita to the orthodox, traditional Jew is a command, given to

Moses on Mount Sinai. The reference to Shechita in the Pentateuch or Torah (five books of Moses) is somewhat cryptic:

“...thou shall kill of thy herd and of thy flocks, which the Lord hath given thee, as I commanded thee...” (Deuteronomy XII:21).

This may suggest that people were familiar with the rules for slaughter (Grandin and Regenstein 1994) or that they were outlined in an earlier command given to Moses at Sinai (Homa 1971). The correct practice of Shechita was conveyed via the oral law and is the only permissible form of animal slaughter according to the traditional body of Jewish law, the Halacha (derives from the Hebrew halach meaning "going" or the "correct way") (Rosen 2004). The Halacha was passed down through the generations orally, until eventually it was written down in a series of volumes (Mishnah and Gemarah) collectively referred to as the Talmud (Grandin and Regenstein 1994). Included in the Talmud and the oral law are details on the treatment of animals generally and prior to slaughter, and the preparation of meat to ensure that it is Kosher (i.e. in accordance with dietary laws). The Pentateuch states that the blood contains the life of the animal. The prohibition against eating blood is stated many times in the Pentateuch:

“...ye shall eat no manner of blood, whether it be of fowl or of beast, in any of your dwellings”. (Leviticus VII:26)

“Whatsoever soul, it be that eateth any manner of blood, even that soul shall be cut off from his people”. (Leviticus VII:27)

“...only be sure that thou eat not the blood: for the blood is life, and thou mayest not eat the life with the flesh...” (Deuteronomy XII:23)

Developed in preindustrial society, Shechita was designed not only to adhere to the commands of the Pentateuch concerning food animal slaughter, but also to ensure the hygienic treatment of meat and the humane treatment of the animals. The Jewish faith places great emphasis on the kind treatment of animals; the Halacha has many examples of thought for the welfare of animals:

“...the obligation to feed one’s animal before feeding oneself...”

(Deuteronomy XI:15) (Rosen 2004).

Shechita is performed by the Shochet, who is a specially trained and approved Jewish male slaughterman of high moral standing, often a rabbi. The Shochet undergoes extensive training on Shechita and the care and maintenance of the Chalaf (Shechita knife). During the act of Shechita the Shochet is to think about the act of taking the animal’s life (NAWAC 2001). The word Chalaf is derived from the Hebrew verb ‘to change’, since it effects a change in the state of the animal from being tref (forbidden) as food while alive to being kosher for consumption (Rosen 2004). The Chalaf is an extremely sharp knife, which is checked before and after use with each animal for any imperfections, such as nicks, which could cause tearing and make the animal tref or not kosher (Grandin 1980). The Chalaf is a knife of adequate length, which is at least twice the width of the animal’s neck (Shragge and Price 2004). The cut is made in one swift movement, cutting soft structures ventral to the vertebral column (as mentioned above), severing both carotid arteries and jugular veins (Homa 1971). There are five rules in the Halacha and Talmud pertaining to the act of Shechita, these are:

- i. Shehiya (Delay): there should be no delay or hesitation in the cut. A delay or hesitation makes the meat tref. The knife must move in a single uninterrupted sweep.
- ii. Derasa (Pressing): there should be no excessive upward or downward pressure of the blade against the neck as it is drawn across the throat beyond that which is necessary to create the incision. Any undue pressure renders the animal tref.
- iii. Halada (Digging): The blade should not be covered by the hide of cattle, wool of sheep or feathers of birds. The incision must not close back upon itself. A long knife is important to prevent this.

- iv. Hagramah (Slipping): The limits within which the knife may be inserted are from the large ring in the windpipe to the top of the upper lobe of the lung when it is inflated. Slaughter above or below these limits renders the animal tref.
- v. Ikkur (Tearing): there must be no tearing of tissues. If any tearing takes place or either the oesophagus or the trachea is torn out or removed from its normal position during Shechita the animal is tref (Grandin 1980; Rosen 2004; Shragge and Price 2004).

The animals were traditionally cast onto the ground on their backs, where the neck was stretched and then the cut performed. However, with the advent of modern high-speed slaughter plants, casting onto the ground of animals is no longer permitted for sanitary reasons (Grandin 1980). In many countries the practice of shackling and hoisting of fully conscious animals by one leg has been prohibited. However this practice is still conducted in other countries such as Uruguay (Bar-Moha 1998) and Chile (Grandin 2004) where meat is exported to Israel. For more information about shackling and hoisting refer to Grandin (1980). Currently the use of upright pens, with chin lifts (ASPCA pen) is recommended in many countries. For more information concerning upright pens refer to (Grandin 1980, 1992, 1994b; Grandin and Regenstein 1994; Grandin 2003a; Shragge and Price 2004). The animal must remain in the chin lift until it is unconscious; this prevents further stimulation of nerve endings in the exposed wound which if stimulated may cause pain – clearly wound management is important (Homa 1971; Gregory 2007).

Once slaughtered the carcass undergoes a postmortem inspection or bedika as detailed in the Talmud. If there are any signs of disease, the carcass is declared tref. Once inspected the rabbi or Shochet puts a kosher mark on the brisket of the carcass, and on the edible offal such as the tongue. The veins, fat and forbidden tissues are removed from the meat. Often the hindquarter part of the carcass is sold into alternative markets as the veins are not easily removed (Anil and Sheard 1994). When the meat is consumed in the home, it is soaked in cold water for half an hour, followed by salting before cooking. Broiling is also accepted (Grandin 1980).

2.3.2 Muslim Slaughter

The Muslim religious method of animal slaughter is referred to as Halal slaughter or al-Dhabh. Halal and other rules regarding treatment of living beings and meat are contained in the Quran. The Quran is the sacred writings of Islam revealed by God to the prophet Muhammad during his life at Mecca and Medina. Halal has been practised by Muslims for the last 1,500 years (Khan 1971). There are variations in the way Halal is practiced; this is possibly due to differences in the interpretation of the Quran and Hadis (the sayings of the prophet Mohammed) (Anil and Sheard 1994). Muslims believe that every soul has to return to the creator and human beings among them will have to explain every action they do in the world. Therefore the Quran promotes kindness to animals as does the Pentateuch. For example one of the sayings of the prophet Muhammad is about a man who was thirsty and accidentally found a well:

“He got down in the well and drank water and when he came out he found a dog panting and licking the mud because of thirst. He remembered his own thirst and felt pity on the dog. He got down in the well, fetched some water and gave it to the dog to drink. God appreciated this act of kindness and forgave him. Those who were listening asked him if there was blessing in treating the animal, to which he replied, to treat kindly any living soul is a source of blessing” (Khan 1971).

Halal slaughter is believed to be humane by Muslims, as it is incomprehensible for them to believe the prophets who taught them the rights of animals and kindness to animals could teach a cruel method (Khan 1971). As with Shechita, Halal slaughter was developed in preindustrial society where the Halal method was more humane than other methods of slaughter. The Quran forbids the consumption of animals killed by beating, strangulation, falls, goring or other damage from animals; it also forbids the consumption of animals dedicated to other religions (Grandin and Regenstein 1994). These rules ensured that animals were slaughtered humanely as stated in the Quran. One of the principal requirements of Halal slaughter, as also with Shechita for Jewish people, is that meat for consumption by Muslims is to

be as bled as thoroughly as possible (Carding 1970) and death can only be the result of the neck incision.

“He has only forbidden you what has died by itself, blood and pork, and anything that has been consecrated to something besides God” (The Cow (al-Baqara), 2:173).

Halal is the only method of slaughter allowed by the Quran. It traditionally involved the casting of an animal onto its side. The carotid arteries, jugular veins and soft tissues ventral to the vertebral column are cut and the animal is left to bleed and undergo involuntary convulsions till these come to a natural end. Any Muslim may slaughter an animal while invoking the name of Allah (Grandin and Regenstein 1994). The blessing of the animal in the name of Allah is to focus on the sanctity of life in the minds of men and prevent men from being cruel to the animal being slaughtered (Khan 1971). During slaughter the animals must not be able to see the knife or another animal being slaughtered (NAWAC 2001), and they must also be facing in the direction of Mecca (NAWAC 2001; Shragge and Price 2004). When Muslims are unable to kill their own animals, it is allowed that they eat meat slaughtered by persons of the book (Christian or Jew) (Grandin and Regenstein 1994). In slaughter plants Halal slaughter is allowed to take place in the presence of a Muslim religious leader reciting the appropriate blessings. However, in larger plants Muslim slaughtermen are often employed (Grandin and Regenstein 1994). Unlike Shechita, there are no special knives for Halal slaughter. Normally a long curved blade is used. The blade is to be sharpened every time before slaughter as taught by the Prophet Mohammed (Khan 1971). It is important with Halal, as with Shechita, that the animal to be slaughtered is both free from injury and disease. It is for this reason that stunning was forbidden as it caused injury to the animal. However, the use of non-penetrative concussion and head-only electrical stunning prior to Halal slaughter has received approval from some Muslim authorities (Grandin and Regenstein 1994; Grandin 1994a). The important factor with head-only electrical stunning is that after stunning the animal must be able to regain consciousness and be injury free. For such electrical stunning the Muslim standard in New Zealand is that the animal must be able to regain consciousness in less than a minute and must be able to eat within five minutes (Grandin and Regenstein 1994). Halal meat from

New Zealand is exported to the Middle East where the markets have some of the most stringent Halal requirements (Grandin 1994b). The stunning of animals for Halal has reduced possible suffering in many countries, but the lack of stringent training and a specific razor sharp knife such as that used with Shechita, means that animals that are not stunned possibly may undergo suffering due to the pain and stress caused by inadequate slaughter practice. For example, Grandin (1994b) observed that cattle undergoing Halal slaughter often reacted violently to multiple hacking cuts made with a knife that was too short. Unlike Shechita-slaughtered meat, Halal meat is not deveined, soaked or salted prior to cooking (Anon 2004).

2.4 Emergency Slaughter

Emergency slaughter is performed to prevent or minimise the further suffering of an individual animal or to prevent, eradicate or contain the spread of an exotic pathogen that may pose a risk to animal or human health or the economy. The principle is to ensure the quickest possible death with the methods available. Emergency slaughter is performed in circumstances where there is likely to be an unacceptable delay in either treating the condition that is causing pain and/or suffering, where the pain is untreatable, where transportation would aggravate the condition and cause further suffering to the animal (AWAC 1996) or in situations where time may be of the essence to contain a disease outbreak (Galvin et al 2005). The methods used for emergency slaughter are dependent on the situation, species and skills of the operators involved, and include: a blow to the head; shooting (rifle, shotgun, penetrative and non-penetrative captive bolt stunning); decapitation; maceration; gassing (CO₂, CO, N, and combinations with inert gases); lethal injection; and bleeding (VNI or thoracic sticking) (Galvin et al 2005). Bleeding without physical destruction of the brain or stunning should only be performed in situations where equipment such as firearms or other stunning devices are unavailable or the skills required for their safe and effective use are not present.

2.5 New Zealand Legislation Concerning the Slaughter of Animals without Prior Stunning

In most countries there is strict legislation regarding the use, consumption and treatment of animals. This is particularly true with legislation regarding the slaughter of livestock for human consumption. In New Zealand, the Animal Welfare Act 1999 sets out the basic obligations relating to the care and slaughter or euthanasia of animals. The act takes a more preventative approach compared to previous legislation, which was almost solely concerned with punishing cruelty (Animal Protection Act 1960). One of the obligations, Section 12(c) of the Act, states that: A person commits an offence who, being the owner of, or a person in charge of, an animal, kills the animal in such a manner that the animal suffers unreasonable or unnecessary pain or distress (NAWAC 2001).

Unlike the Animal Protection Act 1960 the Animal Welfare Act 1999 does not provide guidance on how these obligations can be met. This is found in the codes of welfare. The codes contain minimum animal welfare standards covering all aspects of slaughter. A breach of a minimum standard is not prosecutable; rather a breach of one of the obligations of the Act is prosecutable under the Act. However, the failure to meet a minimum standard in a code of welfare can be used as evidence to support prosecution under the Act. It is important to note that the New Zealand Animal Welfare Act 1999 does not apply outside of New Zealand. There is no legislation to control the importation of products that do not fully comply with New Zealand law. Live animals for export once they have arrived in their destination are also not covered by the Animal Welfare Act 1999.

Currently the animal welfare aspects of slaughter, including religious slaughter are governed by the Slaughter of Stock, Game and Poultry Regulations 1969 under the Meat Act 1981 (the Slaughter Regulations). However, this is being transferred to the Animal Welfare Act 1999, as the Meat Act 1981 is replaced by the Animal Products Act 1999. It is important to note that the Regulations will continue to have effect during the transitional period until the new codes of welfare for the commercial slaughter of animals are issued by the Minister of Agriculture (NAWAC 2001). Currently the code of welfare for the commercial slaughter of animals has been recommended by the National Animal Welfare Advisory Committee (NAWAC) to

the Minister of Agriculture and Forestry New Zealand. At the time of writing, the Minister had not finalised his views on the code.

The New Zealand Bill of Rights Act 1990 protects, affirms and promotes human rights and fundamental freedoms in New Zealand. Under the section covering civil and political rights is outlined the right of:

“manifestation of religion and belief – Every person has the right to manifest that person’s religion or belief in worship, observance, practice, or teaching, either individually or in a community with others, and either in public or in private” (Rishworth et al 2003).

The NAWAC position on slaughter is stated within the draft code for the commercial slaughter of animals 2006. Minimum standard no.7 (a) states that:

“Prior to slaughter, all animals must be stunned so that they are immediately rendered insensible to pain and maintained in that state until death supervenes” (NAWAC 2006).

This standard is currently met by Halal slaughter, which involves the use of head-only electrical stunning. However, under section 73(3) of the Animal Welfare Act 1999, NAWAC recommends that a dispensation be granted to allow Shechita slaughter in order to meet the domestic needs of the New Zealand Jewish community. NAWAC considers that Shechita does not meet the minimum standard for commercial slaughter. However, under the New Zealand Bill of Rights Act, it is the right of every person to be able to manifest that person’s religion or belief in worship, observance, practice or teaching. Shechita is an important part of the Jewish faith. Prohibition of Shechita is often considered as a violation of Jewish rights. The dispensation in the Draft Code for the Commercial Slaughter of Animals 2006 states that:

Notwithstanding Minimum Standard 7(a), Shechita slaughter for goats, sheep, cattle and poultry may be carried out for the purpose of producing animal products for human consumption in New Zealand (NAWAC 2006).

The dispensation by NAWAC is covered in Minimum Standard 22 and is conditional upon the following requirements being met during Shechita:

- *Goats, sheep and poultry may be Shechita slaughtered without prior stunning.*
- *A competent Shochet must slaughter the animals. The Shochet must practise under the jurisdiction of a recognised Rabbinical authority. Before conducting Shechita in New Zealand for the first time, the Shochet must provide to the Director-General of the Ministry of Agriculture and Forestry written evidence of his current competency from that Rabbinical authority*
- *All cattle slaughtered by Shechita must be stunned no more than 5 seconds after cutting of the throat.*
- *Any animal declared by the Shochet to be non-kosher must be stunned immediately.*
- *The method of restraint of the animal must be by a method that is approved by the Director-General of the Ministry of Agriculture and Forestry in consultation with NAWAC and representatives of the New Zealand Jewish community.*
- *In the case of poultry, the neck must be inspected to ensure that both carotid arteries are completely severed (NAWAC 2006).*

Currently these provisions are in place allowing Shechita slaughter, but at present they have been voluntarily suspended in commercial slaughter plants pending the Minister's decision on the Codes of Welfare for Commercial Slaughter of Animals.

Emergency slaughter is governed by the Code of Recommendations and Minimum Standards of Emergency Slaughter of Farm Livestock (AWAC 1996). The code states that during emergency slaughter by VNI it is of extreme importance that the cut be administered quickly to restrained animals, and the cut must ensure that

both carotid arteries are served. The code also states that due to differences in blood supply to the brain in cattle compared to other species that:

“except in extreme circumstances species other than sheep should not be slaughtered by a throat cut only” (AWAC 1996).

2.6 Physiology of Pain

Pain is a multidimensional phenomenon, which involves a variety of different systems and processes of the body. The International Association for the Study of Pain (IASP) definition of pain states that pain is:

“An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”
(IASP 2008).

The definition has been adopted by both the human and veterinary medical professions in recognition of the universal similarities in pain between humans and animals (Murrell and Johnson 2006). The dual sensory and emotional components of pain make it a subjective experience; one person’s experience of pain can be very different from that of another. Most humans are able to communicate their experiences of pain, however non-communicative individuals such as very young children, individuals with communicative difficulties or cognitive impairment and animals are often unable to express the pain they are experiencing. This does not make the pain they experience any less real or the suffering caused by that pain any less. Previously it was believed that only humans were capable of experiencing pain (Flecknell 2000). Even now some authors suggest that ruminants are “probably much less sensitive than man” to pain (Levinger 1995). Due to the phylogenetic similarities in the central nervous system (CNS) between higher animals, especially non-human mammals and humans, there can be little doubt that mammals are capable of experiencing pain or that they can suffer as a result of it (Barnett 1997).

The classical picture of pain was of injury in the peripheral tissues, and nervous signals transmitted to and processing in the thalamus and lower centres of the brain. However, this model has now been expanded to include other types of pain, namely somatic, visceral and neuropathic pain. It also includes the processing within the cerebral cortex and associated brain areas of the impulses generated by noxious stimuli, interpretation of such impulses by these higher brain centres as painful, and the modulation of nociception via descending projections and the gate control theory (Melzack and Wall 1965). This model includes essential components of current understanding of the term nociception.

The cerebral cortex has an integral role in the conscious perception of pain (Talbot et al 1991; Jones et al 1992; Treede et al 1999, 2000; Bromm 2001; Hofbauer et al 2001). It is only after the nociceptive signal reaches the cortical regions associated with consciousness that it is perceived as pain (Johnson 2002), so that without consciousness there is no pain experience (Bromm 2001). The finding that pain is perceived in the cortex makes sense, as often a noxious event is not perceived as being painful (although activating known pain pathways) and therefore is not perceived as pain. The perception of pain is complicated and involves multiple regions of the cerebral cortex (Ibinson et al 2004). The multiple representations reflect the fact that sensory, motor, associative, autonomous, and limbic structures are involved in the processing of the many perceptual and nocifensive components of pain (Bromm 2001).

Some of the cortical regions most frequently activated by pain are the primary and secondary somatosensory cortices (S1 and S2). These regions receive noxious and innocuous somatosensory input from the thalamus and contain neurons that code spatial, temporal, and intensive aspects of innocuous/noxious somatosensory stimuli, characteristics which help in the sensory-discrimination of pain processing (Hofbauer et al 2001). Other regions that appear to be activated, as shown by human brain imaging studies of pain, are the anterior cingulate cortex (ACC) and the insular cortex (IC). Both of these are considered to be components of the “limbic structure” and therefore are considered to be involved in the processing of affective motivational dimension of pain (Hofbauer et al 2001).

2.6.1 Measurement of Pain in Livestock

Investigations examining nociception and pain in animals have used a variety of different measures. These can be broadly grouped into four categories: behavioural, endocrine, autonomic and electrophysiological measures (Johnson et al 2008). All four are indirect measures of pain, due to pain being a subjective experience and the inability of most animals to communicate to humans the intensity of their pain. Despite this, all four measures can and have provided important information on the physiology of pain and responses to noxious sensory input. Much of the previous work investigating nociception and pain in livestock species has been carried out using endocrine responses: dehorning (Wohlt et al 1994; Morisse et al 1995; Petrie et al 1996; Mellor and Stafford 1997; McMeekan et al 1998ab; Sylvester et al 1998ab; Graf and Senn 1999; Grondahl-Nielsen et al 1999; Sutherland et al 2002; Stafford et al 2003); castration and tail docking (Lester et al 1991ab; Wood et al 1991; Kent et al 1995; Molony et al 1995; Sutherland 1999; Sutherland et al 1999; Jongman et al 2000; Mellor et al 2002); mulesing (Jongman et al 2000); slaughter (Tume and Shaw 1992); branding (Lay et al 1992ab; Schwartzkopf-Genswein et al 1997). These studies have made significant contributions to the study of nociception/pain and animal welfare. However, it is important to note that endocrine responses are not direct measures of pain; rather they provide an indirect indication of how unpleasant an experience is emotionally and physically (Mellor and Stafford 2002). Alterations in circulating glucocorticoid concentrations have been commonly used in the investigation of pain-induced distress and its alleviation in a variety of livestock species (Mellor and Stafford 2002). Cortisol concentrations are the most commonly measured index of hypothalamic-pituitary adrenocortical (HPA) axis activity. Investigation of cortisol has increased the scientific understanding of pain-induced distress in a variety of livestock species in response to husbandry practices (Mellor and Stafford 2000, 2004; Stafford and Mellor 2005). However it is important to note that the HPA axis responds to a variety of non-pain related situations and that there is a time lag between peak plasma cortisol concentrations and the causative stimuli (Mellor and Stafford 2000, 2002). In controlled research studies these factors can be accounted for in the experimental design, producing reliable results. However, the complex physiological environment during slaughter makes the measurement of

plasma cortisol concentrations unsuitable for the investigation of the potential acute noxiousness of slaughter.

The measurement of acute catecholamine responses has been suggested as a more useful tool in the investigation of acute responses to noxious stimuli (Mellor and Stafford 2000). Activation of the rapidly-acting sympathetic adrenomedullary system (SAM) has been previously used in the investigation of noxious sensory input (Mellor et al 2002). However like HPA activation, catecholamines (adrenaline and noradrenaline) are released in response to a variety physiological situations including, but not only, pain.

Behavioural changes have been used intensively in the investigation of noxious stimuli (Wood et al 1991; Kent et al 1995; Molony et al 1995; Morisse et al 1995; Graf and Senn 1999; Grondahl-Nielsen et al 1999; Stafford and Mellor 2002; Sylvester et al 2004), and they have often been used in conjunction with changes in the HPA axis or other measures (Morisse et al 1995; Ong et al 1997; Graf and Senn 1999; Grondahl-Nielsen et al 1999). Behavioural measures have some inherent problems, including anthropomorphism and being subjective measures. Rating scales such as the visual analogue scales (VAS) are particularly prone to anthropomorphism (Roughan 2004). Furthermore, it is often difficult to determine the intensity of pain based on a behavioural response (Barnett 1997). These problems have been somewhat mitigated by the use of behaviour-based pain scoring systems specific for particular species (Firth and Haldane 1999; Molony et al 2002; Roughan and Flecknell 2003).

Electrophysiological and neuro-imaging techniques for the assessment of noxiousness and pain have now provided insight into the cortical processing of pain. While still an indirect measure of pain, the electrical activity elicited in the cortex by a noxious stimulus is more representative of the acute cognitive perception of pain (Barnett 1997).

2.7 Electroencephalogram and Evoked Potentials

In 1875 Richard Caton reported in the British Medical Journal both spontaneous and evoked mediated “feeble” electrical currents from the dura of the brain of rabbits and monkeys (Caton 1875), thus representing the first report of the

EEG and evoked potentials (EP), respectively. Richard Caton's work is credited as the pioneering work of EEG and EPs (Niedermeyer 2005). Since then the understanding of the mechanisms, causation and meaning of changes of these electrophysiological measures has increased exponentially. Both EEG and EPs are now widely used clinically and in neurological research in both human and veterinary medicine.

2.7.1 Electroencephalogram

The EEG represents the spontaneous electrical activity of the brain. Changes in the EEG represent near real time changes in brain functional activity and as such the EEG provides increased temporal resolution compared to other functional measures of cortical activity (e.g. Functional magnetic resonance imaging (fMRI), positron emission tomography (PET)).

The EEG is generated by the summation of synchronous activity of inhibitory and excitatory post-synaptic potentials of populations of cortical neurons, with a contribution from glial cells (Murrell and Johnson 2006). Axonal action potentials do not contribute significantly to EEG recordings (Fisch 1999a). The generators of the EEG are thought to be a layer of cells arranged uniformly at 90 degrees to the cortical surface referred to as pyramidal cells. The pyramidal cells are pyramid shaped and have cell bodies located in deeper layers with dendrites projecting up to the pial surface of the cortex, where they spread out within layer I of the cortex (Cunningham 2002). Pyramidal cell axons project to other parts of the CNS and carry the major output signals of the cerebral cortex. Neurons with axons projecting in other directions or deep cortical, thalamic or brain stem neurons make little to no contribution to the generation of the EEG. However deeper brain structures do have strong regulatory influences on cortical function and hence the EEG is hypothesised to indirectly reflect activity in these structures in addition to activity in the cerebral cortex (Murrell and Johnson 2006).

2.7.1.1 Recording of the EEG

The EEG is recorded from electrodes placed on the skin surface or subdermally on the skull, while the ECoG is recorded from electrodes placed on the dura or surface of the cerebral cortex via holes drilled into the skull. The most rudimentary electrode montage for recording EEG is recording from two electrodes; the electrical activity between the two electrodes is summated to produce the EEG waveform. One electrode is the non-inverting (active) electrode and the other is the inverting (reference) electrode. Often an earth electrode is included in the montage. A variety of different metals are used for electrodes in the recording of EEG (silver/silver chloride, tungsten, platinum, iridium and stainless steel). The ideal electrodes for recording EEG are made of pure substances with little surface contamination and low impedance, features which minimises electrode potentials and reduces potential distortion and noise in the EEG (Murrell and Johnson 2006). The most routinely used electrodes are pure silver electrodes coated with a layer of silver chloride. These electrodes have low impedance and are readily available. However, other substances with higher impedance (e.g. stainless steel) are often used when situations dictate. To ensure accurate recordings and minimal noise, electrodes are placed on the surface of the scalp (scalp or adhesive electrodes) or subdermally (figure 2.1). Scalp electrodes are positioned on the skull with a cap and make electrical contact via conducting gel, which forms an electrolytic bridge between the electrode and the scalp. Adhesive electrodes are attached to shaven and degreased areas of the scalp. Subdermal electrodes make direct contact with tissue fluid, improving the interface between the generators of the EEG and the recording system (Murrell and Johnson 2006).

The information collected by the recording electrodes is then amplified using differential amplifiers. These are electronic amplifiers that multiply the difference between the non-inverting and inverting electrodes by a set constant factor or differential gain (figure 2.1). The gain is often set at 1000 times the original voltage. Filtering of the EEG can be either analogue-or-digital. Analogue filtering is often performed after amplification; the continuous EEG time signal is filtered to remove unwanted signal components from the EEG. There are a number of different analogue filters used in EEG analysis such as:

- High pass filters, which cut off frequencies below a set frequency while allowing the passing of the frequencies higher than the set frequency.
- Low pass filters which perform the opposite of high pass filters, in that they cut off frequencies above the set frequency while allowing the frequencies below the set value to pass.
- Band-pass filters are a combination of a high-pass and a low-pass filter.
- Notch filters only remove those frequencies in a specific narrow range, while allowing the passage of most other frequencies unaltered.

Analogue filtering was previously used intensely in analogue EEG recording where the EEG output was recorded with pens on moving paper. With the advent of digital EEG recording many recording systems do not use analogue filters, instead relying on digital filtering alone. There are many advantages and disadvantages of using analogue versus digital filtering in a digital EEG. A major advantage is that high frequency activity can be removed prior to digitising the EEG. This eliminates unnecessary high frequency components and reduces demand on the sampling rate of the analogue-to-digital converter (Fisch 1999b).

Analogue-to-digital converters digitise the continuous EEG time signal to a discrete digital time signal (figure 2.1). This allows the EEG signal to be analysed and filtered digitally using computer-based software. The sampling rate is the rate at which the new EEG digital values are sampled from the analogue EEG signal by the analogue-to-digital converter; this is often set by computer-based acquisition software. The sampling rate is one of the factors that determine how accurately the analogue EEG signal is reproduced in digital form. Higher sampling rates produce a digital EEG trace more representative of the analogue trace, however over-sampling increases the amount of data being processed and stored and can cause problems where storage space is limited in older recording systems. A significant problem in analogue to digital conversion is the phenomenon of aliasing. Aliasing occurs when

the sampling rate is decreased to a point where the EEG signal is not recorded in sufficient detail to reproduce the values between the sampling points (Murrell and Johnson 2006). Once an EEG is aliased it is impossible to recover the original EEG signal. The Nyquist frequency or rate is the lowest possible sampling frequency where the entire EEG signal can be accurately recorded without being aliased. This frequency is at least two times as fast as the fastest frequency in the desired signal (Fisch 1999b; Murrell and Johnson 2006). The Nyquist frequency is calculated using the Nyquist theorem (or equation) (Fisch 1999b).

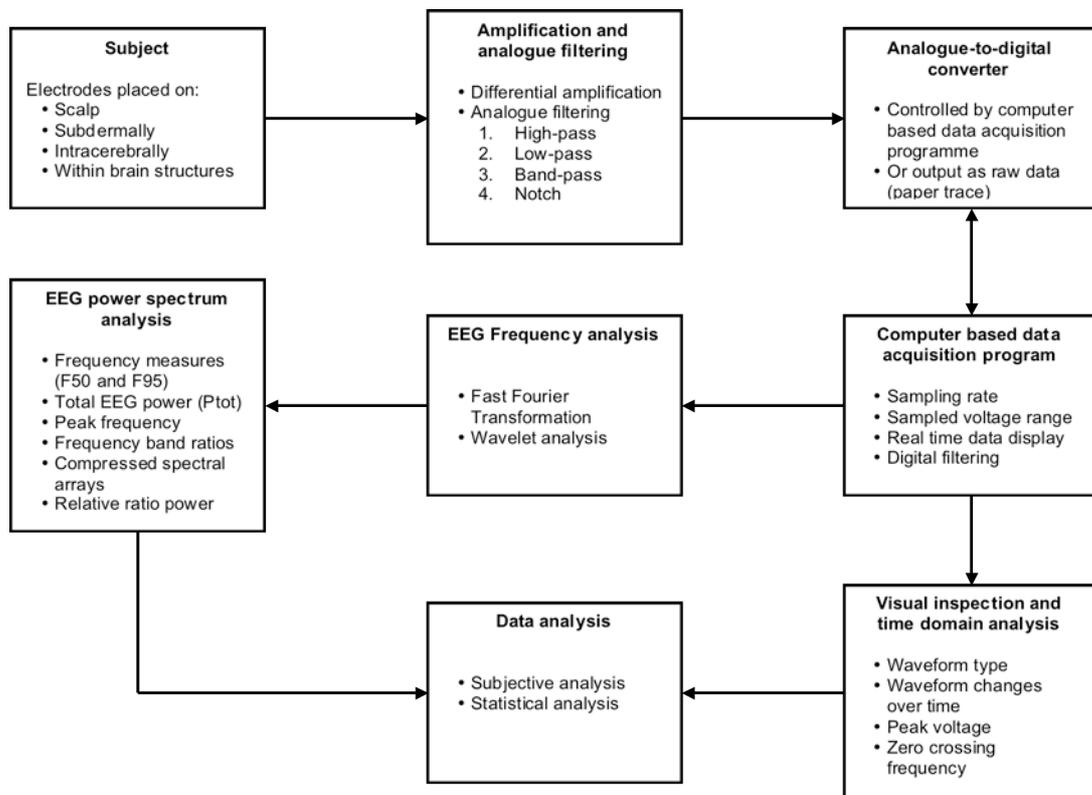


Figure 2.1. Flow chart detailing the important steps in the recording and analysis of the EEG, from a subject, including amplification, analogue filtering, analogue-to-digital conversion and computer-based acquisition to different forms of EEG analysis.

The digital EEG is displayed and recorded by using computer based data acquisition programmes, which display the digital EEG trace for online and offline analysis. The programmes offer a variety of tools for the analysis and manipulation of the trace including: smoothing, digital filtering and spectral analysis. The recorded trace is able to be saved for future analysis, or be exported for further analysis such as frequency analysis with the Fast Fourier Transformation.

2.7.1.2 Interpretation of Noxious Sensory Input with the EEG

The EEG reflects changes in cortical function that can be demonstrated as an alteration in its frequency components (Adrian and Matthews 1934). EEG signals can be viewed from two different standpoints: 1, the time domain, and 2, the frequency domain (EEG power spectrum). Development of the Fast Fourier Transformation (Cooley and Tukey 1965) method has allowed the detailed investigation of the power spectrum of the EEG. The Fast Fourier Transform is used to transform the EEG from amplitude (y) as a function of time (x) to power (y) as a function of frequency (x) or the EEG power spectrum. The EEG power spectrum reveals the signal strength in a particular range of frequencies, and any shifts in those frequencies. Changes in these power spectra have been shown to reflect alterations in the activity of the cerebral cortex associated with cognitive perception of pain (Chen et al 1989). A variety of different EEG descriptors have been used in the analysis of the EEG power spectrum. Figure 2.2 illustrates four of the major descriptors of the EEG power spectrum that have been used in the investigation of noxious sensory input in both humans (Chen et al 1989; Backonja et al 1991; Ferracuti et al 1994; Bromm and Lorenz 1998; Chang et al 2001ab; 2002) and animals (Otto et al 1996; Ong et al 1997; Jongman et al 2000; Murrell et al 2003; Haga and Ranheim 2005; Haga and Dolvik 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2005, 2007; Diesch et al 2008; Kongara 2008). Spectral changes in the EEG are an indirect measure of pain, but as they largely reflect the electrical activity of the cerebral cortex, they are more representative of the cognitive perception and processing of noxious stimuli (Barnett 1997; Johnson et al 2005b).

Median EEG frequency (F50) is a measure of the central location of an EEG power spectrum (figure 2.2a). It is the 50th percentile of the power spectrum. Increases in F50 have been previously associated with nociception in a number of species. Murrell et al (2003, 2005) described increases in F50 after surgical castration in horses and the attenuation of the response with the pre-surgical application of intravenous lignocaine. Similar changes were found in piglets after surgical castration, with attenuated responses in F50 with the prior injection of lignocaine into the spermatic cord of the testes and an increase in F50 in animals without the injection (Haga and Ranheim 2005). Following deer velvet amputation with prior application of

very tight compression bands or local anaesthesia, or without analgesia (control), F50 was shown to increase in the control animals and in compression band animals, while the application of a lignocaine ring block around the antler pedicle prevented the increase in F50 (Johnson et al 2005b). An increase in F50 in response to rubber-ring castration without pain relief has also been observed in lambs of differing postnatal ages (Johnson et al 2005a).

Spectral edge frequency is a measure of the highest frequencies present in the EEG (figure 2.2b). The spectral edge is the frequency that divides the total EEG power into two defined fractions. A variety of percentages have been used to describe spectral edge frequency, among them are 80%, 90% and 95% (Schwilden 2006). 95% spectral edge frequency (F95) divides the EEG power spectrum from 0 to the frequency that contains 95% of the total power, where the rest of the spectrum contains the remaining 5% (Young 2001). 95% Spectral edge frequency has been found to correlate well with depth of halothane anaesthesia in horses (Johnson et al 1993, 1994; Johnson and Taylor 1998), and, in addition, increased in response to the noxious sensory input of antler removal (Johnson et al 2005b).

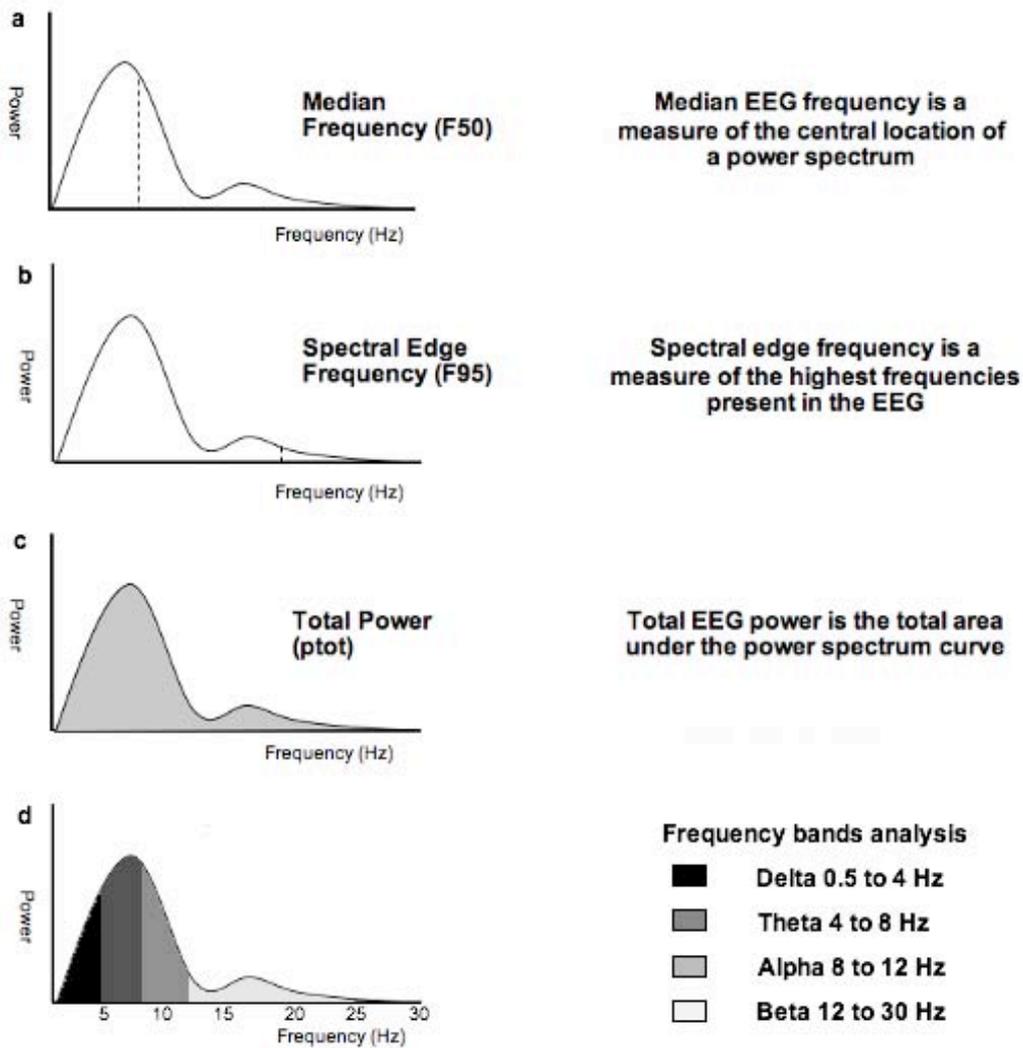


Figure 2.2. Diagrammatic representation of the EEG power spectrum and the four single descriptors used in analysis. a: Median frequency (F50); b: 95% spectral edge frequency (F95); c: Total EEG power (Ptot); and d: Frequency band analysis.

Total power of the EEG power spectrum (Ptot) is defined as the total area under the EEG power spectrum curve (figure 2.2c). It is calculated by the summation of the all the powers of the frequencies under the power spectrum curve. Unlike F50 and F95, Ptot is a measure of total power across all frequency bands recorded in the EEG, not a measure of alterations in frequency. Decreases in Ptot have been previously associated with noxious sensory input in horses (Murrell et al 2003), deer (Johnson et al 2005b), pigs (Haga and Ranheim 2005), rats (McGregor 2005; Murrell et al 2007) and dogs (Kongara 2008).

Generally changes in Ptot relate to changes in F50 and F95, often when Ptot decreases, F50 and F95 increase. As Ptot decreases there is a shift in the distribution of the power in the EEG power spectrum, which results in a higher proportion of the

power, being distributed across the higher end frequencies of the EEG power spectrum. This produces an increase in F50 and F95. However, this relationship does not always hold true as increases in F50 and F95 can occur in the absence of, or even with increases in Ptot (Johnson et al 2005a). This can occur if a large amount of power is contained within a narrow low frequency band, causing an increase in Ptot with little overall affect on F50 or F95. Of the three EEG descriptors, Ptot is the most sensitive to changes in the EEG. Its sensitivity makes Ptot more susceptible to contamination with electrical noise and artefact.

Median frequency, F95 and Ptot can be used to represent changes in a single time period or changes over time. Another method used for the interpretation of the EEG power spectrum is the compressed spectral array (figure 2.3). This gives a 3-dimensional representation of the power spectrum, with time on the x-axis, frequency on the r-axis and power on the y-axis. The compressed spectral array visually represents how frequency and power change over time in relation to each other.

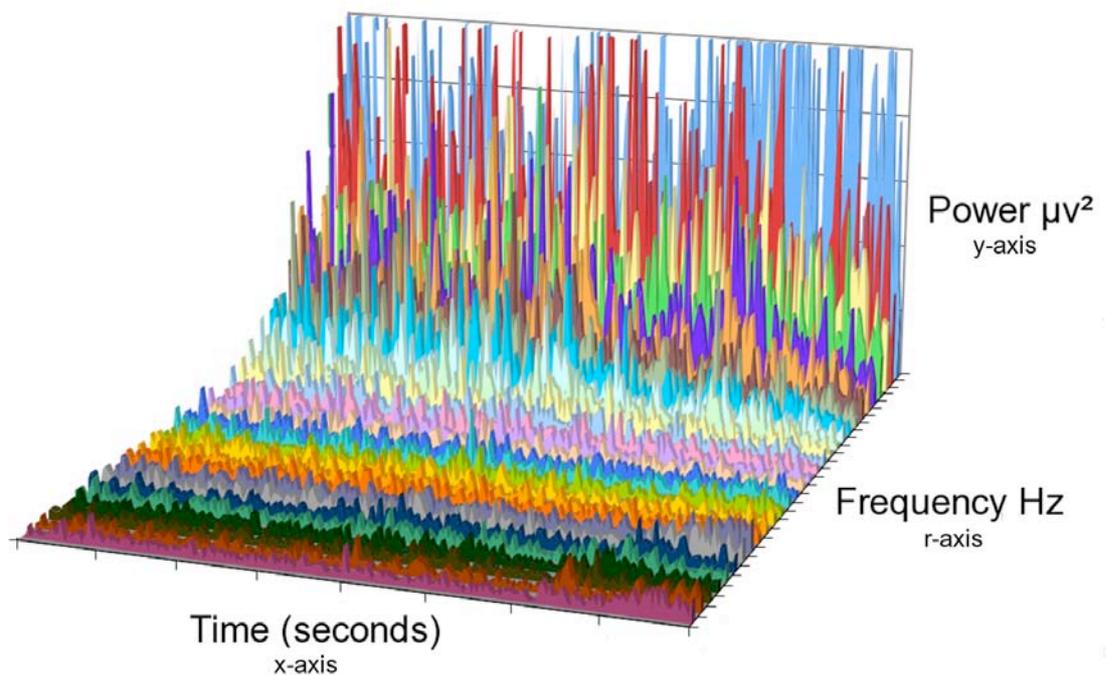


Figure 2.3. Compressed spectral array from the EEG of a anaesthetised calf, with time (seconds) along the x-axis, frequency (Hz) along the r-axis and power (μv^2) on the y-axis.

Many studies use EEG frequency band analysis – a method which investigates the power in a specific frequency band (figure 2.2d). The EEG is typically divided into four different frequency bands: delta (0.5 to 4 Hz), theta (4 to 8 Hz), alpha (8 to

12) and beta (12 to 30). Beta can also be further divided into: beta 1 (12 to 16 Hz); beta 2 (16 to 20 Hz); beta 3 (20 to 28) and some authors make a further division including a fifth frequency band, gamma (26 Hz +). These frequency ranges have been used in a variety of studies in both humans (Chen et al 1989; Backonja et al 1991; Ferracuti et al 1994; Chang et al 2001b, 2002) and animals (Otto et al 1996; Ong et al 1997; Jongman et al 2000; Haga and Dolvik 2005) to investigate noxious sensory input (table 2.1).

Table 2.1. Studies into the effects of experimental pain in humans and animals on EEG frequency band power.

Species	Pain stimulus	Delta	Theta	Alpha	Beta	Reference
Humans	Ice water, hands	↑	ns	↓	↑	Chen et al (1989)
Humans	Ice water, hands	ns	↑	Initial ↓ Later ↑	↑	Backonja et al (1991)
Humans	Ice water, hands	ns	ns	↓	↑	Chen and Rappelsberger (1994) ^a
Humans	Ice water, hands	↑	ns	↓	ns	Ferracuti et al (1994)
Humans	Capsaicin i.m. ^b	↑	ns	↓	↑	Chang et al (2001)
Humans	Hypertonic saline i.m. ^b	ns	ns	↓ Alpha-1 only	ns	Chang et al (2002)
Isoflurane anesthetized horses	Orthopaedic and soft tissue surgery	↓	ns	↑	ns	Otto et al 1996
Adult ewes (sheep)	Electrical stimulation, forelimb	↑	↑	ns	↑ Beta-1 only	Ong et al (1997)
	Castration	↓	↓	↓	↓	
4 week old lambs (sheep)	Mulesing	↓	↓	↓	↓	Jongman et al (2000)
	Tail docking	↓	↓	↓	↓	
Isoflurane anesthetized horses	Castration	ns	ns	ns	ns	Haga and Dolvik (2005)

ns: not significant; i.m. intramuscular; ^a referenced by Bromm and Lorenz (1998); ^b brachioradialis muscle

Experimental studies on healthy human subjects using induced tonic pain, generally produces changes in EEG frequency band power that are relatively

consistent with: increases in low frequency delta power; decreases in alpha power; and increases in high frequency beta power (Chen 2001, 2002) (table 2.1). Studies in animals have produced variable results with little similarity between species. This is potentially caused by non-standardized experimental practices with differences in stimulus types, methodology, and anaesthesia. Furthermore, EEG frequency bands were developed for the analysis of the awake human EEG (Tonner and Bein 2006) and have specific functionality associated with them (Murrell and Johnson 2006). Therefore, assumptions on the function of these specific bands are not readily transferable between species and different recording loci. Furthermore, the definition of the specific frequency ranges of the bands is arbitrary and varies between authors and studies.

2.7.1.3 Minimal Anaesthesia Model

Because of the sensitivity of the EEG to electrical noise and movement, when recording EEG in animals, the animals often have to either have cortically implanted electrodes or be anaesthetised to prevent electrical noise on the EEG. The implantation of cortical electrodes is a time consuming and costly procedure, in which complications from anaesthesia, analgesia and surgery can affect the reliability of recordings. A number of different anaesthesia models have been used in the recording of EEG activity and EPs. The anaesthetic protocols in these studies vary considerably making comparisons between studies and species difficult. The choice of agent has significant effects on the EEG. These can range from depression of amplitude to frequency shifts to burst suppression. Halothane has been shown to cause significantly less EEG depression than isoflurane, sevoflurane and desflurane at equivalent concentrations (Murrell et al 2008). Because of the properties of halothane a number of studies have used models based on halothane anaesthesia.

The minimal anaesthesia model maintains an animal on a stable plane of halothane anaesthesia allowing the recording of cerebrocortical responses to a stimulus by reducing variability of the background cortical electrical activity. Work in ponies examining the EEG power spectrum using the minimal anaesthesia model with the application of different classes of intravenous drugs (ketamine, alfentanil,

midazolam, sarmazenil, guaiphenesin and thiopentone) found that the drugs with recognized analgesic properties reduced F50 to a greater extent than F95 (Johnson and Taylor 1997; Johnson et al 1999, 2000ab, 2003). These results suggested that the changes in F50 were associated with anti-nociceptive properties of a drug (Murrell et al 2003). The minimal anaesthesia model was further refined by work of Murrell et al (2003, 2005) in the examination of the noxious sensory input of surgical castration in horses and the attenuation of those responses with prior application of intravenous lidocaine. The model has now been validated in variety of species, including: horse (Murrell et al 2003, 2005), sheep (Johnson et al 2005a), red deer (*Cervus elaphus*) (Johnson et al 2005b), pig (Haga and Ranheim 2005), Tammar wallaby (*Macropus eugenii*) (Diesch et al 2008), dog (Kongara 2008), chicken (unpublished observations), and rat (McGregor 2005; Murrell et al 2007). As the model employs general anaesthesia it has the additional benefit of allowing the investigation of potentially painful procedures without compromising the welfare of the animals involved which remain unconscious throughout experimentation (Murrell and Johnson 2006).

2.7.2 Evoked Potentials

Since first being described by Caton (1875), EPs are now used for both research and clinical applications in humans and animals. Evoked potentials are time-locked responses of the electrical activity of the CNS to peripheral stimulation. The electrical activity can be of single neurons, populations of neurons or of major structures of the CNS, including the brain stem or cerebral cortex. Sensory evoked potentials are recorded from the CNS following stimulation of sensory organs; common examples include auditory evoked potentials (AEP), visual evoked potentials (VEP) and somatosensory evoked potentials (SEP).

The recording of sensory evoked potentials shares similarities with the recording of EEG. Electrodes are placed on the surface of the cranium, subdermally or cortically and the signal is amplified and filtered (figure 2.4). The signal is either recorded/viewed on a paper trace, oscilloscope or digitised with an analogue-to-digital converter. However, unlike the EEG, which is the spontaneous activity of the EEG,

sensory evoked potentials represent time locked cortical activity generated by a stimulus. The recording and generation of EPs is controlled by a computer-based data acquisition programme. This programme triggers the stimulus and the recording of cortical electrical activity in response to the stimulus. Evoked potentials are small (several microvolts) compared to the spontaneous background EEG and electromyographic (EMG) activity (hundreds of microvolts). To improve the signal to noise ratio, repeated stimulations are summated to produce the EP waveform. The term, “averaged evoked potential” is often used when describing the resultant waveform. In fact, averaging instruments yield the sum of a group of observations, or the sum plus a constant. Consequently, “sum” or “summation” is a more accurate description than “average” or “averaging”.

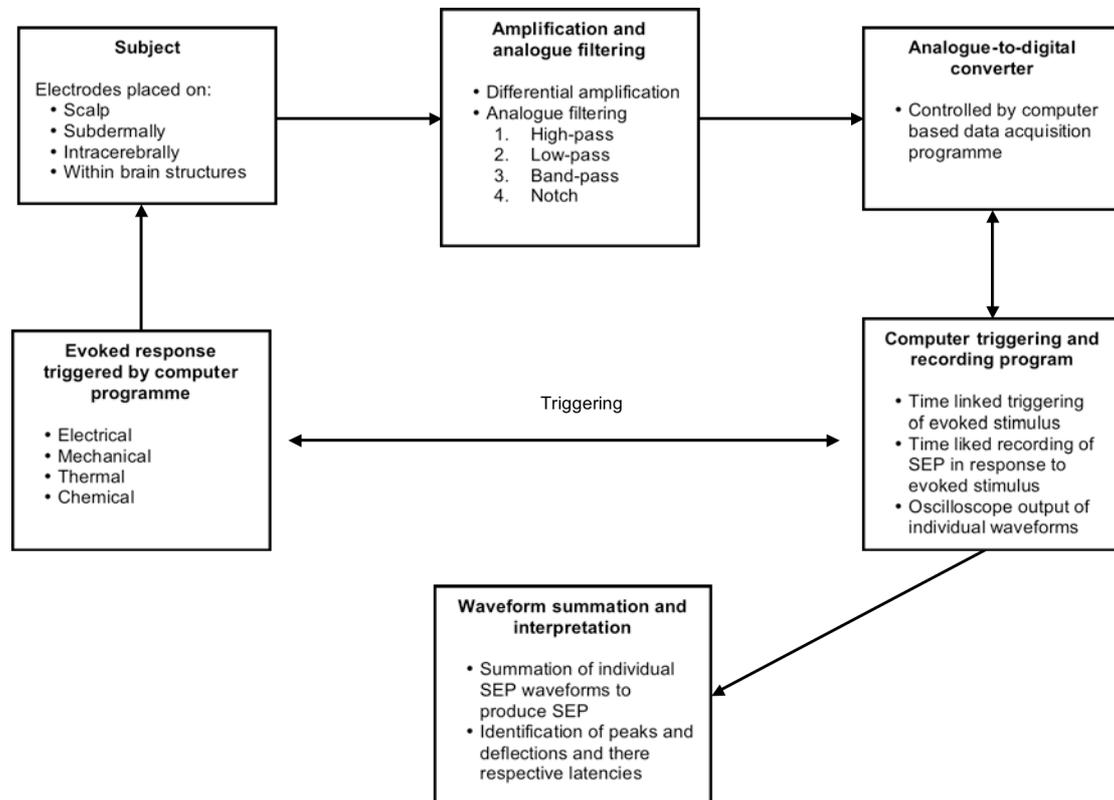


Figure 2.4. Flow chart detailing the major steps in the recording and analysis of the somatosensory evoked potentials, from subject, amplification, analogue filtering, analogue-to-digital conversion, triggering, waveform summation and interpretation.

2.7.2.1 Interpretation of the Evoked Potential Waveform

The summated EP is displayed as a waveform of voltage (y-axis) against time (x-axis) (figure 2.5). The initial voltage peak seen within the first milliseconds of the triggered stimulus is a stimulus artefact (Sebel 1989). The two most examined aspects of EPs are latency and amplitude. Latency is the time in milliseconds from stimulus to a major negative or positive deflection, which is described as either positive (P) or negative (N) (figure 2.5). Deflections (both positive and negative) in the waveform reflect different levels of CNS processing of the triggered stimulus. The latencies are generally described as early, mid and late latencies and correspond to peripheral, thalamocortical (or subcortical) or cortical activity respectively. Amplitude is the measure of positive and negative deflections on an EP waveform, and is measured in microvolts (μV) (figure 2.5).

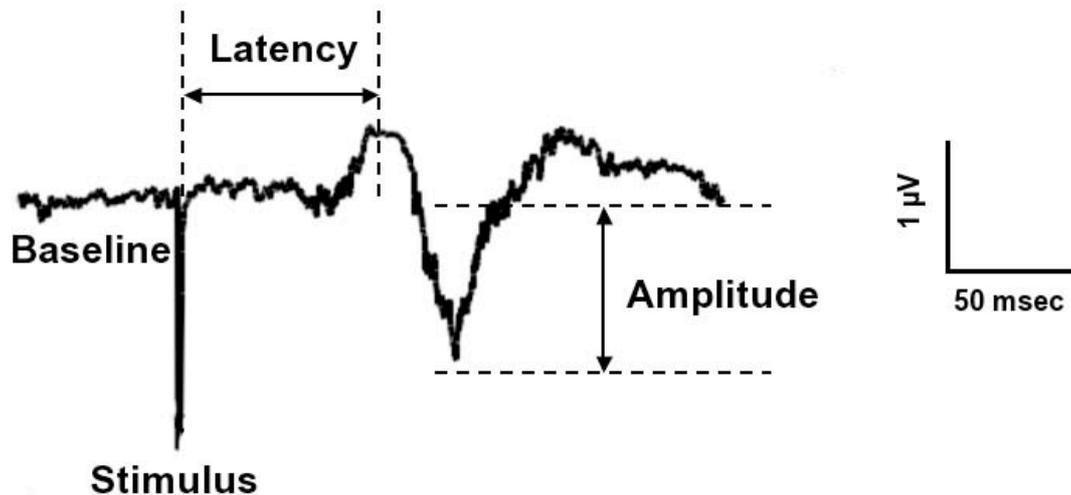


Figure 2.5. Example of a somatosensory evoked potential (SEP) waveform and the measurement of latency (msec) and amplitude (μV). This evoked potential represents the summation of 32 repetitions.

2.8 Time to Insensibility

The time to undoubted insensibility has been an area of much scientific debate with regards to slaughter with or without prior stunning. During slaughter without prior stunning there is potentially a window following VNI and before the onset of cerebral hypoxia and insensibility during which the animal is both conscious and sensible to pain and distress. Determining when consciousness is lost or sufficiently dulled to minimise pain and suffering is problematic (Mellor and Littin 2004). Electrophysiological measures of cortical function such as EEG, SEP and VEP cannot detect the point at which consciousness is lost; rather they provide information on the onset of undoubted insensibility. Although the spontaneous EEG is difficult to interpret in terms of specifying a precise time point for a change in cortical function that reflects dulling or loss of consciousness, the loss of EPs reflects a degree of hypoxic brain damage, which is inconsistent with the maintenance of sensibility (Daly et al 1988).

The effectiveness of different stunning techniques depends on both the time it takes for the technique to induce insensibility and its ability to maintain that insensibility for the full period required for stunning and VNI to cause sufficient cerebral hypoxia for the animal to become irreversibly insensible. A variety of studies have examined the time to undoubted insensibility following both VNI with and without stunning in a variety of domestic animals. Table 2.2 lists some major studies examining the onset of undoubted insensibility after VNI in cattle, sheep, pigs and poultry and the different parameters investigated. There is a range in all species and this is shortest and narrowest in sheep (2 to 14 seconds), then in pigs (13 to 25 seconds), poultry (12 to 26 seconds) and longest and widest in cattle (2 to 385 seconds) (Levinger 1961; Nangeroni and Kennett 1964; Blackmore et al 1979; Groß 1979; Blackmore and Newhook 1981; Newhook and Blackmore 1982b; Blackmore et al 1983; Gregory and Wotton 1984bc; Wotton and Gregory 1986; Daly et al 1988; Bager et al 1992; Barnett et al 2007). In contrast, acute arrest of cerebral blood circulation in normal healthy young men with the use of a cervical pressure cuff has been demonstrated to produce a loss of consciousness on average in 6.8 seconds (range of 6.4 to 6.9) (Rossen et al 1943; Estrella et al 1992).

Table 2.2. Major studies investigating the time to insensibility after ventral neck incision (VNI) slaughter without prior stunning in various species and the parameter reported in publications.

Species	Parameter	Time to insensibility (seconds)	Reference
Cattle	EEG, B	2-10	Levinger (1961)
	EEG	3.5-5	Nangeroni and Kennett (1964)
	EEG, B	10	Groß (1979)
	EEG, B	28-168	Blackmore and Newhook (1981); Blackmore et al (1983)
	EEG	34-85	Newhook and Blackmore (1982b)
	B	20-385	Blackmore (1984)
	VEP	11.5-23	Gregory and Wotton (1984c)
	SEP, VEP	19-113	Daly et al (1988)
	ECoG	10-52	Bager et al (1992)
Sheep	EEG	3.3-6.2	Nangeroni and Kennett (1964)
	EEG	3-10	Blackmore et al (1979)
	EEG	2-7	Blackmore and Newhook (1981); Newhook and Blackmore (1982a)
	VEP	14	Gregory and Wotton (1984b)
	B	8-11	Blackmore (1984)
Pigs	EEG, B	13-25	Blackmore and Newhook (1981)
	VEP	18	Wotton and Gregory (1986)
Poultry	TLP	12-26	Barnett et al (2007)

B: behaviour; ECoG: electrocorticogram; EEG: electroencephalogram; SEP: somatosensory evoked potentials; TLP: time to loss of posture; VEP: visually evoked potentials.

Studies investigating the time to insensibility in cattle can be broadly divided into two groups. The first three studies used changes in spontaneous EEG and behaviour to investigate the time to insensibility in cattle after Shechita slaughter (Levinger 1961; Nangeroni and Kennett 1964; Groß 1979). The reported times to insensibility ranged from 2 to 10 seconds, which is similar to the range reported in sheep (Nangeroni and Kennett 1964; Blackmore et al 1979; Blackmore and Newhook 1981; Newhook and Blackmore 1982a; Blackmore 1984; Gregory and Wotton 1984b). These three were some of the earlier studies to use electrophysiological techniques to assess the onset of insensibility during slaughter of cattle. They described the EEG in the initial 2 seconds after Shechita as having high frequency activity of approximately 20 Hz with a shift to high amplitude, low frequency activity afterwards. The authors state that during the first 2 to 10 seconds after VNI the

animals are most likely conscious but provided Shechita slaughter is performed correctly it is “very probably painless” (Groß 1979). Meanwhile the remaining studies suggest a longer time interval to insensibility in cattle with a wide range of between 10 and 385 seconds (Blackmore and Newhook 1981; Newhook and Blackmore 1982b, 1983; Gregory and Wotton 1984c; Daly et al 1988; Bager et al 1992). However, the range is more generally regarded as being 5 to 60 seconds (Mellor and Littin 2004). Although these studies utilized a variety of electrophysiological and behavioural techniques to investigate the time to undoubted insensibility, including spontaneous EEG, EEG spectral analysis, ECoG, SEP, VEP and behaviour, they similarly concluded that:

- The time to undoubted insensibility was longest in cattle and shortest in sheep. The causation of this is potentially due to the anatomy of the blood supply to the brain in different species and the occlusion of the carotid arteries during and after slaughter in cattle.
- There was a large amount of variability between individual cattle in the time to undoubted insensibility.
- In cattle there is a window after VNI in which the animals are conscious.
- During this window the animals are sensible to any pain and distress caused by slaughter.

The stunning of animals prior to slaughter is a practice designed to render the animals completely insensible to pain and distress prior to VNI, during VNI and until death supervenes. It is also practiced to improve slaughterhouse worker safety and to improve line efficiency. A variety of different methods are used, including mechanical, electrical and gas stunning. Each has its own advantages and disadvantages in particular species and environments. Mechanical methods of stunning include free bullet, penetrative captive bolt (PCB) and NPCB. All three methods cause physical damage to the brain, the first two by the projectile entering the cranium. However, with NPCB stunning the mushroom-shaped head does not penetrative the cranium. Insensibility is caused by the kinetic energy of the rapidly

moving bolt being transferred to the brain causing the disruption of cortical function. The effectiveness of both PCB and NPCB stunning in inducing insensibility has been examined in a number of livestock species. Tables 2.3, 2.4 and 2.5 examine some of the results of these studies in cattle, sheep and poultry, respectively.

Table 2.3. Studies of the time to insensibility in cattle with different stunning techniques: penetrative captive bolt (PCB); non-penetrative captive bolt (NPCB); electrical stunning. The parameter used and time to insensibility are indicated.

Method	Parameter	Time to insensibility (seconds)	Reference
PCB	EEG, B	Immediate (zero baseline after 23 seconds)	Groß (1979)
	EEG	Immediate	Fricker and Riek (1981)
	EEG	Immediate (frontal and occipital stun), 21 ± 6 (nape of neck)	Lambooy and Spanjaard (1981)
	EEG	Immediate	Blackmore and Newhook (1982)
	VEP ^a	VEPs recorded after stunning at all velocities in some animals	Daly et al (1986)
NPCB	EEG	Immediate	Blackmore and Newhook (1982)
	B, LRR	Immediate for 80% of animals stunned	Blackmore (1979)
Electrical stunning	EEG	Immediate (head to back)	Blackmore and Newhook (1982)
	CR, RB	Immediate, return of rhythmic breathing, corneal, palpebral reflexes within 50 seconds	Wotton et al (2000)

B: behaviour; CR: corneal reflex; EEG: electroencephalogram; LRR: loss of righting reflex; RB: return of rhythmic breathing; VEP: visually evoked potentials. ^a different bolt velocities.

Electrical stunning is designed to pass a sufficiently large current through the animal's head, via a pair of electrodes placed on either side of the cranium, to cause depolarisation of cortical neurons resulting in desynchronised neuronal activity, an epileptiform EEG trace and insensibility (Blackmore and Delany 1988). The time to insensibility after electrical stunning has been examined in a number of species. Tables 2.3 and 2.4 detail some of the important studies of the time to loss of sensibility in cattle and sheep, respectively. Captive bolt and electrical stunning have been demonstrated to reliably produce instant insensibility when performed correctly. However when performed incorrectly or with un-maintained equipment there is the potential for significant welfare compromise.

Table 2.4. Studies of the time to insensibility in sheep with different stunning techniques: penetrative captive bolt (PCB); non-penetrative captive bolt (NPCB); electrical stunning. The parameter used and time to insensibility are indicated.

Method	Parameter	Time to insensibility (seconds)	Reference
PCB (poll)	VEP	Immediate with recovery of VEP 49.6 ± 16.7	Daly and Whittington (1986)
PCB	SEP, VEP	Immediate (VEP persisted in one animal for 35 seconds)	Daly et al (1986)
NPCB	B, LRR	Immediate for 95% of animals stunned (highest impulse)	Blackmore (1979)
Electrical stunning	EEG	Immediate (duration 18-42)	Blackmore and Newhook (1982)
	TEP	Immediate, possible analgesia after electrical stunning	Gregory and Wotton (1988)
Head to back	VEP, SEP, EEG	Immediate	Anil and McKinstry (1991)
Head only	EEG, RB	Immediate with recovery of RB in 29.5 ± 1.55	Velarde et al (2002)
Head only and head to back	EEG	Immediate (possible recovery of head only if VNI delayed)	Gregory and Wotton (1984a)

B: behaviour; EEG: electroencephalogram; LRR: loss of righting reflex; SEP: somatosensory evoked potentials; RB: return of rhythmic breathing; TEP: tooth evoked potential; VEP: visually evoked potentials.

Table 2.5. Studies of the time to insensibility in poultry with different stunning techniques, penetrative captive bolt (PCB) and non-penetrative captive bolt (NPCB). The parameter used and time to insensibility are indicated.

Method	Parameter	Time to insensibility (seconds)	Reference
PCB (different positions, bolt sizes, air pressure)	B, EEG, VEP	Immediate (only perpendicular effective in instant stunning/killing)	Raj and O'Callaghan (2001)
NPCB	VEP	Immediate	Gregory and Wotton (1990)

B: behaviour; EEG: electroencephalogram; VEP: visually evoked potentials.

2.9 Blood Supply to the Bovine Brain

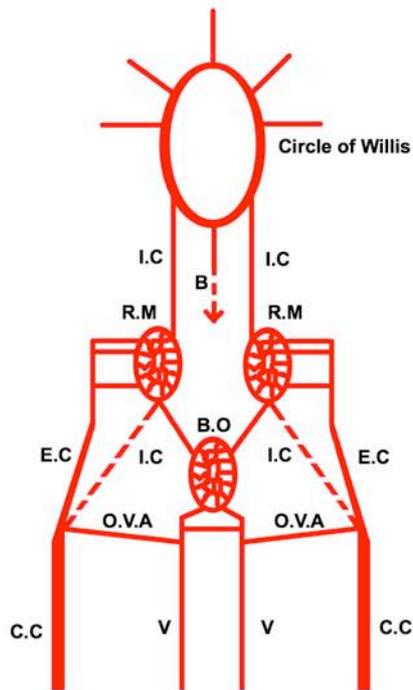
Oxygenated blood leaves the left ventricle of the heart from the aorta. The aorta gives off branches leading to the head, neck and forelimbs before becoming the thoracic aorta, which supplies blood to the rest of the body (Blackmore and Delany 1988). The brachiocephalic artery branches from the aorta. It divides into the right and left subclavian arteries, which pass around the first ribs to supply the forelimbs and structures of the neck. The bicarotid trunk, another major branch of the aorta, passes cranially. It then divides into the left and right common carotid arteries, which continue up the neck together with the vagus nerves and deep to the jugular veins (Blackmore and Delany 1988). The vertebral arteries, which branch from the subclavian arteries, run cranio-dorsally to pass through the successive transverse foramina of the sixth-to-first cervical vertebrae (Dyce et al 1996).

The common carotid arteries at the level of the first cervical vertebra divide into the internal and external carotid arteries and the occipital artery. In older animals the internal carotid makes little or no contribution to cerebral blood supply (Baldwin 1964, 1971a). In young calves the internal carotid is poorly developed, because its lumen becomes obliterated during development (Baldwin and Bell 1963). The external carotid arteries branch to form the occipital arteries, linguofacial trunk, parotid arteries, masseteric branch, caudal auricular arteries, and superficial temporal artery, before transition into the maxillary artery (Schummer et al 1981).

The maxillary artery runs along the base of the skull, supplying blood to many parts of the head. In ruminants the maxillary artery gives off dorsally directed rete branches. These branches link with the arterial network at the base of the skull, the rete mirabile (Schummer et al 1981) (figure 2.6). The rete mirabile is an anastomatic or a plexuses network, which is formed by many closely wound, anastomosing arteries (Dyce et al 1996); i.e. they are vessels that have established connections between arteries, veins or lymph vessels. The rete mirabile in cattle is intra-cranial, lying sub-durally in the cavernous sinus (Baldwin 1964). The rete mirabile in cattle is much more complex than that in sheep with more side-to-side anastomosis in cattle (Baldwin 1971). On the dorsal medial surface of the rete a single vessel emerges to pierce the dural membrane to supply blood to the circular artery at the base of brain,

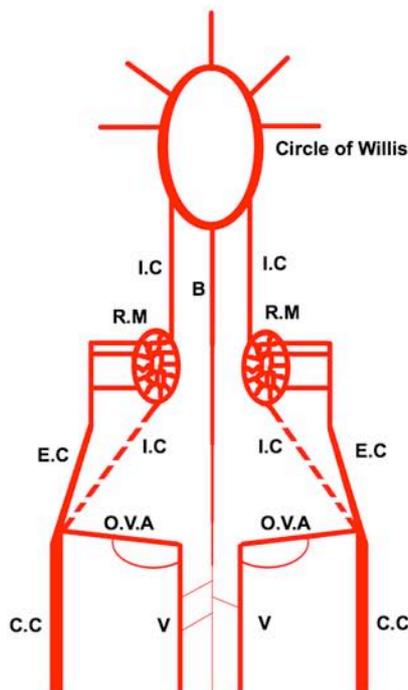
the Circle of Willis (Baldwin 1964). The Circle of Willis communicates directly with both the left and the right rete mirabile and supplies blood to the entire brain.

Blood is supplied to the rete mirabile principally by the maxillary artery, but also by the vertebral arteries. The vertebral arteries travel cranially within the vertebrae. In both cattle and sheep they communicate directly with the common carotid arteries via the occipito-vertebral anastomosis (figure 2.6). This is a vessel in which blood from the vertebral arteries can contribute to the rete mirabile and hence to cerebral perfusion. However, in cattle the vertebral arteries can also directly communicate with the rete mirabile via the basi-occipital plexus (figure 2.6). The basi-occipital plexus is a rete of vessels in which the both the left and right vertebral arteries make connections. Projections from the plexus connect with both left and right rete mirabiles. This path allows the supply of blood via the vertebral arteries to the brain in cattle in absence of connections with the carotid arteries (Baldwin and Bell 1963; Baldwin 1964).



Cattle

- I.C Internal carotid artery
- B Basilar artery
- R.M Rete mirabile
- B.O Basi-occipital plexus
- E.C External carotid artery
- O.V.A Occipito-vertebral anastomosis
- V Vertebral artery
- C.C Common carotid artery



Sheep

- I.C Internal carotid artery
- B Basilar artery
- R.M Rete mirabile
- E.C External carotid artery
- O.V.A Occipito-vertebral anastomosis
- V Vertebral artery
- C.C Common carotid artery

Figure 2.6. Diagrammatic illustration of the blood supply to the brain in cattle and sheep. Adapted from Baldwin (1971); Blackmore and Delany (1988).

2.9.1 Vertebral Arteries during Slaughter in Cattle

The variation in time to undoubted insensibility between cattle and other species may be due to the unique anatomical differences in cerebral perfusion in cattle compared to other species. Cerebral perfusion in cattle is primarily supplied by a mixture of both carotid and vertebral arterial blood (figure 2.6). In sheep, humans, dogs, cats and rabbits the vertebral arteries only make small contributions to specific areas (anterior spinal cord, cerebellum) of the brain. Cattle are unique in that the vertebral blood flows to the entire brain as well as anterior structures (Baldwin 1971). During slaughter by VNI only the common carotid arteries are severed, leaving the vertebral arteries intact within the vertebrae. In cattle, as noted above, the vertebral arteries communicate directly to the rete mirabile by the basi-occipital plexus as well as communicating via the occipito-vertebral anastomosis to the common carotids (Baldwin and Bell 1963). In sheep, as also noted, the connection between the rete mirabile and the vertebral arteries via the basi-occipital plexus is absent. Ventral neck incision severs the common carotid arteries, while leaving the vertebral arteries intact. It has been suggested that in cattle the blood supplied via the vertebral arteries following VNI is sufficient to prolong the period of sensibility. There has been considerable scientific debate concerning the role of the vertebral arteries in maintaining cerebral perfusion after VNI in cattle.

After VNI the severed common carotid arteries can become occluded, resulting in impendence of blood flow from the cut cephalic and cardiac ends (Gregory et al 2006). There are four possible mechanisms of occlusion of the severed common carotid arteries:

- When the artery is cut shedding of the intima of the artery wall might occur. This could allow blood to enter between the concentric layers of the arterial wall, possibly causing engorgement and restriction of blood loss.

- Upon severance, the artery may retract within the surrounding connective tissue sheath, resulting in blood flowing around the outer wall of the artery and collecting within the connective tissue sheath. This could cause both a physical barrier and compression of the artery as the tissue sheath engorges with blood.
- Platelets could aggregate at the severed end of the carotid arteries producing a white clot, which may restrict blood loss.
- The artery may go into annular spasm if severance leads to smooth muscle spasm in the arterial wall, an effect that could be promoted by the release of vasoactive amines from platelets. Annular spasm could cause constriction of the severed ends of the carotid arteries, restricting blood loss (Anil et al 1995a; Wotton 2004; Gregory et al 2006).

Levinger (1995) and Rosen (2004) have stated that following the incision during Shechita slaughter, blood would flow via the path of least-resistance to the severed cephalic ends of the jugular veins and carotid arteries. However this would be dependent on the absence of occlusion of the cut cephalic ends of these blood vessels. Several authors have suggested that the vertebral arteries can supply sufficient blood to the brain following such carotid occlusion to maintain or prolong the time to loss of cerebrocortical function (Newhook and Blackmore 1982b; Blackmore 1984; Blackman et al 1986; Bager et al 1988; Anil et al 1995ab; Wotton 2004). Conversely, other authors have stated that the vertebral arteries would be insufficient to maintain adequate blood supply to maintain normal brain function (Shaw et al 1990; Levinger 1995; Rosen 2004). The prevalence of carotid occlusion in cattle (n=387), calves (n=189) and sheep (n=411) after stunning and VNI slaughter was reported as 16%, 25% and 0%, respectively (Gregory et al 2006). This suggests that carotid occlusion is not a rare or isolated occurrence in cattle, especially in calves where significant carotid occlusion was found in 1 in 4 calves slaughtered by transection of neck tissues.

2.10 Pain During Slaughter

The issue of pain during and after slaughter by VNI without prior stunning has elicited significant discussion within scientific and religious communities and by governments and sectors of the general public. As already discussed (section 2.9), the time to the onset of insensibility of cattle is considerably longer (5 to 60 seconds) when compared to sheep (2 to 14 seconds). Most authors concede that after slaughter by VNI there is a period where cattle are conscious prior to the onset of insensibility (Levinger 1961; Nangeroni and Kennett 1964; Blackmore et al 1979; Groß 1979; Grandin 1980; Blackmore and Newhook 1981; Newhook and Blackmore 1982b; Blackmore et al 1983; Gregory and Wotton 1984c; Wotton and Gregory 1986; Daly et al 1988; Bager et al 1992). The act of VNI involves the transection of skin, muscle, trachea, oesophagus, sensory nerves and connective tissues. All these tissues are well endowed with sensory neurons and nociceptors which, when cut, would be expected to activate nociceptive pathways leading to perceived pain by the still conscious animals prior to the onset of hypoxia-induced insensibility.

Several authors have suggested that VNI without stunning would cause a form of sensory shock, including pain, due to afferent injury discharges from severed sensory neuron as well as nociceptors. Gregory (2005) has suggested that the animal would experience a combination of all sensory inputs, producing shock, and that there is no reason to expect one sensory input would take precedence over others. Nevertheless, damaged nerves may still communicate with undamaged nerves, and undamaged nociceptors and other sensory nerves in and around the wound could be responsive to further stimulation (e.g. from pressure of the incision or withdrawal of the knife, and air currents), resulting in potential nociceptor stimulation (Gregory 2007).

Some authors have theorised that during Shechita slaughter the exquisite sharpness of the Chalaf, combined with the smooth motion of the incision, causes minimal stimulation of the incised tissues. These authors go on to suggest that the stimulation is typically below the level adequate to activate pain pathways (Levinger 1995; Rosen 2004). This argument is based on anecdotal observations and analogies to human experiences where pain is not perceived after tissue damage, for example

surgeons cutting themselves with scalpels during surgery without being aware of it (Levinger 1995), or soldiers on the battlefield unaware of significant tissue damage. Grandin (1980) stated that both Shechita and Halal slaughter, if performed correctly with humane restraint are probably the least painful techniques of throat-cutting for conscious animals. However, there is no convincing scientific evidence to support the suggestion that a cut performed with a Chalaf prevents nociception and the perception of pain in animals. As discussed previously (section 2.9), Levinger (1961), Nangeroni and Kennett (1964) and Groß (1979) described a period after Shechita slaughter of maintained consciousness (2 to 10 seconds). The authors conceded that the animals were conscious during this period, but they stated that the animals did not experience pain or distress from the tissue damage of slaughter as the EEG changes were of “low grade” and because of the absence of body movements. However, they did report that in the initial 2 seconds after shechita there was EEG activity of high frequency. This activity is potentially representative of a state of cortical irritability and hyperactivity that has been previously associated with noxious sensory input in a variety of species (Murrell and Johnson 2006).

Furthermore, Rosen (2004) in a viewpoint article suggested that following Shechita slaughter the collapse in jugular venous pressure impaired the maintenance of brain structure resulting in “a kind of ‘implosion’ of the brain”. The suggestion is that the ‘implosion’ of the brain tissue results in the disruption of normal cortical function. However, the author did not provide sufficient explanation for the mechanisms of this event or any scientific evidence confirming the statement. Until scientifically tested this theory remains highly speculative.

Levinger (1995) stated that it is the release of the chemical substances during cellular damage that causes stimulation of nociceptors and that there is a long interval (“several seconds”) between the release of these substances and the perception of pain. He further suggested that if the brain is capable of only a brief period of functional activity after Shechita, it is possible that the ability for the perception of pain has ceased prior to cortical processing of the stimulus. This theory is erroneous on several counts: firstly, nociceptors are able to be stimulated not just by chemical but also by thermal and mechanical stimuli; secondly, it is apparent that the author is referring to the mechanisms of inflammation-mediated pain, while this type of pain would be of little consequence during slaughter, where, due to the timeframe of action, nociception would still occur from damaged and undamaged neurons in

proximity to the tissue damage; thirdly, stimulation of myelinated A delta and unmyelinated C fibres with conduction velocities of between 4 to 30 and 0.4 to 1.8 ms, respectively (Bromm and Lorenz 1998) would result in rapid transmission of impulses to higher centres of the CNS, well within the timeframe required for perception of pain before the onset of insensibility; and lastly, the author ignores the considerable body of work that established there is a range in the time to insensibility in cattle of between 5 to 60 seconds (Levinger 1961; Nangeroni and Kennett 1964; Blackmore et al 1979; Groß 1979; Blackmore and Newhook 1981; Newhook and Blackmore 1982b, 1983; Gregory and Wotton 1984c, Daly et al 1988; Bager et al 1992; Mellor and Littin 2004).

2.11 References

- Adrian ED, Matthews BH.** The interpretation of potential waves in the cortex. *Journal of Physiology* 81, 440-71, 1934
- Anil H, Sheard PR.** Welfare implications of religious slaughter. *Meat Focus International* 3, 1994
- Anil MH, McKinstry JL.** Reflexes and loss of sensibility following head-to-back electrical stunning in sheep. *The Veterinary Record* 128, 106-7, 1991
- Anil MH, McKinstry JL, Gregory NG, Wotton SB, Symonds H.** Welfare of Calves .2. Increase in vertebral artery blood-flow following exsanguination by neck sticking and evaluation of chest sticking as an alternative slaughter method. *Meat Science* 41, 113-23, 1995a
- Anil MH, McKinstry JL, Wotton SB, Gregory NG.** Welfare of calves .1. investigations into some aspects of calf slaughter. *Meat Science* 41, 101-12, 1995b
- Anon.** American meat institute fact sheet: religious slaughter. <http://www.meatami.com/content/presscenter/factsheets/Infokits/FactSheetReligiousSlaughter.pdf>, 2004
- AWAC.** Code of recommendations and minimum standards for the emergency slaughter of farm livestock, Code of Animal Welfare No.19, Ministry of Agriculture and Forestry, Wellington, 1996
- Backonja M, Howland EW, Wang J, Smith J, Salinsky M, Cleeland CS.** Tonic changes in alpha power during immersion of the hand in cold water. *Electroencephalography and clinical Neurophysiology* 79, 192-203, 1991
- Bager F, Devine CE, Gilbert KV.** Jugular blood flow in calves after head-only electrical stunning and throat-cutting. *Meat Science* 22, 237-43, 1988
- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP.** Onset of insensibility at slaughter in calves - effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162-73, 1992
- Baldwin BA, Bell FR.** The anatomy of the cerebral circulation of the sheep and ox. The dynamic distribution of the blood supplied by the carotid and vertebral arteries to cranial regions. *Journal of Anatomy* 97, 203-15, 1963
- Baldwin BA.** The anatomy of the arterial supply to the cranial regions of the sheep and ox. *American Journal of Anatomy* 115, 101-18, 1964

- Baldwin BA.** Anatomical and physiological factors involved in slaughter by section of the carotid arteries. In: Ewer TK (ed) *UFAW Symposium, Humane Killing and Slaughterhouse Techniques*. Pp 34-43. The Universities Federation for Animal Welfare, 1971
- Bar-Moha Y.** Onward conquistadors.
<http://www.grandin.com/ritual/kosher.meat.uruguay.html>, 1998
- Barnett JL.** Measuring pain in animals. *Australian Veterinary Journal* 75, 878-9, 1997
- Barnett JL, Cronin GM, Scott PC.** Behavioural responses of poultry during Kosher slaughter and their implications for the birds' welfare. *Veterinary Record* 160, 45-9, 2007
- Battye J.** Ethical issues and animal use in science. In: 'Ethical Approaches to Animal-Based Science, Proceedings of the Joint ANZCCART/NAEAC Conference'. Auckland, New Zealand. (eds) Mellor DJ, Fisher M, Sutherland G. Pp 11-8. (ANZCCART, The Royal Society of New Zealand, Wellington, New Zealand), 1998
- Blackman NL, Cheetham K, Blackmore DK.** Differences in blood-supply to the cerebral-cortex between sheep and calves during slaughter. *Research in Veterinary Science* 40, 252-4, 1986
- Blackmore DK.** Non-penetrative percussion stunning of sheep and calves. *Veterinary Record* 105, 372-5, 1979
- Blackmore DK, Newhook JC, Petersen GV.** Electrical stunning and humane slaughter. *New Zealand Veterinary Journal* 27, 224-, 1979
- Blackmore DK, Newhook JC.** Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* 29, 219-22, 1981
- Blackmore DK, Newhook JC.** Electroencephalographic studies of stunning and slaughter of sheep and calves .3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Science* 7, 19-28, 1982
- Blackmore DK, Newhook JC, Grandin T.** Time of onset of insensibility in 4-week-old to 6-week-old calves during slaughter. *Meat Science* 9, 145-9, 1983
- Blackmore DK.** Differences in behaviour between sheep and cattle during slaughter. *Research in Veterinary Science* 37, 223-6, 1984
- Blackmore DK, Delany MW.** Slaughter of stock, a practical review and guide, Veterinary Continuing Education Massey University, Palmerston North, 1988
- Bromm B, Lorenz J.** Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology* 107, 227-53, 1998

- Bromm B.** Brain images of pain. *News in Physiological Sciences* 16, 244-9, 2001
- Carding T.** Welfare of animals slaughtered for meat, World Federation for the Protection of Animals, Zurich, 1970
- Caton R.** The electric currents of the brain. *The British Medical Journal*, 278, 1875
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN.** Topographic effects of tonic cutaneous nociceptive stimulation on human electroencephalograph. *Neuroscience Letters* 305, 49-52, 2001a
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN.** Different EEG topographic effects of painful and non-painful intramuscular stimulation in man. *Experimental Brain Research* 141, 195-203, 2001b
- Chang PF, Arendt-Nielsen L, Chen ACN.** Differential cerebral responses to aversive auditory arousal versus muscle pain: specific EEG patterns are associated with human pain processing. *Experimental Brain Research* 147, 387-93, 2002
- Chen ACN, Dworkin SF, Haug J, Gehrig J.** Topographic brain measures of human pain and pain responsivity. *Pain* 37, 129-41, 1989
- Chen ACN.** New perspectives in EEG/MEG brain mapping and PET/fMRI neuroimaging of human pain. *International Journal of Psychophysiology* 42, 147-59, 2001
- Chen ACN.** EEG/MEG brain mapping of human pain: recent advances. *International Congress Series* 1232, 5-16, 2002
- Cooley JW, Tukey JW.** An algorithm for the machine calculation of complex fourier series. *Mathematics of Computation* 19, 297-301, 1965
- Cunningham JG.** Textbook of veterinary physiology, 3 edn. W.B. Saunders Company, Philadelphia, 2002
- Daly CC, Whittington PE.** Concussive methods of pre-slaughter stunning in sheep - effects of captive bolt stunning in the poll position on brain-function. *Research in Veterinary Science* 41, 353-5, 1986
- Daly CC, Gregory NG, Wotton SB, Whittington PE.** Concussive methods of pre-slaughter stunning in sheep - assessment of brain-function using cortical evoked-responses. *Research in Veterinary Science* 41, 349-52, 1986
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325-9, 1988

- Diesch TJ, Mellor DJ, Johnson CB, Lentle RG.** Responsiveness to painful stimuli in anaesthetised newborn and young animals of varying neurological maturity (lambs, rat pups and wallaby joeys). In: '6th World Congress on Alternatives & Animal Use in the Life Sciences, 2007'. Tokyo, Japan Pp 549-52, 2008
- Dun CS.** Stress reactions of cattle undergoing ritual slaughter using two methods of restraint. *Veterinary Record* 126, 522-5, 1990
- Duncan IJH.** Poultry welfare: science or subjectivity? *British Poultry Science* 43, 643-52, 2002
- Duncan JH, Fraser D.** Understanding animal welfare. In: Appleby MC, Hughes BO (eds). *Animal Welfare*. CAB International, Oxon, 1997
- Dyce KM, Sack WO, Wensing CJG.** Textbook of veterinary anatomy, 2 edn. W.B. Saunders Company, Philadelphia, 1996
- Estrella M, Forster BS, Whinnery JE.** Statistical analysis of the human strangulation experiments: comparison to +Gz-induced loss of consciousness. Naval Air Warfare Centre – Aircraft Division, Warminster, 1992
- FAWC.** Report on the welfare of farmed animals at slaughter or killing. Part 1. red meat animals. Farm Animal Welfare Council, London, 2003
- Ferracuti S, Seri S, Mattia D, Cruccu, G.** Quantitative EEG modification during the cold water pressor test: hemispheric and hand differences. *International Journal of Psychophysiology* 17, 261-8, 1994
- Firth AM, Haldane SL.** Development of a scale to evaluate postoperative pain in dogs. *Journal of the American Veterinary Medical Association* 214, 651-9, 1999
- Fisch BJ.** The source of the EEG. In: *Fisch and Spehlmann's EEG Primer*. Pp 3-14. Elsevier, Amsterdam, 1999a
- Fisch BJ.** Digital and analog EEG instruments: parts and functions. In: *Fisch and Spehlmann's EEG Primer*. Pp 35-72. Elsevier, Amsterdam, 1999b
- Flecknell PA.** Animal pain - an introduction. In: Flecknell P, Waterman-Pearson A (eds). *Pain Management in Animals*. Pp 1-8. W.B. Saunders, London, 2000
- Fricker CH, Riek W.** Die betäubung von rindern vor dem schlachten mit hilfe des bolzenschuß-apparates. *Fleischwirtschaft* 61, 124-7, 1981
- Galvin JW, Blokhuis H, Chimbombi MC, Jong D, Wotton S.** Killing of animals for disease control purposes. *Revue Scientifique Et Technique-Office International Des Epizooties* 24, 711-22, 2005

- Graf B, Senn M.** Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. *Applied Animal Behaviour Science* 62, 153-71, 1999
- Grandin T.** Problems with Kosher slaughter. *International Journal for the Study of Animal Problems* 1, 375-90, 1980
- Grandin T.** Humanitarian aspects of Shechita in the United States. *Judaism* 39, 436-46, 1990
- Grandin T.** Observations of cattle restraint devices for stunning and slaughtering. *Animal Welfare* 1, 85-90, 1992
- Grandin T.** Euthanasia and slaughter of livestock. *Journal of the American Veterinary Medical Association* 204, 1354-60, 1994a
- Grandin T.** Farm animal-welfare during handling, transport, and slaughter. *Journal of the American Veterinary Medical Association* 204, 372-7, 1994b
- Grandin T, Regenstein JM.** Religious slaughter and animal welfare: a discussion for meat scientists. *Meat Focus International*, 115-23, 1994
- Grandin T.** Slaughterhouse design and religious slaughter. In: DVD. 2003a
- Grandin T.** Transferring results of behavioural research to industry to improve animal welfare on the farm, ranch and the slaughter plant. *Applied Animal Behaviour Science* 81, 215-28, 2003b
- Grandin T.** Preface to the Israeli article about kosher slaughter in South America. Fort Collins, <http://www.grandin.com/ritual/kosher.meat.uruguay.html>, 2004
- Grandin T.** Maintenance of good animal welfare standards in beef slaughter plants by use of auditing programs. *JAVMA-Journal of the American Veterinary Medical Association* 226, 370-3, 2005
- Gregory N.** Stunning and slaughter. In: *Animal Welfare and Meat Production*. Pp 191-212. CABI Publishing, Wallingford, 2007
- Gregory NG, Wotton SB.** Sheep slaughtering procedures .3. Head-to-back electrical stunning. *British Veterinary Journal* 140, 570-5, 1984a
- Gregory NG, Wotton SB.** Sheep slaughtering procedures .2. Time to loss of brain responsiveness after exsanguination or cardiac-arrest. *British Veterinary Journal* 140, 354-60, 1984b
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141-3, 1984c

- Gregory NG, Wotton SB.** Sheep slaughtering procedures .5. Responsiveness to potentially painful stimuli following electrical stunning. *British Veterinary Journal* 144, 573-80, 1988
- Gregory NG, Wotton SB.** Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *Veterinary Record* 126, 570-2, 1990
- Gregory NG.** Slaughter, ethics and the law. In: Jensen W, Devine C, Dikeman M (eds). *Encyclopedia of Meat Sciences*. Pp 1233-6. Elsevier Ltd, Oxford, 2004
- Gregory NG.** Recent concerns about stunning and slaughter. *Meat Science* 70, 481-91, 2005
- Gregory NG, Shaw FD, Whitford JC, Patterson-Kane JC.** Prevalence of ballooning of the severed carotid arteries at slaughter in cattle, calves and sheep. *Meat Science* 74, 655-7, 2006
- Gregory N.** Stunning and slaughter. In: *Animal Welfare and Meat Production*. Pp 191-212. CABI Publishing, Wallingford, 2007
- Grondahl-Nielsen C, Simonsen HB, Lund JD, Hesselholt M.** Behavioural, endocrine and cardiac responses in young calves undergoing dehorning without and with use of sedation and analgesia. *Veterinary Journal* 158, 14-20, 1999
- Groß R.** Elektroencephalographische und elektrokardiographische verlaufsuntersuchungen nach bolzenschußbetäubung und nach töten durch entbluten in der form des rituellen schlachtens. durch die tierärztliche hochschule, Hannover, 1979
- Haga HA, Dolvik NI.** Electroencephalographic and cardiovascular variables as nociceptive indicators in isoflurane-anaesthetized horses. *Veterinary Anaesthesia and Analgesia* 32, 128-35, 2005
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1-9, 2005
- Hofbauer RK, Rainville P, Duncan GH, Bushnell MC.** Cortical representation of the sensory dimension of pain. *Journal of Neurophysiology* 86, 402-11, 2001
- Homa B.** The Jewish methods of slaughter. In: Ewer TK (ed) *UFAW Symposium, Humane Killing and Slaughterhouse Techniques*. Pp 44-49. The Universities Federation for Animal Welfare, 1971
- IASP.** IASP pain terminology. 27 April 2008, http://www.iasp-pain.org/AM/Template.cfm?Section=General_Resource_Links&Template=/CM/HTMLDisplay.cfm&ContentID=3058#Pain, 2008

- Ibinson JW, Small RH, Algaze A, Roberts CJ, Clark DL, Schmalbrock P.** Functional magnetic resonance imaging studies of pain. *Anesthesiology* 101, 960-9, 2004
- Johnson CB, Young SS, Taylor PM.** Use of frequency spectral analysis of the equine electroencephalogram as an indication of the depth of halothane anesthesia. In: Sebel PS, Bonke B, Winograd E (eds). *Memory and Awareness in Anaesthesia*. Pp 289-95. P T R Prentice Hall, Englewood Cliffs, 1993
- Johnson CB, Young SS, Taylor PM.** Analysis of the frequency spectrum of the equine electroencephalogram during halothane anaesthesia. *Research in Veterinary Science* 56, 373-8, 1994
- Johnson CB, Taylor PM.** Effects of alfentanil on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Research in Veterinary Science* 62, 159-63, 1997
- Johnson CB, Taylor PM.** Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* 81, 748-53, 1998
- Johnson CB, Bloomfield M, Taylor PM.** Effects of ketamine on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Veterinary Surgery* 28, 380-5, 1999
- Johnson CB, Bloomfield M, Taylor PM.** Effects of guaiphenesin on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Veterinary Anaesthesia and Analgesia* 27, 6-12, 2000a
- Johnson CB, Bloomfield M, Taylor PM.** Effects of thiopentone on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Veterinary Anaesthesia and Analgesia* 27, 82-8, 2000b
- Johnson CB.** Pain - definition and physiology. In: *Proceedings of the New Zealand Society of Animal Production*. 2002
- Johnson CB, Bloomfield M, Taylor PM.** Effects of midazolam and sarmazenil on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Journal of Veterinary Pharmacology and Therapeutics* 26, 105-12, 2003
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433-7, 2005a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and analgesia* 32, 61-71, 2005b

- Johnson CB, Murrell JC, Gibson TJ, Mellor DJ.** Innovative refinements to anaesthesia techniques can deliver pain research without pain. In: '6th World Congress on Alternatives & Animal Use in the Life Sciences, 2007'. Tokyo, Japan Pp 97-100, 2008
- Jones AKP, Friston K, Frackowiak RSJ.** Localization of responses to pain in human cerebral cortex. *Science* 255, 215-6, 1992
- Jongman EC, Morris JP, Barnett JL, Hemsworth PH.** EEG changes in 4-week-old lambs in response to castration, tail docking and mulesing. *Australian Veterinary Journal* 78, 339-43, 2000
- Kent JE, Molony V, Robertson IS.** Comparison of the burdizzo and rubber ring methods for castrating and tail docking lambs. *Veterinary Record* 136, 192-6, 1995
- Khan GM.** The Muslim methods of slaughter. In: Ewer TK (ed) *UFAW Symposium, Humane Killing and Slaughterhouse Techniques*. Pp 50-57. The Universities Federation for Animal Welfare, 1971
- Kongara K.** Studies on renal safety and preventive analgesic efficacy of Tromadox and parecoxib in dogs. PhD thesis, Massey University, Palmerston North, 2008
- Lambooy E, Spanjaard W.** Effect of the shooting position on the stunning of calves by captive bolt. *Veterinary Record* 109, 359-61, 1981
- Lay DC, Friend TH, Grissom KK, Bowers CL, Mal ME.** Effects of freeze or hot-iron branding of angus calves on some physiological and behavioral indicators of stress. *Applied Animal Behaviour Science* 33, 137-47, 1992a
- Lay DC, Friend TH, Bowers CL, Grissom KK, Jenkins OC.** A comparative physiological and behavioral-study of freeze and hot-iron branding using dairy-cows. *Journal of Animal Science* 70, 1121-5, 1992b
- Lester SJ, Mellor DJ, Ward RN.** Effects of repeated handling on the cortisol responses of young lambs castrated and tailed surgically. *New Zealand Veterinary Journal* 39, 147-9, 1991a
- Lester SJ, Mellor DJ, Ward RN, Holmes RJ.** Cortisol responses of young lambs to castration and tailing using different methods. *New Zealand Veterinary Journal* 39, 134-8, 1991b
- Levinger IM.** Untersuchungen zum schächtproblem. Dr thesis, University of Zürich, Zürich, 1961
- Levinger IM.** Shechita in the light of the year 2000, Critical review of the scientific aspects of methods of slaughter and Shechita, Maskil L'David, Jerusalem, 1995

- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. MSc thesis, Massey University, Palmerston North, NZ, 2005
- McMeekan CM, Mellor DJ, Stafford KJ, Bruce RA, Ward RN, Gregory NG.** Effects of local anaesthesia of 4 to 8 hours' duration on the acute cortisol response to scoop dehorning in calves. *Australian Veterinary Journal* 76, 281-5, 1998a
- McMeekan CM, Stafford KJ, Mellor DJ, Bruce RA, Ward RN, Gregory NG.** Effects of regional analgesia and/or a non-steroidal anti-inflammatory analgesic on the acute cortisol response to dehorning in calves. *Research in Veterinary Science* 64, 147-50, 1998b
- Mellor DJ.** How can animal-based scientists demonstrate ethical integrity? In: 'Ethical Approaches to Animal-Based Science, Proceedings of the Joint ANZCCART/NAEAC Conference'. Auckland, New Zealand. (eds) Mellor DJ, Fisher M, Sutherland G. Pp 19-31. (ANZCCART, The Royal Society of New Zealand, Wellington, New Zealand), 1998
- Mellor, DJ, and Battye, J.** Mutual respect overcomes mutual disregard: working to resolve the GE impasse. *Reflections on the Use of Human Genes in Other Organisms: Ethical, Spiritual and Cultural Dimensions*. (The Bioethics Council, Ministry for the Environment, Wellington). Pp 15-19, 2004
- Mellor DJ, Stafford KJ.** Interpretation of cortisol responses in calf disbudding studies. *New Zealand Veterinary Journal* 45, 126-7, 1997
- Mellor DJ, Stafford KJ.** Integrating practical, regulatory and ethical strategies for enhancing farm animal welfare. *Australian Veterinary Journal* 79, 762-8, 2001
- Mellor DJ, Stafford KJ, Todd SE, Lowe TE, Gregory NG, Bruce RA, Ward RN.** A comparison of catecholamine and cortisol responses of young lambs and calves to painful husbandry procedures. *Australian Veterinary Journal* 80, 228-33, 2002
- Mellor DJ, Stafford KJ.** Acute castration and tailing distress and its alleviation in lambs. *New Zealand Veterinary Journal* 48, 33-43, 2000
- Mellor DJ, Stafford KJ.** Physiological indices of pain-induced distress. In: *Proceedings of the New Zealand Society of Animal Production*. 2002
- Mellor DJ, Stafford KJ.** Physiological and behavioural assessment of pain in ruminants: Principles and caveats. *ATLA-Alternatives to Laboratory Animals* 32, 267-71, 2004
- Mellor DJ, Littin KE.** Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13, S127-S32, 2004

- Melzack R, Wall PD.** Pain mechanisms: a new theory. *Science* 150, 971-9, 1965
- Molony V, Kent JE, Robertson IS.** Assessment of acute and chronic pain after different methods of castration of calves. *Applied Animal Behaviour Science* 46, 33-48, 1995
- Molony V, Kent JE, McKendrick IJ.** Validation of a method for assessment of an acute pain in lambs. *Applied Animal Behaviour Science* 76, 215-38, 2002
- Morisse JP, Cotte JP, Huonnic D.** Effect of dehorning on behavior and plasma-cortisol responses in young calves. *Applied Animal Behaviour Science* 43, 239-47, 1995
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-21, 2005
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Murrell JC, Mitchinson SL, Waters D, Johnson CB.** Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* 98, 366-71, 2007
- Murrell JC, Waters D, Johnson CB.** Comparative effects of halothane, isoflurane, sevoflurane and desflurane on the electroencephalogram of the rat. *Laboratory Animals* 42, 161-70, 2008
- Nangeroni LL, Kennett PD.** Electroencephalographic studies of the effects of Shechita slaughter on cortical function in ruminants. Cornell University, New York, 1964
- NAWAC.** Discussion paper on the animal welfare standards to apply when animals are commercially slaughtered in accordance with religious requirement. Ministry of Agriculture and Forestry, Wellington, 2001
- NAWAC.** Animal welfare (commercial slaughter) code of welfare 2006 public draft. 2006
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .1. The onset of permanent insensibility in sheep during slaughter. *Meat Science* 6, 221-33, 1982a

- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295-300, 1982b
- Niedermeyer E.** Historical aspects. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Pp 1-16. Lippincott Williams & Wilkins, Philadelphia, 2005
- Ong RM, Morris JP, Odwyer JK, Barnett JL, Hemsworth PH, Clarke IJ.** Behavioural and EEG changes in sheep in response to painful acute electrical stimuli. *Australian Veterinary Journal* 75, 189-93, 1997
- Otto KA, Voigt S, Piepenbrock S, Deegen E, Short CE.** Differences in quantitated electroencephalographic variables during surgical stimulation of horses anesthetized with isoflurane. *Veterinary Surgery* 25, 249-55, 1996
- Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA, Ward RN.** Cortisol responses of calves to two methods of disbudding used with or without local anaesthetic. *New Zealand Veterinary Journal* 44, 9-14, 1996
- Raj ABM, O'Callaghan M.** Evaluation of a pneumatically operated captive bolt for stunning/killing broiler chickens. *British Poultry Science* 42, 295-9, 2001
- Rishworth P, Huscroft G, Optican S, Mahoney R.** The New Zealand bill of rights, Oxford University Press, New York, 2003
- Rosen R, Kabat H, Anderson JP.** Acute arrest of cerebral circulation in man. *Archives of Neurology and Psychiatry* 50, 510-28, 1943
- Rosen SD.** Viewpoint article; Physiological insights into Shechita. *Veterinary Record* 154, 759-65, 2004
- Roughan J.** Personal communication. In. 2004
- Roughan JV, Flecknell PA.** Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *European Journal of Pain* 7, 397-406, 2003
- Russell WMS, Burch RL.** The principles of humane experimental technique, Methuen & Co Ltd, London, 1959
- Schummer A, Wilkens H, Vollmerhaus B, Habermehl K-H.** The circulatory system, the skin, and the cutaneous organs of the domestic mammals, Verlag Paul Parey, Berlin, 1981
- Schwartzkopf-Genswein KS, Stookey JM, dePassille AM, Rushen J.** Comparison of hot-iron and freeze branding on cortisol levels and pain sensitivity in beef cattle. *Canadian Journal of Animal Science* 77, 369-74, 1997

- Schwilden H.** Concepts of EEG processing: from power spectrum to bispectrum, fractals, entropies and all that. *Best Practice & Research Clinical Anaesthesiology* 20, 31-48, 2006
- Sebel PS.** Somatosensory, visual and motor evoked potentials in anaesthetized patients. In: Jones JG (ed) *Bailliere's Clinical Anaesthesiology, International Practice and Research, Depth of Anaesthesia*. Pp 587-602. Bailliere Tindall, London, 1989
- Shaw FD, Bager F, Devine CE.** The role of the vertebral arteries in maintaining spontaneous electrocortical activity after electrical stunning and slaughter in calves. *New Zealand Veterinary Journal* 38, 14-6, 1990
- Shimshony A, Chaudry MM.** Slaughter of animals for human consumption. *Revue Scientifique Et Technique-Office International Des Epizooties* 24, 693-710, 2005
- Shragge JE, Price MA.** Religious slaughter. In: Jensen W, Devine C, Dikeman M (eds). *Encyclopedia of Meat Sciences*. Pp 1162-7. Elsevier Ltd, Oxford, 2004
- Spedding C.** The environment and animal welfare: ethical issues. In: 'Ethical Approaches to Animal-Based Science, Proceedings of the Joint ANZCCART/NAEAC Conference'. Auckland, New Zealand. (eds) Mellor DJ, Fisher M, Sutherland G. Pp 3-10. (ANZCCART, The Royal Society of New Zealand, Wellington, New Zealand), 1998
- Spedding C.** Animal welfare, Earthscan Publications Ltd, London, 2000
- Stafford KJ, Mellor DJ.** Monitoring pain in animals using behaviour. In: *Proceedings of the New Zealand Society of Animal Production*. 2002
- Stafford KJ, Mellor DJ, Todd SE, Ward RN, McMeekan CM.** The effect of different combinations of lignocaine, ketoprofen, xylazine and tolazoline on the acute cortisol response to dehorning in calves. *New Zealand Veterinary Journal* 51, 219-26, 2003
- Stafford KJ, Mellor DJ.** Dehorning and disbudding distress and its alleviation in calves. *The Veterinary Journal* 169, 337-49, 2005
- Sutherland MA, Mellor DJ, Stafford KJ, Gregory NG, Bruce RA, Ward RN, Todd SE.** Acute cortisol responses of lambs to ring castration and docking after the injection of lignocaine into the scrotal neck or testes at the time of ring application. *Australian Veterinary Journal* 77, 738-41, 1999
- Sutherland MA.** Alleviation of the distress caused by ring castration plus tailing of lambs and dehorning of calves. Massey University, Palmerston North, 1999

- Sutherland MA, Mellor DJ, Stafford KJ, Gregory NG, Bruce RA, Ward RN.** Cortisol responses to dehorning of calves given a 5-h local anaesthetic regimen plus phenylbutazone, ketoprofen, or adrenocorticotrophic hormone prior to dehorning. *Research in Veterinary Science* 73, 115-23, 2002
- Swanson JC.** Farm animal well-being and intensive production systems. *Journal of Animal Science* 73, 2744-51, 1995
- Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA, Ward RN.** Acute cortisol responses of calves to four methods of dehorning by amputation. *Australian Veterinary Journal* 76, 123-6, 1998a
- Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA, Ward RN.** Acute cortisol responses of calves to scoop dehorning using local anaesthesia and/or cauterization of the wound. *Australian Veterinary Journal* 76, 118-22, 1998b
- Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA, Ward RN.** Behavioural responses of calves to amputation dehorning with and without local anaesthesia. *Australian Veterinary Journal* 82, 697-700, 2004
- Talbot JD, Marrett S, Evans AC, Meyer E, Bushnell MC, Duncan GH.** Multiple representations of pain in human cerebral-cortex. *Science* 251, 1355-8, 1991
- Tonner PH, Bein B.** Classic electroencephalographic parameters: median frequency, spectral edge frequency etc. *Best Practice & Research Clinical Anaesthesiology* 20, 147-59, 2006
- Treede R-D, Apkarian AV, Bromm B, Greenspan JD, Lenz FA.** Cortical representation of pain: functional characterization of nociceptive areas near the lateral sulcus. *Pain* 87, 113-9, 2000
- Treede RD, Kenshalo DR, Gracely RH, Jones AKP.** The cortical representation of pain. *Pain* 79, 105-11, 1999
- Tume RK, Shaw FD.** Beta-endorphin and cortisol concentrations in plasma of blood-samples collected during exsanguination of cattle. *Meat Science* 31, 211-7, 1992
- Velarde A, Ruiz-de-la-Torre JL, Rosello C, Fabrega E, Diestre A, Manteca X.** Assessment of return to consciousness after electrical stunning in lambs. *Animal Welfare* 11, 333-41, 2002
- Wohlt JE, Allyn ME, Zajac PK, Katz LS.** Cortisol increases in plasma of holstein heifer calves from handling and method of electrical dehorning. *Journal of Dairy Science* 77, 3725-9, 1994

Wood GN, Molony V, Fleetwoodwalker SM, Hodgson JC, Mellor DJ. Effects of local-anesthesia and intravenous naloxone on the changes in behaviour and plasma-concentrations of cortisol produced by castration and tail docking with tight rubber rings in young lambs. *Research in Veterinary Science* 51, 193-9, 1991

Wotton S. Slaughter without prestunning. *The Veterinary Record* 155, 64, 2004

Wotton SB, Gregory NG. Pig slaughtering procedures - time to loss of brain responsiveness after exsanguination or cardiac-arrest. *Research in Veterinary Science* 40, 148-51, 1986

Wotton SB, Gregory NG, Whittington PE, Parkman ID. Electrical stunning of cattle. *Veterinary Record* 147, 681-4, 2000

Young SS. Computerized data acquisition and analysis for the life sciences: a hands-on guide, Cambridge University Press, Cambridge, 2001

CHAPTER 3

**VALIDATION OF THE ACUTE
ELECTROENCEPHALOGRAPHIC RESPONSES OF
CALVES TO NOXIOUS STIMULUS WITH SCOOP
DEHORNING**



Published scientific-peer-reviewed paper

Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Validation of the Acute Electroencephalographic Responses of Calves to Noxious Stimulus with Scoop Dehorning. *New Zealand Veterinary Journal* 55, 152-7, 2007

3.1 Abstract

AIM: To validate use of the electroencephalogram (EEG) and a minimal anaesthesia model for assessment of noxious sensory input caused by scoop dehorning of calves.

METHODS: Twenty Friesian heifers weighing 125-178 kg were maintained under light general anaesthesia using an established protocol (minimal anaesthesia model). They were then dehorned using amputation either with (DH+LA) or without (DH) a lidocaine ring block. Changes in the EEG and electrocardiogram (ECG) in response to scoop dehorning were recorded. Median frequency (F50), 95% spectral edge frequency (F95) and total power (Ptot) were derived from the EEG data.

RESULTS: There were significant increases in F50 ($p<0.01$) and F95 ($p<0.01$), and a decrease in Ptot ($p<0.01$), following dehorning in the DH group, but there were no changes in the DH+LA group. Transient bradycardia in the first 75 seconds following dehorning was recorded in the DH group compared to pre-treatment values and to the DH+LA group ($p<0.001$). However, tachycardia was evident by 15 minutes after dehorning in the DH group.

CONCLUSIONS: The results validate use of the EEG and a minimal anaesthesia model for assessment of noxious sensory inputs in cattle.

3.2 Introduction

It is recognised that the cerebral cortex has an integral role in the conscious perception of pain (Talbot et al 1991; Jones et al 1992; Treede et al 1999). The electroencephalogram (EEG) has long been known to reflect changes in cortical function by an alteration in the components of its frequency (Adrian and Matthews 1934). Development of the Fast Fourier Transformation method (Cooley and Tukey 1965) has allowed statistical analysis of specific variables in the power spectra of EEGs. Changes in the power spectra of the EEG have been shown to reflect alterations in the activity of the cerebral cortex associated with cognitive perception of pain (Chen et al 1989). Spectral analysis of the EEG has been used both in human (Chen et al 1989; Bromm and Lorenz 1998; Chang et al 2001ab, 2002) and animal (Ong et al 1997; Jongman et al 2000; Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Kongara 2008) studies to examine the acute noxiousness of painful events, e.g. ice water baths, surgery, castration, tail docking, mulesing.

Although spectral changes in the EEG are indirect measures of pain, they reflect cortical electrical activity and hence are more likely to reflect the cognitive perception and processing of noxiousness (Barnett 1997; Johnson et al 2005a). Spectral EEG variables that have been found to be strongly correlated with noxious stimulation are median frequency (F50) and total power (P_{tot}) of the EEG (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Kongara 2008), and 95% spectral edge frequency (F95) is correlated with increasing depth of halothane anaesthesia (Johnson et al 1993, 1994; Johnson and Taylor 1998). Median frequency is the frequency below which 50% of the total power of the EEG is located. 95% spectral edge frequency is the frequency below which 95% of the total power of the EEG is located. Increases in F50 and F95 have been previously associated with nociception (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Kongara 2008). Total power is defined as the total area under the power spectrum curve (Murrell and Johnson 2006). A decrease in P_{tot} has been previously associated with nociception (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Kongara 2008). Combined with the use

of a minimal anaesthesia model, therefore, EEG spectral analysis could potentially be a useful tool in the quantification of noxious sensory inputs in cattle.

The minimal anaesthesia model maintains an animal on a stable plane of anaesthesia, allowing the recording of cerebrocortical responses to a stimulus by reducing variability in background cerebrocortical electrical activity due to extraneous stimuli. To date, this technique has been used to investigate responses to noxious sensory stimuli in the horse (Murrell et al 2003, 2005), sheep (Johnson et al 2005a), red deer (*Cervus elaphus*) (Johnson et al 2005b), pig (Haga and Ranheim 2005), tammar wallaby (*Macropus eugenii*) (Diesch et al 2008), dog (Kongara 2008) and rat (McGregor 2005; Murrell et al 2007). The model has the additional benefit of allowing the investigation of painful procedures without compromising the welfare of the animal. However, the use of this model and spectral analysis for the detection of cerebrocortical changes in response to noxious stimulation are a novel and untested methodology in cattle. Validation is required before assessments of the apparent noxiousness, or otherwise, of other potentially noxious events can be made.

The dehorning of calves is a routine husbandry practice, which is a noxious insult (Stafford and Mellor 2005). The acute noxiousness of dehorning is well established in terms of induced changes in concentrations of plasma cortisol (Morisse et al 1995; Petrie et al 1996; McMeekan et al 1997, 1998ab; Sylvester et al 1998ab; Grøndahl-Nielsen et al 1999; Sutherland et al 2002a; Stafford et al 2003), concentrations of catecholamine (Mellor et al 2002), and behaviour (Morisse et al 1995; Graf and Senn 1999; Grøndahl-Nielsen et al 1999; Stafford et al 2000; Sylvester et al 2004). The application of a cornual nerve block has been demonstrated to virtually eliminate escape behaviours seen during dehorning (Sylvester et al 2004) and to significantly reduce the acute plasma cortisol response (Morisse et al 1995; Petrie et al 1996; McMeekan et al 1998ab; Sylvester et al 1998a; Graf and Senn 1999; Grøndahl-Nielsen et al 1999; Sutherland et al 2002ab). The use of amputation dehorning is therefore a useful technique to validate indices of noxiousness, and has the additional advantage of representing a predominantly somatic noxious insult, without visceral components. The bovine horn is primarily innervated by the cornual nerve, which is a branch of the trigeminal nerve (Budras et al 2003). As the trigeminal nerve is the largest somatic sensory nerve of most mammals (Brodal 1957; Kruger and Young 1981; Butler and Hodos 2005), EEG responses to dehorning will reflect somatic, as opposed to visceral, stimulation. This may reduce complications in

interpretation of the EEG due to differences in cortical processing of somatic and visceral noxious stimuli (Aziz et al 2000; Dunckley et al 2005; Johnson et al 2005a).

The aim of this study was to validate the use of the minimal anaesthesia model to assess noxious somatic stimuli in cattle by evaluating responses to scoop dehorning with or without prior application of a lidocaine ring block. It was hypothesised that dehorning would cause changes in the EEG that are associated with noxious stimulation and that the application of a lidocaine ring block would mitigate this response.

3.3 Materials and Methods

3.3.1 Animals

All calves were sourced from a dairy unit at Massey University, and the study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand. Friesian heifers of 6 to 9 months of age, weighing 125-178 kg were used in the study. Prior to experimentation, calves were kept in accordance with normal dairy farming practices. Calves were randomly allocated to one of two treatment groups: scoop dehorning alone (DH) (n=10) or scoop dehorning with lidocaine ring nerve block (DH+LA) (n=10). The animals were penned overnight, and had access to water but not food the day prior to the study.

3.3.2 Anaesthesia

Anaesthesia was induced using a mixture of 3.4 (standard deviation (SD) 0.3) mg/kg ketamine (Parnell laboratories, Auckland, NZ) and 4.1 (SD 1.0) mg/kg propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid injection into a jugular vein. Following endotracheal intubation with a 16-mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia),

anaesthesia was maintained using inhalation of halothane (Halothane-Vet; Merial NZ Limited, Manukau City, New Zealand) in oxygen (BOC, Palmerston North, NZ), delivered via a precision vaporiser (Fluothane; MedSource Ltd, Ashburton, NZ) and a circle breathing system (VMS Anaesthesia Machine; Matrix Medial Inc, New York, USA). Calves were allowed to breath spontaneously throughout the experiment. End tidal halothane tension ($F_{E'}\text{HAL}$) was maintained at 0.9%. End tidal carbon dioxide tension, heart rate and respiratory rate were monitored using an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Hamburg, Germany). All subsequent procedures were carried out under general anaesthesia.

3.3.3 EEG and ECG Recording

Subdermal 27-G stainless-steel needle electrodes (Medelec; Radiometer, Auckland, NZ) were placed in a three-electrode montage, adapted from the description by Mayhew and Washbourne (1990). The non-inverting electrode was placed in the midline between the medial canthi of the eyes, the inverting electrode over the left mastoid process, and the ground electrode caudal to the poll; see Murrell and Johnson (2006) for further explanation. The impedance was not controlled throughout the study, however intermittent recordings of impedance were made. Electrode impedance was in the range of 2 to 4 k Ω . A base apex electrode configuration was used to record electrocardiogram (ECG).

The EEG and ECG were amplified using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota, Florida, USA). The EEG was recorded with a gain of 1,000 and pass-band of 0.1 to 500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10 to 500 Hz. Both EEG and ECG data were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

3.3.4 Experimental Procedure

Once anaesthetised, calves were placed in right lateral recumbency on a padded airbed, and the head supported with a vacu-support surgical support (Shoof International Ltd, Cambridge, NZ). Lidocaine (Nopaine; Phoenix Pharm Distributors Ltd, Auckland, NZ) ring blocks (20 ml per animal) were administered around the base of the left horn, as described by Graf and Senn 1999, of calves in the DH+LA group after induction of general anaesthesia. Calves in the DH group did not receive lidocaine ring blocks. Ten minutes were allowed for equilibration of general anaesthesia, after which a 15-minute pre-treatment trace was recorded (Figure 3.1). The left horn of each calf in the two groups was removed using the scoop dehorner (Barnes Dehorner, Stones, Missouri, USA). The same trained operator performed all dehorning during the experiment. The time of removal of the horn was recorded. Dehorning equipment was cleaned between horns and animals. Data were recorded for 15 minutes after dehorning for every animal.

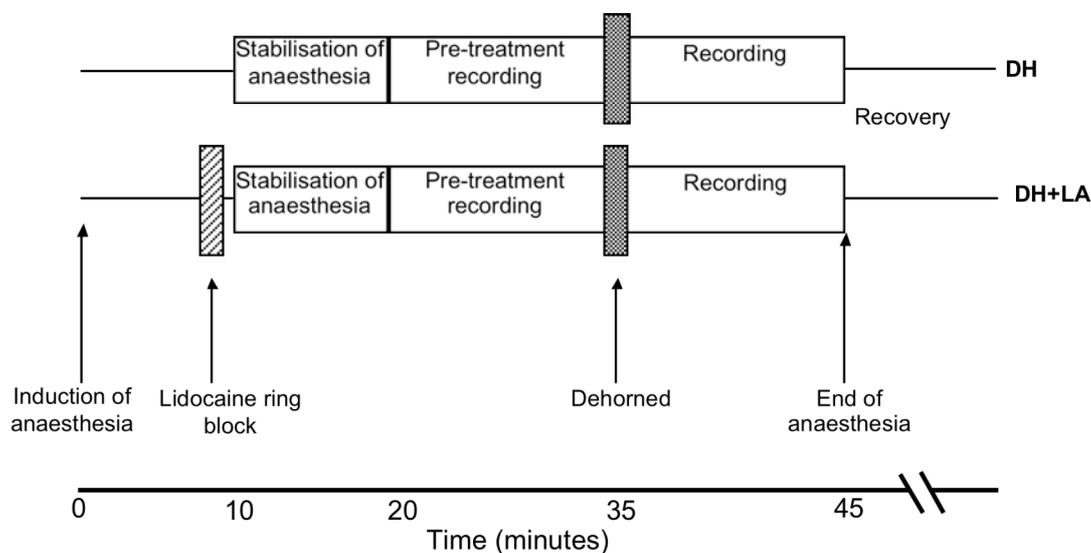


Figure 3.1. Diagram of the experimental design for scoop dehorning alone (DH) or scoop dehorning with lidocaine ring block (DH+LA) groups over time (minutes) from induction of anaesthesia (0 minutes) to recovery (45 minutes).

After the experiment, all remaining horns were removed by scoop dehorning with the prior administration of a 5-ml lidocaine cornual nerve block. Additionally, calves were given the long-acting antibiotic Depocillin (12 mg/kg) (Procaine

Penicillin; Intervet Ltd, Upper Hutt, NZ) and systemic pain relief comprising 3 mg/kg ketoprofen 10% (Merial NZ Ltd, Manukau City, NZ) administered intravenously prior to recovery from anaesthesia. Calves were continuously monitored in darkened pens during this period of recovery. After recovery, the animals were returned to their herd.

3.3.5 EEG and ECG Analysis

EEG epochs contaminated by artefacts, overscale or underscale were manually rejected from analysis of raw EEG data, using Chart 4.2.3 (ADInstruments Ltd). The F50, F95 and Ptot were calculated for consecutive non-overlapping 1-second epochs, using purpose-written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North, NZ, 2002). Data were multiplied using a Welch window. Fast Fourier Transformation was applied to each epoch, generating sequential power spectra with 1Hz frequency bins. Subsequent analysis was performed using Microsoft Excel 2002 (Microsoft Corporation, Redmond, USA). Variables derived from 2 seconds before to 5 seconds after dehorning were excluded from EEG analysis, to prevent contamination caused by movement artefact due to the surgical procedure.

Heart rates were calculated from ECG data, as the percentage change from their pre-treatment values (100%), pooled into their respective treatment groups (DH, DH+LA), and comparisons made between treatments and to pre-treatment values. Pre-treatment values originated from the mean of the initial 200 seconds of data recording. Care was taken to exclude values from within 100 seconds of dehorning in order to prevent potential inclusion of data affected by movement during dehorning.

3.3.6 Statistical Analysis

Data were analysed using Minitab 14.2 (Minitab Incorporated Pennsylvania, USA). An Anderson-Darling test for normality was performed on all data. Between-group comparisons for EEG data were made using area-under-the-curve analysis of 10-second blocks and a one-way analysis of variance (ANOVA) unstacked. Between-

group comparisons for heart rate were made between individual time points using a one-way ANOVA (unstacked). Because of the importance of this technique as a basis for further studies a conservative P value was chosen to indicate significance. Post-hoc comparisons to a pre-treatment value were made using Dunnett's test, for both EEG and heart rate data. Values of $p < 0.01$ were taken to indicate significance in all analyses.

3.4 Results

Mean F50 (Figure 3.2) and mean F95 (Figure 3.3) were significantly increased following dehorning in DH compared to DH+LA calves ($p < 0.01$), and compared to their respective pre-treatment values in DH only ($p < 0.01$). Mean Ptot (Figure 3.4) was significantly decreased following dehorning in the DH compared to the DH+LA group ($p < 0.01$), and when compared to its pre-treatment values in the DH group ($p < 0.01$). All EEG responses in the DH group following dehorning were transient and returned to pre-treatment values within 2 minutes after dehorning. There were no significant differences from pre-treatment values for any EEG variable in DH+LA calves. One calf was excluded from EEG analysis (DH+LA) due to an unacceptable level of contamination of the EEG.

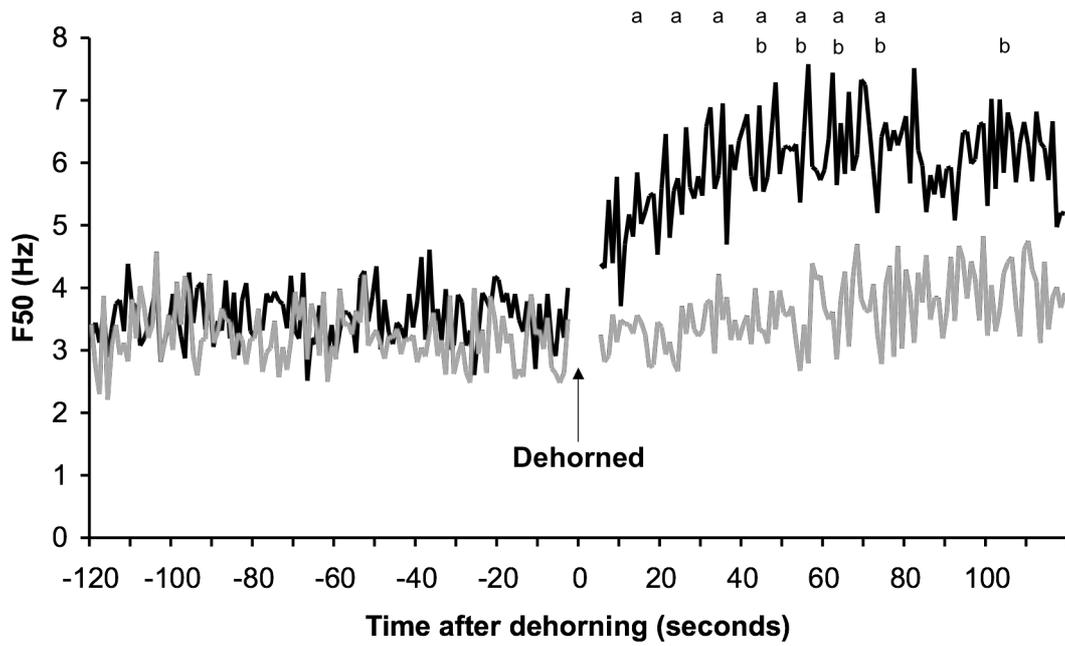


Figure 3.2. Median frequency (F50) (mean) of the electroencephalogram (EEG) before and after dehorning in calves dehorned with (—) (DH+LA) or without (---) a lidocaine ring block (DH) (a = values significantly different between treatment groups, $p < 0.01$; b = values significantly different from pre-treatment DH, $p < 0.01$), 0 seconds = time point of dehorning.

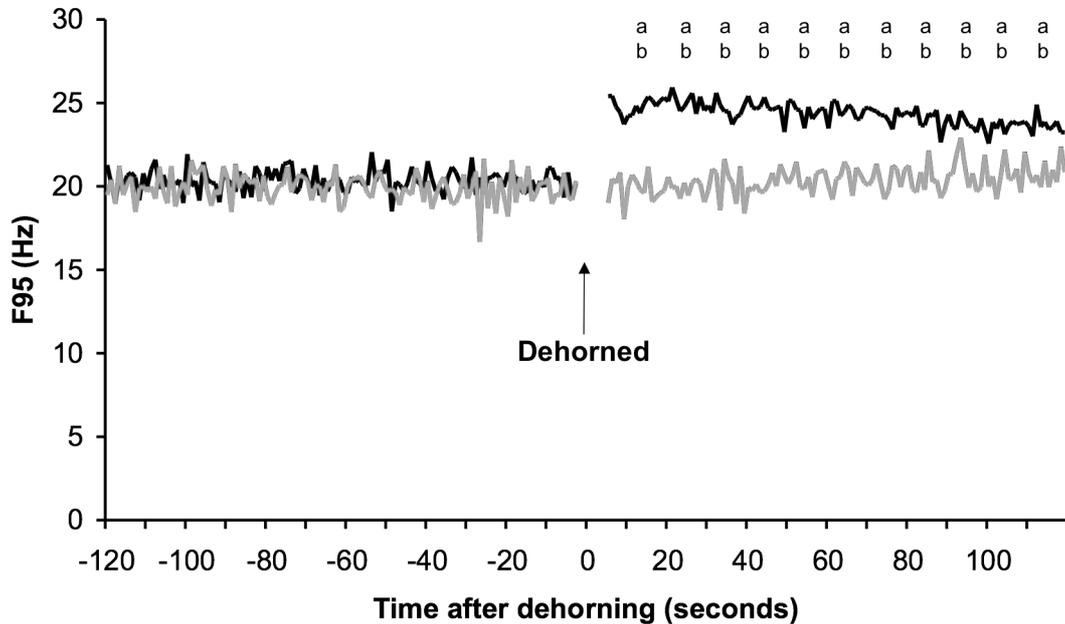


Figure 3.3. Mean 95% spectral edge frequency (F95) of the electroencephalogram (EEG) before and after dehorning in calves dehorned with (—) (DH+LA) or without (---) a lidocaine ring block (DH) (a = values significantly different between treatment groups, $p < 0.01$; b = values significantly different from pre-treatment DH, $p < 0.01$), 0 seconds = time point of dehorning.

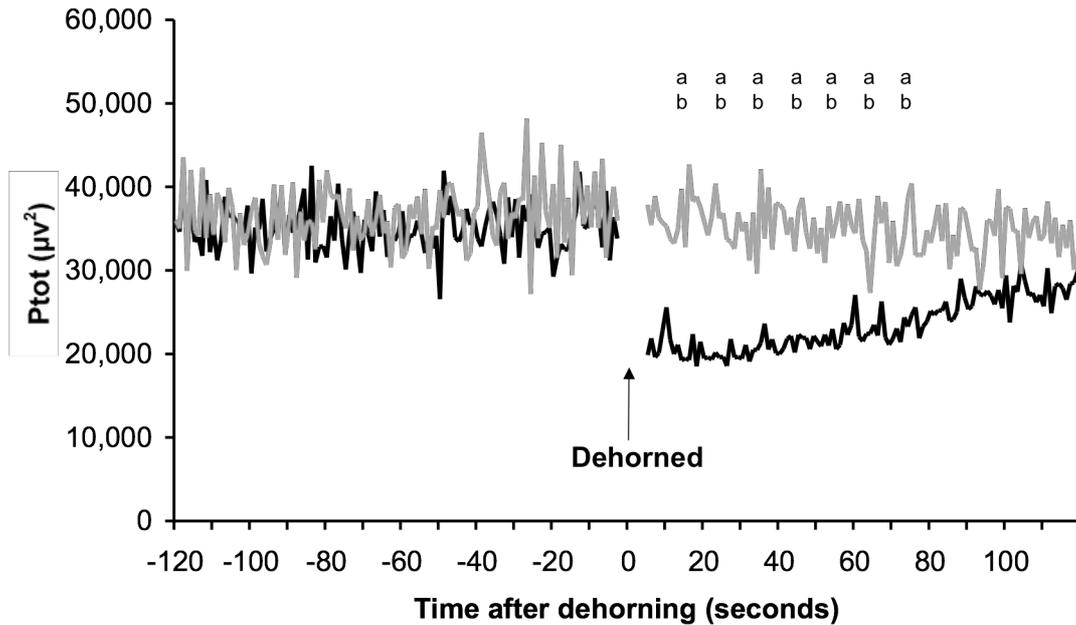


Figure 3.4. Mean total power (P_{tot}) of the electroencephalogram (EEG) before and after dehorning in calves dehorned with (—) (DH+LA) or without (---) a lidocaine ring block (DH) (a = values significantly different between treatment groups, $p < 0.01$; b = values significantly different from pre-treatment DH, $p < 0.01$), 0 seconds = time point of dehorning.

ECG data from three calves (two DH+LA and one DH) were excluded from heart rate analysis due to an unacceptable level of contamination. Following dehorning, heart rate immediately decreased in DH calves compared to pre-treatment values ($p < 0.01$) (Table 3.1). Heart rate was significantly lower in the DH compared to the DH+LA group ($p < 0.001$) during the first 75 seconds after dehorning (Table 3.1). This decrease was transient and returned to pre-treatment values by 90 seconds after dehorning. After the initial changes, heart rate in both groups increased with increasing variability (Table 3.1). This tachycardia became significant in DH, but not DH+LA calves, compared to pre-treatment values ($p < 0.01$) by 15 minutes after treatment.

Table 3.1. Mean \pm standard error of the mean of heart rate (beats per minute) as a percentage of individual pre-treatments at time points after the start of dehorning for scoop dehorning alone (DH) or scoop dehorning with lidocaine ring block (DH+LA) groups.

Time (seconds)	DH+LA (% of pre-treatment)	DH (% of pre-treatment)	P-value (difference between treatments)
-15 (pre-treatment) ^a	100.14 \pm 0.59 ^c	98.81 \pm 0.77	0.187
0 (dehorned) ^b	101.68 \pm 2.41	73.98 \pm 3.67 ^d	<0.001
15	101.94 \pm 2.96	72.99 \pm 3.58 ^d	<0.001
30	102.10 \pm 3.19	73.66 \pm 3.49 ^d	<0.001
45	101.29 \pm 3.23	76.60 \pm 2.50 ^d	<0.001
60	100.48 \pm 3.15	77.15 \pm 2.11 ^d	<0.001
75	99.93 \pm 3.11	77.71 \pm 2.20 ^d	<0.001
90	109.76 \pm 5.54	104.07 \pm 4.86	0.458
105	109.76 \pm 5.75	105.19 \pm 5.31	0.619
120	108.53 \pm 5.92	104.80 \pm 5.15	0.642
135	108.43 \pm 6.12	103.92 \pm 5.15	0.587
150	108.32 \pm 6.28	103.23 \pm 5.12	0.546
165	108.02 \pm 6.47	102.84 \pm 5.17	0.548
180	107.99 \pm 6.62	102.46 \pm 5.14	0.527
300 (5 minutes)	108.18 \pm 7.02	107.33 \pm 5.80	0.928
600 (10 minutes)	115.77 \pm 7.95	119.78 \pm 7.84	0.726
900 (15 minutes)	117.12 \pm 9.71	122.91 \pm 7.64 ^d	0.652

^a Pre-treatment

^b Dehorned

^c There were no significant differences ($P < 0.01$) between pre-treatment and any of the post-treatment values in the DH+LA group

^d Value differed significantly from pre-treatment level ($p < 0.01$)

3.5 Discussion

The results of this study validate the use of spectral analysis of the EEG in minimally anaesthetised cattle for the assessment of noxious stimuli. The study further validates the existing body of evidence of the acute noxiousness of dehorning and its attenuation with the application of a lidocaine ring block (Stafford and Mellor 2005).

These findings are consistent with previous work investigating the acute noxiousness of routine husbandry practices, utilising a similar minimal anaesthesia protocol (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab). Increases in F50 and F95, and a decrease in Ptot, following velvet antler amputation, and attenuation of these changes by prior local anaesthetic nerve block have been observed in deer (Johnson et al 2005b). Surgical castration of horses (Murrell et al 2003) and piglets (Haga and Ranheim 2005) elicited similar changes in F50 and Ptot. The application of intravenous lidocaine prior to surgical castration in horses (Murrell et al 2005) blunted F50 and Ptot changes previously observed without analgesia (Murrell et al 2003). Injection of lidocaine into the spermatic cord or the testes of piglets similarly blunted the EEG response to castration (Haga and Ranheim 2005). An increase in F50 in response to rubber-ring castration was observed in lambs of differing ages (Johnson et al 2005a). These studies and the observed increase in F50 following dehorning in the DH group support the hypothesis that the F50 responds preferentially to noxious stimuli (Murrell et al 2003; Johnson et al 2005a). This is further supported by work in conscious sheep investigating behaviour and EEG bandwidth spectral changes in response to electrical stimulation (Ong et al 1997). These workers demonstrated that behavioural responses associated with noxious stimulation were associated with increased activity in the middle frequency ranges of the EEG (delta 2, theta 2, alpha 1 and alpha 2), which correlate with F50. The current experiment was carried out under general anaesthesia, which abolished somatic motor responses to the noxious stimuli thereby minimising the possibility of related artefacts.

EEG spectral analysis has been demonstrated to respond predictably to changes in the partial pressure of inhalation anaesthetic agents (Johnson and Taylor 1998). Spectral edge frequency has been shown to correlate with changes in FE'HAL

in the horse (Johnson et al 1994; Johnson and Taylor 1998). It is unlikely that alterations in FE'HAL produced the reported spectral power changes in the current study. End-tidal halothane partial pressure was maintained at 0.9-0.95% and 10 minutes were allowed for equilibrium of anaesthesia, followed by 15 minutes of recordings pre-treatment. Over this period, F95 remained stable after which changes in spectral power occurred rapidly following dehorning. Changes seen in the EEG in response to noxious stimulation are blunted by the actions of general anaesthesia (Antognini and Carstens 1999; Orth et al 2005). However, in the current experiment, significant changes were still recorded in the DH group following dehorning.

Changes in the frequency spectrum of the EEG following dehorning without a lidocaine ring block represent the acute phase of the response to noxious stimulation or first pain. All EEG indices examined in the current experiment returned to pre-treatment levels within 2 minutes of dehorning. This is in contrast to the elevated cortisol levels and behavioural responses to amputation dehorning, which have been shown to last for 7-9 hours (Petrie et al 1996; McMeekan et al 1997, 1998ab; Sylvester et al 1998a) or 6 hours (Sylvester et al 2004). Persistent pain following this acute phase of dehorning is highly probable and is supported by the time taken for wound healing and by reductions in weight gain after dehorning (Stafford and Mellor 2005). The short duration of the EEG response to noxious stimulation could be due to the actions of the general anaesthesia, pain modulation or anti-nociceptive mechanisms within the central nervous system (CNS) following noxious stimulation (Fields and Basbaum 1999). Evidence of the cortex influencing pain by interrupting the transmission of noxious signals from the spinal cord is well established with the descending pain modulation system (Ohara et al 2005). However, these factors would also be expected to influence the plasma cortisol and behavioural responses to noxious stimulation, and this was not evident in the previous investigations. Background electrical activity of the cortex could potentially dominate and mask the chronic response of the EEG to a noxious stimulus over time, making subsequent detection and analysis of such a response unfeasible following the acute noxious response.

Propofol and ketamine were chosen for induction of general anaesthesia due to their short duration of action and rapid excretion. Ketamine has analgesic properties (Branson 2001), which may have blunted the somatic responses to noxious stimulation (Johnson et al 2005b). As the doses of ketamine given were not

significantly different between the treatment groups any such influence would have been common to both treatment groups. In addition there were no changes in EEG variables over the course of the per-treatment, indicating that the effects of the induction agents had waned by the time of the onset of data collection.

Transient bradycardia was observed in the DH group immediately following dehorning. Acute bradycardia in response to a noxious stimulus in anaesthetised animals has been observed previously with velvet antler removal in deer (Johnson et al 2005b), and castration of lambs (Johnson et al 2005a) and piglets (Haga and Ranheim 2005). These results are in contrast to previous studies where only tachycardia was reported following a noxious stimuli (Lay et al 1992ab; Grøndahl-Nielsen et al 1999; Peers et al 2002). Heart rate in the current study was recorded continuously during the 15 minutes before and after dehorning. This allowed detection of short-lived bradycardia (Table 3.1), whereas intermittent recording, as used in previous investigations may have overlooked this transient effect. Similar transient bradycardia was reported in conscious human infants undergoing routine immunisation (Johnston and Strada 1986). Its presence in anaesthetised calves and conscious human infants suggests that this response is not due to anaesthesia but is rather an acute response to nervous stimulation.

Cornual nerve block is routinely used during dehorning in mature cattle (Stafford and Mellor 2005). Although the cornual nerve supplies the primary innervation, other nerves are also involved. The infratrochlear and frontal nerves, branches of the trigeminal nerve (King and Riley 1980) can both carry sensory fibres from the bovine horn. Possible innervation from cutaneous branches of spinal nerves C1 and C2 can also innervate the caudal aspect of the horn of mature cattle (King and Riley 1980; Hall and Clarke 2001). Complete blockade of the bovine horn is not always achieved following the application of lidocaine to the cornual nerve (Sylvester et al 1998a). The use of a lidocaine ring block compared to cornual nerve block has the advantage of providing complete blockade of sensory input from the bovine horn.

In conclusion, the current experiment validates the use of this novel approach for investigating painful husbandry procedures in cattle and demonstrates the usefulness of this technique in animal welfare research. These findings shed new light on the noxiousness of scoop dehorning, and confirm and add to the existing body of evidence relating to the noxiousness of scoop dehorning and its alleviation with a lidocaine ring block prior to the procedure.

3.6 References

- Adrian ED, Matthews BHC.** The interpretation of potential waves in the cortex. *Journal of Physiology* 81, 440-71, 1934
- Antognini JF, Carstens E.** Isoflurane blunts electroencephalographic and thalamic-reticular formation responses to noxious stimulation in goats. *Anesthesiology* 91, 1770-9, 1999
- Aziz Q, Thompson DG, Ng VWK, Hamdy S, Sarkar S, Brammer MJ, Bullmore ET, Hobson A, Tracey I, Gregory L, Simmons A, Williams SCR.** Cortical processing of human somatic and visceral sensation. *Journal of Neuroscience* 20, 2657-63, 2000
- Barnett JL.** Measuring pain in animals. *Australian Veterinary Journal* 75, 878-9, 1997
- Branson KR.** Injectable anesthetics. In: Adams HR (ed) *Veterinary Pharmacology and Therapeutics*. Pp 216-67. Iowa State University Press/Ames, Iowa, 2001
- Brodal A.** The trigeminal nerve. *The Cranial Nerves*. Pp 79-96. Blackwell Scientific Publications, Oxford, UK, 1962
- Bromm B, Lorenz J.** Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology* 107, 227-53, 1998
- Budras K-D, Habel RE, Wunsche A, Buda S.** Central nervous system and cranial nerves. *Bovine Anatomy. An Illustrated Text*. Pp 50-57. Schlutersche, Hannover, Germany, 2003
- Butler AD, Hodos W.** Functional organization of the cranial nerves. *Comparative Vertebrate Neuroanatomy*, 2nd Edtn. Pp 173-182, John Wiley & Sons Inc, Hoboken, USA, 2005
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN.** Topographic effects of tonic cutaneous nociceptive stimulation on human electroencephalograph. *Neuroscience Letters* 305, 49-52, 2001a
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN.** Different EEG topographic effects of painful and non-painful intramuscular stimulation in man. *Experimental Brain Research* 141, 195-203, 2001b
- Chang PF, Arendt-Nielsen L, Chen ACN.** Differential cerebral responses to aversive auditory arousal versus muscle pain: specific EEG patterns are associated with human pain processing. *Experimental Brain Research* 147, 387-93, 2002

- Chen ACN, Dworkin SF, Haug J, Gehrig J.** Topographic brain measures of human pain and pain responsiveness. *Pain* 37, 129-41, 1989
- Cooley JW, Tukey JW.** An algorithm for the machine calculation of complex fourier series. *Mathematics of Computation* 19, 297-301, 1965
- Diesch TJ, Mellor DJ, Johnson CB, Lentle RG.** Responsiveness to painful stimuli in anaesthetised newborn and young animals of varying neurological maturity (lambs, rat pups and wallaby joeys). In: '6th World Congress on Alternatives & Animal Use in the Life Sciences, 2007'. Tokyo, Japan Pp 549-52, 2008
- Dunckley P, Wise RG, Aziz Q, Painter D, Brooks J, Tracey I, Chang L.** Cortical processing of visceral and somatic stimulation: differentiating pain intensity from unpleasantness. *Neuroscience* 133, 533-42, 2005
- Fields HL, Basbaum AI.** Central nervous system mechanisms of pain modulation. In: Wall PD, Melzack R (eds). *Textbook of Pain*. Pp 309-29. Churchill Livingstone, Edinburgh, UK, 1999
- Graf B, Senn M.** Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. *Applied Animal Behaviour Science* 62, 153-71, 1999
- Grøndahl-Nielsen C, Simonsen HB, Lund JD, Hesselholt M.** Behavioural, endocrine and cardiac responses in young calves undergoing dehorning without and with use of sedation and analgesia. *Veterinary Journal* 158, 14-20, 1999
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1-9, 2005
- Hall LW, Clarke KW.** Anaesthesia of cattle. *Veterinary Anaesthesia*, 10 Edtn. Pp 315-39. WB Saunders, London, UK, 2001
- Johnson CB, Taylor PM.** Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* 81, 748-53, 1998
- Johnson CB, Young SS, Taylor PM.** Use of frequency spectral analysis of the equine electroencephalogram as an indication of the depth of halothane anaesthesia. In: Sebel PS, Bonke B, Winograd E (eds). *Memory and Awareness in Anesthesia*. Pp 289-95. PTR Prentice Hall, Englewood Cliffs, UK, 1993
- Johnson CB, Young SS, Taylor PM.** Analysis of the frequency spectrum of the equine electroencephalogram during halothane anaesthesia. *Research in Veterinary Science* 56, 373-8, 1994

- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433-7, 2005a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61-71, 2005b
- Johnston CC, Strada ME.** Acute pain response in infants: a multidimensional description. *Pain* 24, 373-82, 1986
- Jones AKP, Friston K, Frackowiak RSJ.** Localization of responses to pain in human cerebral cortex. *Science* 255, 215-6, 1992
- Jongman EC, Morris JP, Barnett JL, Hemsworth PH.** EEG changes in 4-week-old lambs in response to castration, tail docking and mulesing. *Australian Veterinary Journal* 78, 339-43, 2000
- King AS, Riley VA.** *A guide to the physiological and clinical anatomy of the head.* Department of Veterinary Anatomy, University of Liverpool, Liverpool, UK, 1980
- Kongara K.** Studies on renal safety and preventive analgesic efficacy of Tromadex and parecoxib in dogs. *PhD thesis*, Massey University, Palmerston North, NZ, 2008
- Kruger L, Young RF.** Specialized features of the trigeminal nerve and its central connections. In: Samii M, Jannetta PJ (eds). *The Cranial Nerves*. Pp 273-301. Springer-Verlag, Berlin, Germany, 1981
- Lay DC, Friend TH, Bowers CL, Grissom KK, Jenkins OC.** A comparative physiological and behavioral-study of freeze and hot iron branding using dairy-cows. *Journal of Animal Science* 70, 1121-5, 1992a
- Lay DC, Friend TH, Grissom KK, Bowers CL, Mal ME.** Effects of freeze or hot-iron branding of angus calves on some physiological and behavioural indicators of stress. *Applied Animal Behaviour Science* 33, 137-47, 1992b
- Mayhew IG, Washbourne JR.** A method of assessing auditory and brainstem function in horses. *British Veterinary Journal* 146, 509-18, 1990
- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- McMeekan CM, Mellor DJ, Stafford KJ, Bruce RA, Ward RN, Gregory NG.** Effects of shallow scoop and deep scoop dehorning on plasma cortisol concentrations in calves. *New Zealand Veterinary Journal* 45, 72-4, 1997

- McMeekan CM, Mellor DJ, Stafford KJ, Bruce RA, Ward RN, Gregory NG.** Effects of local anaesthesia of 4 to 8 hours' duration on the acute cortisol response to scoop dehorning in calves. *Australian Veterinary Journal* 76, 281-5, 1998a
- McMeekan CM, Stafford KJ, Mellor DJ, Bruce RA, Ward RN, Gregory NG.** Effects of regional analgesia and/or a non-steroidal anti-inflammatory analgesic on the acute cortisol response to dehorning in calves. *Research in Veterinary Science* 64, 147-50, 1998b
- Mellor DJ, Stafford KJ, Todd SE, Lowe TE, Gregory NG, Bruce RA, Ward RN.** A comparison of catecholamine and cortisol responses of young lambs and calves to painful husbandry procedures. *Australian Veterinary Journal* 80, 228-33, 2002
- Morisse JP, Cotte JP, Huonnic D.** Effect of dehorning on behaviour and plasma-cortisol responses in young calves. *Applied Animal Behaviour Science* 43, 239-47, 1995
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-21, 2005
- Murrell JC, Johnson C.B.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Murrell JC, Mitchinson SL, Waters D, Johnson CB.** Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* 98, 366-71, 2007
- Ohara PT, Vit J -P, Jasmin L.** Cortical modulation of pain. *Cellular and Molecular Life Sciences* 62, 44-52, 2005
- Ong RM, Morris JP, O'Dwyer JK, Barnett JL, Hemsworth PH, Clarke IJ.** Behavioural and EEG changes in sheep in response to painful acute electrical stimuli. *Australian Veterinary Journal* 75, 189-93, 1997
- Orth M, Barter L, Dominguez C, Atherley R, Carstens E, Antognini JF.** Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation. *British Journal of Anaesthesia* 95, 477-84, 2005

- Peers A, Mellor DJ, Wintour EM, Dodic M.** Blood pressure, heart rate, hormonal and other acute responses to rubber-ring castration and tail docking of lambs. *New Zealand Veterinary Journal* 50, 56-62, 2002
- Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA, Ward RN.** Cortisol responses of calves to two methods of disbudding used with or without local anaesthetic. *New Zealand Veterinary Journal* 44, 9-14, 1996
- Stafford KJ, Mellor DJ, Ward RN, Cann B.** Behavioural response of calves to amputation dehorning with or without local anaesthesia. *Proceedings of the New Zealand Society of Animal Production* 60, 234-6, 2000
- Stafford KJ, Mellor DJ, Todd SE, Ward RN, McMeekan CM.** The effect of different combinations of lignocaine, ketoprofen, xylazine and tolazoline on the acute cortisol response to dehorning in calves. *New Zealand Veterinary Journal* 51, 219-26, 2003
- Stafford KJ, Mellor DJ.** Dehorning and disbudding distress and its alleviation in calves. *Veterinary Journal* 169, 337-49, 2005
- Sutherland MA, Mellor DJ, Stafford KJ, Gregory NG, Bruce RA, Ward RN.** Cortisol responses to dehorning of calves given a 5-h local anaesthetic regimen plus phenylbutazone, ketoprofen, or adrenocorticotropic hormone prior to dehorning. *Research in Veterinary Science* 73, 115-23, 2002a
- Sutherland MA, Mellor DJ, Stafford KJ, Gregory NG, Bruce RA, Ward RN.** Effect of local anaesthetic combined with wound cauterisation on the cortisol response to dehorning in calves. *Australian Veterinary Journal* 80, 165-7, 2002b
- Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA, Ward RN.** Acute cortisol responses of calves to scoop dehorning using local anaesthesia and/or cautery of the wound. *Australian Veterinary Journal* 76, 118-22, 1998a
- Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA, Ward RN.** Acute cortisol responses of calves to four methods of dehorning by amputation. *Australian Veterinary Journal* 76, 123-6, 1998b
- Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA, Ward RN.** Behavioural responses of calves to amputation dehorning with and without local anaesthesia. *Australian Veterinary Journal* 82, 697-700, 2004
- Talbot JD, Marrett S, Evans AC, Meyer E, Bushnell MC, Duncan GH.** Multiple representations of pain in human cerebral cortex. *Science* 251, 1355-8, 1991
- Treede RD, Kenshalo DR, Gracely RH, Jones AKP.** The cortical representation of pain. *Pain* 79, 105-11, 1999

CHAPTER 4
ELECTROENCEPHALOGRAPHIC RESPONSES OF CALVES TO SLAUGHTER BY VENTRAL NECK INCISION WITHOUT PRIOR STUNNING



A paper based on this chapter has been published in the New Zealand Veterinary Journal.

Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, Johnstone AC, Mellor DJ. Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning. *New Zealand Veterinary Journal* 57, 77-83, 2009

4.1 Abstract

AIM: The aim of the study was to investigate the extent to which slaughter by ventral neck incision (VNI) without prior stunning may be perceived as a noxious stimulus. The study utilised EEG variables that have previously been associated with noxious stimulation in calves anaesthetised with halothane in oxygen.

METHODS: Fourteen Angus steers weighing 109-162 kg were maintained under a stable light plane of halothane anaesthesia using the minimal anaesthesia model. The EEG was recorded bilaterally for five minutes prior to and five minutes following VNI. A single incision was made in the ventral aspect of the neck severing all tissues ventral to the vertebral column including the major blood vessels supplying and draining the head.

RESULTS: During the 30 seconds following VNI, 95% spectral edge frequency (F95) and total EEG power (P_{tot}) showed significant changes compared to pre-treatment values ($P < 0.05$). Median frequency (F50) increased significantly in recordings from the right side of the cranium ($P < 0.05$).

CONCLUSIONS: This study is the first investigation of the noxiousness of slaughter by VNI using EEG spectral analysis. It was concluded from the results that there is a period following slaughter where the VNI represents a noxious stimulus.

4.2 Introduction

Calves slaughtered for human consumption in New Zealand and the United Kingdom must be rendered completely insensible prior to slaughter with the application of a mechanical or electrical stun and maintained in that state until death (FAWC 2003; NAWAC 2006). In certain situations livestock are slaughtered without prior stunning, common examples being emergency and religious slaughter.

During ventral neck incision (VNI) slaughter a single incision with an exquisitely sharp blade is made in the ventral aspect of the neck severing the major blood vessels supplying and draining the brain. The incision also transects skin, muscle, trachea, oesophagus, sensory nerves and connective tissues (Mellor and Littin 2004). There are a number of potential welfare concerns regarding neck-cut slaughter without prior stunning, including possible pain due to the incision itself and pain and distress during the time before the onset of undoubted insensibility.

The time to undoubted insensibility with or without stunning has been a topic of much detailed research in a variety of species. This has involved investigations of cortical electrical activity in terms of changes of EEG/ECoG in amplitude and waveform type (Levinger 1961; Nangeroni and Kennett 1964; Blackmore and Newhook 1981; Newhook and Blackmore 1982ab; Blackmore et al 1983; Gregory and Wotton 1984b; Bager et al 1992; Anil et al 1995ab; Cook et al 1996), changes in the power spectrum of the EEG (Bager et al 1992), changes in brain function in terms of visual (Gregory and Wotton 1984a; Daly et al 1988; Anil et al 1995a) and somatosensory (Daly et al 1988; Anil et al 1995b) evoked potentials, loss of evoked eye response (Levinger 1961; Barnett et al 2007) and changes in behaviour (Levinger 1961; Blackmore 1984; Grandin 1994; Barnett et al 2007). There is insufficient understanding of conscious processes to be able to interpret the significance of these events in terms of loss of consciousness, but the window before the onset of undoubted insensibility can be reliably assessed (Mellor and Littin 2004). Most authorities consider this window to be between approximately 5 and 60 seconds in duration in cattle (Mellor and Littin 2004), although it has been postulated that the rapid decompression of the cerebral vault results in implosion of the brain leading to a more rapid loss of sensibility (Rosen 2004). During this window between neck incision and insensibility, the animal could experience pain and/or distress.

Until recently no tools were available for assessing pain in animals during this window (Mellor et al 2000; Rutherford 2002). Recent advances in the quantitative interpretation of the EEG have identified changes in cerebrocortical function in response to noxious stimulation (Murrell and Johnson 2006). Changes in the frequency spectrum of the human EEG have been shown to reflect alterations in activity associated with the cognitive perception of pain (Chen et al 1989). Chapter 3 reported EEG responses to scoop dehorning in minimally anaesthetised calves. Median frequency (F50), 95% spectral edge frequency (F95) and total power (Ptot) of the EEG were found to correspond with the noxious stimulus of dehorning. All EEG responses were abolished by the prior application of local anaesthetic blockade (Chapter 3). Similar changes have now been identified during anaesthesia in 7 species of mammals (Murrell et al 2003; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Diesch et al 2008; Kongara 2008).

Changes in F95 have also been associated with increasing depth of halothane anaesthesia (Johnson et al 1993, 1994; Johnson and Taylor 1998). The minimal anaesthesia model involves maintaining the animal on a stable light plane of halothane anaesthesia where the animal is unconscious, but still able to demonstrate EEG responses to noxious stimulation (Murrell and Johnson 2006). This model allows the investigation of cerebrocortical responses to noxious stimuli without compromising the welfare of the animal.

The aim of this study was to examine EEG responses to slaughter by VNI without prior stunning in order to ascertain the noxiousness or otherwise of this manipulation. After slaughter, a cohort of brains was examined histologically to identify any structural changes following VNI.

4.3 Materials and Methods

4.3.1 Animals

Calves were sourced from a commercial stock agent and kept in accordance with normal farming practices. Prior to the study animals were penned overnight with access to water but not food. Twelve Angus steers weighing 109-162 kg were allocated to receive a VNI. Another group of 10 Friesian heifers and bulls weighing 134-207 kg were allocated to receive a sham incision (SI) designed to mimic the action of the VNI without tissue damage. The study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand (Protocol no. 04/86).

4.3.2 Anaesthesia

Anaesthesia was induced using a mixture of ketamine 3.7 (SD 0.5) mg/kg (Parnell laboratories, Auckland, NZ) and propofol 6.9 (SD 3.4) mg/kg (DBL, Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid injection into a jugular vein. Following endotracheal intubation with a 16 mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia), anaesthesia was maintained using inhalation of halothane (Halothane-Vet, Merial NZ Limited) in oxygen (BOC, Palmerston North, NZ) delivered via a precision vaporizer (Fluothane, MedSource Ltd, Ashburton, NZ) and a circle breathing system (VMS Anaesthesia Machine, Matrix Medial Inc, New York, USA). End tidal halothane tension (F_E' HAL) was maintained at 0.9%. End tidal carbon dioxide tension, heart rate and respiratory rate were monitored using an anaesthetic agent monitor (Hewlett Packard M1025B, Hamburg, Germany). All subsequent procedures were carried out under general anaesthesia.

4.3.3 EEG and ECG Recording

Five subdermal 27-gauge stainless steel needle electrodes (Medelec, Radiometer Auckland New Zealand) were placed in a bilateral fronto-zygomatic electrode montages, as adapted from Mayhew and Washbourne (1990). The non-inverting (active) electrodes (n=2) were placed in the midline between the medial canthi of the eyes, the inverting (reference) electrodes (n=2) over the left and right mastoid processes and a common ground electrode (n=1) caudal to the poll. A base apex electrode configuration was used to record ECG.

The EEG and ECG were amplified with isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers, World Precision Instruments Sarasota Florida USA). The EEG was recorded with a gain of 1,000 and pass-band of 0.1 to 500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10 to 500 Hz. Both EEG and ECG data were digitised at a rate of 1 kHz (Powerlab/4sp, ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

4.3.4 Experimental Procedure

Once anaesthetised, calves were placed in dorsal recumbency on a specially designed bed (Massey University Mechanical Services, Palmerston North, New Zealand) with the head securely held in position on a purpose built head frame (Massey University Mechanical Services, Palmerston North, New Zealand). This frame reduced movement of the head during VNI and SI and provided tension to the neck to ensure that the cut edges of the incision did not come into contact with each other after the cut was made. The femoral artery of the right leg was cannulated (18-gauge BD Insyte Intravenous Catheter; Becton Dickinson Infusion Therapy Systems Inc, Utah, USA), for direct monitoring of arterial blood pressure. The arterial blood pressure transducer (Spectramed Medical Products, Singapore) was re-calibrated against a mercury column (Baumanometer, W.A. Baum Co. Inc., New York, USA) for each animal. Fifteen minutes were allowed for equilibration of general anaesthesia (figure 4.1).

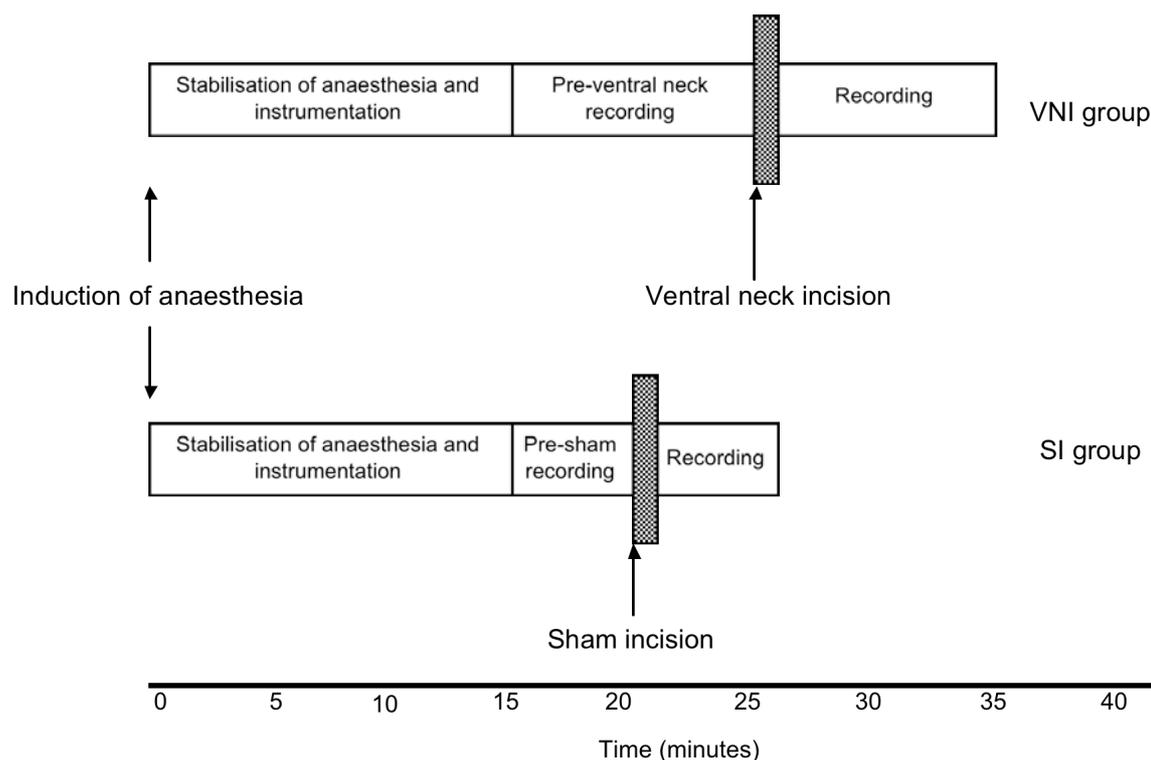


Figure 4.1. Diagram of the experimental design in minutes for ventral neck incision (group VNI) and sham incision (group SI). Anaesthesia occurred at 0 minutes and ventral neck incision (VNI) at 25 minutes.

VNI calves

In the VNI animals a 5-minute pre-treatment EEG recording was made immediately followed by slaughter by a single incision to the ventral aspect of the neck below the level of the larynx with an extremely sharp flat edged knife, with a blade 245 mm long by 28 mm wide (Granton Ragg Ltd, Sheffield, England). The knife was used exclusively for the neck incision and was re-sharpened after each use with a Tru Hone sharpener (model no. LCF; Tru Hone Corporation, Ocala, Florida, USA). Data were recorded for 5 minutes following slaughter, after which the wound was inspected for complete severance of the major blood vessels and for any significant signs of carotid arterial occlusion.

SI calves

In the SI animals a 5-minute pre-treatment recording was made (figure 4.1), after which a sham incision was undertaken using a broom handle drawn across the neck with a similar action and position to that of VNI, but causing no tissue damage.

Data were recorded for 5 minutes following SI. After completion of this experiment the SI calves went on to be used in a different experiment.

4.3.5 EEG and ECG Analysis

Electroencephalographic epochs contaminated by artefacts such as over- and under-scale or large single spikes, were manually rejected from analysis using Chart 5.4.2 (ADInstruments Ltd, Sydney, Australia). The F50, F95 and Ptot were calculated for consecutive non-overlapping 1-second epochs using purpose-written software (Spectral Analyser: Craig Johnson, Massey University, Palmerston North, New Zealand, 2002). Fast Fourier Transformation was applied to each epoch generating sequential power spectra with 1 Hz frequency bins. Subsequent analysis and generation of compressed spectral arrays were performed with Microsoft Excel Mac 2004 (Microsoft Corporation, Redmond, USA). Variables derived from 2 seconds before to 5 seconds after VNI were excluded from EEG analysis to prevent contamination by movement artefact due to the act of VNI. Data from EEG spectral analysis were displayed as specific EEG indices (F50, F95 and Ptot) or compressed spectral arrays, which incorporate alterations in power and frequency over time, and were derived from the EEG power spectra. All EEG data are represented as mean \pm SD.

In addition, EEG traces were inspected visually and classified into one of five categories: out of range; active EEG; transitional EEG; high amplitude low frequency EEG (Bager et al 1992) (HALF); isoelectric EEG. Active EEG represents normal cerebrocortical activity in anaesthetised calves. Transitional EEG was classified as having an amplitude of less than half of that of the pre-treatment EEG. High amplitude low frequency EEG (HALF) was classified as a waveform with rhythmic activity of high amplitude and low frequency. Isoelectric EEG was classified as a trace with an amplitude of less than 1/8 of that of normal pre-stunning EEG with little or no low frequency components. Heart rate was calculated from ECG data, using the rate meter function in Chart (ADInstruments Ltd), and is represented as mean \pm SEM.

4.3.6 Histopathology

Following the completion of the experiment, heads were removed from VNI animals and perfused for 5 minutes with heparinised sodium lactate and for 30 minutes with buffered formalin (10%), via the carotid arteries (Multiparin, Heparin Sodium, CP Pharmaceuticals Ltd, Wrexham, UK; sodium lactate, Hartmann's solution, Baxter Healthcare Ltd, Toongabbie, Australia). Brains were extracted from the skulls and immediately placed in buffered formalin (10%) for future histological analysis. Samples were taken for slide preparation from the obex, spinal cord, pons, cerebellum, midbrain, thalamus, and short gyri of the insula and primary somatosensory cortices (caudal to the central sulcus). Blocks of tissue from the desired areas were placed in ethanol baths and embedded with paraffin. Sections were cut at 5 μ m and stained with hematoxylin and eosin (H&E) using routine histological techniques. All slides were examined under light microscopy.

4.3.7 Statistical Analysis

EEG data were calculated and displayed as percentage changes from pre-treatment values. Data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA) and Prism 4.0c (GraphPad Software Incorporated, San Diego, CA, USA). The distribution of the data was tested for normality with the Anderson-Darling test for EEG indices. Analysis of differences between pre-treatment values and EEG indices were performed on consecutive non-overlapping blocks of 30 seconds with a Mann-Whitney non-parametric test (F95 and Ptot) or a one-way analysis of variance (ANOVA) unstacked (F50). Correlations between actual blood pressure and EEG indices and between left and right-sided EEG were made using a two-tailed Spearman's rank coefficient test for non-parametric data. Blood pressure is displayed as mean values \pm SEM.

4.4 Results

Two calves were excluded from analysis due to inadequate VNI, as a result of incomplete severance of the carotid arteries. In addition, four EEG traces were excluded from analysis due to unacceptable levels of contamination with electrical noise.

In the initial 30 seconds following VNI, F95 increased from pre-treatment values of 101 (SD 9)% to 111 (SD 12)% ($P < 0.05$) and remained stable for 150 seconds following VNI in both right and left-sided EEG (figure 4.2). After this period, bursts of periodic activity were visible bilaterally. The response in Ptot was biphasic (figure 4.3). Initially it increased from pre-treatment values of 95 (SD 23)% to 160 (SD 91)% and 297 (SD 152)% on the right and left cerebral hemispheres respectively ($p < 0.0521$; $P < 0.0022$). By 60 seconds after VNI, Ptot had decreased to 60 (SD 23)% and 65 (SD 27)% of pre-treatment values for the right and left sides respectively ($P < 0.05$). After 150 seconds, Ptot began to exhibit bursts of periodic activity in individual animals. In the initial 30 seconds following VNI, F50 increased from pre-treatment values of 110 (SD 43) to 125 (SD 84) ($P = 0.036$) on the right side (figure 4.4).

Visual assessment of the EEG showed that the mean duration of out of range data following VNI was 2 (SD 1) seconds (figure 4.5). The mean duration of active EEG for anaesthetised calves following VNI was 34 (SD 16) seconds. Transitional EEG was observed in most, but not all animals, as waveforms changed from active EEG to HALF. The mean duration of transitional EEG for the 9 animals in the group that displayed this kind of EEG activity was 70 (SD 50) seconds. The mean time to onset of HALF was 76 (SD 26) seconds with a mean duration of 144 (SD 73) seconds. This activity occurred at similar time points to the periodic activity seen in Ptot (figure 4.3). The mean time to the onset of isoelectric EEG was 192 (SD 71) seconds. An example of a compressed spectral array is illustrated in figure 4.6. This gives a visual representation of the changes in EEG variables described above.

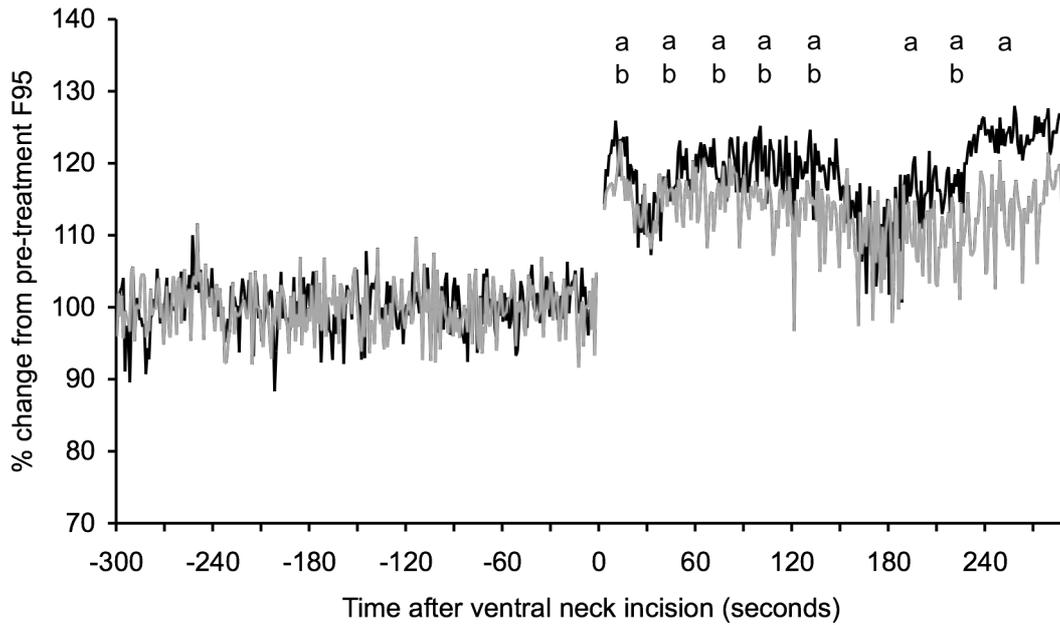


Figure 4.2. Mean 95% spectral edge frequency (F95) following ventral neck incision (VNI) at 0 seconds. Electroencephalogram (EEG) from the right (—) and from the left (---) of the cranium. a = significant difference from pre-treatment right ($P < 0.05$), b = significant difference from pre-treatment left cerebral hemisphere ($P < 0.05$).

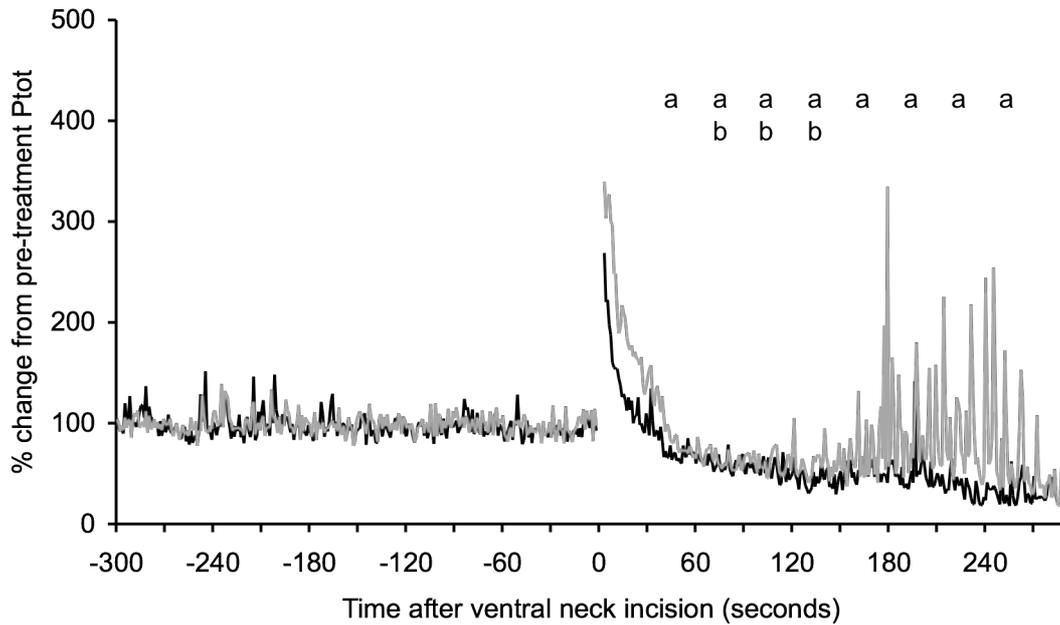


Figure 4.3. Mean total power (Ptot) of the electroencephalogram (EEG) following ventral neck incision (VNI) 0 seconds. EEG from the right (—) and from the left (---) of the cranium. a = significant difference from pre-treatment right ($P < 0.05$), b = significant difference from pre-treatment left cerebral hemisphere ($P < 0.05$).

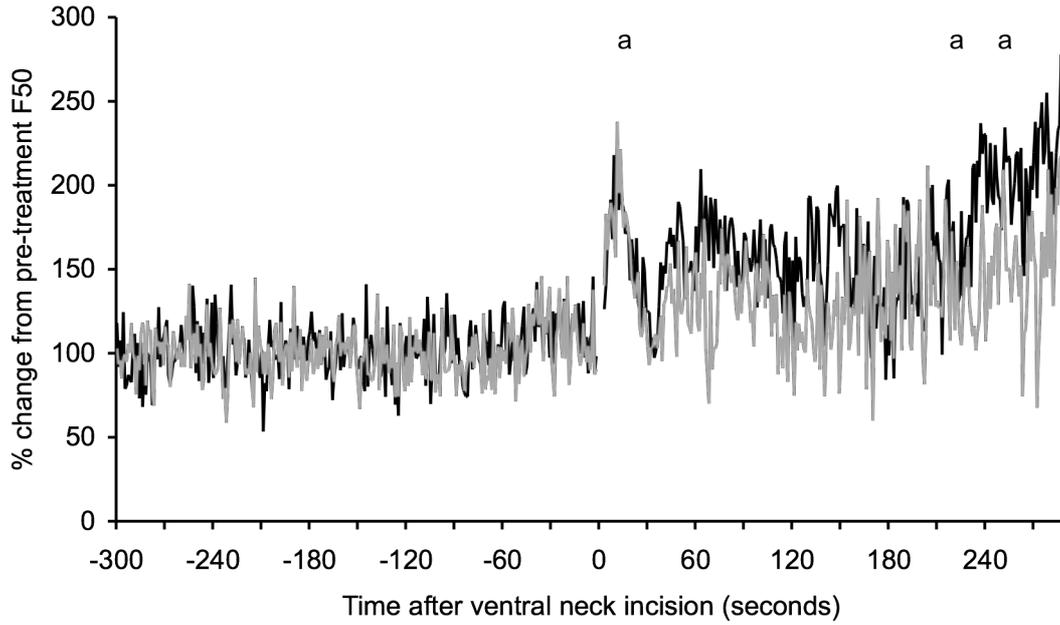


Figure 4.4. Median frequency (F50) (mean) following ventral neck incision (VNI) at 0 seconds. Electroencephalogram (EEG) from the right (—) and from the left (—) of the cranium. a = significant difference from pre-treatment right ($P<0.05$), b = significant difference from pre-treatment left cerebral hemisphere ($P<0.05$).

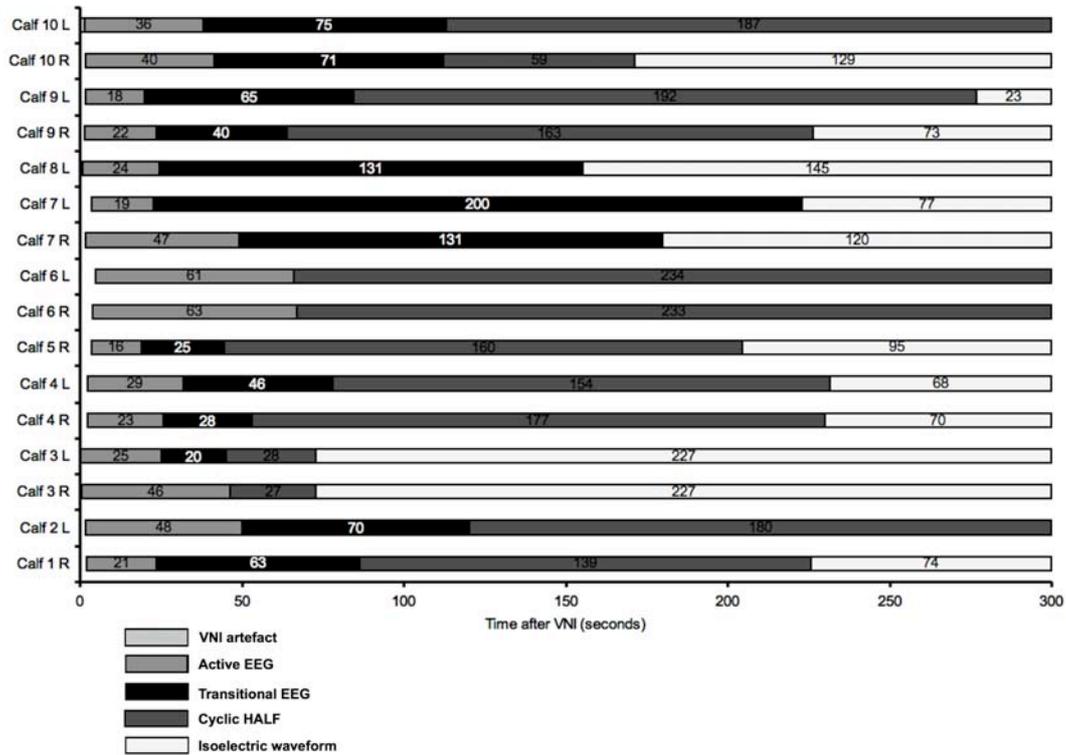


Figure 4.5. Characteristics of the spontaneous electroencephalogram (EEG) in individual animals over time (seconds) after ventral neck incision (VNI). The spontaneous traces were visually inspected and were either classified as: VNI artefact, active EEG, transitional EEG, HALF EEG, and isoelectric EEG. R = EEG recorded from the right and L = EEG recorded from the left hand side of the cranium. The time duration in seconds are displayed on the individual bars; clear gaps was out of range.

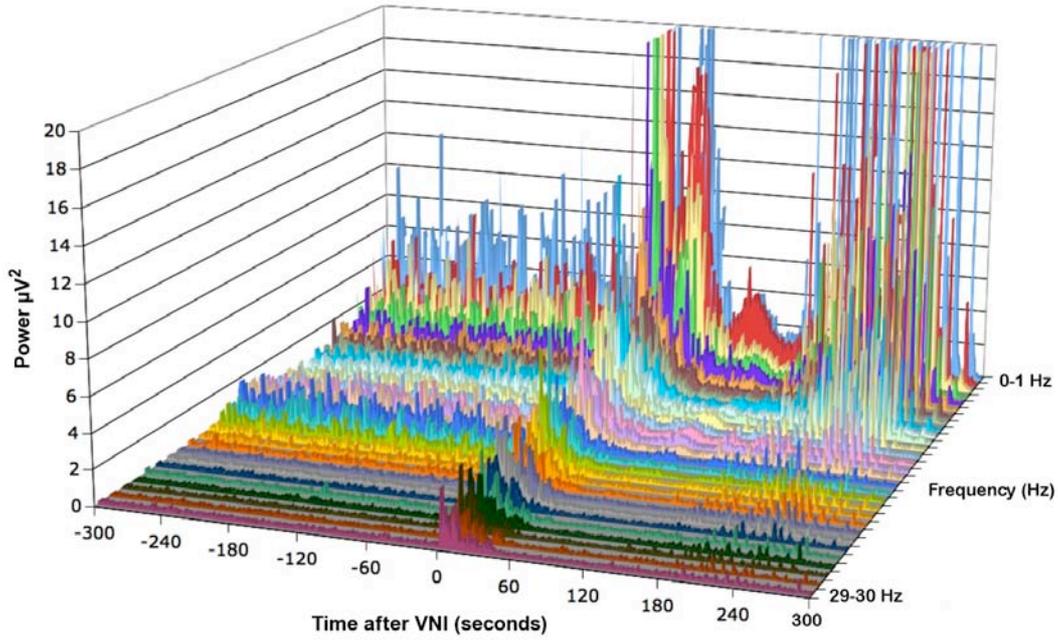


Figure 4.6. The compressed spectral array from calf 4 left cerebral hemisphere, representing a typical array in response to ventral neck incision (VNI) at 0 seconds.

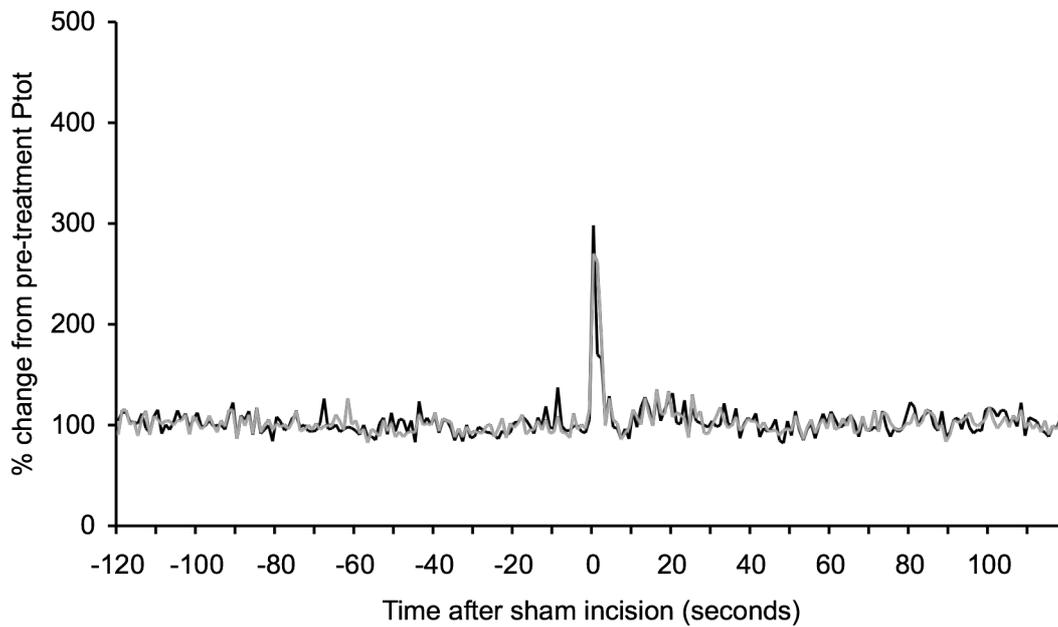


Figure 4.7. Mean Ptot following application of a non-noxious sham incision (SI) at 0 seconds. Electroencephalogram (EEG) from the right (—) and from the left (—) of the cranium.

After SI there were no significant differences in F50, F95 and Ptot from pre-treatment values. However, there was a transient increase in Ptot immediately following application of the sham incision (figure 4.7). This had a mean duration of 3 seconds.

After VNI blood pressure decreased from a pre-treatment value of 112 (SD 33) mmHg to 22 (SD 8) mmHg and 4 (SD 9) mmHg at time points of 40 and 110 seconds, respectively (figure 4.8). The decrease in blood pressure was significant from pre-treatment values from 15 seconds onwards. Following VNI heart rate decreased from pre-treatment values (table 4.1). Bradycardia was significant ($P < 0.05$) 30 and 60 seconds after VNI. From 140 seconds after VNI, tachycardia developed.

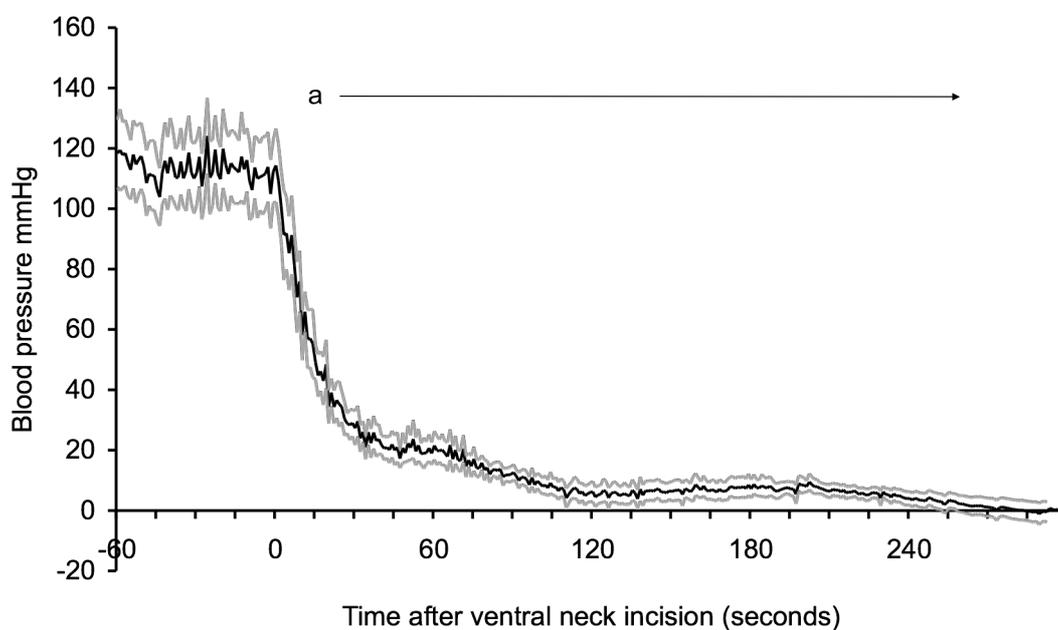


Figure 4.8. Mean \pm standard error of the mean (—), femoral arterial blood pressure (mmHg) before and after ventral neck incision (VNI). a = significant difference from pre-treatment values ($P < 0.05$).

Positive correlations ($P < 0.001$) were found between decreasing blood pressure and changes in Ptot following VNI ($r = 0.6866$ right-sided and $r = 0.5355$ left-sided). Other EEG indices displayed weak correlations with blood pressure following VNI.

Table 4.1. Mean \pm standard error of the mean, changes in heart (beats per minute) at time points after ventral neck incision (VNI).

Time after VNI (seconds)	Heart rate (\pm SEM) (bpm)	P-value (difference from pre-treatment values)
-15 ^a	129.98 \pm 9.36	na
0 ^b	129.05 \pm 9.29	0.9162
15	127.67 \pm 20.34	0.3184
30	104.29 \pm 5.92	0.0313 ^c
45	105.34 \pm 5.85	0.0520
60	102.30 \pm 5.60	0.0313 ^c
75	104.96 \pm 5.37	0.0740
90	105.06 \pm 4.69	0.0520
105	105.37 \pm 5.08	0.0519
120	104.71 \pm 7.12	0.0831
135	111.88 \pm 7.46	0.2271
150	125.14 \pm 7.20	0.7929
165	135.30 \pm 8.38	0.8748
180	140.72 \pm 9.05	0.3720
300 (5 minutes)	137.64 \pm 10.91	0.5625

^a Pre-treatment^b Ventral neck incision (VNI)^c Significant difference from pre-treatment values ($p < 0.05$)

No signs of occlusion were seen at either the cephalic or cardiac ends of the carotid arteries following VNI during or after the period of data collection. Gross examination of the brains following VNI showed no abnormal haemorrhage. Histological examination revealed no internal haemorrhage or lesions of any kind (table 4.2). Slides taken from the obex, pons, midbrain and somatosensory cortex had dilated vessels or vacuoles, indicating good penetration of the fixation agent immediately following death. However in the cerebellum of five of the calves, there were mild changes in white matter with artefact around glial cells, potentially indicating poor fixation of the cerebellum. A number of slides had ‘chatter’, folding and creasing artefacts, but these were not sufficient to hinder histological examination.

Table 4.2. Histology results for calves after ventral neck incision (VNI). Sections taken from obex, spinal cord, pons, cerebellum, midbrain, thalamus, insula and somatosensory cortex. Chatter = processing artefact.

Calve No.	Obex,	Spinal cord	Pons	Cerebellum	Midbrain	Thalamus	Somatosensory cortex	Insula
1	Normal minor chatter	Normal	Normal	Normal, mild changes in white matter, poorly stained	Normal	Normal	Normal,	Normal
2	Normal	Normal	Focal area of neuronal change	Normal	Normal, two large neurons with vacuoles	Normal	Normal	Normal, creasing artefact
3	Normal	Mild diffuse glycosis	Normal	Normal, mild changes in white matter	Normal	Normal	Normal, meninges extended in cortex (due to fixation)	Normal
4	Infrequent holes in solitary white matter & reticular formation	Normal	Normal	Normal, mild changes in white matter	Normal	Normal	Normal	Normal
5	Infrequent holes	Normal	Distended veins, normal	Increased number of cells in the meninges (younger animal)	Normal, large blood vessels (older animal)	Normal	Normal, lots of capillary holes from fixation	Normal
6	Infrequent holes in solitary white matter & reticular formation	Normal	Normal	Normal, mild changes in white matter	Normal	Normal	Normal	Normal
7	Normal	Numerous vacuoles, some shredding in the grey matter of ventral horn	Vacuoles in the white matter	Artefact around some glial cells in the cerebellum roof & white matter, fibre loosening throughout cerebellum white matter	Normal	Normal	Normal	Normal

4.5 Discussion

Slaughter by VNI produced responses in all measured EEG indices that have been previously associated with noxious stimulation in calves (Chapter 3).

Increases in F95 have been seen in response to noxious stimulation (Stanski et al 1987; Barter et al 2005; Dominguez et al 2005; Johnson et al 2005b; McGregor 2005; Orth et al 2005; Chapter 3) and also with increasing depth of halothane anaesthesia (Johnson et al 1993, 1994; Johnson and Taylor 1998). In the current study F_E'HAL was tightly controlled during pre-treatment recording periods. However incision of the neck resulted in the severance of the trachea and endotracheal tube. Whilst this stopped the delivery of halothane to the animal, the concurrent interruption of the major component of cerebral arterial blood supply would have reduced cerebral perfusion and so limited the extent to which any reduction in body F_E'HAL was reflected in the brain. The EEG changes following VNI were of short duration so that the observed changes in F95 were not likely to be a response to decreasing concentrations of halothane in the brain.

The initial increase in P_{tot} after VNI contrasts with previous studies that have generally shown decreases in P_{tot} following a noxious stimulus (Antognini et al 2001; Murrell et al 2003; Haga and Ranheim 2005; Johnson et al 2005b; Chapter 3). However, an initial increase in P_{tot} has been observed in lambs of different ages undergoing rubber ring castration (Johnson et al 2005a). In the current study, the SI group demonstrated a transient increase in P_{tot} comparable in onset to that reported following VNI, but of a lesser duration and magnitude. This suggests that the increase observed after VNI may be partially caused by movement of the animal during incision of the neck. The remainder of the increase following VNI may be related to the loss of skin, muscle and connective tissue tension and to contracture of the ventral muscles of the neck that typically follows this reduction of tension.

After the initial increase in P_{tot} following VNI, P_{tot} significantly decreased. A decrease in power content of specific ECoG frequency bands (2-8 Hz and 8-30 Hz) in conscious cattle has also been reported after slaughter without stunning (Bager et al 1992). This reported effect may be a cortical response to noxious inputs due to slaughter by VNI or, conversely, to loss of mid to high frequency activity resulting in a reduction of functional cerebrocortical activity. Changes in P_{tot} following slaughter

should be interpreted with caution as decreases in P_{tot} have been previously associated with noxious stimulation in cattle (Chapter 3) and with reductions in cortical function during stunning (Chapter 6). In the current experiment it is highly probable that the calves were responding both to the noxious component of slaughter while gradually losing cortical function over time due to hypoxia. This makes interpretation of P_{tot} in isolation of other results difficult and potentially misleading.

The later spikes of increased activity in P_{tot} are linked with both the onset of HALF activity seen on visual assessment of EEG traces and the increase in power of the predominantly lower frequencies (0-10 Hz) of the compressed spectral array. Levinger (1961) found similar activity to that of HALF in the EEG of sheep; 2-4 Hz between 22-44 seconds following Shechita. In humans, hypoxia induced syncope has been shown to occur after a period of slowing of background rhythms accompanied by high amplitude delta activity (Brenner 1997), thereby resembling HALF. Additionally, during cardiac arrest in humans, slow waves of increasing amplitude and decreasing frequency appear in the EEG with a duration of 7 to 13 seconds (Aichner and Bauer 2005). The results from the current study are consistent with these observations and suggest that cerebral hypoxia was the primary cause of this activity.

Changes in F50 have previously been associated with noxious stimulation in a variety of species (Otto et al 1996; Murrell et al 2003; Barter et al 2005; Dominguez et al 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Orth et al 2005; Murrell et al 2007; Kongara 2008; Chapter 3). Those seen in this study were similar to changes in F95, but were less in magnitude. The inherent variability associated with F50 may have reduced its statistical power to a point where it was difficult to identify changes in the challenging environment following VNI.

Median frequency and F95 are both measures of the frequency components of the EEG; they provide no indication of the power or amplitude of the EEG signal. As such, they should always be interpreted in the light of major changes in total signal power (P_{tot}). As the EEG becomes isoelectric, changes in F50 and F95 increasingly represent background noise rather than reflecting cortical function. Thus, there is a period after VNI when the EEG represents real activity, and not background. During this period, changes in F50 and F95 may be interpreted as being responses to noxious stimulation, in particular, during the period when active EEG is still present. As noted here, active EEG following VNI was defined as being similar in waveform type and amplitude to that of the pre-treatment EEG. It represents the period of functional

cerebrocortical activity after VNI during which an animal, if conscious, may perceive noxious stimulation as painful and distressing.

Compressed spectral array provides a more detailed representation of changes in both the frequency and power contents of the EEG of individual animals following VNI. There were increases in the higher frequencies (24-30 Hz) of the compressed spectral arrays with durations of 30 to 60 seconds. These changes correlate with those observed in F95 following VNI. Furthermore the duration of the increases in high frequency activity corresponds to the period of active EEG reported from visual inspection of the EEG waveform. Therefore any increases in F50 and F95 during this window of EEG activity would represent responses to the noxious stimulus of VNI.

In the current study active EEG is not synonymous with sensibility as all animals were anaesthetised during the entire experimental period. This means that any conclusions concerning consciousness need to be carefully considered. Based on previous work it appears that after VNI, sensibility and the associated cognitive ability to perceive pain and experience distress is not lost immediately (Mellor and Littin 2004). Measures of undoubted insensibility have demonstrated that this period varies considerably between species and individuals. There is considerable variation in the reported time to undoubted insensibility in cattle: 28-168 (Blackmore and Newhook 1981; Blackmore et al 1983), 3.5-5 (Nangeroni and Kennett 1964), 34-85 (Newhook and Blackmore 1982b), 19-113 (Daly et al 1988), 10-52 (Bager et al 1992), 2-10 (Levinger 1961), 11.5-23 (Gregory and Wotton 1984b), 20-385 (Blackmore 1984) seconds. The variation within species may be attributed to the use of different experimental techniques and differing criteria used to define insensibility and death (Shaw et al 1990). Changes in the EEG attributable to noxiousness in the current study are within the reported window of possible sensibility in the majority of studies following VNI (i.e. 5 to 60 seconds) (Mellor and Littin 2004).

In the current experiment there were no signs of occlusion at either cephalic or cardiac ends of the cut carotid arteries during or after the data collection period. This suggests that the prolonged periods of functional cortical activity seen in individual calves may be due to the animals having sufficient oxygen within the brain and delivered by the vertebral arteries for the maintenance of cerebral metabolism in the absence of carotid occlusion. It has been calculated that the human brain has enough oxygen within it for metabolism to persist for about 7.5 seconds following the disruption of supply (McIlwain and Bachelard 1985; Hillman 1993).

Histological examination of the brains taken from calves in the VNI group revealed no detectable abnormal features. Rosen (2004) suggested that following Shechita the collapse in jugular venous pressure, without replacement with carotid blood would result in impaired maintenance of brain structure. Based on the current results there were no indications of loss of structure or of lesions resulting from a sudden decompression of the cranial vault following sectioning of the carotid arteries and jugular veins.

This study is the first to examine quantitatively the noxiousness of sensory input during slaughter by VNI. The results demonstrate that VNI causes EEG changes that are quantitatively and qualitatively similar to those observed following scoop dehorning (Chapter 3). In combination with previous analyses (Mellor and Littin 2004), these changes demonstrate that VNI has strong potential to be perceived as a noxious stimulus and therefore to be painful in conscious animals subjected to this procedure.

4.6 References

- Aichner F, Bauer G.** Cerebral anoxia. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Pp 455-70. Lippincott Williams & Wilkins, Philadelphia, 2005
- Anil MH, McKinstry JL, Gregory NG, Wotton SB, Symonds H.** Welfare of calves .2. Increase in vertebral artery blood-flow following exsanguination by neck sticking and evaluation of chest sticking as an alternative slaughter method. *Meat Science* 41, 113-23, 1995a
- Anil MH, McKinstry JL, Wotton SB, Gregory NG.** Welfare of calves .1. Investigations into some aspects of calf slaughter. *Meat Science* 41, 101-12, 1995b
- Antognini JF, Saadi J, Wang XW, Carstens E, Piercy M.** Propofol action in both spinal cord and brain blunts electroencephalographic responses to noxious stimulation in goats. *Sleep* 24, 26-31, 2001
- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP.** Onset of insensibility at slaughter in calves - effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162-73, 1992
- Barnett JL, Cronin GM, Scott PC.** Behavioural responses of poultry during kosher slaughter and their implications for the birds' welfare. *Veterinary Record* 160, 45-9, 2007
- Barter L, Dominguez CL, Carstens E, Antognini JF.** The effect of isoflurane and halothane on electroencephalographic activation elicited by repetitive noxious c-fiber stimulation. *Neuroscience Letters* 382, 242-7, 2005
- Blackmore DK, Newhook JC.** Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* 29, 219-22, 1981
- Blackmore DK, Newhook JC, Grandin T.** Time of onset of insensibility in 4-week-old to 6-week-old calves during slaughter. *Meat Science* 9, 145-9, 1983
- Blackmore DK.** Differences in behaviour between sheep and cattle during slaughter. *Research in Veterinary Science* 37, 223-6, 1984
- Brenner RP.** Electroencephalography in syncope. *Journal of Clinical Neurophysiology* 14, 197-209, 1997
- Chen ACN, Dworkin SF, Haug J, Gehrig J.** Topographic brain measures of human pain and pain responsivity. *Pain* 37, 129-41, 1989

- Cook CJ, Maasland SA, Devine CE, Gilbert KV, Blackmore DK.** Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. *Research in Veterinary Science* 60, 255-61, 1996
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325-9, 1988
- Diesch TJ, Mellor DJ, Johnson CB, Lentle RG.** Responsiveness to painful stimuli in anaesthetised newborn and young animals of varying neurological maturity (lambs, rat pups and wallaby joeys). In: 6th World Congress on Alternatives & Animal Use in the Life Sciences'. Tokyo, Japan Pp 549-52, 2008
- Dominguez CL, Barter LS, Antognini JF.** Intrathecal picrotoxin minimally alters electroencephalographic responses to noxious stimulation during halothane and isoflurane anaesthesia. *Acta Anaesthesiologica Scandinavica* 49, 763-70, 2005
- FAWC.** Report on the welfare of farmed animals at slaughter or killing. Part 1. red meat animals. Farm Animal Welfare Council, London, 2003
- Grandin T.** Euthanasia and slaughter of livestock. *Journal of the American Veterinary Medical Association* 204, 1354-60, 1994
- Gregory NG, Wotton SB.** Sheep slaughtering procedures .2. Time to loss of brain responsiveness after exsanguination or cardiac-arrest. *British Veterinary Journal* 140, 354-60, 1984a
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141-3, 1984b
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1-9, 2005
- Hillman H.** The possible pain experienced during execution by different methods. *Perception* 22, 745-53, 1993
- Johnson CB, Young SS, Taylor PM.** Use of frequency spectral analysis of the equine electroencephalogram as an indication of the depth of halothane anaesthesia. In: Sebel PS, Bonke B, Winograd E (eds). *Memory and Awareness in Anesthesia*. Pp 289-95. P T R Prentice Hall, Englewood Cliffs, 1993
- Johnson CB, Young SS, Taylor PM.** Analysis of the frequency spectrum of the equine electroencephalogram during halothane anaesthesia. *Research in Veterinary Science* 56, 373-8, 1994

- Johnson CB, Taylor PM.** Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* 81, 748-53, 1998
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433-7, 2005a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61-71, 2005b
- Kongara K.** Studies on renal safety and preventive analgesic efficacy of Tromadex and parecoxib in dogs. PhD thesis, Massey University, Palmerston North, NZ, 2008
- Levinger IM.** Untersuchungen zum schächtproblem. Dr thesis, University of Zürich, Zürich, 1961
- Mayhew IG, Washbourne JR.** A method of assessing auditory and brain-stem function in horses. *British Veterinary Journal* 146, 509-18, 1990
- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- McIlwain H, Bachelard HS.** Biochemistry and the Central Nervous System, 5 edn. Pp 598. Churchill Livingstone, Edinburgh, 1985
- Mellor DJ, Cook CJ, Stafford KJ.** Quantifying some responses to pain as a stressor. In: Moberg GP, Mench JA (eds). *The Biology of Animal Stress Basic Principles and Implications for Animal Welfare*. Pp 171-98. CABI Publishing, Wallingford, 2000
- Mellor DJ, Littin KE.** Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13, S127-S32, 2004
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-21, 2005

- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Murrell JC, Mitchinson SL, Waters D, Johnson CB.** Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* 98, 366-71, 2007
- Nangeroni LL, Kennett PD.** Electroencephalographic studies of the effects of shechita slaughter on cortical function in ruminants. Cornell University, New York, 1964
- NAWAC.** Animal welfare (commercial slaughter) code of welfare 2006 public draft. In. Pp 1-65. (New Zealand) 2006
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves - .1. The onset of permanent insensibility in sheep during slaughter. *Meat Science* 6, 221-33, 1982a
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295-300, 1982b
- Orth M, Barter L, Dominguez C, Atherley R, Carstens E, Antognini JF.** Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation. *British Journal of Anaesthesia* 95, 477-84, 2005
- Otto KA, Voigt S, Piepenbrock S, Deegen E, Short CE.** Differences in quantitated electroencephalographic variables during surgical stimulation of horses anesthetized with isoflurane. *Veterinary Surgery* 25, 249-55, 1996
- Rosen SD.** Viewpoint article; Physiological insights into Shechita. *Veterinary Record* 154, 759-65, 2004
- Rutherford KMD.** Assessing pain in animals. *Animal Welfare* 11, 31-53, 2002
- Shaw FD, Bager F, Devine CE.** The role of the vertebral arteries in maintaining spontaneous electrocortical activity after electrical stunning and slaughter in calves. *New Zealand Veterinary Journal* 38, 14-6, 1990
- Stanski DR, Vuyk J, Ausems M, Arts R, Kramer C, Spierdijk J.** Can the EEG be used to monitor anesthetic depth for alfentanil with N₂O? In: 'Annual Meeting American Society of Anesthesiologists'. (ed Gelman S), 1987

CHAPTER 5

COMPONENTS OF ELECTROENCEPHALOGRAPHIC RESPONSES TO SLAUGHTER: EFFECTS OF CUTTING NECK TISSUES COMPARED TO MAJOR BLOOD VESSELS



A paper based on this chapter has been published in the New Zealand Veterinary Journal.

Gibson TJ, Johnson CB, Murrell JC, Chambers JP, Stafford KJ, Mellor DJ.
Components of electroencephalographic responses to slaughter in halothane-
anaesthetised calves: Effects of cutting neck tissues compared with major
blood vessels. *New Zealand Veterinary Journal* 57, 84-9, 2009

5.1 Abstract

AIM: To identify mechanisms responsible for EEG responses to slaughter by ventral neck incision (VNI) without prior stunning in halothane-anaesthetised calves.

METHODS: Calves were assigned to two groups; neck tissue incision with intact blood circulation through the brain (NTI) and the transection of the major blood vessel of the neck but not most other neck tissues (BVT). The NTI animals had their carotid arteries and jugular veins exposed and cannulated proximal and distal to the proposed site of subsequent neck tissue incision. Blood flow through these vessels was diverted so that cerebral perfusion and drainage were preserved following neck incision. In BVT animals, the carotid arteries and jugular veins were exposed bilaterally by surgical dissection. They were then transected without further damage to the remaining structures of the neck. Changes in EEG and arterial blood pressure were compared in each group before and after NTI or BVT and between groups following treatments.

RESULTS: Neck tissue incision (NTI) resulted in little overall change in median frequency (F50), an increase in 95% spectral edge frequency (F95), and an initial increase in total power (P_{tot}) followed by a transient decrease with an eventual return to pre-treatment values. There was significant between-animal variation in these EEG parameters. Transection of the major blood vessels of the neck (BVT), resulted in a decrease in F50 in most animals, changes in F95 were highly variable and there was a decrease in P_{tot}.

DISCUSSION: The EEG responses seen following NTI and BVT were qualitatively distinct, and suggested that cutting the neck tissues in the NTI animals caused greater noxious sensory input than transection of only the major blood vessels of the neck. These observations support the conclusion that the EEG responses seen after VNI in intact animals are primarily due to noxious stimulation as a result of incision of ventral neck tissues and not mainly as a result of loss of blood flow through the brain.

5.2 Introduction

Slaughter of animals without prior stunning is associated with a number of potential welfare issues. Possible pain and distress during and immediately following ventral neck incision (VNI) and the time to onset of insensibility have both received considerable attention (Levinger 1961; Nangeroni and Kennett 1964; Homa 1971; Levinger 1979; Blackmore and Newhook 1981; Newhook and Blackmore, 1982ab; Blackmore et al 1983, 1984; Gregory and Wotton 1984ab; Daly et al 1988; Bager et al 1992; Grandin 1994; Anil et al 1995ab; Levinger 1995; Cook et al 1996; Rosen 2004; Barnett et al 2007). Pain caused by VNI has been the subject of much debate. It has been suggested that the use of an exquisitely sharp knife produces minimal reactions in animals and therefore that such a neck cut is not perceived by the animal as painful (Levinger 1961; Homa 1971; Levinger 1979; Grandin 1994; Levinger 1995; Rosen 2004). However there is little evidence to support this suggestion. Until recently it was not clear whether or not slaughter of conscious animals by VNI causes pain or distress. This was due to the complexities of measuring pain in animals (Mellor et al 2000; Rutherford 2002) and limitations on the interpretation of behavioural and physiological responses to neck-cut slaughter (Barnett 1997; FAWC 2003; Rosen 2004). The phylogenetic similarities in structure and function of the central nervous systems (CNS) between humans and other mammals leave little doubt that farm animals can indeed experience pain (Barnett 1997). There is also little doubt that these animals are aware prior to, during and for a period after neck-cut slaughter without prior stunning (Levinger 1961; Nangeroni and Kennett 1964; Grandin 1980; Blackmore and Newhook 1981; Newhook and Blackmore 1982b; Blackmore et al 1983; Daly et al 1988; Barnett et al 2007). It is therefore possible that neck-cut slaughter represents a noxious stimulus, which is perceived by the animal as painful prior to the onset of insensibility.

Chapter 4 reported the first experimental investigation of the noxiousness of slaughter by VNI of calves using EEG spectral analysis. In this study it was demonstrated that there is a period following slaughter when the VNI would represent a noxious stimulus, similar in character to that shown to be caused by amputation dehorning in cattle (Chapter 3). However, this EEG response indicating noxious stimulation may be due to stimulation of nociceptors in the incised tissues of the neck,

to the loss of cerebral perfusion by blood caused by transection of the vessels of the neck, or to a combination of both factors. The aim of this study was to investigate the relative contributions of these two factors to the EEG responses associated with VNI slaughter without stunning in halothane-anaesthetised calves.

5.3 Materials and Methods

5.3.1 Animals

Seventeen calves weighing between 109 and 170kg and sourced from a commercial stock agent were allocated to one of two treatments. In one group, VNI was performed while cerebral perfusion was maintained via cannulae that preserved the blood supply around the incision site (NTI, n=10). In the second group, exteriorised carotid arteries and jugular veins were transected without concomitant damage to the other tissues of the neck (BVT, n=7). Prior to the study, animals were penned overnight with access to water but not food. The study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand (Approval No: 04/86).

5.3.2 Anaesthesia, EEG and ECG

Anaesthesia was induced using a mixture of ketamine (Parnell Laboratories, Auckland, NZ) and propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid intravenous injection. The total doses of induction agents in the two groups were: NTI, 3.4 (SD 0.3) and 7.9 (SD 1.2) and BVT, 4.1 (SD 0.4) and 6.6 (SD 1.3) mg/kg of ketamine and propofol, respectively. Anaesthesia was maintained using halothane in oxygen as previously described in Chapter 3. End tidal halothane tension ($F_{E'}\text{HAL}$) was measured and maintained in the range of 0.85-0.95%.

The EEG and the ECG were recorded as previously described in Chapters 3 and 4. EEG data were recorded from the right and left cerebral hemispheres. The EEG and ECG were amplified using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota FL, USA). The EEG was recorded with a gain of 1,000 and pass-band of 0.1 to 500 Hz, except in two animals in the BVT group where the pass-band was 1 to 500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10 to 500 Hz. Data were digitised at a rate of 1kHz and analysed off-line after completion of experimentation.

5.3.3 Experimental Procedure

Group NTI

Following induction of anaesthesia, calves were placed in dorsal recumbency (figure 5.1). Bilateral surgical exposure of the carotid artery and jugular vein was undertaken on sections of the neck proximal and distal to the planned site of neck tissue transection (Chapter 4). Calves were heparinized with a bolus of 170 (SD 14) U/kg of heparin after which an infusion into a femoral vein was begun at a rate of 3.4 (SD 0.3) U/kg.min via a syringe driver (Mi60-2B microinfusion pump, World Precision Instruments, Florida, USA). Surgical cannulation of the exposed vessels was performed using purpose built curved stainless steel cannulae (Massey University Mechanical Services, Palmerston North, New Zealand). Cannulae were connected over the proposed transection site using flexible silicon and PVC tubing. The distance between the proximal and distal cannulation sites was 100-120 mm. The metal cannulae had the following dimensions: arterial, 5 mm internal diameter, 80 mm long; venous 8 mm internal diameter, 100 mm long. The flexible tubing was 500-600 mm long with internal diameters of 5 mm for arteries (silicon) and 9 mm for veins (PVC). After completion of the cannulations, surgical swabs (BSN Medical Ltd, Mount Waverley, Australia) soaked in sodium lactate (Hartmann's solution, Baxter Healthcare Ltd, Toongabbie, Australia) were placed in and around the wounds, after which they were closed with forceps to prevent dehydration of exposed tissues. In the final three animals, the carotid arteries and jugular veins were insulated with aluminium foil.

A 15-minute period was allowed for stabilisation of anaesthesia before data collection commenced. A 5-minute pre-treatment recording of EEG, blood pressure and ECG was undertaken after which NTI was performed on the intact section of neck between the proximal and distal cannulation sites using a sharp flat edged knife, with a blade 245 mm long by 28 mm high (Granton Ragg Ltd, Sheffield, England). The knife was used exclusively for the neck incision and was re-sharpened after every animal with a Tru Hone sharpener (model no. LCF; Tru Hone Corporation, Ocala, Florida, USA). Data were collected for 5 minutes following NTI after which all calves were euthanased with sodium pentobarbitone (Pentobarb 500, National Veterinary Supplies Ltd, Auckland, NZ) injected into one of the exteriorised carotid arteries.

Group BVT

Following induction of anaesthesia, calves were placed in dorsal recumbency (figure 5.1). The carotid arteries and jugular veins were exteriorised bilaterally. Metal plates were placed beneath the vessels to provide a solid cutting surface, leaving the vagosympathetic trunk and other structures undamaged and unexposed within the neck. Surgical swabs (BSN medical Ltd, Mount Waverley, Australia) soaked in sodium lactate (Hartmann's solution, Baxter Healthcare Ltd, Toongabbie, Australia) were positioned over the wound and vessels prior to transection to prevent drying out of the exposed tissues. Following completion of surgery, animals were stabilized for 1 hour. Calves were heparinized with a bolus of 124 (11.5) U/kg of heparin (Multiparin, Heparin Sodium, CP Pharmaceuticals Ltd, Wrexham, UK) injected into a femoral vein. Data were recorded for 15 minutes after which two operators using fresh scalpel blades simultaneously transected both carotid arteries and jugular veins. Data were collected for 5 minutes following vessel transection. All carcasses were weighed after the recording period.

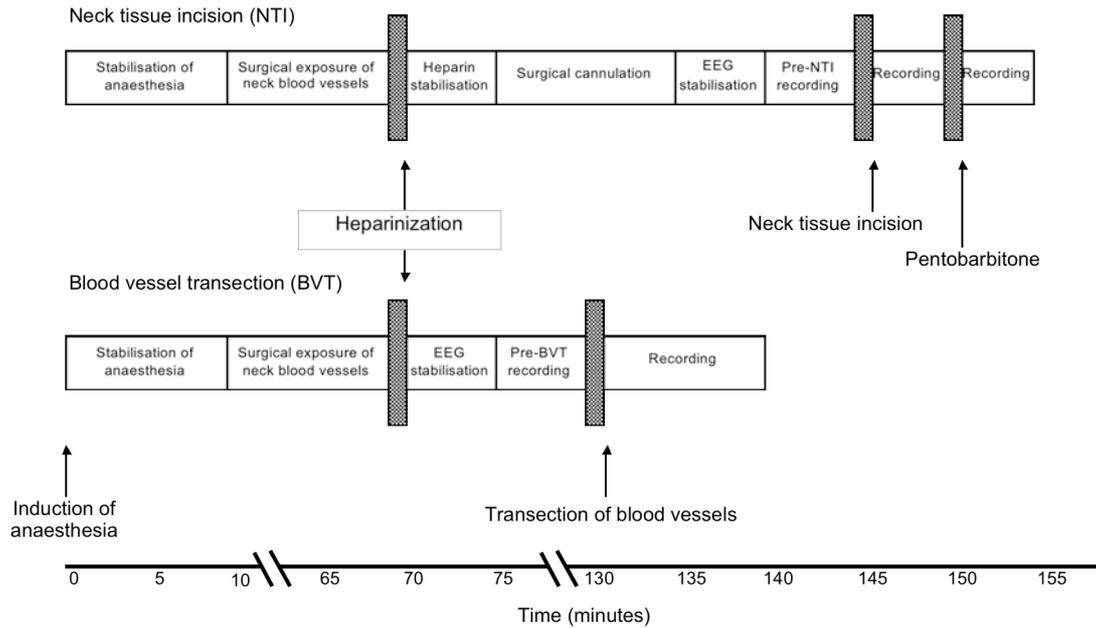


Figure 5.1. Diagram of the experimental design in minutes for neck tissue incision (NTI) and blood vessel transection (BVT). Anaesthesia induction occurred at 0 minutes, and the times to neck incision and vessel transection are indicated. Likewise the timing of heparinization and pentobarbitone euthanasia are indicated with vertical grey blocks.

5.3.4 Data Analysis

EEG and arterial blood pressure analyses were performed after completion of data collection. EEG was inspected visually for signs of over- and under-scale and noise. EEG traces containing significant artefacts were excluded from further analysis. Fast Fourier Transformation was performed using purpose written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North NZ, 2002) as previously described (Chapter 3). All subsequent analysis was performed using Microsoft Excel Mac 2004 (Microsoft Corporation, Redmond, USA).

Data from EEG spectral analysis were displayed as specific EEG indices (F50, F95 and Ptot), that have been found to correlate with noxious stimulation (Chapter 3), and as compressed spectral arrays, which incorporate alterations in power and frequency over time. Data for F50, F95 and Ptot for individual animals are displayed as time series graphs with mean changes overlaid. Data were smoothed using a ten-point moving average.

Blood pressure was recorded from a catheterised femoral artery. Further analysis was performed in Microsoft Excel. Blood pressure data are displayed as the mean \pm SEM.

5.3.5 Statistics

Blood pressure data were analysed using Minitab 14.2 (Minitab Incorporated Pennsylvania, USA). Data were tested for normality using an Anderson-Darling test. Between groups comparisons were made using area-under-the-curve (AUC) analysis and an unstacked one-way analysis of variance (ANVOA). Comparisons with pre-treatment values were made using Dunnett's post hoc test. The level of statistical significance was taken to be $P < 0.05$.

5.4 Results

5.4.1 EEG Power Spectra Indices

Three NTI calves and one BVT calf were excluded from EEG analysis due to depressed cerebrocortical activity during the pre-treatment period in two animals and movement artefact causing 30-40 seconds of out of range data after treatment in the other two. Cerebrocortical depression was presumably due to reduced cerebral perfusion caused by the surgical manipulation of the major blood vessels of the neck. Three further NTI calves were removed from analysis due to $F_{E'}HAL$ being 1.1-1.3% as opposed to the desired level of about 0.85-0.95%. Useable data were therefore available from four NTI and six BVT calves. These small sample sizes precluded statistical analysis of the EEG power spectra data.

Within Group Comparisons

In the NTI group there was significant variability between individuals in F50 (figure 5.2). A transient increase in mean F50 was seen during the initial 40 seconds following NTI, after which it returned to pre-treatment levels. This increase in mean F50 was primarily attributed to a large increase in F50 seen in one animal. Mean F95 increased, as did the F95 in individual animals, such that it remained elevated above pre-treatment values during the recording period (figure 5.3). Ptot showed a transient increase related to movement artefact both in individual animals and as the mean. This was followed by a decrease with a return to pre-treatment values by about 40 seconds after NTI (figure 5.4).

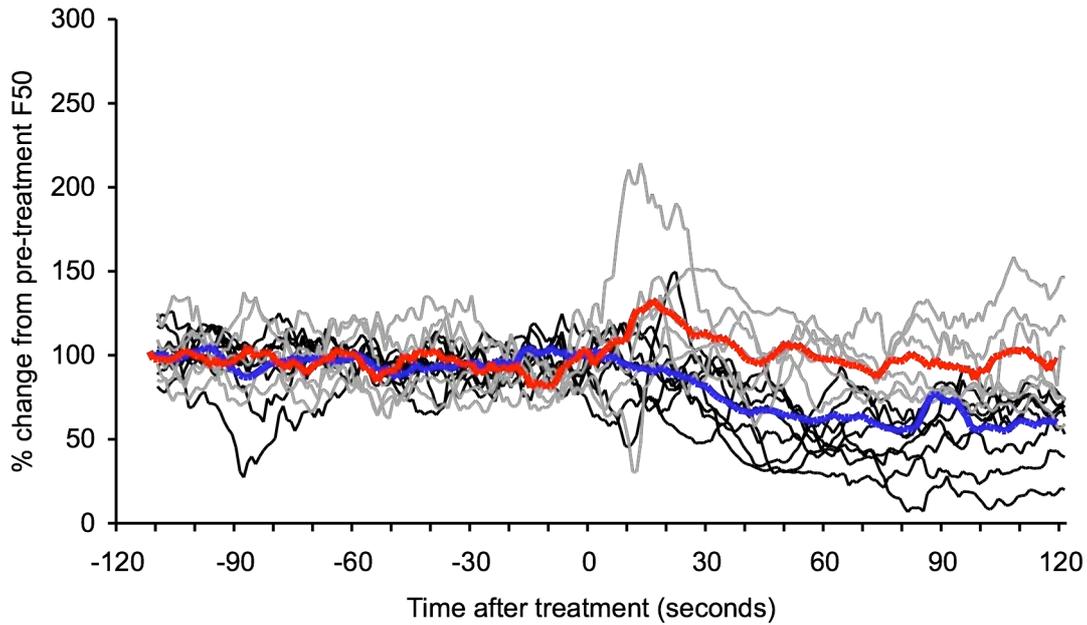


Figure 5.2. Percentage change in median frequency (F50) of the EEG following neck tissue incision (NTI) (—) and blood vessel transection (—) (BVT) at 0 seconds in individual animals, with mean percentage change overlaid for NTI (—) and BVT (—). Data are displayed as 10-point moving averages.

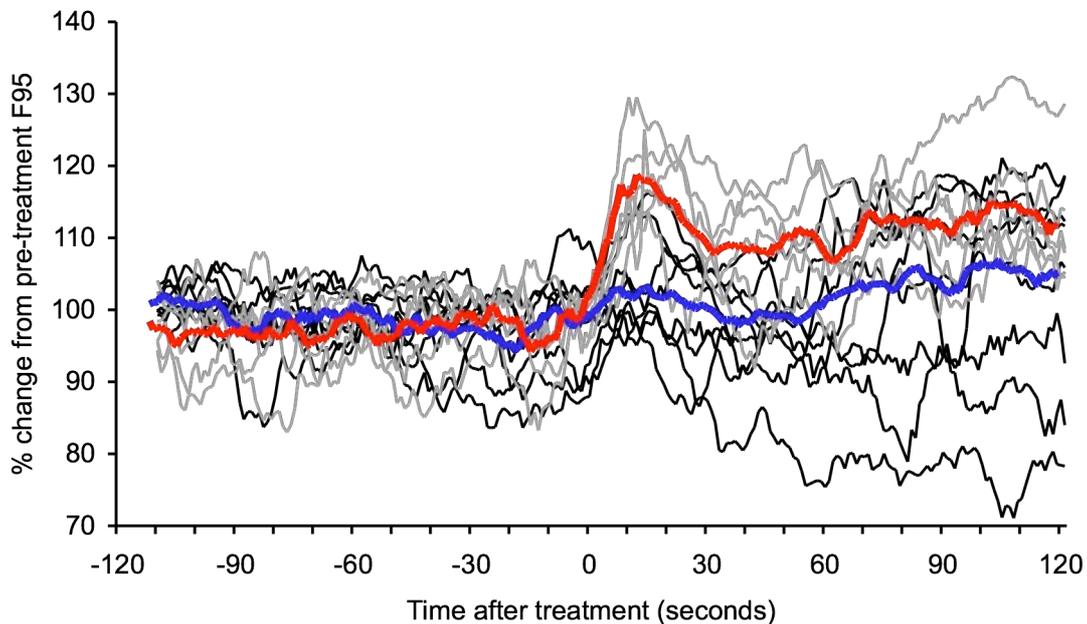


Figure 5.3. Percentage change in 95% spectral edge frequency (F95) of the EEG following neck tissue incision (—) (NTI) and blood vessel transection (—) (BVT) at 0 seconds in individual animals, with mean percentage change overlaid for NTI (—) and BVT (—). Data are displayed as 10-point moving averages.

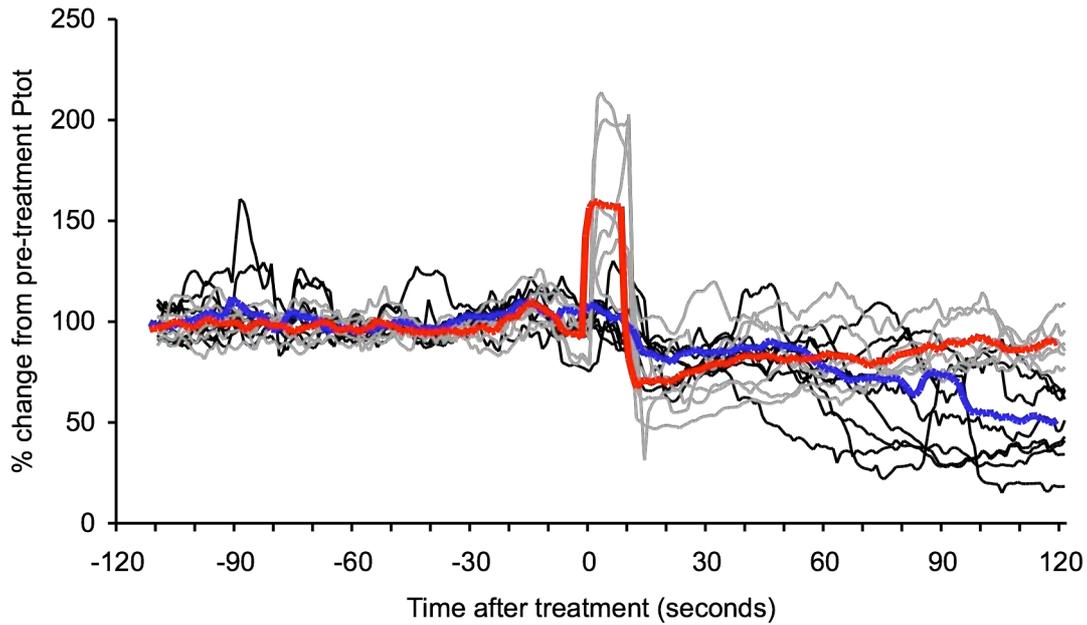


Figure 5.4. Percentage change in total power (P_{tot}) of the EEG power spectrum of the EEG following and neck tissue incision (—) (NTI) and blood vessel transection (—) (BVT) at 0 seconds in individual animals, with mean percentage change overlaid for NTI (—) and BVT (—). Data are displayed as 10-point moving averages.

In the BVT group, mean F50 decreased from pre-treatment values throughout the recording period. This decrease was variable in individual animals (figure 5.2). F95 demonstrated variable responses in individual animals with both increases and decreases (figure 5.3). The mean F95 increased from pre-treatment values, but the overall change was relatively small in magnitude. After transection of the blood vessels, mean P_{tot} showed a continued gradual decrease, with increasing variability in individual animals (figure 5.4).

Between Group Comparisons

The changes in F50, F95 and P_{tot} in the NTI group are distinct from those in the BVT group (figures 5.2, 5.3 and 5.4 respectively). Although variable, F50 in the NTI group increased and then returned to pre-treatment values. In the BVT group, mean F50 continued to decrease through the recording period with no initial increase. In the NTI group F95 increased markedly, whereas in the BVT group there was a small increase, with values remaining close to pre-treatment levels. P_{tot} increased, decreased and then returned to pre-treatment values in the NTI group, whereas in the

BVT group there was little initial response after which P_{tot} decreased throughout the remainder of the recording period.

5.4.2 Compressed Spectral Arrays in Individuals

Following NTI there were variable changes in the compressed spectral arrays in individual animals. Generally NTI resulted in a decrease in frequency power in lower frequencies (0-9 Hz) (figure 5.5). This activity corresponds with the increase in F95 following VNI. Frequency power in the 0-9 Hz band generally returns to pre-treatment values 90 seconds after NTI.

The high pass filter setting of 1.0 Hz in two calves in the BVT group removed the 0-1 Hz frequency band from the compressed spectral array in those animals. Generally in the initial 20-50 seconds after BVT, there were few visually detectable changes from pre-treatment values in the compressed spectral arrays. Thereafter power decreased in most frequencies, with decreases in frequencies between 3-15 Hz being most prominent (figure 5.6).

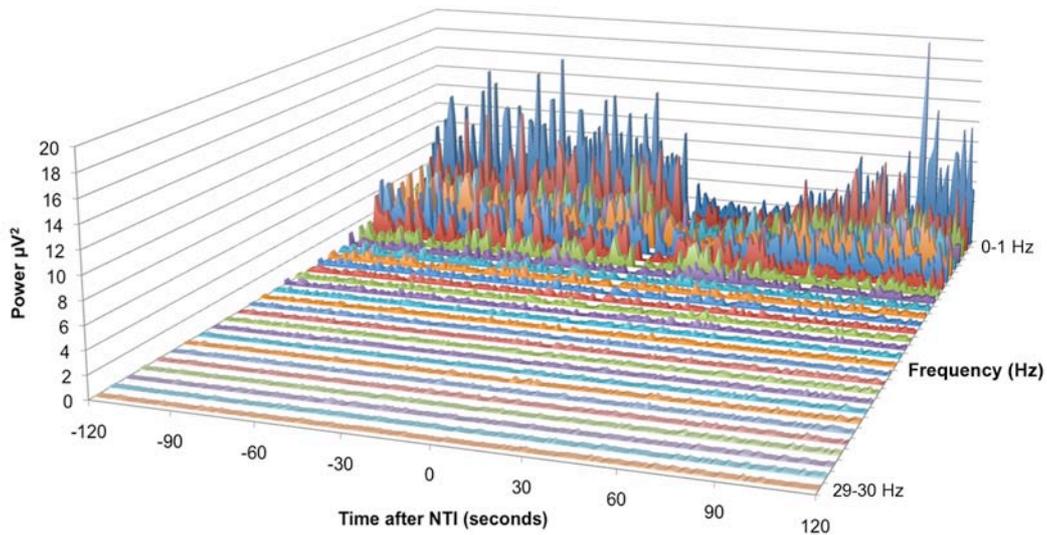


Figure 5.5. An example of the typical response to neck tissue incision (NTI) on the compressed spectral array from calf 3 left cerebral hemisphere. Neck tissue incision occurred at 0 seconds.

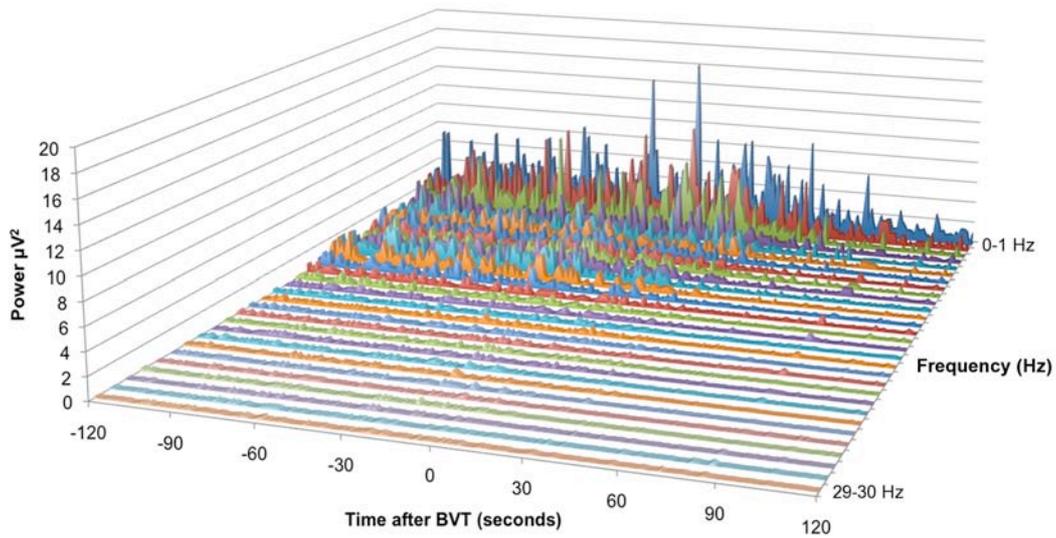


Figure 5.6. An example of the typical response to the transection of the major blood vessels (BVT) supplying the brain on the compressed spectral array from calf 1 right cerebral hemisphere. Transection of blood vessels occurred at 0 seconds.

5.4.3 Blood Pressure Responses

Arterial blood pressure was not recorded in one calf in the NTI group for technical reasons. Mean arterial pressure in the rest decreased from pre-treatment values in both groups (figure 5.7). During the first 30 seconds after NTI, mean arterial blood pressure decreased from 101 mmHg (SD 22) to 78 mmHg (SD 28). This decrease approached but did not achieve statistical significance ($P=0.065$).

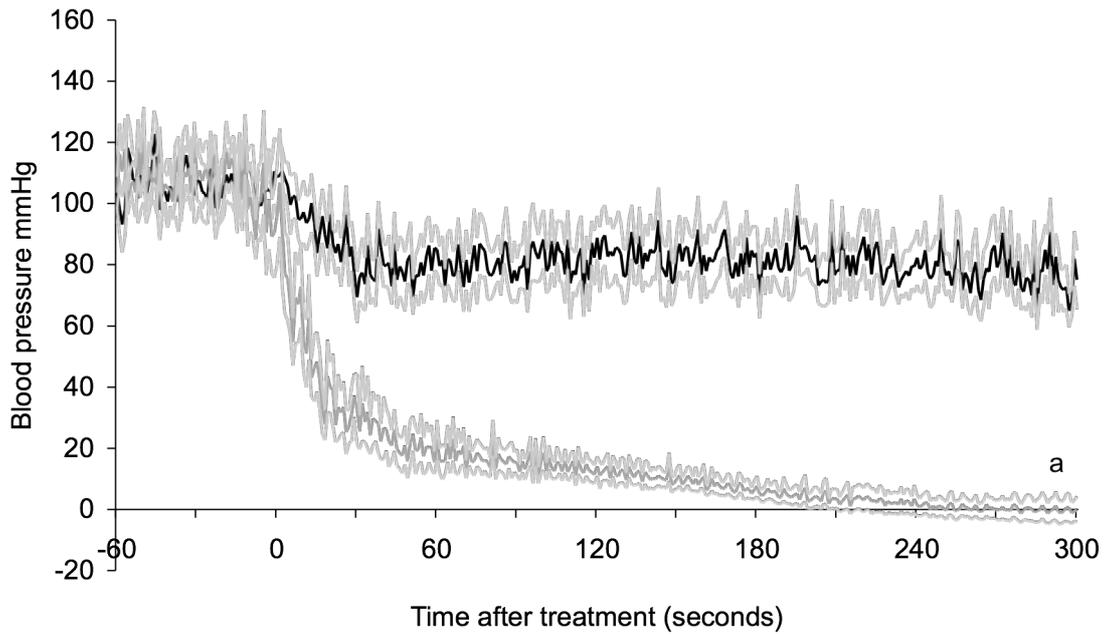


Figure 5.7. Mean changes in blood pressure (mmHg) following neck tissue incision (—) (NTI) and transection of blood vessels (—) (BVT) at 0 seconds (— \pm SEM). a = significant difference between treatments, $P < 0.001$.

Ten seconds after BVT, mean arterial pressure had reduced by half from pre-treatment values of 111 mmHg (SD 24) to 55 mmHg (SD 24). Arterial pressure was 15 mmHg (SD 9) and 11 mmHg (SD 7) at 60 and 120 seconds respectively after vessel transection, both being significantly lower than pre-treatment values ($P < 0.05$). Before treatment (NTI or BVT) there were no significant between-group differences in mean arterial pressure. After treatment values differed significantly between groups ($P < 0.001$).

5.5 Discussion

Chapter 4 reported changes in the EEG of calves in response to VNI involving simultaneous incision of the ventral neck tissues and the major blood vessels. In the current experiment it is apparent that neck tissue incision without interruption of blood supply to the brain (NTI) evoked a cerebrocortical response distinct from that of vessel transection alone (BVT). The changes in F95 following NTI were qualitatively similar to those seen after VNI in intact calves (Chapter 4). This has important implications as it suggests that the EEG response to VNI is likely to be due primarily to noxious sensory input evoked by incision of ventral neck tissues and not mainly to disruption of blood flow through the brain.

The different elements of the EEG responses seen following BVT support this conclusion. The decrease in F50 following bilateral transection of the carotid arteries and jugular veins without severance of other structures of the neck is opposite to changes caused by dehorning-induced noxious sensory input in cattle (Chapter 3) or VNI slaughter (Chapter 4). The decreases in the power content of the compressed spectral arrays in the frequency bands between 3-15 Hz following vessel transection are consistent with the reported decrease in F50. The absence of uniform changes in F95 and the decrease in F50 after vessel transection suggest an absence of noxious sensory input. Total power decreased from pre-treatment values following BVT, with no transient increases as seen after VNI in intact calves (Chapter 4). Total power in the BVT calves did not return to pre-treatment values, unlike that in the NTI group. The decrease in P_{tot} following vessel transection in the BVT calves represents a reduction in and eventual loss of cerebral cortical electrical activity due to ischaemia.

Tissue damage to the neck during NTI would have resulted in activation of nociceptors in and around the damaged tissues. Severance of sensory axons causes a barrage of afferent injury discharges lasting for 2 to 4 seconds after which the depolarised severed axon becomes inactive (Wall et al 1974; Gregory 2004). Nevertheless, damaged neurites may communicate with undamaged nerves. In addition, undamaged nociceptors and other sensory nerves in the region of the wound could be responsive to further stimulation (Gregory 2007), such as by blood coursing over the wound from the cephalic or cardiac ends of the served vessels, pressure from

the incision, air currents and possible mechanical activation during involuntary hypoxic gasping reflexes.

The transient increase in P_{tot} following NTI is similar in morphology to that observed following VNI in intact animals (Chapter 4), but smaller in magnitude. This may be caused by movement artefact during transection of the tissues of the neck. NTI without disruption of perfusion to the brain produced similar movement of the head and neck to that seen during VNI in intact animals (Chapter 4). This conclusion is supported by the absence of an increase in P_{tot} following vessel transection in the BVT group, as the transection of the vessels produced little movement of the head and neck.

Noxious stimulation during surgical exposure and cannulation may have caused hypersensitivity or hyperalgesia in both groups. Central sensitisation (Woolf 1996) may have affected the nociceptive response to NTI. However, as the responses in F95 were similar in shape and magnitude to those previously observed following VNI in intact animals (Chapter 4), the surgical exposure probably did not significantly alter the EEG responses to incision of the neck tissues.

Incision of ventral neck tissues without disruption of blood circulation through the brain resulted in an immediate decrease in blood pressure in the NTI group. The relatively small blood loss from the incision of the neck was not considered to be sufficient to cause the observed decrease in blood pressure. More probably, severance of the vagosympathetic trunk resulted in loss of afferent and efferent vagal activity. Vagal transection may also have resulted in some redistribution of blood flow (Gregory 2005). Vagotomy in dogs following severe hemorrhagic shock prevented the recovery of mean arterial blood pressure, cardiac output and heart rate seen in intact dogs (Schertel et al 1991).

Occlusion of the severed cephalic or cardiac ends of the carotid arteries (due to carotid compression or carotid ballooning) following VNI slaughter has been considered to contribute to delayed loss of sensibility (Bager et al 1988; Anil et al 1995ab; Wotton 2004). In the current study, all calves were heparinized to reduce the likelihood of blood clotting. The formation of blood clots on the severed ends of the carotid arteries or in the surrounding connective tissue sheath could reduce the rate of blood loss. This was not observed in the present calves. In some animals in the BVT group the cardiac ends of the carotid arteries retracted back into the wound with the intact sternomandibularis muscle closing over them. The pressure provided by the

intact sternomandibularis muscles may have been sufficient to retard blood loss. Despite this, changes in blood pressure following BVT were qualitatively and quantitatively similar to those following slaughter by VNI in otherwise intact animals (Anil et al 2006; Chapter 4). This finding strongly supports the view that blood loss in BVT calves closely corresponded to that observed after neck cut slaughter.

The shortened period of stabilisation between the end of surgery and the beginning of pre-treatment recording in the NTI group may have influenced the current results, but was deemed necessary in order that the period of extracorporeal circulation be as short as possible. End tidal halothane tension was maintained above 1.5% during surgical manipulation, after which it was reduced to be within the desired range of 0.85 and 0.95%. In theory $F_E'HAL$ should match that of brain concentration, however the short period of stabilisation may not have been sufficient to allow complete stabilisation of the brain concentration of halothane and thus may have influenced the EEG. Any increase in $F_E'HAL$ at the time of noxious stimulation would have been expected to blunt or obtund EEG responses to the manipulations, thereby underestimating the impact of noxious sensory inputs. This may partially account for the reduced responses seen in the present NTI calves compared to those exposed to VNI (chapter 4).

Blood carried to the head via the extracorporeal circulation in the NTI calves was exposed to the ambient external temperature and this may have resulted in cooling of and a decreased metabolism in the cerebral cortical tissues. Hypothermia has been shown to affect brain electrical activity, with increases in latency and some decreases in amplitude of somatosensory evoked potentials (Hansen and Claassen 2005). During severe hypothermia (body temperature 20°C and below), the amplitude and frequency of the EEG is significantly diminished, resulting in burst suppression (Blume and Sharbrough 2005). Alterations in the EEG following cooling of extracorporeal circulation during cardiopulmonary bypass surgery have produced variable results. Levy (1984) reported a linear relationship between temperature and P_{tot} and no relationship between temperature and F95. Conversely, Bashein et al (1992) found no relationship in any EEG descriptors. In the current study core body temperature did not fall below 33°C in the NTI group. The temperature of the head and brain were not measured. To minimise possible heat loss from the extracorporeal circulation, the vessels were insulated with aluminium foil in the last three animals. There were no qualitative or quantitative differences in the EEG or any measured

EEG descriptors between animals that received the insulation and those that did not. Although cerebral cortical function may have been affected by extracorporeal cooling, the measured EEG indices remained stable during the 10-minute stabilisation period following cannulation and during the 5-minute pre-treatment recording period.

Every attempt was made to minimise the influence of the experimental preparation on the results in the current study, however surgical manipulation, prolonged anaesthesia, hypothermia and artificial redirection of the carotid and jugular blood may have potentially affected cerebral cortical function at the time of treatments in some animals. Data from such animals were excluded from analysis, resulting in a small sample size, which precluded the use of statistics. Despite this the current results demonstrate the causation of the noxious responses previously seen following VNI slaughter (Chapter 4).

Finally, these findings support the conclusion that the acute EEG response seen after VNI slaughter of calves is due primarily to noxious sensory input caused by incision of ventral neck tissues, and not to loss of cerebral perfusion following severance of the carotid arteries and jugular veins.

5.6 References

- Anil MH, McKinstry JL, Gregory NG, Wotton SB, Symonds H.** Welfare of calves .2. Increase in vertebral artery blood-flow following exsanguination by neck sticking and evaluation of chest sticking as an alternative slaughter method. *Meat Science* 41, 113-23, 1995a
- Anil MH, McKinstry JL, Wotton SB, Gregory NG.** Welfare of calves .1. Investigations into some aspects of calf slaughter. *Meat Science* 41, 101-12, 1995b
- Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Weaver HR, Erdogan O, Hughes S, Mason C.** Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: exsanguination and quality parameters. *Animal Welfare* 15, 325-30, 2006
- Bager F, Devine CE, Gilbert KV.** Jugular blood flow in calves after head-only electrical stunning and throat-cutting. *Meat Science* 22, 237-43, 1988
- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP.** Onset of insensibility at slaughter in calves - effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162-73, 1992
- Barnett JL.** Measuring pain in animals. *Australian Veterinary Journal* 75, 878-9, 1997
- Barnett JL, Cronin GM, Scott PC.** Behavioural responses of poultry during kosher slaughter and their implications for the birds' welfare. *Veterinary Record* 160, 45-9, 2007
- Bashein G, Nessly ML, Bledsoe SW, Townes BD, Davis KB, Coppel DB, Hornbein TF.** Electroencephalography during surgery with cardiopulmonary bypass and hypothermia. *Anesthesiology* 76, 878-91, 1992
- Blackmore DK, Newhook JC.** Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* 29, 219-22, 1981
- Blackmore DK, Newhook JC, Grandin T.** Time of onset of insensibility in 4-week-old to 6-week-old calves during slaughter. *Meat Science* 9, 145-9, 1983
- Blackmore DK.** Differences in behaviour between sheep and cattle during slaughter. *Research in Veterinary Science* 37, 223-6, 1984

- Blume WT, Sharbrough FW.** EEG monitoring during cardiopulmonary bypass and open heart surgery. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Lippincott Williams & Wilkins, Philadelphia, 2005
- Cook CJ, Maasland SA, Devine CE, Gilbert KV, Blackmore DK.** Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. *Research in Veterinary Science* 60, 255-61, 1996
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325-9, 1988
- FAWC.** Report on the welfare of farmed animals at slaughter or killing. Part 1. red meat animals. Farm Animal Welfare Council, London, 2003
- Grandin T.** Problems with kosher slaughter. *International Journal for the Study of Animal Problems* 1, 375-90, 1980
- Grandin T.** Euthanasia and slaughter of livestock. *Journal of the American Veterinary Medical Association* 204, 1354-60, 1994
- Gregory N.** Stunning and slaughter. In: *Animal Welfare and Meat Production*. Pp 191-212. CABI Publishing, Wallingford, 2007
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141-3, 1984a
- Gregory NG, Wotton SB.** Sheep slaughtering procedures .2. Time to loss of brain responsiveness after exsanguination or cardiac-arrest. *British Veterinary Journal* 140, 354-60, 1984b
- Gregory NG.** Pain. In: *Physiology and behaviour of animal suffering*. Pp 94-130. Blackwell Publishing, Oxford, 2004
- Gregory NG.** Recent concerns about stunning and slaughter. *Meat Science* 70, 481-91, 2005
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1-9, 2005
- Hansen HC, Claassen J.** EEG and evoked potentials in neuroanesthesia, intraoperative neurological monitoring, and neurointensive care. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Lippincott Williams & Wilkins, Philadelphia, 2005

- Homa B.** The Jewish methods of slaughter. In: Ewer TK (ed) *UFAW Symposium, Humane Killing and Slaughterhouse Techniques*. Pp 44-49. The Universities Federation for Animal Welfare, 1971
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433-7, 2005a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61-71, 2005b
- Levinger IM.** Untersuchungen zum schächtproblem. Dr thesis, University of Zürich, Zürich, 1961
- Levinger IM.** Jewish method of slaughtering animals for food and its influence on blood supply to the brain and on the normal functioning of the nervous system. *Animal Regulation Studies* 2, 111-26, 1979
- Levinger IM.** Shechita in the light of the year 2000, critical review of the scientific aspects of methods of slaughter and Shechita, Maskil L'David, Jerusalem, 1995
- Levy WJ.** Quantitative analysis of EEG changes during hypothermia. *Anesthesiology* 60, 291-7, 1984
- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- Mellor DJ, Cook CJ, Stafford KJ.** Quantifying some responses to pain as a stressor. In: Moberg GP, Mench JA (eds). *The Biology of Animal Stress Basic Principles and Implications for Animal Welfare*. Pp 171-98. CABI Publishing, Wallingford, 2000
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-21, 2005
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006

- Nangeroni LL, Kennett PD.** Electroencephalographic studies of the effects of shechita slaughter on cortical function in ruminants. Cornell University, New York, 1964
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves - .1. The onset of permanent insensibility in sheep during slaughter. *Meat Science* 6, 221-33, 1982a
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295-300, 1982b
- Rosen SD.** Viewpoint article; Physiological insights into shechita. *Veterinary Record* 154, 759-65, 2004
- Rutherford KMD.** Assessing pain in animals. *Animal Welfare* 11, 31-53, 2002
- Schertel ER, Valentine AK, Schmall LM, Allen DA, Muir WW.** Vagotomy alters the hemodynamic response of dogs in hemorrhagic shock. *Circulatory Shock* 34, 393-7, 1991
- Wall PD, Waxman S, Basbaum AI.** Ongoing activity in peripheral nerve: injury discharge. *Experimental Neurology* 45, 576-86, 1974
- Wolf CJ.** Windup and central sensitization are not equivalent. *Pain* 66, 105-8, 1996
- Wotton S.** Slaughter without prestunning. *The Veterinary Record* 155, 64, 2004

CHAPTER 6
ELECTROENCEPHALOGRAPHIC RESPONSE TO
CONCUSSIVE NON-PENETRATIVE CAPTIVE BOLT
STUNNING IN CATTLE



A paper based on this chapter has been published in the New Zealand Veterinary Journal.

Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ.
Electroencephalographic responses to concussive non-penetrative captive-bolt
stunning in halothane-anaesthetised calves. *New Zealand Veterinary Journal*
57, 90-5, 2009

6.1 Abstract

AIM: To investigate the electroencephalographic (EEG) and cardiovascular responses of cattle to non-penetrative captive bolt (NPCB) stunning.

METHODS: Ten calves were anaesthetised with halothane using a minimal anaesthesia protocol. The EEG, blood pressure and electrocardiogram (ECG) were recorded before and after NPCB stunning. Visual inspection of the spontaneous EEG and alterations in total power (P_{tot}) of the EEG power spectrum were used to investigate the effects of stunning.

RESULTS: After stunning cerebrocortical function was significantly altered in all animals. In four animals P_{tot} decreased throughout the entire recording period. In the other five animals P_{tot} responded to stunning in a biphasic manner characterised by an immediate decrease followed by a transient increase and then final decrease to values similar to those of the non-biphasic animals.

DISCUSSION: Non-penetrative captive bolt stunning significantly altered cerebrocortical function in halothane-anaesthetised calves. The changes in cerebrocortical function would be sufficient to produce insensibility within 0 to 14 seconds in awake cattle.

6.2 Introduction

Concussive stunning of livestock prior to slaughter was practiced in China as early as the 15th Century (Mellor and Littin 2004) in order to improve the safety of slaughtermen and facilitate handling of large animals during the slaughter process. Stunning is now primarily practiced to render animals insensible to pain and distress prior to the act of slaughter. In New Zealand, with few exceptions, all animals slaughtered commercially for human consumption must be rendered immediately insensible to pain and distress prior to slaughter and remain so during the entire slaughtering process (NAWAC 1994, 2006). Both mechanical and electrical stunning are in use in New Zealand. Mechanical stunning is widely employed with many species of livestock and is routinely performed around the world during slaughter of animals for human consumption or prior to euthanasia.

There are three commonly used methods of mechanical stunning: non-penetrative captive bolt (NPCB), penetrative captive bolt (PCB) and free bullet. Both the PCB and free bullet methods involve penetration of the cranium. The PCB stunner is positioned in the middle of the animal's forehead and drives a steel bolt through the skull into the brain (Grandin 1980). Unconsciousness is caused by a combination of direct damage to the brain and the kinetic energy delivered to the animal's head (Gregory 1991). Penetrative captive bolt stunning is widely used throughout the world. However, following the identification of a link between bovine spongiform encephalopathy (BSE) and human creutzfeldt-jakob disease (CJD) (Bruce et al 1997; Hill et al 1997), the potential for dispersal of central nervous system (CNS) tissue fragments into distal organs and tissues after PCB stunning (Anil et al 1999; Daly et al 2002; Coore et al 2005) has resulted in a decline in its popularity.

Non-penetrative stunners or percussive stunners operate in a similar way to PCB stunners. Sudden expansion of compressed air or an explosive charge propels the bolt against the forehead of the animal. However, unlike PCB stunners, NPCB stunners have a mushroom-shaped head, which does not penetrate the cranial vault. Insensibility is caused by the transfer of kinetic energy from the rapidly-moving mushroom head to the cranium and brain of the animal (Blackmore 1979).

The time to loss of undoubted sensibility following PCB stunning has received considerable attention (Fricker and Riek 1981; Lambooy and Spanjaard 1981;

Blackmore and Newhook 1982; Daly and Whittington 1986; Daly et al 1986, 1987; Raj and O'Callaghan 2001), but less attention has been given to NPCB stunning. Changes in amplitude and morphology of the EEG and the onset of isoelectric EEG (Blackmore and Newhook 1982), loss of righting reflexes (Blackmore 1979), loss of visual evoked potentials (Gregory and Wotton 1990) and loss of corneal reflexes (Blackmore 1979; Blackmore and Newhook 1982), have all been investigated during NPCB stunning.

The EEG is a representation of the functional activity of the brain (Murrell and Johnson 2006). Subjective assessment of spontaneous EEG has been used to assess functional changes in cerebrocortical activity following NPCB stunning (Blackmore and Newhook 1982), but this approach does not readily lend itself to detailed statistical analysis of EEG changes. Application of the Fast Fourier Transformation (Cooley and Tukey 1965) allows detailed objective statistical interpretation of changes in specific EEG indices derived from EEG power spectra. Objective examination of such changes may provide new information about cerebrocortical function during NPCB stunning in cattle.

Previous studies have reported changes in specific EEG indices (medium frequency (F50), 95% spectral edge frequency (F95) and total power (P_{tot}) of the EEG) that correlate with noxious stimulation (Chapter 3). Increases in F50 and F95, and a decrease in P_{tot}, are associated with nociception (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Kongara 2008; Chapter 3). Both F50 and F95 are measures of frequency and provide no indication of the power or amplitude of the EEG signal. They should always be interpreted with reference to major changes in signal power. As cerebrocortical activity diminishes (e.g. during anaesthesia, ischemia or hypoxia), changes in F50 and F95 increasingly represent changes in the background EEG noise rather than the frequency component of the EEG, such that conclusions on the effects of stunning based on F50 and F95 alone may be erroneous or misleading. In contrast, P_{tot} may be more useful for investigating cortical function after NPCB stunning as decreases in P_{tot} can be used to demonstrate progression towards an isoelectric EEG. As P_{tot} represents the total area under the power spectrum curve (Murrell and Johnson 2006), changes are related to changes in amplitude of the spontaneous EEG.

The aim of this study was to investigate cerebrocortical and cardiovascular responses to concussive NPCB stunning in calves with the aim of evaluating the effectiveness of NPCB stunning in rendering calves insensible prior to slaughter.

6.3 Materials and Methods

6.3.1 Animals

All experimental work was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand (protocol no. 04/86). Ten calves, weighing 109-144 kg, were sourced from a commercial stock agent. The animals were penned overnight, and had free access to water but not food prior to experimentation.

6.3.2 Anaesthesia

Anaesthesia was induced by intravenous injection of a mixture of 4.0 (SD 0.4) mg/kg ketamine (Parnell Laboratories, Auckland, NZ) and 6.5 (SD 2.5) mg/kg propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) into a jugular vein. Calves were intubated with a 16 mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia) and anaesthesia was maintained with halothane (Halothane-Vet; Merial NZ Ltd, Manukau City, NZ) in oxygen (Air Liquide, Palmerston North, NZ) delivered via a precision vaporiser (Fluothane; MedSource Ltd, Ashburton, NZ) and circle breathing circuit (VMS Anaesthesia Machine; Matrix Medical Inc, New York, USA). End-tidal halothane tension ($P_{E'}\text{HAL}$) was maintained between 0.85 and 0.90%. End-tidal carbon dioxide tension, $P_{E'}\text{HAL}$, and respiratory rate were monitored with an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Hamburg, Germany) that sampled airway gases from the proximal end of the endotracheal tube. Calves breathed spontaneously throughout the experimental period.

6.3.3 EEG and ECG Recording

EEG and ECG were measured using the protocol described Chapter 3 and 4. EEG was recorded bilaterally (right cerebral hemisphere and left cerebral hemisphere) and amplified with isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota, Florida, USA). The EEG was recorded with a gain of 1,000 and pass-band of 0.1 to 500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10 to 500 Hz. Both EEG and ECG data were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

6.3.4 Experimental Procedure

Following induction of anaesthesia, calves were placed in dorsal recumbency on a specially designed bed (Massey University Mechanical Services; Palmerston North, NZ) with the head held in position on a purpose-designed head frame (Massey University Mechanical Services; Palmerston North, NZ). A steel bar was positioned on the back of the neck proximal to the horns and poll to reduce movement of the head. This placement of the head left the frontal bone unobstructed for stunning. EEG and ECG instrumentation was performed and the femoral artery was surgically exposed and cannulated with a custom built 18-gauge cannula (Massey University; Palmerston North, NZ) for the measurement of arterial blood pressure. Blood pressure data were amplified using a pressure amplifier (Custom built pressure amplifier; Massey University, Palmerston North, NZ) and digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia). The arterial blood pressure transducer (Spectramed Medical Products, Singapore) was re-calibrated against a mercury column (Baumanometer, W.A. Baum Co. Inc., NY, USA) for each animal. Halothane was stabilized and maintained at a constant tension for at least 15 minutes, after which a 10-minute pre-treatment EEG recording was made (figure 6.1). This was followed by a NPCB stun (CASH Magnum Knocker Concussion Stunner; Accles and Shelvoke Ltd, Birmingham, England), using 4 grain cartridges (Cash cartridges AS25, Black label, .25 cal; Accles & Shelvoke Ltd, Birmingham, England), delivered 30 mm

above the intersection of lines drawn from the medial canthus of each eye to the rostral border of the contralateral ear according to the manufacturer's instructions. Data were recorded for at least five minutes after stunning, at which point all cattle were euthanased with an intravenous injection of sodium pentobarbitone (Pentobarb 500; National Veterinary Supplies Ltd, Auckland, NZ).

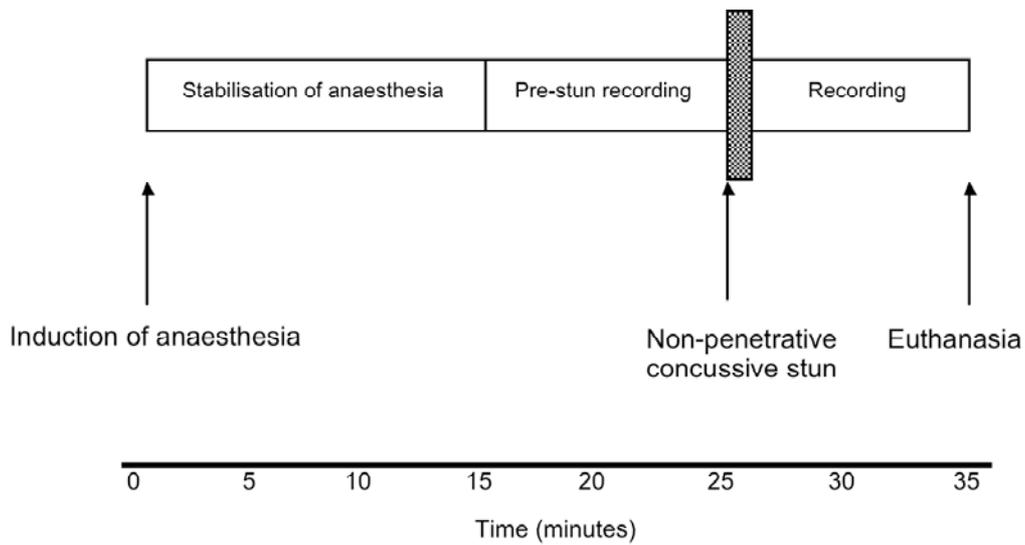


Figure 6.1. Diagram of the experimental design with induction at 0 minutes and non-penetrative captive bolt (NPCB) stunning at 25 minutes (marked with a vertical grey block).

6.3.5 Data and Statistical Analysis

EEG and arterial blood pressure analyses were performed after completion of data collection. Spontaneous EEG was visually inspected for signs of over- and under-scale, out of range data and external noise in the pre-treatment recordings. Sections of EEG recordings containing significant artefact were excluded from further analysis. Fast Fourier Transformation was performed using purpose written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North NZ, 2002) as previously described Chapter 3. Briefly, EEG data were multiplied with a Welch window and Fast Fourier Transformation performed to generate power spectra with 1Hz frequency bins from sequential 1-second epochs. Subsequent analyses were performed with Microsoft Excel Mac 2004 (Microsoft Corporation, Redwood, USA).

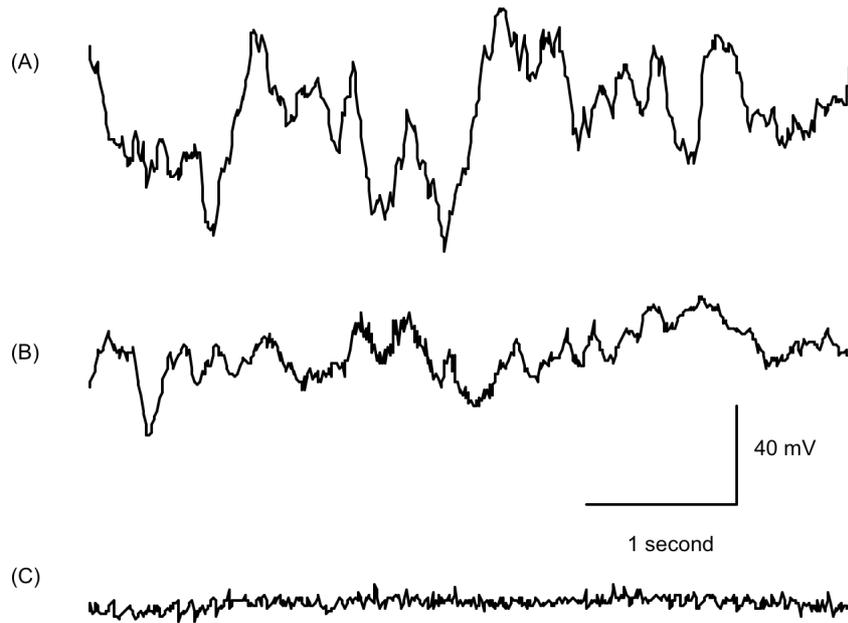


Figure 6.2. EEG trace from a calve showing the course of changes from active EEG (A), to transitional EEG (B) and to an isoelectric EEG (C).

Spontaneous EEG traces were visually inspected and classified into four categories (figure 6.2) of active EEG, transitional EEG, high amplitude low frequency (HALF) EEG and isoelectric EEG. Active EEG represented normal cerebrocortical activity so that in anaesthetised calves it had a similar waveform and amplitude before NPCB stunning as it did, if present, afterwards. Transitional EEG was classified as having an amplitude of less than half of the pre-stunning EEG with a significant frequency change. HALF EEG was a waveform with rhythmic activity of high amplitude and low frequency. Isoelectric EEG was classified as a stable trace consisting of background noise with an amplitude of less than 1/8 of the normal pre-stunning EEG with little to no low frequency component.

EEG data were either displayed as percentage change in P_{tot} from pre-treatment values or as individual compressed spectral arrays. EEG data derived from 2 seconds before to 5 seconds after NPCB stunning were excluded from analysis to prevent contamination by movement artefact. All data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA) and Prism 4.0c (GraphPad Software Incorporated, San Diego, CA, USA). The data distributions were tested for normality with the Anderson-Darling test. Analysis of differences between pre- and post-treatment values for P_{tot} was performed on consecutive non-overlapping 30-second epochs with a Mann-Whitney non-parametric test. Blood pressure and heart

rate were displayed as mean values \pm SEM. Statistical analyses of blood pressure and heart rate were performed on individual time points taken every 15 seconds with a Mann-Whitney non-parametric test. Values of $P < 0.05$ were taken to indicate statistical significance in all analyses.

6.4 Results

Immediately after NPCB stunning, respiration ceased in all calves. Some animals exhibited slow uncoordinated limb movements during the first 5 seconds. The frontal bone of all calves had a 30 mm diameter circular depressed fracture at the site of NPCB impact.

One calf was removed from Ptot analyses due to large periods of time when the EEG was out of range. Animals exhibited one of two different Ptot responses to NPCB stunning. Four calves (figure 6.3) had an immediate decrease in Ptot. The decrease was significant ($P < 0.05$) only on the right hemisphere. The five other calves (figure 6.4) displayed a biphasic response with two distinct periods of Ptot activity. In these animals, the initial decrease was followed by a small transient increase in Ptot that peaked at approximately 80 seconds after NPCB stunning. At completion of the second decrease, Ptot was of a similar magnitude to that seen in animals not displaying biphasic responses. Changes on the left were qualitatively similar to those on the right, but did not achieve statistical significance.

These responses in mean Ptot were mirrored in the compressed spectral arrays of the individual animals. Two examples are provided in figures 6.5 and 6.6. The array in figure 6.5 is from a calf that displayed a simple response to NPCB stunning. Activity in all frequency bands was diminished. The array in figure 6.6 is from a calf that displayed a biphasic response, such that the initial reduction after stunning was followed by a transient recovery of EEG activity that diminished over time.

Figure 6.7 details the time that different EEG patterns were demonstrated by each of the animals in the study. The mean time to initial commencement of an isoelectric trace following stunning was very variable. Overall an isoelectric EEG occurred 60 (SD 87) seconds after NPCB stunning. The mean time to the onset of

transitional EEG following stunning was 8 (SD 14) seconds. Active EEG was only present in 2 calves after NPCB stunning (duration of 6-16 seconds).

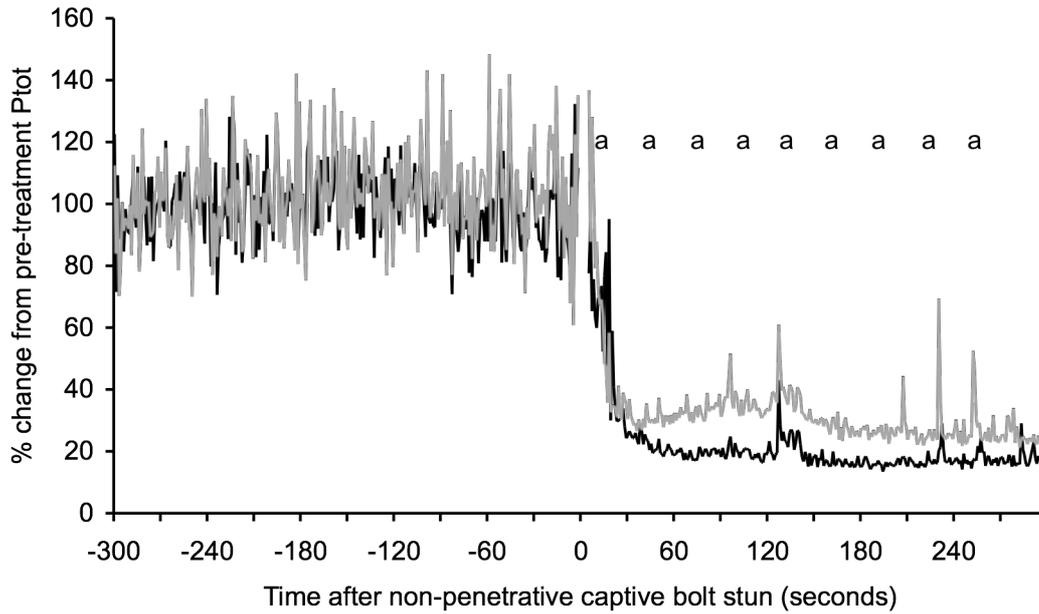


Figure 6.3. Mean changes total power (P_{tot}) of the electroencephalogram (EEG) before and after non-penetrative captive bolt (NPCB) stunning only; simple response ($n=4$). Right cerebral hemisphere (—), left cerebral hemisphere (---). (a) = significant difference from pre-treatment values P_{tot} ($P<0.05$) right cerebral hemisphere.

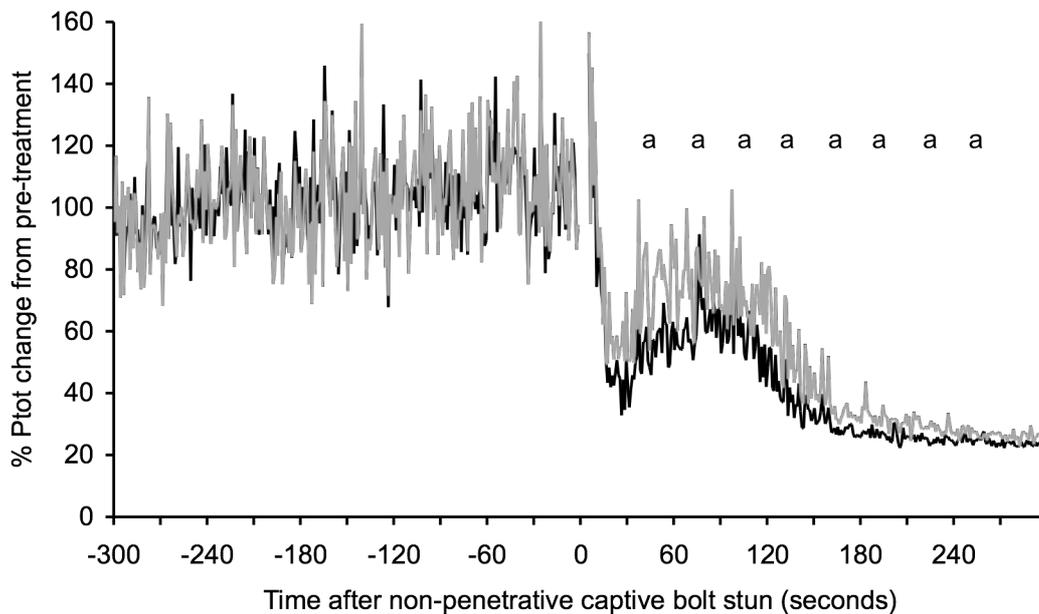


Figure 6.4. Mean changes total power (P_{tot}) of the electroencephalogram (EEG) before and after non-penetrative captive bolt (NPCB) stunning only; biphasic response ($n=5$). Right cerebral hemisphere (—), left cerebral hemisphere (---). (a) = significant difference from pre-treatment values P_{tot} ($P<0.05$) right cerebral hemisphere.

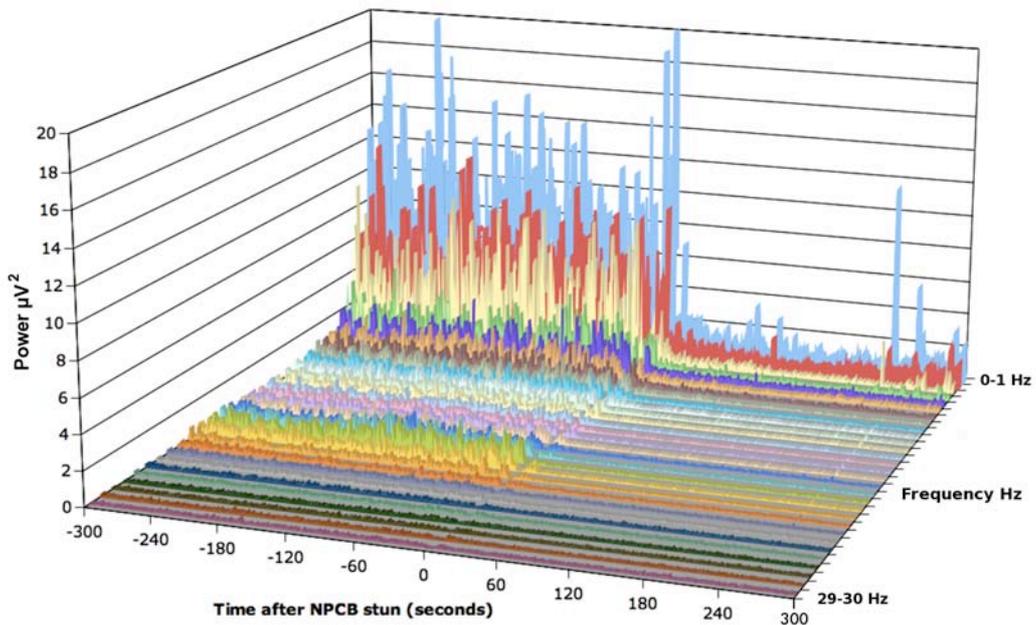


Figure 6.5. Example of the compressed spectral array from a calf with a simple response after non-penetrative captive bolt (NPCB) stunning at 0 seconds.

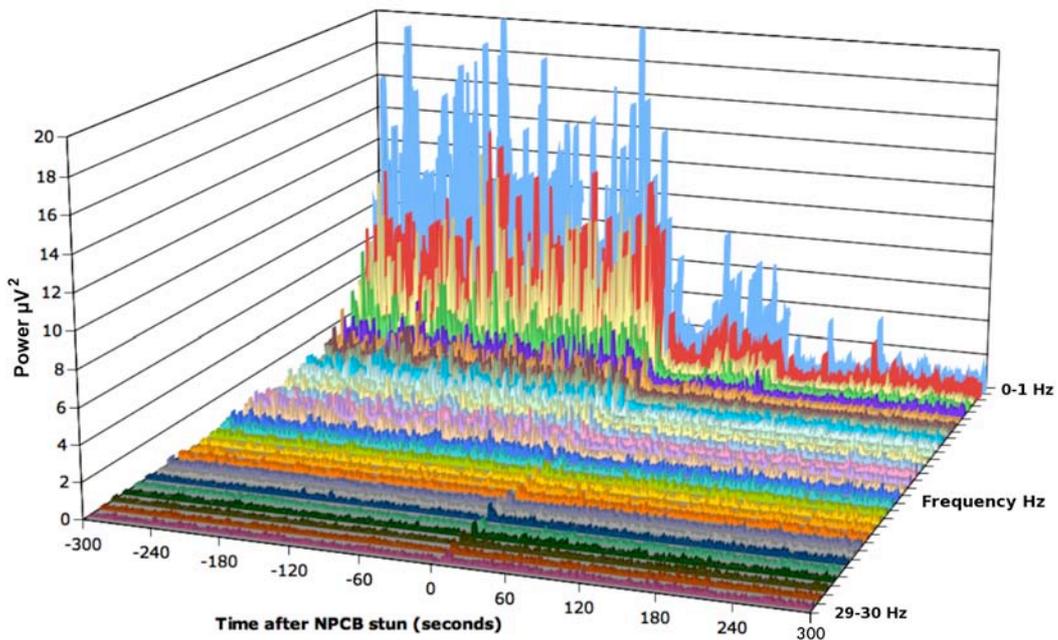


Figure 6.6. Example of the compressed spectral array from a calf with a biphasic response after non-penetrative captive bolt (NPCB) stunning at 0 seconds.

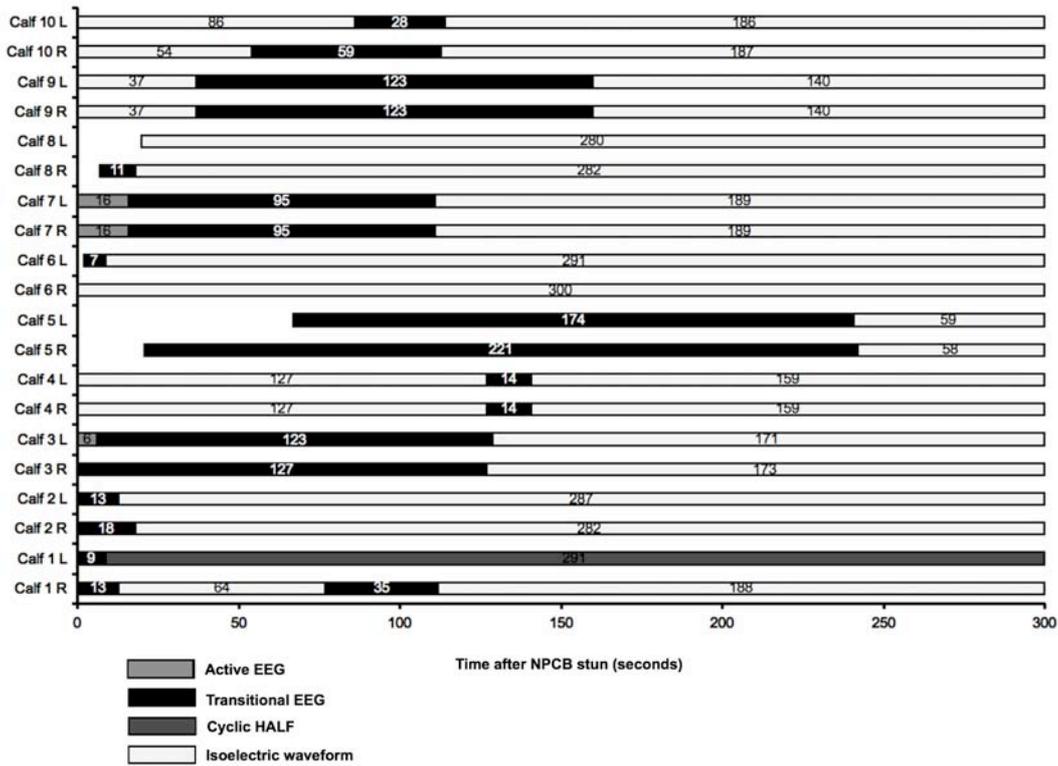


Figure 6.7. Characteristics of the spontaneous electroencephalogram (EEG) in individual animals over time (seconds). Visually examined and classed as either, active, transitional, cyclic EEG and isoelectric. R represents EEG recorded from the right and L the EEG recorded from the left side of the cranium. Time durations in seconds is displayed on the individual bars; clear gaps represent periods where the EEG was out of range.

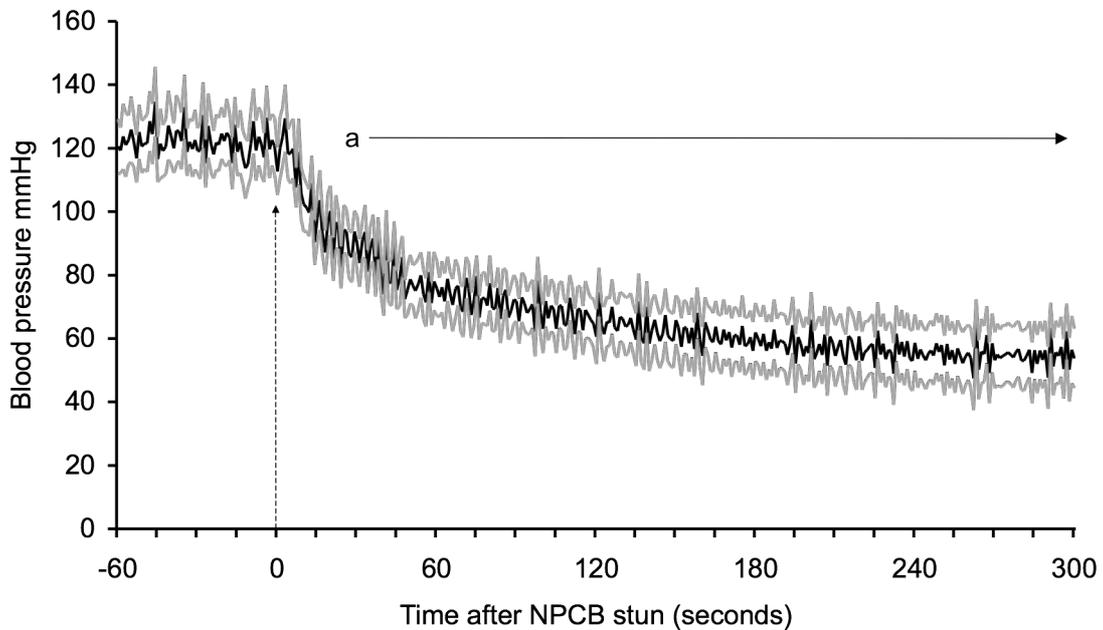


Figure 6.8. Changes in mean femoral arterial blood pressure (mmHg) before and after non-penetrative captive bolt (NPCB) stunning (— \pm SEM). (a) = significant difference from pre-treatment values ($P < 0.05$). The arrow denotes the application of NPCB stun.

Changes in mean arterial blood pressure are illustrated in figure 6.8. Prior to stunning mean arterial blood pressure was 119 (SD 24) mmHg. Following stunning blood pressure fell significantly ($P<0.05$) to 76 (SD 27) mmHg and 61 (SD 27) mmHg at 60 and 120 seconds respectively after stunning. At the conclusion of the recording period (300 seconds after stunning), mean blood pressure was 54 (SD 30) mmHg.

ECG data from four calves were used in the calculation of heart rate (table 6.1). Other ECG traces were rejected due to artefactual contamination. Following stunning mean heart rate significantly decreased ($P<0.05$) from 87 (SD 2) bpm before to 82 (SD 3) bpm at 30 seconds after stunning. Heart rate remained significantly reduced until 285 seconds after stunning, after that there was some recovery. The changes in heart rate were statistically significant, but clinically small in magnitude.

Table 6.1. Mean \pm standard error of the mean of heart rate (beats per minute) at individual time points after non-penetrative captive bolt (NPCB) stunning.

Time after NPCB (seconds)	Heart rate (\pm SEM) (bpm)	P-value (difference from pre-treatment)
-15 ^a	87.25 \pm 1.02	na
0 ^b	na	na
15	82.75 \pm 0.66	0.0163 ^c
30	81.55 \pm 1.20	0.0163 ^c
45	99.75 \pm 19.43	0.2703
60	80.75 \pm 1.11	0.0122 ^c
75	80.5 \pm 1.02	0.0122 ^c
90	81.25 \pm 1.50	0.0163 ^c
105	80.75 \pm 1.68	0.0163 ^c
120	81.50 \pm 1.96	0.0367 ^c
135	81.25 \pm 1.85	0.0216 ^c
150	81.5 \pm 1.83	0.0216 ^c
165	81.5 \pm 1.80	0.0283 ^c
180	80.75 \pm 1.77	0.0163 ^c
300 (5 minutes)	85.5 \pm 2.25	0.6015

^a Pre-treatment

^b Non-penetrative captive bolt (NPCB) stun

^c Significant difference from pre-treatment values ($p<0.05$)

6.5 Discussion

In all cases NPCB stunning instantaneously altered cerebrocortical activity. The initial decrease in P_{tot} seen in all animals represents loss of cerebrocortical function required for the maintenance of sensibility (Blackmore and Delany 1988). This study adds to the existing evidence of the effectiveness of NPCB stunning in achieving rapid insensibility prior to slaughter (Blackmore 1979; Blackmore and Newhook 1982; Gregory and Wotton 1990).

The pattern of changes of EEG activity following NPCB stunning was not uniform between animals. Some animals displayed a small transient increase in P_{tot} after the initial decrease following NPCB stunning. This period of activity correlates with the transitional EEG seen on visual inspection of the spontaneous EEG. Transitional EEG had a different morphology from pre-treatment EEG, active EEG and isoelectric EEG. Other authors have characterised this period in penetrative and non-penetrative captive bolt stunning as being incompatible with sensibility (Blackmore and Delany 1988). In the current study NPCB stunning in one animal was associated with a period of HALF EEG activity similar to the low frequency delta and theta waveforms seen following successful PCB stunning of conscious calves (Lambooy and Spanjaard 1981). This activity has been previously reported in both humans and animals after both clinical and experimental traumatic brain injury (TBI) and is seen during unconsciousness when caused by concussive impacts (Shaw 2002).

It has been suggested that spontaneous EEG and ECoG activity cannot be used to accurately demonstrate the onset and duration of insensibility following penetrative captive bolt stunning in cattle (Blackmore and Delany 1988) as cortically evoked responses were lost immediately after PCB stunning in animals exhibiting apparently normal EEG traces (Blackmore and Delany 1988). Despite this, analysis of EEG traces from conscious animals can provide useful information on the window of cerebral cortical activity within which decisions on the onset of insensibility can be made. Analysis of the EEG provides information on when undoubted insensibility is present, which defines an end point useful in the interpretation of cerebrocortical activity (Blackmore and Newhook 1982).

Changes in the EEG observed in this study were variable between animals, particularly in terms of the time point at which the EEG became isoelectric. The onset

of isoelectric EEG does not correlate with the early part of the decrease in P_{tot} following NPCB stunning, but the onset of the transitional period of EEG does. This period of low amplitude, low frequency EEG is considered to be associated with insensibility because palpebral and corneal reflexes are absent during it (Blackmore and Delany 1988). Moreover, rhythmic respiration ceased immediately in all calves in the current experiment, further indicating effectiveness of stunning (Finnie 1997).

NPCB stunning resulted in both focal and diffuse brain damage (data not shown). Focal damage occurred at the site of impact, characterised by depressed fractures of the skull with adjacent subarachnoid haemorrhage and physical damage to brain tissue. Diffuse brain damage has been seen throughout the brain, manifested as traumatic axon injury, brain swelling and haemorrhage. Finnie (1997), using immunostaining for amyloid precursor protein, demonstrated that NPCB stunning in sheep caused traumatic axon injury throughout the major regions of the brain as early as 1 hour after injury. This was similar in nature to that observed in humans following some forms of TBI. In humans the most common forces involved in concussive TBI are acceleration/deceleration of the freely moving head resulting in rotational damage to the brain. However, in cattle the relatively large and immobile head supported by significant musculature reduces acceleration and deceleration (Finnie 1995, 1997). In the current experiment cattle were stunned while in dorsal recumbency with the head attached to an immobilising frame. This would also have reduced acceleration/deceleration. The striking of the mushroom-shaped bolt against the cranium imparts kinetic energy to the head primarily as contact force (Halliday 1999) with minimal acceleration/deceleration forces. Contact force occurs when the impact energy is imparted without movement of the head. NPCB stunning may have potential as a model of these functional and histological effects of concussive contact force TBI in large mammals.

The probable link between BSE and CJD and the potential for PCB stunning to spread brain tissue fragments to distal organs has generated increased interest in NPCB stunning as a method of rendering animals insensible prior to slaughter. The current study demonstrates the effectiveness of NPCB stunning in disrupting cerebrocortical function. However, the physical damage to the cranium and underlying brain structures observed here presumably accounts for the brain tissue fragments found in jugular blood after both NPCB and PCB stunning (Coore et al 2005). This suggests that while NPCB stunning may be effective in inducing

insensibility, it may not reduce the risk of haematogenous spread of CNS tissue fragments.

Arterial blood pressure decreased following stunning and remained reduced during the experimental recording period. This is in contrast to arterial blood pressure recorded from conscious sheep where a NPCB stun caused a 60% increase in both systolic and diastolic pressures (Blackmore and Newhook 1982; Blackmore and Delany 1988). The increase reported in sheep was maximal 20 seconds after stunning after which the animals were slaughtered by VNI. If the time interval between stunning and sticking had been longer, an overall decrease in blood pressure similar to that reported here may have occurred. Initial bradycardia seen in the current study could have been responsible for the decrease in blood pressure, but this is unlikely as the changes in heart rate, although statistically significant, were biologically inconsequential and would have been insufficient to markedly affect cardiac output and decrease arterial blood pressure. Alternatively, blood pressure may have been reduced by some actions of halothane anaesthesia, but this also seems unlikely as anaesthetic-induced changes in blood pressure are dose dependent (Marshall and Wollman 1980) and $FE'HAL$ was kept at a constant stable tension. Mean heart rate 45 seconds after NPCB stunning increased, this was not significant and was caused by an outlier.

It is possible that halothane anaesthesia modulated the effects of NPCB stunning on the EEG in the current study. Halothane has been demonstrated to cause a progressive slowing of EEG frequencies with increasing tensions (Hansen and Claassen 2005), but as noted, $FE'HAL$ was kept stable throughout the recording period. Alterations in functional activity caused by stunning were of such magnitude and occurred so rapidly that it seems unlikely that alterations in anaesthesia could account for these results.

In conclusion the current study examined the functional EEG effects of NPCB stunning in more detail than has been previously reported. These findings confirm the effectiveness of NPCB stunning in rendering cattle insensible prior to VNI slaughter and extend our understanding of the effects of this technique on functional cortical activity. These findings validate the use of EEG power spectra in investigations of cerebrocortical function prior to and after NPCB stunning.

6.6 References

- Anil MH, Love S, Williams S, Shand A, McKinstry JL, Helps CR, Waterman-Pearson A, Seghatchian J, Harbour DA.** Potential contamination of beef carcasses with brain tissue at slaughter. *The Veterinary Record* 145, 460-2, 1999
- Blackmore DK.** Non-penetrative percussion stunning of sheep and calves. *Veterinary Record* 105, 372-5, 1979
- Blackmore DK, Newhook JC.** Electroencephalographic studies of stunning and slaughter of sheep and calves .3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Science* 7, 19-28, 1982
- Blackmore DK, Delany MW.** Slaughter of stock, a practical review and guide, Veterinary Continuing Education Massey University, Palmerston North, 1988
- Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ.** Transmission to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 389, 498-501, 1997
- Cooley JW, Tukey JW.** An algorithm for the machine calculation of complex fourier series. *Mathematics of Computation* 19, 297-301, 1965
- Coore RR, Love S, McKinstry JL, Weaver HR, Philips A, Hillman T, Hiles M, Helps CR, Anil MH.** Brain tissue fragments in jugular vein blood of cattle stunned by use of penetrating or nonpenetrating captive bolt guns. *Journal of Food Protection* 68, 882-4, 2005
- Daly CC, Gregory NG, Wotton SB, Whittington PE.** Concussive methods of pre-slaughter stunning in sheep - assessment of brain-function using cortical evoked-responses. *Research in Veterinary Science* 41, 349-52, 1986
- Daly CC, Whittington PE.** Concussive methods of pre-slaughter stunning in sheep - effects of captive bolt stunning in the poll position on brain-function. *Research in Veterinary Science* 41, 353-5, 1986
- Daly CC, Gregory NG, Wotton SB.** Captive bolt stunning of cattle - effects on brain-function and role of bolt velocity. *British Veterinary Journal* 143, 574-80, 1987
- Daly DJ, Prendergast DM, Sheridan JJ, Blair IS, McDowell A.** Use of a marker organism to model the spread of central nervous system tissues in cattle and the abattoir environment during commercial stunning and carcass dressing. *Applied and Environmental Microbiology* 68, 791-8, 2002

- Finnie JW.** Neuropathological changes produced by nonpenetrating percussive captive bolt stunning of cattle. *New Zealand Veterinary Journal* 43, 183-5, 1995
- Finnie JW.** Traumatic head injury in ruminant livestock. *Australian Veterinary Journal* 75, 204-8, 1997
- Fricker CH, Riek W.** Die betäubung von rindern vor dem schlachten mit hilfe des bolzenschuß-apparates. *Fleischwirtschaft* 61, 124-7, 1981
- Grandin T.** Mechanical, electrical and anesthetic stunning methods for livestock. *International Journal for the Study of Animal Problems* 1, 242-63, 1980
- Gregory NG, Wotton SB.** Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *Veterinary Record* 126, 570-2, 1990
- Gregory NG.** Humane slaughter. *Outlook on Agriculture* 20, 95-101, 1991
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1-9, 2005
- Halliday AL.** Pathophysiology. In: Marion DW (ed) *Traumatic Brain Injury*. Thieme Medical Publishers, New York, 1999
- Hansen HC, Claassen J.** EEG and evoked potentials in neuroanesthesia, intraoperative neurological monitoring, and neurointensive care. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Lippincott Williams & Wilkins, Philadelphia, 2005
- Hill AN, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J, Doey LJ, Lantos P.** The same prion strain causes vCJD and BSE. *Nature* 389, 448-50, 1997
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433-7, 2005a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and analgesia* 32, 61-71, 2005b
- Kongara K.** Studies on renal safety and preventive analgesic efficacy of tromadox and parecoxib in dogs. PhD thesis, Massey University, Palmerston North, NZ, 2008

- Lambooy E, Spanjaard W.** Effect of the shooting position on the stunning of calves by captive bolt. *Veterinary Record* 109, 359-61, 1981
- Marshall BE, Wollman H.** General anesthetics. In: Gilman's AG, Goodman LS, Gilman A (eds). *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. Pp 277-8. MacMillan Publishing, New York, 1980
- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- Mellor DJ, Littin KE.** Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13, S127-S32, 2004
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-21, 2005
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Murrell JC, Mitchinson SL, Waters D, Johnson CB.** Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* 98, 366-71, 2007
- NAWAC.** Code of recommendations and minimum standards for the welfare of animals at the time of slaughter at licensed and approved premises, Code of Animal Welfare No. 10, Ministry of Agriculture and Forestry, Wellington, 1994
- NAWAC.** Animal welfare (commercial slaughter) code of welfare 2006 public draft. In. Pp 1-65. (New Zealand) 2006
- Raj ABM, O'Callaghan M.** Evaluation of a pneumatically operated captive bolt for stunning/killing broiler chickens. *British Poultry Science* 42, 295-9, 2001
- Shaw NA.** Neurophysiology of concussion. *Progress in Neurobiology* 67, 281-344, 2002

CHAPTER 7

AMELIORATION OF ELECTROENCEPHALOGRAPHIC RESPONSES TO SLAUGHTER BY NON-PENETRATIVE CAPTIVE BOLT STUNNING AFTER VENTRAL NECK INCISION IN HALOTHANE ANAESTHETISED CALVES



A paper based on this chapter has been published in the New Zealand Veterinary Journal.

Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ. Amelioration of electroencephalographic responses to slaughter by non-penetrative captive-bolt stunning after ventral-neck incision in halothane-anaesthetised calves. *New Zealand Veterinary Journal* 57, 96-101, 2009

7.1 Abstract

AIM: To investigate the ability of non-penetrative captive bolt (NPCB) stunning delivered after ventral neck incision (VNI) to ameliorate responses to noxious stimulation associated with slaughter by VNI.

METHODS: Seven calves were minimally anaesthetised using an established anaesthesia protocol. EEG indices of cerebrocortical activity were measured in calves in response to VNI followed 5 seconds later by NPCB stunning (VNI+NPCB). Changes in heart rate and arterial blood pressure were also measured.

RESULTS: After VNI in some calves there were periods of active EEG, which ceased after NPCB stunning in most of the animals. Two calves had periods of active EEG following stunning. Carotid artery occlusion was observed in three calves after VNI+NPCB, but this had no effect on EEG, heart rate and blood pressure compared to other animals.

DISCUSSION: NPCB stunning after VNI resulted in the cessation of functional cortical activity in the majority of calves. This procedure prevents the development of cerebrocortical responses to VNI demonstrated elsewhere, which would be painful in conscious animals subjected to VNI alone.

7.2 Introduction

In New Zealand cattle are stunned prior to slaughter using penetrative captive bolt (PCB), non-penetrative captive bolt (NPCB) or electrical methods. The stun is intended to render the animal completely insensible to any pain and distress caused by VNI slaughter. Chapter 4 demonstrated that VNI without prior stunning causes noxious sensory input in halothane anaesthetised calves by using EEG indices previously shown to be associated with noxious stimulation (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Kongara 2008; Chapter 3). Additional work has demonstrated that these responses were due to transection of neck tissues as apposed to loss of blood flow through the brain (Chapter 5).

These results support the hypothesis that VNI is a noxious procedure and that the animals undergoing slaughter without stunning would experience pain and distress prior to the onset of insensibility. Chapter 6 reported the effectiveness of NPCB stunning in abolishing cerebrocortical activity in cattle. Cerebrocortical activity after NPCB stunning was shown to be incompatible with sensibility, confirming the effectiveness of the NPCB stunning in rendering cattle insensible (Blackmore 1979; Blackmore and Newhook 1982; Gregory and Wotton 1990).

The New Zealand draft commercial slaughter code (NAWAC 2006) includes exemptions, which permit a limited number of species of livestock to be slaughtered without prior stunning in some circumstances. This allows for the needs of specific communities where the meat is to be consumed in New Zealand only. This exemption is conditional on a number of specific requirements including that “all cattle slaughtered without prior stunning must be stunned no more than 5 seconds following cutting of the throat” (draft commercial slaughter code, minimum standard 22(b)). During the 5 seconds prior to stunning, the animal could experience both pain and distress, as based on the previously reported results (Chapters 4 and 5). Stunning within 5 seconds would be expected to render the cattle insensible to further pain and distress (Blackmore 1979; Fricker and Riek 1981; Lambooy and Spanjaard 1981; Blackmore and Newhook 1982; Daly et al 1987; Chapter 6), but as yet there have been no direct studies of post-VNI stunning to confirm this and the recommendation is based on a ‘very high likelihood of benefit’ rationale.

The aim of this study was to examine the cerebrocortical effects of slaughter by VNI with NPCB stunning five seconds after VNI using techniques described previously in Chapters 3 to 6.

7.3 Materials and Methods

7.3.1 Animals

All manipulations in the current experiment were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand (protocol 04/86). Seven Angus steer and bull calves weighing between 134-207 kg were sourced from a commercial stock agent and maintained by routine animal husbandry practices. Calves were penned 24 hours prior to experimentation with access to water but not food.

7.3.2 Anaesthesia and Instrumentation

Anaesthesia was induced by intravenous injection of a mixture of ketamine (Parnell Laboratories, Auckland, NZ) and propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect into a jugular vein. The total doses of induction agents were: 3.9 (SD 0.5) and 8.1 (SD 1.4) mg/kg of ketamine and propofol respectively. Calves were intubated with a 16 mm endotracheal tube (Cook Veterinary Products, Brisbane, Australia). Anaesthesia was maintained with halothane (Halothane-Vet; Merial NZ Ltd, Manukau City, NZ) in oxygen (Air Liquide, Palmerston North, NZ) using a precision vaporiser (Fluothane; MedSource Ltd, Ashburton, NZ) and breathing circuit (VMS Anaesthesia Machine; Matrix Medical Inc, New York, USA). End tidal halothane tension ($F_{E'}\text{HAL}$) was measured and maintained at 0.9% in individual animals with an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Hamburg, Germany). Following induction calves

were placed in dorsal recumbency on a specially designed bed (Massey University Mechanical Services, Palmerston North, New Zealand). The head was securely held in position on a purpose-designed head frame (Massey University Mechanical Services, Palmerston North, New Zealand), which allowed unobstructed access to the neck, and stunning site.

Arterial blood pressure was recorded from a femoral artery as previously described in Chapter 4. EEG was recorded from the right and left cerebral hemispheres. Non-inverting (active) electrodes were placed in the midline between the medial canthi of the eyes, the inverting (reference) electrodes over the left and right mastoid process, and a common ground electrode caudal to the poll. The EEG was amplified with a gain of 1,000 and a pass-band of 0.1 to 500Hz (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota FL, USA). The ECG was recorded with a gain of 1,000 and a pass-band of 1 to 500 Hz. All signals were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia).

7.3.3 Experimental Procedure

After induction of anaesthesia and instrumentation, anaesthesia was stabilized and a 10-minute pre-treatment recording was made (figure 7.1). Calves received VNI followed 5 seconds later by NPCB stunning in accordance with minimum standard no.22b, Public Draft Commercial Slaughter Code of Welfare (NAWAC 2006). Data were recorded for a minimum of 5 minutes following slaughter, after which the wound was inspected for complete severance of major blood vessels and for any carotid occlusion. Carcasses were weighed at the end of the recording period.

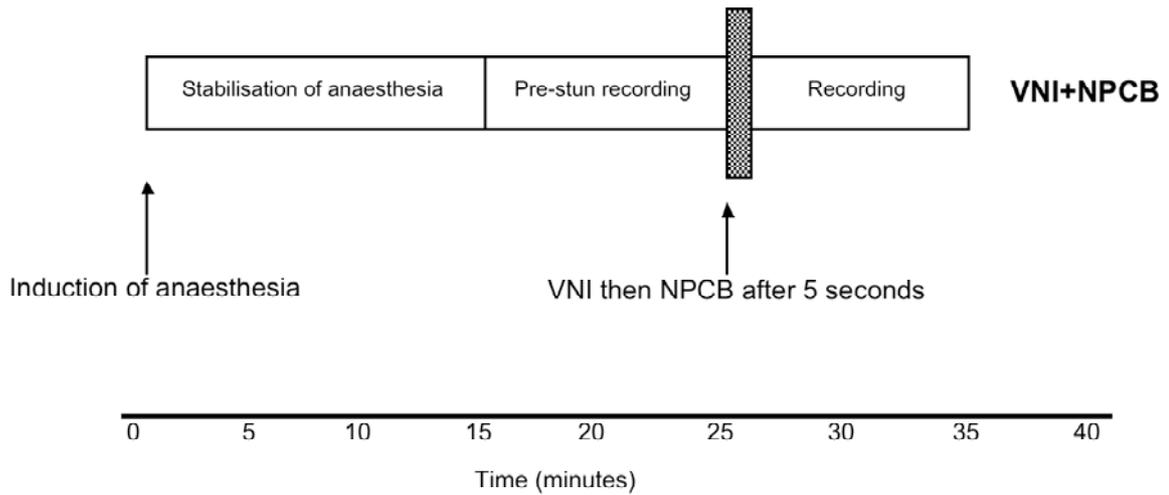


Figure 7.1. Diagram of the experimental design. Anaesthesia was induced at 0 minutes, following which anaesthesia was stabilized. A 10-minute pre-treatment recorded was taken; this was immediately preceded by ventral neck incision with non-penetrative captive bolt stunning (VNI+NPCB) 5 seconds after (marked with a vertical grey block). The electroencephalogram (EEG) was recorded for a further 10 minutes following treatment.

7.3.4 Data Analysis and Statistics

EEG and arterial blood pressure analyses were performed after completion of data collection. EEG was inspected visually for signs of over- and under-scale and external noise. EEG traces containing significant artefacts were excluded from analysis. Fast Fourier Transformation was performed using purpose written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North NZ, 2002) as previously described in Chapter 3. Subsequent analyses were performed with Microsoft Excel Mac 2004 (Microsoft Corporation, Redwood, USA).

EEG was classified into four categories by visual inspection: active EEG; transitional EEG; high amplitude low frequency (HALF) EEG; isoelectric EEG. Active EEG following NPCB stunning in anaesthetised calves represents normal cerebrocortical activity; it is similar in morphology and amplitude to the pre-treatment EEG. Transitional EEG was classified as having an amplitude of less than half of pre-stunning EEG with a significant frequency change. HALF EEG had rhythmic activity of high amplitude and low frequency. Isoelectric EEG was classified as a stable trace consisting of background noise with an amplitude of less than 1/8 of the normal pre-stunning EEG with little to no low frequency component. These are the same classifications as used previously in Chapters 4 and 5.

EEG data were displayed either as percentage changes in P_{tot} from pre-treatment values or as compressed spectral arrays from individual animals. EEG data derived from 2 seconds before VNI to 5 seconds after NPCB were excluded from analysis to prevent contamination by movement artefact. All data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA) and Prism 4.0c (GraphPad Software Incorporated, San Diego, CA, USA). The distribution of the data was tested for normality with the Anderson-Darling test. Analysis of differences between pre-treatment values and P_{tot} was performed on consecutive non-overlapping blocks of 30 seconds with a Mann-Whitney non-parametric test. Mean blood pressure is displayed as mean \pm SEM. Analysis of the correlation between blood pressure and P_{tot} was performed using a two-tailed spearman correlation test for non-parametric data with Prism 4.0c.

Heart rate is presented in table 7.1 as mean values \pm SEM. Statistical analysis for blood pressure and heart rate were performed using the Mann-Whitney non-parametric test on individual time points every 15 seconds. Values of P<0.05 were taken to indicate significance in all analyses.

7.4 Results

EEG data from the left cerebral hemisphere of two calves was rejected from power spectrum analysis due to large periods of external noise contaminating pre-treatment and post-treatment values. Three calves had signs of significant occlusion at the cardiac ends of the severed carotid arteries at the end of data collection. The occlusion was unilateral in two and bilateral in the remaining animal. Calves with either unilateral or bilateral carotid occlusion showed no biological difference in cerebrocortical activity, blood pressure, or heart rate from the other calves in the study. Occlusion was first observed two minutes following VNI. Data from the three occluded animals and the remaining calves were pooled and used in-group analysis. At the site of NPCB impact the frontal bone had a 30 mm diameter depressed fracture in all calves.

After VNI+NPCB, P_{tot} (figure 7.2), increased transiently from pre-treatment values, and this was followed by a gradual decrease to below pre-treatment values.

P_{tot} was significantly decreased from pre-treatment values by 30 seconds after VNI+NPCB ($P < 0.05$). By 90 seconds after VNI+NPCB P_{tot} had decreased to 32 (SD 14)%. Variability in mean P_{tot} in both EEG channels increased 160 seconds after VNI+NPCB with large hypoxia-related bursts of activity on the P_{tot} time series.

Figure 7.3 is an example of a typical compressed spectral array from an individual calf before and after VNI+NPCB. After VNI+NPCB there were major decreases in power in all frequency bands compared to pre-treatment values. The compressed spectral arrays of three animals displayed large hypoxia related bursts of increased power approximately 160 seconds after VNI+NPCB in the low-end frequency (0-4 Hz), activity which correlates with the bursts seen in P_{tot} during the same time period (figure 7.2).

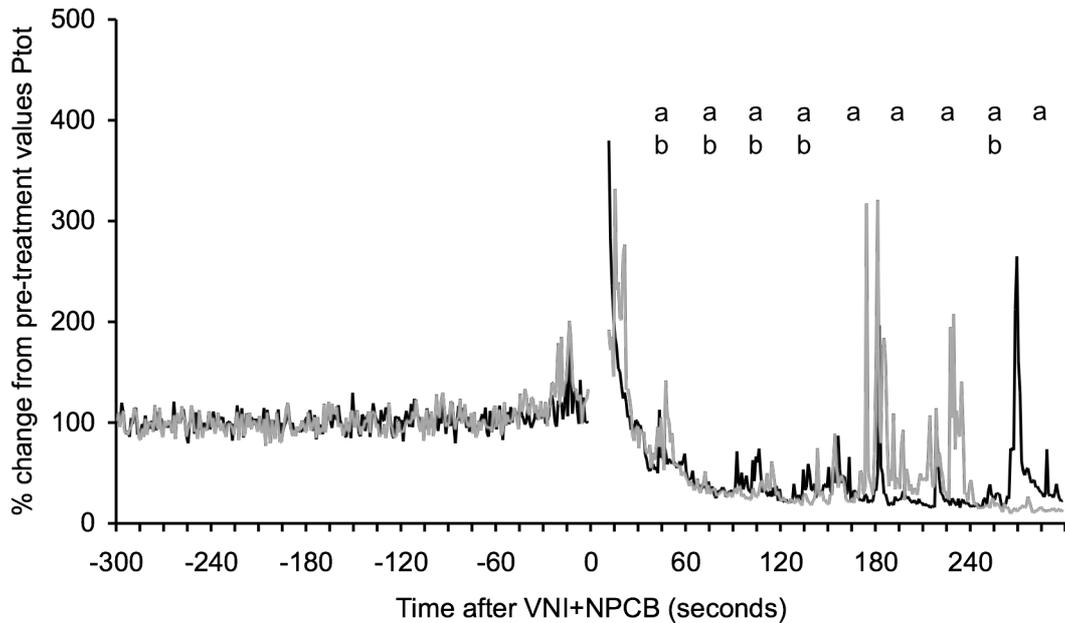


Figure 7.2. Mean changes in total power (P_{tot}) of the electroencephalogram (EEG) before and after ventral neck incision followed 5 seconds later by non-penetrative captive bolt stunning (VNI+NPCB). Right (—) and left (---) cerebral hemisphere. a = significant difference from pre-treatment values, right cerebral hemisphere ($P < 0.05$), b = significant difference from pre-treatment values left cerebral hemisphere ($P < 0.05$).

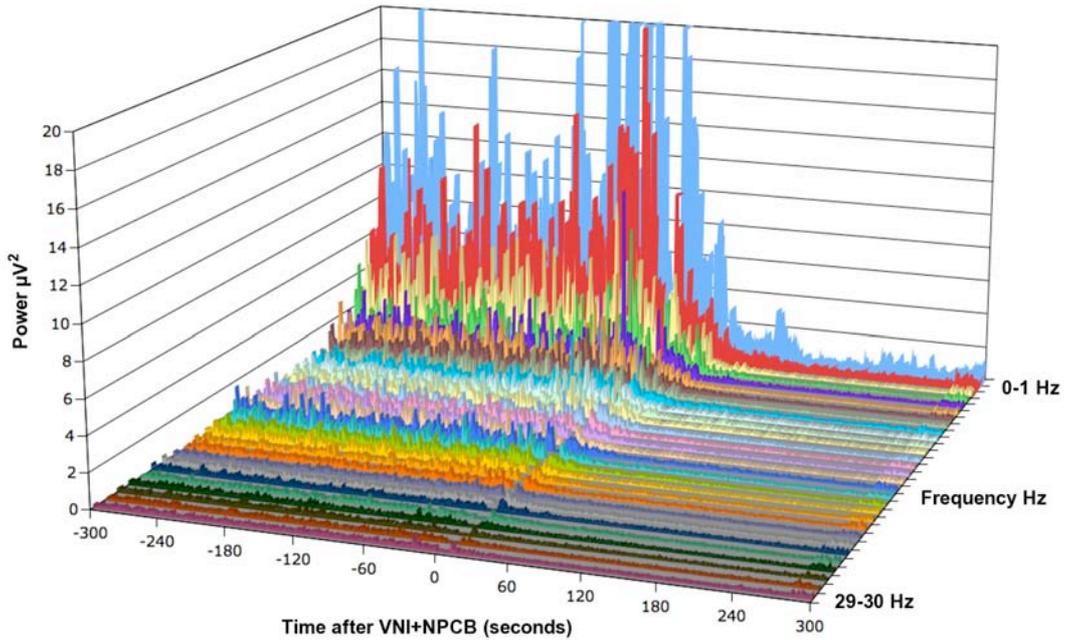


Figure 7.3. A typical example of a compressed spectral array from an individual calf before and after ventral neck incision followed 5-seconds latter by non-penetrative captive bolt stunning (VNI+NPCB).

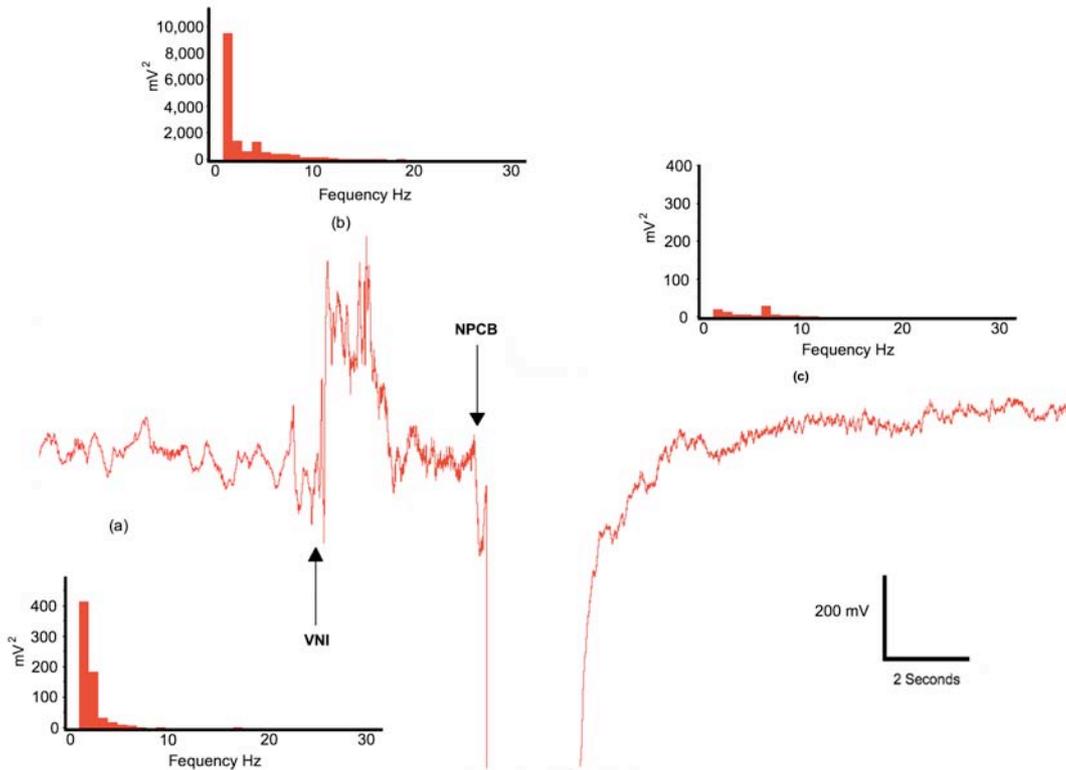


Figure 7.4. An example of the spontaneous electroencephalogram (EEG) of an individual calf before, during and after ventral neck incision (VNI) and non-penetrative captive bolt (NPCB) stunning, with individual power spectrums for two-second epochs during (a) pre-treatment electroencephalogram, (b) VNI artefact, and (c) transitional electroencephalogram after NPCB stunning. Arrows represent the VNI and NPCB.

Figure 7.4 is an example of a 25 second section of EEG with corresponding EEG power spectrum curves (2 second epochs) from an individual calf before and after VNI+NPCB. The initial period (a) illustrates pre-treatment EEG activity, (b) VNI artefact and (c) post-treatment transitional EEG. The first arrow shows the point of VNI and the second the point of the NPCB stun 5 seconds after VNI. After the period of VNI-related artefact, the EEG had similar amplitude to the pre-treatment period but contained a greater proportion of higher frequencies. NPCB stunning resulted in a period of out of range data, followed by an alteration in morphology to transitional EEG. Power across all frequencies was distinctly increased during VNI artefact (b), compared to that before treatment (a) or to that of the post-treatment transitional EEG (c). EEG power, principally of the lower frequencies (0-2 Hz), was significantly decreased in the post-treatment transitional EEG (c) compared to pre-treatment recordings (a).

Figure 7.5 examines the characteristics and time of onset of different periods of cortical activity based on visual inspection of the spontaneous EEG waveform. Following VNI there were periods of active EEG in some calves. After NPCB stunning the waveform was out of range for a brief period in all calves. There was considerable variation in the type of cortical activity following VNI+NPCB. In two calves there were periods of active EEG after NPCB stunning. In the remaining animals, following the out of range data after the stun there was depressed cortical activity in the form of transitional EEG, HALF or isoelectric waveforms.

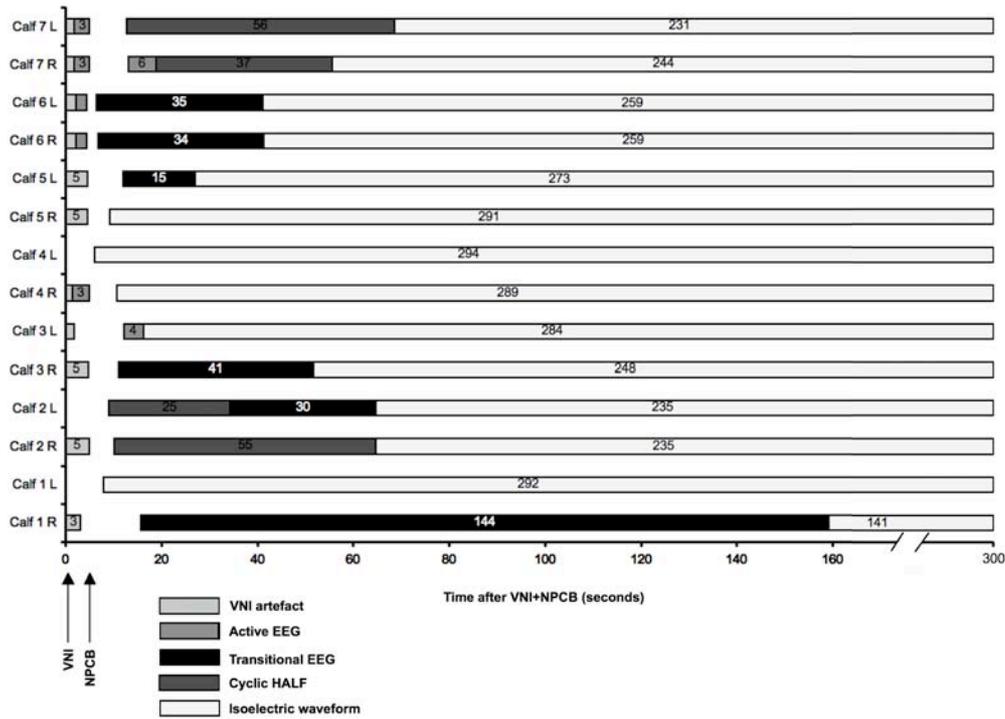


Figure 7.5. Characteristics of the spontaneous electroencephalogram (EEG) in individual animals over time (seconds) after ventral neck incision followed 5 seconds later by non-penetrative captive bolt stunning (VNI+NPCB). The spontaneous traces were visually inspected and were either classified as: VNI artefact, active EEG, transitional EEG, cyclic HALF, and isoelectric waveform. Arrows represent time point of VNI and NPCB. R represents EEG recorded from the right and L the EEG recorded from the left side of the cranium. Time durations in seconds are displayed on the individual bars; clear gaps represent periods where the EEG recording was out of range.

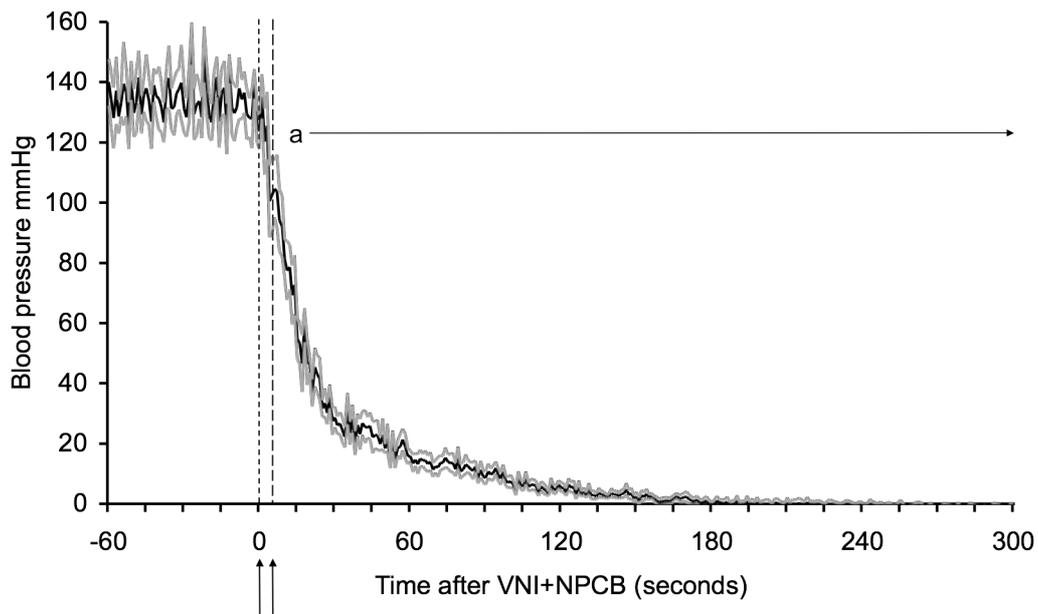


Figure 7.6. Mean \pm standard error of the mean (—) changes in femoral arterial blood pressure (mmHg) before and after ventral neck incision followed 5 seconds later with a non-penetrative captive bolt stun (VNI+NPCB). (a) = significant difference from pre-treatment values ($P < 0.05$). Short dashes represent VNI, long dashes represent NPCB.

Femoral arterial blood pressure decreased significantly after VNI+NPCB ($P < 0.05$) (figure 7.6). From pre-treatment values of 136 mmHg (SD 22), arterial blood pressure was reduced to 29 mmHg (SD 10) 30 seconds post VNI+NPCB. Sixty, 120 and 180 seconds post VNI+NPCB arterial blood pressure was 16 mmHg (SD 5), 5 mmHg (SD 4) and 0 mmHg (SD 3) respectively. The decrease in P_{tot} seen in response to VNI+NPCB was significantly correlated with the fall in blood pressure (right side: $r = 0.5202$ (95% confidence interval (CI) 0.4276 to 0.6019) ($P < 0.0001$); left side: $r = 0.5641$ (CI 0.4771 to 0.6401) ($P < 0.0001$)). There were no significant differences in heart rate from pre-treatment values in the initial 105 seconds after VNI+NPCB (Table 7.1). Thereafter, heart rate significantly decreased from pre-treatment values and continued to decrease throughout the remainder of the recording period ($P < 0.05$).

Table 7.1. Mean \pm standard error of the mean, changes in heart (beats per minute) at time points after ventral neck incision followed 5 seconds later by non-penetrative captive bolt stunning (VNI+NPCB) of calves.

Time after VNI+NPCB (seconds)	Heart rate (bpm) (\pm SEM)	P-value (difference from pre-treatment values)
-15 ^a	100.40 \pm 5.91	na
0 ^b	100.00 \pm 5.30	0.9155
15	89.40 \pm 5.52	0.2045
30	85.80 \pm 6.04	0.0907
45	84.40 \pm 5.43	0.0897
60	82.80 \pm 4.85	0.0907
75	81.60 \pm 5.46	0.0907
90	79.60 \pm 5.65	0.0714
105	78.80 \pm 5.69	0.0714
120	76.80 \pm 5.54	0.0345 ^c
135	71.80 \pm 5.64	0.0196 ^c
150	65.40 \pm 6.76	0.0200 ^c
165	60.60 \pm 7.85	0.0196 ^c
180	59.60 \pm 7.88	0.0200 ^c
300 (5 minutes)	54.80 \pm 5.43	0.0122 ^c

^a Pre-treatment

^b Ventral neck incision followed 5 seconds later by non-penetrative captive bolt stunning (VNI+NPCB)

^c Significant difference from pre-treatment values ($p < 0.05$)

7.5 Discussion

The application of the NPCB stun 5 seconds after VNI produced a decrease in P_{tot} and a change in spontaneous EEG waveforms incompatible with sensibility in the majority of animals. These EEG changes were evident immediately after the short period of artefact.

Following slaughter by VNI without stunning there is a period during which the animal is likely to be sensible (Levinger 1961; Nangeroni and Kennett 1964; Blackmore and Newhook 1981; Newhook and Blackmore 1982; Blackmore et al 1983, 1984; Gregory and Wotton 1984; Daly et al 1988; Bager et al 1992) and, as shown (Chapter 4), would therefore experience pain and distress until insensibility supervenes. The use of NPCB stunning has been demonstrated to alter cerebrocortical function (Chapter 6) and result in insensibility in calves (Blackmore 1979; Blackmore and Newhook 1982; Finnie 1995). In the current experiment there were periods of active EEG after VNI and prior to NPCB in three calves. During this period the calves may have been potentially sensible and responsive to noxious stimuli had they not been anaesthetised. After VNI+NPCB cortical function was altered in all but two animals from active EEG to transitional, cyclic or isoelectric waveforms. These EEG states have been previously demonstrated as incompatible with sensibility (Lambooy and Spanjaard 1981; Blackmore and Delany 1988; Shaw 2002) (Chapter 6).

Based on these results it appears that NPCB stunning can produce insensibility in calves after VNI. This work adds to the growing body of evidence of the effectiveness of NPCB stunning in rendering animals insensible prior to slaughter and validates the use of post VNI stunning as a means of rendering calves insensible to the noxious sensory inputs resulting from VNI. This is supported by the work, which showed that penetrative captive bolt (PCB) stunning performed after slaughter prevented the rise in noradrenaline seen after Shechita slaughter (Petty et al 1994). This suggests that post VNI stunning may reduce the stress response caused by Shechita slaughter. Alternatively the post slaughter stun may have resulted in the inhibition of subconscious reflex responses to hypotension (Gregory 2007). Both explanations provide evidence of the potential effectiveness of a post incision stun in either reducing the stress response or resulting in the major disruption of brain function down to the level of subcortical structures.

In the current study two calves had a period of unilaterally active EEG after VNI+NPCB (duration of 4 and 6 seconds). The period of active EEG was only observed on one hemisphere of each animal with the other hemisphere showing either an isoelectric or cyclic HALF waveform. Incorrect positioning of the stunner on the animal's head, a deflected shot or malfunctioning stunner itself may have been responsible for this period of active EEG. Conversely this period of active EEG could be similar to the short period of excitation previously described following PCB stunning (Blackmore and Delany 1988). Furthermore, after PCB stunning in cattle, visual evoked potentials have been observed following stunning (Daly et al 1987). In the current experiment it is unclear whether this period of active EEG following VNI+NPCB represents cortical activity capable of supporting sensibility, as all calves in the experiment were anaesthetised.

The initial transient increase in P_{tot} following VNI+NPCB mirrored the profile of the response to VNI without prior stunning (Chapter 4). This response was also seen at a lesser magnitude following sham incision (Chapter 4). The similarity between these results suggests that movement artefact during and after VNI was primarily responsible for the transient increase in P_{tot} . NPCB stunning alone did not result in a significant increase in P_{tot} (Chapter 6), rather it resulted in an immediate decrease in P_{tot} , which represented a disruption of cerebrocortical activity. In the current study P_{tot} cannot be used to assess functional cortical activity in the initial period after VNI as the movement response to neck incision masks any such changes.

Carotid occlusion occurred in 3 of the 7 animals in the current study. Carotid occlusion of the severed carotid arteries has the potential to impair bleed-out and provide conditions for the resumption or maintenance of the cortical function necessary for sensibility. Cattle are principally at risk because of the ability of the vertebral arteries to supply blood to the entire bovine brain through the occipito-vertebral anastomosis. The prevalence of carotid occlusion in 576 cattle slaughtered at abattoir in the UK was found to be 16% and 25% for adult cattle and bobby calves respectively (Gregory et al 2006). In the current experiment, however, the blood pressure profile of calves with carotid occlusion was the same as that of un-occluded animals. Furthermore, there were no visually evident or statistically significant differences between EEG responses of calves with occluded and non-occluded carotid arteries. This suggests that VNI+NPCB resulted in either non-reversible stun-induced

insensibility or that the early blood loss was sufficient to cause cerebrocortical anoxia prior to the formation of carotid occlusion.

Mean arterial blood pressure after VNI+NPCB was similar to that seen following VNI alone (Chapter 4), suggesting that there is no basis for the claim that stunning impedes blood loss (Levinger 1995). This supports work in cattle, which has shown that Halal slaughtered cattle (un-stunned) showed no difference in rate of blood loss when compared to cattle stunned percussively prior to slaughter (Anil et al 2006).

In conclusion, NPCB stunning 5 seconds after VNI is effective in disrupting cerebrocortical activity. Based on these and previous results, it is probable that during the 5 seconds before NPCB stunning, calves would be both sensible and responsive to noxious sensory input, but NPCB stunning would render the animals insensible for the remainder of the period of potential sensibility following VNI, and would thus lead to a significant improvement in the welfare of the animals compared to that with VNI alone.

7.6 References

- Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Weaver HR, Erdogan O, Hughes S, Mason C.** Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: exsanguination and quality parameters. *Animal Welfare* 15, 325-30, 2006
- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP.** Onset of insensibility at slaughter in calves - effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162-73, 1992
- Blackmore DK.** Non-penetrative percussion stunning of sheep and calves. *Veterinary Record* 105, 372-5, 1979
- Blackmore DK, Newhook JC.** Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* 29, 219-22, 1981
- Blackmore DK, Newhook JC.** Electroencephalographic studies of stunning and slaughter of sheep and calves .3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Science* 7, 19-28, 1982
- Blackmore DK, Newhook JC, Grandin T.** Time of onset of insensibility in 4-week-old to 6-week-old calves during slaughter. *Meat Science* 9, 145-9, 1983
- Blackmore DK, Delany MW.** Slaughter of stock, a practical review and guide, Veterinary Continuing Education Massey University, Palmerston North, 1988
- Daly CC, Gregory NG, Wotton SB.** Captive bolt stunning of cattle - effects on brain-function and role of bolt velocity. *British Veterinary Journal* 143, 574-80, 1987
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325-9, 1988
- Finnie JW.** Neuropathological changes produced by nonpenetrating percussive captive bolt stunning of cattle. *New Zealand Veterinary Journal* 43, 183-5, 1995
- Fricker CH, Riek W.** Die betäubung von rindern vor dem schlachten mit hilfe des bolzenschuß-apparates. *Fleischwirtschaft* 61, 124-7, 1981
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141-3, 1984

- Gregory NG, Wotton SB.** Comparison of neck dislocation and precussion of the head on visual evoked responses in the chicken's brain. *Veterinary Record* 126, 570-2, 1990
- Gregory NG, Shaw FD, Whitford JC, Patterson-Kane JC.** Prevalence of ballooning of the severed carotid arteries at slaughter in cattle, calves and sheep. *Meat Science* 74, 655-7, 2006
- Gregory N.** Stunning and slaughter. In: *Animal Welfare and Meat Production*. Pp 191-212. CABI Publishing, Wallingford, 2007
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1-9, 2005
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433-7, 2005a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and analgesia* 32, 61-71, 2005b
- Kongara K.** Studies on renal safety and preventive analgesic efficacy of Tromadrox and parecoxib in dogs. PhD thesis, Massey University, Palmerston North, NZ, 2008
- Lambooy E, Spanjaard W.** Effect of the shooting position on the stunning of calves by captive bolt. *Veterinary Record* 109, 359-61, 1981
- Levinger IM.** Untersuchungen zum schächtproblem. Dr thesis, University of Zürich, Zürich, 1961
- Levinger IM.** Shechita in the light of the year 2000, critical review of the scientific aspects of methods of slaughter and Shechita, Maskil L'David, Jerusalem, 1995
- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- Mellor DJ, Littin KE.** Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13, S127-S32, 2004

- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-21, 2005
- Murrell JC, Mitchinson SL, Waters D, Johnson CB.** Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* 98, 366-71, 2007
- Nangeroni LL, Kennett PD.** Electroencephalographic studies of the effects of shechita slaughter on cortical function in ruminants. Cornell University, New York, 1964
- NAWAC.** Animal welfare (commercial slaughter) code of welfare 2006 public draft. In. Pp 1-65. (New Zealand) 2006
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295-300, 1982
- Petty DB, Hattingh J, Ganhao MF, Bezuidenhout L.** Factors which affect blood variables of slaughtered cattle. *Journal of the South African Veterinary Association* 65, 41-5, 1994
- Shaw NA.** Neurophysiology of concussion. *Progress in Neurobiology* 67, 281-344, 2002

CHAPTER 8

**INHERENT VARIABILITY OF ACUTE
ELECTROENCEPHALOGRAPHIC RESPONSES TO
NOXIOUS SUPRAMAXIMAL ELECTRICAL STIMULI IN
CALVES**



Formatted for submission to a peer-reviewed scientific journal.

8.1 Abstract

AIM: The aim of the study was to examine the inherent variability associated with the electroencephalogram (EEG) in the minimal anaesthesia model during investigation of noxious sensory input in calves.

METHODS: Thirty-nine calves were maintained under a stable plane of halothane anaesthesia and a supramaximal electrical stimulus of 70-volts was delivered to the left shoulder.

RESULTS: The supermaximal 70-volt electrical stimulus resulted in significant increases in median frequency (F50) and spectral edge frequency (F95), and a significant decrease in the total power (P_{tot}) of the EEG ($P < 0.05$).

DISCUSSION: Changes in the EEG in response to noxious sensory input with a sample size of 39 animals were quantitatively and qualitative similar to those observed previously in a smaller number ($n=10$) cattle. Increasing the study sample sizes did reduce the inherent variability of the EEG parameters measured, but the improvements in statistical power over smaller sample sizes were minimal.

8.2 Introduction

The minimal anaesthesia model and EEG power spectrum have been used in the investigation of noxious stimuli in a number of different species (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab; McGregor 2005; Murrell et al 2007; Diesch et al 2008; Kongara 2008). This work has allowed the investigation of painful events without compromising the welfare of the animals involved. The model has provided important information on the functional cortical processing of noxious sensory input and allows the detailed statistical quantification of the magnitude of responses to potentially welfare compromising procedures. As more species are investigated the understanding of the dynamics of the response to noxious sensory input in the EEG power spectrum has increased. However, there are some limitations to the model. The EEG and its power spectrum are inherently variable, requiring sufficient sample sizes to detect statistically significance changes or effects. Fundamental research using small laboratory mammals has allowed the use of larger sample sizes, because of the relatively small unit cost of such animals. However, animal welfare research is often performed on species that as individuals are more costly or are otherwise highly valued, such as production animals or companion animals. Also, some of the experimental manipulations require the animals to be euthanased. Cost or the emotional value of such animals may therefore preclude the use of more than the absolute minimum number required to detect an anticipated effect, thereby resulting in the use of small sample sizes. This raises the question of how EEG measurement itself may influence the number of animals required in a study.

The EEG is prone to contamination from movement, electromyographic (EMG) activity, 50 Hz mains electrical noise and radiating electromagnetic radiation. The EEG signal is in the range of 100 mv. Contamination of the signal can result in artefacts of several volts in magnitude or overwhelm the amplifiers and filters, masking EEG data. EEG waveforms that are highly contaminated or have large periods of out of range data, either have these periods removed or the entire trace is rejected from further analysis. This reduces the useable sample size and the statistical power.

Supramaximal electrical stimuli have been previously used in the investigation of noxiousness using EEG spectral analysis in anaesthetized rats (Barter et al 2005; Orth et al 2005; Murrell et al 2007) and dogs (Kongara 2008). The advantages of a supramaximal noxious electrical stimulus and the minimal anaesthesia model are that the stimulus is controlled, quantifiable and reproducible (Murrell et al 2007) and allows investigation of a noxious sensory input without causing major tissue damage. The minimal anaesthesia model has been previously validated in a range of species for the investigation of noxious sensory input: calves (Chapters 3-7), horses (Murrell et al 2003, 2005), sheep (Johnson et al 2005a), red deer (*Cervus elaphus*) (Johnson et al 2005b), rats (McGregor 2005; Murrell et al 2007; Diesch et al 2008), wallabies (*Macropus eugenii*) (Diesch et al 2008), dogs (Kongara 2008) and pigs (Haga and Ranheim 2005). These studies have demonstrated EEG indices that are associated with noxious sensory input: median frequency (F50), 95% spectral edge frequency (F95) and total power (Ptot) of the EEG. Increases in F50, F95 and decreases in Ptot have been generally associated with noxious sensory input (Murrell and Johnson 2006).

The aims of this study were to examine the inherent variability of the EEG response to an acute supramaximal noxious electrical stimulus applied to minimally anaesthetised calves, and, using published information, to compare the EEG response to this stimulus with that in other species.

8.3 Materials and Methods

8.3.1 Animals

Electrical stimulation was performed in all animals in the present study prior to their use in the other experiments described here (Chapters 4, 6 and 7). Prior to electrical stimulation no other major manipulations were performed on the animals, apart from anaesthesia, EEG, electrocardiogram (ECG) and femoral arterial blood pressure instrumentation. Thirty-nine calves of between 6 to 11 months of age, mixed breed and sex, weighing between 102 and 207 kg were sourced from a commercial

stock agent. Calves had access to water but not food 24 hours prior to experimentation. All manipulations were approved by the Massey University Animals Ethics Committee (Protocol No. 04/86 and 06/61).

8.3.2 Anaesthesia

Anaesthesia was induced by the intravenous injection of ketamine (3.7 (SD 0.6) mg/kg) (Parnell Laboratories, Auckland NZ) and propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) (6.6 (SD 2.4) mg/kg) to effect. Endotracheal intubation was performed with a 16 mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia). Anaesthesia was maintained with halothane (Halothane-Vet; Merial NZ Ltd, Manukau City, NZ), (Halothane BP; Nicholas Piramal India Ltd, Chennai, India) vaporized in oxygen (Air Liquide, Palmerston North, NZ) with a precision vaporiser (Fluothane; MedSource Ltd, Ashburton, NZ) and circle breathing system (VMS Anaesthesia Machine; Matrix Medical Inc, New York, USA). End tidal halothane tension ($FE'HAL$), end tidal carbon dioxide tension, heart rate and respiratory rate were monitored using an anaesthetic agent monitor (Hewlett Packard M1025B, Hamburg, Germany). Calves were allowed to breath spontaneously during the experiment and $FE'HAL$ was maintained at 0.90% throughout experimental recording.

8.3.3 EEG, ECG and Arterial Blood Pressure Recoding

The EEG was recorded bilaterally with subdermal 27-G stainless-steel needle electrodes (Viasys Healthcare, Surrey, England) placed in three electrode montages. The non-inverting (active) electrodes were placed in the midline between the medial canthi of the eyes, the inverting (reference) electrodes over the left and right mastoid processes, and a common ground electrode caudal to the poll, this montage recorded EEG from the left and right cerebral hemispheres. This configuration has been previously demonstrated to produce high quality signals with low electrode

impedance (2 to 4 k Ω) (Chapter 3). The EEG, ECG and femoral arterial blood pressure were recorded as previously described (Chapters 3-7)

8.3.4 Experimental Procedure

After induction of anaesthesia calves were weighed and placed in dorsal recumbency on a specially designed bed (Massey University Mechanical Services; Palmerston North, NZ). Two silver/silver chloride stimulating electrodes (custom built, Massey University, Palmerston North, NZ) were placed subdermally 20 mm apart on the left shoulder of each animal. EEG, ECG and femoral arterial cannulation were performed. Ten minutes were allowed for equilibration of general anaesthesia (figure 8.1), after which a 10-minute pre-treatment trace was recorded. Then a 70-volt electrical stimulus was applied to the left shoulder of each animal for 2 seconds with a S48K square pulse electrical stimulator (Grass Technologies, Astro-Med Inc, West Warwick, Rhode Island USA). The EEG was recorded for a further 5 minutes, after which the animals were used in different experiments.

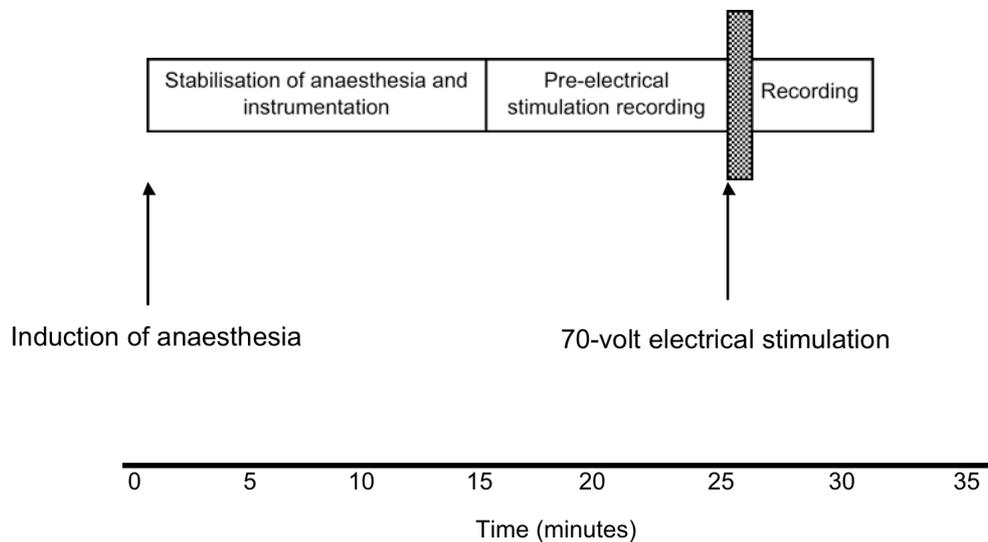


Figure 7. Experimental design, after induction of anaesthesia at 0 minutes, showing stabilisation of anaesthesia, instrumentation, pre-treatment recording, the 70-volt supramaximal electrical stimulus (grey vertical box) and the post-treatment recording.

8.3.5 Data and Statistical Analysis

EEG, ECG and arterial blood pressure data analyses were performed after completion of data collection as previously described (Chapters 3-7). EEG data derived from 2 seconds before to 5 seconds after electrical stimulation were excluded from analysis to prevent contamination by movement and electrical stimulus artefact. All data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA) and Prism 4.0c (GraphPad Software Incorporated, San Diego, CA, USA). The distribution of the data was tested for normality with the Anderson-Darling test. EEG power spectrum indices are graphically represented as the initial acute response (interval plot) or as a time series.

The analysis of differences between post and pre-treatment values for F50, F95 and Ptot was performed on consecutive non-overlapping blocks of 30 seconds with the non-parametric Mann-Whitney test. Blood pressure values had a Gaussian distribution, while heart rate had a non-Gaussian distribution. Blood pressure and heart rate are displayed as mean values \pm SEM. Individual time points every 15 seconds post-treatment were compared with pre-treatment values of blood pressure and heart rate with either a one-sided analysis of variance (ANOVA) or Mann-Whitney test respectively. Values of $P < 0.05$ were taken to indicate statistical significance in all analyses.

8.4 Results

Some EEG traces recorded from the right ($n=5$) and left ($n=6$) cerebral hemispheres were removed from further analysis due to large periods of out of range data at the point of stimulation. Two calves displayed no EEG response to electrical stimulation and were not included in analysis. There was no presence of epileptiform-like activity (high amplitude low frequency) after electrical stimulation in any EEG traces.

The 70-volt electrical stimulus caused an immediate significant increase in F50 and F95 in both hemispheres and a significant decrease in Ptot in the right

cerebral hemisphere ($P < 0.05$) (figure 8.2) from pre-treatment values ($n=32$). F50 increased from pre-treatment values of 100 (SD 20)% to 150 (SD 60)% in the initial 30 seconds following electrical stimulation (figure 8.2 and 8.3). F95 significantly increased from pre-treatment values of 98 (SD 2)% to 108 (SD 9)% during the 30 seconds after electrical stimulation (figure 8.2 and 8.4). Ptot significantly decreased from the pre-treatment values of 95 (SD 9)% to 84 (SD 27)% during the first 30 seconds after electrical stimulation ($P < 0.05$) in the right cerebral hemisphere (figure 8.2 and 8.5). The decrease was transient with a returned to pre-treatment values by 120 seconds, followed by a further decrease between 210 and 270 seconds post electrical stimulation ($P < 0.05$) (figure 8.5).

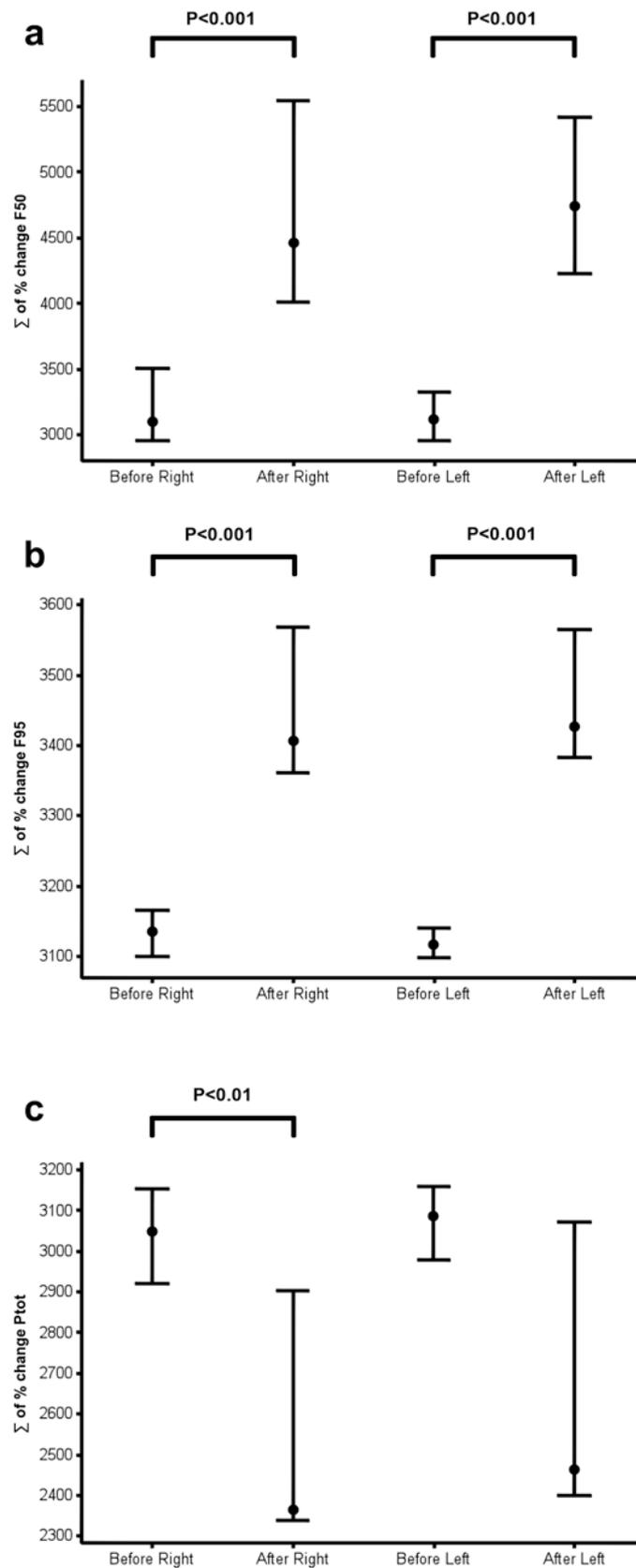


Figure 8.2. Interval plots showing the medians and 95% confidence intervals of the initial EEG response (right and left cerebral hemispheres): data summed (Σ) from 30 seconds before and after application of a 70-volt supramaximal electrical stimulus to the left shoulder of calves. F50 (a), F95 (b) and Ptot (c).

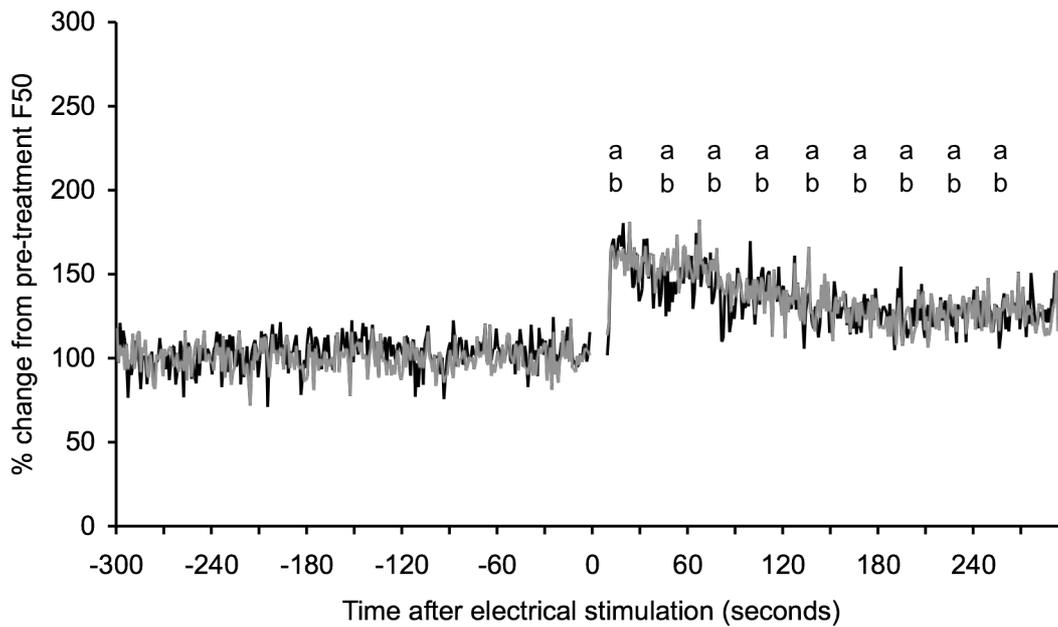


Figure 8.3. Mean changes in median frequency (F50) after a 70-volt supramaximal electrical stimulus applied at 0 seconds to the left shoulder of calves. Right (—) and left (---) cerebral hemisphere. a = significant difference from pre-treatment values, right cerebral hemisphere ($P < 0.05$), b = significant difference from pre-treatment values, left cerebral hemisphere ($P < 0.05$).

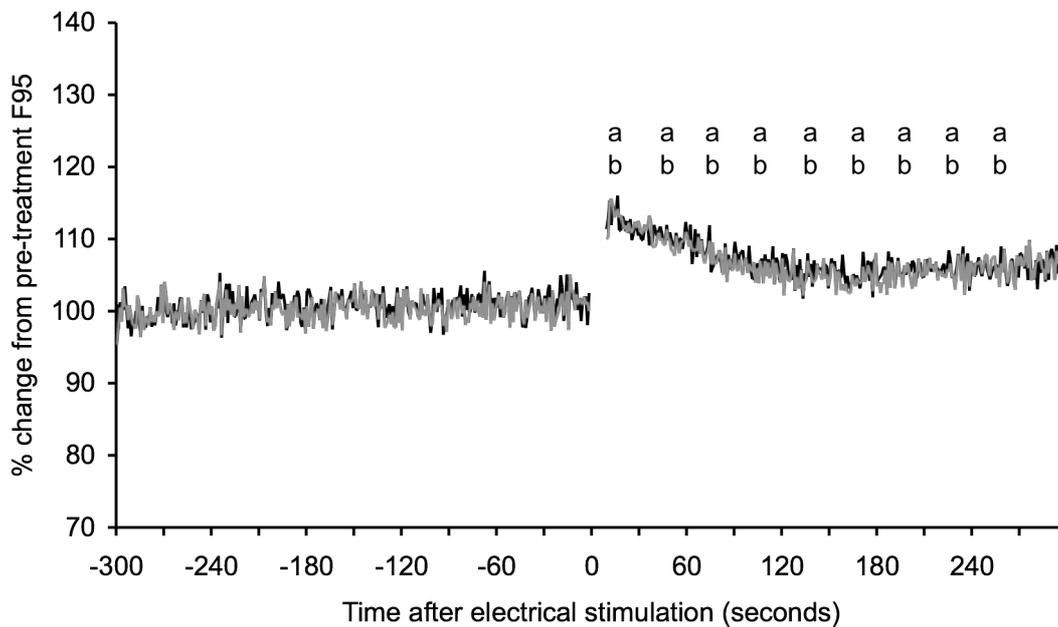


Figure 8.4. Mean changes in 95% spectral edge frequency (F95) after a 70-volt supramaximal electrical stimulus applied at 0 seconds to the left shoulder of calves. Right (—) and left (---) cerebral hemisphere. a = significant difference from pre-treatment values, right cerebral hemisphere ($P < 0.05$), b = significant difference from pre-treatment values, left cerebral hemisphere ($P < 0.05$).

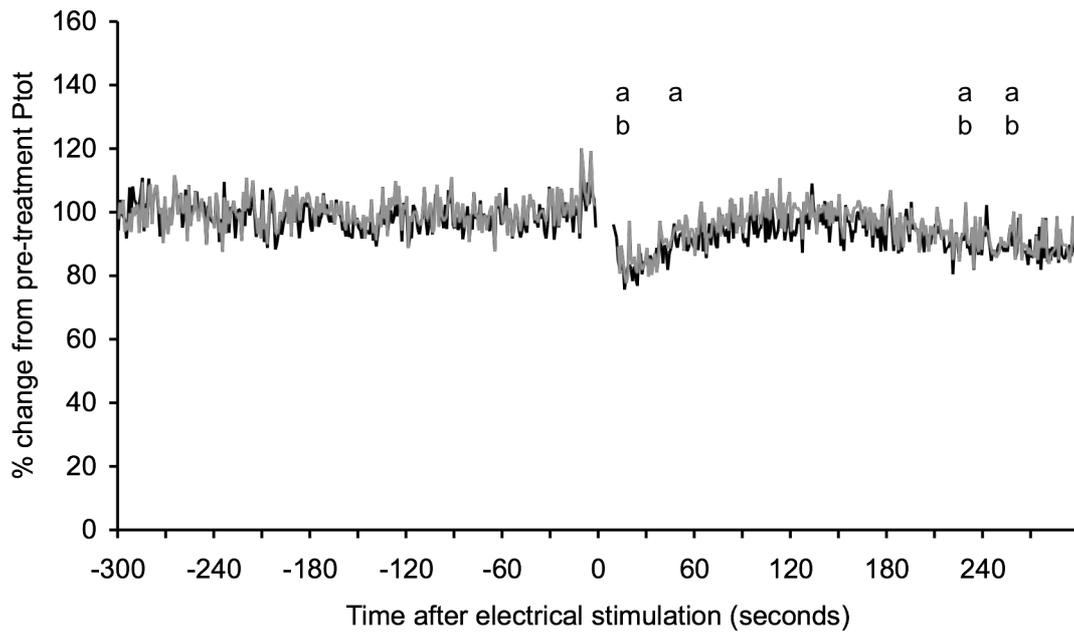


Figure 8.5. Mean changes in total power (P_{tot}) after a 70-volt supramaximal electrical stimulus applied at 0 minutes to the left shoulder of calves. Right (—) and left (---) cerebral hemisphere. a = significant difference from pre-treatment values, right cerebral hemisphere ($P < 0.05$), b = significant difference from pre-treatment values, left cerebral hemisphere ($P < 0.05$).

Heart rate data from 10 calves and blood pressure data from 3 were removed from further analysis due to equipment malfunction, stimulus artefact or unacceptable external noise. After electrical stimulation there was no significant change in either blood pressure (mmHg) ($n=36$) or heart rate (beats per minute (BPM)) ($n=29$) (table 8.1) over the entire recording period.

Table 8.1 Mean \pm standard error of the mean (SEM), changes in heart rate (beats per minute (bpm)) and blood pressure (mmHg) at different time points after a 70-volt supramaximal electrical stimulus. a = pre-treatment value, b = 70-volt supramaximal electrical stimulus.

Time after VNI+NPCB (seconds)	Heart rate (\pm SEM) (bpm)	Heart rate P-values (difference from pre-treatment values)	Blood pressure (\pm SEM) mmHg	Blood pressure P-values (difference from pre-treatment values)
-15 ^a	110.28 \pm 6.60	na	123.99 \pm 4.58	na
0 ^b	98.48 \pm 5.79	na	122.58 \pm 4.77	0.832
15	99.72 \pm 7.41	0.253	119.74 \pm 4.89	0.528
30	101.07 \pm 5.55	0.380	117.77 \pm 4.61	0.342
45	103.00 \pm 5.43	0.499	119.96 \pm 3.96	0.508
60	95.97 \pm 5.60	0.597	119.31 \pm 4.56	0.472
75	97.21 \pm 5.56	0.116	118.85 \pm 3.95	0.399
90	100.48 \pm 5.54	0.150	118.66 \pm 3.75	0.371
105	97.66 \pm 5.84	0.367	122.53 \pm 4.87	0.828
120	96.93 \pm 5.16	0.211	122.21 \pm 3.73	0.764
135	95.17 \pm 5.61	0.113	124.31 \pm 4.31	0.959
150	98.21 \pm 5.23	0.084	119.55 \pm 4.02	0.469
165	96.69 \pm 5.58	0.155	123.99 \pm 4.35	0.999
180	97.66 \pm 5.00	0.131	121.53 \pm 4.47	0.702
300 (5 minutes)	110.28 \pm 6.60	0.084	118.26 \pm 5.03	0.402

^a Pre-treatment values

^b Electrical stimulation

8.5 Discussion

The EEG response to a supramaximal 70-volt electrical stimulus in 32 calves was quantitatively and qualitatively similar to the response observed in calves after dehorning without a local anaesthetic ring block (Chapter 3) and the changes in F95 seen after VNI and neck tissue incision (Chapters 4 and 5 respectively). The responses were also similar to those seen after other forms of noxious stimulation in a number of species (Murrell et al 2003; Haga and Ranheim 2005; Johnson et al 2005ab; Murrell et al 2005, 2007; Kongara 2008). This study demonstrates that the data acquired from small sample sizes is robust in that they are similar to the data generated from studies with larger sample sizes. More specifically, the use of sample sizes of 10 animals produces reliable and reproducible results for EEG responses to noxious stimulation (Chapter 3, 4).

The electrical stimulus in the present experiment was given to the left shoulder while the animals were anaesthetised and in dorsal recumbency. The stimulus initially produced a period of interference in the EEG and ECG. Potentially, sufficient current might have passed through the brain to affect cortical function. However, no epileptiform-like activity was observed after electrical stimulation.

Electrical stunning is used to render some species of farmed animals insensible prior to slaughter. A high voltage current is passed through the brain to render the animal insensible. Epileptiform activity is seen as a sign of a successful stun as it represents cortical dysfunction (Blackmore and Delany 1988; Cook et al 1991; Wotton et al 2000). Experiments into different stunning positions for humane slaughter have demonstrated that neck to brisket stunning with a 400-volt open circuit (current limited to 1.5A) electrical stimulus for 4 seconds does not produce epileptiform-like activity (Cook et al 1991). The neck electrodes in that experiment were more cranial (cervical vertebrae C2 to C5) and delivered a much higher voltage than in the present experiment. Therefore, it is not likely that the 70-volt electrical stimulus used here was sufficient to produce any period of insensibility.

Supramaximal electrical stimulation is often used to investigate noxious sensory input because of its ease of use and reproducibility. Although the stimulus used here was sufficiently noxious to elicit EEG responses, the use of electrical stimulation to investigate noxious sensory inputs has limitations. Electrical

stimulation is non-selective as it activates all peripheral afferent fibres and can bypass normal nociceptive pathways (Murrell et al 2007). Although the electrical stimulus in the present study produced EEG responses indicative of noxiousness it is highly likely that the stimulus recruited afferent fibres carrying both noxious and non-noxious impulses to the cerebral cortex (Murrell et al 2007). Furthermore, in two calves the electrical stimulus was not sufficient to produce noxious-like responses in the EEG. Insufficient recruitment of nociceptors by electrical stimuli can result in impulses not being propagated by afferent nerve fibres. To reduce the likelihood of this occurrence a supramaximal electrical stimulus of 70-volts was used in this experiment. In these two cases, however, movement of the subdermal stimulating electrodes during stimulation may have resulted in an alteration in the fluid-electrode salt bridge thus effecting impedance and producing a non-supramaximal stimulus.

In conclusion, this study demonstrates that EEG responses to noxious sensory input were quantitatively and qualitatively similar to those previously observed in cattle. Increasing sample size (hence statistical power) reduced the inherent variability associated with the EEG, but the improvements in statistical power over smaller sample sizes were minimal. This study confirms and validates the previous results in cattle employing smaller numbers.

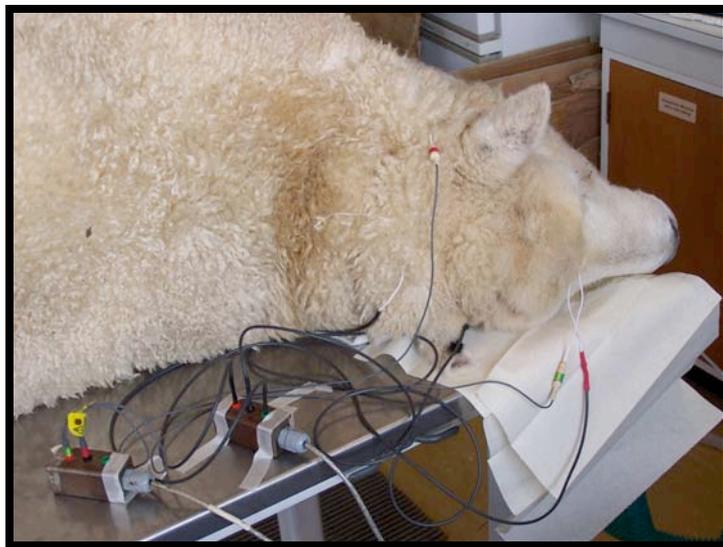
8.6 References

- Barter L, Dominguez CL, Carstens E, Antognini JF.** The effect of isoflurane and halothane on electroencephalographic activation elicited by repetitive noxious c-fiber stimulation. *Neuroscience Letters* 382, 242-7, 2005
- Blackmore DK, Delany MW.** Slaughter of stock, a practical review and guide, Veterinary Continuing Education Massey University, Palmerston North, 1988
- Cook CJ, Devine CE, Gilbert KV, Tavener A, Day AM.** Electroencephalograms and electrocardiograms in young bulls following upper cervical vertebrae-to-brisket stunning. *New Zealand Veterinary Journal* 39, 121-5, 1991
- Diesch TJ, Mellor DJ, Johnson CB, Lentle RG.** Responsiveness to painful stimuli in anaesthetised newborn and young animals of varying neurological maturity (lambs, rat pups and wallaby joeys). In: '6th World Congress on Alternatives & Animal Use in the Life Sciences, 2007'. Tokyo, Japan Pp 549-52, 2008
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1-9, 2005
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433-7, 2005a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and analgesia* 32, 61-71, 2005b
- Kongara K.** Studies on renal safety and preventive analgesic efficacy of Tromadrox and parecoxib in dogs. PhD thesis, Massey University, Palmerston North, 2008
- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-46, 2003

- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-21, 2005
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Murrell JC, Mitchinson SL, Waters D, Johnson CB.** Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* 98, 366-71, 2007
- Orth M, Barter L, Dominguez C, Atherley R, Carstens E, Antognini JF.** Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation. *British Journal of Anaesthesia* 95, 477-84, 2005
- Wotton SB, Gregory NG, Whittington PE, Parkman ID.** Electrical stunning of cattle. *Veterinary Record* 147, 681-4, 2000

CHAPTER 9

PILOT STUDY TO DEVELOP A METHOD OF GENERATING AND RECORDING SOMATOSENSORY EVOKED POTENTIALS WITHOUT SURGICALLY IMPLANTED CORTICAL ELECTRODES



Formatted for submission to a peer-reviewed scientific journal.

9.1 Abstract

AIM: This pilot study aimed to develop a methodology for generating and recording somatosensory evoked potentials (SEPs) in halothane-anaesthetised sheep using transcutaneous electrodes.

METHOD: Two ewes were anaesthetised using the established minimal anaesthesia protocol. Subdermal stimulating electrodes were positioned over the tibial nerve in one ewe and in the other the superficial peroneal nerve was exposed and stimulated. Time-linked SEPs were recorded in response to electrical stimulation of the electrodes.

RESULTS: SEPs were generated with the stimulation of the exteriorised superficial peroneal nerve but not the unexposed tibial nerve. The impact of different halothane settings on SEP latency was assessed in the former animal.

DISCUSSION: Somatosensory evoked potentials were repeatedly generated by stimulation of the exteriorised superficial peroneal nerve and were recorded using transcutaneous electrodes. The affect of increasing halothane settings was minimal subcortically and maximal cortically. This method for the generation and recording of SEPs merits further study in more animals.

9.2 Introduction

Somatosensory evoked potentials (SEPs) are time-locked responses of the electrical activity of the central nervous system (CNS) produced by peripheral stimulation. They represent the cortical processing of impulses in primary sensory pathways within the CNS. Somatosensory evoked potentials have been used to assess or monitor anaesthetic depth (Sebel 1989) and analgesia (Stienen et al 2004), neuronal integrity during surgery (Pelosi et al 2002) and intensive care (Guérit 1999), and traumatic brain injury (Shaw 1986) and loss of sensibility following exsanguination (Daly et al 1988), and to research CNS processing of pain-related inputs (Bromm and Lorenz 1998).

Somatosensory evoked potentials are small compared to the electroencephalogram (EEG) in which they are embedded and they can only be recorded using signal summation to improve the signal-to-noise ratio (Sebel 1989). Cortical responses to peripheral stimulation are in the region of two to four microvolts, whereas background EEG, electromyogram (EMG) and external electrical noise can be of the order of hundreds of microvolts. Surgical implantation of direct-contact cortical electrodes through the bones of the cranium in animals significantly improves the signal-to-noise ratio (Hashiguchi et al 2007), but is invasive, with potential to produce complicating residual effects after surgery (Kubler and Neumann 2005). Moreover, inter-species differences between the shape and size of the cranium (Murrell and Johnson 2006) and a lack of defined stereotaxic coordinates for non-standard laboratory animals complicate precise electrode placement in particular regions of the brain. However, investigation of global, as opposed to regional, cortical responses does not require such precise electrode placement. Accordingly, the aim of the current pilot study was to develop a repeatable method for generating global cortical far-field SEPs without the need to implant cortical recording electrodes.

9.3 Materials and Methods

9.3.1 Animals

Two Romany ewes sourced from the Large Animal Teaching Unit of Massey University, Palmerston North, New Zealand were kept in accordance with normal farming practice. Ewes were penned, with access to water but not food during the 24 hours prior to the study. The study was approved by the Massey University Animal Ethics Committee, Palmerston, New Zealand (Protocol no. 04/86, amendment 31/3/2006).

9.3.2 Anaesthesia

Anaesthesia was induced with a mixture of ketamine (2-3 mg/kg) (Parnell Laboratories, Auckland, NZ) and propofol (5-6 mg/kg) (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia). Once anaesthetised, endotracheal intubation was performed with an 8 mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia). Anaesthesia was maintained with halothane (Halothane-Vet; Merial NZ, Manukau City, NZ) using vaporizer settings of 1.0, 1.7, 2.1 and 2.3%, delivered via a precision vaporizer (Fluothane, MedSource Ltd, Ashburton, NZ) and a circle breathing system (VMS Anaesthesia Machine, Maxtrix Medial Inc, New York, USA). All sheep were allowed to breathe spontaneously throughout the experiment.

9.3.3 EEG and SEP Recording

Subdermal 27 gauge stainless steel needle electrode (Medelec, Radiometer Auckland New Zealand) were placed in a bilateral three-electrode montage (Mayhew and Washbourne 1990). The non-inverting (active) electrode was placed in the midline between the medial canthi of the eyes, the inverting (reference) electrodes

over the left and right mastoid processes and the ground electrode caudal to the poll. The EEG and SEP were recorded simultaneously via shared electrodes and amplifiers.

The EEG was recorded with a gain of 1,000 and pass-band of 0.1 to 500 Hz, using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers, World Precision Instruments Sarasota Florida USA). Signals were simultaneously digitised at a rate of 1 kHz (Powerlab/4sp, ADInstruments Ltd, Sydney, Australia) and recorded on two personal computers (MacBook Pro and iMac, Apple Inc, Cupertino, California, USA). One computer recorded raw EEG using the data acquisition software package Chart 5.5.1 (ADInstruments Ltd) and the other performed signal summation to generate SEPs (Scope 4.01, ADInstruments Ltd). The second computer was also used to trigger an electrical stimulator (Grass S48 Stimulator, Grass Technologies, Astro-Med Inc, West-Warwick, Rhode Island, USA).

Somatosensory evoked potentials were recorded with a recording duration of 500 ms at 4kHz. A stimulating pulse was delivered to either the unexposed tibial nerve (ewe 1) or the exposed superficial peroneal nerve (ewe 2). The pulse had an amplitude of 3-10 V, a duration of 0.5 ms and was delivered at a delay of 100 ms relative to the start of the recording window. Thirty-two successive sweeps were averaged to produce each waveform. Each recording period yielded 3 waveforms. To confirm that the origin of the recorded waveforms did not result from electrical artefact or other possible contamination, a control run was performed on the nerve preparation with a break in the stimulating circuit. Signal summation was performed using Scope, with subsequent analysis performed in Excel 2004 (Mac, Microsoft Corporation, Redmond, Washington, USA).

9.3.4 Experimental Procedure

Ewe 1

Following induction of anaesthesia, the ewe was placed in right lateral recumbency on a surgical table. Two 27 gauge stainless steel stimulating needle electrodes (Medelec, Radiometer Auckland New Zealand) were inserted subcutaneously in the region of the unexposed tibial nerve of the left hind limb,

located cranial to the calcaneal tendon (Daly et al 1988) and set approximately 20 mm apart.

Ewe 2

A single incision was made laterally on the left hind limb, 50 mm distal to the metatarsus. A 50 mm section of the superficial peroneal nerve was exposed, ligated and cut distally. The nerve was placed on a specially designed nerve bath. The bath was positioned over the dissected section of the leg with the superficial peroneal nerve laid over two silver/silver chloride stimulating electrodes (custom built, Massey University, Palmerston North, NZ) placed 5 mm apart. Proximal to the stimulating electrodes the nerve was connected to earth with a surgical swab soaked in Hartmann's solution (Baxter Healthcare Ltd, Toongabbie, NSW, Australia). The nerve and exposed tissues were moistened with Hartmann's solution to prevent dehydration and loss of electrolytes. Following stabilisation of anaesthesia, SEPs were recorded every 10 minutes. Following the end of data collection both animals were euthanized by intravenously injected sodium pentobarbitone (Pentobarb 500, National Veterinary Supplies Ltd, Auckland, NZ) prior to recovery from anaesthesia.

9.4 Results

SEPs could not be generated in Ewe 1. The methodology was subsequently altered for Ewe 2 and SEPs were successfully generated.

The stimulus artefact due to the electrical stimulus was removed from mean SEPs. Mean waveforms are displayed \pm standard error of the mean (SEM). Time point 0 denotes the point of stimulation. Peaks are designated either P (positive deflection) or N (negative deflection), and the subscript number is the time in milliseconds after stimulation. At a halothane vaporizer setting of 1%, electrical stimulation produced six distinct evoked responses in both recorded hemispheres: right cerebral hemisphere, P65, N86, P120, N189, P222, N366; left cerebral hemisphere, P65, N87, P122, N189, P224, N370) (figure 9.1). Disconnection of the stimulating circuit abolished generation of the SEP, which returned upon reconnection.

Increasing the halothane vaporizer setting to 1.7% increased the variability and amplitude in the SEP in both hemispheres. The P74, P180, P199 and P61, P182, P199 peaks from the right and left cerebral hemispheres respectively, were of a similar latency to those observed with a halothane vaporiser setting of 1%. Increasing the vaporiser setting from 1% to 1.7% increased the latency of the negative deflections from N86 (right) and N87 (left) to N114 (right) and N112 (left), respectively.

A vaporizer setting of 2.1% resulted in reduced variability and amplitude in the SEP in both hemispheres. The P64 and P66 peaks from the right and left cerebral hemispheres respectively, were similar in latency to those observed at settings of 1 and 1.7%. Increasing the vaporizer setting from 1.7% to 2.1% increased the latency of the negative deflections from N114 and N112 for the right and left cerebral hemispheres respectively to N142 and N156. A vaporizer setting of 2.3% resulted in the loss of generation of the SEP at all latencies

Following the final recording period at a vaporiser setting 2.3%, decreasing the vaporizer setting to 0.5% halothane resulted in the reappearance of SEPs after 15 minutes. This SEP was compressed but similar in morphology to that at a 1% halothane setting. Following completion of the study the ewe was euthanized with sodium pentobarbitone whilst still under anaesthesia, this abolished generation of SEPs.

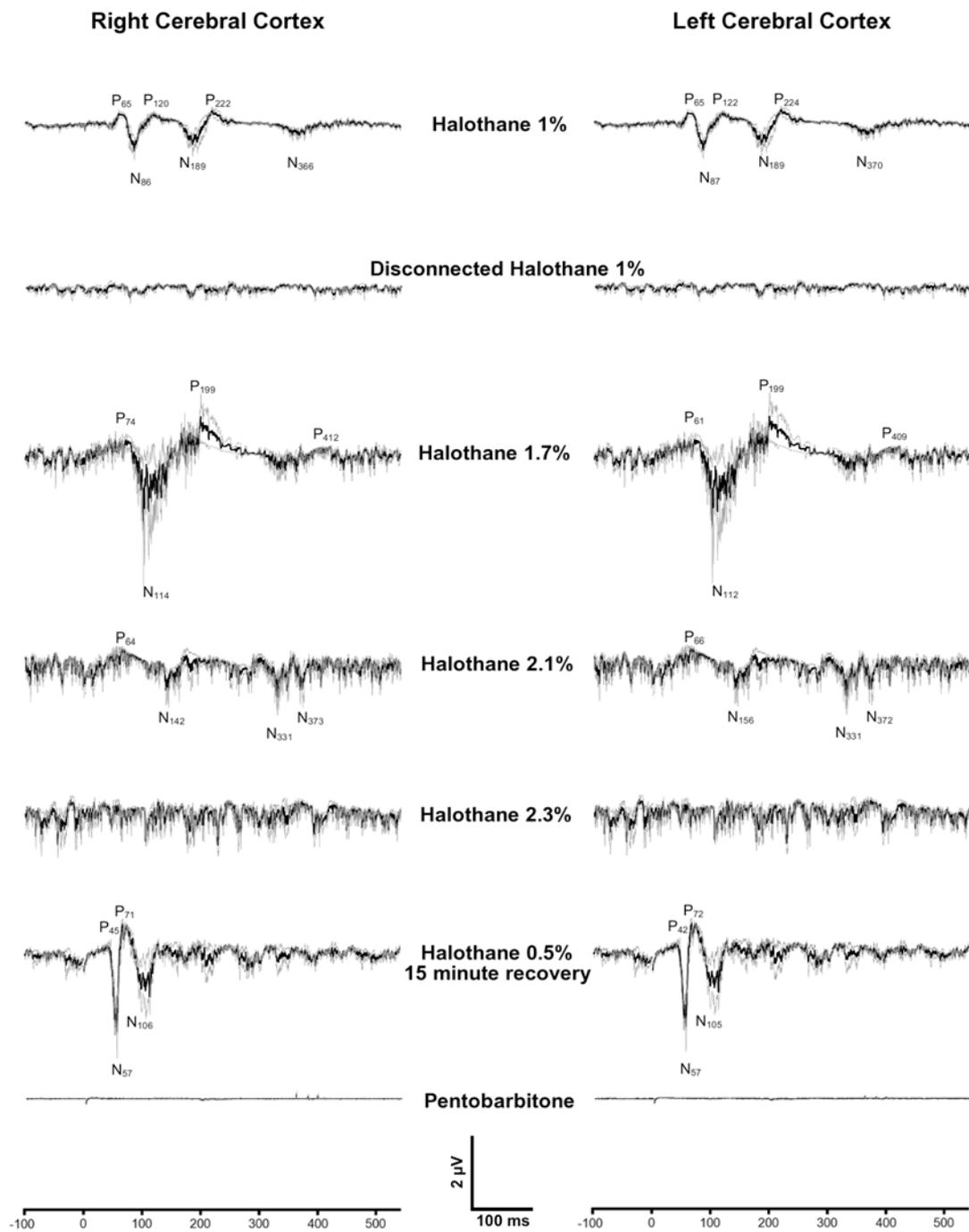


Figure 9.1. Mean somatosensory evoked potentials (—) \pm SEM (—), recorded in response to a 10-volt electrical stimulus to the exposed superficial peroneal nerve of ewe 2, at halothane vapouriser settings of 1% (connected and disconnected), 1.7%, 2.1% and 2.3%, and at 0.5% after 15-minute recovery, and following euthanasia with pentobarbitone. The electrical stimulus was delivered at 0 milliseconds with the stimulus artefact removed for clarity. Peaks are designated either P (positive deflection) or N (negative deflection), and the subscript number is the time in milliseconds after stimulation.

9.5 Discussion

This is a novel model for the recording and generating of SEPs without the need for surgical implantation of cortical electrodes in sheep. The SEPs generated in Ewe 2 with the earthed superficial peroneal nerve were repeatable, with synchronization between the right and left cerebral hemispheres. Stimulation of the tibial nerve with needle electrodes in Ewe 1 produced a waveform where the noise of the stimulus artefact masked any generated SEPs.

Increasing depth of halothane anaesthesia from a vaporiser setting 1.0 to 1.7% in Ewe 2 initially produced increased variability and amplitude in the individually recorded waveforms. However, further increasing halothane concentrations produced depression of amplitude (at 2.1%) and the eventual loss of evoked responses (at 2.3%). Similar positive mid latencies were observed with halothane vaporizer settings of 1%, 1.7% and 2.1%. These latencies represent thalamocortical (or subcortical) processing (Mauguière 1999); they were affected in amplitude and minimally in latency with increasing concentrations of halothane. Pathak et al (1989) found that the dose related effects of halothane on evoked potentials were minimal subcortically at the peripheral nerve and L3 and C6 spinal cord locations and maximal cortically. Long latencies are cortical in origin and increase with increasing concentrations of halothane (Peterson et al 1986). This was observed in the current experiment with increases in the initial negative latencies as the concentrations of halothane were increased from 1% to 1.7% and then to 2.1%.

Recovery from deep halothane anaesthesia resulted in a compressed waveform, but similar morphology in latencies to those at a vaporizer setting of 1%. Care must be taken when examining this waveform, as there is no indication of the brain concentration of halothane. Administration of pentobarbitone overdose resulted in rapid death of the ewe and complete loss of SEP with the recorded waveform representing background electrical noise.

In addition to the dose related effects of halothane, the induction agents could potentially have influenced the generation of SEPs. Ketamine is a dissociative anaesthetic with analgesic properties (Branson 2001) and it has been previously shown to increase cortical SEP amplitude (Schubert et al 1990), and propofol produces a dose-dependent suppression of the amplitude and latency of cortical SEPs

(Logginidou et al 2003). These agents were chosen for their short duration of action (Johnson et al 2005b), but the possibility exists that they could have influenced the resulting SEPs. Propofol's depressive properties could have suppressed/reduced SEP amplitude with 1% halothane. Similarly, following propofol metabolism, ketamine may have increased the amplitude of the waveform at 1.7% halothane. However, the interval between induction and generation of repeatable waveforms was 40 minutes, during which period propofol would have been metabolised (Branson 2001) and any effects of ketamine would be expected to have been waning (Johnson et al 2005b).

Although this model does not involve the surgical implantation of cortical electrodes, it still involves some surgery to expose the superficial peroneal nerve. This surgery is less invasive and complicated than the implantation of direct-contact cortical electrodes as it only involves the partial (50 mm) exposure of the superficial peroneal nerve. A shortcoming of the current experiment was that end tidal halothane tension was not measured. End tidal tension is more representative of the brain concentration of halothane. Furthermore, habituation of the exposed superficial peroneal nerve and the CNS to repeated electrical stimulation could have potentially affected generation of SEP (Stienen et al 2004).

In conclusion, generation of SEP from the repeated stimulation of the earthed exposed superficial peroneal nerve proved to be potentially useful for the investigation of global SEP. Further work is needed to further develop this model and to assess its reliability in more animals.

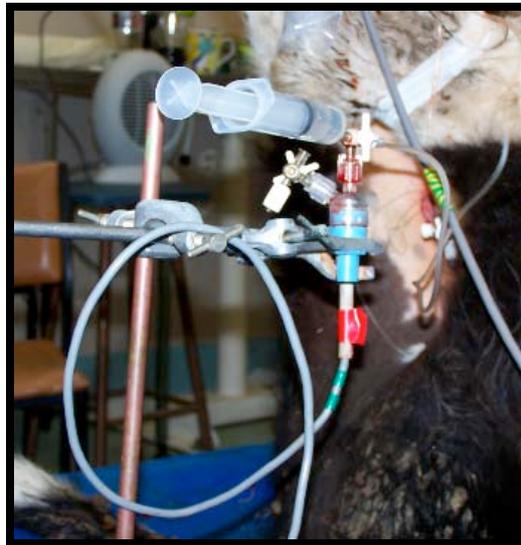
9.6 References

- Branson KR.** Injectable anesthetics. In: Adams HR (ed) *Veterinary Pharmacology and Therapeutics*. Pp 213-67. Iowa State University Press/Ames, Iowa, 2001
- Bromm B, Lorenz J.** Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology* 107, 227-53, 1998
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325-9, 1988
- Guérit JM.** Medical technology assessment EEG and evoked potentials in the intensive care unit. *Neurophysiologie Clinique* 29, 301-17, 1999
- Hashiguchi K, Morioka T, Yoshida F, Miyagi Y, Nagata S, Sakata A, Sasaki T.** Correlation between scalp-recorded electroencephalographic and electrocorticographic activities during ictal period. *Seizure* 16, 238-47, 2007
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61-71, 2005b
- Kubler A, Neumann N.** Brain-computer interfaces - the key for the conscious brain locked into a paralyzed body. In: *Boundaries of Consciousness: Neurobiology and Neuropathology*. Pp 513-25, 2005
- Logginidou HG, Li B-H, Li D-P, Lohmann JS, Schuler HG, DiVittore NA, Kreiser S, Cronin AJ.** Propofol suppresses the cortical somatosensory evoked potential in rats. *Anesthesia and Analgesia* 97, 1784-8, 2003
- Mauguière F.** Somatosensory evoked potentials: normal responses, abnormal waveforms and clinical applications in neurological diseases. In: Eiedermeyer E, Lopes da Silva F (eds). *Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*. Pp 1014-58. Lippincott Williams & Wilkins, Baltimore, 1999
- Mayhew IG, Washbourne JR.** A method of assessing auditory and brainstem function in horses. *British Veterinary Journal* 146, 509-18, 1990
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Pathak KS, Amaddio MD, Scoles PV, Shaffer JW, Mackay W.** Effects of halothane, euflurane, and isoflurane in nitrous oxide on multilevel somatosensory evoked potentials. *Anesthesiology* 70, 1989

- Pelosi L, Lamb J, Grevitt M, Mehdian SMH, Webb JK, Blumhardt LD.** Combined monitoring of motor and somatosensory evoked potentials in orthopaedic spinal surgery. *Clinical Neurophysiology* 113, 1082-91, 2002
- Peterson DO, Drummond JC, Todd MT.** Effects of halothane, euflurane, isoflurane, and nitrous oxide on somatosensory evoked potentials in humans. *Anesthesiology* 65, 35-40, 1986
- Schubert A, Licina MG, Lineberry PJ.** The effects of ketamine on human somatosensory evoked potentials and its modification by nitrous oxide. *Anesthesiology* 72, 33-9, 1990
- Sebel PS.** Somatosensory, visual and motor evoked potentials in anaesthetized patients. In: Jones JG (ed) *Bailliere's Clinical Anaesthesiology, International Practice and Research, Depth of Anaesthesia*. Pp 587-602. Bailliere Tindall, London, 1989
- Shaw NA.** Somatosensory evoked potentials after experimental head injury in the awake rat. *Journal of the Neurological Sciences* 74, 257-70, 1986
- Stienen PJ, van den Brom WE, de Groot HNM, Venker-van Haagen AJ, Hellebrekers LJ.** Difference between primary somatosensory cortex- and vertex-derived somatosensory-evoked potentials in the rat. *Brain Research* 1030, 256-66, 2004

CHAPTER 10

INVESTIGATION OF CORTICAL FUNCTION WITH THE USE OF SOMATOSENSORY-EVOKED POTENTIALS IN RESPONSE TO VENTRAL NECK INCISION OR NON-PENETRATIVE CAPTIVE BOLT STUNNING IN CALVES



Formatted for submission to a peer-reviewed scientific journal.

10.1 Abstract

AIM: The aim of the study was to examine the effects of either ventral neck incision slaughter (VNI) or non-penetrative captive bolt stunning (NPCB) on the propagation of somatosensory evoked potentials (SEP) in cattle.

METHODS: Somatosensory evoked potentials were generated by stimulation of the exposed superficial peroneal nerve as previously described (Chapter 9). The electroencephalogram (EEG), SEP and blood pressure were recorded in response to either VNI (n=9) or NPCB (n=7) stunning in calves.

RESULTS: The generation and recording of SEPs was problematic with only two complete data sets recorded (one from each group). Following VNI, SEPs were lost after 16 to 48 seconds, which correlated with the period of 'active' EEG activity observed in the spontaneous EEG. Evoked potentials were still present 160 seconds after NPCB stunning in one calf, but during this period the spontaneous EEG was either transitional or isoelectric.

DISCUSSION: Although significant difficulties were experienced in the generation of SEP in cattle, the recorded data sets in one calf combined with the changes in the spontaneous EEG suggest that after VNI there is a period where this animal may have been sensible to pain and distress had it not been anaesthetised.

10.2 Introduction

During slaughter without prior stunning there exists the potential for significant welfare compromise due to possible pain during incision of neck tissues and to pain and distress experienced by the animal prior to the onset of insensibility. The time to onset of undoubted insensibility after slaughter with or without stunning has been researched in a number of farmed species. These studies have investigated changes in: behaviour (Levinger 1961; Groß 1979; Blackmore and Newhook 1981; Blackmore et al 1983; Blackmore 1984), spontaneous EEG (Levinger 1961; Nangeroni and Kennett 1964; Blackmore et al 1979; Groß 1979; Blackmore and Newhook 1981; Fricker and Riek 1981; Lambooy and Spanjaard 1981; Blackmore and Newhook 1982; Newhook and Blackmore 1982ab; Blackmore et al 1983), EEG power spectrum (Bager et al 1992; Velarde et al 2002), loss of posture (Barnett et al 2007), loss of righting reflex (Blackmore 1979) and evoked potentials (Gregory and Wotton 1984ab; Daly et al 1986; Wotton and Gregory 1986; Daly et al 1988).

Electrophysiological indices of cortical function have been used to investigate the time to insensibility in cattle. The two most studied are alterations in the spontaneous EEG and cortically evoked potentials. Changes in the spontaneous EEG of cattle have been used to investigate the time to loss of sensibility after VNI (Levinger 1961; Nangeroni and Kennett 1964; Groß 1979; Blackmore and Newhook 1981; Newhook and Blackmore 1982b; Blackmore et al 1983) and the effectiveness of penetrative and non-penetrative captive bolt (PCB and NPCB, respectively) stunning in inducing and maintaining insensibility (Groß 1979; Fricker and Riek 1981; Lambooy and Spanjaard 1981; Blackmore and Newhook 1982). Although the EEG represents the functional electrical activity of the cerebral cortex, alterations in this functional activity are not directly synonymous with sensibility and there is currently insufficient understanding of conscious processes to be able to interpret alterations in the EEG in terms of loss of consciousness. Furthermore, interpretation of the EEG waveform is subjective and precise determination of when a change in waveform type occurs is problematic. Despite this, investigations of spontaneous EEG can provide important information on the general onset of undoubted insensibility.

An alternative is to assess cortical function during stunning and slaughter by generating and recording cortical evoked potentials. Evoked potentials have been

suggested as a more reliable indicator of brain disturbance following stunning than the EEG (Gregory 2007). Evoked potentials are not intended to represent sensibility or consciousness. Rather their loss represents an indication of an insult to the brain sufficient to cause failure of a primary sensory pathway within the CNS, and a failure of function at such a rudimentary level undoubtedly reflects profound brain disturbance inconsistent with sensibility (Daly et al 1987, 1988). Visually evoked potentials (VEPs) (Gregory and Wotton 1984a; Daly et al 1988) and somatosensory evoked potentials (SEPs) (Daly et al 1988) have been previously used to investigate the time to undoubted insensibility after slaughter and stunning. These studies recorded SEPs and VEPs with direct-contact cortical electrodes. This preparation reduces the signal-to-noise ratio (Hashiguchi et al 2007), but surgical placement of electrodes is invasive (Kubler and Neumann 2005) and may have residual effects on the generation and recording of evoked potentials.

Chapter 9 described the development of a model for generating and recording SEPs in halothane-anaesthetised sheep. The model involved the stimulation of the exposed earthed superficial peroneal nerve. This allowed the recording of SEPs without cortically implanted electrodes and maintains consistency with previous EEG studies in cattle (Chapters 3-8). This model combined with the minimal anaesthesia model (Murrell and Johnson 2006) could potentially provide further information on the time to insensibility in cattle and provide a point for comparison with work investigating the potential noxiousness of slaughter (Chapters 3-4).

The aim of the study was to examine the effects of VNI or NPCB stunning on the propagation of SEPs using the model developed in sheep (chapter 9).

10.3 Materials and Methods

10.3.1 Animals

Sixteen calves weighing between 118 to 149 kg were maintained using normal husbandry practices. During the 24 hours prior to experimentation calves had access to water but not food. The animals were allocated to two treatment groups: the effects

of VNI slaughter (VNI group) (n=9) or NPCB stunning (NPCB group) (n=7) on the propagation of SEP. The study was approved by the Massey University Animal Ethics Committee (Protocol no. 04/86).

10.3.2 Anaesthesia and Blood Pressure

Anaesthesia was induced with a mixture of ketamine (3.9 (SD 0.3) mg/kg) (Parnell Laboratories, Auckland NZ) and propofol (6.5 (SD 2.0) mg/kg) (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered intravenously into a jugular vein. Anaesthesia was maintained by inhalation of halothane in oxygen as previously described (Chapters 3-9). End tidal halothane tension (F_E' HAL) was maintained in the range of 0.85-0.95%. The measurement of blood pressure was performed as described elsewhere (Chapters 4-9).

10.3.3 Superficial Peroneal Nerve Preparation

Animals were placed in right lateral recumbency for surgical exposure of the superficial peroneal nerve. A single incision was made laterally on the left hind limb, 50 mm distal to the metatarsus. Proximal to its bifurcation into lateral and medial branches, a 100 mm section of the superficial peroneal nerve was exposed, ligated and cut distally. The nerve was placed on a purpose built nerve bath (figure 10.1). The bath was attached to the leg with Velcro straps. The nerve was laid over two silver/silver chloride stimulating electrodes placed 5 mm apart (figure 10.1). Proximal to the stimulating electrodes the nerve was connected to earth with a surgical swab soaked in Hartmann's solution (Baxter Healthcare Ltd, Toongabbie, NSW, Australia). The nerve and exposed tissues were moistened with Hartmann's solution to prevent dehydration and loss of electrolytes. Stimulating electrode cables and the nerve were secured to the leg to prevent movement during SEP recording (figure 10.1).



Figure 10.1. Superficial peroneal nerve preparation. A: the exposed superficial peroneal nerve layed over two stimulating electrodes (positive distal and negative proximal). A surgical swap, located proximally, was soaked in Hartmann's solution, was earthed and acted as the nerve bath. B: the finished preparation with moistend swaps preventing deydration.

10.3.4 EEG and SEP

The EEG and SEP were recorded from the left and right cerebral hemispheres as previously described (Chapters 3-9). They were amplified using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota FL, USA). The EEG and SEP was recorded with a gain of 1,000 and pass-band of 0.1 to 500 Hz. Data were digitised at a rate of 1kHz and recorded on two personal computers (MacBook Pro and iMac, Apple Inc, Cupertino, California, USA). EEG was recorded with the data acquisition software package Chart 5.5.1 (ADInstruments Ltd, Sydney, Australia).

Somatosensory evoked potentials were recorded on the second computer with the software package Scope 4.01 (ADInstruments, Ltd). Scope was used to trigger the electrical stimulator (Grass S48 Stimulator, Grass Technologies, Astro-Med Inc, West-Warwick, Rhode Island, USA) with a square wave pulse of 0.5 ms in duration and 3-volts in amplitude. The exposed peroneal nerve was stimulated with a 20-volt pulse having a duration of 0.5 ms. Somatosensory evoked potentials were recorded with a 100 ms delay and a recording duration of 500 ms at 4 kHz. Thirty-two successive sweeps were summated with Scope 4.01 to produce each waveform, with

subsequent analysis performed in Excel 2004 (Mac, Microsoft Corporation, Redmond, Washington, USA).

10.3.5 Experimental Procedure

Calves in the NPCB group remained in right lateral recumbency during experimentation, while calves in the VNI group were placed in dorsal recumbency after exposure and instrumentation of the superficial peroneal nerve. The neck was stretched and the head secured in position in the head frame. After stabilisation of anaesthesia for nerve preparation surgery and repositioning (VNI only) (figure 10.2), pre-treatment SEP traces were recorded every 10-minutes until three complete traces had been generated. After a period of re-stabilisation following pre-treatment SEP generation, calves received either VNI (as described previously chapters 4 and 5) or NPCB stunning (as described in chapter 6). SEPs were recorded continuously for 8 minutes. Calves that received NPCB stunning were euthanized by intravenously injected sodium pentobarbitone (Pentobarb 500; National Veterinary Supplies Ltd, Auckland, NZ) at the conclusion of data collection.

10.3.6 Interruption of EEG and SEP Waveforms

The spontaneous EEG was inspected visually and classified into one of six categories: out of range; active EEG; transitional EEG; high amplitude low frequency EEG (HALF); isoelectric EEG; and high amplitude high frequency EEG (HAHF) (Chapters 4-7). Active EEG represents normal cerebrocortical activity in anaesthetised calves. Transitional EEG was classified as having an amplitude of less than half of pre-treatment EEG. High amplitude low frequency EEG (HALF) was classified as a waveform with rhythmic activity of high amplitude and low frequency. High amplitude high frequency EEG was classified as a burst of activity similar in frequency as sleep spindles but of much higher amplitude. Isoelectric EEG was classified as a trace with an amplitude of less than 1/8 of the pre-stunning EEG with little or no low frequency components.

Somatosensory evoked potentials were measured for alterations in latency and amplitude. Latency was determined for either positive (P) or negative (N) deflections and was measured in milliseconds on the SEP waveform. The latencies represented different levels of CNS processing of the evoked stimulus. Waveforms with a signal to noise ratio <2 (based on baseline recording taken 100 ms prior to stimulation) were excluded from further analysis (Murrell et al 2007).

10.3.7 Statistics

Means and standard deviations (SD) were calculated using standard statistical techniques. Blood pressure was displayed as mean values. The distribution of blood pressure was tested for normality with the Anderson-Darling test and found to have a non-normal distribution. Statistical analysis of blood pressure was performed on individual values taken every 15 seconds using a Mann-Whitney non-parametric test. Values of $P < 0.05$ were taken to indicate statistical significance.

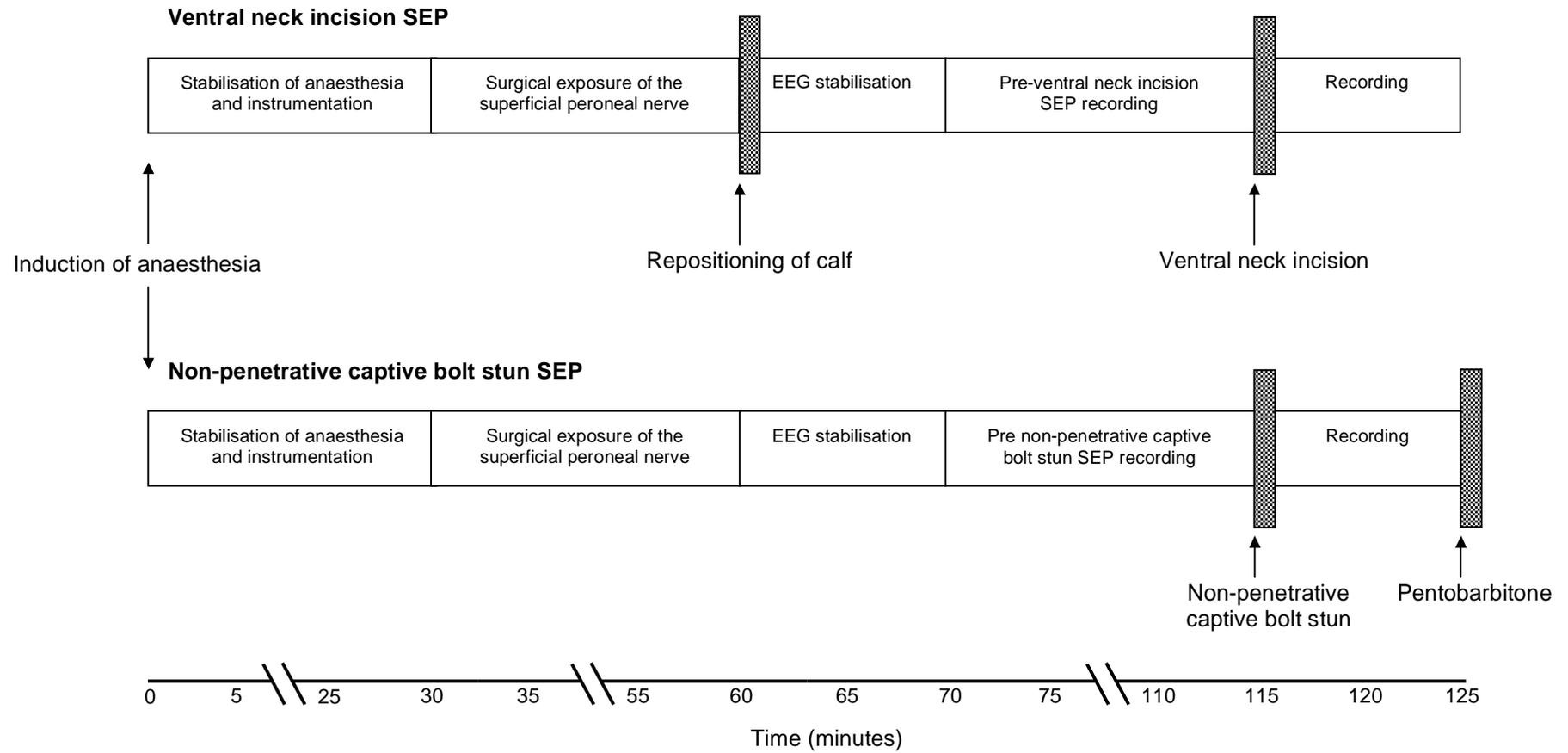


Figure 10.2. Diagrammatic representation of the experimental design for both ventral neck incision (VNI) and non-penetrative captive bolt (NCPB) stunning groups. Calves in both groups were anaesthetized at 0 minutes. Treatments occurred approximately 115 minutes from induction of anesthesia.

10.4 Results

The generation of SEP was highly variable in individual animals in both treatment groups. Only two complete data sets were recorded with repeatable pre-treatment SEPs generated. The remaining animals either had non-repeatable SEPs or the signal-to-noise ratio was <2 . Somatosensory evoked potentials were recorded from calf 8 in the VNI group and calf 7 from the NPCB group. Figure 10.3 provides details of the waveforms recorded from these two animals. Calf 9 from the VNI group had only the right carotid artery cut, leaving the left carotid artery intact.

10.4.1 SEP

VNI Calf 8

Pre-treatment SEPs were successfully generated with repeatable positive (P200) peaks. Generation of pre-treatment SEPs was highly problematic in the VNI group. After VNI, the P200 deflection gradually decreased in amplitude (figure 10.3) until it disappeared. It was difficult to judge the exact time when evoked responses disappeared completely, but it occurred between 16-48 seconds after the VNI. However, activity at 16-32 and 32-48 seconds after VNI was below the signal to noise threshold set prior to experimentation. Reducing the summation period did not reveal with greater precision the point at which the P200 was lost.

NPCB Calf 7

Pre-treatment SEPs were successfully generated and were repeatable with positive (P110, P197, P304) and negative (N156, N250) deflections. After NPCB stunning (figure 10.3) the initial 0-16 seconds of SEPs were unusable with a large DC shift, and were therefore subsequently excluded from further analysis. After this initial period, there was a initial transient decrease in amplitude and a latency change from pre-treatment recordings with a prominent positive deflection P227 increasing in

amplitude over time. Somatosensory evoked potentials were still present 160 seconds after the stun.

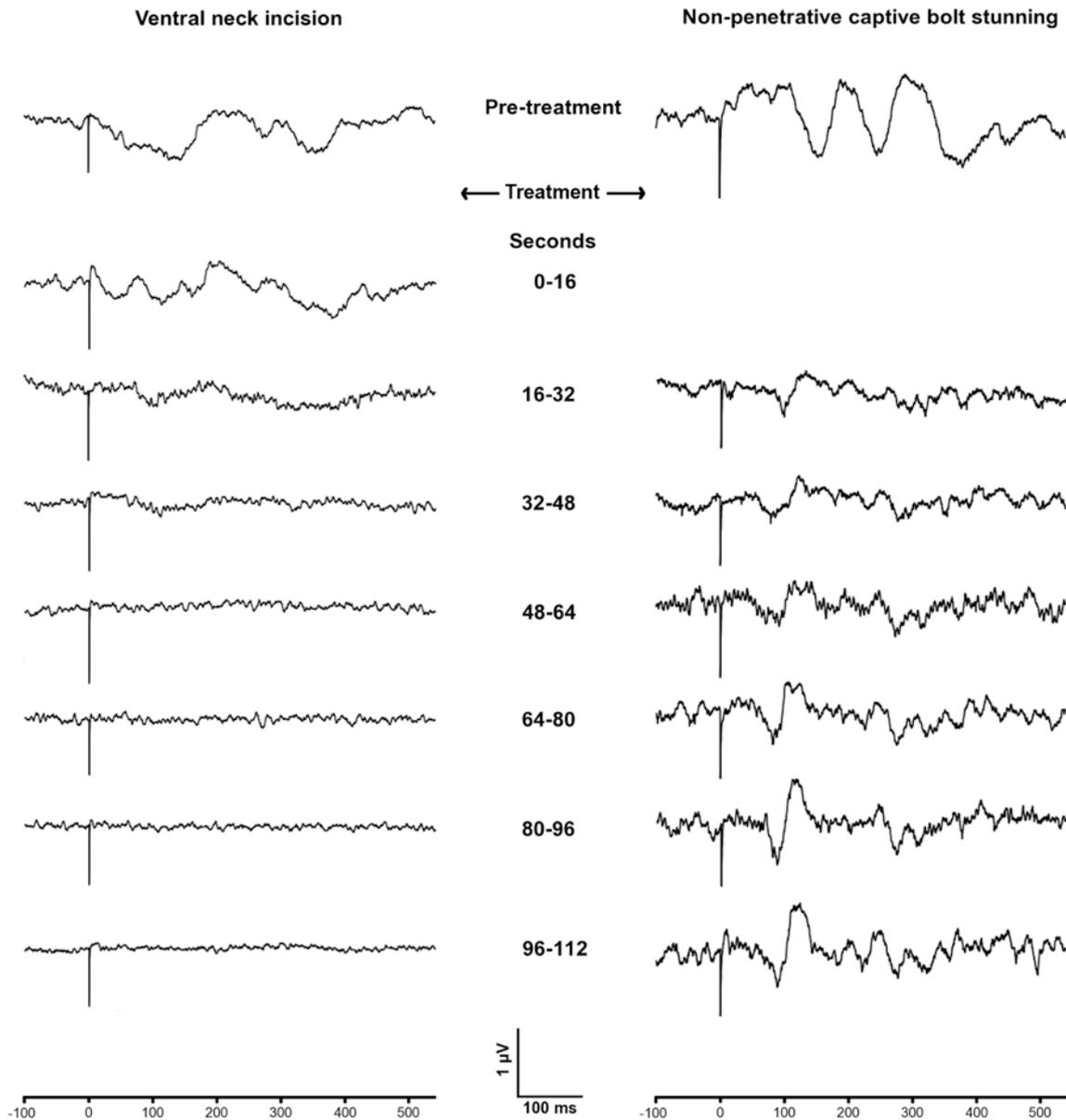


Figure 10.3. Examples of somatosensory evoked potentials (SEP) from two calves after either ventral neck incision slaughter (calf 8 VNI group) or non-penetrative captive stunning (calf 7 NPCB group). These were the only data sets where pre-treatment SEPs were able to be generated. Each waveform is the summation of 32 traces. There was a stimulus delay of 100 ms with the stimulus being delivered at 0 minutes.

10.4.2 Spontaneous EEG

The EEG was recorded simultaneously during generation of SEP waveforms. Data sets were obtained from 9 animals from the VNI group and 7 from the NPCB. One EEG trace was excluded from analysis in the NPCB group due to unacceptable levels of contamination with electrical noise.

VNI Group

Assessment of the spontaneous EEG showed that the mean duration of VNI artefact was 3 (SD 2) seconds after VNI (figure 10.4). The mean duration of active EEG was 14 (SD 9) seconds. Active EEG was recorded in all nine animals but not from both hemispheres in each case. Transitional EEG was observed for between 7 and 136 seconds in six animals. The mean time to onset of HALF was 36 (SD 38) seconds with a mean duration of 121 (SD 100) seconds. The mean time to onset of the isoelectric trace was 93 (SD 80) seconds. In calf 8, after an initial period of VNI artefact (6 and 3 seconds for the right left hemispheres respectively), had periods of active EEG with durations of 16 and 19 seconds for the right and left cerebral hemispheres, respectively. These periods of active EEG merged into HALF activity at 22 seconds for both hemispheres. Only calf 4 in the left cerebral hemisphere showed a period of HAHF EEG activity.

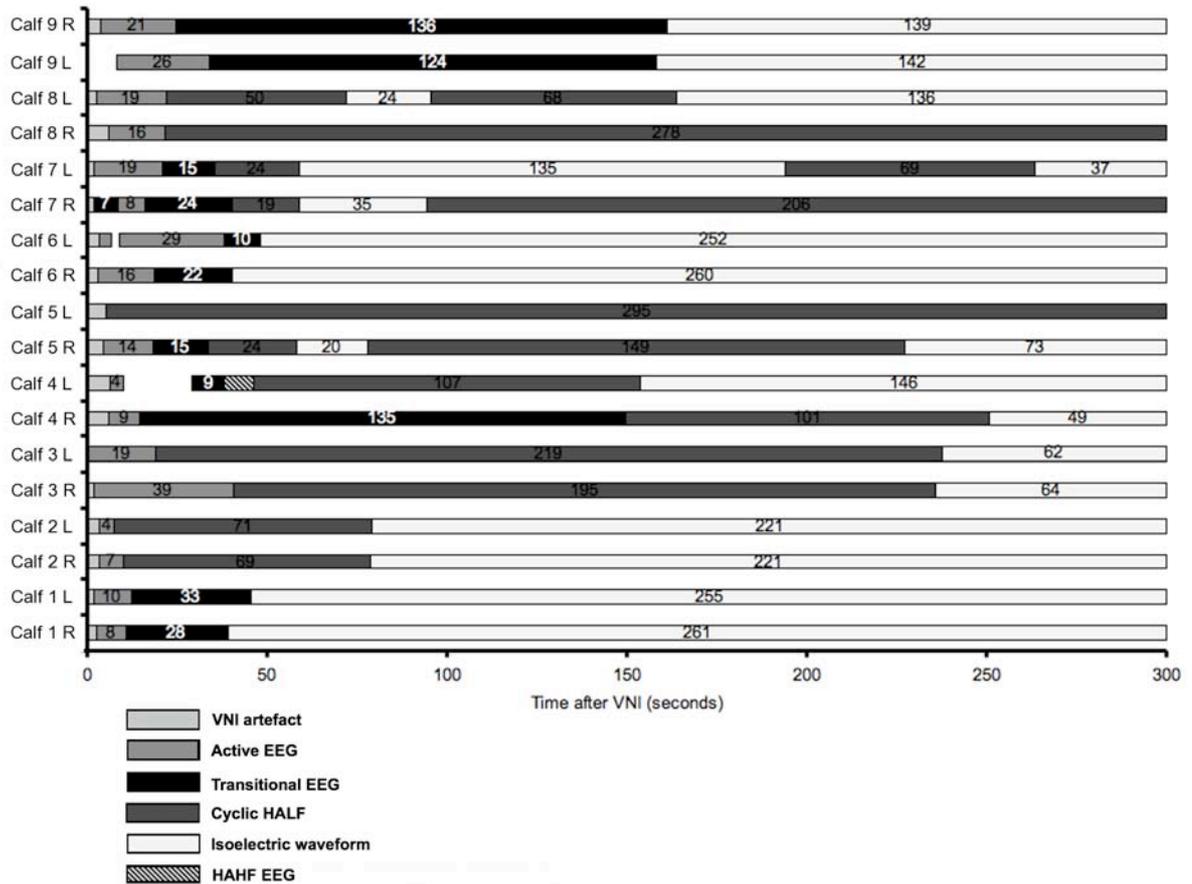


Figure 10.4. Changes in the characteristics of spontaneous electroencephalogram (EEG) in individual animals after ventral neck incision (VNI). The traces were visually inspected and were classified as: VNI artefact, active EEG, transitional EEG, cyclic HALF activity, isoelectric waveform and high amplitude high frequency HAHF. R represents EEG recorded from the right and L the EEG recorded from the left side of the cranium. The time durations in seconds are displayed on the individual bars; clear gaps represent periods where the EEG recording was out of range.

NPCB Group

Stunning produced periods of out of range data in five animals with a mean duration of 12 (SD 13) seconds (figure 10.5). Active EEG was only observed in two animals after NPCB with a mean duration of 11 (SD) seconds. Transitional EEG was observed in six of the animals (duration 131 (SD 90) seconds), with the onset of the isoelectric waveform at 109 (SD 80) seconds. The EEG of calve 7 after NPCB exhibited transitional activity for periods of 45 and 83 seconds for the right and left hemispheres, respectively, before changing to isoelectric waveforms.

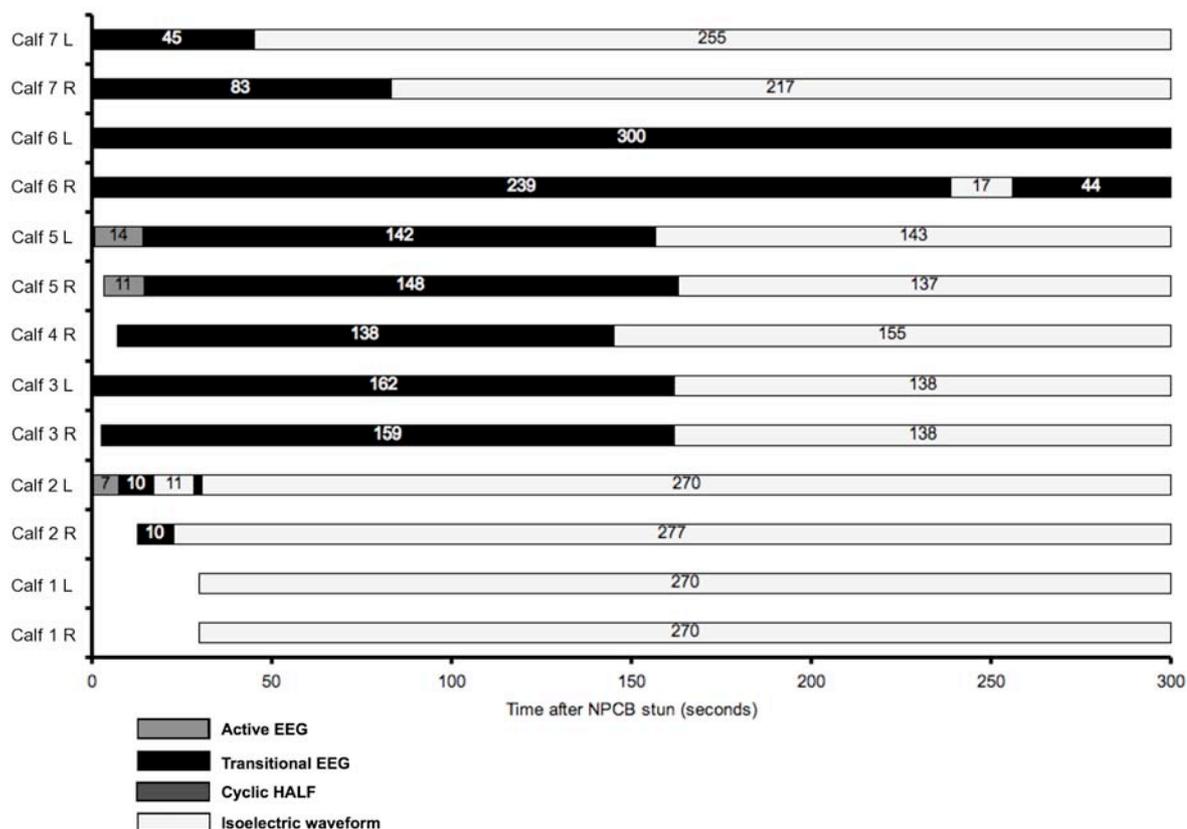


Figure 10.5. Characteristics of the spontaneous electroencephalogram (EEG) in individual animals after non-penetrative captive bolt stunning (NPCB). The traces were visually inspected and were classified as: active EEG, transitional EEG, cyclic HALF activity and isoelectric waveforms. R represents EEG recorded from the right and L the EEG recorded from the left side of the cranium. The time durations in seconds are displayed on the individual bars; clear gaps represent periods where the EEG recording was out of range.

10.4.3 Blood Pressure

Following incision in the VNI group mean blood pressure (figure 10.6) significantly decreased from pre-treatment values of 117 mmHg (SD 19) to 10 mmHg (SD 10) and 8 (SD 10) after 60 and 120 seconds respectively ($P < 0.05$). There was no qualitative difference in blood pressure values between Calf 9 (one carotid cut) and the remaining animals in the group. Mean blood pressure in the NPCB group significantly decreased from pre-treatment values of 120 mmHg (SD 27) to 65 mmHg (SD 30) and 47 mmHg (SD 32) after 60 and 120 seconds respectively ($P < 0.05$).

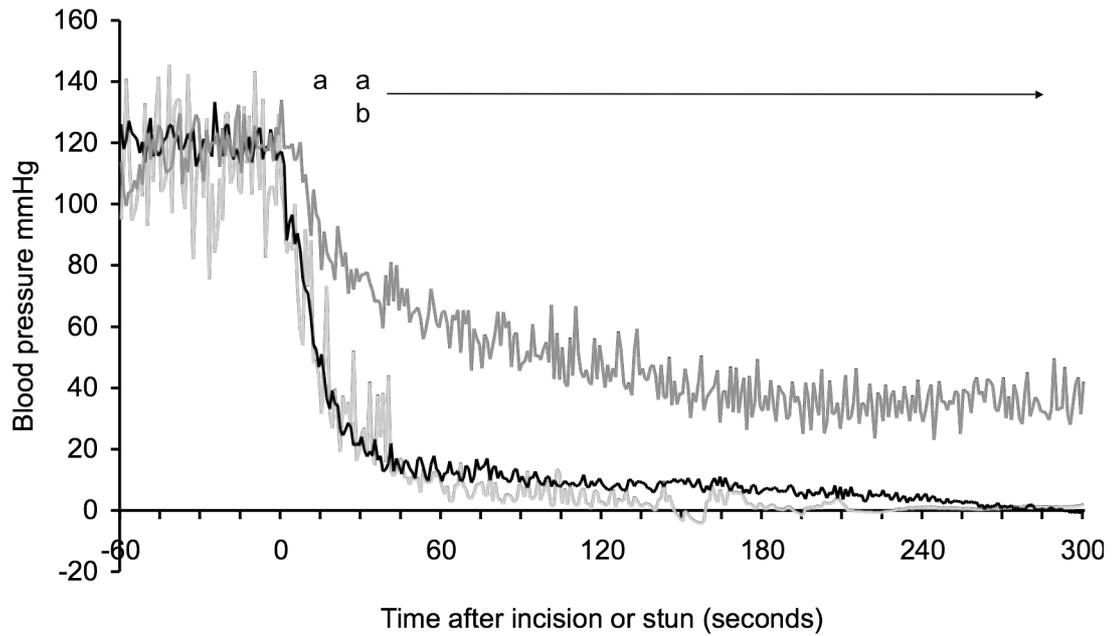


Figure 10.6. Mean blood pressure after ventral neck incision (VNI) (—) and non-penetrative captive bolt (NPCB) stunning only (—). Calf 9 from the VNI group had one only carotid cut (—). a = significant difference from pretreatment values in the VNI group. b = significant difference from pretreatment values in the NPCB stunning group

10.5 Discussion

Somatosensory evoked potentials after VNI disappeared in calf 8 between 16 to 48 seconds. The spontaneous EEG in this animal contained periods of active EEG, the cessation of which corresponded with the loss of evoked potentials. It is highly probable that had this animal been conscious it would have been sensible and experienced pain and distress during this period (Chapters 3-4). In the current experiment there was a wide range in the duration of active EEG after VNI. Previous work in cattle has established that the persistence of evoked responses (SEPs and VEPs) in individual animals varied widely after Shechita, ranging from 16 to 126 seconds (Gregory and Wotton 1984a; Daly et al 1988).

Non-penetrative captive bolt stunning initially decreased SEP amplitude but positive deflections were still present 160 seconds after stunning. Dependent on the amount of brain damage induced, NPCB stunning can cause either permanent or temporary insensibility (Blackmore and Delany 1988). Potentially the SEP-like activity reported at 160 seconds post stun could represent recovery of primary sensory pathways, possibly indicating a return of cerebral function. This could result from incorrect stunning position or poor maintenance of the stun gun affecting bolt velocity and thus stunner performance (Gregory 2007). However, at this stage after stunning the spontaneous EEG was changing from transitional to isoelectric and there were no other EEG or physical responses suggesting a possible return of sensibility. Transitional-like activity has been previously characterised as being incompatible with the maintenance of sensibility (Blackmore and Delany 1988). Burst suppression of the EEG during deep propofol anaesthesia is somewhat comparable to an isoelectric EEG. Huotari et al (2004) demonstrated the generation of four separate cortical responses to noxious stimulation of the median nerve in burst-suppressed propofol-anaesthetised humans. Non-penetrative captive bolt stunning in this experiment often produced an isoelectric EEG trace, which potentially produced a similar condition for the generation of evoked responses to that seen during propofol-induced burst suppression.

Difficulties were experienced in the current experiment in generation of SEP. Previous studies have used intracortically implanted electrodes, which reduce the signal-to-noise ratio. Subdermal needle electrodes were used in the current

experiment to maintain consistency with previous EEG studies in cattle (Chapters 3-8). However, intracortically implanted electrodes may have improved the recording of SEP in the current experiment. Prior to this experiment, a pilot study was undertaken in sheep to develop the methodology for generation of SEPs (Chapter 9). It was from this that the methodology of isolating the superficial peroneal nerve in the hind limb was developed. Somatosensory evoked potentials were reliably generated using this methodology in one sheep. However, generation of pre-treatment SEPs in calves was problematic. Habituation of the exposed superficial peroneal nerve to repeated electrical stimulation might have affected SEP generation. Stienen et al (2004) have demonstrated the habituation of SEPs recorded from the vertex of rats to rapidly increasing stimulus frequencies.

The decrease in blood pressure seen here in the NPCB stun group was similar to that observed in the previous NPCB stunning experiment (Chapter 5). The changes in blood pressure in the calf with only one carotid artery cut were both qualitatively and quantitatively similar to those in the other animals in the VNI group and similar to the previous experiments involving VNI whether or not the animals were stunned (Chapters 4 and 7).

High amplitude high frequency activity (HAHF) was recorded in the hemisphere of one calf 38 seconds after VNI. This activity has not been previously observed in the EEG of calves after VNI (chapter 4, 5 and 7). The short duration of this activity and its absence in other animals suggests external noise or electrical artefact from the stimulation of the superficial peroneal nerve.

In conclusion, the generation of SEPs from cattle in response to either VNI or NBSP was problematic. However, based on alterations in the spontaneous EEG of all calves studied, there was a period following VNI in which the animals, if conscious, would potentially have been sensible and could have perceived pain and distress. This conclusion is supported by the limited SEP data obtained from one calf exposed to VNI.

10.6 References

- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP.** Onset of insensibility at slaughter in calves - effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162-73, 1992
- Barnett JL, Cronin GM, Scott PC.** Behavioural responses of poultry during kosher slaughter and their implications for the birds' welfare. *Veterinary Record* 160, 45-9, 2007
- Blackmore DK.** Non-penetrative percussion stunning of sheep and calves. *Veterinary Record* 105, 372-5, 1979
- Blackmore DK, Newhook JC, Petersen GV.** Electrical stunning and humane slaughter. *New Zealand Veterinary Journal* 27, 224-, 1979
- Blackmore DK, Newhook JC.** Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* 29, 219-22, 1981
- Blackmore DK, Newhook JC.** Electroencephalographic studies of stunning and slaughter of sheep and calves .3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Science* 7, 19-28, 1982
- Blackmore DK, Newhook JC, Grandin T.** Time of onset of insensibility in 4-week-old to 6-week-old calves during slaughter. *Meat Science* 9, 145-9, 1983
- Blackmore DK.** Differences in behaviour between sheep and cattle during slaughter. *research in veterinary science* 37, 223-6, 1984
- Blackmore DK, Delany MW.** Slaughter of stock, a practical review and guide, Veterinary Continuing Education Massey University, Palmerston North, 1988
- Daly CC, Gregory NG, Wotton SB, Whittington PE.** Concussive methods of pre-slaughter stunning in sheep - assessment of brain-function using cortical evoked-responses. *Research in Veterinary Science* 41, 349-52, 1986
- Daly CC, Gregory NG, Wotton SB.** Captive bolt stunning of cattle - effects on brain-function and role of bolt velocity. *British Veterinary Journal* 143, 574-80, 1987
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with shechita slaughter. *Veterinary Record* 122, 325-9, 1988
- Fricker CH, Riek W.** Die betäubung von rindern vor dem schlachten mit hilfe des bolzenschuß-apparates. *Fleischwirtschaft* 61, 124-7, 1981

- Gregory N.** Stunning and slaughter. In: *Animal Welfare and Meat Production*. Pp 191-212. CABI Publishing, Wallingford, 2007
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141-3, 1984a
- Gregory NG, Wotton SB.** Sheep Slaughtering Procedures .2. Time to loss of brain responsiveness after exsanguination or cardiac-arrest. *British Veterinary Journal* 140, 354-60, 1984b
- Groß R.** Elektroencephalographische und elektrokardiographische verlaufsuntersuchungen nach bolzenschußbetäubung und nach töten durch entbluten in der form des rituellen schlachtens. durch die Tierärztliche Hochschule, Hannover, 1979
- Hashiguchi K, Morioka T, Yoshida F, Miyagi Y, Nagata S, Sakata A, Sasaki T.** Correlation between scalp-recorded electroencephalographic and electrocorticographic activities during ictal period. *Seizure* 16, 238-47, 2007
- Huotari AM, Koskinen M, Suominen K, Alahuhta S, Remes R, Hartikainen KM, Jantti V.** Evoked EEG patterns during burst suppression with propofol. *British Journal of Anaesthesia* 92, 18-24, 2004
- Kubler A, Neumann N.** Brain-computer interfaces - the key for the conscious brain locked into a paralyzed body. In: *Boundaries of Consciousness: Neurobiology and Neuropathology*. Pp 513-25, 2005
- Lambooy E, Spanjaard W.** Effect of the shooting position on the stunning of calves by captive bolt. *Veterinary Record* 109, 359-61, 1981
- Levinger IM.** Untersuchungen zum schächtproblem. Dr thesis, University of Zürich, Zürich, 1961
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Murrell JC, Mitchinson SL, Johnstone AC, Johnson CB, Barnes GRG.** Is it possible to generate cerebral evoked potentials with a mechanical stimulus from the duodenum in rats? *Journal of Neuroscience Methods* 162, 215-21, 2007
- Nangeroni LL, Kennett PD.** Electroencephalographic studies of the effects of Shechita slaughter on cortical function in ruminants. Cornell University, New York, 1964
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .1. The onset of permanent insensibility in sheep during slaughter. *Meat Science* 6, 221-33, 1982a

- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .2. The Onset of Permanent Insensibility in Calves During Slaughter. *Meat Science* 6, 295-300, 1982b
- Shaw NA.** Neurophysiology of concussion. *Progress in Neurobiology* 67, 281-344, 2002
- Stienen PJ, van den Brom WE, de Groot HNM, Venker-van Haagen AJ, Hellebrekers LJ.** Difference between primary somatosensory cortex- and vertex-derived somatosensory-evoked potentials in the rat. *Brain Research* 1030, 256-66, 2004
- Velarde A, Ruiz-de-la-Torre JL, Rosello C, Fabrega E, Diestre A, Manteca X.** Assessment of return to consciousness after electrical stunning in lambs. *Animal Welfare* 11, 333-41, 2002
- Wotton SB, Gregory NG.** Pig slaughtering procedures - time to loss of brain responsiveness after exsanguination or cardiac-arrest. *Research in Veterinary Science* 40, 148-51, 1986

CHAPTER 11

GENERAL DISCUSSIONS AND CONCLUSIONS

This thesis presents the first body of work aimed at quantitatively investigating the potential noxiousness of slaughter by ventral neck incision (VNI) without prior stunning. Changes in spontaneous electroencephalogram (EEG), EEG power spectrum, somatosensory evoked potentials (SEP), blood pressure and heart rate were used to examine effects of VNI in cattle. The experimental chapters are the first to investigate the potential noxiousness of slaughter using the minimal anaesthesia model and alterations in the EEG power spectrum that have been previously associated with noxious sensory input in a variety of mammalian species (Murrell and Johnson 2006).

The thesis demonstrates that the tissue damage associated with slaughter by VNI causes noxious sensory input that would be perceived by the animal as pain. Combined with previous work investigating the time to undoubted insensibility (Levinger 1961; Nangeroni and Kennett 1964; Blackmore and Newhook 1981; Newhook and Blackmore 1982ab; Blackmore et al 1983; Blackmore 1984; Gregory and Wotton 1984ab; Daly et al 1988; Bager et al 1992; Grandin 1994a; Anil et al 1995ab; Cook et al 1996; Barnett et al 2007), it is apparent that there is a window following slaughter without stunning where the animal is conscious and would suffer in response to the pain caused by slaughter and the associated distress prior to hypoxia-induced insensibility. Chapters 3 to 10 detail the experiments and the interpretation undertaken to arrive at this conclusion.

Surgical dehorning was used as a validation technique for the minimal anaesthesia model and EEG power spectrum methodology in cattle. The results demonstrated a 'typical' response to somatic noxious stimulation, similar to that seen in red deer following velvet antler removal (Johnson et al 2005b). Dehorning caused: an immediate increase in F95 that decreased over the next two minutes; an increase in F50 that developed more gradually over the first minute following dehorning and persisted during the second minute; an immediate decrease in P_{tot} which then

gradually increased over the next two minutes. All EEG responses were abolished by the prior application of local anaesthetic blockade

When considering the results of the dehorning, VNI and stunning studies, it must be remembered that there are important differences in the model between these studies. The physiological environment during VNI represents the most challenging use of the minimal anaesthesia model to date. In particular, in addition to any noxious stimulation, dramatic alterations in cerebral blood flow are an integral part of the manipulation and will have their own effects on the EEG variables measured. Conversely the physiological environment during stunning is very different from that of VNI or dehorning. Alterations in EEG activity are a combination of stunning induced cerebral dysfunction and hypoxia as well as any potential noxious stimulation. This discussion will firstly consider the EEG changes in the light of these underlying physiological alterations and which of the EEG changes seen may be due to the different physiological effects, and secondarily it will examine how these studies interrelate.

11.1 Noxiousness of Slaughter

Ventral neck incision caused changes in the EEG representative of noxious sensory input (Chapter 4). This study also defined a period during which changes in the EEG power spectrum were representative of noxious experiences as apposed to loss of cortical function. Ventral neck incision caused an increase in F95. Similar increases in response to noxious sensory input have been previously described in other anaesthetized animals (Antognini et al 2001; Barter et al 2005; Johnson et al 2005b; Orth et al 2005; Chapter 3). However, the causation of the noxious responses was not apparent, as the changes in the EEG power spectrum may have been due to the tissue damage associated with the transection of neck tissues or the loss of blood flow to and from the brain, or a combination of the two.

The question was investigated in two groups of cattle, where cerebral blood flow remained intact during neck incision (NTI) or carotid and jugular blood flows were disrupted without major damage to neck tissues (BVT) (Chapter 5). Cutting of the neck tissues without markedly disrupting cerebral blood flow produced an

increase in F95. This response was therefore largely due to the noxious sensory input associated with tissue damage and not to potentially confounding effects of cerebral ischaemia and the onset of hypoxia. In BVT animals, where only the exteriorised blood vessels of the neck were transected, P_{tot} fell to 50% of pre-treatment values after 2 minutes. Shortly after this time the EEG became irregular with bursts of seizure-like activity, which were presumably related to the onset of hypoxaemia. Taken together, the results from these two groups show that it was largely the tissue damage, not mainly the loss of blood flow through the brain, that caused the noxious sensory input in cattle during VNI slaughter without prior stunning.

One of the most striking findings in P_{tot} was a large and immediate increase seen after VNI. This was seen in all the groups where the neck was incised (including where stunning was carried out after incision) (Chapters 4, 5 and 7) and to a lesser extent in the sham incision group (Chapter 4). It was absent in the BVT group (Chapter 5) and in the non-penetrative captive bolt (NPCB) stun only group (Chapter 6). This initial increase seems most likely to be an artefact due primarily to movements of the animal inevitably generated by the act of VNI, as cutting through the ventral strap muscles of the neck caused a large amount of movement of the head relative to the body. Moreover, VNI would cause electrical changes in the cut muscles and these would be expected to create far field potentials recordable by the EEG electrodes.

Histological examination of brains from animals slaughtered by VNI showed no evidence to support the hypothesis of brain ‘implosion’ (Rosen 2004) which, it was suggested, would result from loss of maintenance of brain structure following the sudden interruption of cerebral blood supply with severance of the carotid arteries and jugular veins.

11.2 Modulation of the EEG Responses to VNI with NPCB Stunning

Non-penetrative captive bolt stunning in calves was investigated alone (Chapter 6) and after VNI (Chapter 7). In these chapters the dangers of interpreting

F95 and F50 without considering EEG power were discussed. Stunning caused an immediate decrease in P_{tot} and a change in waveform type in the spontaneous EEG. These changes represented an alteration in normal cerebrocortical function associated with physical damage, subarachnoid haemorrhage, traumatic axon injury and brain swelling. The NPCB stun abolished normal cerebrocortical activity in cattle with a progression towards an isoelectric EEG trace.

The modulation of the noxious sensory input of VNI with post NPCB stunning was investigated in calves. Stunning performed 5 seconds after VNI abolished the EEG responses indicative of noxious sensory input reported in Chapters 4 and 5, i.e. NPCB stunning caused a decrease in P_{tot} after the initial increase associated with movement during VNI. Analysis of the spontaneous EEG revealed an immediate change in waveform type following stunning in most animals to states associated with loss of cortical function.

11.3 Cortical Function after NPCB Stunning or VNI Slaughter

The alterations in waveform type in the spontaneous EEG can be used to demonstrate the progression towards an isoelectric trace, or states that are incompatible with the maintenance of sensibility, but they are not an indication of insensibility and cannot accurately define when undoubted insensibility begins. Somatosensory evoked potentials were used in cattle to investigate the time to undoubted insensibility after VNI or NPCB stunning. Prior to the study in cattle (Chapter 10), a pilot study was carried out in sheep (Chapter 9). The methodology developed in sheep was used in the cattle. Significant difficulties were experienced in the generation and recording of SEP in cattle. However from the data sets recorded, combined with alterations in the spontaneous EEG, it was found in one animal that after VNI the generation of evoked potentials was lost between 16 to 48 seconds. The loss of evoked potentials represented the failure of a primary sensory pathway within the CNS. This suggested that prior to the loss of SEPs after VNI the mechanisms needed for sensibility were still present. However, interpretation of this was hindered by the generation of evoked potentials in an animal after NPCB stunning where the spontaneous EEG was in states previously associated with insensibility.

11.4 Experimental Limitations

Care was taken when designing and performing the present research to minimise and account for potential factors, which could limit the validity and the interpretation of the results. Specific limiting factors in the individual experiments have been discussed in their respective chapters. However, a number of common limitations are discussed below.

Electrical interference (noise) is a constant problem when conducting electrophysiological research. The EEG is prone to contamination with 50 Hz noise from the mains, from radiating interference (for example 100 Hz noise from fluorescent lights) and from materials and objects storing charge and acting as capacitors. The high frequency (>40 Hz) electrical noise rarely affects the desired frequency range in the EEG, as much of this external noise can be removed with digital filters. However, noise does preclude real time visual inspection of the EEG, which can cause problems during instrumentation and data recording, as there is no indication of the quality of the recorded signal.

A number of methods were used to minimise the impact of external electrical interference. Isolated transformers were used to isolate electrophysiological recording equipment, amplifiers/filters were further isolated from mains with batteries, the animals were isolated, and the equipment and animals were earthed to common points to prevent ground loops. During experimentation intermittent noise (150 Hz) was recorded on the EEG. The source was traced to the formation of an earth bridge between the dripping saliva of the anaesthetised calf and surface water on the surgery floor, which was draining into a metal drain. Isolation of the saliva prevented further noise. Much of the radiating electrical noise can be minimized or removed with the use of an electrically shielded room or a faraday cage. However, this was impractical given the location, equipment, methodological constraints and the potential for physical interference during dehorning, VNI and NPCB.

Alterations in the EEG power spectrum have been used in a variety of studies to investigate brain electrical activity in response to stimuli other than noxious sensory input. The potential exists that the EEG activity in response to noxious sensory input may be shared by other stimuli and not exclusively related to pain,

suggesting that the changes are non-specific and related to sensory processing in general (Chang et al 2001a).

Experiments in humans have correlated changes in the EEG power spectrum with the cognitive perception of pain (Chen et al 1989). Further studies have investigated the different EEG topographic effects of painful and non-painful intramuscular stimulation in humans (Chang et al 2001b) and the differential cerebral responses to aversive auditory arousal versus muscle pain (Chang et al 2002). These studies have demonstrated that painful and non-painful muscular stimulation evoked distinct EEG activation and that EEG activation in response to pain was distinct to aversive auditory arousal (Chang et al 2001b, 2002). Furthermore investigation of changes in the EEG power spectrum in repose to cognitive processing of other stimuli unrelated to pain has demonstrated specific EEG responses that are distinct to that of noxious sensory input or pain. For example the euphoria induced by marihuana (Lukas et al 1995) and ethanol usage (Lukas et al 1986; Lukas and Mendelson 1988) in man results in a rapid transient increase in EEG alpha power (8-13 Hz). This is potentially similar to the frequencies corresponding to F50 but this does not correlate with the observed changes in F95 in response to noxious stimulation.

In all the studies calves of differing breeds and sex were used in experimentation. This variation may have affected the results with differing pain thresholds between sexes (Sheffield et al 2000) and breeds (Levinger 1995; Archer et al 2004). Sex and breed were not standardized, as the mixed sample population was considered to be more representative of the animals during commercial slaughter. All calves in the experiments were selected to be within a predetermined age/weight range in order to reduce complications associated with anaesthesia while allowing the animals to be effectively handled and managed.

In chapters 4, 6 and 7 a 70-volt electrical stimulus was applied to the right shoulder of each animal prior to VNI or NPCB. An EEG or somatic response to this noxious electrical stimulus served as an entry requirement into the study. However, the stimulus might have sensitised or caused hyperalgesia to further noxious sensory input potentially compromising the validity of the results. Despite this, it was deemed essential to have this entry requirement to exclude the possibility of non-responsiveness due to excess anaesthesia. In chapter 5 where significant manipulation of the neck tissues was performed electrical stimulation was not carried out, but the responses in the NTI group were found to be qualitatively and quantitatively similar to

those caused by VNI, described in chapter 4, where an electrical stimulus was used prior to experimentation.

In experiments described in chapters 4 and 5 where VNI or NTI was performed, the animals were positioned in dorsal recumbency, exposing and extending the neck providing a good surface for incision. However, the slaughter of un-stunned cattle in dorsal recumbency is prohibited in many countries (Grandin 1994b) because of the stress involved in inverting the animal (Dun 1990; Grandin and Regenstein 1994; Shragge and Price 2004). Furthermore, when inverted the calves may aspirate blood (Grandin and Regenstein 1994; Gregory et al 2009), potentially resulting in further distress. Upright restraining systems have replaced inverted systems in many countries (Gregory 2005). The purpose of this body of work described in this thesis was to investigate the potential noxiousness of slaughter by VNI, and not to examine different methods of restraint. Because of this, the experimental benefits associated with slaughtering in dorsal recumbency, and in order to facilitate management of anaesthesia in the calves, the need for comparability in restraint system as used during commercial operations was considered to be less important.

In the studies involving NPCB stunning (chapters 6 and 7) there were some problems with miss firing of the stunner caused by a faulty spring in the firing mechanism; this could have caused ineffective stunning in some cases which may have affected the results. During these experiments no measure of stunner effectiveness was used. This might have forestalled such problems. The maintenance and testing of stunning devices is an important welfare issue during slaughter as decreasing bolt velocity (Daly et al 1987) and other stunner malfunctions can cause significant welfare compromise (Gregory 2007). In the studies involving NPCB stunning the equipment was cleaned at the end of each day and serviced by a commercial company at the conclusion of the experiments. The lack of a method for testing the effectiveness of the NPCB stunner was a significant limitation of studies. Currently there is no commercially based product available for testing NPCB stunners, however a rudimentary device is available for testing penetrative captive bolt (PCB) stunners (Cash Stuncheck; Accles and Shelvoke Ltd, West Midlands, UK).

In chapter 7, the ability of NPCB stunning to modulate the noxious effects of VNI was examined. A NPCB stun was applied 5 seconds after VNI. The 5-second

interval between VNI and NPCB was chosen because it was recommended in the public draft of the Animal Welfare (Commercial Slaughter) Code of Welfare 2006 (NAWAC 2006). In the current experiment the interval was of insufficient duration to observe noxious responses on the EEG because of out of range data and movement artefact caused by VNI. In hindsight, a NPCB stun 10 to 15 seconds after VNI would probably have been sufficient to observe EEG activity due to the incision prior to stunning and the modulation of that response with NPCB stunning.

It is important to note that the technique of slaughter without stunning examined in this thesis was not either Shechita or Halal slaughter. The techniques used were designed to be a close approximation of these methods. Without the involvement of the Jewish or Muslim slaughtermen and their specific skills and equipment the results of the experiments cannot be classified as in response to religious slaughter. There has been criticism (Rosen 2004) of studies that have not employed Jewish slaughtermen for the investigation of slaughter without stunning. These have been called incomparable with Shechita slaughter. However both Shechita and Halal slaughter involve the sectioning of the intact tissues of the ventral neck of un-stunned, conscious animals. Despite the fact that the methods for slaughter used in the current experiments were not Shechita or Halal they do involve similar tissue destruction to the ventral aspect of the neck and unless proven otherwise it seems likely that the EEG response will be representative.

11.5 Future Work

The present study suggests several avenues for future research with a number of significant issues regarding slaughter by VNI and NPCB stunning needing further investigation.

Firstly, after Shechita slaughter the Shochet inspects the wound for signs of a clean cut, complete severance of the carotid arteries and for any indications of carotid occlusion. Observations have been made of some Shochet's placing their hands into the open wound of cattle immediately after the cut (FAWC 2003), to ensure severance of neck vessels. This has the potential to cause further noxious stimulation to the damaged tissues of the neck and vessels, causing an additional noxious sensory barrage producing further pain and distress prior to hypoxia-induced insensibility. An experiment similar to that in chapter 4 could be performed where the wound is manipulated post VNI incision. Care would need to be taken not to cause additional movement that could produce artefacts in the EEG. Cortically-implanted electrodes may reduce the likelihood of such artefacts.

The role of the vertebral arteries in potentially maintaining blood flow to the brain after VNI slaughter, especially in combination with carotid occlusion, has been a subject of much debate. Studies have examined the time to loss of sensibility with the surgical ligation of the vertebral arteries (Shaw et al 1990) or measurement of vertebral blood flow (Anil et al 1995a) or pressure (Williamson et al 1958), with conflicting results. The current experiments examining VNI (Chapters 4, 5 and 7) were performed with the vertebral arteries intact. An experiment designed to investigate the modulation of the noxious sensory input of VNI with the ligation of the vertebral arteries using the minimal anaesthesia model could potentially provide useful information on the role of the vertebral arteries in maintaining cerebral blood supply sufficient for the perception of pain.

Another area for future study is investigation of vertebral arterial blood pressure during and after slaughter by VNI. Rosen (2004) in a viewpoint article stated that after Shechita slaughter blood from the vertebral arteries would flow in the direction of least resistance, towards the distal stumps of the severed carotids, bypassing the brain. However, this would be dependent on the presence or absence of carotid occlusion. The measurement of vertebral blood pressure, and the effects of

carotid occlusion on it, before and after VNI could be used to examine this hypothesis.

The large immediate increase in P_{tot} after VNI was hypothesised to be in response to movement during slaughter. This artefact may have masked potential biological responses to VNI slaughter. The causation of the increase in P_{tot} after VNI warrants further study. Cortically implanted electrodes could minimise the effects of movement during VNI. Furthermore, pre-VNI surgical dissection of the ventral strap muscles in isolation of the carotid arteries, jugular veins and other neck tissues could minimise movement artefact caused by the loss of muscular tone (i.e. neck and tissue movement) during VNI slaughter. The use of neuromuscular-blocking agents would also potentially reduce muscular based movement responses in P_{tot} . However there are a number of welfare concerns with the use of neuromuscular-blocking agents in slaughter-based research, including the potential for paralysis of individual animals that are not under complete anaesthesia during VNI slaughter. This would need to be carefully managed.

As mentioned previously there is currently no commercially-based product available for testing the performance of NPCB stunners. A device that is able to provide an indication of performance in the slaughterhouse would inevitably improve the welfare of animals during stunning with the identification of defects in equipment. Prior to the development of such a device, it is essential that the characteristics of NPCB stunning are examined with the development of models that: investigate the forces involved; how the energy is transferred to the cranium; the effect of differing bolt velocities; the effect of stunning location; and the effects of variation in cranium shape and bone density. Further investigations are needed into the functional activity of the brain (electrophysiological and neuro-imaging) in response to NPCB stunning to better understand the mechanisms of concussive insensibility in livestock, data from which would aid in the development of a testing device.

In chapters 9 and 10 the generation and recording of SEPs was investigated in sheep and cattle, respectively, using the subdermal electrodes and electrical stimulation of the exposed superficial peroneal nerve. The generation and recording of SEPs in cattle was problematic. The reasons for this unreliability, and whether or not similar unreliability would occur in sheep when sufficient numbers are investigated, merit further work.

11.6 Final Conclusion

In conclusion, the combination of these results demonstrates that incision of the neck tissues constitutes a substantial noxious stimulus, which in unanaesthetised animals would be likely to be experienced as pain. The use of NPCB stunning produced states of cortical activity that are incompatible with the maintenance of sensibility and the ability to experience pain resulting from VNI slaughter.

11.7 References

- Anil MH, McKinstry JL, Gregory NG, Wotton SB, Symonds H.** Welfare of Calves .2. Increase in vertebral artery blood-flow following exsanguination by neck sticking and evaluation of chest sticking as an alternative slaughter method. *Meat Science* 41, 113-23, 1995a
- Anil MH, McKinstry JL, Wotton SB, Gregory NG.** Welfare of calves .1. Investigations into some aspects of calf slaughter. *Meat Science* 41, 101-12, 1995b
- Antognini JF, Saadi J, Wang XW, Carstens E, Piercy M.** Propofol action in both spinal cord and brain blunts electroencephalographic responses to noxious stimulation in goats. *Sleep* 24, 26-31, 2001
- Archer N, Johnston AM, Khalid M.** Differences in the acute pain responses of two breeds of lamb following castration and tail docking with the rubber ring method. *Animal Welfare* 13, 135-41, 2004
- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP.** Onset of insensibility at slaughter in calves - effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162-73, 1992
- Barnett JL, Cronin GM, Scott PC.** Behavioural responses of poultry during kosher slaughter and their implications for the birds' welfare. *Veterinary Record* 160, 45-9, 2007
- Barter L, Dominguez CL, Carstens E, Antognini JF.** The effect of isoflurane and halothane on electroencephalographic activation elicited by repetitive noxious c-fiber stimulation. *Neuroscience Letters* 382, 242-7, 2005
- Blackmore DK, Newhook JC.** Insensibility during slaughter of pigs in comparison to other domestic stock. *New Zealand Veterinary Journal* 29, 219-22, 1981
- Blackmore DK, Newhook JC, Grandin T.** Time of onset of insensibility in 4-week-old to 6-week-old calves during slaughter. *Meat Science* 9, 145-9, 1983
- Blackmore DK.** Differences in behaviour between sheep and cattle during slaughter. *Research in Veterinary Science* 37, 223-6, 1984
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN.** Topographic effects of tonic cutaneous nociceptive stimulation on human electroencephalograph. *Neuroscience Letters* 305, 49-52, 2001a
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN.** Different EEG topographic effects of painful and non-painful intramuscular stimulation in man. *Experimental Brain Research* 141, 195-203, 2001b

- Chang PF, Arendt-Nielsen L, Chen ACN.** Differential cerebral responses to aversive auditory arousal versus muscle pain: specific EEG patterns are associated with human pain processing. *Experimental Brain Research* 147, 387-93, 2002
- Chen ACN, Dworkin SF, Haug J, Gehrig J.** Topographic brain measures of human pain and pain responsivity. *Pain* 37, 129-41, 1989
- Cook CJ, Maasland SA, Devine CE, Gilbert KV, Blackmore DK.** Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. *Research in Veterinary Science* 60, 255-61, 1996
- Daly CC, Gregory NG, Wotton SB.** Captive bolt stunning of cattle - effects on brain-function and role of bolt velocity. *British Veterinary Journal* 143, 574-80, 1987
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter - conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325-9, 1988
- Dun CS.** Stress reactions of cattle undergoing ritual slaughter using two methods of restraint. *Veterinary Record* 126, 522-5, 1990
- FAWC.** Report on the welfare of farmed animals at slaughter or killing. Part 1. red meat animals. Farm Animal Welfare Council, London, 2003
- Grandin T.** Euthanasia and slaughter of livestock. *Journal of the American Veterinary Medical Association* 204, 1354-60, 1994a
- Grandin T.** Farm animal-welfare during handling, transport, and slaughter. *Journal of the American Veterinary Medical Association* 204, 372-7, 1994b
- Grandin T, Regenstein JM.** Religious slaughter and animal welfare: a discussion for meat scientists. *Meat Focus International*, 115-23, 1994
- Gregory N.** Stunning and slaughter. In: *Animal Welfare and Meat Production*. Pp 191-212. CABI Publishing, Wallingford, 2007
- Gregory NG.** Recent concerns about stunning and slaughter. *Meat Science* 70, 481-91, 2005
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141-3, 1984a
- Gregory NG, Wotton SB.** Sheep slaughtering procedures .2. Time to loss of brain responsiveness after exsanguination or cardiac-arrest. *British Veterinary Journal* 140, 354-60, 1984b

- Gregory NG, von Wenzlawowicz M, von Holleben K.** Blood in the respiratory tract during slaughter with and without stunning in cattle. *Meat Science* 82, 13-6, 2009
- Johnson CB, Young SS, Taylor PM.** Use of frequency spectral analysis of the equine electroencephalogram as an indication of the depth of halothane anesthesia. In: Sebel PS, Bonke B, Winograd E (eds). *Memory and Awareness in Anesthesia*. Pp 289-95. P T R Prentice Hall, Englewood Cliffs, 1993
- Johnson CB, Taylor PM.** Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* 81, 748-53, 1998
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and analgesia* 32, 61-71, 2005b
- Levinger IM.** Untersuchungen zum schächtproblem. Dr thesis, University of Zürich, Zürich, 1961
- Levinger IM.** Shechita in the light of the year 2000, critical review of the scientific aspects of methods of slaughter and Shechita, Maskil L'David, Jerusalem, 1995
- Lukas SE, Mendelson JH, Benedikt RA, Jones, B.** EEG Alpha Activity Increased During Transient Episodes of Ethanol-Induced Euphoria. *Pharmacology Biochemistry & Behavior* 25, 889-95, 1986
- Lukas SE, Mendelson, JH.** Electroencephalographic Activity and Plasma ACTH during Ethanol-Induced Euphoria. *Biological Psychiatry* 23, 141-8, 1988
- Lukas SE, Mendelson JH, Benedikt R.** Electroencephalographic correlates of marihuana-induced euphoria. *Drug and Alcohol Dependence* 37, 131-40, 1995
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325-35, 2006
- Nangeroni LL, Kennett PD.** Electroencephalographic studies of the effects of shechita slaughter on cortical function in ruminants. Cornell University, New York, 1964
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves - .1. The onset of permanent insensibility in sheep during slaughter. *Meat Science* 6, 221-33, 1982a
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves .2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295-300, 1982b

- NAWAC.** Animal welfare (commercial slaughter) code of welfare 2006 public draft. In. Pp 1-65. (New Zealand) 2006
- Orth M, Barter L, Dominguez C, Atherley R, Carstens E, Antognini JF.** Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation. *British Journal of Anaesthesia* 95, 477-84, 2005
- Rosen SD.** Viewpoint article; Physiological insights into Shechita. *Veterinary Record* 154, 759-65, 2004
- Shaw FD, Bager F, Devine CE.** The role of the vertebral arteries in maintaining spontaneous electrocortical activity after electrical stunning and slaughter in calves. *New Zealand Veterinary Journal* 38, 14-6, 1990
- Sheffield D, Biles PL, Orom H, Maixner W, Sheps DS.** Race and sex differences in cutaneous pain perception. *Psychosomatic Medicine* 62, 2000
- Shragge JE, Price MA.** Religious slaughter. In: Jensen W, Devine C, Dikeman M (eds). *Encyclopaedia of Meat Sciences*. Pp 1162-7. Elsevier Ltd, Oxford, 2004
- Williamson AJ, Nangeroni LL, Dukes HH.** Blood pressure and blood flow in the vertebral arteries of ruminants. Cornell University, New York, 1958

APPENDIX 1

EEG SPECTRAL ANALYSIS PROGRAMME CODE

This code was used for the spectral analyses of the EEG with the Fast Fourier Transformation (FFT) (Cooley and Tukey 1965) in Chapters 3 to 8. The code outputs the EEG power spectrum over time, power and frequency and also outputs: median frequency (F50), 95% spectral edge frequency (F95) and total power (Ptot) of the EEG power spectrum. It is written in the C programming language.

```
/*
 * Spectral Analyser
 * © 2002 Craig Johnson
 *
 * Now with DC Offset Removal as Standard (new for 2006)
 *
 */

#include <stdio.h>
#include <stdlib.h>
#include <time.h>
#include <math.h>
#include <string.h>
#include "nr.h"
#include "nrutil.h"
#include "floater.h"

#define EPS 1.0e-3
#define NP 1024

int main(void)
{
    int i, n=NP/2, rounder;
    float *data, *size, tmpfloat, window [1024], halfWay, top, totalPower, tester, intTester,
    median, edge, spectrum [31], sum, average;
    char dataFile [100], writeFile[100], holder [100], append [3] = "OUT";
    char test;

    FILE *inputFile, *outputFile;

    for (i=0;i<=1023;i++) /* Window function (Welch window)*/
        {
            top = (i+1)-n;
            halfWay = top/n;
            window[i] =1-pow(halfWay,2);
        }

    printf ("Spectral Analyser © Craig Johnson 2002\n\n");
    test = 'x';
    dataFile [0] = 'n';

    while (1)
```

```

        {
vectors */      data = vector (1,NP);                /* allocates memory for the
size = vector (1,NP/2+1);

        printf ("Enter the name of the next file:\t");
                                                    /*
Find the name of the next file */
        scanf ("%s",&dataFile);
        printf ("\n\n");

*/      if ((inputFile = fopen (dataFile,"r"))== NULL)      /* opens the input file
        {
            printf ("\nCannot Open This File\n");
            return 1;
        }

        strncpy (writeFile,dataFile,100);
        strncat (writeFile,append,3);                /* names the output
file */      outputFile = fopen (writeFile,"w");        /* opens the output file */

        fprintf (outputFile,"0\t1\t2\t3\t4\t5\t6\t7\t8\t9\t10");
        fprintf (outputFile,"\t11\t12\t13\t14\t15\t16\t17\t18\t19\t20");
        fprintf (outputFile,"\t21\t22\t23\t24\t25\t26\t27\t28\t29");
        fprintf (outputFile,"\tMedian\tSE\tPtot\n");

while (!feof (inputFile))
    {
        for (i=0;i<=11;i++) data [i] = 0; /* zero padding at start */
        {
            data [i] = 0;
        }
        for (i=12;i<=1011;i++) /* input from the file */
        {
            if (feof(inputFile)) break;
            fgets (holder,100,inputFile);
            /*changer = Floater (holder);
            data [i] = changer;*/
            sscanf(holder,"%f",&tmpfloat);
            data[i]=tmpfloat;
        }
        for (i=1012;i<=1023;i++) /* zero padding at end */
        {
            data [i] = 0;
        }

        sum =0;
    }

```

```

for (i=0;i<=1023;i++) /* Find average value*/
    {
        sum = sum + data [i];
    }

average = sum/ 1024;

for (i=0;i<=1023;i++) /* Subtract average value*/
    {
        data [i] = data [i] - average;
    }

for (i=0;i<=1023;i++) /* Multiply data by Window function*/
    {
        data [i] = data [i] * window [i];
    }

realft (data,NP,1); /* The FFT algorithm */

for (i=2;i<=32;i++)
    {
        spectrum[i]=sqrt(SQR(data[2*i-1])+SQR(data[2*i])); /* Places the real
component of the FFT into size[] */
    }

for (i=0;i<=29;i++) /* Shuffles FFT to correct frequency*/
    {
        spectrum[i]=spectrum[i+2];
    }
for (i=0;i<=29;i++)
    {
        totalPower = totalPower + spectrum[i]; /* Calculates the total power */
    }
intTester = totalPower * 100;
rounder = intTester;
intTester = rounder;
totalPower = intTester / 100;

tester = totalPower / 2; /* Calculates the median frequency */
i = 0;
do
    {
        tester = tester - spectrum [i];
        i++;
    } while (tester > spectrum [i]);
median = i + (tester / spectrum [i]);
intTester = median * 100;
rounder = intTester;
intTester = rounder;
median = intTester / 100;

tester = totalPower * 0.95; /* Calculates the spectral edge frequency */
i = 0;
while (tester > spectrum [i])
    {
        tester = tester - spectrum [i];
        i++;
    }

```

```
edge = i + (tester / spectrum [i]);
intTester = edge * 100;
rounder = intTester;
intTester = rounder;
edge = intTester / 100;

if (!feof(inputFile))
    {

for (i=0;i<=29;i++)      /* Writes a line to the output file */
    {
    fprintf (outputFile,"%f\t",spectrum [i]);
    }

fprintf (outputFile,"%f\t",median);
fprintf (outputFile,"%f\t",edge);
fprintf (outputFile,"%f\t",totalPower);
fprintf (outputFile,"\n");
    }

median = edge = totalPower = 0;

}

/* end of file loop */

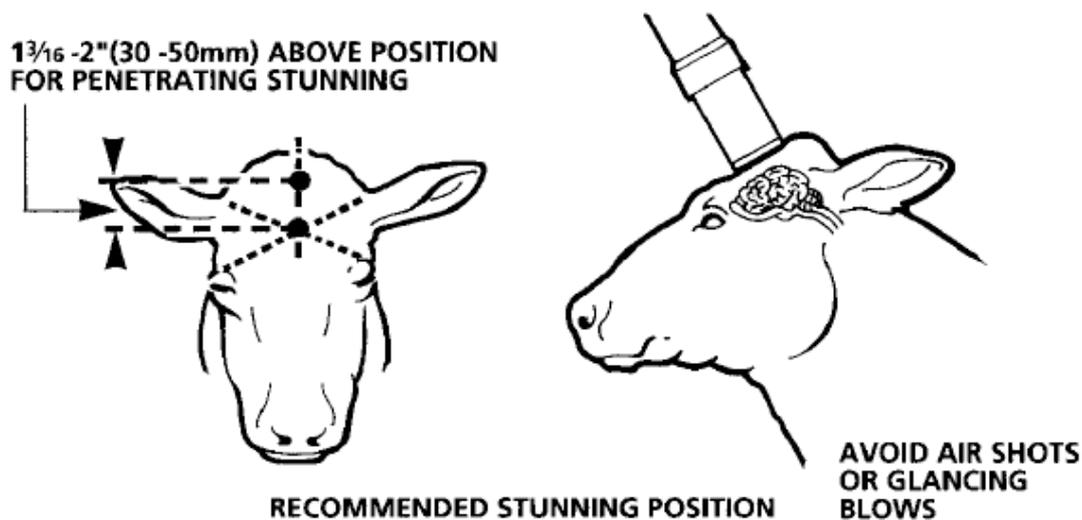
fclose (inputFile);
fclose (outputFile);

}
/* End of the while control loop */
free_vector (size,1,NP/2+1);
free_vector (data,1,NP);
}
```

APPENDIX 2

STUNNING POSITION IN CATTLE

Manufacturer Instructions for the placement and firing of the CASH Magnum Knocker Concussion Stunner (Accles and Shelvoke Ltd, Birmingham, England) were followed in Chapters 6, 7 and 10.



The muzzle was pressed firmly on the animal's head in accordance to the manufacturer recommended stunning position, 30 mm above the intersection of lines drawn from the medial canthus of each eye to the rostral border of the contralateral ear (Accles and Shelvoke Ltd).

APPENDIX 3

PUBLISHED SCIENTIFIC PEER-REVIEWED PAPERS

Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Validation of the Acute Electroencephalographic Responses of Calves to Noxious Stimulus with Scoop Dehorning. *New Zealand Veterinary Journal* 55, 152-7, 2007

152

New Zealand Veterinary Journal 55(4), 152-157, 2007

Scientific Article

Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning

TJ Gibson^{*†§}, CB Johnson^{*†}, KJ Stafford^{*†}, SL Mitchinson[†] and DJ Mellor^{*‡}

Abstract

AIM: To validate use of the electroencephalogram (EEG) and a minimal anaesthesia model for assessment of noxious sensory input caused by scoop dehorning of calves.

METHODS: Twenty Friesian heifers weighing 125–178 kg were maintained under light general anaesthesia using halothane and an established protocol (minimal anaesthesia model). They were then dehorned using a scoop dehorner either with (DH+LA) or without (DH) a lignocaine ring block, and changes in the EEG and electrocardiogram (ECG) recorded. Median frequency (F50), 95% spectral edge frequency (F95) and total power (Ptot) were derived from the EEG data.

RESULTS: There were significant increases in the F50 ($p < 0.01$) and F95 ($p < 0.01$), and a decrease in Ptot ($p < 0.01$) following dehorning in the DH group, but there were no changes in the DH+LA group. Transient bradycardia in the first 75 sec following dehorning was recorded in the DH group compared with both pre-treatment values in the same group and with the DH+LA group ($p < 0.001$). Tachycardia was evident by 15 min after dehorning in the DH but not the DH+LA group.

CONCLUSIONS: The results validate use of the EEG and a minimal anaesthesia model for assessment of noxious sensory inputs in cattle.

KEY WORDS: Bradycardia, calves, dehorning, electroencephalogram, lignocaine ring block, minimal anaesthesia model, nociception, pain, tachycardia

Introduction

The cerebral cortex has an integral role in the conscious perception of pain (Talbot et al 1991; Jones et al 1992; Treede et al 1999). The EEG has long been known to reflect changes in cortical function by an alteration in the components of its frequency (Adrian and Matthews 1934). Development of the Fast Fourier Transformation method (Cooley and Tukey 1965) has allowed statistical analysis of specific variables in the power spectra of EEGs. Changes in the power spectra of the EEG have been shown to reflect alterations in the activity of the cerebral cortex associated with cognitive perception of pain (Chen et al 1989). Spectral analysis of EEGs has been used both in human (Chen et al 1989; Bromm and Lorenz 1998; Chang et al 2001a,b, 2002) and animal

(Ong et al 1997; Jongman et al 2000; Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005a,b; McGregor 2005) studies to examine the acute noxiousness of painful events, e.g. ice water baths, surgery, castration, tail docking, mulesing.

Although spectral changes in the EEG are indirect measures of pain, they reflect cortical electrical activity and hence are more likely to reflect the cognitive perception and processing of noxiousness (Barnett 1997; Johnson et al 2005a). Spectral EEG variables that have been found to be strongly correlated with noxious stimulation are F50 and Ptot (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005a,b; McGregor 2005), and F95 is correlated with increasing depth of halothane anaesthesia (Johnson et al 1993, 1994; Johnson and Taylor 1998). The F50 is the frequency below which 50% of the total power of the EEG is located. The F95 is the frequency below which 95% of the total power of the EEG is located. Increases in F50 and F95 have been previously associated with nociception (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005a,b; McGregor 2005). The Ptot is defined as the total area under the power spectrum curve (Murrell and Johnson 2006). A decrease in Ptot has been previously associated with nociception (Murrell 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005a,b; McGregor 2005). Combined with the use of a minimal anaesthesia model, therefore, EEG spectral analysis could potentially be a useful tool in the quantification of noxiousness in cattle.

The minimal anaesthesia model maintains an animal on a stable plane of anaesthesia, allowing the recording of cerebrocortical responses to a stimulus by reducing variability in background cerebrocortical electrical activity due to extraneous stimuli. To date, this technique has been used to investigate responses to noxious sensory stimuli in horses (Murrell et al 2003, 2005), sheep (Johnson et al 2005a), red deer (*Cervus elaphus*) (Johnson et al 2005b), pigs (Haga and Ranheim 2005), Tammar wallaby (*Macropus eugenii*) (Diesch et al 2005, non-peer-reviewed) and rats (McGregor 2005). The model has the additional benefit of allowing the investigation of painful procedures without compromising the welfare of the animal. However, the use of this model and spectral analysis for the detection of cerebrocortical changes in response to noxious stimulation are a novel and untested methodology in cattle. Validation is required before assessments of the apparent noxiousness, or otherwise, of other noxious events can be made.

ANOVA	Analysis of variance
DH	Dehorned only
DH+LA	Dehorned plus lignocaine ring block
ECG	Electrocardiogram/electrocardiographic
EEG	Electroencephalogram/electroencephalographic
F50	Median frequency
F95	95% Spectral edge frequency
Ptot	Total power (of the electroencephalogram)
SD	Standard deviation

* Animal Welfare Science and Bioethics Centre, Massey University, Private Bag 11222, Palmerston North, New Zealand.

† Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand.

‡ Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North, New Zealand.

§ Author for correspondence. Email: t.g.gibson@massey.ac.nz

Amputation (scoop) dehorning of calves is a routine husbandry practice, which is a noxious insult (Stafford and Mellor 2005). The acute noxiousness of dehorning is well established in terms of induced changes in concentrations of plasma cortisol (Morisse et al 1995; Petrie et al 1996; McMeekan et al 1997, 1998ab; Sylvester et al 1998ab; Grøndahl-Nielsen et al 1999; Sutherland et al 2002a; Stafford et al 2003), concentrations of catecholamine (Mellor et al 2002), and behaviour (Morisse et al 1995; Graf and Senn 1999; Grøndahl-Nielsen et al 1999; Stafford et al 2000; Sylvester et al 2004). The application of a corneal nerve block has been demonstrated to virtually eliminate escape behaviours seen during dehorning (Sylvester et al 2004) and to significantly reduce the acute plasma cortisol response (Morisse et al 1995; Petrie et al 1996; McMeekan et al 1998ab; Sylvester et al 1998a; Graf and Senn 1999; Grøndahl-Nielsen et al 1999; Sutherland et al 2002ab). The use of amputation dehorning is therefore a useful technique to validate indices of noxiousness, and has the additional advantage of representing a predominantly somatic noxious insult, without visceral components. The bovine horn is primarily innervated by the corneal nerve, which is a branch of the trigeminal nerve (Budras et al 2003). As the trigeminal nerve is the largest somatic sensory nerve of most mammals (Brodal 1962; Kruger and Young 1981; Butler and Hodos 2005), EEG responses to dehorning will reflect somatic, as opposed to visceral, stimulation. This may reduce complications in interpretation of the EEG due to differences in cortical processing of somatic and visceral noxious stimuli (Aziz et al 2000; Dunckley et al 2005; Johnson et al 2005a).

The aim of this study was to validate the use of the minimal anaesthesia model to assess noxious somatic stimuli in cattle by evaluating responses to scoop dehorning with or without prior application of a lignocaine ring block. It was hypothesised that dehorning would cause changes in the EEG that are associated with noxious stimulation and that the application of a lignocaine ring block would mitigate this response.

Materials and methods

Animals

All calves were sourced from a dairy unit at Massey University, and the study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand. Friesian heifers 6–9 months old, weighing 125–178 kg, were used in the study. Prior to experimentation, calves were kept in accordance with normal dairy farming practices. Calves were randomly allocated to one of two treatment groups: scoop dehorning alone (DH; n=10), or scoop dehorning with lignocaine ring nerve block (DH+LA; n=10). The animals were penned overnight, and had access to water but not food the day prior to the study.

Anaesthesia

Anaesthesia was induced using a mixture of 3.4 (standard deviation (SD) 0.3) mg/kg ketamine (Parnell Laboratories, Auckland, NZ) and 4.1 (SD 1.0) mg/kg propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid injection into a jugular vein. Following endotracheal intubation with a 16-mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia), anaesthesia was maintained using inhalation of halothane (Halothane-Vet; Merial NZ Ltd, Manukau City, NZ) in oxygen (BOC, Palmerston North, NZ), delivered via a precision vaporiser (Fluothane; MedSource Ltd, Ashburton, NZ) and a circle breathing system (VMS Anaesthesia Machine; Matrix Medial Inc, New York, USA). Calves were allowed to breath

spontaneously throughout the experiment. End-tidal halothane tension was maintained at 0.9%. End-tidal carbon dioxide tension, heart rate and respiratory rate were monitored using an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Hamburg, Germany). All subsequent procedures were carried out under general anaesthesia.

EEG and ECG recording

Subdermal 27-G stainless-steel needle electrodes (Medelec; Radiometer, Auckland, NZ) were placed in a three-electrode montage, adapted from the description by Mayhew and Washbourne (1990). The non-inverting electrode was placed in the midline between the medial canthi of the eyes, the inverting electrode over the left mastoid process, and the ground electrode caudal to the poll (see Murrell and Johnson (2006) for further explanation). Electrode impedance was not controlled throughout the study but intermittent recordings of impedance were made which were in the range of 2 to 4 k Ω . A base-apex electrode configuration was used to record the ECG.

The EEG and ECG were amplified using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota FL, USA). The EEG was recorded with a gain of 1,000 and pass-band of 0.1 to 500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10 to 500 Hz. Both EEG and ECG data were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

Experimental procedure

Once anaesthetised, calves were placed in right lateral recumbency on a padded airbed, and the head supported with a vacuum support surgical support (Shoof International Ltd, Cambridge, NZ). Lignocaine (Nopaine; Phoenix Pharm Distributors Ltd, Auckland, NZ) ring blocks (20 ml per animal) were administered around the base of the left horn, as described by Graf and Senn (1999), of calves in the DH+LA group after induction of anaesthesia. Calves in the DH group did not receive lignocaine ring blocks. Ten minutes were allowed for equilibration of general anaesthesia, after which a 15-min pre-treatment trace was recorded (Figure 1). The left horn of each calf in the two groups was removed using the scoop dehorner (Barnes Dehorners, Stones MO, USA). The same trained operator performed all dehorning during the experiment. The time of removal of the horn was recorded. Dehorning equipment was cleaned between horns and animals. Data were recorded for 15 min after dehorning for every animal.

After the experiment, all remaining horns were removed by scoop dehorning with the prior administration of a 5-ml lignocaine corneal nerve block. Additionally, calves were given the long-acting antibiotic procaine penicillin (12 mg/kg; Depocillin; Intervet Ltd, Upper Hutt, NZ) and systemic pain relief comprising 3 mg/kg ketoprofen 10% (Merial NZ Ltd) administered intravenously prior to recovery from anaesthesia. Calves were continuously monitored in darkened pens during this period of recovery. After recovery, the animals were returned to their herd.

EEG and ECG analysis

EEG epochs contaminated by artefacts, overscale or underscale were manually rejected from analysis of raw EEG data, using Chart 4.2.3 (ADInstruments Ltd). The F50, F95 and Ptot were calculated for consecutive non-overlapping 1-sec epochs, using purpose-written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North, NZ, 2002). Data were multiplied using a Welch window. Fast Fourier Transformation was applied to each epoch, generating sequential power spectra with 1-Hz frequency bins. Subsequent analysis was performed using Microsoft Excel 2002 (Microsoft Corporation, Redwood, USA).

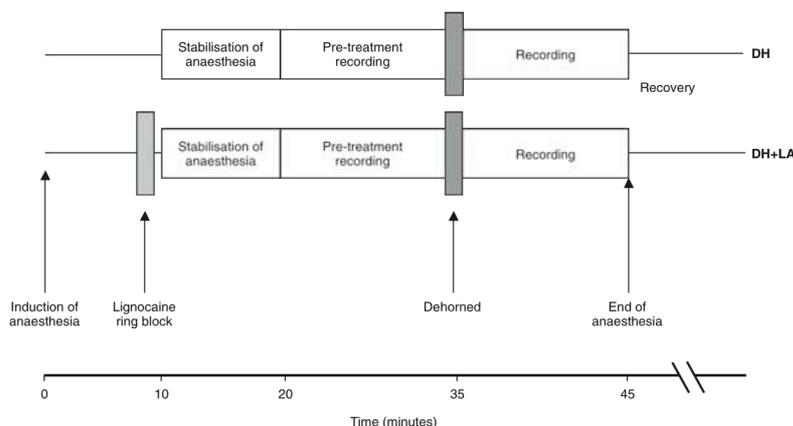


Figure 1. Diagram of the experimental design for scoop dehorning alone (DH) or scoop dehorning with lignocaine ring block (DH+LA) in groups of calves over time (minutes) from induction of anaesthesia (0 minutes) to recovery (45 minutes).

Variables derived from 2 sec before to 5 sec after dehorning were excluded from EEG analysis, to prevent contamination caused by movement artefact due to the surgical procedure.

Heart rates were calculated from ECG data, as the percentage change from their pre-treatment values (100%), pooled into their respective treatment groups (DH, DH+LA), and comparisons made between treatments and with pre-treatments. Pre-treatment values originated from the mean of the initial 200 sec of data recording. Care was taken to exclude values from within 100 sec of dehorning, in order to prevent potential inclusion of data affected by movement during dehorning.

Statistical analysis

Data were analysed using Minitab 14.2 (Minitab Incorporated, Pennsylvania, USA). An Anderson-Darling test for normality was performed on all data. Between-group comparisons for EEG data were made using area-under-the-curve analysis of 10-sec blocks and a one-way analysis of variance (ANOVA) unstacked. Between-group comparisons for heart rate were made between individual time points using a one-way ANOVA (unstacked). *Post-hoc* comparisons with a pre-treatment value were made using Dunnett's test, for both EEG and heart-rate data. Values of $p < 0.01$ were taken to indicate significance in all analyses. The intention of this study was to validate the minimal anaesthesia technique in cattle, prior to its use to investigate the effects of a number of other manipulations, which may or may not represent noxious stimuli; because of the importance of this technique as a basis for our further studies, a conservative p -value ($p < 0.01$) was chosen to indicate significance.

Results

Mean F50 (Figure 2) and mean F95 (Figure 3) were significantly increased following dehorning in DH compared with DH+LA calves ($p < 0.01$), and compared with their respective pre-treatment values in DH only ($p < 0.01$). Mean P_{tot} (Figure 4) was significantly decreased following dehorning in the DH compared with the DH+LA group ($p < 0.01$), and when compared with its pre-treatment values in the DH group ($p < 0.01$). All EEG re-

sponses in the DH group following dehorning were transient and returned to pre-treatment values within 2 min after dehorning. There were no significant differences from pre-treatment values for any EEG variable in DH+LA calves. One calf was excluded from EEG analysis (DH+LA) due to an unacceptable level of contamination of the EEG.

ECG data from three calves (two DH+LA and one DH) were excluded from heart-rate analysis due to an unacceptable level of contamination. Following dehorning, heart rate immediately decreased in DH calves compared with pre-treatment values ($p < 0.01$; Table 1). Heart rate was significantly lower in the DH compared with the DH+LA group ($p < 0.001$) during the first 75 sec after dehorning (Table 1). This decrease was transient and returned to pre-treatment values by 90 sec after dehorning. After the initial changes, heart rate in both groups increased with increasing variability (Table 1). This tachycardia became significant in DH, but not DH+LA, calves compared with pre-treatment values ($p < 0.01$) by 15 min after treatment.

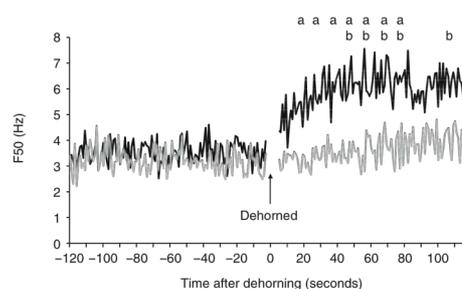


Figure 2. Mean of the median frequency (F50) of the electroencephalogram before and after dehorning in calves dehorned with (—) or without (---) a lignocaine ring block. a = Values significantly different between treatment groups ($p < 0.01$); b = values significantly different from baseline dehorned without lignocaine ring block ($p < 0.01$); 0 seconds = time point of dehorning.

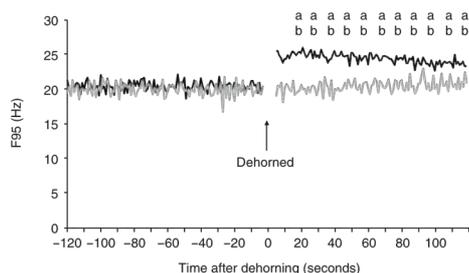


Figure 3. Mean 95% spectral edge frequency (F95) of the electroencephalogram before and after dehorning in calves dehorned with (—) or without (---) a lignocaine ring block. a = Values significantly different between treatment groups ($p < 0.01$); b = values significantly different from baseline dehorned without lignocaine ring block ($p < 0.01$); 0 seconds = time point of dehorning.

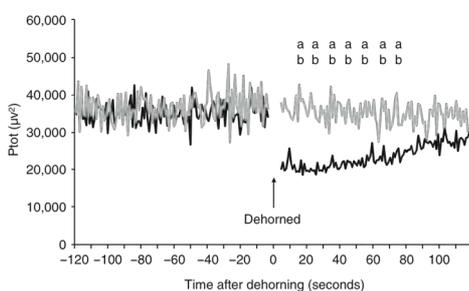


Figure 4. Mean total power (Ptot) of the electroencephalogram before and after dehorning in calves dehorned with (—) or without (---) a lignocaine ring block. a = Values significantly different between treatment groups ($p < 0.01$); b = values significantly different from baseline dehorned without lignocaine ring block ($p < 0.01$); 0 seconds = time point of dehorning.

Discussion

The results of this study validate the use of spectral analysis of the EEG in minimally anaesthetised cattle for the assessment of a noxious stimulus. The study further validates the existing body of evidence of the acute noxiousness of dehorning and its attenuation with the application of a lignocaine ring block (Stafford and Mellor 2005).

Our findings are consistent with previous work that investigated the acute noxiousness of routine husbandry practices that used a similar minimal anaesthesia protocol (Murrell et al 2003, 2005; Haga and Ranheim 2005; Johnson et al 2005ab). Increases in F50 and F95, and a decrease in Ptot, following velvet antler amputation, and attenuation of these changes by prior local anaesthetic nerve block, have been observed in deer (Johnson et al 2005b). Surgical castration of horses (Murrell et al 2003) and piglets (Haga and Ranheim 2005) elicited similar changes in F50 and Ptot. The application of intravenous lignocaine prior to surgical castration in horses (Murrell et al 2005) attenuated changes in F50 and Ptot previously observed without analgesia (Murrell et al 2003). Injection of lignocaine into the spermatic cord or the testes of piglets similarly attenuated the EEG response to castration (Haga and Ranheim 2005). An increase in F50 in response to rubber-ring castration was observed in lambs of differing ages

Table 1. Mean \pm standard error of the mean of heart rate (beats per minute) as a percentage of individual pre-treatments at time points after the start of dehorning for scoop dehorning alone (DH) or scoop dehorning with lignocaine ring block (DH+LA) groups of calves.

Time (sec)	DH+LA (% of pre-treatment)	DH (% of pre-treatment)	P-value (difference between treatments)
-15 ^a	100.14 \pm 0.59 ^c	98.81 \pm 0.77	0.187
0 ^b	101.68 \pm 2.41	73.98 \pm 3.67 ^d	<0.001
15	101.94 \pm 2.96	72.99 \pm 3.58 ^d	<0.001
30	102.10 \pm 3.19	73.66 \pm 3.49 ^d	<0.001
45	101.29 \pm 3.23	76.60 \pm 2.50 ^d	<0.001
60	100.48 \pm 3.15	77.15 \pm 2.11 ^d	<0.001
75	99.93 \pm 3.11	77.71 \pm 2.20 ^d	<0.001
90	109.76 \pm 5.54	104.07 \pm 4.86	0.458
105	109.76 \pm 5.75	105.19 \pm 5.31	0.619
120	108.53 \pm 5.92	104.80 \pm 5.15	0.642
135	108.43 \pm 6.12	103.92 \pm 5.15	0.587
150	108.32 \pm 6.28	103.23 \pm 5.12	0.546
165	108.02 \pm 6.47	102.84 \pm 5.17	0.548
180	107.99 \pm 6.62	102.46 \pm 5.14	0.527
300 (5 min)	108.18 \pm 7.02	107.33 \pm 5.80	0.928
600 (10 min)	115.77 \pm 7.95	119.78 \pm 7.84	0.726
900 (15 min)	117.12 \pm 9.71	122.91 \pm 7.64 ^d	0.652

^a Pre-treatment

^b Dehorned

^c There were no significant differences ($p < 0.01$) between pre-treatment and any of the post-treatment values in the DH+LA group

^d Value differed significantly from pre-treatment level ($p < 0.01$)

(Johnson et al 2005a). Those studies, and the observed increase in F50 following dehorning in the DH group, support the hypothesis that the F50 responds preferentially to noxious stimuli (Murrell et al 2003; Johnson et al 2005a). This is further supported by work in conscious sheep, that investigated behaviour and spectral changes in EEG bandwidth in response to electrical stimulation (Ong et al 1997). Those workers demonstrated that behavioural responses associated with noxious stimulation were associated with increased activity in the middle frequency ranges of the EEG (delta 2, theta 2, alpha 1 and alpha 2), which correlate with F50. The current experiment was carried out under general anaesthesia, which abolished somatic motor responses to the noxious stimuli, reducing the possibility of artefact.

Results from EEG spectral analysis changed predictably in response to changes in the partial pressure of inhalation anaesthetic agents (Johnson and Taylor 1998). Changes in spectral edge frequency correlated with changes in end-tidal halothane partial pressure in the horse (Johnson et al 1994; Johnson and Taylor 1998). It is unlikely that alterations in end-tidal halothane partial pressure produced the reported changes in spectral power in the current study. End-tidal halothane partial pressure was maintained at 0.9–0.95% and 10 min were allowed for equilibrium of anaesthesia, followed by 15 min of recordings pre-treatment, prior to dehorning. Over this period F95 remained stable, after which changes in spectral power occurred rapidly following dehorning. Changes seen in the EEG in response to noxious stimulation were attenuated by the actions of general anaesthesia in other studies (Antognini and Carstens 1999; Orth et al 2005). However, in the current experiment, significant changes were still recorded in the DH group following dehorning.

Changes in the frequency spectrum of the EEG following dehorning without a lignocaine ring block represented the acute phase of the response to noxious stimulation or first pain. All EEG indices examined in the current experiment returned to pre-treatment levels within 2 min of dehorning. This is in contrast to the elevated cortisol levels and behavioural responses to amputation dehorning, which have been shown to last for 7–9 h (Petrie et al 1996; McMeekan et al 1997, 1998ab; Sylvester et al 1998a) or 6 h (Sylvester et al 2004). Persistent pain following this acute phase of dehorning is highly probable and is supported by the time taken for wound healing and by reductions in weight gain after dehorning (Stafford and Mellor 2005). The short duration of the EEG response to noxious stimulation could have been due to the actions of the general anaesthesia, pain modulation or anti-nociceptive mechanisms within the central nervous system following noxious stimulation (Fields and Basbaum 1999). Evidence of the cortex influencing pain by interrupting the transmission of noxious signals from the spinal cord is well established with the descending pain modulation system (Ohara et al 2005). However, these factors would also be expected to influence the plasma cortisol and behavioural responses to noxious stimulation, which was not evident in the previous investigations. Background electrical activity of the cortex could potentially dominate and mask the chronic response of the EEG to a noxious stimulus over time, making subsequent detection and analysis of such a response unfeasible following the acute noxious response.

Propofol and ketamine were chosen for induction of general anaesthesia due to their short duration of action and rapid excretion. Ketamine has analgesic properties (Branson 2001), which may have attenuated the somatic responses to noxious stimulation (Johnson et al 2005b). As the doses of ketamine given were not significantly different between the treatment groups, any such influence would have been common to both treatment groups. In addition, there were no changes in EEG variables during the baseline recording period, indicating that the effects of the induction agents had waned by the time of the onset of data collection.

Transient bradycardia was observed in the DH group immediately following dehorning. Acute bradycardia in response to a noxious stimulus in anaesthetised animals has been observed previously with velvet antler removal in deer (Johnson et al 2005b), and castration of lambs (Johnson et al 2005a) and piglets (Haga and Ranheim 2005). These results are in contrast to previous studies where only tachycardia was reported following noxious stimuli (Lay et al 1992ab; Grøndahl-Nielsen et al 1999; Peers et al 2002). Heart rate in the current study was recorded continuously during the 15 min before and after dehorning. This allowed detection of short-lived bradycardia (Table 1), whereas intermittent recording, as used in previous investigations, may have overlooked this transient effect. Similar transient bradycardia was reported in conscious human infants undergoing routine immunisation (Johnston and Strada 1986). Its presence in anaesthetised calves and conscious human infants suggests that this response was not due to anaesthesia but was rather an acute response to nervous stimulation.

Cornual nerve block is routinely used during dehorning in mature cattle (Stafford and Mellor 2005). Although the cornual nerve supplies the primary innervation, other nerves are also involved. The infratrochlear and frontal nerves, branches of the trigeminal nerve (King and Riley 1980), can both carry sensory fibres from the bovine horn. Possible innervation from cutaneous branches of spinal nerves C1 and C2 can also innervate the caudal aspect of the horn of mature cattle (King and Riley 1980; Hall and Clarke 2001). Complete blockade of the bovine horn was not always achieved following the application of lignocaine to the cornual

nerve (Sylvester et al 1998a). The use of a lignocaine ring block rather than a cornual nerve block had the advantage of providing complete blockade of sensory input from the bovine horn.

In conclusion, the current experiment validates the use of this novel approach for investigating acute painful husbandry procedures in cattle such as dehorning, and demonstrates the usefulness of this technique in animal welfare research. These findings confirm and add to the existing body of evidence relating to the noxiousness of scoop dehorning, and its alleviation using a lignocaine ring block prior to the procedure.

Acknowledgements

The authors are grateful for the joint funding of the study by the Ministry of Agriculture and Forestry of New Zealand and the Department of Environment, Food and Rural Affairs of the United Kingdom. The authors also thank all the members of the neurophysiological group, Massey University, for their help with experimental work and helpful discussion, and members of the Massey University Dairy Unit 1 for assistance during the experiment. TJ Gibson was the recipient of a C Alma Baker Postgraduate Scholarship.

References

- Adrian ED, Matthews BHC. The interpretation of potential waves in the cortex. *Journal of Physiology* 81, 440–71, 1934
- Antognini JF, Carstens E. Isoflurane blunts electroencephalographic and thalamic-reticular formation responses to noxious stimulation in goats. *Anesthesiology* 91, 1770–9, 1999
- Aziz Q, Thompson DG, Ng VWK, Hamdy S, Sarkar S, Brammer MJ, Bullmore ET, Hobson A, Tracey I, Gregory L, Simmons A, Williams SCR. Cortical processing of human somatic and visceral sensation. *Journal of Neuroscience* 20, 2657–63, 2000
- Barnett JL. Measuring pain in animals. *Australian Veterinary Journal* 75, 878–9, 1997
- Branson KR. Injectable anesthetics. In: Adams HR (ed). *Veterinary Pharmacology and Therapeutics*. Pp 213–67. Iowa State University Press, Ames IA, USA, 2001
- Brodal A. The trigeminal nerve. *The Cranial Nerves*. Pp 79–96. Blackwell Scientific Publications, Oxford, UK, 1962
- Bromm B, Lorenz J. Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology* 107, 227–53, 1998
- Budras K-D, Habel RE, Wunsche A, Buda S. Central nervous system and cranial nerves. *Bovine Anatomy. An Illustrated Text*. Pp 50–7. Schlutersche, Hannover, Germany, 2003
- Butler AD, Hodoss W. Functional organization of the cranial nerves. *Comparative Vertebrate Neuroanatomy*. 2nd Edtn. Pp 173–82. John Wiley & Sons Inc, Hoboken, USA, 2005
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN. Topographic effects of tonic cutaneous nociceptive stimulation on human electroencephalograph. *Neuroscience Letters* 305, 49–52, 2001 a
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN. Different EEG topographic effects of painful and non-painful intramuscular stimulation in man. *Experimental Brain Research* 141, 195–203, 2001 b
- Chang PF, Arendt-Nielsen L, Chen ACN. Differential cerebral responses to aversive auditory arousal versus muscle pain: specific EEG patterns are associated with human pain processing. *Experimental Brain Research* 147, 387–93, 2002
- Chen ACN, Dworkin SF, Haug J, Gehrig J. Topographic brain measures of human pain and pain responsibility. *Pain* 37, 129–41, 1989
- Cooley JW, Tukey JW. An algorithm for the machine calculation of complex fourier series. *Mathematics of Computation* 19, 297–301, 1965

- Diesch TJ, Johnson CB, Mellor DJ, Lentle RJ. Development of brain function and responsiveness in the tamar wallaby (*Macropus eugenii*). P 70. *Medical Science Congress*. Queenstown, NZ, 2005
- Dundley P, Wise RG, Aziz Q, Painter D, Brooks J, Tracey I, Chang L. Cortical processing of visceral and somatic stimulation: differentiating pain intensity from unpleasantness. *Neuroscience* 133, 533–42, 2005
- Fields HL, Basbaum AI. Central nervous system mechanisms of pain modulation. In: Wall PD, Melzack R (eds). *Textbook of Pain*. Pp 309–29. Churchill Livingstone, Edinburgh, UK, 1999
- Graf B, Senn M. Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. *Applied Animal Behaviour Science* 62, 153–71, 1999
- Gron Dahl-Nielsen C, Simonsen HB, Lund JD, Hesselholt M. Behavioural, endocrine and cardiac responses in young calves undergoing dehorning without and with use of sedation and analgesia. *Veterinary Journal* 158, 14–20, 1999
- Haga HA, Ranheim B. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1–9, 2005
- Hall LW, Clarke KW. Anaesthesia of cattle. *Veterinary Anaesthesia*. 10th Edn. Pp 315–39. WB Saunders, London, UK, 2001
- Johnson CB, Taylor PM. Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* 81, 748–53, 1998
- Johnson CB, Young SS, Taylor PM. Use of frequency spectral analysis of the equine electroencephalogram as an indication of the depth of halothane anaesthesia. In: Sebel PS, Bonke B, Winograd E (eds). *Memory and Awareness in Anaesthesia*. Pp 289–95. PTR Prentice Hall, Englewood Cliffs, UK, 1993
- Johnson CB, Young SS, Taylor PM. Analysis of the frequency spectrum of the equine electroencephalogram during halothane anaesthesia. *Research in Veterinary Science* 56, 373–8, 1994
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ. Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433–7, 2005 a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA. Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61–71, 2005 b
- Johnston CC, Strada ME. Acute pain response in infants: a multidimensional description. *Pain* 24, 373–82, 1986
- Jones AKP, Friston K, Frackowiak RSJ. Localization of responses to pain in human cerebral cortex. *Science* 255, 215–6, 1992
- Jongman EC, Morris JP, Barnett JL, Hemsforth PH. EEG changes in 4-week-old lambs in response to castration, tail docking and mulesing. *Australian Veterinary Journal* 78, 339–43, 2000
- King AS, Riley VA. *A Guide to the Physiological and Clinical Anatomy of the Head*. Department of Veterinary Anatomy, University of Liverpool, Liverpool, UK, 1980
- Kruger L, Young RF. Specialized features of the trigeminal nerve and its central connections. In: Samii M, Jannetta PJ (eds). *The Cranial Nerves*. Pp 273–301. Springer-Verlag, Berlin, Germany, 1981
- Lay DC, Friend TH, Bowers CL, Grissom KK, Jenkins OC. A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. *Journal of Animal Science* 70, 1121–5, 1992 a
- Lay DC, Friend TH, Grissom KK, Bowers CL, Mal ME. Effects of freeze or hot-iron branding of Angus calves on some physiological and behavioural indicators of stress. *Applied Animal Behaviour Science* 33, 137–47, 1992 b
- Mayhew IG, Washbourne JR. A method of assessing auditory and brainstem function in horses. *British Veterinary Journal* 146, 509–18, 1990
- McGregor MJ. Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- McMeekan CM, Mellor DJ, Stafford KJ, Bruce RA, Ward RN, Gregory NG. Effects of shallow scoop and deep scoop dehorning on plasma cortisol concentrations in calves. *New Zealand Veterinary Journal* 45, 72–4, 1997
- McMeekan CM, Mellor DJ, Stafford KJ, Bruce RA, Ward RN, Gregory NG. Effects of local anaesthesia of 4 to 8 hours' duration on the acute cortisol response to scoop dehorning in calves. *Australian Veterinary Journal* 76, 281–5, 1998 a
- McMeekan CM, Stafford KJ, Mellor DJ, Bruce RA, Ward RN, Gregory NG. Effects of regional analgesia and/or a non-steroidal anti-inflammatory analgesic on the acute cortisol response to dehorning in calves. *Research in Veterinary Science* 64, 147–50, 1998 b
- Mellor DJ, Stafford KJ, Todd SE, Lowe TE, Gregory NG, Bruce RA, Ward RN. A comparison of catecholamine and cortisol responses of young lambs and calves to painful husbandry procedures. *Australian Veterinary Journal* 80, 228–33, 2002
- Morrisse JB, Cotte JP, Huonnic D. Effect of dehorning on behaviour and plasma-cortisol responses in young calves. *Applied Animal Behaviour Science* 43, 239–47, 1995
- Murrell JC, Johnson CB. Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325–35, 2006
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE. Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138–46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE. Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212–21, 2005
- Ohara PT, Vit J-P, Jasmin L. Cortical modulation of pain. *Cellular and Molecular Life Sciences* 62, 44–52, 2005
- Ong RM, Morris JB, O'Dwyer JK, Barnett JL, Hemsforth PH, Clarke IJ. Behavioural and EEG changes in sheep in response to painful acute electrical stimuli. *Australian Veterinary Journal* 75, 189–93, 1997
- Orth M, Barter L, Dominguez C, Atherley R, Carstens E, Antognini JF. Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation. *British Journal of Anaesthesia* 95, 477–84, 2005
- Peers A, Mellor DJ, Wintour EM, Dodic M. Blood pressure, heart rate, hormonal and other acute responses to rubber-ring castration and tail docking of lambs. *New Zealand Veterinary Journal* 50, 56–62, 2002
- Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. Cortisol responses of calves to two methods of disbudding used with or without local anaesthetic. *New Zealand Veterinary Journal* 44, 9–14, 1996
- Stafford KJ, Mellor DJ. Dehorning and disbudding distress and its alleviation in calves. *Veterinary Journal* 169, 337–49, 2005
- Stafford KJ, Mellor DJ, Ward RN, Cann B. Behavioural response of calves to amputation dehorning with or without local anaesthesia. *Proceedings of the New Zealand Society of Animal Production* 60, 234–6, 2000
- Stafford KJ, Mellor DJ, Todd SE, Ward RN, McMeekan CM. The effect of different combinations of lignocaine, ketoprofen, xylazine and tolazoline on the acute cortisol response to dehorning in calves. *New Zealand Veterinary Journal* 51, 219–26, 2003
- Sutherland MA, Mellor DJ, Stafford KJ, Gregory NG, Bruce RA, Ward RN. Cortisol responses to dehorning of calves given a 5-h local anaesthetic regimen plus phenylbutazone, ketoprofen, or adrenocorticotropic hormone prior to dehorning. *Research in Veterinary Science* 73, 115–23, 2002 a
- Sutherland MA, Mellor DJ, Stafford KJ, Gregory NG, Bruce RA, Ward RN. Effect of local anaesthetic combined with wound cauterisation on the cortisol response to dehorning in calves. *Australian Veterinary Journal* 80, 165–7, 2002 b
- Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. Acute cortisol responses of calves to scoop dehorning using local anaesthesia and/or cautery of the wound. *Australian Veterinary Journal* 76, 118–22, 1998 a
- Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA, Ward RN. Acute cortisol responses of calves to four methods of dehorning by amputation. *Australian Veterinary Journal* 76, 123–6, 1998 b
- Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA, Ward RN. Behavioural responses of calves to amputation dehorning with and without local anaesthesia. *Australian Veterinary Journal* 82, 697–700, 2004
- Talbot JD, Marrett S, Evans AC, Meyer E, Bushnell MC, Duncan GH. Multiple representations of pain in human cerebral cortex. *Science* 251, 1355–8, 1991
- Treede RD, Kenshalo DR, Gracely RH, Jones AKP. The cortical representation of pain. *Pain* 79, 105–11, 1999

Submitted 23 November 2006

Accepted for publication 13 March 2007

Johnson CB, Murrell JC, Gibson TJ, Mellor DJ. Innovative refinements to anaesthesia techniques can deliver pain research without pain. In: '6th World Congress on Alternatives & Animal Use in the Life Sciences, 2007'. Tokyo, Japan Pp 97-100, 2008

AATEX 14, Special Issue, 97-100
Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences
August 21-25, 2007, Tokyo, Japan

Innovative refinements to anaesthesia techniques can deliver pain research without pain

Craig Brian Johnson¹, Jo Murrell², Troy John Gibson¹ and David James Mellor³

¹Institute of Veterinary, Animal and Biomedical Sciences ²Department of Clinical Veterinary Sciences, University of Bristol
³Animal Welfare Sciences and Bioethics Centre, Massey University

Corresponding author: Craig Brian Johnson
Institute of Veterinary, Animal and Biomedical Sciences, Massey University
Palmerston North, New Zealand
C.B.Johnson@massey.ac.nz

Abstract

Research into analgesia has traditionally not been possible without subjecting animals to pain. The practice of inflicting pain in some animals in order to relieve pain in others leads to an obvious ethical dilemma. Over the last 15 years we have developed and refined a novel approach to anaesthesia that allows the cerebral cortex of an anaesthetised animal to respond to noxious stimuli in a similar manner to that of a conscious animal experiencing pain. Under these conditions, changes in specific electroencephalographic variables seen in response to noxious stimulation and their attenuation by different methods of analgesia have allowed various analgesic strategies to be directly compared with each other. Our approach has enabled analgesia research to be undertaken for the first time without subjecting animals to pain. We have studied pain and analgesia in this way in cattle, deer, sheep, horses, rats, dogs and wallabies. This paper will outline our new approach to analgesia research and discuss the advantages of this novel technique over more traditional approaches. We will draw on examples of applied analgesia research from several species of mammals in which our techniques have been applied.

Keywords: pain, analgesia, anaesthesia, refinement

Introduction

Pain has been described as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP, 1979). The dual sensory and experiential aspects of pain make it the most subjective of the sensory modalities. It has been suggested that the degree of sensory stimulation is a less important factor in the degree of perceived pain than the prior state of the central nervous system that receives the stimulation (Vossen et al. 2006). This subjectivism has made pain difficult to quantify objectively and this has led to the widespread use of a variety of subjective pain scales in human patients. Although subjective pain scoring has proved to be a very powerful tool, its use is limited to those patients that are able to describe their pain. Non-communicative patients such as very young children, adults with various forms of cognitive and communicative impairment and animals are not suitable candidates for these methods. The need to assess pain in these groups has fuelled a continuing search for objective measures that correlate well with subjective pain scores.

Objective measures of pain and nociception can be broadly divided into four categories:

- Autonomic responses
- Endocrine stress responses
- Behavioural responses
- Neurophysiological responses

This paper will discuss the latter category and in particular the analysis of electroencephalographic responses to noxious stimulation during controlled general anaesthesia, the so-called minimal anaesthesia model (Murrell and Johnson 2006).

The relationship between pain and the EEG

Prior to the mid 1990s, the perception of pain was thought to be a function of the limbic structure (Lico et al. 1974). The development of functional magnetic resonance imaging allowed the areas of the brain involved in the processing of pain to be firmly identified. Cerebral structures, particularly the insula cortex and anterior cingulate gyrus were found to be specifically responsive to pain in human volunteers (Craig et al. 1996). The discovery of the inherent role

Craig Brian Johnson, et al.

of the cerebral cortex in turn lead to renewed interest in electroencephalographic analysis as a means of measuring pain and nociception.

Principles of EEG analysis

Electroencephalograms are often analysed using the Fast Fourier Transform (FFT). The following is a very brief explanation of this methodology. Any signal or waveform whose statistical descriptors (mean frequency, relative frequency components etc.) do not change over time is said to be stationary. Signal analysis theory states that any stationary signal can be considered to be the sum of an infinite number of sine waves of different frequencies and strengths. Fast Fourier Transformation transposes a signal in the time domain into the frequency domain, that is it converts a conventional signal into a power spectrum, a histographic representation of the original signal (Fig 1). For a more detailed explanation of FFT analysis, see Young (2001).

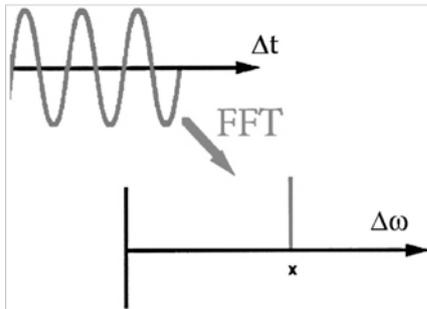


Fig. 1. Graphical representation of Fast Fourier Transformation (FFT) of a time domain signal (Δt) to a frequency domain signal ($\Delta \omega$). The point x in the frequency domain represents the frequency of the signal.

Fast Fourier transformation of one short epoch of EEG (typically one second), gives an indication of the frequencies present at that time. The power spectra of consecutive epochs can be displayed adjacent to each other to give an indication of how the frequency components change over time. This is a compressed spectral array (CSA). Typical CSAs are illustrated in Fig 2. A CSA gives a good visual representation of EEG changes, but in order to perform statistical analysis, it is necessary to derive mathematical descriptors from this waveform. The most frequently used descriptors are: median frequency (F50: the statistical median), which gives a general view of the CSA; 95% spectral edge (F95: the 95th percentile), which responds to changes in high frequencies; total EEG power (ptot: the area under the curve), which responds to the lower frequencies. For more details

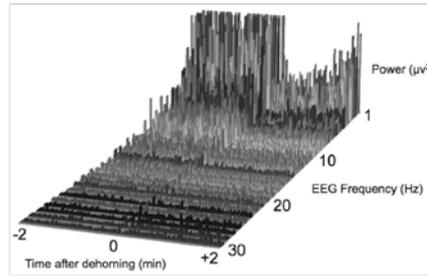


Fig. 2. Compressed spectral array of EEG during scoop dehorning. This represents the EEG response to dehorning (at time 0) in an anaesthetised heifer. An immediate reduction in low frequency power and increase in high frequency power lasting for approximately two minutes can be seen. Data from Gibson et al. (2007).

on these variables, see Murrell and Johnson (2006).

The minimal anaesthesia model

The minimal anaesthesia model takes advantage of the finding that under carefully controlled conditions of general anaesthesia, noxious stimulation can result in EEG changes (Murrell et al, 2003) that are similar to those seen in conscious animals (Ong et al. 1997). In conscious human volunteers, these changes have been shown to correlate well with subjective perception of pain (Chen et al. 1989). This means that we can compare the pain perception resulting from different noxious stimuli in animals that are anaesthetised. By definition they cannot feel pain as they are anaesthetised, but the EEG changes give us an indication of the degree of pain that they would have perceived were they consciously aware. This gives us, for the first time, a method of investigating pain in animals that does not require us to subject experimental animals to pain. Even if animals form part of a negative control group and receive no analgesia in addition to general anaesthesia, they are not conscious throughout the study and can be given appropriate analgesia before they recover from the general anaesthetic.

To date the minimal anaesthesia model has been used to investigate pain in 8 species of mammal: horses (Murrell et al. 2003); sheep (Johnson et al. 2005a); red deer (Johnson et al. 2005b); cattle (Gibson et al. 2007); pigs (Haga et al. 2005); rats (Murrell et al. 2007); wallabies (Diesch et al. 2008); dogs (data in preparation). Two examples of the practical applications of this model will be discussed below:

- Scoop dehorning in calves
- Velvet antler removal in red deer

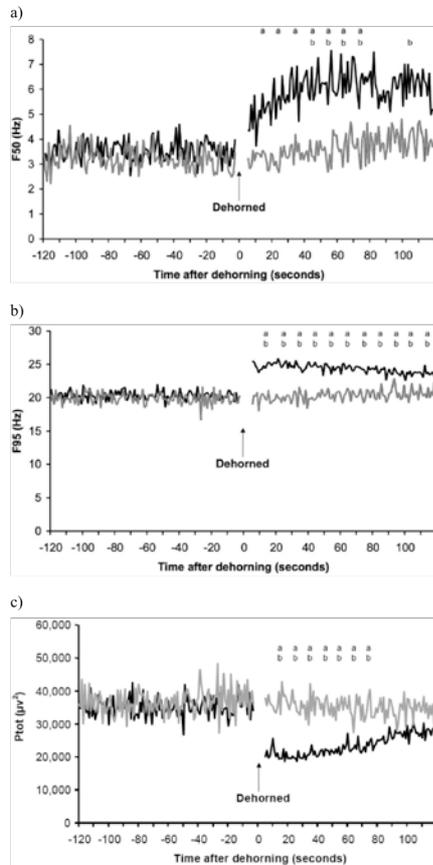


Fig. 3. Changes in: a) median frequency (F50); b) 95% spectral edge frequency (F95); c) total EEG power (Ptot) following scoop dehorning at time 0 in anaesthetised cattle. Data from Gibson et al. (2007) reproduced with permission.

Scoop dehorning in calves (Gibson et al. 2007)

Changes in F50, F95 and p_{tot} in the two minutes following scoop dehorning in cattle are illustrated in Fig 3. Dehorning resulted in an immediate increase in F95 and decrease in p_{tot} and a more gradual increase in F50. The addition of a local anaesthetic ring block prevented these EEG responses demonstrating the effectiveness of local anaesthetic ring block as an analgesic technique for this procedure. The experiment validated the use of the minimal anaesthetic technique in cattle and has formed the basis for further studies (data currently under analysis).

Velvet antler removal in red deer (Johnson et al. 2005b)

This study compared the use of local anaesthetic ring block or antler pedicle compression to no analgesia for the surgical removal of velvet antler in red deer. Antler pedicle compression was proposed as a method of field analgesia for velvet harvesting. This study demonstrated that antler pedicle compression was not as analgesic as local anaesthetic ring block and in addition that the application of the compressive band was itself a significant noxious stimulus. As a result of these and other studies, the National Animal Welfare Advisory Committee declined to recommend the approval of antler pedicle compression in New Zealand and it has not been adopted for use in the field. This is an example of how results generated using the minimal anaesthesia model have been used to influence animal welfare policy at a national level in New Zealand.

Conclusions

In conclusion, the minimal anaesthesia model offers significant advantages over other methodologies available to pain researchers. All animals are anaesthetised throughout the period of data collection. This means that a control group with no additional analgesia can be included into studies against which to compare the effects of proposed techniques of analgesia. Together with the very tight degree of control afforded by the conditions of general anaesthesia, this increases the statistical power of research studies and allows significant effects to be identified using fewer animals than would be possible with other experimental techniques. Experimental animals can be given analgesia using appropriate clinical techniques after the completion of data collection, but before they recover from general anaesthesia. This represents significant reduction in the numbers of animals used and refinement in terms of the welfare impact to those animals in an area of research where replacement is not currently a realistic option.

The ability to give better analgesia to experimental animals than they would be expected to receive under routine animal husbandry conditions means that for the first time, pain research can be carried out whilst simultaneously improving the welfare of the animals involved in the studies.

Acknowledgements

I am indebted to all past and present members of Massey University Comparative Analgesia Group "Team Ouch" for their enthusiasm and dedication to our studies. Research studies discussed in this paper were funded in part or in whole by: Ministry of Agriculture and Forestry (NZ); Department of the Environment, Food and Rural Affairs (UK); Velvet Antler Removal New Zealand.

Craig Brian Johnson, et. al.

References

- Chen ACN, Dworkin SF and Haug J (1989). Topographic brain measures of human pain and pain responsivity. *Pain* **37** 129-141.
- Craig AD, Reiman EM, Evans A and Bushnell MC (1996). Functional imaging of an illusion of pain. *Nature* **384** 258-260.
- Diesch TJ, Johnson CB, Mellor DJ and Lentle RG (2008). Proceedings of 6th World Congress on Alternatives and Animal Use in the Life Sciences, Tokyo Japan, pp. 97-100.
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL and Mellor DJ (2007). Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* **55** 152-157.
- Haga, H. & Ranheim, B. (2005) Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* **32** 1-9.
- IASP (1979). <http://www.iasp-pain.org>
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S and Mellor DJ (2005a). Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* **53** 433-437.
- Johnson CB, Woodbury WM, Caulkett N & Wilson P (2005b). Comparison of lidocaine and antler pedicle compression for analgesia during antler removal in red deer (*Cervus elaphus*) anaesthetised by halothane in oxygen: EEG effects. *Journal of Veterinary Anaesthesia and analgesia* **32** 16-71.
- Lico MC, Hoffmann A & Covian MR (1974). Influence of some limbic structures upon somatic and autonomic manifestations of pain. *Physiology and Behaviour* **12** 805-11
- Murrell JC, Johnson CB, White K, Taylor PM, Haberham Z and Waterman-Pearson AE (2003). Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* **30** 138-146.
- Murrell JC and Johnson CB (2006). Neurophysiological techniques to assess pain in animals (Review). *Journal of Veterinary Pharmacology and Therapeutics* **29** 325-335.
- Murrell JC, Waters D, Mitchinson SL and Johnson CB (2007). Comparative effect of thermal, mechanical and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* **98** 366-371.
- Ong RM, Morris JP, O'Dwyer JK, Barnett JL, Hemsworth PH, Clarke IJ (1997). Behavioural and EEG changes in sheep in response to painful acute electrical stimuli. *Australian Veterinary Journal* **75** 189-93.
- Young SS (2001). *Computerized data acquisition and analysis for the life sciences*. Cambridge, UK. Cambridge University Press. ISBN: 0-521-56570-7.
- Vossen HGM, van Os J, Hermens H, Lousberg R (2006). Evidence That Trait-Anxiety and Trait-Depression Differentially Moderate Cortical Processing of Pain. *Clinical Journal of Pain* **22** 725-729.

Mellor DJ, Gibson TJ, Johnson CB. A re-evaluation of the need to stun calves prior to slaughter by ventral-neck incision. *New Zealand Veterinary Journal* 57, 74-6, 2009

New Zealand Veterinary Journal 57(2), 74-76, 2009

74

Review Article

A re-evaluation of the need to stun calves prior to slaughter by ventral-neck incision: An introductory review

DJ Mellor^{*†§}, TJ Gibson^{*‡#} and CB Johnson^{*‡}

Abstract

Commercial slaughter of farm livestock usually employs an extensive incision that severs the soft tissues of the neck including the major blood vessels supplying and draining the brain. It is intended to cause a catastrophic decrease in cerebral blood flow with rapid onset of unconsciousness or insensibility. The tissues of the neck are innervated with nociceptive nerve fibres and their transection will cause a barrage of sensory impulses. Consciousness, and therefore the ability of the animal to feel pain and experience distress after the incision, may persist for 60 seconds or longer in cattle. These observations suggest that livestock may experience pain and distress during the period before they become unconscious (insensible). Psychological shock and fear may also be associated with the extensive tissue damage and blood loss. Pre-incision stunning has been adopted as a precautionary measure to prevent suffering. However, the question remains: How intense and noxious are these experiences? Recent methodological developments related to quantitative analysis of the electroencephalogram (EEG) allow the experience of pain to be assessed more directly than has hitherto been possible. This methodology has now been applied to the question of the slaughter of calves by ventral-neck incision. The new information demonstrates clearly for the first time that the act of slaughter by ventral-neck incision is associated with noxious stimulation that would be expected to be perceived as painful in the period between the incision and loss of consciousness. These data provide further support for the value of stunning in preventing pain and distress in animals subjected to this procedure.

KEY WORDS: *Calves, distress, insensibility, nociception, pain, pre-incision stunning, slaughter, ventral-neck incision*

Introduction

Most farm livestock in industrialised countries are slaughtered in commercial slaughterhouses. The entire process includes mustering, yarding and loading at the farm of origin, transport to the slaughterhouse, lairage, movement to the point of slaughter, restraint during slaughter, and finally the act of slaughter itself

(Shimshony and Chaudry 2005). Substantial improvements in the humaneness of this process have occurred through the application of science over many years. Thus, many forms of animal welfare compromise that can occur during these stages have been identified and may now be minimised by the application of scientifically developed and validated methods that are incorporated into codes of practice (Grandin 1998; Gregory 1998; Mellor and Littin 2004; Shimshony and Chaudry 2005). Here, we focus on humane aspects of the act of slaughter as an introduction to four studies on this subject published concurrently (Gibson *et al.* 2009abcd).

The slaughter of farm livestock usually employs ventral incision of the throat or neck. This raises a number of questions when the incision is conducted in conscious animals, as occurs, for example, during emergency slaughter (Galvin *et al.* 2005) and some forms of religious slaughter (Levinger 1995; Rosen 2004; Shimshony and Chaudry 2005; Anil *et al.* 2006). Is the neck incision itself painful? How much pain is generated within the cut tissues? Does the sudden drop in blood flow to the brain cause distress? How long does the animal remain conscious after the neck incision? What measures can be adopted to minimise any pain and distress and reduce the duration of consciousness?

Potential for noxious sensory input

The neck incision severs major blood vessels supplying and draining the brain, and is intended to cause a catastrophic decrease in cerebral blood flow leading to a rapid onset of disordered brain function and unconsciousness or insensibility (reviewed by Mellor and Littin 2004). The neck incision is usually very extensive. It transects skin, muscle, trachea, oesophagus, carotid arteries, jugular veins, other blood vessels, sensory and motor nerves, and connective tissue. All of these soft tissues are innervated with nociceptive nerve fibres such that transection of the tissues themselves and the nerves within them will cause a barrage of impulses to travel to the brain. There is also the possibility of psychological shock from the massive stimulation of all sensory nerves at the time of the incision.

Consciousness, and therefore the ability of the animal to feel pain and experience distress, is not lost immediately after the neck incision. Various parameters have been used to arrive at this conclusion, including particular behaviours, EEG, electrocorticograms, evoked responses following sound, light and electrical stimulation, cranial-nerve reflex activity, release of cerebral neurotransmitters, breathing characteristics, heart rate, blood pressure, blood flow, and blood levels of oxygen, carbon dioxide and metabolite (Mellor and Littin 2004; Gibson *et al.* 2009a). To

* Animal Welfare Science and Bioethics Centre, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

† Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

‡ Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

Current address: Veterinary Clinical Sciences, Royal Veterinary College, North Mymms, Herts AL9 7TA, United Kingdom.

§ Author for correspondence. Email: d.j.mellor@massey.ac.nz

EEG Electroencephalogram(s)

date, however, interpretation of the behavioural and physiological changes, especially in terms of the presence or absence of pain and distress and as an indication of when consciousness first becomes dulled enough to reduce any noxious experience to acceptably low levels, has been problematical. Nevertheless, the period before the onset of undoubted unconsciousness or insensibility can be assessed with some confidence. On the basis of the physiological criteria listed above, the shortest period of consciousness in any species tested is apparently 3 seconds and the longest is more than 60 seconds, and there is a range in each species. This range is apparently narrower in sheep (5 to 22 seconds; most sheep 5–7 seconds) and goats (3 to <7 seconds) than in cattle and poultry (5 to 60 or more seconds) (Newhook and Blackmore 1982; Anonymous 1987; Daly *et al.* 1988; Bager *et al.* 1992; Levinger 1995; Barnett *et al.* 2007).

These observations therefore suggest that conscious farm livestock slaughtered by neck incision may experience pain and distress during the period before they become unconscious (insensible) due to stimulation of nociceptors in the extensively damaged neck tissues. Psychological shock and fear may also be associated with the extensive tissue damage and blood loss. However, the question remains: How intense and noxious are these experiences?

Minimising or avoiding noxious sensory input

To date, opinion on this question has been divided. Some scientists have claimed that provided an exquisitely sharp knife with no physical blemishes in the blade is used to complete a swift, clean incision with a free bleed-out significant pain and distress are avoided (e.g. Levinger 1995; Rosen 2004). In contrast, in accord with dominant international scientific opinion (Anonymous 2001), other scientists have held that conscious (non-stunned) animals are likely to experience an unreasonable level of pain and distress during the neck incision and after it until they become insensible (e.g. Gregory 1998). Until recently, however, neurophysiological methodology has not allowed this question to be addressed directly.

Accordingly, a precautionary approach has usually been adopted, so that pre-incision stunning, designed to render the animal unconscious before the neck is cut, has been employed widely internationally (Gregory 1998; Anonymous 2001). Unconscious animals cannot experience pain or any other sensations during the neck incision or subsequently from the cut tissues, nor can they experience any distress due to shock and rapid blood loss. Three main methods of stunning, all of which have been validated scientifically (Blackmore *et al.* 1993; Gregory 1998; Shimshony and Chaudry 2005), are currently employed. The first employs concussion by captive bolt or percussive bolt, and the second involves an electric current applied to the head only or in a head-to-back configuration, and both of these immediately induce disordered brain function and unconsciousness. The third method employs carbon dioxide, and this induces unconsciousness fairly quickly by its anaesthetic effects on brain function. It is noteworthy that pre-incision percussive stunning (using a sledge-hammer), which dates back to the 18th Century in Europe and the United States of America and to the early 15th Century in China (Mellor and Littin 2004), was originally introduced to minimise risks to slaughtermen and to facilitate handling of large animals, such as cattle, during slaughter (Warring 1974; Gregory 1989). It was

only later that the beneficial humane consequences of a properly executed percussive stun became widely appreciated.

Re-evaluating the question of noxious sensory input

Recent methodological developments related to quantitative analysis of different components of the EEG now allow the likely experience of pain due to noxious sensory input to be assessed more directly than has hitherto been possible (Murrell and Johnson 2006). Building on changes in the EEG specifically associated with the human experience of pain (Chen *et al.* 1989; Bromm and Lorenz 1998; Chang *et al.* 2001a,b, 2002), the use of similar features of the EEG to detect the perception of noxious sensory input associated with surgery, castration, tail docking, velvet antler removal, and dehorning has now been validated in calves, red deer (*Cervus elaphus*), horses, sheep, pigs and rats (Murrell *et al.* 2003, 2005; Haga and Ranheim 2005; Johnson *et al.* 2005a,b; McGregor 2005; Gibson *et al.* 2007). The methodology involves a minimal anaesthesia model where maintenance of an animal at a stable plane of anaesthesia allows the recording of cerebrocortical responses to a stimulus by reducing variability in background cerebrocortical electrical activity due to extraneous stimuli. The model has the additional benefit of allowing painful procedures to be investigated without compromising the welfare of the animals because they remain lightly anaesthetised throughout.

This methodology has now been applied to the slaughter of calves by ventral-neck incision without prior stunning and to other variants of this practice. The results are discussed by Gibson *et al.* (2009abcd). The impact of ventral-neck incision alone is reported in the first paper (Gibson *et al.* 2009a), demonstrating that the incision was associated with significant noxious sensory input that would have been likely to be perceived as pain in conscious animals. The question of whether this noxious sensory input was due primarily to the cutting of neck tissues or to interruption of blood flow to and from the brain is addressed in the second paper (Gibson *et al.* 2009b), demonstrating that the predominant noxious stimulus was transection of neck tissue. The third paper reports on the impact of non-penetrative captive-bolt stunning on the features of the EEG, which were assessed quantitatively for the first time (Gibson *et al.* 2009c), and showed that the vast majority of animals were rendered insensible before data were able to be collected from about 3 seconds after stunning. The fourth paper assesses the extent to which applying a non-penetrative captive-bolt stun 5 seconds after the ventral-neck incision ameliorated the noxious sensory input caused by the incision (Gibson *et al.* 2009d), and showed that the stun prevented the subsequent development of responses in the EEG to noxious sensory input in most of the animals.

Conclusions

This new information demonstrates clearly for the first time that the act of slaughter by ventral-neck incision is associated with noxious stimulation that would be likely to be perceived as painful in the period between the incision and loss of consciousness. In cattle, this can be as long as 60 seconds or more (Newhook and Blackmore 1982). The effects of captive-bolt stunning in producing rapid unconsciousness and ameliorating changes in the EEG

associated with neck incision have also been clearly demonstrated. Taken together, these papers (Gibson *et al.* 2009abcd) provide the most comprehensive electrophysiological picture to date of the events surrounding slaughter by neck incision, and provide further support for the value of stunning in preventing pain and distress in animals subjected to this procedure.

Acknowledgements

We are grateful to the Department for Environment, Food and Rural Affairs (DEFRA, United Kingdom) and the Ministry of Agriculture and Forestry (MAF Biosecurity, New Zealand) for financially supporting this programme, and to Drs David Pritchard and Kirk Thompson (DEFRA) and Dr David Bayvel (MAF) for contributions to experimental design during the planning stage. We are also grateful for the input of three anonymous international referees who commented on the four manuscripts before their submission to the *New Zealand Veterinary Journal* and its full peer-reviewing process. TJ Gibson was the recipient of a C Alma Baker Postgraduate Scholarship.

References

- Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Weaver HR, Erdogan O, Hughes S, Manson C. Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: exsanguination and quality parameters. *Animal Welfare* 15, 325–30, 2006
- *Anonymous. *Humane Slaughter of Animals for Food*. Universities Federation for Animal Welfare, Potters Bar, UK, 1987
- *Anonymous. *Discussion Paper on the Animal Welfare Standards to Apply when Animals are Slaughtered in Accordance with Religious Requirements*. National Animal Welfare Advisory Committee, MAF Biosecurity New Zealand, Wellington, NZ, 2001
- Bager F, Braggins TJ, Devine CE, Graahuis AE, Mellor DJ, Tavener A, Updell MP. Onset of insensibility at slaughter in calves: effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indices of cerebral metabolism. *Research in Veterinary Science* 52, 162–73, 1992
- Barnett JS, Cronin GM, Scott PC. Behavioural responses of poultry during kosher slaughter and their implications for the birds' welfare. *Veterinary Record* 160, 45–9, 2007
- Blackmore DK, Cook CJ, Devine CE, Gilbert KV, Tavener A, Langdon S, Isaacs S, Maasland SA. Electrical stunning of red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 41, 126–30, 1993
- Bromm B, Lorenz J. Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology* 107, 227–53, 1998
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN. Topographic effects of tonic cutaneous nociceptive stimulation on human electroencephalograph. *Neuroscience Letters* 305, 49–52, 2001 a
- Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN. Different EEG topographic effects of painful and non-painful intramuscular stimulation in man. *Experimental Brain Research* 141, 195–203, 2001 b
- Chang PF, Arendt-Nielsen L, Chen ACN. Differential cerebral responses to aversive auditory arousal versus muscle pain: specific EEG patterns are associated with human pain processing. *Experimental Brain Research* 147, 387–93, 2002
- Chen ACN, Dworkin SF, Haug J, Gehrig J. Topographic brain measures of human pain and pain responsiveness. *Pain* 37, 129–41, 1989
- Daly CC, Kallweit E, Ellendorf F. Cortical function in cattle during slaughter: conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325–9, 1988
- Galvin JW, Blokhuis H, Chimbombi MC, Jong D, Wotton S. Killing animals for disease control purposes. *Review Scientifique et Technique de l'Office International des Epizooties* 24, 711–22, 2005
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55, 152–7, 2007
- Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, Johnstone AC, Mellor DJ. Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning. *New Zealand Veterinary Journal* 57, 77–83, 2009 a
- Gibson TJ, Johnson CB, Murrell JC, Chambers JP, Stafford KJ, Mellor DJ. Components of electroencephalographic responses to slaughter in halothane-anaesthetised calves: Effects of cutting neck tissues compared with major blood vessels. *New Zealand Veterinary Journal* 57, 84–9, 2009 b
- Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ. Electroencephalographic responses to concussive non-penetrative captive-bolt stunning in halothane-anaesthetised calves. *New Zealand Veterinary Journal* 57, 90–5, 2009 c
- Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ. Amelioration of electroencephalographic responses to slaughter by non-penetrative captive-bolt stunning after ventral-neck incision in halothane-anaesthetised calves. *New Zealand Veterinary Journal* 57, 96–101, 2009 d
- *Grandin T. Solving livestock handling problems in slaughter plants. In: Gregory NG (ed). *Animal Welfare and Meat Science*. Pp 42–63. CAB International Publishing, Wallingford, Oxon, UK, 1998
- Gregory NG. Slaughtering methods and equipment. *Veterinary History* 6, 73–84, 1989
- *Gregory NG. Physiology of stress, distress, stunning and slaughter. *Animal Welfare and Meat Science*. Pp 64–92. CAB International Publishing, Wallingford, Oxon, UK, 1998
- Haga HA, Ranheim B. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1–9, 2005
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ. Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433–7, 2005 a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA. Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61–71, 2005 b
- *Levinger IM. *Shechita in the Light of the Year 2000: A Critical Review of the Scientific Aspects of Methods of Slaughter and Shechita*. Maskil L'David, Jerusalem, Israel, 1995
- *McGregor MJ. Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- Mellor DJ, Littin KE. Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13 (Supplement), 127–32, 2004
- Murrell JC, Johnson CB. Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325–35, 2006
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE. Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138–46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE. Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212–21, 2005
- Newhook JC, Blackmore DK. Electroencephalographic studies of stunning and slaughter of sheep and calves. Part 2. *Meat Science* 6, 295–300, 1982
- Rosen SD. Opinion piece: Physiological insights into Shechita. *Veterinary Record* 154, 759–65, 2004
- Shimshony A, Chaudry MM. Slaughter of animals for human consumption. *Review Scientifique et Technique de l'Office International des Epizooties* 24, 693–710, 2005
- Warring R. Electrical stunning: a review of the literature. *Veterinary Bulletin* 46, 617–35, 1974

Submitted 09 September 2008

Accepted for publication 18 February 2009

*Non-peer-reviewed

Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, Johnstone AC, Mellor DJ. Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning. *New Zealand Veterinary Journal* 57, 77-83, 2009

77

New Zealand Veterinary Journal 57(2), 77-83, 2009

Scientific Article

Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning

TJ Gibson^{*†‡}, CB Johnson^{*‡§}, JC Murrell^{‡#}, CM Hulls^{*¥}, SL Mitchinson^{*‡}, KJ Stafford^{*‡}, AC Johnstone[‡] and DJ Mellor^{*¥}

Abstract

AIM: To investigate whether the electroencephalographic (EEG) responses to slaughter by ventral-neck incision without prior stunning may be perceived as painful in halothane-anaesthetised calves.

METHODS: Fourteen Angus steers were minimally anaesthetised with halothane, using an established anaesthesia protocol. EEG indices were recorded bilaterally for 5 minutes prior to and 5 minutes following ventral-neck incision. A single incision was made in the ventral aspect of the neck, severing all tissues ventral to the vertebral column including the major blood vessels supplying and draining the head. Changes in the median frequency (F50), 95% spectral edge frequency (F95) and total power of the EEG (Ptot) were used to investigate the effects of ventral-neck incision. At the completion of the experiment, brains of calves were examined histologically.

RESULTS: During the 30 seconds following ventral-neck incision, the F95 and Ptot showed significant changes ($p < 0.05$) compared with pre-treatment values. The F50 increased significantly from recordings from the right side of the cranium. No gross or histological abnormalities were detected in the brains following slaughter.

CONCLUSIONS: This study is the first investigation of the noxiousness of slaughter by ventral-neck incision, using EEG spectral analysis. It demonstrated that there is a period following slaughter where ventral-neck incision represents a noxious stimulus.

KEY WORDS: Calves, compressed spectral array, electroencephalogram, emergency slaughter, minimal anaesthesia, nociception, pain, slaughter, ventral-neck incision

Introduction

Calves slaughtered for human consumption in New Zealand must be rendered completely insensible, prior to slaughter, with the application of a mechanical or electrical stun and maintained in that state until death (Anonymous 2006). In certain situations, livestock are slaughtered without prior stunning, common examples of this being emergency and religious slaughter. The United Kingdom Farm Animal Welfare Council recently recommended that further research should be undertaken, following which the status of religious slaughter be re-examined (Anonymous 2003).

During slaughter by ventral-neck incision, a single incision with an extremely sharp blade is made in the ventral aspect of the neck, severing the major blood vessels to the brain. The incision also transects skin, muscle, trachea, oesophagus, sensory nerves and connective tissues (Mellor and Littin 2004). There are a number of potential welfare concerns regarding neck-cut slaughter without prior stunning, including possible pain due to the incision itself and distress during the time before the onset of undoubted insensibility.

The time to undoubted insensibility following ventral-neck incision with or without stunning has been a topic of much detailed research in a variety of species. This has involved investigations of cortical electrical activity in terms of changes in the EEG/electrocorticogram in amplitude and waveform type (Nangeroni and Kennett 1964; Newhook and Blackmore 1982; Gregory and Wotton 1984; Anil *et al.* 1995a), changes in the power spectrum of the EEG (Bager *et al.* 1992), changes in brain function in terms of visual (Gregory and Wotton 1984; Daly *et al.* 1988; Anil *et al.* 1995b) and somatosensory (Daly *et al.* 1988; Anil *et al.* 1995a) evoked potentials, loss of evoked eye response (Levinger 1961; Barnett *et al.* 2007), and changes in behaviour (Levinger 1961; Blackmore 1984; Grandin 1994; Barnett *et al.* 2007). There is insufficient understanding of conscious processes to be able to interpret the significance of these events in terms of loss of consciousness, but the window before the onset of undoubted insensibility can be reliably assessed (Mellor and Littin 2004). Most authorities consider this window to be between approximately 5 and 60 seconds or more in duration in cattle (Mellor and Littin 2004), although it has been postulated that the rapid decompression of the cerebral vault results in implosion of the brain, leading to a more rapid loss of sensibility (Rosen 2004). During this

* Animal Welfare Science and Bioethics Centre, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

† Current address: Veterinary Clinical Sciences, Royal Veterinary College, North Mymms, Herts AL9 7TA, United Kingdom.

‡ Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

Current address: Department of Clinical Veterinary Science, Division of Companion Animals, University of Bristol, Bristol BS8 1TH, United Kingdom.

¥ Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

§ Author for correspondence. Email: c.b.johnson@massey.ac.nz

ECG Electrocardiogram(s)/electrocardiographic
 EEG Electroencephalogram(s)/electroencephalographic
 F50 Median frequency
 F95 95% Spectral edge frequency
 Ptot Total power of the electroencephalogram

window between neck incision and insensibility the animal could experience pain and/or distress.

Until recently no tools were available for assessing pain in animals during this window (Mellor *et al.* 2000; Rutherford 2002). Recent advances in the quantitative interpretation of the EEG have identified changes in cerebrocortical function in response to noxious stimulation (Murrell and Johnson 2006). Changes in the frequency spectrum of the human EEG have been shown to reflect alterations in activity associated with the cognitive perception of pain (Chen *et al.* 1989). We have recently reported EEG responses to scoop dehorning in minimally anaesthetised calves (Gibson *et al.* 2007). The F50 and F95 are the frequency below which 50% and 95%, respectively, of the total power of the EEG is located, and the Ptot is the total area under the power spectrum curve (Murrell and Johnson 2006). Increases in F50 and F95 and a decrease in Ptot have been previously associated with nociception in animals (Murrell *et al.* 2003; Johnson *et al.* 2005a; Gibson *et al.* 2007) and also with pain in man (Chen *et al.* 1989). Changes in F50, F95 and Ptot were found to correlate with the noxious stimulus of dehorning, and these responses were abolished by the prior application of local anaesthetic blockade (Gibson *et al.* 2007). Similar changes have now been identified in seven species of mammals during surgical stimulation under anaesthesia (Murrell *et al.* 2003; Haga and Ranheim 2005; Johnson *et al.* 2005ab; McGregor 2005).

Changes in F95 have also been associated with increasing depth of anaesthesia (Johnson *et al.* 1993, 1994; Johnson and Taylor 1998). The minimal anaesthesia model involves maintaining the animal on a stable light plane of halothane anaesthesia, where the animal is unconscious but still able to demonstrate EEG responses to noxious stimulation (Murrell and Johnson 2006). This model allows the investigation of cerebrocortical responses to noxious stimuli without compromising the welfare of the animal (Gibson *et al.* 2007).

The aim of this study was to examine EEG responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning, in order to ascertain the noxiousness or otherwise of this manipulation. Following slaughter, histological examination of brains was carried out to identify any structural changes following ventral-neck incision.

Materials and methods

Animals

Calves were sourced from a commercial stock agent and kept in accordance with normal farming practices. Fourteen Angus steers weighing 109–162 kg were allocated to receive ventral-neck incision. Another group of 10 Friesian heifers and bulls weighing 134–207 kg were allocated to receive a sham incision designed to mimic the action of the ventral-neck incision without tissue damage. The sham incision data were collected prior to the use of these animals in another study. Prior to the study, animals were penned overnight with free access to water but not food. The study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Anaesthesia

Anaesthesia was induced using a mixture of 3.7 (SD 0.5) mg/kg ketamine (Parnell Laboratories, Auckland, NZ) and 6.9 (SD 3.4)

mg/kg propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid injection into a jugular vein. Following intubation with a 16-mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia), anaesthesia was maintained using inhalation of halothane (Halothane-Vet; Merial NZ Limited, Manukau City, NZ) in oxygen (BOC, Palmerston North, NZ) delivered via a precision vaporiser (Fluothane; Med-Source Ltd, Ashburton, NZ) and a circle breathing system (VMS Anaesthesia Machine; Matrix Medial Inc, New York, USA). End-tidal halothane tension was maintained at 0.9%. End-tidal CO₂ tension, end-tidal halothane tension, heart rate and respiratory rate were monitored using an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Hamburg, Germany). All subsequent procedures were carried out under general anaesthesia.

EEG and electrocardiographic (ECG) recording

Subdermal 27-G stainless-steel needle electrodes (Medelec, Radiometer, Auckland, NZ) were placed in a bilateral fronto-zygomatic electrode montage, as adapted from the method described by Mayhew and Washbourne (1990). The non-inverting (active) electrodes were placed in the midline between the medial canthi of the eyes, the inverting (reference) electrodes over the left and right mastoid processes, and a common ground electrode caudal to the poll. A base apex electrode configuration was used to record ECG.

The EEG and ECG were amplified using isolated differential signal amplifiers (Iso-Dam Isolated Physiological Signal Amplifiers; World Precision Instruments, Sarasota FL, USA). The EEG was recorded with a gain of 1,000 and a pass-band of 0.1–500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10–500 Hz. Both EEG and ECG data were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

Experimental procedure

Once anaesthetised, calves were placed in dorsal recumbency on a specially designed platform (Massey University Mechanical Services, Massey University, Palmerston North, NZ) with the head securely held in position on a purpose-built head frame (Massey University Mechanical Services). This frame reduced movement of the head during ventral-neck incision and sham incision and provided tension to the neck to ensure that the cut edges did not come into contact with each other after incision. The femoral artery of the right leg was cannulated (18-G BD Insyte Intravenous Catheter; Becton Dickinson Infusion Therapy Systems Inc, Utah, USA) for direct monitoring of arterial blood pressure. The arterial blood pressure transducer (Spectramed Medical Products, Singapore) was re-calibrated against a mercury column (Baumanometer; WA Baum Co Inc, New York, USA) for each animal. Fifteen minutes were allowed for equilibration of general anaesthesia before collection of data commenced.

Ventral-neck incision

A 5-minute pre-treatment EEG recording was made, immediately followed by a single incision to the ventral aspect of the neck below the level of the larynx using a sharp, flat-edged knife 245 mm long by 28 mm wide (Granton Ragg Ltd, Sheffield, England). The knife was used exclusively for the neck incision and was re-sharpened after each use, using a Tru Hone sharpener (Model No. LCF; Tru Hone Corporation, Ocala FL, USA). The incision was always carried out by the same operator. Data were recorded for 5 minutes following slaughter, after which the wound was inspected for complete severance of the major blood vessels and for

any significant signs of occlusion of carotid arteries. Calves were weighed immediately after induction of anaesthesia and all carcasses were weighed at the end of the recording period, to allow estimation of blood loss.

Sham incision

In the sham incision animals, a 5-minute pre-treatment recording was made, after which sham incision was undertaken using a broom handle drawn across the neck with a similar action and position to that of ventral-neck incision, but causing no tissue damage. Data were recorded for 5 minutes following sham incision. After completion of this study these calves were used in a different experiment.

Analysis of EEG and ECG data

EEG epochs contaminated by artefacts such as over- and under-scale or large single spikes were manually rejected from analysis using Chart 5.4.2 (ADInstruments Ltd). The F50, F95 and Ptot were calculated for consecutive non-overlapping 1-second epochs using purpose-written software (Spectral Analyser; Craig Johnson, Massey University, Palmerston North, NZ, 2002). Fast Fourier Transformation was applied to each epoch, generating sequential power spectra with 1-Hz frequency bins. Subsequent analysis and generation of compressed spectral arrays were performed using Microsoft Excel Mac 2004 (Microsoft Corporation, Redmond, USA). Variables derived from 2 seconds before to 5 seconds after ventral-neck incision were excluded from EEG analysis to prevent contamination by movement artefact due to the act of ventral-neck incision.

Data from EEG spectral analysis are displayed as specific EEG indices (F50, F95 and Ptot), or compressed spectral arrays, which incorporated alterations in power and frequency over time, and were derived from the EEG power spectra.

EEG traces were inspected visually and classified into one of five categories, *viz* out of range, active EEG, transitional EEG, high-amplitude low-frequency EEG (Bager *et al.* 1992), and isoelectric EEG. Active EEG represented normal cerebrocortical activity in anaesthetised calves. Transitional EEG was classified as having an amplitude of less than half of pre-treatment EEG. High-amplitude low-frequency EEG was classified as a waveform with rhythmic activity of high amplitude and low frequency. Isoelectric EEG was classified as a stable trace consisting of background noise with an amplitude of <1/3 of the normal pre-stunning EEG with little to no low-frequency component.

Heart rate was calculated from ECG data, using the rate meter function in Chart (ADInstruments Ltd).

Histopathology

Following ventral-neck incision and completion of the experiment, heads were removed from calves and perfused for 5 minutes with heparinised sodium lactate and for 30 minutes with buffered formalin (10%), via the carotid arteries (Multipartin, Heparin Sodium; CP Pharmaceuticals Ltd, Wrexham, UK; and sodium lactate, Hartmann's Solution; Baxter Healthcare Ltd, Toongabbie, Australia). Brains were extracted from the skulls and immediately placed in 10% buffered formalin, for future histological examination. Samples were taken from the obex, spinal cord, pons, cerebellum, midbrain, thalamus, and short gyri of the insula and primary somatosensory cortices (caudal to the central sulcus). Blocks of tissue from those areas were placed in ethanol baths, routinely processed, and embedded with paraffin. Sections were cut at 5 µm and stained with H&E, then examined histologically.

Statistical analysis

EEG data were calculated and displayed as percentage changes from pre-treatment values. All data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA) and Prism 4.0c (GraphPad Software Incorporated, San Diego CA, USA). The distribution of the data was tested for normality using the Anderson-Darling test (Anderson and Darling 1952) or the Kolmogorov-Smirnov test for EEG indices and frequency bands, respectively. Analysis of differences between pre- and post-treatment values for EEG indices was performed on consecutive non-overlapping 30-second epochs using a Mann-Whitney non-parametric test (F95 and Ptot), or a one-way ANOVA (F50). Analysis of the correlation between blood pressure and EEG indices and between left- and right-sided EEG was performed using a two-tailed Spearman's rank coefficient test for non-parametric data. Analysis of blood pressure and heart rate data was performed on individual time points taken every 15 seconds, using a Mann-Whitney non-parametric test. Blood loss was calculated as a percentage of liveweight.

Results

Two calves were excluded from analysis because of inadequate ventral-neck incision due to incomplete severance of the carotid arteries. In addition, four EEG traces (left side from Calves 1 and 5 and right side from Calves 2 and 8) were excluded from analysis due to unacceptable levels of contamination with external noise. Figures illustrate EEG from the left and right sides separately in order to better illustrate the variability of these signals.

In the initial 30 seconds following ventral-neck incision, mean F95 increased from a pre-treatment value of 101 (SD 9)% to 111 (SD 12)% ($p < 0.05$) and remained stable for 150 seconds (Figure 1). After this period, bursts of periodic activity were visible bilaterally. The response in Ptot was biphasic (Figure 2). Initially, it increased from a mean pre-treatment value of 95 (SD 23)% to 160 (SD 91)% and 297 (SD 152)% on the right and left cerebral hemispheres, respectively ($p < 0.052$; $p < 0.002$, respectively). By 60 seconds after ventral-neck incision, mean Ptot had decreased to 60 (SD 23)% and 65 (SD 27)% of pre-treatment values for the

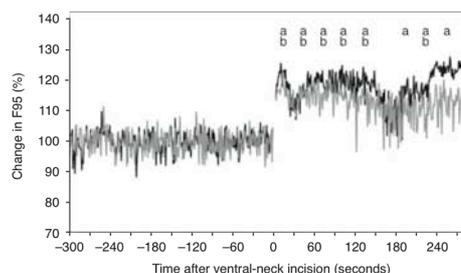


Figure 1. Percentage change in the mean 95% spectral edge frequency (F95), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following ventral-neck incision at time point 0. ^aSignificant difference from pre-treatment values, right cerebral hemisphere ($p < 0.05$). ^bSignificant difference from pre-treatment values, left cerebral hemisphere ($p < 0.05$).

right and left sides, respectively ($p < 0.05$). After 150 seconds, Ptot began to exhibit bursts of periodic activity in individual animals. In the initial 30 seconds following ventral-neck incision, mean F50 increased from a pre-treatment value of 110 (SD 43)% to 125 (SD 84)% ($p = 0.036$) on the right side. Changes in F50 on the left side were similar to those on the right, but of a lesser magnitude and did not reach statistical significance (Figure 3).

Visual assessment of the EEG showed that the mean duration of out-of-range data following ventral-neck incision was 2 (SD 1) seconds. The mean duration of active EEG for anaesthetised calves following ventral-neck incision was 34 (SD 16) seconds. Transitional EEG was observed in most, but not all, animals as waveforms changed from active EEG to high-amplitude low-frequency EEG. The mean duration of transitional EEG in the nine animals in the group that displayed it was 70 (SD 50) seconds. The mean time to onset of high-amplitude low-frequency EEG was 76 (SD 26) seconds, and this EEG pattern had a mean duration of 144 (SD 73) seconds. This activity occurred at similar time points to the periodic activity seen in Ptot (Figure 2). The mean time to the onset of isoelectric EEG was 192 (SD 71) seconds.

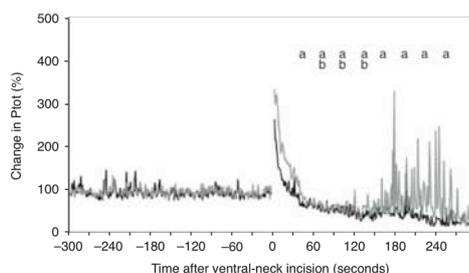


Figure 2. Percentage change in the mean total power (Ptot), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following ventral-neck incision at time point 0. ^aSignificant difference from pre-treatment values, right cerebral hemisphere ($p < 0.05$). ^bSignificant difference from pre-treatment values, left cerebral hemisphere ($p < 0.05$).

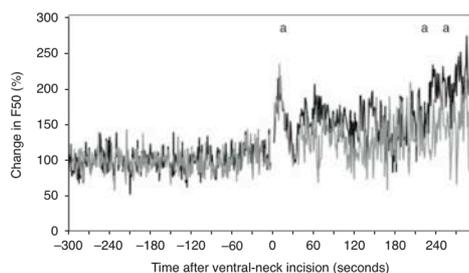


Figure 3. Percentage change in the mean median frequency (F50), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following ventral-neck incision at time point 0. ^aSignificant difference from pre-treatment values, right cerebral hemisphere ($p < 0.05$).

After sham incision there were no significant differences in F50, F95 and Ptot from pre-treatment values (Ptot illustrated in Figure 4). However, there was a transient increase in Ptot immediately following application of the sham incision. This increase had a mean duration of 3 seconds (Figure 4).

After ventral-neck incision mean blood pressure decreased from a pre-treatment value of 112 (SEM 9.5) mm Hg to 22 (SEM 2.3) mm Hg and 4 (SEM 2.6) mm Hg at time points of 40 and 110 seconds, respectively (Figure 5). The blood pressure was significantly lower than pre-treatment values from 15 seconds onwards. Heart rate decreased from pre-treatment values following ventral-neck incision (Table 1). This decrease was significant 30 and 60 seconds after ventral-neck incision ($p < 0.05$). Tachycardia developed from 140 seconds after ventral-neck incision onwards. Estimated blood loss, as a percentage of liveweight, reached 4.5 (SEM 0.4)% after 120 seconds.

Positive correlations ($p < 0.001$) were found between decreasing blood pressure and changes in Ptot following ventral-neck incision ($r = 0.6866$ right-sided; $r = 0.5355$ left-sided). Other EEG indices displayed weak correlations with blood pressure following ventral-neck incision.

No signs of occlusion were seen at either the cephalic or cardiac ends of the carotid arteries following ventral-neck incision during or after the period of data collection. Gross examination of the brains following ventral-neck incision showed no abnormal haemorrhage. Histological examination revealed no internal

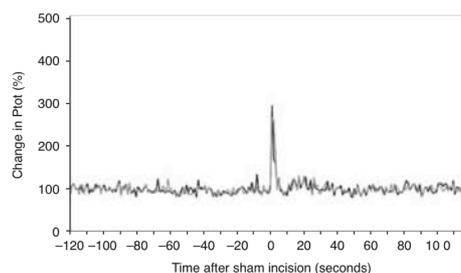


Figure 4. Percentage change in the mean total power (Ptot), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following application of a non-noxious sham incision at time point 0.

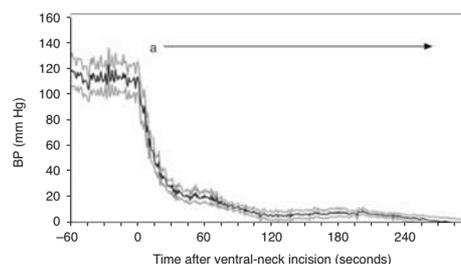


Figure 5. Mean \pm SEM femoral arterial blood pressure (BP; mm Hg) of halothane-anaesthetised calves before and after ventral-neck incision. ^aSignificant difference from pre-treatment values ($p < 0.05$).

Table 1. Mean (\pm SEM) heart rate (beats per minute; bpm) of halothane-anaesthetised calves at individual time points after ventral-neck incision.

Time after ventral-neck incision (seconds)	Heart rate (\pm SEM) (bpm)	P-value ^a
-15 ^b	130.0 \pm 9.36	na
0 ^c	129.1 \pm 9.29	0.916
15	127.7 \pm 20.34	0.318
30	104.3 \pm 5.92	0.031
45	105.3 \pm 5.85	0.052
60	102.3 \pm 5.60	0.031
75	105.0 \pm 5.37	0.074
90	105.1 \pm 4.69	0.052
105	105.4 \pm 5.08	0.052
120	104.7 \pm 7.12	0.083
135	111.9 \pm 7.46	0.227
150	125.1 \pm 7.20	0.793
165	135.3 \pm 8.38	0.875
180	140.7 \pm 9.05	0.372
300 (5 minutes)	137.6 \pm 10.91	0.563

^a Significance of difference from pre-treatment values^b Pre-treatment value^c Ventral-neck incision

na = not applicable

haemorrhage nor any identifiable lesions. Slides taken from the obex, pons, midbrain and somatosensory cortex had dilated vessels or vacuoles, indicating good penetration of the fixation agent immediately following death. However, in the cerebellum of five of the calves, there were mild changes in white matter with artefact around glial cells, potentially indicating poor fixation of the cerebellum. A number of sections had 'chatter', folding and creasing artefacts, but these were not sufficient to hinder histological examination.

Discussion

Slaughter by ventral-neck incision produced responses in all EEG indices measured that have been previously associated with noxious stimulation in calves (Gibson *et al.* 2007).

Increases in F95 have been seen in response to noxious stimulation (Johnson *et al.* 2005b; Gibson *et al.* 2007) and also with decreasing depth of halothane anaesthesia (Johnson *et al.* 1993, 1994; Johnson and Taylor 1998). In the current study, end-tidal halothane tension was tightly controlled during pre-treatment recording periods. However, incision of the neck resulted in severance of the trachea and endotracheal tube. Whilst this stopped the delivery of halothane to the animal, the concurrent interruption of the major component of cerebral arterial blood supply would have reduced cerebral perfusion and so limited the extent to which any reduction in body end-tidal halothane tension was reflected in the brain. The germane EEG changes following ventral-neck incision were of short duration so that the observed changes in F95 were not likely to be a response to decreasing concentrations of halothane in the brain.

The initial increase in Ptot after ventral-neck incision contrasts with previous studies that have generally shown decreases in Ptot following a noxious stimulus (Murrell *et al.* 2003; Haga and

Ranheim 2005; Johnson *et al.* 2005b; Gibson *et al.* 2007). However, an initial increase in Ptot was observed in lambs of different ages undergoing rubber-ring castration (Johnson *et al.* 2005a). In the current study, the sham-incision group demonstrated a transient increase in Ptot comparable in onset to that seen following ventral-neck incision, but of a lower duration and magnitude. This suggests that the increase observed after ventral-neck incision may be partially caused by movement of the animal during incision of the neck. The remainder of the increase following ventral-neck incision may be related to the loss of skin, muscle and connective tissue tension and to contracture of the ventral muscles of the neck that typically follows this reduction of tension.

After the initial increase in Ptot following ventral-neck incision, Ptot significantly decreased. A decrease in power content of specific electrocorticographic frequency bands (2–8 Hz and 8–30 Hz) in conscious cattle has also been reported after slaughter without stunning (Bager *et al.* 1992). This reported effect may be a cortical response to noxious inputs due to slaughter by ventral-neck incision or, conversely, to loss of mid- to high-frequency activity resulting in a reduction of functional cerebrocortical activity. Changes in Ptot following slaughter should be interpreted with caution as decreases in Ptot have been previously associated with noxious stimulation in cattle (Gibson *et al.* 2007), and with reductions in cortical function during stunning (Gibson *et al.* 2009). In the current study, it is highly probable that the calves were responding both to the noxious component of slaughter while gradually losing cortical function over time caused by hypoxia.

The later spikes of increased activity in Ptot were linked with both the onset of high-amplitude low-frequency EEG activity seen on visual assessment of EEG traces and the increase in power of the predominantly lower frequencies (0–10 Hz) of the compressed spectral array. Levinger (1961) found similar activity to that of high-amplitude low-frequency EEG in sheep, i.e. 2–4 Hz between 22–44 seconds following Shechita slaughter (slaughter in accordance with Hebrew ritual practice). In humans, hypoxia-induced syncope has been shown to occur after a period of slowing of background rhythms accompanied by high-amplitude delta activity (Brenner 1997), thereby resembling high-amplitude low-frequency EEG. Additionally, during cardiac arrest in humans slow waves of increasing amplitude and decreasing frequency appeared in the EEG, with a duration of 7–13 seconds (Aichner and Bauer 2005). Our results are consistent with these observations and suggest that cerebral hypoxia was the primary cause of this activity.

Changes in F50 have previously been associated with noxious stimulation in a variety of species (Murrell *et al.* 2003; Johnson *et al.* 2005ab; McGregor 2005; Gibson *et al.* 2007). Similar changes were seen unequivocally on the right side of the brain in this study. The inherent variability associated with F50 may have reduced its statistical power to a point where, in the challenging environment following ventral-neck incision, it was difficult to statistically identify these changes on the left side.

Median frequency and F95 are both measures of the frequency components of the EEG, but they provide no indication of the power or amplitude of the EEG signal. Accordingly, they should always be interpreted in the light of major changes in total signal power (Ptot). As the EEG becomes isoelectric, changes in F50 and F95 increasingly represent background noise rather than reflecting cortical function. Thus, there is a period after ventral-neck incision when the EEG represents real activity and not background

noise. During this period, changes in F50 and F95 may be interpreted as being responses to noxious stimulation, in particular during the period when active EEG is still present. In the study presented here, active EEG following ventral-neck incision was defined as being similar in waveform type and amplitude to that before ventral-neck incision. It represents the period of functional cerebrocortical activity during which an animal, if conscious, may perceive noxious stimulation as painful and distressing.

The compressed spectral array provides a more detailed representation of changes in both the frequency and power contents of the EEG of individual animals following ventral-neck incision. There were increases in the higher frequencies (24–30 Hz) of the compressed spectral arrays, with durations of 30–60 seconds. These changes correlate with those observed in F95 following ventral-neck incision. Furthermore, the duration of the increases in high-frequency activity correspond to the period of active EEG reported from visual inspection of the EEG waveform.

In the current study, active EEG was not synonymous with sensibility as all animals were anaesthetised during the entire experimental period. This requires any conclusions concerning consciousness to be carefully considered. Based on previous work it appears that after ventral-neck incision sensibility and the associated cognitive ability to perceive pain and experience distress are not lost immediately (Mellor and Littin 2004). Measures of undoubted insensibility have demonstrated that this period varies considerably between species and individuals. There is considerable variation in the reported time to undoubted insensibility in cattle, *viz* 2–10 seconds (Levinger 1961), 34–85 seconds (Newhook and Blackmore 1982), or 19–113 seconds (Daly *et al.* 1988). Although some of this variation may be due to the use of different experimental techniques and differing criteria to define insensibility and death (Shaw *et al.* 1990), individual differences in cerebrocortical responses are also likely to be involved. Changes in the EEG attributable to noxiousness in the current study are within the reported window of possible sensibility in the majority of studies following ventral-neck incision.

In the current study, there were no signs of occlusion at either cephalic or cardiac ends of the cut carotid arteries during or after the data collection period. This suggests that the prolonged periods of functional cortical activity seen in individual calves may have been due to the animals having sufficient oxygen stored in the brain and delivered by the vertebral arteries for the maintenance of cerebral metabolism in the absence of carotid occlusion. It has been calculated that the human brain has enough oxygen stored for metabolism to persist for about 7 seconds following the disruption of supply (Hillman 1993).

Blood loss estimated from liveweight change was 4.5% of liveweight 120 seconds following ventral-neck incision in the current study. The pattern of bleed-out was both qualitatively and quantitatively similar to that reported in conscious adult cattle undergoing Halal slaughter (Anil *et al.* 2006). Blood loss was 3% of liveweight in that study, falling within the error bars of the current study.

Histological examination of the brains taken from calves in the ventral-neck incision group displayed no detectable abnormal features. Rosen (2004) suggested that following Shechita the collapse in jugular venous pressure, without replacement with carotid

blood, would result in impaired maintenance of brain structure. Based on the current results there were no indications of loss of structure or of lesions from the suggested sudden decompression of the cranial vault following sectioning of the carotid arteries and jugular veins.

This study is the first to examine quantitatively the noxiousness of slaughter by ventral-neck incision. The results demonstrated that ventral-neck incision caused EEG changes which were quantitatively and qualitatively similar to those observed following scoop dehorning (Gibson *et al.* 2007). In combination with previous analyses (Mellor and Littin 2004), these changes demonstrated that ventral-neck incision has strong potential to be perceived as a noxious stimulus and therefore to be painful in conscious animals subjected to this procedure.

Acknowledgements

The authors would like to thank the members of the Comparative Neuroscience Group, the Large Animal Teaching Unit, and the Small Animal Production Unit, Massey University, for assistance during the experiment. This study was jointly funded by the Department for Environment, Food and Rural Affairs of the United Kingdom and the Ministry of Agriculture and Forestry of New Zealand. TJ Gibson was the recipient of a C Alma Baker Postgraduate Scholarship.

References

- [†]Aichner F, Bauer G. Cerebral anoxia. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Pp 455–70. Lippincott Williams & Wilkins, Philadelphia, USA, 2005
- Anderson TW, Darling DA. Asymptotic theory of certain "goodness-of-fit" criteria based on stochastic processes. *Annals of Mathematical Statistics* 23, 193–211, 1952
- Anil MH, McKinstry JL, Wotton SB, Gregory NG. Welfare of calves. 1. Investigations into some aspects of calf slaughter. *Meat Science* 41, 101–12, 1995 a
- Anil MH, McKinstry JL, Gregory NG, Wotton SB, Symonds H. Welfare of calves. 2. Increase in vertebral artery blood flow following exsanguination by neck sticking and evaluation of chest sticking as an alternative slaughter method. *Meat Science* 41, 113–23, 1995 b
- Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Weaver HR, Erdogan O, Hughes S, Manson C. Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: exsanguination and quality parameters. *Animal Welfare* 15, 325–30, 2006
- [†]Anonymous. *Report on the Welfare of Farmed Animals at Slaughter or Killing, Part 1. Red Meat Animals*. Farm Animal Welfare Council, London, UK, 2003
- [†]Anonymous. *Animal Welfare (Commercial Slaughter) Code of Welfare 2006 Public Draft*. National Animal Welfare Advisory Committee, Wellington, NZ, 2006
- Bager F, Braggins TJ, Devine CE, Graafhuis AE, Mellor DJ, Tavener A, Upsdell MP. Onset of insensibility at slaughter in calves – effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indexes of cerebral metabolism. *Research in Veterinary Science* 52, 162–73, 1992
- Barnett JL, Cronin GM, Scott PC. Behavioural responses of poultry during Kosher slaughter and their implications for the birds' welfare. *Veterinary Record* 160, 45–9, 2007
- Blackmore DK. Differences in behaviour between sheep and cattle during slaughter. *Research in Veterinary Science* 37, 223–6, 1984

[†]Non-peer-reviewed

- Brenner RP.** Electroencephalography in syncope. *Journal of Clinical Neurophysiology* 14, 197–209, 1997
- Chen ACN, Dworkin SF, Haug J, Gehrig J.** Topographic brain measures of human pain and pain responsivity. *Pain* 37, 129–41, 1989
- Daly CC, Kallweit E, Ellendorf F.** Cortical function in cattle during slaughter – conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325–9, 1988
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ.** Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55, 152–7, 2007
- Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ.** Electroencephalographic responses to concussive non-penetrative captive-bolt stunning in halothane-anaesthetised calves. *New Zealand Veterinary Journal* 57, 90–5, 2009
- Grandin T.** Euthanasia and slaughter of livestock. *Journal of the American Veterinary Medical Association* 204, 1354–60, 1994
- Gregory NG, Wotton SB.** Sheep slaughtering procedures. 2. Time to loss of brain responsiveness after exsanguination or cardiac arrest. *British Veterinary Journal* 140, 354–60, 1984
- Haga HA, Ranheim B.** Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1–9, 2005
- Hillman H.** The possible pain experienced during execution by different methods. *Perception* 22, 745–53, 1993
- Johnson CB, Taylor PM.** Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* 81, 748–53, 1998
- Johnson CB, Young SS, Taylor PM.** Use of frequency spectral analysis of the equine electroencephalogram as an indication of the depth of halothane anaesthesia. In: Sebel PS, Bonke B, Winograd E (eds). *Memory and Awareness in Anesthesia*. Pp 289–95. PTR Prentice Hall, Englewood Cliffs, USA, 1993
- Johnson CB, Young SS, Taylor PM.** Analysis of the frequency spectrum of the equine electroencephalogram during halothane anaesthesia. *Research in Veterinary Science* 56, 373–8, 1994
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433–7, 2005 a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61–71, 2005 b
- Levinger IM.** Untersuchungen zum Schächtproblem. [Investigations of the Shechita problem.] *Dr thesis*, University of Zürich, Zürich, Switzerland, 1961
- Mayhew IG, Washbourne JR.** A method of assessing auditory and brain-stem function in horses. *British Veterinary Journal* 146, 509–18, 1990
- McGregor MJ.** Electroencephalographic responses to somatic and visceral noxious stimulation. *MSc thesis*, Massey University, Palmerston North, NZ, 2005
- Mellor DJ, Littin KE.** Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13 (Supplement), 127–32, 2004
- Mellor DJ, Cook CJ, Stafford KJ.** Quantifying some responses to pain as a stressor. In: Moberg GP, Mench JA (eds). *The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare*. Pp 171–98. CABI Publishing, Wallingford, UK, 2000
- Murrell JC, Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325–35, 2006
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138–46, 2003
- Nangeroni LL, Kennett PD.** *Electroencephalographic Studies of the Effects of Shechita Slaughter on Cortical Function in Ruminants*. Cornell University, New York, USA, 1964
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves. 2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295–300, 1982
- Rosen SD.** Opinion piece: Physiological insights into Shechita. *Veterinary Record* 154, 759–65, 2004
- Rutherford KMD.** Assessing pain in animals. *Animal Welfare* 11, 31–53, 2002
- Shaw FD, Bager F, Devine CE.** The role of the vertebral arteries in maintaining spontaneous electrocortical activity after electrical stunning and slaughter in calves. *New Zealand Veterinary Journal* 38, 14–16, 1990

Submitted 13 February 2008

Accepted for publication 13 February 2009

*Non-peer-reviewed

Gibson TJ, Johnson CB, Murrell JC, Chambers JP, Stafford KJ, Mellor DJ. Components of electroencephalographic responses to slaughter in halothane-anaesthetised calves: Effects of cutting neck tissues compared with major blood vessels. *New Zealand Veterinary Journal* 57, 84-9, 2009

New Zealand Veterinary Journal 57(2), 84-89, 2009

84

Scientific Article

Components of electroencephalographic responses to slaughter in halothane-anaesthetised calves: Effects of cutting neck tissues compared with major blood vessels

TJ Gibson^{*†‡}, CB Johnson^{*‡§}, JC Murrell^{‡#}, JP Chambers^{*‡}, KJ Stafford^{*‡} and DJ Mellor^{*‡}

Abstract

AIM: To identify whether cutting neck tissues or cutting major blood vessels initiates the mechanisms responsible for electroencephalographic (EEG) responses to slaughter by ventral-neck incision without prior stunning in halothane-anaesthetised calves.

METHODS: Calves were assigned to two groups, *viz* transection of neck tissues with intact blood circulation through the brain (n=10), or transection of the major blood vessels of the neck but not most other neck tissues (n=7). They were minimally anaesthetised with halothane, using an established anaesthesia protocol. The animals in the neck-tissue transection group had their carotid arteries and jugular veins exposed and cannulated proximal and distal to the proposed site of subsequent ventral-neck incision; this diverted blood flow through these vessels so that cerebral perfusion and drainage were preserved. In animals in the blood-vessel transection group, the carotid arteries and jugular veins were exposed bilaterally by surgical dissection. They were then transected without further damage to the remaining structures of the neck. Changes in the median frequency (F50), 95% spectral edge frequency (F95), total power of the EEG (Ptot), and arterial blood pressure were compared within each group before and after neck-tissue or blood-vessel transection, and between groups following treatments.

RESULTS: Neck-tissue transection resulted in little overall change in the F50, an increase in the F95, and an initial increase in Ptot followed by a transient decrease and eventual return to pre-treatment values. There was between-animal variation in these EEG parameters. Transection of the major blood vessels of the neck resulted in a decrease in F50 in most animals; changes in F95 were highly variable, and there was a decrease in Ptot.

CONCLUSIONS: The EEG responses seen following neck-tissue and blood-vessel transection were qualitatively distinct, and suggested that cutting neck tissues caused greater noxious sensory input than transection of only the major blood vessels of the neck. These observations support the conclusion that the EEG responses seen after ventral-neck incision in intact animals are primarily due to noxious stimulation as a result of incision

of ventral-neck tissues and not mainly as a result of loss of blood flow through the brain.

KEYWORDS: Calves, compressed spectral array, electroencephalogram, emergency slaughter, median frequency, minimal anaesthesia, nociception, pain, slaughter, total power of the EEG power spectrum, transection, ventral-neck incision, 95% spectral edge frequency

Introduction

Slaughter of animals without prior stunning is associated with a number of potential welfare issues. Possible pain and distress during and immediately following ventral-neck incision and the time to onset of insensibility have both received considerable attention (Levinger 1961; Newhook and Blackmore 1982ab; Gregory and Wotton 1984ab). Pain caused by ventral-neck incision has been the subject of much debate. It has been suggested that the use of an exquisitely sharp knife produces minimal behavioural reactions in animals and therefore that such a neck cut is not perceived by the animal as painful (Levinger 1961; Grandin 1994; Rosen 2004). However, there is little neurophysiological evidence to support this suggestion. Until recently it was not clear whether or not slaughter of conscious animals by ventral-neck incision causes pain or distress. This was due to the complexities of measuring pain in animals (Mellor *et al.* 2000; Rutherford 2002) and limitations on the interpretation of behavioural and physiological responses to slaughter by neck incision alone (Barnett 1997; Anonymous 2003; Rosen 2004). The phylogenetic similarities in structure and function of the central nervous systems (CNS) between humans and other mammals leave little doubt that farm animals can indeed experience pain (Barnett 1997). There is also little doubt that these animals are aware prior to, during, and for a period after, slaughter by neck incision without prior stunning (Levinger 1961; Newhook and Blackmore 1982ab; Daly *et al.* 1988). It is therefore possible that slaughter by neck incision alone represents a noxious stimulus which is perceived by the animal as painful prior to the onset of insensibility.

We have reported previously the first experimental investigation using EEG spectral analysis of the noxiousness of slaughter by ventral-neck incision of calves (Gibson *et al.* 2009). In that study,

* Animal Welfare Science and Bioethics Centre, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

† Current address: Veterinary Clinical Sciences, Royal Veterinary College, North Mymms, Herts AL9 7TA, United Kingdom.

‡ Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

Current address: Department of Clinical Veterinary Science, Division of Companion Animals, University of Bristol, Bristol BS8 1TH, United Kingdom.

¥ Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

§ Author for correspondence. Email: c.b.johnson@massey.ac.nz

CNS	Central nervous system(s)
EEG	Electroencephalogram(s)/electroencephalographic
F50	Median frequency
F95	95% Spectral edge frequency
Ptot	Total power of the electroencephalogram
PVC	Polyvinyl chloride

we demonstrated that there is a period following slaughter when ventral-neck incision would represent a noxious stimulus as measured by specific EEG responses. Those responses were similar in character to those shown to be caused by amputation dehorning in cattle (Gibson *et al.* 2007). The response to ventral-neck incision may be due to stimulation of nociceptors in the incised tissues of the neck, to the loss of cerebral perfusion by blood caused by transection of the vessels of the neck, or to a combination of both factors. The aim of this study was to investigate the relative contributions of these two factors to the EEG responses of halothane-anaesthetised calves associated with slaughter by ventral-neck incision without prior stunning.

Materials and methods

Animals

Seventeen mixed-breed calves weighing 109–170 kg were sourced from a commercial stock agent and kept in accordance with normal farming practices. They were allocated to one of two treatments. In one group, ventral-neck incision was performed while cerebral perfusion was maintained via cannulae that preserved the blood supply around the incision site (neck-tissue transection group; $n=10$). In the second group, exteriorised carotid arteries and jugular veins were transected without concomitant damage to the other tissues of the neck (blood-vessel transection group; $n=7$). Prior to the study, animals were penned overnight (approximately 16 hours) with free access to water but not food. The study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Anaesthesia

Anaesthesia was induced using a mixture of ketamine (Parnell Laboratories, Auckland, NZ) and propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid injection into a jugular vein. The total doses of induction agents were 3.4 (SD 0.3) and 7.9 (SD 1.2) mg/kg for the neck-tissue transection group, and 4.1 (SD 0.4) and 6.6 (SD 1.3) mg/kg for the blood-vessel transection group, of ketamine and propofol, respectively. Anaesthesia was maintained using halothane in oxygen as described previously by Gibson *et al.* (2007). End-tidal halothane tension was maintained between 0.85 and 0.95%. Three neck-tissue transection calves were removed from analysis as their end-tidal halothane tension was not in this range at the time of transection.

EEG recording

Placement of electrodes and recording of EEG data were as described by Gibson *et al.* (2007, 2009). EEG data were recorded from the right and left cerebral hemispheres. The EEG were amplified using isolated differential signal amplifiers (Iso-Dam Isolated Physiological Signal Amplifiers; World Precision Instruments, Sarasota FL, USA). The EEG was recorded with a gain of 1,000 and a pass-band of 0.1–500 Hz, except in two animals in the blood-vessel transection group where the pass-band was 1–500 Hz. EEG data were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

Experimental procedure

Neck-tissue transection group

Once anaesthetised, calves were placed in dorsal recumbency on a specially designed platform (Massey University Mechanical Serv-

ices, Massey University, Palmerston North, NZ) with the head securely held in position on a purpose-built head frame (Massey University Mechanical Services). Bilateral surgical exposure of the carotid artery and jugular vein was undertaken on sections of the neck proximal and distal to the planned site of ventral-neck incision (Gibson *et al.* 2009). Calves were heparinised with a bolus of 170 (SD 14) U/kg heparin (Multiparin, Heparin Sodium; CP Pharmaceuticals Ltd, Wrexham, UK) after which an infusion into a femoral vein was begun at a rate of 3.4 (SD 0.3) U/kg/minute via a syringe driver (Mi60-2B Microinfusion Pump; World Precision Instruments). Surgical cannulation of the exposed vessels was performed using purpose-built curved stainless-steel cannulae (Massey University Mechanical Services) connected over the proposed transection site using flexible silicon and polyvinyl chloride (PVC) tubing. The distance between the proximal and distal cannulation sites was 100–120 mm. The metal cannulae had the following dimensions: arterial 5 mm internal diameter, 80 mm long; venous 8 mm internal diameter, 100 mm long. The flexible tubing was 500–600 mm long and had internal diameters of 5 mm for arteries (silicon) and 9 mm for veins (PVC). After completion of the cannulations, surgical swabs (BSN Medical Ltd, Mount Waverley, Australia) soaked in sodium lactate (Hartmann's Solution; Baxter Healthcare Ltd, Toongabbie, Australia) were placed in and around the wounds, which were then closed with forceps to prevent dehydration of exposed tissues. In the final three animals, the carotid arteries and jugular veins were insulated with aluminium foil to reduce heat loss by convection and radiation. Fifteen minutes were allowed for equilibration of general anaesthesia before collection of data commenced.

A 5-minute pre-treatment recording of EEG and blood pressure (recorded from a catheterised femoral artery; Gibson *et al.* 2009) was made, immediately followed by neck-tissue transection by a smooth incision on the intact section of neck between the proximal and distal cannulation sites using a sharp, flat-edged knife 245 mm long by 28 mm wide (Granton Ragg Ltd, Sheffield, England). The knife was used exclusively for incision of the neck and was re-sharpened after each use, using a Tru Hone sharpener (Model No. LCF; Tru Hone Corporation, Ocala FL, USA). The incision was always carried out by the same operator. Data were collected for 5 minutes following neck-tissue transection, after which all calves were subject to euthanasia with sodium pentobarbitone (Pentobarb 500; National Veterinary Supplies Ltd, Auckland, NZ) injected into one of the exteriorised carotid arteries.

Blood-vessel transection group

This group was handled in a similar manner to the neck-tissue transection group, with the following exceptions. Metal plates were placed beneath bilaterally exteriorised carotid arteries and jugular veins to provide a solid cutting surface, leaving the vagosympathetic trunk and other structures undamaged and unexposed within the neck. Following completion of surgery, animals were stabilised for 1 hour. Calves were heparinised with a bolus of 124 (11.5) U/kg heparin (Multiparin, Heparin Sodium; CP Pharmaceuticals Ltd) injected into a femoral vein. Data were recorded for 15 minutes, after which two operators using fresh scalpel blades simultaneously transected both carotid arteries and jugular veins. Data were collected for 5 minutes following transection of the vessels.

Analysis of EEG data

Analyses of EEG and arterial blood pressure were performed after completion of data collection. EEG traces were inspected visu-

ally for signs of over- and under-scale and external noise. Traces containing significant artefact were excluded from further analysis. Artefacts which were detected and excluded included loss of high-frequency components of the EEG during the pre-treatment period (two animals) and movement artefact causing more than 30–40 seconds of out-of-range data (two animals). Calculation of F50, F95 and Ptot indices and Fast Fourier Transformation was performed using purpose-written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North, NZ, 2002) as described previously (Gibson *et al.* 2007). All subsequent analysis was performed using Microsoft Excel Mac 2004 (Microsoft Corporation, Redmond, USA).

Data from EEG spectral analysis are displayed as percentage change in specific EEG indices. The mean values from the 5-minute period prior to neck-tissue transection and the 15-minute period prior to blood-vessel transection were used as baselines to calculate percentage changes. Data were smoothed using a 10-point moving average.

Statistical analysis

Blood pressure data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA). The distribution of the data was tested for normality using the Anderson-Darling test (Anderson and Darling 1952). Between-group comparisons were made using area-under-the-curve analysis and ANOVA. Comparisons with pre-treatment values were made using Dunnett's *post-hoc* test. The level of statistical significance was taken to be $p < 0.05$.

Results

EEG power spectra indices

Useable data were available from four neck-tissue transection and six blood-vessel transection calves. These small sample sizes precluded statistical analysis of the EEG power spectra data.

Within-group comparisons

In the neck-tissue transection group, there was much variability between individuals in F50 (Figure 1). A transient increase in the mean F50 was seen during the initial 40 seconds following neck-tissue transection, after which it returned to pre-treatment levels. This increase was primarily attributed to a large increase in F50 seen in one animal. The mean F95 increased, as did the F95 in individual animals, such that it remained elevated above pre-treatment values during the recording period (Figure 2). Ptot showed a transient increase related to movement artefact. This was followed by a decrease and a return to pre-treatment values by about 40 seconds after neck-tissue transection (Figure 3).

In the blood-vessel transection group, the mean F50 decreased from pre-treatment values throughout the recording period. This decrease was variable in individual animals (Figure 1). Variable responses in F95 were observed in individual animals, with both increases and decreases (Figure 2). The mean F95 increased from pre-treatment values but the overall change was relatively small in magnitude. After transection of the blood vessels, the mean Ptot showed a continued gradual decrease, and there was increasing variability in individual animals (Figure 3).

Between-group comparisons

The changes in F50, F95 and Ptot in the neck-tissue transection group were distinct from those in the blood-vessel transection

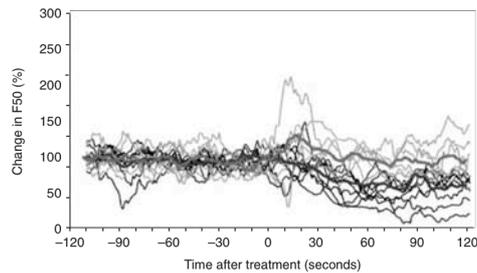


Figure 1. Percentage change in the median frequency (F50), relative to pre-treatment values, of the electroencephalogram of halothane-anaesthetised calves following neck-tissue transection of individual animals (grey line) and the group mean (thick grey line), or blood-vessel transection of individual animals (black line) and the group mean (thick black line). Data are displayed as 10-point moving averages.

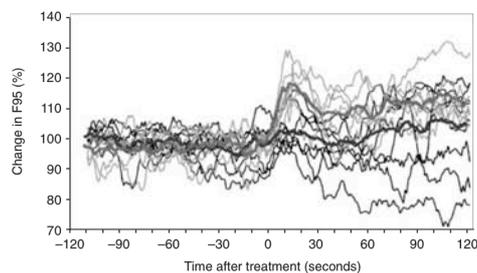


Figure 2. Percentage change in the 95% spectral edge frequency (F95), relative to pre-treatment values, of the electroencephalogram of halothane-anaesthetised calves following neck-tissue transection of individual animals (grey line) and the group mean (thick grey line), or blood-vessel transection of individual animals (black line) and the group mean (thick black line). Data are displayed as 10-point moving averages.

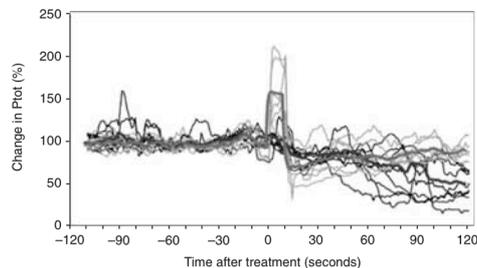


Figure 3. Percentage change in the total power (Ptot), relative to pre-treatment values, of the electroencephalogram of halothane-anaesthetised calves following neck-tissue transection of individual animals (grey line) and the group mean (thick grey line), or blood-vessel transection of individual animals (black line) and the group mean (thick black line). Data are displayed as 10-point moving averages.

group (Figures 1, 2 and 3). Although variable, F50 in the neck-tissue transection group increased and then returned to pre-treatment values. In the blood-vessel transection group, the mean F50 continued to decrease throughout the recording period and there was no initial increase. In the neck-tissue transection group, F95 increased whereas in the blood-vessel transection group, there was

a small increase but F95 remained close to pre-treatment values. Ptot showed a transient increase related to movement artefact. This was followed by a decrease, with a return to pre-treatment values by about 40 seconds after neck-tissue transection, whereas in the blood-vessel transection group there was little initial response, after which Ptot decreased throughout the remainder of the recording period.

Blood pressure responses

Arterial blood pressure was not recorded in one calf in the neck-tissue transection group for technical reasons. Mean arterial pressure in the other three animals decreased from pre-treatment values in both groups (Figure 4). During the first 30 seconds after neck-tissue transection, mean arterial blood pressure decreased from 101 (SEM 12.7) to 78 (SEM 16.1) mm Hg ($p=0.065$; $n=3$).

Ten seconds after blood-vessel transection, mean arterial pressure had reduced by half from pre-treatment values of 111 (SEM 9.8) to 55 (SEM 9.8) mm Hg. Mean arterial pressure was 15 (SEM 3.7) and 11 (SEM 2.9) mm Hg at 60 and 120 seconds, respectively, after vessel transection, both being lower than pre-treatment values ($p<0.05$). Before treatment there were no significant between-group differences in mean arterial pressure; after treatment, values differed significantly between groups ($p<0.001$).

Discussion

Changes in the EEG of calves in response to ventral-neck incision involving simultaneous incision of the ventral-neck tissues and the major blood vessels have been previously reported by us (Gibson *et al.* 2009). In the current study, it was apparent that neck-tissue transection without interruption of blood supply to the brain evoked a cerebrocortical response distinct from that of blood-vessel transection alone. Changes in F95 following neck-tissue transection were qualitatively similar to those seen after ventral-neck incision in intact calves (Gibson *et al.* 2009). This has important implications as it suggests that the EEG response to ventral-neck incision is likely to be due primarily to noxious sensory input evoked by incision of ventral-neck tissues and not mainly to disruption of blood flow through the brain. The different elements of the EEG responses seen following blood-vessel transection support this conclusion. The decrease in F50 following bilateral transection of the carotid arteries and jugular veins without severance of other structures of the neck was opposite to

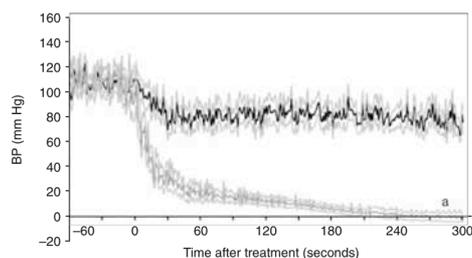


Figure 4. Mean \pm SEM (either side of data lines) femoral arterial blood pressure (BP; mm Hg) of halothane-anesthetised calves following neck-tissue transection (grey line) or blood-vessel-transection (black line) at time point 0. ^a Significant difference between treatment groups ($p<0.001$).

changes caused by dehorning-induced noxious sensory input in cattle (Gibson *et al.* 2007) or slaughter of calves by ventral-neck incision (Gibson *et al.* 2009). The absence of uniform changes in F95 and the decrease in F50 after vessel transection suggest an absence of noxious sensory input. Ptot decreased from baseline values following blood-vessel transection, with no transient increase as seen after ventral-neck incision in intact calves (Gibson *et al.* 2009). Ptot did not return to pre-treatment values as occurred in the neck-tissue transection group. The decrease in Ptot following vessel severance in the blood-vessel transection group represents a reduction in and eventual loss of cerebral cortical electrical activity due to ischaemia.

Tissue damage to the neck during neck-tissue transection would have resulted in activation of nociceptors in and around the damaged tissues. Severance of sensory axons causes a barrage of afferent injury discharges lasting for 2–4 seconds, after which the depolarised severed axon becomes inactive (Wall *et al.* 1974; Gregory 2004). Undamaged nociceptors and other sensory nerves in the region of the wound could be responsive to further stimulation (Gregory 2007), such as by blood coursing over the wound from the cephalic or cardiac ends of the severed vessels, pressure from the incision, air currents, and possible mechanical activation during involuntary hypoxic gasping reflexes.

The transient increase in Ptot following neck-tissue transection was similar in trend to that observed following ventral neck-incision in intact animals (Gibson *et al.* 2009), however the magnitude of increase was relatively smaller following neck-tissue transection. This may have been caused by movement artefact during transection of the tissues of the neck. Neck-tissue transection without disruption of perfusion to the brain produced similar movement of the head and neck to that seen during ventral-neck incision in intact animals (Gibson *et al.* 2009). This conclusion is supported by the absence of an increase in Ptot following vessel severance in the blood-vessel transection group, as cutting the vessels produced little movement of the head and neck.

Noxious stimulation during surgical exposure and cannulation may have caused hypersensitivity or hyperalgesia in both groups. Central sensitisation (Woolf 1996) may have affected the nociceptive response to neck-tissue transection. However, as the responses in F95 were qualitatively similar to those previously observed following ventral-neck incision in intact animals (Gibson *et al.* 2009) the surgical exposure probably did not significantly alter the EEG responses to incision of the ventral-neck tissues.

Transection of ventral-neck tissues without disruption of blood circulation through the brain resulted in an immediate decrease in blood pressure in the neck-tissue transection group. The relatively small blood loss from the incision of the neck was not considered to be sufficient to cause the observed decrease in blood pressure. More probably, severance of the vagosympathetic trunk resulted in loss of afferent and efferent vagal activity. Vagal transection may also have resulted in some redistribution of blood flow (Gregory 2005).

Occlusion of the severed cephalic or cardiac ends of the carotid arteries, due to compression or ballooning of the carotid artery, following slaughter by ventral-neck incision was considered to contribute to delayed loss of sensibility (Bager *et al.* 1988; Anil *et al.* 1995ab; Wotton 2004). In the current study, all calves were heparinised to reduce the likelihood of blood clotting. The formation of blood clots on the severed ends of the carotid arteries or in the surrounding connective tissue sheath could reduce the rate of blood loss; this was not observed in the calves in the present

study. In some animals in the blood-vessel transection group, the cardiac ends of the carotid arteries retracted back into the wound and the intact sternomandibularis muscles closed over them. The pressure provided by those muscles may have been sufficient to retard blood loss. Despite this, changes in blood pressure following blood-vessel transection were qualitatively and quantitatively similar to those following slaughter by ventral-neck incision in otherwise intact animals (Anil *et al.* 2006; Gibson *et al.* 2009). This finding strongly supports the view that blood loss in calves in the blood-vessel transection group closely corresponded to that observed after slaughter by neck incision.

The shortened period of stabilisation between the end of surgery (equilibration of general anaesthesia) and the beginning of pre-treatment recording in the neck-tissue transection group, compared with the previous study (Gibson *et al.* 2009), may have influenced the current results but was deemed necessary in order that the period of extracorporeal circulation be as short as possible. End-tidal halothane tension was maintained above 1.5% during surgical manipulation, after which it was reduced to be within the desired range of 0.85–0.95%. In theory, end-tidal halothane tension should match that of the brain, however the short period of equilibration may not have been sufficient to allow complete stabilisation of the tension of halothane in the brain and thus may have influenced the EEG. Evidence from the baseline periods is that the EEG variables were stable prior to the neck-tissue transection, indicating that this was not the case and that the animals were at a stable plane of anaesthesia at the time of transection. Any increase in end-tidal halothane tension at the time of noxious stimulation would have been expected to blunt or obtund EEG responses to the manipulations, thereby underestimating the impact of noxious sensory inputs. This may partially account for the reduced responses seen in the present calves in the neck-tissue transection group compared with those in our previous study (Gibson *et al.* 2009).

Blood carried to the head via the extracorporeal circulation in the calves in the neck-tissue transection group was exposed to the ambient external temperature and this may have resulted in cooling of and a decreased metabolism in the cerebral cortical tissues. Hypothermia has been shown to affect electrical activity of the brain, with increases in latency and some decreases in amplitude of somatosensory-evoked potentials (Hansen and Claassen 2005). During severe hypothermia (body temperature <20°C), the amplitude and frequency of the EEG are significantly diminished, resulting in burst suppression (Blume and Sharbrough 2005). Alterations in the EEG following cooling of extracorporeal circulation during cardiopulmonary bypass surgery have produced variable results. Levy (1984) reported a linear relationship between temperature and Prot and no relationship between temperature and F95. Conversely, Bashein *et al.* (1992) found no relationship in any EEG descriptors. To minimise possible heat loss from the extracorporeal circulation in the current study the vessels were covered with aluminium foil in the last three animals. There were no qualitative or quantitative differences in the EEG or any measured EEG descriptors between animals that had the vessels covered and those that did not. Although cerebral cortical function may have been affected by extracorporeal cooling, the measured EEG indices remained stable during the 10-minute equilibration period following cannulation and during the 5-minute pre-treatment recording period.

Every attempt was made to minimise the influence of the experimental preparation on the results in the current study, however surgical manipulation, prolonged anaesthesia, hypothermia and

artificial redirection of the carotid and jugular blood flow may have affected cerebrocortical function at the time of treatment in some animals. Data from such animals were excluded from analysis, resulting in the small sample sizes, which precluded the use of statistical analyses of EEG data. Despite this, the current study demonstrated the causation of the noxious responses previously seen following slaughter via ventral-neck incision (Gibson *et al.* 2009).

The magnitude of EEG changes in the present study were somewhat imprecise because of the small number of animals involved in the study. In addition, changes in the animals' physiological status consequent on the manipulations to which they were subjected were necessarily different between the two groups. For example, the changes in EEG variables in the first study (Gibson *et al.* 2009) indicate responses to noxious stimulation against a background of failing CNS function due to the interruption of the cerebral blood supply. In the present study, changes in EEG variables in the neck-tissue transection group reflect a response to noxious stimulation with continuing CNS function. In this sense, they look more similar to the responses to dehorning in cattle (Gibson *et al.* 2007). The changes in the blood-vessel transection group reflect an interruption of cerebral perfusion and so are set against a background of failing CNS function. In this case, the EEG variables do not show the initial changes characteristic of a response to noxious stimulation. Given the relative complexities of the two preparations, we think it most likely that manipulations prior to neck-tissue transection interfered with the animal's ability to respond to a noxious stimulus more than the manipulations prior to blood-vessel transection. The blood-vessel transection group did not show signs of response to noxious stimulation and so it is very unlikely that such interruption of cerebral perfusion would constitute a noxious stimulus. Despite the complexity of the neck tissue-transection preparation, there were still signs of a CNS response to noxious stimulation, and we feel that this is a significant finding.

Finally, these findings support the conclusion that the acute EEG response seen after slaughter of calves by ventral-neck incision was due primarily to noxious sensory input caused by incision of ventral-neck tissues, and not to loss of cerebral perfusion following severance of the carotid arteries and jugular veins.

Acknowledgements

The authors would like to thank Corrin Hulls, Leanne McCracken, Kavitha Kongara, Nicki Grint, Amanda Mellhone and Zoe Matthews for their assistance with experimental work. This study was jointly funded by the Department for Environment, Food and Rural Affairs of the United Kingdom and the Ministry of Agriculture and Forestry of New Zealand. TJ Gibson was the recipient of a C Alma Baker Postgraduate Scholarship.

References

- Anderson TW, Darling DA. Asymptotic theory of certain "goodness-of-fit" criteria based on stochastic processes. *Annals of Mathematical Statistics* 23, 193–21, 1952
- Anil MH, McKinstry JL, Wotton SB, Gregory NG. Welfare of calves. 1. Investigations into some aspects of calf slaughter. *Meat Science* 41, 101–12, 1995 a

- Anil MH, McKinstry JL, Gregory NG, Wotton SB, Symonds H. Welfare of calves. 2. Increase in vertebral artery blood flow following exsanguination by neck sticking and evaluation of chest sticking as an alternative slaughter method. *Meat Science* 41, 113–23, 1995 b
- Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Weaver HR, Erdogan O, Hughes S, Manson C. Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: exsanguination and quality parameters. *Animal Welfare* 15, 325–30, 2006
- *Anonymous. *Report on the Welfare of Farmed Animals at Slaughter or Killing, Part 1. Red Meat Animals*. Farm Animal Welfare Council, London, UK, 2003
- Bager F, Devine CE, Gilbert KV. Jugular blood flow in calves after head-only electrical stunning and throat-cutting. *Meat Science* 22, 237–43, 1988
- Barnett JL. Measuring pain in animals. *Australian Veterinary Journal* 75, 878–9, 1997
- Bashein G, Nessler ML, Bledsoe SW, Townes BD, Davis KB, Coppel DB, Hornbein TF. Electroencephalography during surgery with cardiopulmonary bypass and hypothermia. *Anesthesiology* 76, 878–91, 1992
- *Blume WT, Sharbrough FW. EEG Monitoring during cardioid endarterectomy and open heart surgery. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Lippincott Williams & Wilkins, Philadelphia, USA, 2005
- Daly CC, Kallweit E, Ellendorf F. Cortical function in cattle during slaughter – conventional captive bolt stunning followed by exsanguination compared with Shechita slaughter. *Veterinary Record* 122, 325–9, 1988
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55, 152–7, 2007
- Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, Johnstone AC, Mellor DJ. Electroencephalographic responses of halothane-anesthetised calves to slaughter by ventral-neck incision without prior stunning. *New Zealand Veterinary Journal* 57, 77–83, 2009
- Grandin T. Euthanasia and slaughter of livestock. *Journal of the American Veterinary Medical Association* 204, 1354–60, 1994
- *Gregory NG. Pain. *Physiology and Behaviour of Animal Suffering*. Pp 94–130. Blackwell Publishing, Oxford, UK, 2004
- Gregory NG. Recent concerns about stunning and slaughter. *Meat Science* 70, 481–91, 2005
- *Gregory NG. Stunning and slaughter. *Animal Welfare and Meat Production*. Pp 191–212. CABI Publishing, Wallingford, UK, 2007
- Gregory NG, Wotton SB. Sheep slaughtering procedures. 2. Time to loss of brain responsiveness after exsanguination or cardiac arrest. *British Veterinary Journal* 140, 354–60, 1984 a
- Gregory NG, Wotton SB. Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141–3, 1984 b
- *Hansen HC, Claassen J. EEG and evoked potentials in neuroanesthesia, intraoperative neurological monitoring, and neurointensive care. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Lippincott Williams & Wilkins, Philadelphia, USA, 2005
- Levinger IM. Untersuchungen zum Schächtproblem. [Investigations of the Schechita problem.] *Dr thesis*, University of Zürich, Zürich, Switzerland, 1961
- Levy WJ. Quantitative analysis of EEG changes during hypothermia. *Anesthesiology* 60, 291–7, 1984
- *Mellor DJ, Cook CJ, Stafford KJ. Quantifying some responses to pain as a stressor. In: Moberg GP, Mench JA (eds). *The Biology of Animal Stress Basic Principles and Implications for Animal Welfare*. Pp 171–98. CABI Publishing, Wallingford, UK, 2000
- Newhook JC, Blackmore DK. Electroencephalographic studies of stunning and slaughter of sheep and calves. 1. The onset of permanent insensibility in sheep during slaughter. *Meat Science* 6, 221–33, 1982 a
- Newhook JC, Blackmore DK. Electroencephalographic studies of stunning and slaughter of sheep and calves. 2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295–300, 1982 b
- Rosen SD. Opinion piece; Physiological insights into Shechita. *Veterinary Record* 154, 759–65, 2004
- Rutherford KMD. Assessing pain in animals. *Animal Welfare* 11, 31–53, 2002
- Wall PD, Waxman S, Basbaum AI. Ongoing activity in peripheral nerve: injury discharge. *Experimental Neurology* 45, 576–86, 1974
- Woolf CJ. Windup and central sensitization are not equivalent. *Pain* 66, 105–8, 1996
- Wotton S. Slaughter without prestunning. *Veterinary Record* 155, 64, 2004

Submitted 13 February 2008

Accepted for publication 16 February 2009

*Non-peer-reviewed

Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ. Electroencephalographic responses to concussive non-penetrative captive-bolt stunning in halothane-anaesthetised calves. *New Zealand Veterinary Journal* 57, 90-5, 2009

New Zealand Veterinary Journal 57(2), 90-95, 2009

90

Scientific Article

Electroencephalographic responses to concussive non-penetrative captive-bolt stunning in halothane-anaesthetised calves

TJ Gibson^{*†‡}, CB Johnson^{*‡§}, JC Murrell^{‡#}, SL Mitchinson^{*‡}, KJ Stafford^{*‡} and DJ Mellor^{*‡¥}

Abstract

AIM: To investigate the electroencephalographic (EEG) and cardiovascular responses of halothane-anaesthetised calves to non-penetrative captive-bolt stunning.

METHODS: Ten calves were minimally anaesthetised with halothane, using an established anaesthesia protocol. The EEG, blood pressure and electrocardiogram (ECG) were recorded before and after non-penetrative captive-bolt stunning. Visual inspection and alterations in the total power of the EEG (Ptot) were used to investigate the effects of stunning.

RESULTS: Captive-bolt stunning significantly altered cerebrocortical function in all animals. In four animals, Ptot decreased immediately following stunning and remained low throughout the recording period. In another five animals, Ptot responded to stunning in a biphasic manner characterised by an immediate decrease followed by a transient increase and then a final decrease to values similar to those of the non-biphasic animals.

CONCLUSIONS: Non-penetrative captive-bolt stunning significantly altered cerebrocortical function in halothane-anaesthetised calves. The changes in cerebrocortical function would be sufficient to produce insensibility within 0 to 14 seconds in conscious animals.

KEY WORDS: Calves, compressed spectral array, concussive stunning, electroencephalogram, minimal anaesthesia, non-penetrative stunning, slaughter

Introduction

Concussive stunning of livestock prior to slaughter was practised in China as early as the 15th Century (Mellor and Littin 2004), in order to improve the safety of slaughtermen and facilitate handling of large animals during the slaughter process. Stunning is now primarily practised to render animals insensible to pain and distress prior to the act of slaughter. In New Zealand, with few

exceptions, all animals slaughtered commercially for human consumption must be rendered immediately insensible to pain and distress prior to slaughter and remain so during the entire slaughter process (Anonymous 1994, 2006). Both mechanical and electrical stunning are in use in New Zealand. Mechanical stunning is widely employed with many species of livestock and is routinely performed around the world during slaughter of animals for human consumption or prior to euthanasia.

The time to loss of undoubted sensibility following penetrative captive-bolt stunning has received considerable attention (Fricker and Riek 1981; Blackmore and Newhook 1982; Daly *et al.* 1986), but less attention has been given to non-penetrative captive-bolt stunning. Changes in amplitude and morphology of the EEG and the onset of isoelectric EEG (Blackmore and Newhook 1982), loss of righting reflexes (Blackmore 1979), loss of visual evoked potentials (Gregory and Wotton 1990) and loss of corneal reflexes (Blackmore 1979; Blackmore and Newhook 1982) have all been investigated during non-penetrative captive-bolt stunning.

The pattern of changes of EEG activity following non-penetrative captive-bolt stunning includes a period of transitional EEG seen on visual inspection of the EEG traces. Transitional EEG had a different morphology from both pre-treatment active EEG and isoelectric EEG, and has been characterised as being incompatible with sensibility (Blackmore and Delany 1988). High-amplitude low-frequency EEG activity similar to the low-frequency delta and theta waveforms seen following successful penetrative captive-bolt stunning of conscious calves (Lambooy and Spanjaard 1981) may also be present. This activity has been reported in both humans and animals after clinical and experimental traumatic brain injury, and is seen during unconsciousness caused by concussive impacts (Shaw 2002).

It has been suggested that spontaneous EEG and electrocorticographic activity cannot be used to accurately demonstrate the onset and duration of insensibility following penetrative captive-bolt stunning in cattle as cortically evoked responses were lost immediately after such stunning in animals exhibiting apparently normal EEG traces (Blackmore and Delany 1988). Despite this, analysis of such traces from conscious animals can provide useful information on the window of cerebral cortical activity within which decisions on the onset of insensibility can be made. Analysis of the EEG provides information on when undoubted insensibility is present, which defines an end point useful in the interpretation of cerebrocortical activity (Blackmore and Newhook 1982).

* Animal Welfare Science and Bioethics Centre, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

† Current address: Veterinary Clinical Sciences, Royal Veterinary College, North Mymms, Herts AL9 7TA, United Kingdom.

‡ Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

Current address: Department of Clinical Veterinary Science, Division of Companion Animals, University of Bristol, Bristol BS8 1TH, United Kingdom.

¥ Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

§ Author for correspondence. Email: c.b.johnson@massey.ac.nz

ECG Electrocardiogram(s)/electrocardiographic
EEG Electroencephalogram(s)/electroencephalographic
Ptot Total power of the electroencephalogram

The EEG is a representation of the functional activity of the brain (Murrell and Johnson 2006). Subjective evaluation of raw EEG has been used to assess functional changes in cerebrocortical activity following non-penetrative captive-bolt stunning (Blackmore and Newhook 1982), but this approach does not readily lend itself to detailed statistical analysis of EEG changes. Application of the Fast Fourier Transformation (Cooley and Tukey 1965) allows detailed objective statistical interpretation of changes in specific EEG indices derived from EEG power spectra. Objective examination of such changes may provide new information about cerebrocortical function during non-penetrative captive-bolt stunning in cattle.

The aim of this study was to investigate cerebrocortical and cardiovascular responses to concussive non-penetrative captive-bolt stunning in halothane-anaesthetised calves, in order to evaluate the effectiveness of this method in rendering calves insensible prior to slaughter.

Materials and methods

Animals

Ten mixed-breed calves weighing 109–144 kg were sourced from a commercial stock agent and kept in accordance with normal farming practices. Prior to the study, the animals were penned overnight with free access to water but not food. The study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Anaesthesia

Anaesthesia was induced using a mixture of 4.0 (SD 0.4) mg/kg ketamine (Parnell Laboratories, Auckland, NZ) and 6.5 (SD 2.5) mg/kg propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid injection into a jugular vein. Following intubation with a 16-mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia), anaesthesia was maintained using inhalation of halothane (Halothane-Vet; Merial NZ Ltd, Manukau City, NZ) in oxygen (BOC, Palmerston North, NZ) delivered via a precision vaporiser (Fluothane; MedSource Ltd, Ashburton, NZ) and circle breathing system (VMS Anaesthesia Machine; Matrix Medical Inc, New York, USA). End-tidal halothane tension was maintained between 0.85 and 0.90%. End-tidal CO₂ tension, end-tidal halothane tension and respiratory rate were monitored using an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Hamburg, Germany) that sampled airway gases from the proximal end of the endotracheal tube. Calves breathed spontaneously throughout the experimental period.

EEG and ECG recording

Placement of electrodes and recording of EEG and ECG data were as described by Gibson *et al.* (2007, 2009). EEG data were recorded from the right and left cerebral hemispheres. The EEG and ECG were amplified using isolated differential signal amplifiers (Iso-Dam Isolated Physiological Signal Amplifiers; World Precision Instruments, Sarasota FL, USA). The EEG was recorded with a gain of 1,000 and a pass-band of 0.1–500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10–500 Hz. Both EEG and ECG data were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

Experimental procedure

Once anaesthetised, calves were placed in dorsal recumbency on a specially designed platform (Massey University Mechanical Services; Massey University, Palmerston North, NZ) with the head held securely in position on a purpose-designed head frame (Massey University Mechanical Services). A steel bar was positioned on the back of the neck proximal to the horns and poll to reduce movement of the head. This placement of the head left the frontal bone unobstructed for stunning. EEG and ECG instrumentation was attached and a femoral artery surgically exposed and cannulated, as described by Gibson *et al.* (2009). Blood pressure data were amplified using a pressure amplifier (Custom-built Pressure Amplifier; Massey University Mechanical Services) and digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd). The arterial blood pressure transducer (Spectramed Medical Products, Singapore) was re-calibrated against a mercury column (Baumanometer; WA Baum Co Inc, New York, USA) for each animal. Fifteen minutes were allowed for equilibration of general anaesthesia before collection of data commenced.

A 10-minute pre-treatment EEG recording was made, immediately followed by a non-penetrative captive-bolt stun (CASH Magnum Knocker Concussion Stunner; Acles and Shelvoke Ltd, Birmingham, England), using 4-grain cartridges (Cash Cartridges AS25, Black label, .25 cal; Acles and Shelvoke Ltd), delivered 30 mm above the intersection of lines drawn from the medial canthus of each eye to the rostral border of the contralateral ear, according to the manufacturer's instructions. Data were recorded for 5 minutes following stunning, after which all calves were subject to euthanasia with an I/V injection of sodium pentobarbitone (Pentobarb 500; National Veterinary Supplies Ltd, Auckland, NZ). After euthanasia, tissue damage caused by the non-penetrative captive bolt was visually assessed externally, and after midline longitudinal transections of the skulls of all calves. Brains were extracted from the skulls, fixed in 10% buffered formalin, and routinely processed for histological examination, as described previously (Gibson *et al.* 2009).

Analysis of EEG and ECG data

Analyses of EEG and arterial blood pressure were performed after completion of data collection. EEG traces were inspected visually for signs of over- and under-scale, out-of-range data and external noise. Traces containing significant artefact were excluded from further analysis. Fast Fourier Transformation was performed using purpose-written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North, NZ, 2002), as described previously by Gibson *et al.* (2007). Briefly, EEG data were multiplied with a Welch window, and Fast Fourier Transformation performed to generate power spectra with 1-Hz frequency bins, from sequential 1-second epochs. Subsequent analyses were performed using Microsoft Excel Mac 2004 (Microsoft Corporation, Redwood, USA).

EEG traces were inspected visually and classified into one of four categories, *viz* active EEG, transitional EEG, high-amplitude low-frequency EEG, and isoelectric EEG (Figure 1). Active EEG represented normal cerebrocortical activity so that in anaesthetised calves it had similar waveform and amplitude before non-penetrative captive-bolt stunning as afterwards. Transitional EEG was classified as having an amplitude of less than half of the pre-stunning EEG with a significant frequency change. High-amplitude low-frequency EEG was classified as a waveform with rhythmic activity of high amplitude and low frequency. Isoelectric EEG

was classified as a stable trace consisting of background noise with an amplitude of $<1\%$ of the normal pre-stunning EEG with little to no low-frequency component.

Heart rate was calculated from ECG data, using the rate meter function in Chart (ADInstruments Ltd).

Statistical analysis

EEG data were calculated and are displayed as percentage changes in Ptot from pre-treatment values. EEG data derived from 2 seconds before to 5 seconds after non-penetrative captive-bolt stunning were excluded from analysis to prevent contamination by movement artefact. All data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA) and Prism 4.0c (GraphPad Software Incorporated, San Diego CA, USA). The distribution of the data was tested for normality using the Anderson-Darling test (Anderson and Darling 1952). Analysis of differences between pre- and post-treatment values for Ptot was performed on consecutive non-overlapping 30-second epochs using a Mann-Whitney non-parametric test. Analysis of blood pressure and heart rate data was performed on individual time points taken every 15 seconds, using a Mann-Whitney non-parametric test. The level of statistical significance was taken to be $p < 0.05$.

Results

Immediately after non-penetrative captive-bolt stunning, respiration ceased in all calves. Some animals exhibited slow uncoordinated limb movements during the first 5 seconds. The frontal bone of all calves had a 30-mm diameter circular depressed fracture at the site of impact of the non-penetrative captive bolt, with adjacent subarachnoid haemorrhage and physical damage to brain tissue. Diffuse damage was also seen throughout the brain, manifested as traumatic axon injury, brain swelling and haemorrhage.

One calf was removed from Ptot analyses due to large periods of time when the EEG was out of range; the cause of this artefact was not investigated. Animals exhibited one of two different Ptot responses to non-penetrative captive-bolt stunning. Four calves had an immediate decrease in Ptot (Figure 2a). The decrease was significant ($p < 0.05$) only in the right hemisphere. The other five calves displayed a biphasic response, with two distinct periods

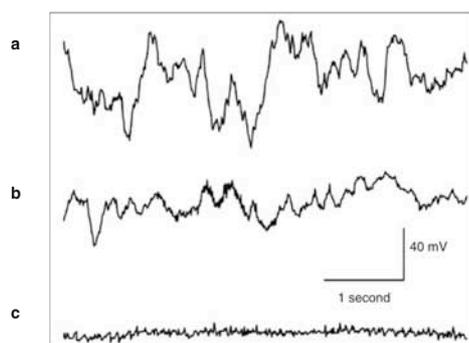


Figure 1. Electroencephalographic (EEG) trace from a halothane-anaesthetised calf showing the course of changes from (a) active EEG, to (b) transitional EEG, and to (c) an isoelectric EEG.

of Ptot activity (Figure 2b). In these animals, the initial decrease was followed by a small transient increase in Ptot, which peaked at approximately 80 seconds after non-penetrative captive-bolt stunning. At completion of the second decrease, the Ptot had a similar magnitude to that seen in animals not displaying biphasic responses.

The time to initial commencement of an isoelectric EEG following stunning was very variable (Figure 3). On average, an isoelectric EEG occurred 60 (SD 87) seconds after non-penetrative captive-bolt stunning. The mean time to the onset of transitional EEG following stunning was 8 (SD 14) seconds.

Changes in mean arterial blood pressure are illustrated in Figure 4. Prior to stunning, mean arterial blood pressure was 119 (SEM 7.6) mm Hg. Blood pressure fell to 76 (SEM 8.5) and 61 (SEM 8.5) mm Hg at 60 and 120 seconds, respectively, after stunning ($p < 0.05$). At the conclusion of the recording period (300 seconds after stunning), mean blood pressure was 54 (SEM 9.5) mm Hg.

ECG data from four calves were used in the calculation of heart rate (Table 1). Other ECG traces were rejected due to artefactual contamination. Following stunning, the mean heart rate decreased from 87 (SEM 1.0) beats per minute before to 82 (SEM 1.0) beats per minute at 30 seconds after stunning ($p < 0.05$). Heart rate remained significantly reduced until 285 seconds after stunning, after which there was some recovery. The changes in heart rate were statistically significant, but clinically small in magnitude.

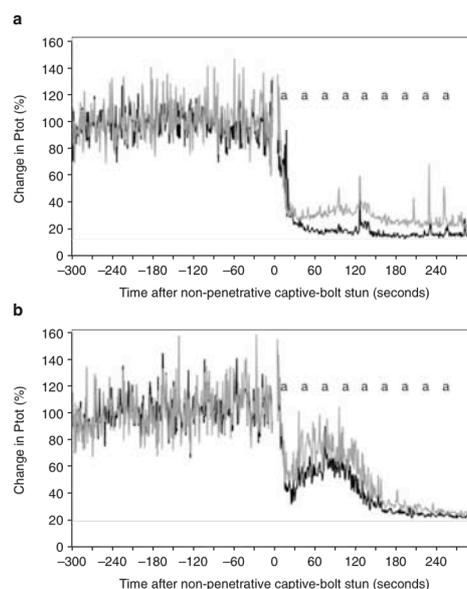


Figure 2. Percentage change in the mean total power (Ptot), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves before and after non-penetrative captive-bolt stunning. (a) Non-biphasic response ($n=4$), and (b) biphasic response ($n=5$). * Significant difference from pre-treatment values, right cerebral hemisphere ($p < 0.05$).

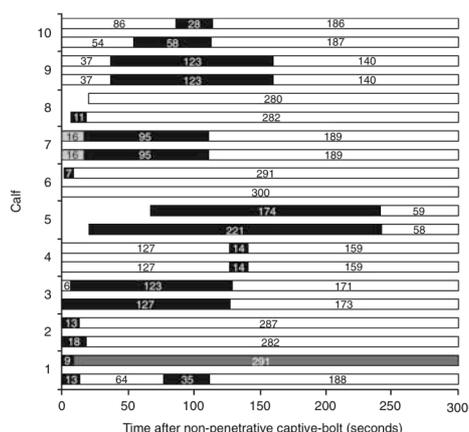


Figure 3. Characteristics of the raw electroencephalogram (EEG) of individual halothane-anaesthetised calves over time (seconds) following non-penetrative captive-bolt stunning. EEG traces were inspected visually and classified as either active (□), transitional (■), high-amplitude low-frequency (▨), or isoelectric (■). Time durations in seconds are displayed on the individual bars; clear gaps represent periods where the EEG recording was out of range.

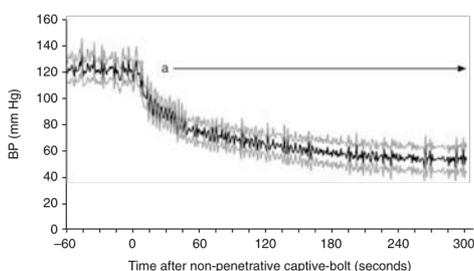


Figure 4. Mean \pm SEM femoral arterial blood pressure (BP; mm Hg) of halothane-anaesthetised calves before and after non-penetrative captive-bolt stunning. ^a Significant difference from pre-treatment values ($p < 0.05$).

Discussion

In the vast majority of calves in the study presented here, non-penetrative captive-bolt stunning virtually instantaneously altered cerebrocortical activity. The initial decrease in Ptot seen in all animals represents loss of cerebrocortical function required for the maintenance of sensibility (Blackmore and Delany 1988). This study adds to the existing evidence of the effectiveness of non-penetrative captive-bolt stunning in achieving rapid insensibility prior to slaughter (Blackmore 1979; Blackmore and Newhook 1982; Gregory and Wotton 1990).

Analysis of Ptot is valuable for investigating cortical function after non-penetrative captive-bolt stunning as decreases in Ptot can be used to demonstrate progression towards an isoelectric EEG. Ptot represents the total area under the power spectrum curve (Murrell and Johnson 2006) so changes are related to changes in amplitude of the raw EEG.

Table 1. Mean (\pm SEM) heart rate (beats per minute; bpm) of halothane-anaesthetised calves at individual time points after non-penetrative captive-bolt stunning.

Time after non-penetrative captive-bolt stun (seconds)	Heart rate (\pm SEM) (bpm)	P-value ^a
-15 ^b	87.3 \pm 1.02	na
0 ^c	na	na
15	82.8 \pm 0.66	0.016
30	81.6 \pm 1.20	0.016
45	99.8 \pm 19.43	0.270
60	80.8 \pm 1.11	0.012
75	80.5 \pm 1.02	0.012
90	81.3 \pm 1.50	0.016
105	80.8 \pm 1.68	0.016
120	81.5 \pm 1.96	0.037
135	81.3 \pm 1.85	0.022
150	81.5 \pm 1.83	0.022
165	81.5 \pm 1.80	0.028
180	80.8 \pm 1.77	0.016
300 (5 minutes)	85.5 \pm 2.25	0.602

^a Significance of difference from pre-treatment values

^b Pre-treatment value

^c Non-penetrative captive-bolt stun
na = not applicable

The pattern of changes of EEG activity following non-penetrative captive-bolt stunning was not uniform between animals. Some animals displayed a small transient increase in Ptot after the initial decrease following stunning. This period of activity correlates with the transitional EEG seen on visual inspection of the EEG. In the current study, non-penetrative captive-bolt stunning in one animal was associated with a period of high-amplitude low-frequency EEG activity similar to the low-frequency delta and theta waveforms seen following successful penetrative captive-bolt stunning of conscious calves (Lambooy and Spanjaard 1981). There were also variation between animals in the time point at which the EEG became isoelectric. The onset of isoelectric EEG does not correlate with the early part of the decrease in Ptot following non-penetrative captive-bolt stunning, but the onset of the transitional period of EEG does. This period of high-amplitude low-frequency EEG is considered to be associated with insensibility because of the absence of palpebral and corneal reflexes (Blackmore and Delany 1988). Moreover, rhythmic respiration ceased immediately in all calves in the current study, further indicating effective stunning (Finnie 1997).

Non-penetrative captive-bolt stunning resulted in both focal and diffuse brain damage in the calves in the current study. Finnie (1997), using immunostaining for amyloid precursor protein, demonstrated that non-penetrative captive-bolt stunning in sheep caused traumatic axon injury throughout the major regions of the brain. This was similar in nature to that observed in humans following some forms of traumatic brain injury. In humans, the most common forces involved in concussive traumatic brain injury are acceleration/deceleration of the freely moving head, resulting in rotational damage to the brain. However, in cattle, the relatively large and immobile head supported by significant musculature reduces acceleration and deceleration (Finnie 1995, 1997). In the current study, calves were stunned while in dorsal recumbency with the head attached to an immobilising frame. This would also have reduced acceleration/deceleration in a similar

manner. The striking of the mushroom-shaped bolt against the cranium imparts kinetic energy to the head primarily as contact force (Halliday 1999), with minimal acceleration/deceleration forces. Contact force occurs when the impact energy is imparted without movement of the head. Non-penetrative captive-bolt stunning may have potential as a model of these functional and histological effects of concussive contact-force traumatic brain injury in large mammals.

The probable link between bovine spongiform encephalopathy and Creutzfeldt-Jakob disease (Bruce *et al.* 1997; Hill *et al.* 1997) and the potential for penetrative captive-bolt stunning to spread brain tissue fragments to distal organs (Anil *et al.* 1999; Daly *et al.* 2002; Coore *et al.* 2005) has generated increased interest in non-penetrative captive-bolt stunning as a method of rendering animals insensible prior to slaughter. There are three commonly used methods of mechanical stunning, *viz* non-penetrative captive bolt, penetrative captive bolt, and free bullet. Both the penetrative captive-bolt and free-bullet methods involve penetration of the cranium. The penetrative captive-bolt stunner is positioned in the middle of the animal's forehead and drives a steel bolt through the skull into the brain (Grandin 1980). Unconsciousness is caused by a combination of direct damage to the brain and the energy delivered to the animal's head (Gregory 1991). Penetrative captive-bolt stunning is widely used throughout the world but, for the reasons outlined above, it is declining in popularity.

The current study demonstrated the effectiveness of non-penetrative captive-bolt stunning in disrupting cerebrocortical function. However, the physical damage to the cranium and underlying brain structures observed here presumably accounts for the fragments of brain tissue found in jugular blood after both non-penetrative and penetrative captive-bolt stunning (Coore *et al.* 2005). This suggests that while non-penetrative captive-bolt stunning may be effective in inducing insensibility, it may not reduce the risk of haematogenous spread of fragments of central nervous system tissue.

Arterial blood pressure decreased following stunning and remained reduced during the recording period. This is in contrast to arterial blood pressure recorded from conscious sheep where a non-penetrative stun caused a 60% increase in both systolic and diastolic pressures (Blackmore and Newhook 1982; Blackmore and Delany 1988). The increase reported in sheep was maximal 20 seconds after stunning, after which the animals were slaughtered by ventral-neck incision. If the time interval between stunning and neck incision had been longer, an overall decrease in blood pressure similar to that reported here may have occurred. Initial bradycardia seen in the current study could have been responsible for the decrease in blood pressure, but this is unlikely as the changes in heart rate, although statistically significant, were biologically negligible and would have been insufficient to markedly affect cardiac output and decrease arterial blood pressure. Alternatively, blood pressure may have been reduced by some actions of halothane anaesthesia, but this also seems unlikely as anaesthetic-induced changes in blood pressure are dose-dependent (Marshall and Wollman 1980), and end-tidal halothane was kept at a constant stable tension.

It is possible that halothane anaesthesia modulated the effects of non-penetrative captive-bolt stunning on the EEG in the current study. Halothane has been demonstrated to cause a progressive slowing of EEG frequencies with increasing tensions (Hansen and Claassen 2005), but as noted, end-tidal halothane tension

was kept stable throughout the recording period. Alterations in functional activity caused by stunning were of such magnitude and occurred so rapidly that it seems unlikely that alterations in anaesthesia could account for these results.

In conclusion, the current study examined the effects of non-penetrative captive-bolt stunning on the EEG in more detail than has been previously reported. These findings confirm the effectiveness of this method in rendering cattle insensible prior to slaughter, and extend our understanding of the effects of this technique.

Acknowledgements

The authors would like to thank Corrin Hulls, Leanne McCracken, and the members of the Large Animal Teaching Unit and the Small Animal Production Unit, Massey University, for assistance during the experiment. This study was jointly funded by the Department for Environment, Food and Rural Affairs of the United Kingdom and the Ministry of Agriculture and Forestry of New Zealand. TJ Gibson was the recipient of a C Alma Baker Postgraduate Scholarship.

References

- Anderson TW, Darling DA. Asymptotic theory of certain "goodness-of-fit" criteria based on stochastic processes. *Annals of Mathematical Statistics* 23, 193–21, 1952
- Anil MH, Love S, Williams S, Shand A, McKinstry JL, Helps CR, Waterman-Pearson A, Seghatchian J, Harbour DA. Potential contamination of beef carcasses with brain tissue at slaughter. *Veterinary Record* 145, 460–2, 1999
- *Anonymous. *Code of Recommendations and Minimum Standards for the Welfare of Animals at the Time of Slaughter at Licensed and Approved Premises. Code of Animal Welfare No. 10.* Ministry of Agriculture and Fisheries, Wellington, NZ, 1994
- *Anonymous. *Animal Welfare (Commercial Slaughter) Code of Welfare 2006 Public Draft.* National Animal Welfare Advisory Committee, Wellington, NZ, 2006
- Blackmore DK. Non-penetrative percussion stunning of sheep and calves. *Veterinary Record* 105, 372–5, 1979
- *Blackmore DK, Delany MW. *Slaughter of Stock. A practical Review and Guide.* Veterinary Continuing Education Massey University, Palmerston North, NZ, 1988
- Blackmore DK, Newhook JC. Electroencephalographic studies of stunning and slaughter of sheep and calves. 3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Science* 7, 19–28, 1982
- Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCordle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ. Transmission to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 389, 498–501, 1997
- Cooley JW, Tukey JW. An algorithm for the machine calculation of complex Fourier series. *Mathematics of Computation* 19, 297–301, 1965
- Coore RR, Love S, McKinstry JL, Weaver HR, Philips A, Hillman T, Hiles M, Helps CR, Anil MH. Brain tissue fragments in jugular vein blood of cattle stunned by use of penetrating or nonpenetrating captive bolt guns. *Journal of Food Protection* 68, 882–4, 2005
- Daly CC, Gregory NG, Wotton SB, Whittington PE. Concussive methods of pre-slaughter stunning in sheep – assessment of brain-function using cortical-evoked responses. *Research in Veterinary Science* 41, 349–52, 1986

*Non-peer-reviewed

- Daly DJ, Prendergast DM, Sheridan JJ, Blair IS, McDowell A. Use of a marker organism to model the spread of central nervous system tissues in cattle and the abattoir environment during commercial stunning and carcass dressing. *Applied and Environmental Microbiology* 68, 791–8, 2002
- Finnie JW. Neuropathological changes produced by non-penetrating percussive captive bolt stunning of cattle. *New Zealand Veterinary Journal* 43, 183–5, 1995
- Finnie JW. Traumatic head injury in ruminant livestock. *Australian Veterinary Journal* 75, 204–8, 1997
- Fricker CH, Riek W. Die betäubung von rindern vor dem schlachten mit hilfe des bolzenschuß-apparates. [Using a captive bolt pistol to stun cattle before slaughter.] *Fleischwirtschaft* 61, 124–7, 1981
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55, 152–7, 2007
- Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, Johnstone AC, Mellor DJ. Electroencephalographic responses of halothane-anesthetised calves to slaughter by ventral-neck incision without prior stunning. *New Zealand Veterinary Journal* 57, 77–83, 2009
- Grandin T. Mechanical, electrical and anesthetic stunning methods for livestock. *International Journal for the Study of Animal Problems* 1, 242–63, 1980
- Gregory NG. Humane slaughter. *Outlook on Agriculture* 20, 95–101, 1991
- Gregory NG, Wotton SB. Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *Veterinary Record* 126, 570–2, 1990
- Halliday AL. Pathophysiology. In: Marion DW (ed). *Traumatic Brain Injury*. Pp 29–38. Thieme Medical Publishers, New York, USA, 1999
- Hansen HC, Claassen J. EEG and evoked potentials in neuroanesthesia, intraoperative neurological monitoring, and neurointensive care. In: Niedermeyer E, Silva FLD (eds). *Electroencephalography, Basic Principles, Clinical Applications, and Related Fields*. Pp 1137–64. Lippincott Williams & Wilkins, Philadelphia, USA, 2005
- Hill AN, Desbruslais M, Joiner S, Side KCL, Gowland I, Collinge J, Doey LJ, Lantos P. The same prion strain causes vCJD and BSE. *Nature* 389, 448–50, 1997
- Lambooy E, Spanjaard W. Effect of the shooting position on the stunning of calves by captive bolt. *Veterinary Record* 109, 359–61, 1981
- Marshall BE, Wollman H. General anesthetics. In: Gilman AG, Goodman LS, Gilman A (eds). *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. Pp 277–8. MacMillan Publishing, New York, USA, 1980
- Mellor DJ, Littin KE. Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13 (Supplement), 127–32, 2004
- Murrell JC, Johnson CB. Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics* 29, 325–35, 2006
- Shaw NA. Neurophysiology of concussion. *Progress in Neurobiology* 67, 281–344, 2002

Submitted 13 February 2008

Accepted for publication 17 February 2009

*Non-peer-reviewed

Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ. Amelioration of electroencephalographic responses to slaughter by non-penetrative captive-bolt stunning after ventral-neck incision in halothane-anaesthetised calves. *New Zealand Veterinary Journal* 57, 96-101, 2009

New Zealand Veterinary Journal 57(2), 96-101, 2009

96

Scientific Article

Amelioration of electroencephalographic responses to slaughter by non-penetrative captive-bolt stunning after ventral-neck incision in halothane-anaesthetised calves

TJ Gibson^{*†‡}, CB Johnson^{*‡§}, JC Murrell^{‡#}, SL Mitchinson^{*‡}, KJ Stafford^{*‡} and DJ Mellor^{*‡¥}

Abstract

AIM: To investigate the ability of non-penetrative captive-bolt stunning delivered after ventral-neck incision to ameliorate responses to noxious stimulation associated with slaughter by ventral-neck incision in halothane-anaesthetised calves.

METHODS: Seven calves were minimally anaesthetised with halothane, using an established anaesthesia protocol. Electroencephalographic (EEG) indices of cerebrocortical activity were measured in response to ventral-neck incision followed 5 seconds later by non-penetrative captive-bolt stunning. Changes in heart rate and arterial blood pressure were measured and instances of occlusion of the carotid arteries were also noted.

RESULTS: After ventral-neck incision there were periods of an active EEG in some calves, which ceased after non-penetrative captive-bolt stunning in most of the animals. Two calves had periods of active EEG following stunning. Arterial blood pressure decreased significantly after neck incision in all calves, including three with occlusion of the carotid arteries.

CONCLUSIONS: Non-penetrative captive-bolt stunning after ventral-neck incision resulted in the cessation of functional cortical activity in the majority of calves. This procedure prevented the development of cerebrocortical responses to ventral-neck incision, demonstrated elsewhere, which would be painful in conscious animals subjected to this procedure. In addition, instances of carotid arterial occlusion had no significant effect on the decrease in arterial blood pressure.

KEY WORDS: *Calves, compressed spectral array, concussive stunning, electroencephalogram, minimal anaesthesia, non-penetrative stunning, slaughter*

Introduction

In New Zealand, cattle are stunned prior to slaughter using penetrative captive-bolt, non-penetrative captive bolt, or electrical

methods. The stun is intended to render the animal completely insensible to any pain and distress that may be caused by slaughter by ventral-neck incision. Using EEG indices previously shown to be associated with noxious stimulation in farm animals (Murrell *et al.* 2003, 2005; Johnson *et al.* 2005ab; Gibson *et al.* 2007) and pain in man (Chen *et al.* 1989), we demonstrated that ventral-neck incision without prior stunning caused noxious sensory input in halothane-anaesthetised calves (Gibson *et al.* 2009a). Additionally, we demonstrated that those responses were due primarily to transection of neck tissues as opposed to loss of blood flow through the brain (Gibson *et al.* 2009b).

The time to undoubted insensibility following ventral-neck incision with or without stunning has been a topic of much detailed research in a variety of species, as reviewed previously (Gibson *et al.* 2009a). However, the window before the onset of undoubted insensibility has been assessed and most authorities consider this window to be between approximately 5 and 60 seconds, or more, in duration in cattle (Mellor and Littin 2004). During this period between neck incision and insensibility the animal could experience pain and/or distress.

A recent study examining slaughter by ventral-neck incision (Gibson *et al.* 2009a) supports the hypothesis that this is a noxious process and that animals undergoing slaughter without stunning would experience pain and/or distress prior to the onset of insensibility. Cerebrocortical activity after non-penetrative captive-bolt stunning has been shown to render cattle insensible (Blackmore 1979; Blackmore and Newhook 1982; Gregory and Wotton 1990). Using quantitative EEG parameters, we have confirmed that such stunning abolishes cerebrocortical activity in cattle (Gibson *et al.* 2009c).

The New Zealand Public Draft Commercial Slaughter Code (Anonymous 2006) includes exemptions which permit a limited number of species of livestock to be slaughtered without prior stunning in some circumstances. This allows for the needs of specific communities where the meat is to be consumed in New Zealand only. This exemption is conditional on a number of specific requirements, including Minimum Standard 22(b) of the Draft Commercial Slaughter Code that "all cattle slaughtered without prior stunning must be stunned no more than 5 seconds following cutting of the throat". During the 5 seconds prior to stunning, the animal could experience both pain and distress, as suggested by results reported previously (Gibson *et al.* 2009ab). Stunning within 5 seconds would be expected to render the animals insensible to further pain and distress (Gibson *et al.* 2009c), but as yet

* Animal Welfare Science and Bioethics Centre, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

† Current address: Veterinary Clinical Sciences, Royal Veterinary College, North Mymms, Herts AL9 7TA, United Kingdom.

‡ Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

Current address: Department of Clinical Veterinary Science, Division of Companion Animals, University of Bristol, Bristol BS8 1TH, United Kingdom.

¥ Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

§ Author for correspondence. Email: c.b.johnson@massey.ac.nz

ECG Electrocardiogram(s)/electrocardiographic
EEG Electroencephalogram(s)/electroencephalographic
Ptot Total power of the electroencephalogram

there have been no direct studies of stunning after ventral-neck incision to confirm this, and the recommendation is based on a 'very high likelihood of benefit' rationale.

The aim of this study was to examine the cerebrocortical effects of slaughter by ventral-neck incision followed 5 seconds later by non-penetrative captive-bolt stunning, using techniques described previously (Gibson *et al.* 2007, 2009abc).

Materials and methods

Animals

Seven Angus calves (steers and bulls) weighing 134–207 kg were sourced from a commercial stock agent and kept in accordance with normal farming practices. Prior to the study, animals were penned for 24 hours with free access to water but not food. This study was approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Anaesthesia

Anaesthesia was induced using a mixture of 3.9 (SD 0.5) mg/ml ketamine (Parnell Laboratories, Auckland, NZ) and 8.1 (SD 1.4) mg/kg propofol (DBL; Mayne Pharma Pty Ltd, Melbourne, Australia) administered to effect by rapid injection into a jugular vein. Following intubation with a 16-mm cuffed endotracheal tube (Cook Veterinary Products, Brisbane, Australia), anaesthesia was maintained using inhalation of halothane (Halothane-Vet; Merial NZ Ltd, Manukau City, NZ) in oxygen (BOC, Palmerston North, NZ) delivered via a precision vaporiser (Fluothane; MedSource Ltd, Ashburton, NZ) and a circle breathing system (VMS Anaesthesia Machine; Matrix Medical Inc, New York, USA). End-tidal halothane tension was maintained at 0.9% in individual animals, using an anaesthetic agent monitor (Hewlett Packard M1025B; Hewlett Packard, Hamburg, Germany).

EEG and electrocardiographic (ECG) recording

Placement of electrodes and recording of EEG and ECG data were as described previously by Gibson *et al.* (2009a). EEG data were recorded from the right and left cerebral hemispheres. The EEG and ECG were amplified using isolated differential signal amplifiers (Iso-Dam Isolated Physiological Signal Amplifiers; World Precision Instruments, Sarasota FL, USA). The EEG was recorded with a gain of 1,000 and a pass-band of 0.1–500 Hz. The ECG was recorded with a gain of 1,000 and a pass-band of 10–500 Hz. Both EEG and ECG data were digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia) and analysed off-line after completion of the experiment.

Experimental procedure

Once anaesthetised, calves were placed in dorsal recumbency on a specially designed platform (Massey University Mechanical Services; Massey University, Palmerston North, NZ) with the head securely held in position on a purpose-designed head frame (Massey University Mechanical Services), which allowed unobstructed access to the neck and stunning site. Arterial blood pressure was recorded from a femoral artery, as described by Gibson *et al.* (2009a). Fifteen minutes were allowed for equilibration of general anaesthesia before collection of data commenced.

A 10-minute pre-treatment EEG recording was made, immediately followed by ventral-neck incision (as per Gibson *et al.* 2009a). This was followed 5 seconds later by non-penetrative captive-bolt stunning, in accordance with Minimum Standard No.

22b of the Public Draft Commercial Slaughter Code of Welfare (Anonymous 2006). Data were recorded for 5 minutes following slaughter, after which the wound was inspected for complete severance of major blood vessels and for any significant signs of occlusion of carotid arteries.

Analysis of EEG and ECG data

Analyses of EEG and arterial blood pressure were performed after completion of data collection. EEG traces were inspected visually for signs of over- and under-scale and external noise. Traces containing significant artefact were excluded from further analysis. Fast Fourier Transformation was performed using purpose-written software (Spectral Analyser; CB Johnson, Massey University, Palmerston North, NZ, 2002), as described previously (Gibson *et al.* 2007). All subsequent analysis was performed using Microsoft Excel Mac 2004 (Microsoft Corporation, Redwood, USA).

EEG were inspected visually and classified into one of four categories, *viz* active EEG, transitional EEG, high-amplitude low-frequency EEG, and isoelectric EEG. Active EEG represented normal cerebrocortical activity so that in anaesthetised calves it had similar waveform and amplitude before non-penetrative captive-bolt stunning as afterwards. Transitional EEG was classified as having an amplitude of less than half of the pre-stunning EEG with a significant frequency change. High-amplitude low-frequency EEG was classified as a waveform with rhythmic activity of high amplitude and low frequency. Isoelectric EEG was classified as a stable trace consisting of background noise with an amplitude of <1% of the normal pre-stunning EEG with little to no low-frequency component.

Heart rate was calculated from ECG data, using the rate meter function in Chart (ADInstruments Ltd).

Statistical analysis

EEG data were calculated and are displayed either as percentage changes in the total power of the EEG (P_{tot}) from pre-treatment values or as compressed spectral arrays from individual animals. EEG data derived from 2 seconds before ventral-neck incision to 5 seconds after non-penetrative captive-bolt stunning were excluded from analysis to prevent contamination by movement artefact. All data were analysed using Minitab 14.2 (Minitab Incorporated, State College PA, USA) and Prism 4.0c (GraphPad Software Incorporated, San Diego CA, USA). The distribution of the data was tested for normality using the Anderson-Darling test (Anderson and Darling 1952). Analysis of differences between pre- and post-treatment values for P_{tot} was performed on consecutive non-overlapping 30-second epochs, using a Mann-Whitney non-parametric test. Analysis of the correlation between blood pressure and P_{tot} was performed using a two-tailed Spearman's rank coefficient test for non-parametric data.

Analysis of blood pressure and heart rate data was performed on individual time points taken every 15 seconds, using a Mann-Whitney non-parametric test. The level of statistical significance was taken to be $p < 0.05$.

Results

The frontal bone of all calves had a 30-mm diameter circular depressed fracture at the site of impact of the non-penetrative captive bolt. EEG data from the left cerebral hemisphere of two calves were rejected from power spectrum analysis due to large periods

of external noise contaminating pre- and post-treatment values. Three calves had signs of significant occlusion at the cardiac ends of the severed carotid arteries at the end of data collection. Calves with occlusion of carotid arteries showed no biological difference in cerebrocortical activity, blood pressure or heart rate from the other calves in the study. Occlusion was first observed 2 minutes following ventral-neck incision. Data from the three occluded animals and the remaining calves were pooled and used in analysis of the group.

After ventral-neck incision and non-penetrative captive-bolt stunning, Ptot increased from pre-treatment values, and then gradually decreased to below pre-treatment values (Figure 1). The decrease from pre-treatment values was significant by 30 seconds after neck incision ($p < 0.05$). By 90 seconds after incision, mean Ptot had decreased to 32 (SD 14)% of pre-treatment values. Variability in mean Ptot in both EEG channels increased 160 seconds after incision, with large bursts of activity on the Ptot time series.

An example of a typical compressed spectral array from an individual calf before and after ventral-neck incision and non-penetrative captive-bolt stunning is illustrated in Figure 2. After incision and stunning there were major decreases in power in all frequency bands compared with pre-treatment values. The com-

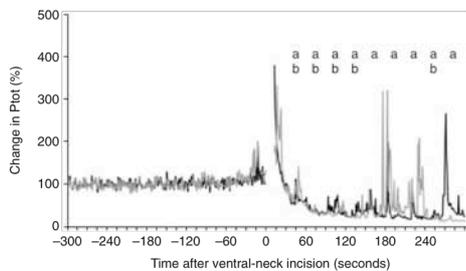


Figure 1. Percentage change in the mean total power (Ptot), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves before and after ventral-neck incision followed 5 seconds later by non-penetrative captive-bolt stunning. ^a Significant difference from pre-treatment values, right cerebral hemisphere ($p < 0.05$). ^b Significant difference from pre-treatment values, left cerebral hemisphere ($p < 0.05$).

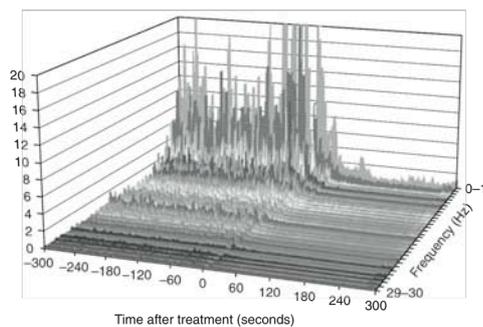


Figure 2. A typical example of a compressed spectral array of the electroencephalogram from an individual halothane-anaesthetised calf before and after ventral-neck incision followed 5 seconds later by non-penetrative captive-bolt stunning.

pressed spectral arrays of three animals displayed large bursts of increased power approximately 160 seconds after incision in the low-end frequency (0–4 Hz), activity which correlated with the bursts seen in Ptot during the same time period (Figure 1).

An example of a 25-second section of EEG with corresponding EEG power spectrum curves (2-second epochs) from an individual calf before and after ventral-neck incision and non-penetrative captive bolt-stunning is illustrated in Figure 3, demonstrating pre-treatment EEG activity, ventral-neck incision artefact, and post-treatment transitional EEG. The first arrow shows the time of ventral-neck incision and the second the time of the non-penetrative captive-bolt stunning 5 seconds after the incision. After the period of incision-related artefact, the EEG had similar amplitude to the pre-treatment period but contained a greater proportion of higher frequencies. Non-penetrative captive-bolt stunning resulted in a period of out-of-range data, followed by an alteration in morphology to transitional EEG. Power across all frequencies was increased during ventral-neck incision artefact, compared with that before treatment or that of the post-treatment transitional EEG. EEG power, principally of the lower frequencies (0–2 Hz), was significantly decreased in the post-treatment transitional EEG compared with pre-treatment recordings.

Figure 4 details the characteristics and time of onset of different periods of cortical activity based on visual inspection of the EEG waveform. Following neck incision there were periods of active EEG in some calves. After stunning the waveform was out of range for a brief period in all calves. There was a considerable variation in type of cortical activity following incision and stunning. In two calves, there were periods of active EEG after stunning. In the remaining animals, following the out-of-range data after the stun there was depressed cortical activity in the form of transitional EEG, high-amplitude low-frequency or isoelectric EEG.

Femoral arterial blood pressure significantly decreased after ventral neck-incision and non-penetrative captive-bolt stunning ($p < 0.05$) (Figure 5). From mean pre-treatment values of 136 (SEM 8.3) mm Hg, arterial blood pressure was reduced to 29 (SEM 3.8) mm Hg 30 seconds after incision. Sixty, 120 and 180 seconds after the incision mean arterial blood pressure was 16 (SEM 1.9), 5 (SEM 1.5), and 0 (SEM 1.1) mm Hg, respectively. The decrease in Ptot seen in response to ventral-neck incision and non-penetrative captive-bolt stunning was significantly correlated with the fall in blood pressure ($r = 0.5202$ (95% CI = 0.4276–0.6019), $p < 0.0001$, right-sided; $r = 0.5641$ (95% CI = 0.4771–0.6401), $p < 0.0001$, left-sided). After ventral-neck incision and non-penetrative captive-bolt stunning there were no significant differences in heart rate from pre-treatment values in the initial 105 seconds after incision (Table 1). Thereafter, heart rate significantly decreased from pre-treatment values and continued to decrease throughout the remainder of the recording period ($p < 0.05$).

Discussion

The application of non-penetrative captive-bolt stunning 5 seconds after ventral-neck incision produced EEG waveforms in the majority of animals, that were incompatible with continued sensibility. This change in the EEG was evident immediately after the short period of artefact, no later than 10 seconds after the stun. The use of a non-penetrative captive-bolt stun 5 seconds after the ventral-neck incision abolished any subsequent cerebral cortical

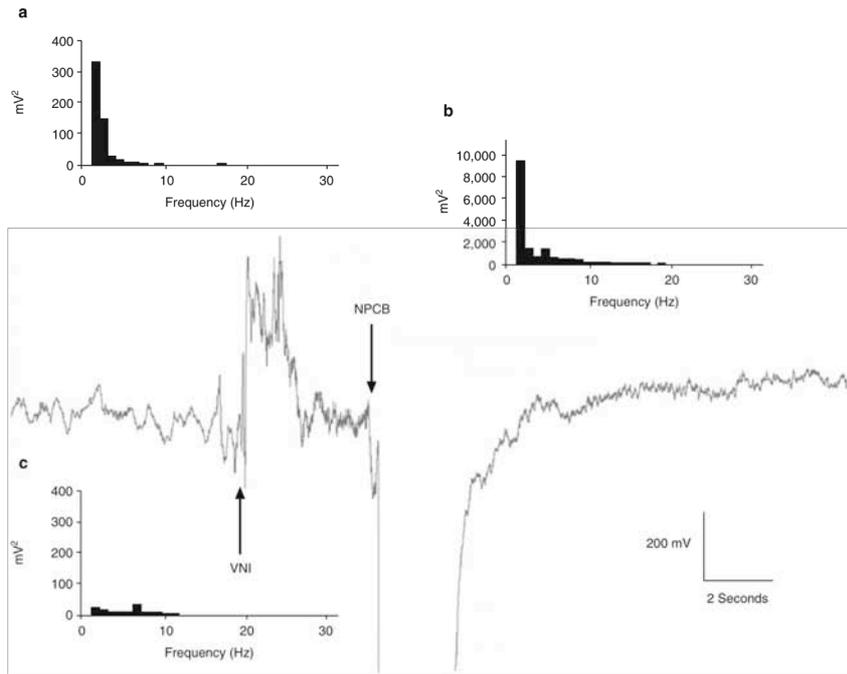


Figure 3. An example of the raw electroencephalogram (EEG) of an individual halothane-anaesthetised calf before, during, and after ventral-neck incision (VNI) and non-penetrative captive-bolt stunning (NPCB), with individual power spectra for 2-second epochs during (a) pre-treatment EEG, (b) VNI artefact, and (c) transitional EEG after NPCB. Arrows represent the times of VNI and NPCB.

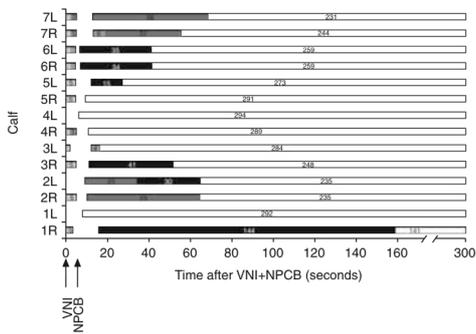


Figure 4. Characteristics of the raw electroencephalogram (EEG) of individual halothane-anaesthetised calves over time (seconds) after ventral-neck incision (VNI) followed 5 seconds later by non-penetrative captive-bolt stunning (NPCB). EEG traces were inspected visually and classified as either VNI artefact (□), active EEG (■), transitional EEG (▒), high-amplitude low-frequency EEG (░), or isoelectric EEG (—). Time durations in seconds are displayed on the individual bars; clear gaps represent periods where the EEG recording was out of range. Arrows represent the times of VNI and NPCB. L = left cerebral hemisphere; R = right cerebral hemisphere.

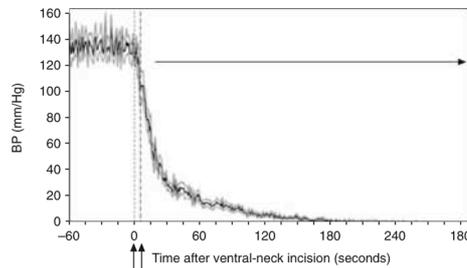


Figure 5. Mean \pm SEM femoral arterial blood pressure (BP; mm Hg) of halothane-anaesthetised calves before and after ventral-neck incision followed 5 seconds later by non-penetrative captive-bolt stunning. ^a Significant difference from pre-treatment values ($p < 0.05$). Short dashes represent the time of the ventral-neck incision and long dashes represent the time of non-penetrative captive-bolt stunning.

responses indicative of noxious sensory input such as those previously reported after slaughter by ventral-neck incision without stunning (Gibson *et al.* 2009a).

Following slaughter by ventral-neck incision without stunning there is a period during which the animal is likely to be sensible (Levinger 1961; Newhook and Blackmore 1982ab; Gregory and Wotton 1984) and would therefore experience pain (Gibson *et*

Table 1. Mean (\pm SEM) heart rate (beats per minute; bpm) of halothane-anaesthetised calves at individual time points after ventral-neck incision followed 5 seconds later by non-penetrative captive-bolt stunning.

Time after ventral-neck incision (seconds)	Heart rate (\pm SEM) (bpm)	P-value ^a
-15 ^b	100.4 \pm 5.91	na
0 ^c	100.0 \pm 5.30	0.916
15	89.4 \pm 5.52	0.205
30	85.8 \pm 6.04	0.091
45	84.4 \pm 5.43	0.090
60	82.8 \pm 4.85	0.091
75	81.6 \pm 5.46	0.091
90	79.6 \pm 5.65	0.071
105	78.8 \pm 5.69	0.071
120	76.8 \pm 5.54	0.035
135	71.8 \pm 5.64	0.020
150	65.4 \pm 6.76	0.020
165	60.6 \pm 7.85	0.020
180	59.6 \pm 7.88	0.020
300 (5 minutes)	54.8 \pm 5.43	0.012

^a Significance of difference from pre-treatment values

^b Pre-treatment value

^c Ventral-neck incision followed 5 seconds later by non-penetrative captive-bolt stunning

na = not applicable

al. 2009a) and distress until insensibility supervenes. The use of non-penetrative captive-bolt stunning has been demonstrated to abolish cerebrocortical function (Gibson *et al.* 2009c) and result in immediate insensibility in calves (Blackmore 1979; Blackmore and Newhook 1982; Finnie 1995). After non-penetrative captive-bolt stunning, which followed ventral-neck incision, cortical function was immediately altered in all but two of the animals in the current study, from active EEG to transitional, high-amplitude low-frequency or isoelectric EEG. These EEG states are incompatible with sensibility (Lambooy and Spanjaard 1981).

Based on these results it appears that non-penetrative captive-bolt stunning can produce insensibility in calves after ventral-neck incision. This work adds to the growing body of evidence of the effectiveness of this method in rendering animals insensible prior to slaughter and validates the use of such stunning following ventral-neck incision to render calves insensible to the noxiousness of the incision.

In the current study, two calves had a period of unilaterally active EEG after ventral-neck incision and non-penetrative captive-bolt stunning, lasting for 4 and 6 seconds after the stun. The period of active EEG was only observed within one hemisphere of each animal. The other hemisphere showed either an isoelectric or high-amplitude low-frequency EEG. Incorrect positioning of the stunner on the animal's head, a deflected shot, or incorrect function of the stunner itself may have been responsible for this period of active EEG.

The initial transient increase in P_{tot} following ventral-neck incision and non-penetrative captive-bolt stunning mirrored the profile of the response to ventral-neck incision without prior stunning (Gibson *et al.* 2009a). This response was also seen to a lower magnitude following sham incision (Gibson *et al.* 2009a). The similarity between these results suggests that movement artefact

during and after ventral-neck incision was primarily responsible for the transient increase in P_{tot}. Non-penetrative captive-bolt stunning alone did not result in a significant increase in P_{tot}; rather, it resulted in an immediate decrease in P_{tot}, which represented a loss of functional cerebrocortical activity (Gibson *et al.* 2009c). In the current study, P_{tot} could not be used to assess functional cortical activity in the initial period after ventral-neck incision as the movement response to neck incision masked any such changes.

Occlusion of the carotid arteries occurred in 3/7 animals in the current study. Occlusion of the severed carotid arteries has the potential to impair bleed-out and provide conditions for the resumption or maintenance of cortical function necessary for sensibility. Cattle are principally at risk because of the ability of the vertebral arteries to supply blood to the entire bovine brain through the occipito-vertebral anastomosis. The prevalence of carotid arterial occlusion in 576 cattle slaughtered at abattoirs in the United Kingdom was found to be 16% and 25% for adult cattle and bobby calves, respectively (Gregory *et al.* 2006). In the current study, however, the blood-pressure profile of calves with occluded carotid arteries was the same as that of non-occluded animals. Furthermore, there were no visually evident or statistically significant differences between EEG responses of calves with occluded and non-occluded carotid arteries. This suggests that ventral-neck incision and non-penetrative captive-bolt stunning resulted in either non-reversible stun-induced insensibility or that the early blood loss was sufficient to cause cerebrocortical anoxia prior to the onset of occlusion of the carotid arteries.

Changes in mean arterial blood pressure after ventral-neck incision and non-penetrative captive-bolt stunning were similar to those seen following ventral-neck incision alone (Gibson *et al.* 2009a), suggesting that there is no basis for the claim that stunning impedes blood loss (Levinger 1995). This supports work in cattle which has shown that Halal-slaughtered cattle (un-stunned) showed no difference in the rate of blood loss when compared with cattle stunned percussively prior to slaughter (Anil *et al.* 2006).

In conclusion, non-penetrative captive-bolt stunning 5 seconds after ventral-neck incision was effective in abolishing the cerebrocortical responses that would be seen subsequently if the stun were not performed (Gibson *et al.* 2009a). Based on these and previous results (Gibson *et al.* 2009a), it is probable that during the 5 seconds before non-penetrative captive-bolt stunning calves would be both sensible and responsive to noxious stimulation, but non-penetrative captive-bolt stunning would render these animals insensible for the remainder of the period of potential sensibility following ventral-neck incision.

Acknowledgements

The authors would like to thank Corrin Hulls, Leanne McCracken, Neil Ward, Mike Hogan, and members of the Large Animal Teaching Unit and the Small Animal Production Unit, Massey University, for assistance during the experiment. This study was jointly funded by the Department for Environment, Food and Rural Affairs of the United Kingdom and the Ministry of Agriculture and Forestry of New Zealand. TJ Gibson was the recipient of a C Alma Baker Postgraduate Scholarship.

References

- Anderson TW, Darling DA.** Asymptotic theory of certain "goodness-of-fit" criteria based on stochastic processes. *Annals of Mathematical Statistics* 23, 193–21, 1952
- Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Weaver HR, Erdogan O, Hughes S, Manson C.** Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: exsanguination and quality parameters. *Animal Welfare* 15, 325–30, 2006
- ***Anonymous.** *Animal Welfare (Commercial Slaughter) Code of Welfare 2006 Public Draft*. National Animal Welfare Advisory Committee, Wellington, NZ, 2006
- Blackmore DK.** Non-penetrative percussion stunning of sheep and calves. *Veterinary Record* 105, 372–5, 1979
- Blackmore DK, Newhook JC.** Electroencephalographic studies of stunning and slaughter of sheep and calves. 3. The duration of insensibility induced by electrical stunning in sheep and calves. *Meat Science* 7, 19–28, 1982
- Chen ACN, Dworkin SF, Haug J, Gehrig J.** Topographic brain measures of human pain and pain responsivity. *Pain* 37, 129–41, 1989
- Finnie JW.** Neuropathological changes produced by non-penetrating percussive captive bolt stunning of cattle. *New Zealand Veterinary Journal* 43, 183–5, 1995
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ.** Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55, 152–7, 2007
- Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, Johnstone AC, Mellor DJ.** Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning. *New Zealand Veterinary Journal* 57, 77–83, 2009 a
- Gibson TJ, Johnson CB, Murrell JC, Chambers JP, Stafford KJ, Mellor DJ.** Components of electroencephalographic responses to slaughter in halothane-anaesthetised calves: Effects of cutting neck tissues compared with major blood vessels. *New Zealand Veterinary Journal* 57, 84–9, 2009 b
- Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ.** Electroencephalographic responses to concussive non-penetrative captive-bolt stunning in halothane-anaesthetised calves. *New Zealand Veterinary Journal* 57, 90–5, 2009 c
- Gregory NG, Wotton SB.** Time to loss of brain responsiveness following exsanguination in calves. *Research in Veterinary Science* 37, 141–3, 1984
- Gregory NG, Wotton SB.** Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *Veterinary Record* 126, 570–2, 1990
- Gregory NG, Shaw FD, Whitford JC, Patterson-Kane JC.** Prevalence of ballooning of the severed carotid arteries at slaughter in cattle, calves and sheep. *Meat Science* 74, 655–7, 2006
- Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ.** Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53, 433–7, 2005 a
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetized red deer (*Cervus elaphus*): electroencephalographic responses. *Journal of Veterinary Anaesthesia and Analgesia* 32, 61–71, 2005 b
- Lambooy E, Spanjaard W.** Effect of the shooting position on the stunning of calves by captive bolt. *Veterinary Record* 109, 359–61, 1981
- Levinger IM.** Untersuchungen zum Schächtproblem. [Investigations of the Schechita problem.] *Dr thesis*, University of Zürich, Zürich, Switzerland, 1961
- ***Levinger IM.** *Schechita in the Light of the Year 2000. Critical Review of the Scientific Aspects of Methods of Slaughter and Schechita*. Maskil L'David, Jerusalem, Israel, 1995
- Mellor DJ, Litin KE.** Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. *Animal Welfare* 13 (Supplement), 127–32, 2004
- Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL, Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138–46, 2003
- Murrell JC, White KL, Johnson CB, Taylor PM, Doherty TJ, Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212–21, 2005
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves. 1. The onset of permanent insensibility in sheep during slaughter. *Meat Science* 6, 221–33, 1982 a
- Newhook JC, Blackmore DK.** Electroencephalographic studies of stunning and slaughter of sheep and calves. 2. The onset of permanent insensibility in calves during slaughter. *Meat Science* 6, 295–300, 1982 b

Submitted 13 February 2008

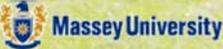
Accepted for publication 17 February 2009

*Non-peer-reviewed

APPENDIX 4

SCIENTIFIC CONFERENCE PRESENTATIONS

Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Electroencephalographic responses of calves to scoop dehorning. In: 'Medical Sciences Congress'. Millennium Hotel Queenstown, New Zealand, 2005




ELECTROENCEPHALOGRAPHIC RESPONSES OF CALVES TO SCOOP DEHORNING

TJ Gibson¹, CB Johnson¹, KJ Stafford^{1,3}, SL Mitchinson¹, and DJ Mellor^{2,3}
 IVABS¹, IFNH², Animal Welfare Science and Bioethics Centre³, Massey University, Palmerston North

Introduction

Dehorning calves is a routine farm practice. Pain-induced distress caused by dehorning and different strategies for its alleviation have been well documented (1). Pain-induced distress measures have a limitation when determining noxiousness, as these changes can be elicited from painless but stressful stimuli. Spectral analysis of the electroencephalogram (EEG) has been associated with noxiousness in both humans (2) and animals (3,4,5,6). Changes in median frequency (F50), 95% spectral edge frequency (F95) and total power of the EEG frequency spectrum (Ptot) have been demonstrated to be associated with noxious stimulation (3,4,5,6). While spectral changes in the EEG are indirect measures of pain, they reflect cortical electrical activity and so are more likely to reflect the cognitive perception of noxiousness. EEG spectral analysis used in conjunction with the minimal anesthesia model (3,4,5,6) presents an ethically attractive model for the investigation of noxiousness during welfare comprising procedures. The aim of the study is to investigate the cerebrocortical responses of minimally anaesthetized calves to scoop dehorning with or without a local anesthetic lidocaine ring block, using EEG variables (F50, F95, Ptot) associated with noxious stimulation.

Materials and Methods

- Twenty Friesian heifer calves were maintained under halothane anesthesia throughout the entire study period in accordance with the minimal anesthesia model (3,4,5,6).
- Individual horns were either removed with a scoop dehorner with (DH + LA) or without (DH) a lidocaine ring block.
- One channel of the EEG and ECG were recorded using transcutaneous needle electrodes.
- Fast Fourier Transformation (FFT) was performed on continuous 1-second epochs of EEG data.
- Between group comparisons were made using area under the curve analysis with One-way ANOVA (unstacked). Post hoc comparisons to baseline were made with Dunnett's test.
- This study was approved by the Massey University Animal Ethics Committee (Protocol No: 04/86).

Results

There was a significant decrease in Ptot ($P < 0.01$) (figure 1) following dehorning in the DH group compared to the DH + LA group. There were significant increases in F95 ($P < 0.001$) (figure 2A) and F50 ($P < 0.001$) (figure 2B) following dehorning in the DH group compared to the DH + LA group. DH F50, F95 and Ptot showed significant differences ($P < 0.01$) compared to baseline. The DH + LA group showed no significant differences from baseline for any EEG variable.

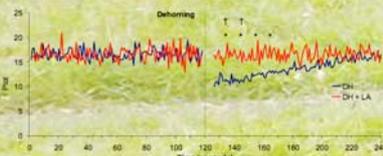


Figure 1. Ptot before and after dehorning, DH (blue) and DH + LA (red). * Significant difference between treatment groups, $P < 0.01$. † Significant difference from baseline for DH (red), $P < 0.01$.

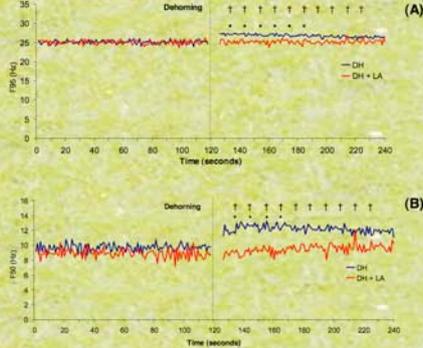


Figure 2. (A) F95 and (B) F50 before and after dehorning for DH (blue) and DH + LA (red). * Values significantly difference between treatment groups, $P < 0.001$. † Values significant difference from baseline for DH (red), $P < 0.01$.

Table 1. Heart rate as a % of baseline for DH + LA and DH (mean \pm SEM). * Values significantly different between treatments, $P < 0.01$. † Values significantly different from baseline DH, $P < 0.01$.

Time (seconds)	DH + LA (% of baseline)	DH (% of baseline)
-15	100.14 \pm 0.59	98.81 \pm 0.77
0 (dehorned)	101.68 \pm 2.41	73.98 \pm 3.67*†
15	101.94 \pm 2.96	72.99 \pm 3.58*†
30	102.10 \pm 3.19	73.66 \pm 3.49*†
45	101.29 \pm 3.23	76.60 \pm 2.50*†
60	100.48 \pm 3.15	77.15 \pm 2.11*†
75	99.93 \pm 3.11	77.71 \pm 2.20*†
90	109.76 \pm 5.54	104.07 \pm 4.86
105	109.20 \pm 5.75	105.19 \pm 5.31

Heart rate as a % of baseline (table 1) in the DH group significantly decreased following dehorning when compared to DH + LA ($P < 0.01$) and the baseline ($P < 0.01$). Heart rate as a % of baseline in the DH + LA group showed no significant differences from the baseline value.

Conclusions

This is the first investigation of the noxiousness of dehorning using EEG spectral analysis. This technique confirms that scoop dehorning is a noxious stimulus and that application of a lidocaine ring block abolishes any cerebrocortical response. Spectral analysis of the EEG combined with the minimal anesthesia model appears to be a useful tool for assessing noxious sensory inputs in cattle. The minimal anesthesia model involves anaesthetised animals that therefore are unconscious to pain during the investigation of potentially welfare comprising procedures.

References

1. K. J. Stafford and D. J. Mellor. The Veterinary Journal 169, 337 (2005).
2. A. C. N. Chen, S. F. Dawkins, J. Hulp, and J. Squire. Pain 37, 123 (1989).
3. C. B. Johnson, P. R. Wilson, W. M. Woodbury, and N. A. Caulcutt. Journal of Veterinary Anaesthesia and Analgesia 32, 61 (2005).
4. C. B. Johnson, K. J. Stafford, S. P. Sylvester, R. N. Ward, S. Mitchinson and D. J. Mellor. New Zealand Veterinary Journal (In Press).
5. J. C. Murrell, C. B. Johnson, K. L. White, P. M. Taylor, Z. L. Heberlein, and A. E. Waterman-Pearson. Veterinary Anaesthesia and Analgesia 30, 128 (2003).
6. J. C. Murrell, K. L. White, C. B. Johnson, P. M. Taylor, T. J. Caherty, and A. E. Waterman-Pearson. Veterinary Anaesthesia and Analgesia 32, 212 (2005).




Gibson TJ, Murrell JC, Stafford KJ, Mellor DJ, Johnston AC, Johnson CB. Functional effects of concussive traumatic brain injury on the cerebral cortex in cattle with a non-penetrative captive bolt. In: 'Medical Sciences Congress'. Rotorua, New Zealand, 2006

FUNCTIONAL EFFECTS OF CONCUSSIVE TRAUMATIC BRAIN INJURY ON THE CEREBRAL CORTEX IN CATTLE WITH A NON-PENETRATIVE CAPTIVE BOLT.

TJ Gibson¹, JC Murrell¹, KJ Stafford¹, DJ Mellor², AC Johnstone¹ and CB Johnson¹

1. IVABS Massey University, Palmerston North New Zealand
2. IFNHH, Massey University, Palmerston North New Zealand

t.g.gibson@massey.ac.nz

Impact acceleration has been investigated as a model of cerebral concussion in a variety of species (Cernak 2005). Non-penetrative captive bolt stunning used to render cattle insensible is similar and may be useful in identification of the functional and histological effects of concussive traumatic brain injury in large mammals. Subjective assessment of raw electroencephalogram (EEG) does not lend itself to statistical analysis. However spectral analysis of the EEG allows objective interpretation of changes. In conjunction with somatosensory evoked potentials (SEP), EEG spectral analysis may provide insight into the functional effects of concussive traumatic brain injury. EEG and SEP studies were carried out in 14 calves under general anaesthesia. Concussive trauma was applied to a site 30 mm above the junction between the frontal and parietal bones on the midline using a non-penetrative captive bolt. Either EEG (n=10) or SEP (n=4) were recorded bilaterally using a temporal montage. A cohort of brains were fixed and examined histologically. Preliminary analysis demonstrated that following concussive trauma, total power of the EEG significantly ($P < 0.001$) decreased from baseline values. SEPs were abolished following concussion and did not return during the recording period. This study indicates that the non-penetrative captive bolt causes traumatic brain injury with significant changes in cerebrocortical function.

Cernak I. Animal Models of Head Trauma. *The Journal of the American Society for Experimental NeuroTherapeutics* 2, 410-22, 2005

The study described was approved by the Massey University Animal Ethics Committee. Supported by MAF (NZ) and DEFRA (UK); TJG was a C. Alma Baker Trust Scholar.

Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Application of EEG to Assessment of Noxious Sensory Input. In: 'New Zealand Pain Society Annual Scientific Meeting'. Convention Centre, Palmerston North, New Zealand p 20, 2007

Application of EEG to assessment of noxious sensory input

T.J. Gibson¹, C.B. Johnson^{1,2}, K.J. Stafford^{1,2}, S.L. Mitchinson¹ and D.J. Mellor²

Speaker: Troy J Gibson

¹Institute of Veterinary, Animal and Biomedical Sciences, and ²Animal Welfare Science and Bioethics Centre, Massey University, Palmerston North, New Zealand.

The physiological, endocrinological and behavioural responses to dehorning in cattle are well documented (1). These changes have limitations when attempting to quantify the degree of pain perceived by the animal (2). The aim of this study was to validate use of the electroencephalogram (EEG) and a minimal anaesthesia model for assessment of noxious sensory input caused by amputation dehorning of calves. Twenty Friesian heifers weighing 125-177 kg were maintained under light general anaesthesia using an established protocol (minimal anaesthesia model). They were then dehorned using an amputation scoop either with (DH+LA) or without (DH) a lidocaine ring block. Changes in the EEG and electrocardiogram (ECG) in response to dehorning were recorded. Median frequency (F50), 95% spectral edge frequency (F95), total power (Ptot) and compressed spectral arrays were derived from the EEG data. There were significant increases in F50 ($p < 0.01$) and F95 ($p < 0.01$), and a decrease in Ptot ($p < 0.01$) following dehorning in the DH group, but there were no changes in the DH+LA group. Transient bradycardia in the first 75 seconds following dehorning was recorded in the DH group compared to pre-treatment values and compared to the DH+LA group ($p < 0.001$). However, tachycardia was evident by 15 minutes after dehorning in the DH group. The results validate use of the EEG and a minimal anaesthesia model for assessment of noxious sensory inputs in cattle and show that the ring-block application of lidocaine local anaesthesia abolishes the measured cerebrocortical responses.

1. Stafford KJ, Mellor DJ. 2005. *The Veterinary Journal* 169: 337-349
2. Barnett JL. 1997. *Australian Veterinary Journal* 75: 878-879

All of the studies described were approved by the Massey University Animal Ethics Committee. Supported by MAF (NZ) and DEFRA (UK); TJG was a C. Alma Baker Trust Scholar.

Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL, Mellor DJ. Electroencephalographic responses of calves to scoop dehorning. In: 'Proceedings of the Society of Dairy Cattle Veterinarians of the New Zealand Veterinary Association'. Christchurch, New Zealand p 216, 2007

Dairy cattle posters

6. ELECTROENCEPHALOGRAPHIC RESPONSES OF CALVES TO SCOOP DEHORNING

TJ Gibson^{ab}, CB Johnson^{ab}, KJ Stafford^{ab}, SL Mitchinson^{ab} and DJ Mellor^{ac}. *Topic leader, ^aAWSB Centre; ^bIVABS; ^cIFNHH, Massey University, Palmerston North, New Zealand.

The physiological, endocrinological and behavioural responses associated with dehorning in cattle are well documented (1). These changes have limitations when attempting to quantify the degree of pain perceived by the animal (2).

The aim of this study was to investigate the noxiousness of scoop dehorning in calves as assessed using electroencephalographic (EEG) variables associated with noxious stimulation (3, 4). Twenty calves were dehorned under minimal general anaesthesia (3, 4) with (local) or without (control) lidocaine local anaesthesia (1).

There were significant increases in median frequency ($P < 0.01$) and 95% spectral edge frequency ($P < 0.001$) following dehorning in the control group compared to the local group. There was a significant decrease in total EEG power ($P < 0.01$) following dehorning in the control group compared to the local group. The local group showed no significant differences from baseline for all EEG variables. This study is the first investigation of the noxiousness of dehorning using EEG spectral analysis. We conclude that scoop dehorning is a noxious stimulus and that the application of local anaesthesia using lidocaine abolishes the measured cerebrocortical responses.

1. Stafford KJ, Mellor DJ. 2005. *The Veterinary Journal* 169: 337-49
2. Barnett JL. 1997. *Australian Veterinary Journal* 75: 878-9
3. Johnson CB, Stafford KJ, Sylvester SP, Ward RN, Mitchinson S, Mellor DJ. 2005. *New Zealand Veterinary Journal* 53: 433-7
4. Johnson CB, Wilson PR, Woodbury WM, Caulkett NA. 2005. *Journal of Veterinary Anaesthesia and Analgesia* 32: 61-71

All of the studies described were approved by the Massey University Animal Ethics Committee. Supported by MAF (NZ) and DEFRA (UK); TJG was a C.Alma Baker Trust Scholar