The Minimal Anaesthesia Model: Development and Refinement of the Concept and Subsequent Practical Applications

A Collection of Papers and a Monograph Presented in Application for the Degree of Doctor of Science at Massey University, Manawatu

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July 2020

Declaration

I declare that the material to be examined in this thesis has not been submitted by me to any other university for the award of any degree.

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July 2020

Acknowledgements

Over the course of my career I have been very lucky to have had the help, ecouragement and oversight of many supervisors, senior colleagues and mentors who have contributed in many ways to the path that I have taken. Despite the risk of missing out those who deserve to be mentioned, I feel that I cannot proceed without expressing my deepest gratitude to Polly Taylor, Avril Waterman-Pearson, David Mellor, Simon Young, Joe Mayhew, Jackie Brealey, Ron Jones, Leighton Richards and Peter Davie. I have also been fortunate to work closely with some exceptional colleagues including Jo Murrell, Ngaio Beausoleil, Paul Chambers, Neil Ward, Julia Washbourne and Alan Jones. Many of these people have also become close friends.

Much of my research has involved the participation of postgraduate students. There is no substitute for the enthusiasm and focus that a postgraduate student brings to a research project and it has been a privilege to have been able to contribute to the careers of these students as a supervisor. I have subsequently seen many of them develop into mature researchers in their own right and seeing them contribute to animal welfare in their many and varied ways makes me very proud to have been associated with them. The part played by others in the work recorded here is indicated by their authorship or in the acknowledgement section of the papers.

The research with which I have been involved has necessitated the use of animals as experimental subjects. It is my belief that my work has improved the welfare of animals overall, but I do feel the need to acknowledge the costs that some of my experimental subjects have borne. My closest association was with Houghton, Ride, Badger, Jason, Kelly, Scooby, Talisman and Welly the eight ponies that were the subjects of my doctoral studies. In mentioning them by name I am also remembering the other animals with whom I have worked over my career.

It is not my family's usual practice to publically thank each other for the mutual love and support that we share, but on this occasion I feel the need to make an exception. I am blessed with three children and currently three grandchildren who are each a great joy to me. I would like to make especial mention of my wife who has made many sacrifices on my behalf and provided immeasurable support to me over the course of our shared journey. This manuscript is dedicated to her.

Introduction

I have been fortunate to have worked in animal welfare science during a very exciting time in the discipline. Over the course of my career, the concept of welfare has moved from a five freedoms approach emphasising the absence of harms to a five domains model which considers the importance of positive welfare and integrates the affective state of animals into its considerations. This change in emphasis has allowed the concept of a life worth living to become the benchmark for considerations of the animal welfare and led to the increasing recognition of sentience in non-human animals.

My contribution has been primarily in the development of a new technique, the minimal anaesthesia model, that enables the quantitative measurement of a mammal's perception of pain when it is subjected to a noxious stimulus. This technique clearly links an animal's affective state with changes in physiological variables derived from the EEG (Electroencephalogram) and as such has been a component of the literature that forms the basis for our present understanding of affective state in animals.

Summary of Research

This thesis will cover my work in the development of the minimal anaesthesia model and its applications in several areas where I have used it as an applied tool. Some of the early studies that form the basis of the minimal anaesthesia contributed to my PhD and where these are cited, their inclusion is for context only, they are not intended to form part of this assessment. They are indicated with a # in the reference list. I have made contributions in 5 main areas and they will each be covered separately.

Development and validation of a minimal anaesthesia model of pain perception

In the ten years following my PhD I worked on ways of using the electroencephalogram as an indicator of potential pain perception in anaesthetised animals. The model that I developed has become known as the minimal anaesthesia model. It is entirely original and uses data recorded from the electroencephalogram of anaesthetised animals to measure responses to stimuli and indicate if these stimuli would be perceived as painful if the animal were conscious. This model enables pain research to be undertaken without inflicting pain on the experimental animals involved because they are anaesthetised for the duration of the experiment. All data are collected prior to the animal's recovery from anaesthesia and so pain relief can be provided using standard clinical techniques even when the animals concerned form part of a control group. In addition to the refinement represented by the ability to always provide pain relief, the inclusion of a control group that does not receive analgesia during data collection gives studies performed using this model enhanced statistical power and enables an overall reduction in the number of animals used in such studies. To date the minimal analgesia model has been adapted for use in 13 different species of mammal. The National Animal Ethics Advisory Committee's 3Rs award in 2006 was given in recognition of the development of this model.

During my anaesthesia residency at the Animal Health Trust (Newmarket, UK) I demonstrated a change in certain EEG-derived variables to changing partial pressures of halothane (1). Subsequently during my PhD at the University of Cambridge, I characterised the EEG response to other inhalation anaesthetics (2) and also a number of injectable anaesthetics (3-7). One of the interesting findings was that the ratio between an agent's effect on the F50 (median frequency of the EEG) and F95 (95% spectral edge frequency of the EEG) appeared to be an indication of the potency of the agent as an analgesic. Potent analgesics such as alfentanil (3) appeared to reduce this ratio to a greater extent than narcotics such as thiopentone (5). Subsequent work after my move to Bristol University (8,9) indicated that surgical stimulation presented under minimal general anaesthesia with halothane resulted in an EEG response that was opposite to that elicited by analgesic agents and that surgical stimulation in the presence of analgesic agents resulted in a response of reduced magnitude. The initial validation of this technique was undertaken in horses, but I have since carried out studies demonstrating the model in 12 other species of mammals and similar validations to that in the horse have been demonstrated in four of these: cattle (10); rat (11); red deer (12). A more detailed discussion of the development of this model can be found in my review of the model as applied to slaughter (13).

In order to be able to properly apply a research methodology it is important to understand its characteristics and limitations. Over the course of the minimal anaesthesia model's development, I have conducted a number of experiments (11, 14-16) to investigate this aspect with the aim of better understanding the model itself and so being better able to interpret data obtained when using the model. The appropriate use of electrophysiological and other tools to assess pain perception in the research environment has become a particular interest of mine which I have explored in two review articles (17, 18).

Welfare issues associated with killing of animals (especially in slaughter, but also in other contexts)

One of the benefits of the minimal analgesia model compared to other research techniques used in pain research is that data are recorded with no time lag. In contrast, techniques that rely on changes in the endocrine system, for example, are associated with a delay that can be as long as 30 minutes between stimulus and response. The instant response seen with this model means that it can be used to record pain responses even in animals that are physiologically unstable. A major application of this feature of the model has been its use to evaluate the welfare impacts of techniques used to kill animals in different circumstances. My use of the minimal anaesthesia model to investigate slaughter in cattle without prior stunning (19, 20) demonstrated 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 (21). Additional studies carried out in conjunction with this demonstrated that stunning with a captive bolt resulted in immediate cessation of EEG activity (22) and that such stunning was protective against the response to ventral neck incision (23). This research has resulted in legislative changes in New Zealand, the proclamation of a fatwa (Islamic religious teaching statement) in Jordan and a reconsideration of stunning prior to slaughter in several countries. Commercial slaughter in New Zealand now requires stunning prior to bleeding and in the UK, the proportion of

cattle that are stunned prior to halal slaughter has risen from 0% in 2007 to 80% in 2014 (an increase of 91 million animals in 2014 alone). This work has also generalized from consideration of cattle only to include sheep and goats (24). The original studies demonstrating the noxious effect of slaughter without stunning in cattle (19, 20, 22, 23) were awarded the inaugural award of the Humane Slaughter Association of the UK for significant advances in the humane slaughter of farmed livestock.

Since the need for stunning prior to slaughter was demonstrated, I have used the minimal anesthesia model to investigate novel methods of slaughter including diathermic syncope (25) and modifications of head-only electrical stunning (26). This research is progressively widening the scope of stunning by developing techniques which are acceptable to a greater proportion of the Islamic World and is increasingly involving researchers from the Muslim community. Direct collaboration with scientists from these communities (27) increases the impact of the results of our studies and also encourages the development of centres of rigorous animal welfare science in Islamic and developing countries.

In addition to slaughter, I have used the model to investigate the welfare implications of killing animals in other contexts. Examples of these studies include:

Decapitation as a method of killing rats and chickens (28, 29)
Carbon dioxide as a method of killing rats (30, 31)
Captive bolt as a method of killing billy goat kids (32)
Carbon dioxide and inert gas euthanasia for neonatal piglets (33, 34)
Intraperitoneal pentabarbitone euthanasia for neonatal piglets (35)
Deep sedation and magnesium sulphate to kill sheep in the field (36)

Development of awareness and pain perception in neonates

Neonatal mammals develop the ability to perceive pain by means of complex interactions of structural and biochemical factors that govern the function of the central nervous system. The onset of neurological milestones such as vision, hearing and complex behaviour occur at different times (relative to birth) in altricial and precocial species and I have demonstrated that the onset of the ability to perceive pain also occurs at different times in these different groups (37-43). In addition to pain perception at the time of injury, it has been shown that when humans are subjected to painful procedures such as circumcision very early in life, they can develop a long-lasting increased susceptibility to pain (hyperalgesia) that can persist into their adult life. Using sheep as a model, I have demonstrated that this hyperalgesia is dependent on the timing of the stimulus (44) and that a single noxious stimulus delivered prior to the onset of pain perception causes persistent changes in brain function (45) as well as altered responses to future painful stimuli when compared to the same stimulus delivered after the onset of pain perception (44). I have demonstrated physical (46), molecular (47, 48) and functional (45) changes in the nervous system that may be responsible for these findings. These results have important implications for the need to provide pain relief for noxious stimuli even before animals develop the ability to perceive pain. In New Zealand they have resulted in changes to the Code of Welfare for Painful Husbandry

Procedures which now contains a gold standard that analgesia should be provided for animals at all ages.

Pain perception and analgesia in birds

My studies investigating perioperative analgesia in birds have highlighted some of the differences in responses to pain relief shown in these animals compared to mammals (49-52). These differences have caused me to develop an interest in the central nervous system pathways of pain in birds and I have begun to compare the electrical responses of the avian brain in response to anaesthesia (53, 54) and noxious stimulation (55) to those of mammals. Whilst these studies are still at an early stage, I am hopeful that they will enable greater understanding of the mechanisms by which birds perceive pain and enable us to assess the effects of pain relief in birds with more confidence than we can at present.

Advances to analgesia in clinical contexts

Analgesia is an integral component of clinical anaesthesia and like all clinical disciplines is subject to ongoing improvements as new agents and techniques are developed and made available. Over the course of my career I have undertaken studies with the aim of assessing novel analgesic techniques with a focus on the relief of acute surgical pain in a range of animals:

Horses, mules and donkeys (56-60) My early clinical studies with horses (56-58) and some of the papers that form part of my PhD (3, 4) investigated the effects of analgesic agents in the horse at a time when the provision of analgesia (especially opioids) in these animals was controversial. The first papers developing the minimal anaesthesia model used horses (8, 9) and following these studies, the EEG response to surgical stimulation in horses has been compared with those in mules and donkeys (59, 60). Of note from these later studies is the finding that donkeys show an EEG response to noxious stimulation that is similar to horses despite very different pain-related behaviour in this species. These findings refute the view (commonly held at the time of publication) that donkeys perceive less pain than horses and so require less analgesia. Rather it appears that pain behaviour in donkeys is less overtly obvious than that in horses, suggesting that care should be taken to ensure adequate analgesia when working with this species.

Red deer (12, 61) These studies documented the extent to which the application of a tight band applied around the antler pedicle provided analgesia for velvet antler removal. This technique had been postulated for use as a part of routine velvet antler harvest, but my results demonstrating that the band was noxious at the time of application and provided inferior analgesia to lidocaine ring block resulted in the Ministry for Primary Industries not approving the technique and it being abandoned by the New Zealand Deer Industry.

Sheep (62-64) My early work investigating the development of awareness and pain perception in neonates used lambs as a model. These studies demonstrated that lambs are capable of pain perception from an early age (37, 39) and that noxious stimulation in very young lambs can lead to prolonged hyperalgesia (44). These findings resulted in changes to the Code of Welfare for Painful Husbandry

Procedures indicating that adequate analgesia would be a future requirement for such procedures. Since this time the first regulations requiring such analgesia have been brought into force. These developments enabled me to investigate methods of pain relief for castration and tail docking that would be both effective and practical in the commercial environment and this research is ongoing.

Dogs (65-70) *and Cats* (71, 72) These are amongst the most common animals to undergo major surgical procedures in veterinary practice and techniques of analgesia are constantly being revised and improved. In these studies, I utilised the minimal anaesthesia model to in combination with other research methodologies to develop the perioperative use of novel drugs (65-68, 70-72) and new ways of using existing drugs (69). These studies contribute to the continual improvement of clinical practice to provide safer and more effective pain relief for small animals.

Birds (49-52) Throughout my career, my interest in physiological aspects of pain perception has gone hand in hand with applied research aiming to develop better methods of analgesia for use in clinical and animal husbandry situations. This is particularly illustrated in my developing interest in avian pain, the theoretical aspects of which were covered above. These studies originated from clinical questions about the effects of analgesics in birds. In addition to making progress investigating the features of avian pain (53-55), my studies in this area have provided information about different potential analgesics (49-52) that is enabling avian clinicians to make more informed decisions about how to treat pain in birds.

Pigs (73) Piglets were one of the animal models used to investigate the effects of pain in neonates and are also a species in which noxious procedures are often carried out in very young animals. This study is an example of how the theoretical aspects of my research have often reflected back into animal husbandry practice in the form of studies comparing proposed ways to mitigate the noxiousness of common painful husbandry procedures.

Ox (Bos indicus)(74, 75) Bos indicus cattle are very difficult animals in which to detect behavioural signs of pain to the extent that the need for analgesia following castration has been questioned. The studies investigating castration in these animals (74, 75) demonstrated central signs of response to noxious stimulation for the first time in this species and also the ability of local anaesthesia to ameliorate these responses.

Ox (Bos taurus)(76) Disbudding of horn buds in cattle has recently been subject to regulation under the Animal Welfare Act and pain relief is now mandatory for this procedure. This paper investigates the use of liquid nitrogen as an alternative to thermocautery for the removal of horn buds. Whilst liquid nitrogen does not appear to be a useful alternative to thermocautery, this paper illustrates the impetus that the new regulation has given into studies seeking less painful alternatives to traditional methods of horn bud removal.

Concluding Remarks

The research presented in this thesis represents both my development of the minimal anaesthesia model and its application in a number of theoretical and applied areas of animal welfare science. The use of this methodology, especially

when combined with other techniques such as behavioural analysis, has proven to be a very powerful way to investigate the perception of noxious stimuli. In particular it enables clear links between physical responses and the underlying affective state of the animal to be made. These links have both expanded our understanding of the development and mechanisms of pain perception in the central nervous system of mammals and also enabled the extent to which animal husbandry procedures such as castration, tail docking and killing are painful to be measured. These latter applied studies have been used as the basis for significant changes to the ways in which painful procedures are carried out. They have contributed to new ways of providing pain relief in a variety of contexts and to changing legislation to ensure that pain relief is used in practice.

List of Publications

Publications marked # report work which was submitted as part of my PhD studies and appear in this list for context only.

1 CB Johnson, SS Young and PM Taylor (1994). Analysis of the frequency spectrum of the equine electroencephalogram during halothane anaesthesia. *Research in Veterinary Science* **56** (3) 373-378

2# CB Johnson and PM Taylor (1998). Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* **81** 748-753.

3# CB Johnson and PM Taylor (1997).Effects of alfentanil on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Research in Veterinary Science* **62** 159-163.

4# CB Johnson and PM Taylor (1999). Effects of ketamine on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Veterinary Surgery* **28** 380-385.

5# CB Johnson, M Bloomfield and PM Taylor (2000). Effects of thiopentone on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Journal of Veterinary Anaesthesia and analgesia* **27** 82-88.

6# CB Johnson, M Bloomfield and PM Taylor (2000). Effects of guaiaphenesin on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Journal of Veterinary Anaesthesia and analgesia* **27** 6-12.

7# CB Johnson, M Bloomfield and PM Taylor (2003). Effects of midazolam and sarmazenil on the equine electroencephalogram during anaesthesia with halothane in oxygen. *Journal of Veterinary Pharmacology and Therapeutics* **26** 105-112.

8 JC Murrell, CB Johnson, KL White, PM Taylor ZL Haberham and AE Waterman-Pearson (2003). Changes in the EEG during castration in horses and ponies anaesthetised with halothane. *Journal of Veterinary Anaesthesia and Analgesia* **30** 138-146.

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10 TJ Gibson, CB Johnson, KJ Stafford, SL Mitchinson and DJ Mellor (2007). Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* **55** 152-157.

11 JC Murrell, SL Mitchinson, L Lesperance, S Sivakumaran and CB Johnson (2010). Electroencephalography during ovariohysterectomy in rats anaesthetised with halothane. *Veterinary Anaesthesia and Analgesia* **37** 14-24.

12 CB Johnson, WM Woodbury, N Caulkett & P Wilson (2005). 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.

13 CB Johnson, TJ Gibson, KJ Stafford and DJ Mellor (2012). Pain perception at slaughter. *Animal Welfare* **21** 113-122.

14# CB Johnson, J Washbourne and PM Taylor (1995). Origin of the equine middle latency auditory evoked potential. *Journal of Veterinary Anaesthesia* **22** 15-18

15 JC Murrell, D Waters, SL Mitchinson and CB Johnson (2007). Comparative effect of thermal, mechanical and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* **98** 366-371.

16 JC Murrell, D Waters and CB Johnson (2008). Comparative effects of halothane, isoflurane, sevoflurane and desflurane on the electroencephalogram of the rat. *Laboratory Animals* **42** 161-170.

17 JC Murrell and CB Johnson (2006). Neurophysiological techniques to assess pain in animals (Review). *Journal of Veterinary Pharmacology and Therapeutics* **29** 325-335.

18 CB Johnson (2016). Research Tools for the Measurement of Pain and Nociception. *Animals* ***6*** 71; doi:10.3390/ani6110071

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

20 TJ Gibson, CB Johnson, JC Murrell, JP Chambers, KJ Stafford and DJ Mellor (2009). Components of EEG responses to slaughter: effects of cutting neck tissues compared to major blood vessels. *New Zealand Veterinary Journal* **57**

84-89.

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25 JL Rault, P Hemsworth, P Cakebread, DJ Mellor and CB Johnson (2014). Evaluation of microwave energy as a humane stunning technique based on electroencephalography (EEG) of anesthetized cattle. *Animal Welfare* **23** 391-400.

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28 K Kongara, AE McIlhone, NJ Kells and CB Johnson (2014). Electroencephalographic evaluation of decapitation of the anaesthetized rat. *Laboratory Animals* **48** 15.

29 AE McIlhone, NJ Beausoleil, DJ Mellor, SL Mitchinson and CB Johnson (2009). Effect of decapitation on the electroencephalogram of anaesthetised chickens. Proceedings of the 10th World Congress of Veterinary Anaesthesia, Glasgow. 69.

30 NJ Kells, NJ Beausoleil, AE McIlhone, CB Johnson (2018). Electroencephalographic responses of anaesthetised rats to carbon dioxide inhalation. *Animal Welfare* **27** 215-223.

31 P Hawkins, MJ Prescott, L Carbone, N Dennison, C Johnson, I Makowska, N Marquardt, G Readman, DM Weary, HDR Golledge (2016). A Good Death?

Report of the Second Newcastle Consensus Meeting on Laboratory Animal Euthanasia. *Animals* **6** 50 doi:10.3390/ani6090050

32 MA Sutherland, TJ Watson, CB Johnson and ST Millman (2016). Evaluation of the efficacy of a non-penetrating captive bolt to euthanise neonatal goats up to 48 hours of age. *Animal Welfare* **25** 471-479.

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34 J-L Rault, NJ Kells, CB Johnson, Dennis, M Sutherland, and DC. Lay Jr (2015). Nitrous oxide as a humane method for piglet euthanasia: behavior and electroencephalography (EEG). *Physiology and Behaviour* **151** *29-37.*

35 NJ Kells, NJ Beausoleil, MA Sutherland, CB Johnson (2018) Electroencephalographic responses of anaesthetised pigs to intraperitoneal injection of sodium pentobarbital. *Animal Welfare* **27** 205-214.

36 KJ Stanger, NJ Kells, AD Fisher, T Jubb, J-L Rault and C Johnson (2019). Evaluation of euthanasia of sheep with intravenous saturated salt solutions to enable the collection of whole, intact brains. *Animal Welfare* **28** 397-406.

37 CB Johnson, KJ Stafford, SP Sylvester, RN Ward, S Mitchinson and DJ Mellor (2005). Effects of age on the electroencephalographic response to castration in lambs anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* **53** 433-437.

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42 NJ Kells, NJ Beausoleil , JP Chamber, MA Sutherland, RS Morrison and CB Johnson (2017). Electroencephalographic responses of anaesthetized pigs (*Sus scrofa*) to tail docking using clippers or cautery iron performed at 2 or 20 days of age. *Journal of Veterinary Anaesthesia and Analgesia* doi: 10.1016/j.vaa.2017.02.003.

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56 CB Johnson, PM Taylor, SS Young and JC Brearley (1993). Postoperative analgesia using phenylbutazone, flunixin or carprofen in horses. *Veterinary Record* **33** 336-338

57 M Raekallio, PM Taylor, CB Johnson, R-M Tulamo and M Ruprah (1996). Intra-articular Morphine in Normal Ponies. *Journal of Veterinary Anaesthesia* **23** 23-26.

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