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# Pain perception at slaughter

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## Abstract

*Recent developments related to quantitative analysis of the electroencephalogram (EEG) have allowed the experience of pain to be assessed more directly than has hitherto been possible. Variables derived from the EEG of animals anaesthetised using our minimal anaesthesia model respond to noxious stimulation in a manner similar to those from conscious animals. This methodology has been used in a variety of applications including the evaluation of analgesic options for painful husbandry procedures and investigation of developmental aspects of the perception of pain. We have now applied the minimal anaesthesia model to the question of the slaughter of calves by ventral-neck incision. A series of studies evaluated the magnitude of EEG response to the noxious stimulus of ventral-neck incision and the physiological mechanisms that underlie this response. We also investigated the EEG effects of stunning by non-penetrating captive bolt and the ability of such stunning to ameliorate the response to ventral-neck incision. The results demonstrate 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 painful in the period between the incision and subsequent loss of consciousness. These data provide further support for the value of stunning in preventing pain and distress in animals subjected to this procedure. We discuss the development of the minimal anaesthesia model and its adaptation for use in the investigation of slaughter by ventral-neck incision as well as considering the contributions of these studies to the ongoing development of international policy concerning the slaughter of animals.*

**Keywords**: *animal welfare, cattle, EEG, pain, slaughter, stunning*

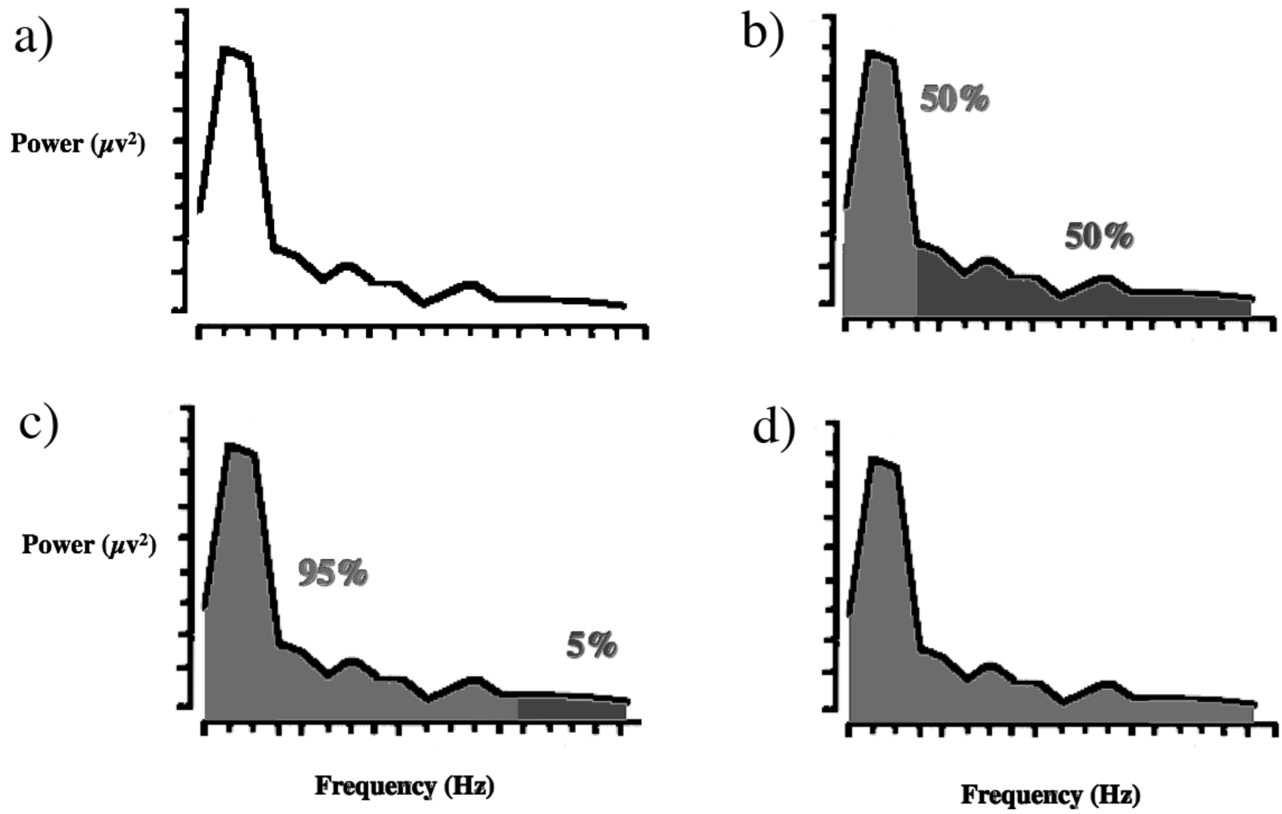
## Introduction

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 the transection of these fibres 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 s or longer in cattle. These observations suggest that livestock may experience pain and distress during the period before they become insensible, but there have previously been no experimental techniques capable of assessing pain perception during this period.

In the early 1990s, there was an increased interest in the possibility of using the electroencephalogram (EEG) to monitor the adequacy of anaesthesia. Studies prior to this time had failed to identify quantifiable EEG changes that correlated with anaesthesia, but the rapid development of personal computers in the previous decade enabled formal signal analysis techniques such as Fast Fourier Transformation (FFT) to become widely available. In the field of veterinary anaesthesia there was particular interest in applying these techniques to horses, because this species had a much higher anaesthetic-related mortality than other common domestic animals (Johnston *et al* 2002).

Early studies investigating the usefulness of the EEG in the clinical arena suggested that FFT analysis may be a useful adjunct to monitoring and against that background the first author began to investigate the EEG effects of inhalational (Johnson *et al* 1994; Johnson & Taylor 1998) and injectable (Johnson 1996; Johnson & Taylor 1997, 1999; Johnson *et al* 2000a,b, 2003) anaesthetic agents in the horse. The EEG was divided into short segments and FFT analysis carried out on each segment to derive a power spectrum. This was then further analysed to derive the median frequency of the EEG (F50), the 95% spectral edge frequency of the EEG (F95) and later the total EEG power (ptot) (Figure 1). Changes in F50, F95 and ptot over time could then be compared in studies where the concentration of individual

**Figure 1**




Power spectrum of (a) electroencephalogram, (b) illustrating derivation of median and spectral edge, (c) frequencies and (d) total power.

**Table 1 EEG effects of injectable anaesthetic agents in the horse.**

| Drug | Effect of F50 (%) | Effect of F95 (%) |
|---|---|---|
| Ketamine | 31 | 21* |
| Detomidine | 13 | 17* |
| Alfentanil | 36** | 26*** |
| Thiopentone | 3 | 27** |
| Midazolam | 21 | 40** |
| GGE | 5 | |
| Sarmazenil | –39 | –56** |

Reductions in F50 and F95. Asterisks indicate significant difference from baseline: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. See text for references.

anaesthetic agents were altered to isolate the EEG effects of these particular drugs. The three EEG variables chosen were used to give an indication of the central location (F50), high frequency components (F95) and low frequency components (ptot) of the EEG power spectrum.

Pharmacokinetic/pharmacodynamic studies of seven injectable drugs used in anaesthesia demonstrated that, in general, increasing plasma concentrations of sedative drugs reduced F50 and F95 (Johnson 1996; Johnson & Taylor 1997, 1999; Johnson *et al* 2000a,b, 2003), but drugs with an analgesic action had greater relative effect on the F50 (Table 1). Anaesthetic agents with no analgesic activity (such as thiopentone) had little effect on F50, whereas agents with potent analgesic effects (such as alfentanil) had a greater effect on F50 than F95.

This apparent link between F50 and plasma concentration of analgesic agents reflected some reports in the literature that pain perception in conscious animals and humans resulted in an increase in EEG frequencies (Ong *et al* 1997; Debenedittis & Degonda 2003). In particular, it was hypothesised that in human volunteers, EEG changes correlated with the magnitude of pain perception rather than the magnitude of the noxious stimulus (Chen *et al* 1989). Our results made us wonder if similar EEG responses to noxious stimuli would be seen in anaesthetised animals and if they could be useful as a correlate of the pain that an animal would perceive if it were conscious.

## Origins of minimal anaesthesia model

In the late 1990s, we investigated the EEG effects of the noxious stimulus of castration in horses (Murrell *et al* 2003), and also the extent to which analgesic agents given in addition to general anaesthesia could alter this response (Murrell *et al* 2005). The noxious stimulation of castration increased F50 and F95 and reduced ptot, whilst concurrent administration of analgesics obtunded this response. The results of these studies together with our earlier studies and other reports in the literature demonstrated the following:



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• Analgesic agents given during general anaesthesia have a characteristic effect on the equine EEG;

• Noxious stimulation under general anaesthesia has the reverse effect;

• Analgesic agents obtund the response to noxious stimulation;

• These changes mirror those seen in conscious animals subjected to noxious stimulation and to humans in pain.

Taken together, these findings indicated that the EEG response may be a useful means of quantification of the degree to which a stimulus would be painful if perceived by a conscious animal. This characteristic EEG response to noxious stimulation under anaesthesia has been termed the minimal anaesthesia model (Murrell & Johnson 2006).

## Development of the minimal anaesthesia model

Over the following few years, the minimal anaesthesia model was adapted to investigate a variety of noxious stimuli in a wide range of mammals. To date, it has been used in horses (Murrell *et al* 2003), sheep (Johnson *et al* 2005b, 2009), red deer (Johnson *et al* 2005a), dogs (Kongara *et al* 2010), rats (Diesch *et al* 2007; Murrell *et al* 2007), wallabies (Diesch *et al* 2010), pigs (Haga & Ranheim 2005) and cattle (Gibson *et al* 2007, 2009a,b,c,d). The anaesthetic technique developed in horses was adapted to these species. The minimal anaesthesia technique has been used to investigate the response to a number of potentially noxious stimuli in these species (Table 2).

**Table 2 Animals and techniques studied using the minimal anaesthesia model.**

| Species | Aspect investigated | Reference |
|---|---|---|
| Horse | Castration | Murrell *et al* (2003) |
| | Effect of analgesics | Murrell *et al* (2005) |
| Sheep | Castration (ontological changes) | Johnson *et al* (2005b) |
| | | Johnson *et al* (2009) |
| Red deer | Velvet antler removal | Johnson *et al* (2005a) |
| Dog | Effect of novel analgesics | Kongara *et al* (2010) |
| Rat | Ontological changes | Diesch *et al* (2009) |
| | Effect of different stimuli | Murrell *et al* (2007) |
| Wallaby | Ontological changes | Diesch *et al* (2010) |
| Pig | Castration | Haga & Ranheim (2005) |
| | Tail docking | Kells *et al*, in prep |
| | Ontological changes | Kells *et al*, in prep |
| Cattle | Dehorning | Gibson *et al* (2007) |
| | Slaughter | Gibson *et al* (2009a) |
| | | Gibson *et al* (2009b) |
| | | Gibson *et al* (2009c) |
| | | Gibson *et al* (2009d) |

## Benefits of the minimal anaesthesia model

The minimal anaesthesia model has a number of advantages over more traditional research methodologies used to evaluate animal pain. Collecting the data from anaesthetised animals means that the animals that are involved in the study do not need to be subjected to pain. All animals can be given analgesia using conventional clinical techniques after the data have been collected, but before they recover from general anaesthesia. This also means that a control group with no additional analgesia can be included in the study without increasing the ethical cost to the animals in that group. The use of anaesthetised animals also reduces the responses to other stimuli and so results in less variability in the recorded data. Taken together, these factors mean that smaller groups of animals are needed with this technique than with other more conventional techniques used in pain research. Thus, the minimal anaesthesia model provides benefits in terms of two of the Three Rs, reduction and refinement.

## Validation of the minimal anaesthesia model in cattle

In order to utilise the minimal anaesthesia technique to investigate the potential noxiousness of slaughter without prior stunning in cattle, it was first necessary to validate the technique in cattle using a stimulus that was known to be noxious (Gibson *et al* 2007). Twenty dairy replacement heifers were anaesthetised using the minimal anaesthesia model. The animals were divided into two groups, one received a local anaesthetic ring block in addition to general anaesthesia (GA + LA) and the other group did not (GA). Animals were then dehorned using a scoop dehorner, a technique that has previously been demonstrated to constitute a noxious stimulus (Stafford & Mellor 2005). The EEG from all animals was analysed for a period of 15 min before and 15 min immediately following dehorning.

There was no EEG response to dehorning in the GA + LA group, but a characteristic response in the GA group. This response is illustrated in Figure 2. This characteristic response is manifest as an immediate increase in F95, an immediate decrease in ptot and an increase in F50 that is not immediate, but develops over the first minute following the application of the noxious stimulus.

## Potential challenges for minimal anaesthesia model in slaughter studies

The use of the minimal anaesthesia model to investigate the perception of slaughter by ventral-neck incision marked a significant advance in the application of this model for a number of reasons. Firstly, all previous studies using this model had investigated the effects of a transient noxious stimulus in a physiologically stable experimental subject. In these cases, the EEG always returned to baseline values following the response to the noxious stimulus and the noxious stimulus itself was the only physiological change that the experimental subject underwent. When investigating slaughter, the noxious


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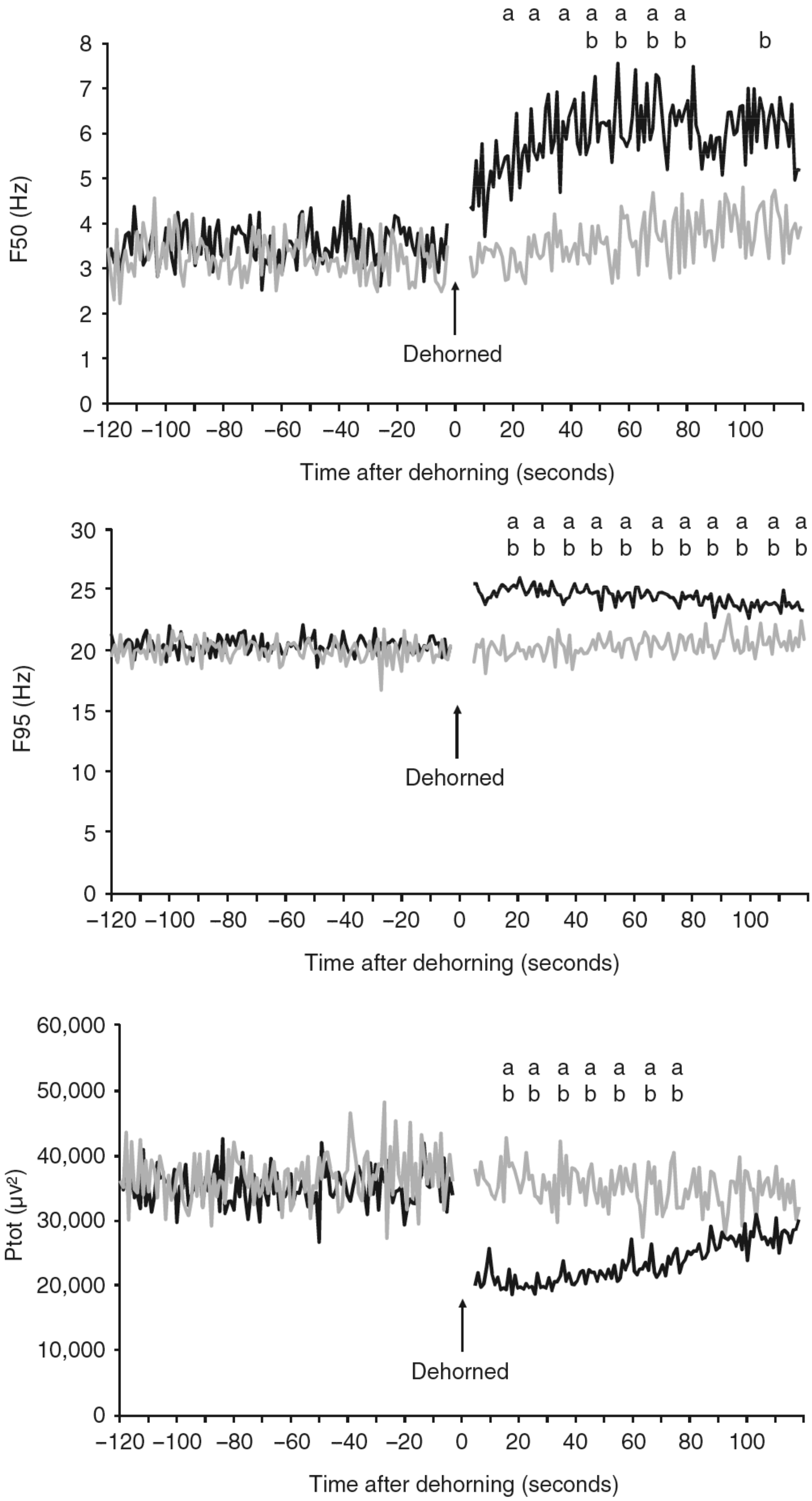
**Figure 2**




Electroencephalographic response of dairy replacement heifers to scoop dehorning under minimal anaesthesia with (grey) or without (black) additional local anaesthetic ring block for (top) median frequency, (middle) spectral edge frequency and (bottom) total power. [a] values significantly different between groups; [b] values significantly different from baseline in general anaesthesia only group ($P$ = 0.01). (From Figures 2, 3 and 4 from Gibson *et al* 2007).



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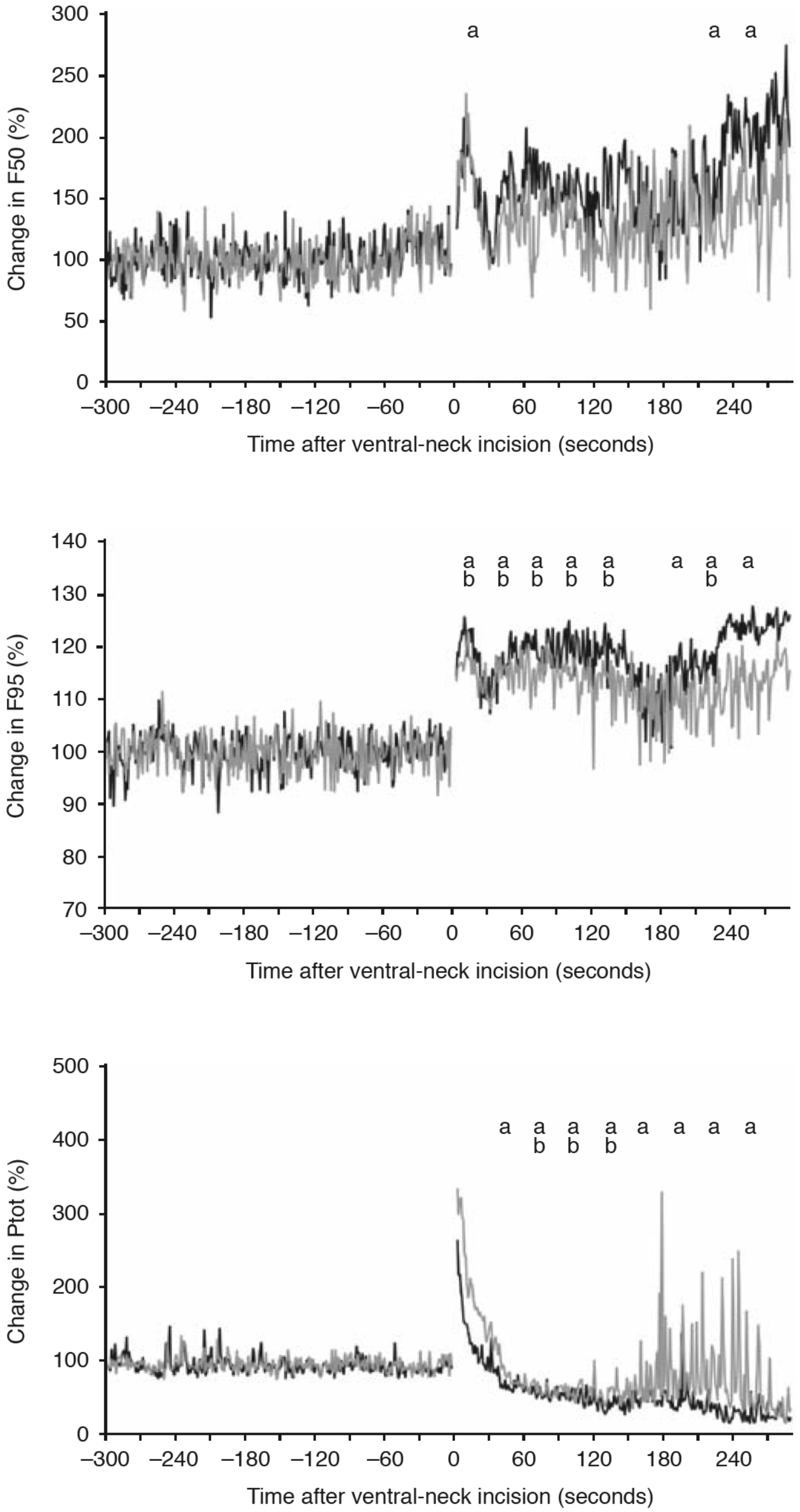
**Figure 3**




Electroencephalographic responses to ventral-neck incision of right (black line) and left (grey line) cerebral hemispheres for (top) median frequency, (middle) spectral edge frequency and (bottom) total power.
[a] values significantly different from baseline right cerebral hemisphere; [b] values significantly different from baseline left cerebral hemisphere ($P = 0.05$). (From Figures 1, 2 and 3 from Gibson *et al* 2007a).

stimulus itself sections the major blood vessels of the neck and thereby induces a physiological instability that ultimately results in the animal's death and also alters a number of important physiological variables, such as cerebral bloodflow and cerebral oxygenation. Following the delivery of the noxious stimulus, the animal never returns to its baseline physiological state.

This complex stimulus and the resultant changes in physiological state mean that the data recorded during slaughter always need to be considered against this background of change. For this reason, a single experimental protocol recording data from animals slaughtered by ventral-neck incision was not sufficient to indicate the potential for such slaughter to be perceived as noxious. In addition, it was necessary to design experimental procedures that could separate the effects of any noxiousness from the effects due to the changes in background physiology.

In addition to the study investigating the EEG effects of slaughter by ventral-neck incision, two additional experimental protocols were designed, one aimed at delivering the noxious stimulus without the attendant physiological disturbance and the other aimed at delivering the physiological disturbances without any noxious stimulation. By considering the results of all three studies together it was hoped that it would be possible to isolate and identify any EEG changes due to noxious stimulation from the mixed response to ventral-neck incision.

## Experimental approaches to effects of slaughter

Previous studies have indicated that the duration of awareness in cattle following slaughter by ventral-neck incision may be up to about 90 s (Levinger 1961; Newhook & Blackmore 1982; Daly *et al* 1988). For this reason, only data recorded in the first 120 s following each manipulation were utilised for comparison of the effects of the experiments. Three experiments were carried out to identify the EEG effects of slaughter by ventral-neck incision:

### EEG effect of slaughter (Gibson *et al* 2009a)

Anaesthetised animals were placed in dorsal recumbency and instrumented to record EEG. After a period of stabilisation and a 5-min baseline recording, a single incision was made in the ventral aspect of the neck, sectioning both carotid arteries and jugular veins. EEG was recorded for a further 5 min. This experimental procedure resulted in an immediate increase in F95, no change in F50 and an increase in ptot followed by a decrease. The EEG changes due to ventral-neck incision are illustrated in Figure 3.

### EEG effect of noxious stimulation alone (Gibson *et al* 2009b)

Following anaesthesia, the jugular veins and carotid arteries were dissected out from the neck both cranial and caudal to the site of proposed ventral-neck incision. Vessels were cannulated at both sites and connected by PVC and silastic tubing which carried the bloodflow and bypassed the site of ventral-neck incision. After this surgical preparation was complete, a period of 15 min was allowed for anaesthesia to stabilise before baseline recording was begun. The small number of data sets obtained from this study precluded formal statistical analysis, but overall a transient increase in F50, a sustained increase in F95 and an initial increase followed by a decrease in ptot were seen. The EEG changes due to noxious stimulus alone are illustrated in Figure 4.

### EEG effect of physiological disturbances alone (Gibson *et al* 2009b)

Following anaesthesia, the jugular veins and carotid arteries were dissected out from the neck and exteriorised at a single site on each side and metal plates placed beneath the vessels to provide a solid cutting surface. A period of 1 h was allowed after the completion of surgery for the stabilisation of anaesthesia before baseline recording was begun. The small number of data sets obtained from this study precluded formal statistical analysis, but overall there was a gradual decrease in F50 through the period after blood vessel transection with an increase in F95 which was small compared to the noxious stimulus alone group and a gradual decrease in ptot. The EEG changes due to vessel transection alone are illustrated in Figure 4.

## Interpretation of results of experimental studies

Comparison of the EEG changes seen with ventral-neck incision and those seen with each of the other two experimental procedures can be interpreted to indicate the following:

### F50

The increase seen following noxious stimulation is opposite to the decrease seen following vessel incision. The absence of change seen with ventral-neck incision would appear to be due to the summation of these two processes.

### F95

The increase seen following ventral-neck incision appears to be largely due to the effect of noxious stimulation rather than the interruption of bloodflow.

### Ptot

The immediate and large though transient increase is difficult to explain from the data presented in these studies. We suspect that it is due to the electrical effects of the contracture of the strap muscles of the neck that are seen in all groups where these muscles are cut. The decrease in the period following this appears to be due to noxious stimulation rather than interruption of bloodflow.

When the EEG effects of ventral-neck incision are compared to those of the characteristic response to noxious stimulation seen with dehorning, it appears that there is a response that is qualitatively similar to the characteristic response even though this is somewhat masked by the effects of the concurrent physiological alterations.

## Effects of stunning on EEG

In a separate study, we investigated the EEG effects of stunning with a concussive captive bolt (Gibson *et al* 2009c). Stunning resulted in one of two different responses.



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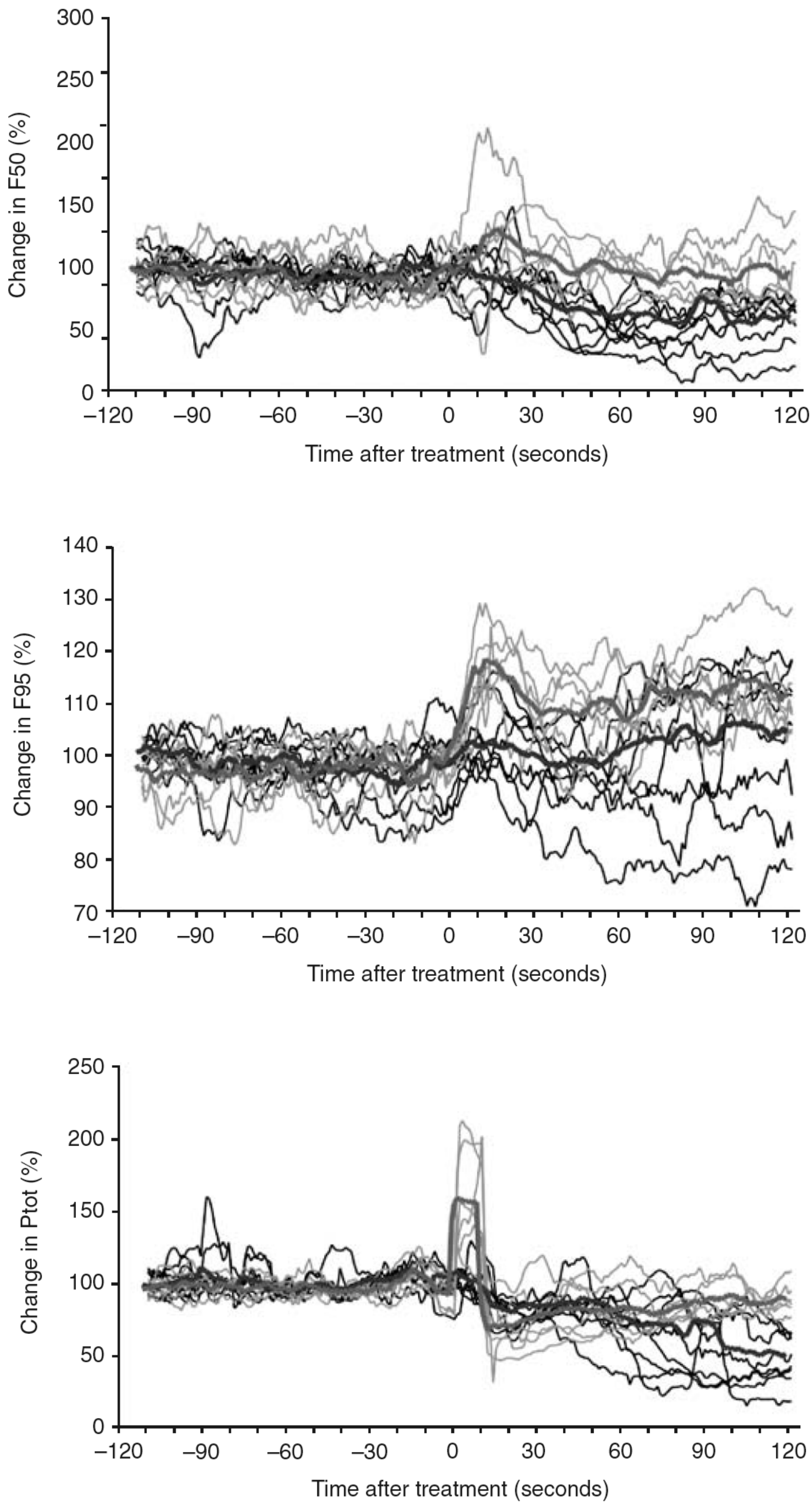
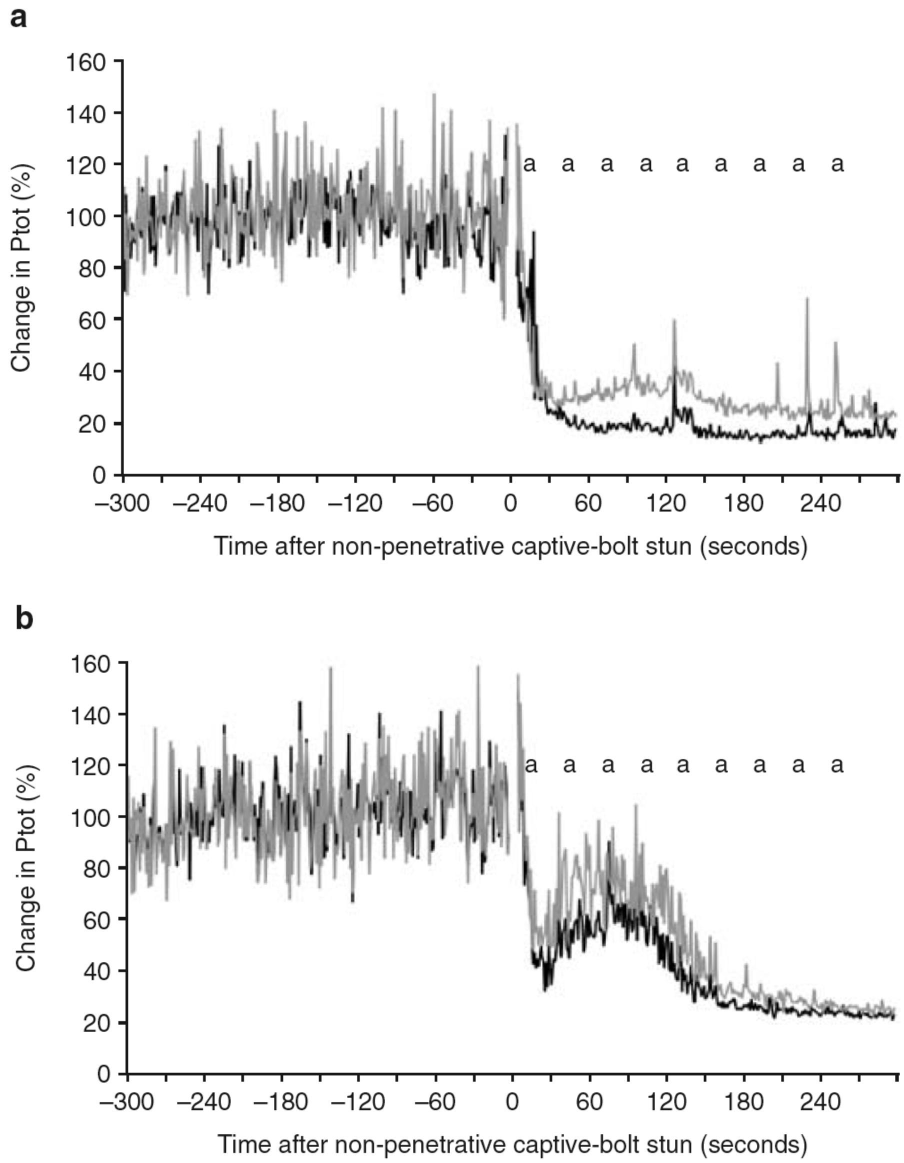
**Figure 4**




Electroencephalographic responses to neck-tissue transection of individual animals (light 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) for (top) median frequency, (middle) spectral edge frequency and (bottom) total power. Data are displayed as 10-point moving averages.
(From Figures 1, 2 and 3 from Gibson *et al* 2009b).

**Figure 5**




Percentage electroencephalographic changes relative to pre-treatment values in total power of the right (black line) and left (grey line) side of the electroencephalogram for (a) non-biphasic response (n = 4), (b) biphasic response (n = 5). [a] Significant difference from pre-treatment values, right cerebral hemisphere ($P < 0.05$).
(From Figure 2 from Gibson *et al* 2009c).

In one (non-biphasic) there was an almost instantaneous change to isoelectric EEG and in the other (biphasic) there was an almost instantaneous change to transitional EEG followed by a subsequent change to isoelectric EEG. Since both isoelectric EEG and transitional EEG are associated with an absence of awareness (Blackmore & Delany 1988), it is concluded that concussive captive-bolt stunning resulted in an almost immediate loss of awareness. The EEG changes due to stunning in the two groups are illustrated in Figure 5.

## Can stunning prevent EEG response to slaughter?

In a final study, we investigated the ability of stunning with a concussive captive bolt delivered 5 s after ventral-neck incision to obtund EEG responses to ventral-neck incision (Gibson *et al* 2009d). This study demonstrated that concussive captive-bolt stunning following ventral-neck incision was effective in abolishing the cerebrocortical responses that would be seen subsequently if the stun were not applied.



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## Political sequelae to slaughter studies

Following the publication of these studies the issue of slaughter without prior stunning has been considered by a number of governments and state authorities. For example, in New Zealand, changes were made to prevent the commercial slaughter of animals without prior stunning. As a result of a recent legal challenge an interim settlement was reached with the New Zealand Jewish Authorities. This settlement permits the non-stunning slaughter of a limited number of poultry for domestic consumption (Anon 2010). This exemption does not extend to cattle. In the European Union, changes to food labelling are being considered that could ensure that all meat products originating from animals that were not stunned prior to slaughter will be clearly labelled as such. The authors are aware that Fatwas have been issued by three Middle Eastern Islamic authorities indicating that animals should be stunned prior to slaughter.

## Conclusion

The following conclusions can be drawn from these studies:

• The minimal anaesthesia model for pain evaluation has been validated in cattle by scoop dehorning (a known noxious stimulus) — the response was abolished by local anaesthesia;

• Ventral-neck incision elicits a cerebrocortical response that would be experienced as pain for the duration of consciousness;

• Severing only the exposed vasculature did not elicit a response indicating this is not responsible for the cerebrocortical response;

• Transecting the neck tissues elicited a response indicating that this is primarily responsible for the cerebrocortical response;

• Stunning causes an immediate cessation of all cerebrocortical activity associated with continued awareness;

• Stunning can abolish any cerebrocortical effects due to ventral-neck transection.

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