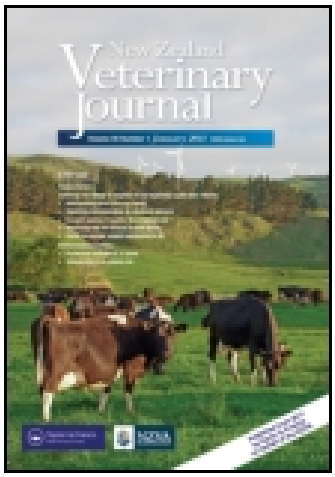

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Scientific Article

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

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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 pain 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

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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; MedSource 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 CO_2 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 resharpened 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 underscale 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 <⅛ 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 (Multiparin, 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

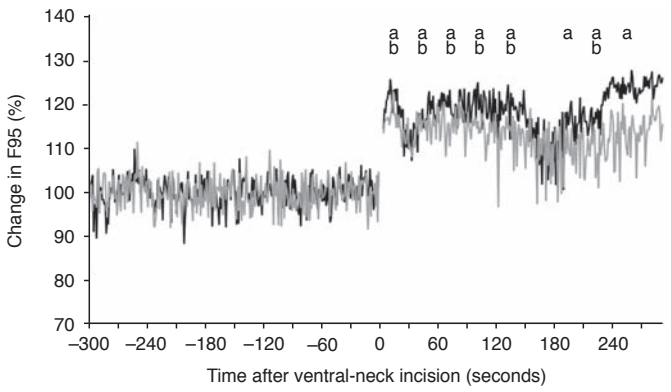

Figure 1. Percentage change in the mean 95% spectral edge frequency (F95), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following ventral-neck incision at time point 0. [a]Significant difference from pre-treatment values, right cerebral hemisphere ($p<0.05$). [b]Significant difference from pre-treatment values, left cerebral hemisphere ($p<0.05$).

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.

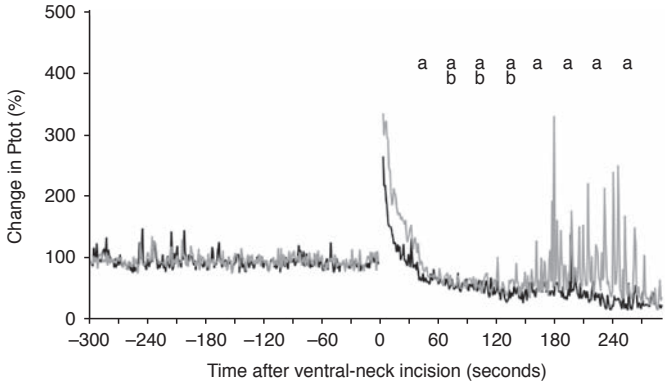

Figure 2. Percentage change in the mean total power (Ptot), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following ventral-neck incision at time point 0. [a] Significant difference from pre-treatment values, right cerebral hemisphere (p<0.05). [b] Significant difference from pre-treatment values, left cerebral hemisphere (p<0.05).

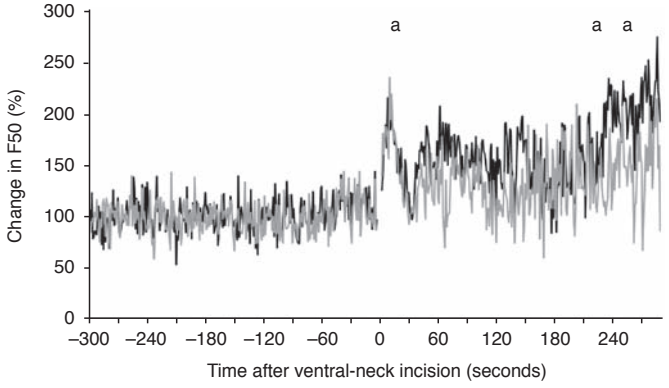

Figure 3. Percentage change in the mean median frequency (F50), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following ventral-neck incision at time point 0. [a] Significant difference from pre-treatment 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 (S 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

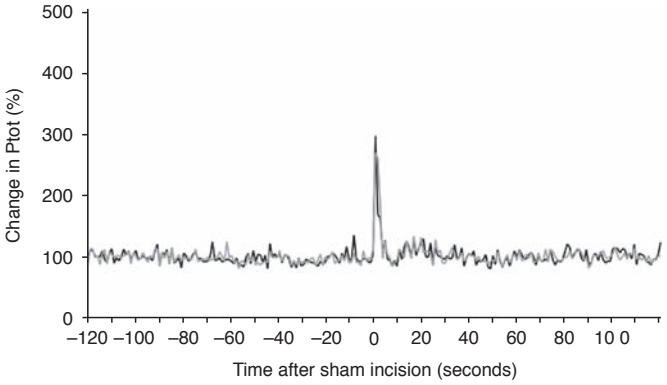

Figure 4. Percentage change in the mean total power (Ptot), relative to pre-treatment values, of the right (black line) and left (grey line) side of the electroencephalogram of halothane-anaesthetised calves following application of a non-noxious sham incision at time point 0.

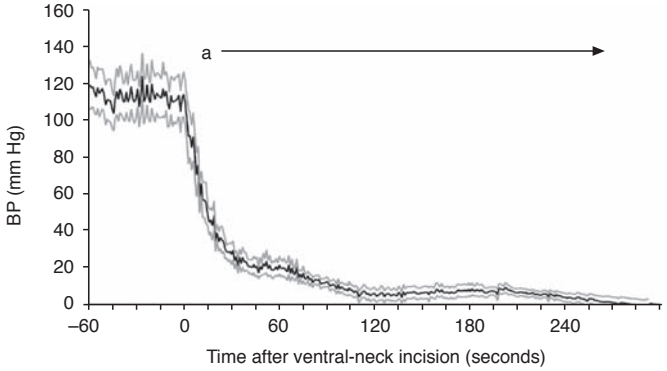

Figure 5. Mean ± SEM femoral arterial blood pressure (BP; mm Hg) of halothane-anaesthetised calves before and after ventral-neck incision. [a] Significant difference from pre-treatment values (p<0.05).

Table 1. Mean (± 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 (± SEM) (bpm)	P-value[a]
–15[b]	130.0 ± 9.36	na
0[c]	129.1 ± 9.29	0.916
15	127.7 ± 20.34	0.318
30	104.3 ± 5.92	0.031
45	105.3 ± 5.85	0.052
60	102.3 ± 5.60	0.031
75	105.0 ± 5.37	0.074
90	105.1 ± 4.69	0.052
105	105.4 ± 5.08	0.052
120	104.7 ± 7.12	0.083
135	111.9 ± 7.46	0.227
150	125.1 ± 7.20	0.793
165	135.3 ± 8.38	0.875
180	140.7 ± 9.05	0.372
300 (5 minutes)	137.6 ± 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.

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