Scientific Article

Components of electroencephalographic responses to slaughter in halothane-anaesthetised calves: Effects of cutting neck tissues compared with major blood vessels

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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.

KEY WORDS: *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,

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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 Services, 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 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

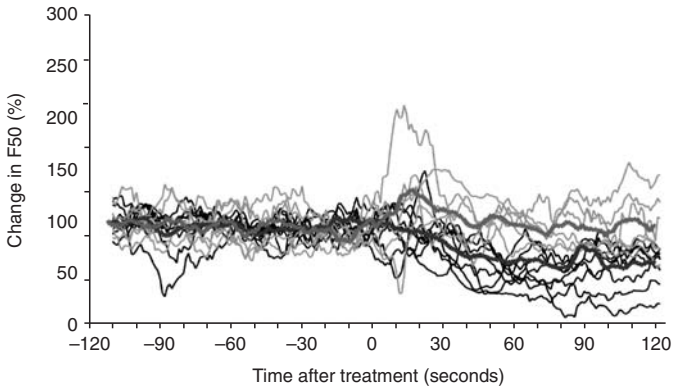

Figure 1. Percentage change in the median frequency (F50), relative to pre-treatment values, of the electroencephalogram of halothane-anaesthetised calves following neck-tissue transection of individual animals (grey line) and the group mean (thick grey line), or blood-vessel transection of individual animals (black line) and the group mean (thick black line). Data are displayed as 10-point moving averages.

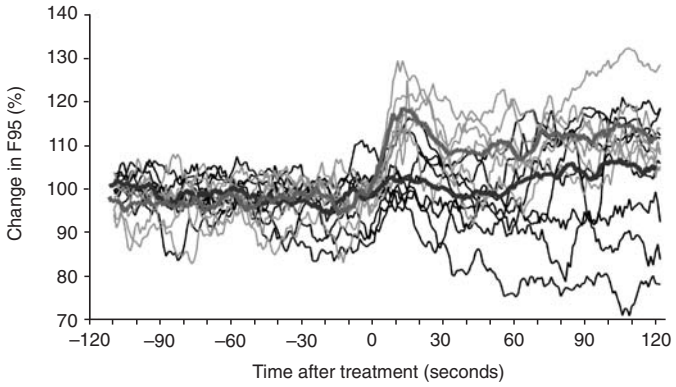

Figure 2. Percentage change in the 95% spectral edge frequency (F95), relative to pre-treatment values, of the electroencephalogram of halothane-anaesthetised calves following neck-tissue transection of individual animals (grey line) and the group mean (thick grey line), or blood-vessel transection of individual animals (black line) and the group mean (thick black line). Data are displayed as 10-point moving averages.

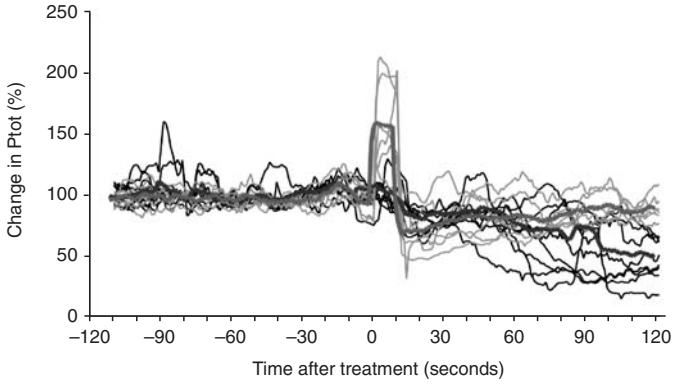

Figure 3. Percentage change in the total power (Ptot), relative to pre-treatment values, of the electroencephalogram of halothane-anaesthetised calves following neck-tissue transection of individual animals (grey line) and the group mean (thick grey line), or blood-vessel transection of individual animals (black line) and the group mean (thick black line). Data are displayed as 10-point moving averages.

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 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.

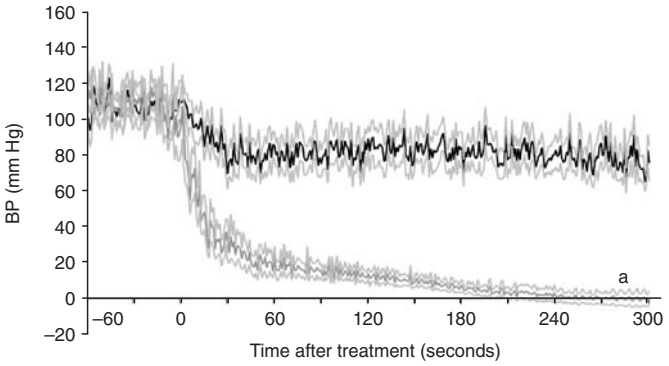

Figure 4. Mean ± SEM (either side of data lines) femoral arterial blood pressure (BP; mm Hg) of halothane-anaesthetised calves following neck-tissue transection (grey line) or blood-vessel-transection (black line) at time point 0. [a] Significant difference between treatment groups ($p<0.001$).

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 Ptot 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.

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