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The effect of electrical head-to-chest stunning on the EEG in sheep

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Abstract

Head-to-body stunning is regarded as 'best practice' stunning for sheep. The benefits are loss of consciousness followed by cardiac arrest, death, prevention of animal movements post stun/kill and improved meat quality. Commercial equipment places electrodes on the head and back, which is known to cause pelt burning, thus reducing the value of the skins. The aim was to demonstrate that passing current at 1.5 A and 50 Hz from the top of the head to the chest in lambs for 3.1 s would result in epilepsy. Electroencephalographic (EEG) and electrocardiographic (ECG) activity was recorded in sheep using non-invasive electrodes. Measurements in this trial were successfully performed on three lambs (live weight 25 to 39 kg) which were anaesthetised and given neuromuscular blockers to inhibit muscle activity. EEG information showed that the head-to-chest stunning produced an epileptic-like episode, which was followed by an isoelectric output. ECG recordings showed that ventricular fibrillation (VF) was induced and coincided with the epileptic brain activity observed. No animals regained brain activity or sinus heart rhythm after applying the stated stunning conditions. As a conclusion, it is postulated that modified stunning equipment passing an electrical current from the top of the head to the sternum in lambs (1.5 A, 50 Hz; 3.1 s) may induce an epileptic seizure and VF.

Keywords: *animal welfare, EEG, electrical stunning, head-to-back, head-to-chest, sheep*

Introduction

In the European Union sheep may only be slaughtered for human consumption after stunning in accordance with the methods and specific requirements set out in EC regulation 1099/2009. The loss of consciousness and sensibility shall be maintained until the death of the animal. With electrical head-to-body stunning, the risk that animals could regain consciousness during bleeding is almost eliminated, even by poor bleeding, due to induction of epilepsy and ventricular fibrillation (VF) (Blackmore & Petersen 1981; Gregory & Wotton 1984; Anil & McKinstry 1992), provided that VF leads to permanent cardiac arrest. An additional advantage is reduction of clonic activity, which facilitates handling of the carcase and, as the heart stops functioning during stunning, problems with blood spots around ruptured capillaries in the carcase are minimised (Gregory 1998). The method has been in use for years, using commercially available equipment where one electrode is placed on the sheep's head, the other on the back, in line with the shoulder and above the heart. Such equipment may, however, cause pelt burning (Gilbert *et al* 1984; Haluk Anil 2012) and a substantial reduction in value of the skin.

The aim of this study is to demonstrate that passing 1.5 A at 50 Hz from the top of the head to the sternum may induce epilepsy and VF in sheep. The literature discusses various ways to achieve head-to-body stunning and inducing VF, without impairing the quality of the skins (Gregory 1998), including head-to-chest/brisket and head-to-leg. To the authors' knowledge, there is no published work proving that placing electrodes over the head and sternum and applying alternating current (1.5 A at 50 Hz), will induce epilepsy.

Materials and methods

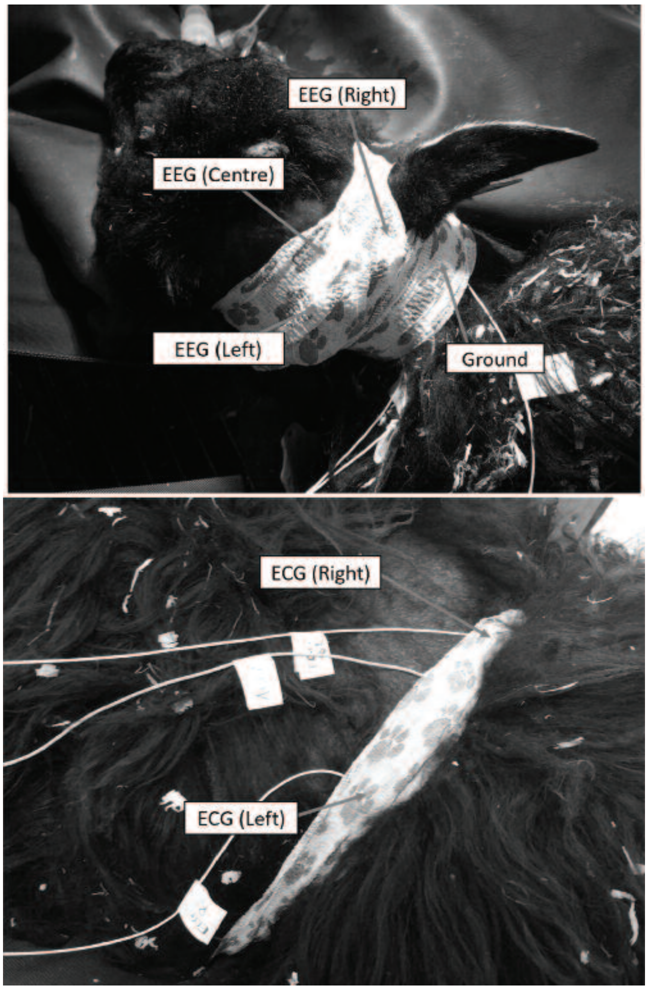
Approval

The experimental work reported in this paper was approved by the Norwegian Food Safety Authority (NFSA/Mattilsynet), application ID 12276.

Study animals

Lambs (n = 4) were transported to the Faculty of Veterinary Medicine, the Norwegian University of Life Sciences in Oslo, Norway. They were housed for four days in two pens with a concrete floor; bedding was provided and hay and water supplied *ad libitum*. One day, prior to the experimental work, the animals were weighed and shorn on the head and back. Live weights were between 25 and 39 kg. The number of animals used was based upon practical considerations, particularly availability of time and financial resources; four animals was considered sufficient to demonstrate the principle of head-to-chest stunning.

Figure 1



Positions of the electrodes for (upper) electroencephalograph (EEG) and ground, in addition to (lower) electrocardiograph (ECG).

Experimental set-up and instrumentation

For each animal a venous catheter was placed in the jugular vein, and then anaesthesia was induced by a 1:1 mixture, mean (range), intravenous dose of propofol (Propofol-Lipuro, Braun, Germany) 4.0 (3.6–4.3) mg kg^{-1} and ketamine (Ketamine Le Vet, Le Vet Beheer BV, The Netherlands) 4.0 (3.6–4.3) mg kg^{-1}. Next, endotracheal intubation was performed, and anaesthesia maintained using sevoflurane (SevoFlo, Zoetis, Finland) in 100% oxygen. The animals were then placed in a restraint that supported their head, mid-section and rear, leaving the legs free to move without touching the floor. Vital signs were monitored and positive pressure ventilation provided throughout the procedure. A neuromuscular blocker was administered (0.4 mg kg^{-1} mivacurium, Mivacron, Aspen Pharma Trading Ltd, Republic of Ireland) to inhibit muscular movement.

The head and neck of each animal was shaved, in addition to an area on the back, just in front of the hind legs, to enable cleaning and with placement of electroencephalographic (EEG) and electrocardiographic (ECG) electrodes, respectively. For EEG and ECG measurements, non-invasive AgCl water-based electrodes were placed on the animal. To ensure good contact, each electrode was soaked in water for > 5 min prior to application. Three EEG electrodes were placed on the head, with two placed on the centre-line between the ears with approximately 7–8 cm spacing, and an additional one placed at the mid-point of these electrodes, but approximately 2–3 cm forward of the centre-line. Two ECG electrodes were applied to the shaved area on the back of the animal, with approximately 15 cm spacing between them. One further electrode (ground) was placed on the animal's neck. All the electrodes were held in place using Petflex® flexible cohesive bandages (Andover Healthcare, USA) (50-mm width). Electrode positioning is illustrated in Figure 1.

Electrode resistance was < 30 kΩ, and all were connected via a 1-m shielded cable to a multi-channel instrumentation amplifier (16 channel Porti, TMSi, The Netherlands). The amplifier uses a Bluetooth interface to stream live data (1 kHz sampling rate) to a bespoke interface (Polybench, TMSi), where data are both displayed in real-time as well as being saved for later processing and interpretation. EEG and ECG data for all animals were collected for a minimum of 4 min post-anaesthesia induction; 2 min prior to stunning to collect baseline data and 2 min post-stunning.

Stunning

All animals were stunned with an electronic high frequency constant amperage stunner from FREUND (STUN-E513, Germany), using modified electrodes on the head and chest. The chest electrode was placed over the sternum and kept in place by the weight of the animal resting in the restraining 'hammock'. The head electrode was manually applied to the top of the head; above the eyes and in front of the ears. The stunning electrodes (which are illustrated in Figure 2) delivered a sinusoidal alternating current (AC) at 50 Hz (1.5 A, 400 V_{peak}) for 3.1 s. Prior to stunning, the head and chest electrode sites on the animal were treated with water soluble contact gel to optimise the effectiveness of the stun.

Ethical considerations

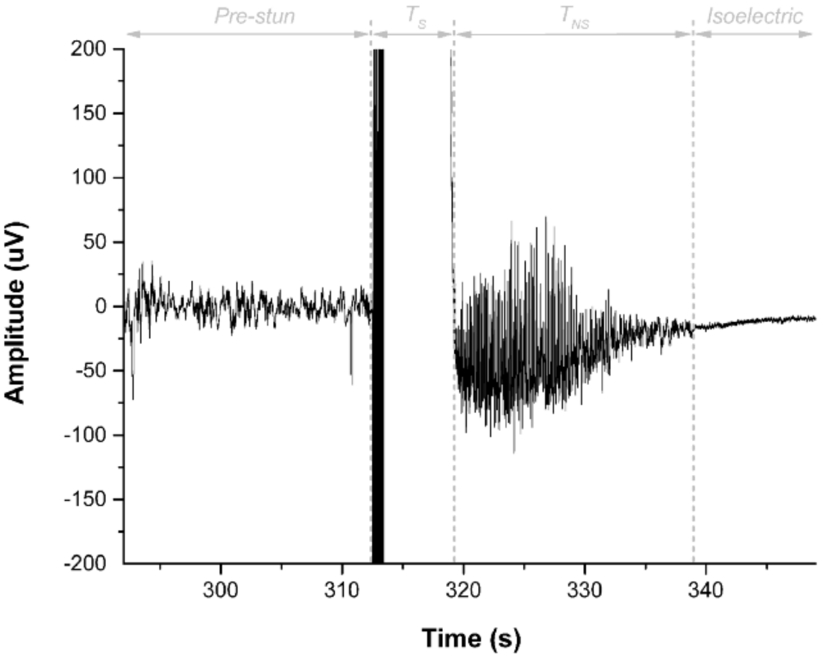
A number of studies make physical observations of animals undergoing electrical stunning procedures to assess consciousness (Blackmore & Newhook 1982; Verhoeven *et al* 2015). In this work, such observations were not made since the animals were anaesthetised and the use of a neuromuscular blocker inhibited movements or reflexes that would typically be assessed. Effect of the neuromuscular block was ensured by lack of response to a train of four stimulations of the facial nerve, but no further physical examination or observation of reflexes were made. To ensure unconsciousness prior to using neuromuscular blocker, the anaesthetic depth was assessed and the end-tidal sevoflurane monitored.

Figure 2

The chest and head stunning electrodes used for this work, shown left and right, respectively.

Figure 3

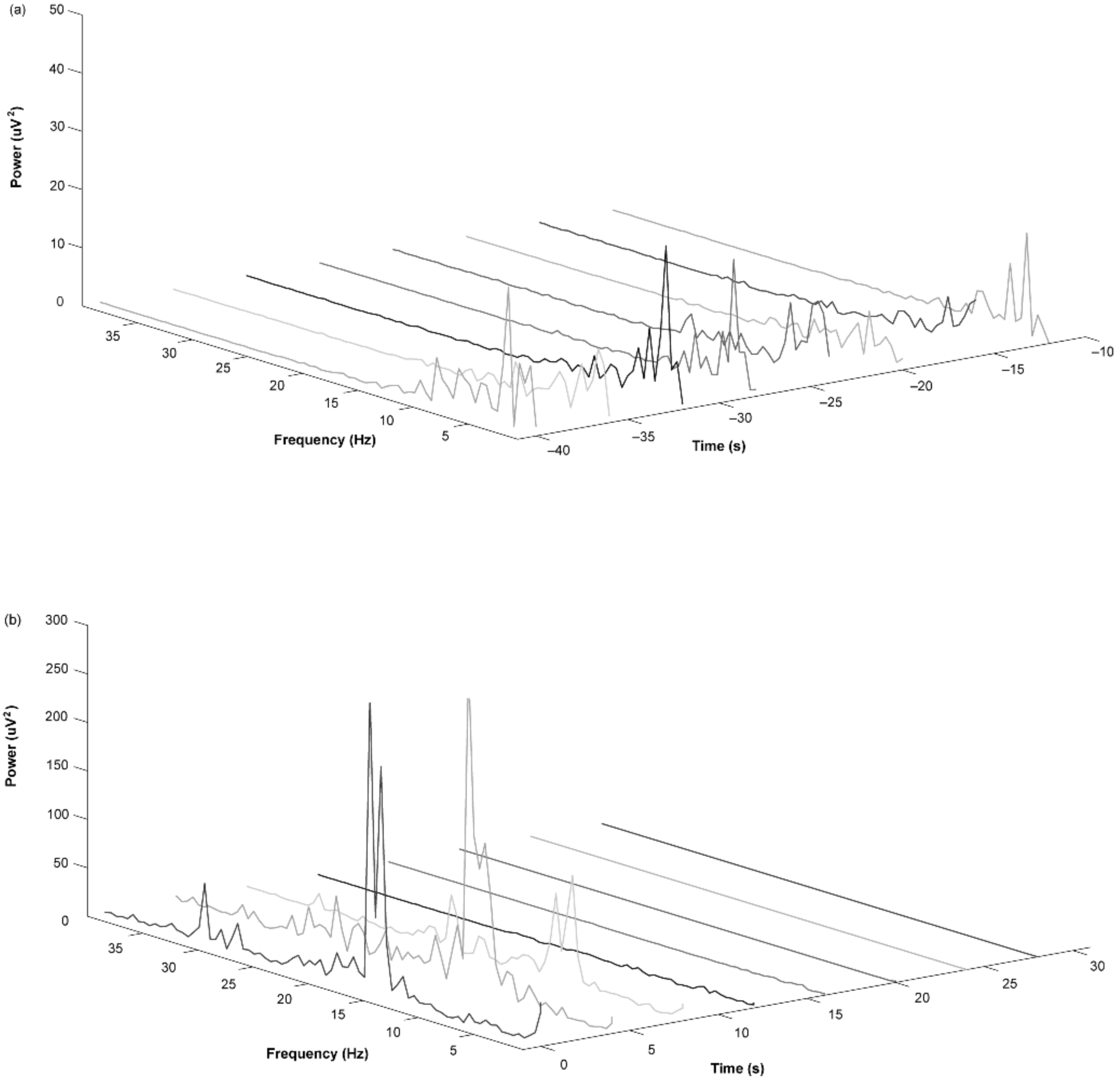
An example electroencephalogram (EEG) produced during the experimental work, which illustrates the typical signal traits in regard to pre-stun, the amplifier recovery period (T_R), a period of high signal amplitude (T_E) prior to an isoelectric output.



Data analysis

Raw EEG and ECG data were collected using PolyBench (TMSi). EEG data were post-processed using a band pass filter between 0.5 and 30 Hz. This post-processed EEG signal was considered in terms of the pre- and post-stunning signal amplitude and used to calculate the time required for the Porti amplifer to recover from input saturation after electrical stunning (T_R). The period during which epileptic activity occurred was also calculated (T_E), which ends when the signal becomes isoelectric. An isoelectric EEG indicates loss of sensibility. A number of methods have been used to define the point at which an EEG signal becomes isoelectric; Devine *et al* (1986), for example, say that T_E ends when the EEG signal amplitude falls below 10 uV. This work adopts the approach of Verhoeven *et al* (2015), who define isoelectric output to be the point where the EEG signal is < 10% of the pre-stun levels. This choice is based on the use of non-invasive electrodes — most earlier studies (Blackmore 1989; Cook *et al* 1995; Sánchez-Barrera *et al* 2014) use invasive systems which tend to exhibit more favourable signal properties, such as higher amplitude and quality (Ball *et al* 2009).

Figure 4



Fast Fourier Transform (FFT) output with respect to time for (a) the period 40 to 10 s prior to stunning, and (b) in the post-stunning period, after T_R has elapsed. These data represent an example from one of the animals under test.

In addition, a Fast Fourier Transform (FFT) of the EEG data sets was performed; each epoch represented a 4-s time-period. This enabled consideration of frequency composition with respect to time, and computation of total spectral power. Total spectral power is presented in the form of delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta (13–30 Hz) as described by Tatum (2014), and is calculated as an average of all successfully measured animals.

ECG data were also band pass filtered and used to determine pre-stun heart rate in each animal. Visual comparisons of ECG traces were also made to show the heart activity immediately post-stun, as well in periods 30 and 60 s post-stun.

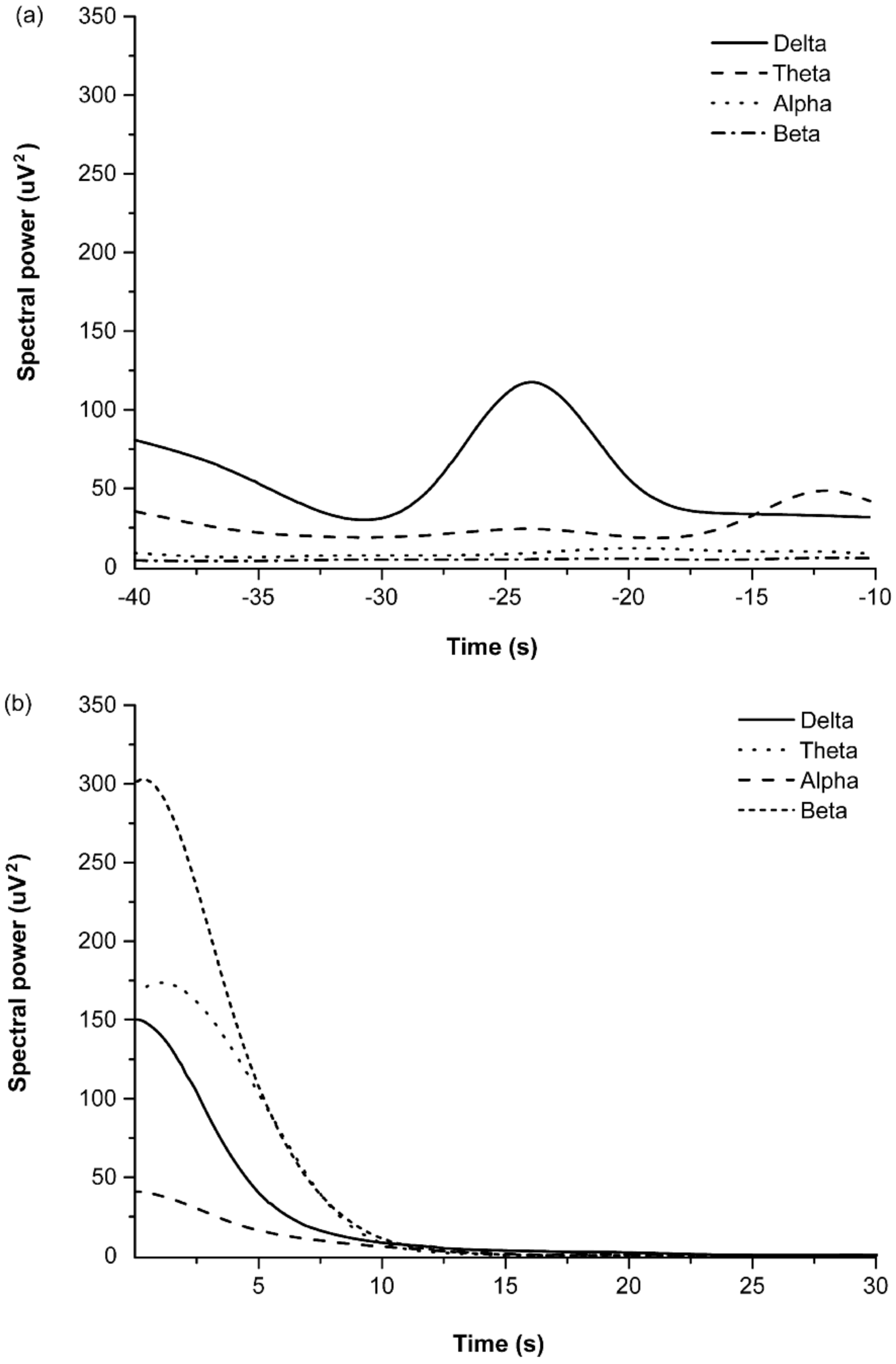
Results

Electroencephalogram activity

In three of the four animals tested, the EEG signal obtained was observed to be free of movement artefacts. An example of the EEG output observed is shown in Figure 3; notably it exhibits similar traits to other recent publications (Sánchez-Barrera *et al* 2014; Llonch *et al* 2015) where EEG measurements have also been used to assess the effectiveness of electrical stunning.

T_R lasted 9.2 s (σ = 2.1). The EEG from one of the animals remained saturated for a period of 28 s, and

Figure 5



Total spectral power (a) before and (b) after stunning, separated into delta, theta, alpha and beta frequency bands prior to and post-stunning.

Table 1 Total spectral power mean (± SD) values pre- and post-stunning.

Time (mins)	Delta	Theta	Alpha	Beta
Pre-stunning spectral power (uV^2)				
–40	80 (± 65)	35 (± 13)	8.7 (± 6.8)	4.2 (± 2.4)
–36	64 (± 58)	22 (± 5.8)	5.3 (± 1.3)	3.5 (± 2.1)
–32	26 (± 10)	18 (± 1)	7.7 (± 4.8)	4.8 (± 2.4)
–28	29 (± 10)	19 (± 7.2)	7.1 (± 3.6)	4.7 (± 2.7)
–24	160 (± 19)	27 (± 17)	8.0 (± 5.7)	4.8 (± 2.2)
–20	36 (± 18)	17 (± 9.5)	13 (± 13)	5.6 (± 3.9)
–16	33 (± 14)	18 (± 7.7)	9.1 (± 6.9)	3.8 (± 1.5)
–12	33 (± 16)	63 (± 45)	10 (± 5.9)	6.7 (± 4)
–8	29 (± 10)	18 (± 9.9)	6 (± 4.2)	4.4 (± 3)
–4	42 (± 20)	21 (± 11)	12 (± 10)	5.4 (± 3.5)
Post-stunning spectral power (uV^2)				
0	205 (± 19)	51 (± 17)	210 (± 22)	420 (± 54)
4	38 (± 14)	16 (± 18)	140 (± 19)	120 (± 16)
8	10 (± 7.1)	8.4 (± 9.8)	9.4 (± 12)	16 (± 21)
12	5.6 (± 4.9)	3.4 (± 4.1)	1.5 (± 1.9)	2.1 (± 2.5)
16	2.6 (± 1.8)	0.3 (± 0.2)	0.2 (± 0.1)	0.2 (± 0.2)
20	3.1 (± 3.6)	1 (± 1.2)	0.3 (± 0.3)	0.3 (± 0.4)
24	0.7 (± 0.3)	0.3 (± 0.1)	0.1 (± 0)	0.1 (± 0)
28	0.5 (± 0.3)	0.2 (± 0)	0.2 (± 0.1)	0.1 (± 0)
32	0.5 (± 0.1)	0.3 (± 0.2)	0.3 (± 0.1)	0.1 (± 0)

motion artefacts were noted in post-processing of the data collected. Therefore, EEG information from this animal is omitted from the paper. Epileptic activity (T_E) lasted for 21.2 s ($\sigma = 5.6$) and was followed by what appeared to be an isoelectric EEG. In total, the time between electrical stunning being applied and isoelectric output was 30.4 s ($\sigma = 6.6$).

Output from FFT analysis is shown in Figure 4, and Figure 5 illustrates the total spectral power in the delta, theta, alpha and beta frequency bands. Both sets of figures illustrate the case 40–10 s prior to stunning commencing, in addition to the 30-s period immediately after T_R elapses. These periods are shown to avoid artefacts present from electrode placement prior to stunning, in addition to the amplifier saturation period (T_R). Total spectral power mean and standard deviation values, as illustrated in Figure 5, are provided in Table 1.

Both Figures 4(a) and 5(a) confirm that prior to stunning most of the EEG and, therefore, brain activity, is concentrated in the delta and theta bands. However, immediately after T_R, there is a shift in recorded activity toward the alpha and beta bands (peaks of 173 and 302 uV^2, respectively), as shown in 4(b) and 5(b) which coincides with the period T_E. Activity in all bands then subsides to minimal levels — after 30 s all bands exhibit spectral power < 1 uV^2.

Electrocardiogram activity

All four animals produced a clear ECG signal. Animal heart rate in the period immediately prior to stunning was 82 bpm ($\sigma = 8.5$). In all animals, the stunning method resulted in loss of the sinus rhythm, which was determined through visual inspection of the ECG signal; examples are shown prior to stunning, in addition to the periods T_{R+0} to T_{R+10}, T_{R+20} to T_{R+30} and T_{R+50} to T_{R+60} in Figure 6(a) to (d), respectively. As time progresses, post-stunning, the VF signal amplitude is observed to decay. Based on previous experience (Mason *et al* 2017), it is noted the VF signal may continue for many minutes post-stunning, typically with a gradually decaying amplitude.

Discussion

For electrical stunning to be effective, the loss of consciousness and sensibility should be maintained until the death of the animal. In this study, anaesthetised animals were exposed to AC at 50 Hz (1.5 A, 400 V_{peak}) for 3.1 s via the use of electrodes placed on the head and over the sternum. Where EEG information was successfully obtained, it was shown that the head-to-chest stunning method produced an epileptic-like episode followed by an isoelectric output. Presentation of the EEG signals in terms of FFT (Figure 4) and total spectral power (Figure 5) demonstrated a reduction in brain activity post-stunning. ECG recordings also show that VF was induced and coincided with the epileptic brain activity observed. No animals regained brain activity or sinus heart rhythm after stunning.

Animal welfare implications and conclusion

The aim of this work was to assess whether head-to-chest electrical stunning could provide a suitable alternative to the widely used method in Norway of head-to-back. The EEG and ECG results obtained show that head-to-chest stunning can be effective for inducing epileptiform seizure and ventricular fibrillation. None of the animals in this experiment were observed to regain consciousness or sensibility. However, it is not possible to provide statistical information regarding the likelihood of a single animal not having a seizure and only having ventricular fibrillation, as that would require testing a large number of animals under commercial conditions. This would be rather complicated to achieve due to the likelihood of motion and muscular artefacts becoming dominant factors within the EEG recordings — a similar situation exists for head-to-body stunning.

Acknowledgements

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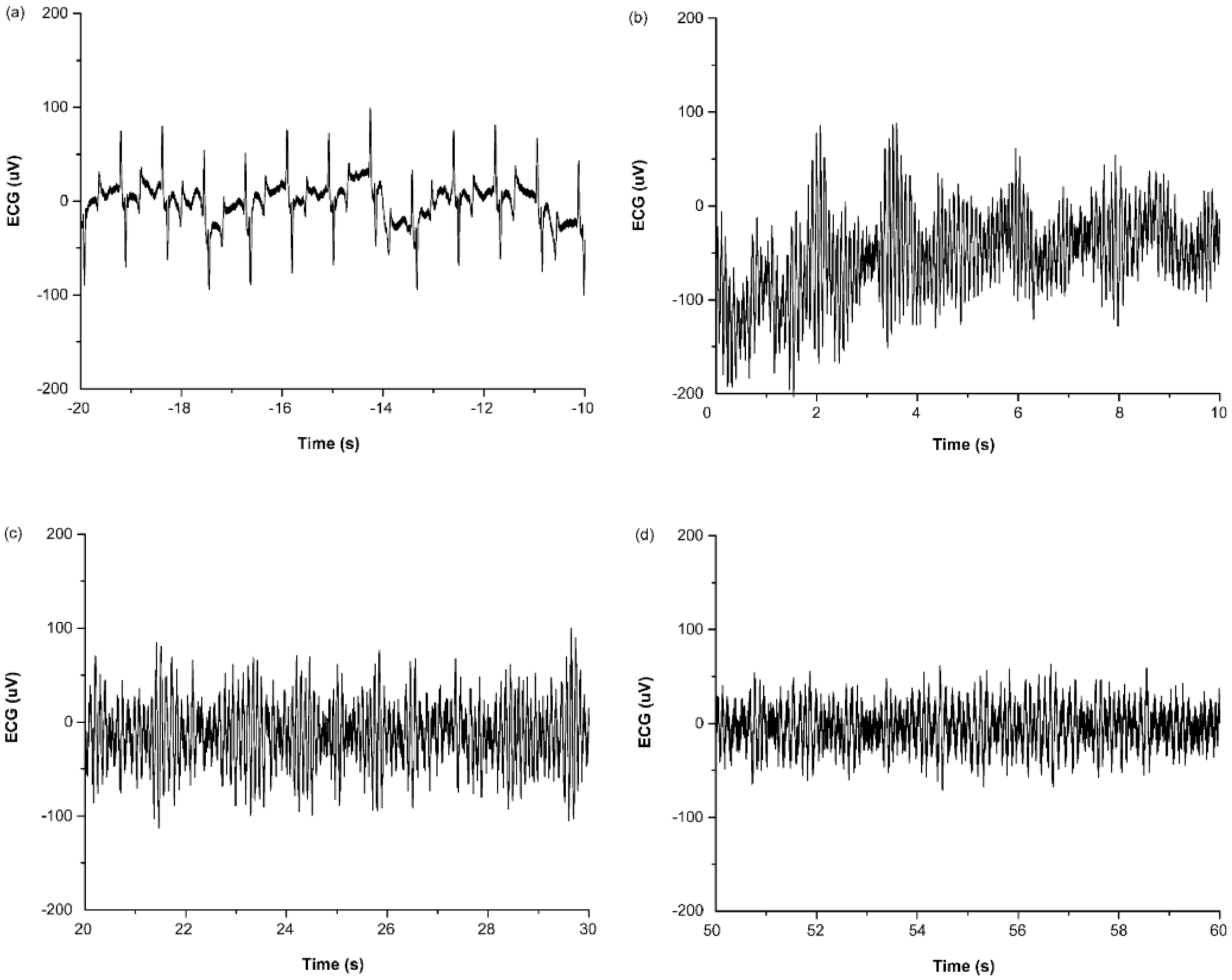
Figure 6



Electrocardiogram (ECG) output recorded (a) prior to stunning, (b) T_{R+0} to T_{R+10}, (c) T_{R+20} to T_{R+30} and (d) T_{R+50} to T_{R+60}.

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