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Electroencephalographic assessment of pneumatically powered penetrating and non-penetrating captive-bolt stunning of bulls

Troy John Gibson[a,*], Steffan Edward Octávio Oliveira[b,c], Filipe Antonio Dalla Costa[b,c], Neville George Gregory[a]

[a] Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, London, United Kingdom
[b] Programa de Pós–graduação em Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, 14.884–900 Jaboticabal, SP, Brazil
[c] Grupo de Estudos e Pesquisas em Etologia e Ecologia Animal, Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, 14.884–900 Jaboticabal, SP, Brazil

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ABSTRACT

The electroencephalographic (EEG) responses of 31 bulls (zebu crossbred cattle) stunned with either pneumatically powered Jarvis penetrating (PCB) or non-penetrating captive bolt (NPCB) was examined. Animals were organized into two treatment groups: PCB ($n = 20$); and NPCB ($n = 11$) stunning, both using airline pressures of 220 psi (1517 kPa). All bulls shot with PCB ($n = 20/20$) had patterns of EEG activity that were inconsistent with consciousness. Of the cattle shot with NPCB 82% ($n = 9/11$) showed waveforms suggesting complete unconsciousness. After stunning two NPCB bulls had periods of normal EEG activity and maintenance (Ptot, delta, theta, beta) or increased (alpha) spectral power compared to pre-treatment values, indicating incomplete concussion. The study showed that pneumatic PCB stunning was effective in rendering all bulls unconscious, while NPCB was less effective. This highlights the potential animal welfare risks associated with NPCB compared to PCB stunning of mature bulls in commercial abattoirs.

1. Introduction

Brazil is the world's largest beef exporter (Cerri et al., 2016), with 40.4 million head of cattle slaughtered in 2014 (FAOSTAT, 2014). The majority of these animals are stunned prior to slaughter by either pneumatically powered penetrating captive bolt (PCB) or non-penetrating captive bolt (NPCB). Despite the widespread use of pneumatically powered devices both in Brazil and elsewhere, there is very limited published information on their effectiveness in inducing reliable long lasting or irrecoverable unconsciousness in cattle. Recent work by Oliveira, Gregory, Dalla Costa, Gibson, and Paranhos da Costa (2017) and Oliveira et al. (2018) has examined the role of airline pressure on velocity, and the behavioural signs and reflexes associated with incomplete concussion following pneumatic PCB and NPCB stunning of different classes of Brazilian beef cattle. Variations in airline pressure were found not to be the major determinant of successful stunning, rather it was the related velocity and the resulting kinetic energy that was delivered to the brain that was found to be more important. Based on behavioural responses these studies suggested that only airline pressures of 190 psi (1310 kPa) and above should be used for stunning cattle with pneumatically powered PCB (Oliveira et al., 2017; Oliveira, Gregory, et al., 2018). A further study by the same authors found that for PCB stunned cattle shot at lower airline pressures and velocities, there was only superficial damage to the brainstems and cerebrums, and that damage increased in severity with increasing airline pressure (Oliveira, Dalla Costa, Gibson, Dalla Costa, & Gregory, 2018). Atkinson (2016) examining behavioural signs and reflexes in an audit of Swedish cattle abattoirs, reported that in one abattoir pneumatic PCB stunning produced adequate stuns in 96% of bulls, compared to only 64% with a cartridge powered PCB. Furthermore, the authors reported in a limited macroscopic examination of 3 heads (2 pneumatic and 1 cartridge PCB) more extensive haemorrhage with the pneumatic compared to the cartridge PCB over the surface of the brain and heavier bleeding around the brainstem (Atkinson, 2016). Oliveira, Dalla Costa, et al. (2018), in a study of 40 Nelore cattle shot with airline pressures of 160, 175, 190 psi for PCB and 220 psi (1103, 1207, 1310 and 1517 kPa respectively) for NPCB, reported that increasing airline pressures for PCB resulted in more extensive brain damage. At the lowest pressure (160 psi; 1103 kPa) two shots had failed to perforate the skull (20%). Only 190 psi (1310 kPa) caused lacerations in the midbrain and pons. Meanwhile pneumatically powered NPCB produced more extensive haemorrhage around the cerebrum but failed to cause macroscopic

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[*] Corresponding author.
E-mail address: tgibson@rvc.ac.uk (T.J. Gibson).

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damage to the brainstem.

Despite the lack of information on pneumatically powered devices, there has been widespread research on the time to loss of consciousness for cartridge powered PCB and NPCB devices for *Bos taurus* cattle. These studies have focused on changes in amplitude and morphology of the EEG and the onset of isoelectric EEG (Gibson et al., 2009a, 2009b; Verhoeven, Gerritzen, Hellebrekers, & Kemp, 2016), changes in the EEG power spectra (Gibson et al., 2009a, 2009b; Zulkifli et al., 2014), loss of righting reflexes (Blackmore, 1979), loss of evoked potentials (Daly, Gregory, & Wotton, 1987; Daly, Kallweit, & Ellendorf, 1988) and loss of brainstem reflexes (Atkinson, Velarde, & Algers, 2013; Blackmore, 1979; Gouveia, Ferreira, Roque de Costa, Vaz-Pires, & Martins da Costa, 2009; Gregory, Lee, & Widdicombe, 2007; Verhoeven et al., 2016; von Wenzlawowicz, von Holleben, & Eser, 2012).

The EEG is a representation of the functional activity of the brain. Unlike assessment of behaviour during and after stunning, assessment of the EEG provides a more direct indication of the disruption of brain function and in some situations is considered a more reliable indicator of when undoubted unconsciousness is present. The aim of the study was to examine the electroencephalographic responses of mature bulls to PCB and NPCB captive bolt stunning.

2. Materials and methods

The study was carried out during routine stunning and slaughter at a Brazilian beef abattoir. This project was approved by the Universidade Estadual Paulista Committee of Ethical Use of Animals and the Royal Veterinary College Clinical Research Ethical Review Board. Thirty one cattle were used in the study, these were all mature crossbred finished bulls (approximately 30 months old, non-breeding Zebu/Hereford, Angus, Braford crossbred) (over 550 kg liveweight). Cattle were shot by two slaughtermen with either the pneumatically powered penetrating (USSS-1, Jarvis Products Corporation®; PCB) or the non-penetrating (USSS-2A, Jarvis Products Corporation®; NPCB) captive bolt guns. Animals were organized into two treatment groups: PCB ($n = 20$); and NPCB ($n = 11$) stunning, both operating at airline pressures of 220 psi (1517 kPa).

Prior to EEG recording electrode placement and captive bolt stunning all animals were individually restrained in a stunning pen equipped with a head yoke and chin lift (Beckhauser Ltd., Brazil). The animals heads and necks remained restrained during electrode placement, pre-treatment recording (20 s), stunning and post stunning (60 s). Restraint of the head and neck aided stunning, prevent displacement of recording electrodes, reduce movement artefact and electrical noise. After the completion of EEG recording, the electrodes were removed and the animals ejected out of the stunning pen, shackled and hoisted and then bled in accordance with routine procedure of the abattoir. If the stunning operator observed animals showing behavioural signs of incomplete concussion, they were reshot while restrained in the stunning pen in accordance with the standard operating procedure of the abattoir. However, due to the line speed, design of the stunning pen and positioning of the researchers it was not possible to record behavioural and brainstem indices of consciousness/unconsciousness.

For EEG recording all animals acted as their own controls with comparisons made between pre and post stunning and between treatments. One channel of EEG was recorded using three 24-gauge stainless steel subdermal electrodes (Neuroline Subdermal, Ambu Inc., Glen Burnie, MD, USA). The electrodes were placed in the skin in a three-electrode montage with: active (non-inverting) left of midline in-line with the back of the eyes; reference (inverting), over the left caudal aspect of the frontal bone (top of head) in-line with the front of the ears; and ground electrode caudal to the poll. Electrodes were further secured in position with duct tape to prevent displacement during stunning. Interelectrode impedance ranged between 1.0 and 1.4 kΩ (MkIII Checktrode, UFI, Morro Bay, CA, USA). Electroencephalogram signals were amplified and filtered with an analogue filter (Bio Amp, ADInstruments Ltd., Sydney, Australia) with low and high pass filters of 200 and 0.1 Hz, respectively. The signals were digitalised (1 kHz) with a 4/20 PowerLab (ADInstruments Ltd., Sydney, Australia) digital to analogue converter and recorded on a Sony laptop (Sony USA Inc. New York, NY, USA) for off-line analysis.

Electroencephalogram epochs contaminated by artefacts such as over- and underscale, large single spikes, or EMG were manually rejected from analysis using Chart 8.1.5 (ADInstruments Ltd). All waveforms were digitally filtered with a pass band of 0.1 to 30 Hz and traces were inspected visually and compared to baseline using the classification systems developed by Gibson, et al. (2009a). They were classified into one of five categories: (1) Movement artefact; (2) Normal EEG; (3) Transitional EEG, (4) High Amplitude Low Frequency (HALF) EEG and (5) Isoelectric EEG. Normal EEG represents activity that is similar in amplitude and frequency to baseline period. Transitional EEG was classified as suppressed activity of having either an amplitude of less than half of that of the pre-treatment EEG. HALF EEG was a waveform of high amplitude and low frequency. Isoelectric EEG was classified as a trace with an amplitude of $< 1/8$ (12.25%) of that of normal pre-stunning EEG with little or no low frequency components. The EEG power spectra of uncontaminated epochs were analyzed. Fast Fourier Transformation with a Welch window was applied to 2 s epochs (pre-treatment and every 5 s post stunning), generating sequential power spectra with 1-Hz frequency bins. Subsequent analysis was performed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA).

Electroencephalogram spectral data were calculated and are displayed as percentage changes in total power (Ptot), delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (12–30 Hz) power from pre-treatment values. Data contamination by movement artefact was rejected from analysis. All data were analyzed using Prism 7.0c (GraphPad Software Incorporated, San Diego CA, USA). The distribution of the data was tested for normality using the D'Agostino & Pearsons normality test. Analysis of differences between EEG classifications was performed using a one-way ANOVA and the post hoc Tukey's multiple comparison test. Spectral data was analyzed with either a two-way ANOVA (post-hoc Tukey's multiple comparisons test) or the Kruskal-Wallis test (post-hoc Dunn's multiple comparisons test) depending on the distribution. The level of statistical significance was taken to be $p < .05$.

3. Results

The pattern of changes in EEG activity following captive bolt stunning, between and within captive bolt gun treatments groups, was not uniform (Figs. 1 and 2). For all animals, there were periods of movement artefact in the EEG immediately after shooting. The duration of the initial period of movement artefact varied between the treatments, with cattle shot with the PCB having a mean initial duration of 2.3 ± 0.2 (range 1–5) seconds and NPCB 1.6 ± 0.4 (range 1–4) seconds, however the difference was not significant ($p = .117$). For both treatment groups, in most animals movement artefact was followed by transitional EEG, with further bursts of movement artefact and transitional EEG before changing into isoelectric waveforms. There was no significant difference in the mean duration of transitional (PCB 9.1 ± 1.3; NPCB 13.0 ± 3.0 s ($p = .169$)) or isoelectric (PCB 15.8 ± 1.8; NPCB 18.3 ± 4.3 s ($p = .539$)) EEG between the treatment groups. The mean time to onset of isoelectric EEG was 11.6 ± 1.7 (range 1–27) and 8.2 ± 4.0 (range 1–26) seconds for PCB and NPCB respectively, however the difference was not significant ($p = .378$). There were periods of high amplitude low frequency (HALF) activity in 10 cattle in both treatment groups ($n = 5$ PCB; $n = 5$ NPCB), this either preceded or followed transitional activity.

Two animals shot with the NPCB (NPCB 8 and NPCB 11) had periods of apparently normal EEG activity after stunning (Fig. 2). In NPCB 8, this period lasted for 3 s, after which it received a second shot (Fig. 2, marked with arrow). After the second shot the EEG reverted to

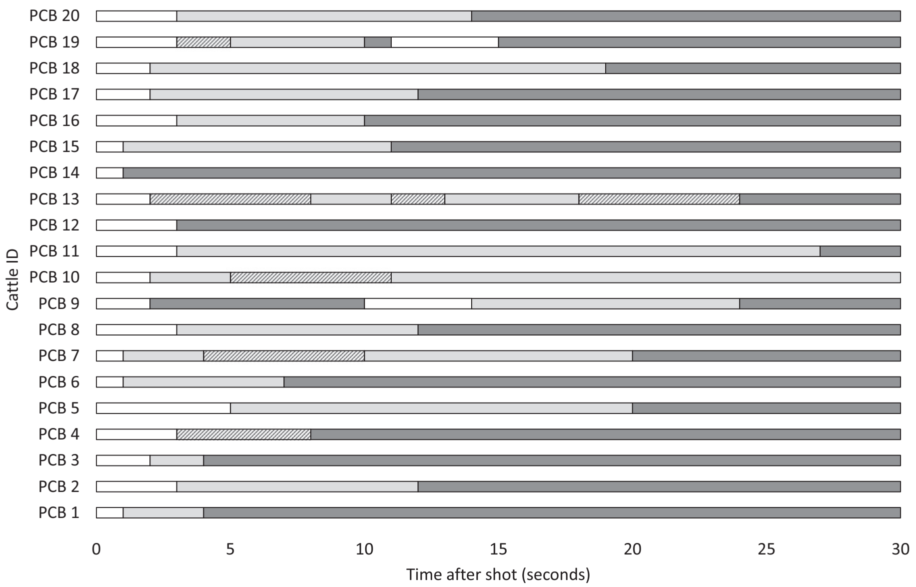

Fig. 1. Characteristics of the EEG in individual bulls shot with a pneumatically powered penetrating captive bolt gun (PCB; time point 0). White bars represent movement artefact; light grey transitional EEG; cross hatched high amplitude, low frequency (HALF); and dark grey isoelectrical EEG.

transitional activity. For NPCB 11 the period of apparently normal EEG lasted for 20 s before changing into HALF activity. This animal did not receive a second shot and was slaughtered and processed as per the abattoir's standard operation. These two animals belonged to the group of 8 animals that were shot by the second operator. It was anecdotally noted that this slaughterman was less experienced, less skilled and had more cases of repeat shooting in animals where EEG data was not recorded.

After PCB and NPCB (with incompletely concussed NPCB 8 and 11 removed) there was a significant decrease in Ptot (PCB $p < .004$; NPCB $p < .001$), as a percentage change from pre-treatment values (Fig. 3). Within 5 s of stunning Ptot had been reduced to 55% and 49% of pre-treatment values for PCB and NPCB respectively. Values continued to decrease to 32% and 36% of pre-treatment for PCB and NPCB respectively by the end of the 30 s data recording period. There were no significant differences between treatments in Ptot values. Ptot for NPCB 8 and 11 continued to be elevated compared to the rest of the NPCB group throughout the data recording period (Fig. 3); only decreasing to 54% of pre-treatment values by 30 s after the first shot.

After stunning, power in delta (PCB $p = .002$; NPCB $p < .001$) and beta (PCB $p = .002$; NPCB $p < .001$) frequency bands significantly decreased from pre-treatment values with no significant difference between treatments ($p = .799$) (Fig. 4A and D). In PCB shot animals theta power initially increased 5 s post stunning, then significantly decreased from pre-treatment values from 10 s ($p = .011$) (Fig. 4B). There was no significant difference between theta NPCB pre-treatment and post stunning power values ($p = .9$), there was also no difference between treatments ($p = .689$). Alpha frequency power for PCB significantly decreased from 15 s after the shot ($p = .015$) (Fig. 4C). Meanwhile for NPCB alpha power did not significantly change from pre-treatment values at any time point during the 30 s of the recording period ($p = .999$).

Delta power for NPCB 8 and 11 decreased to 44% of pre-treatment values 5 s after the shot, before increasing to 70% and remaining elevated compared to the rest of the NPCB group until 30 s after the shot. The power of theta and beta after stunning for NPCB 8 and 11 increased

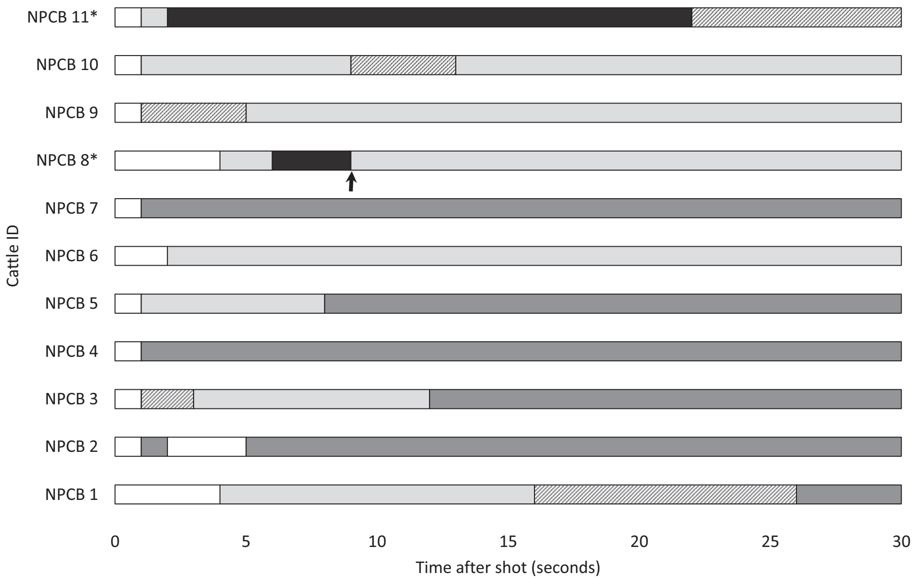

Fig. 2. Characteristics of the EEG in individual bulls shot with a pneumatically powered non-penetrating captive bolt gun (NPCB; time point 0). White bars represent movement artefact; light grey transitional EEG; cross hatched high amplitude, low frequency (HALF); dark grey isoelectrical EEG; and black bars represent normal active EEG activity (non-complete concussion). *NPCB 8 and 11 had periods of normal active EEG. ↑ denotes application of a second captive bolt shot to NPCB 8.

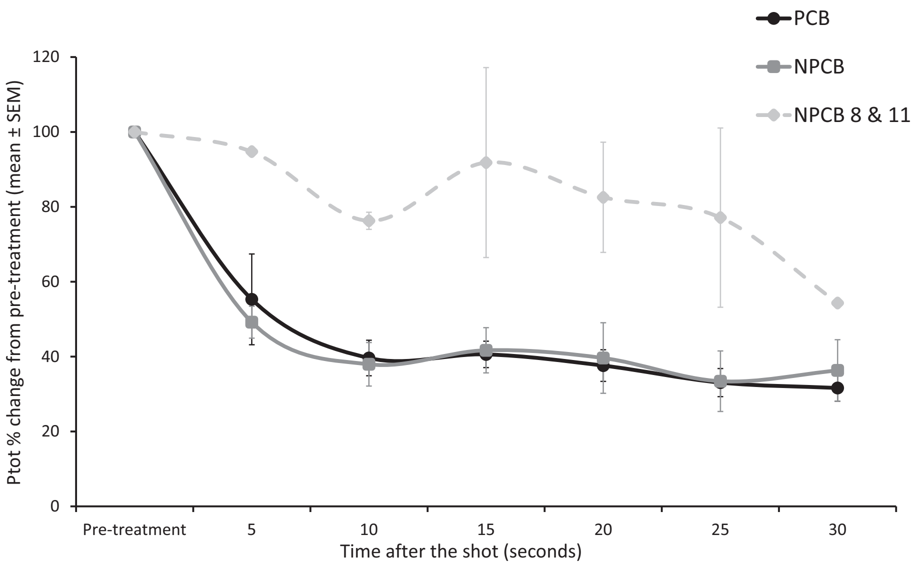

Fig. 3. Mean (± SEM) changes in total power (Ptot) of the electroencephalogram (EEG) of bulls before and after stunning with pneumatically powered penetrating captive bolt (PCB, black line), non-penetrating captive bolt (NPCB, grey line excluding NPCB 8 & 11) and NPCB 8 & 11 (dashed light grey line). Note this excludes periods of movement artefact.

compared to the rest of the group. Theta remained elevated (144–195%) during the entire recording period and beta (77–103%) returned to similar levels as the rest of the group by 30 s (56%). For NPCB 8 and 11, alpha power was biphasic with increases at 5 (269%) and 15 (152%) seconds after shooting, before decreasing to values similar to the rest of the treatment group.

4. Discussion

To the authors knowledge this is the first published examination of

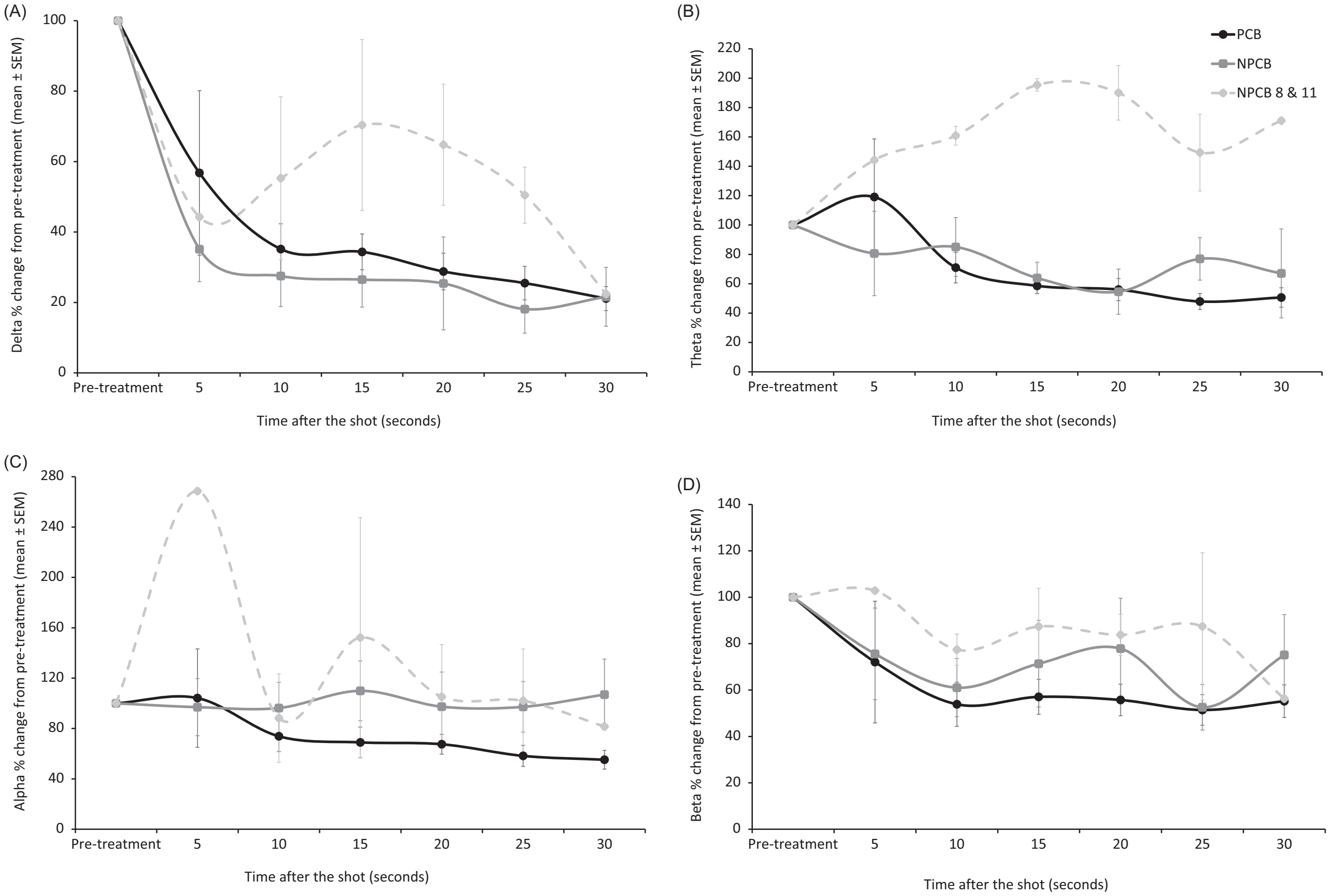

Fig. 4. Mean (± SEM) power of delta (A), theta (B), alpha (C) and beta (D) frequency bands of the electroencephalogram (EEG) of bulls before and after stunning with pneumatically powered penetrating captive bolt (PCB, black line), non-penetrating captive bolt (NPCB, grey line excluding NPCB 8 & 11) and NPCB 8 & 11 (dashed light grey). Note this excludes periods of movement artefact.

EEG activity in mature finished bulls (non-breeding) during pneumatically powered PCB and NPCB stunning in an abattoir operating under commercial conditions. All bulls shot with PCB ($n = 20/20$) had patterns of EEG activity (raw and spectral) that were inconsistent with consciousness. Of the bulls shot with NPCB only 82% ($n = 9/11$) showed waveforms suggesting complete unconsciousness.

Two bulls (NPCB 8 and 11) had periods of normal EEG activity after stunning, and maintenance (Ptot, delta, theta, beta) or increased (alpha) spectral power compared to pre-treatment, indicating incomplete concussion. One of these bulls was reshot within 9 s (NPCB 8) and the other (NPCB 11) had an extended period of normal EEG activity lasting 20 s, before changing to HALF. This last animal was not reshot and was ejected out of the stunning pen for shackling, hoisting and bleeding. It is possible that this animal could have regained or maintained some level of consciousness during part of the slaughter process. The maintenance of beta and increased power of alpha in the two incompletely concussed animals further suggests continued brain function with a shift towards de-synchronization and potential arousal, that could be associated with pain and distress (Gibson et al., 2009; Murrell & Johnson, 2006).

The two incompletely concussed animals were in a group of eight bulls that were shot on the last day of data collection by the second operator. The authors noted that this slaughterman was less experienced, less skilled and had more cases of repeat shooting in animals in which EEG data were not recorded. In the study it was not possible to record behavioural/brainstem indices of consciousness/unconsciousness and shot position. If these factors were recorded it would have been possible to test for associations between EEG activity and shot position plus behavioural indices. It also would have allowed the determination of failed shots due to the method of stunning from operator related factors. Despite this, having failed shots from an inexperienced but trained and certified slaughterman, highlights the difficulty associated with the stunning of bulls and the intolerance for variation in shot placement when using NPCB. Gregory et al. (2007) reported that young bulls were significantly more difficult to stun when using 4.5 g cartridge powered PCB, with 15.1% of young bulls having a shallow depth of concussion.

The EEG waveforms of successfully stunned bulls generally followed a pattern of transitional or HALF activity before becoming isoelectric. Transitional EEG had a different morphology from both pre-treatment active and isoelectric EEG, and has been characterised as being incompatible with consciousness/sensibility in mammalian (Blackmore & Delany, 1988; Gibson et al., 2009a, 2009b) and avian species (Gibson, Rebelo, Gowers, & Chancellor, 2018). The reported HALF EEG activity seen in 10 bulls, has been previously reported in PCB shot mature cattle (Daly et al., 1988), and is similar to the low-frequency delta and theta waveforms seen following successful PCB and NPCB stunning of calves (Groß, 1979; Lambooy & Spanjaard, 1981; Lambooy, Spanjaard, & Eikelenboom, 1981) and heifers/steers/cow (Fricker & Riek, 1981; Zulkifli et al., 2014). This activity has been reported in both humans (Bauer, 2005) and animals (Dennis Jr., Dong, Weisbrod, & Elchlepp, 1988) after clinical or experimental traumatic brain injury, and is seen during unconsciousness caused by concussive impacts (Shaw, 2002).

Following transitional and HALF activity, the EEG in 95% ($n = 19/20$) and 55% ($n = 6/11$) of bulls in the PCB and NPCB groups, respectively, became isoelectric and remained so until the end of the 30 s data collection period. An isoelectric waveform or electrocerebral silence is a flat EEG state that indicates complete and near irrecoverable brain dysfunction leading to brain death (Bauer, 2005). The remaining animals were either in transitional (PCB n = 1/20; NPCB $n = 4/11$) or HALF (NPCB n = 1/11) brain states. Although transitional and HALF activity are inconsistent with the presence of consciousness, they are intermediate brain states and it is possible that if the recording period and stun-to-stick interval were greater, brain activity would have altered. However, it is improbable that if after a prolonged period of transitional EEG that normal activity would have returned, but it is possible that the onset of isoelectric EEG would have been delayed. The duration of the data recording window post stun was beyond the control of the authors and was determined by the line speed of the abattoir. After this point EEG recording electrodes were removed, and the animal ejected from the pen, shackled and bled.

The time to onset of transitional activity was generally related to the decrease in Ptot, delta and beta power from baseline in successfully stunned bulls in both treatment groups (PCB and NPCB). Previous studies on concussive trauma have reported increased delta and theta power in addition to depression of higher frequency activity as indicators of concussion (Lambooy, 1982; Lambooy et al., 1981; Verhoeven et al., 2016; Zulkifli et al., 2014). In the current study, an increase in delta power was not observed in the successfully stunned bulls in either treatment group. However, theta power increased slightly during the 5 s post stunning period in bulls shot with PCB, before steadily decreasing in line with the NPCB group. This initial increase in PCB theta power was not significantly different to pre-treatment or NPCB group values. The differences between previous studies and the current results do not indicate incomplete concussion in these bulls, rather it shows suppression of EEG activity and near complete dysfunction of the brain following stunning. Williams and Denny-Brown (1941) reported a comparable immediate reduction in EEG activity (including low frequency) approaching isoelectric in cats subjected to concussive trauma to the head. Gibson et al. (2018) reported a decrease in Ptot following NPCB stunning of turkeys, where delta activity compared to other frequency bands makes a larger contribution to Ptot values. In the current study movement artefact immediately after stunning was removed prior to spectral analysis, this may have invariably removed some of the low frequency activity that other authors have reported.

After stunning beta power decreased in successfully stunned bulls in both treatment groups. Five seconds after stunning alpha power remained relatively unchanged compared to pre-treatment values, before decreasing for PCB shot bulls. However, alpha power remained elevated for NPCB shot bulls after stunning. The level of suppression of alpha and beta waveforms although not significant was less in successfully stunned NPCB compared to PCB bulls. These results suggest that although rendered unconscious the level of induced concussion with NPCB was less than that observed with PCB stunning. This is further supported by the two NPCB bulls that had periods of recovered EEG post shooting. It has been suggested that NPCB stunning is not as effective at stunning adult cattle as PCB (EFSA, 2004; Gerritzen & Gibson, 2016). Recent work by the authors examining behavioural (Oliveira, Gregory, et al., 2018) and pathological (Oliveira, Dalla Costa, et al., 2018) differences between pneumatic PCB and NPCB responses/damage confirmed that NPCB stunning with an airline pressure of 210–220 psi (1448–1517 kPa) was less effective at inducing unconsciousness and causing damage to brainstem structures. Increasing damage to these structures has been previously reported as being associated with unconsciousness in cattle and other ruminant species (Derscheid et al., 2015; Gibson et al., 2012, 2015; Gregory, Spence, Mason, Tinarwo, & Heasman, 2009; Grist, Lines, Knowles, Mason, & Wotton, 2018). NPCB is now not a permitted method for the stunning of mature cattle in the European Union (EU, 2009).

In conclusion, the study found that pneumatic PCB captive bolt stunning was effective in rendering 100% of mature finished bulls unconscious. Meanwhile pneumatic NPCB was only successful in inducing unconsciousness in 82% of the bulls, with two animals having periods post shooting of normal EEG activity. It could not be determined if this was an error associated with the stunner or operator's inexperience. Despite this, incomplete stunning will lead to compromised welfare in the form of pain and distress. This paper further highlights the animal welfare risks associated with NPCB stunning of mature bulls.

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