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Comparison of penetrating and non-penetrating captive bolt in an alternative occipital approach in calves

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Abstract

The objective of this study was to describe the effect of penetrating or non-penetrating captive bolt using an occipital approach in 4–5 month old, Holstein steers weighing between 100–200 kg. Twelve calves were divided into two treatment groups; penetrating captive bolt (PCB; n = 6) and non-penetrating captive bolt (NPCB; n = 6). This sample size was chosen out of convenience and in conjunction with a separate study. Each calf was sedated with xylazine hydrochloride, then a captive-bolt device, outfitted with a standard penetrating bolt or a non-penetrating bolt, was placed flush on the dorsal midline of the cranium at the external occipital protuberance and aimed downward as though to intersect the intermandibular area. Following impact, indicators for loss of consciousness, such as respiration, righting response, corneal reflex, movement and vocalisation were recorded and characterised along with electrocardiogram and electroencephalogram recordings. After a 5-min observation period, all calves were administered potassium chloride. All calves experienced immediate and sustained loss of consciousness. The mean (± SEM) time to cessation of respiration was 60 (± 53.67) and 0 (± 0.0) s for PCB and NPCB, respectively. The mean time to cessation of convulsions was 310.4 (± 79.74) and 180.0 (± 60.24) s, respectively, and the mean number of convulsions was 2.75 (± 1.03) and 2.0 (± 0.837) for PCB and NPCB, respectively. Isoelectric EEG patterns were observed in 3/5 PCB and 3/4 NPCB with mean time to onset of isoelectric pattern in 69.0 (± 52.24) and 113.5 (± 56.87) s. Both treatments induced a successful stun, which suggests these techniques are appropriate for humane euthanasia in calves of this age.

Keywords: *animal welfare, cattle, euthanasia, non-penetrating captive bolt, occipital approach, penetrating captive bolt*

Introduction

The use of captive-bolt devices is routine for stunning livestock and removal of sensibility to pain and distress prior to humane euthanasia or slaughter. The purpose of these devices is to apply concussive trauma to the calvarium in order to damage vital brain regions necessary for cortical integration, regulation of consciousness, respiration and cardiovascular function. Penetrating captive-bolt (PCB) devices, when discharged, eject a bolt through the skull into the brain. The ideal shot placement results in disruption of the brainstem and/or at least one cerebral cortical hemisphere (Leary *et al* 2013). Typically, loss of consciousness is instantaneous and appears to occur upon contact of the bolt with the skull (Daly & Whittington 1989; Gregory & Shaw 2000). The purpose of extending the bolt into the brain is to induce irreversible tissue damage within the target areas. In contrast, non-penetrating captive bolt (NPCB), when discharged, rapidly accelerates a flat, mallet-shaped bolt which, upon impact with the skull, rapidly transfers its kinetic energy into the skull and underlying brain tissue. This device does not however send a projectile into the brain to induce additional damage but relies on the transfer of kinetic energy. The ensuing concussive shock-waves and rapid impact of the brain against the antipodal wall of the calvarium induces the majority of the underlying injuries (Prins *et al* 2013) In addition, impact with NPCB often induces a depressed skull fracture leading to further tissue damage (Collins *et al* 2017).

The effectiveness of PCB in stunning cattle of various weights and ages has been well-documented and this technique has become the standard for stunning in slaughter facilities throughout the United States (Grandin 1991; Gregory *et al* 2007; Welty 2007; Leary *et al* 2013; United States Department of Agriculture, Food Safety and Inspection Service [USDA FSIS] 2013). However, there appears to be a preference in the literature toward using NPCB in smaller livestock, such as sheep and goats and/or neonates with fewer reports of the efficacy of these devices in adult cattle or older calves. Gilliam *et al* (2018) reported that NPCB was successful in euthanasing neonatal calves.

In addition, Gibson *et al* (2009a,b) extensively evaluated the physiologic and electroencephalographic response of older anaesthetised calves to NPCB and concluded the technique was sufficient to induce stunning. Grist *et al* (2018a,b,c) reported in a series of publications on the efficacy of NPCB in neonatal goats, piglets and lambs. Impacting adult goat cadavers with NCPB resulted in multiple fractures radiating from the site of impact and substantial damage to the cerebral hemispheres and brainstem (Collins *et al* 2017). There are, by comparison, fewer reports of the use NPCB in adult cattle and these studies have produced mixed results. Finnie *et al* (1995) provided a brief report on a review of behavioural and gross necropsy findings of cattle subjected to NPCB at slaughter and concluded the technique was acceptable when coupled with secondary means of euthanasia such as exsanguination. In contrast, Oliveria *et al* (2018) found that adult Nelore cattle were more likely to return to normal rhythmic respiration and require additional impacts with NPCB.

The most commonly employed anatomical landmarks for impact with captive bolt in cattle is a point, midline over the frontal bone, where two lines originating from the base of the horn and extending to the lateral canthus of the contralateral eye intersect. Both the European Union and United States Department of Agriculture (USDA) guidelines indicate that this approach is the most appropriate for cattle and the only approach recommended for adult cattle (Panel 2004; USDA FSIS 2013). An alternative approach, acceptable for use in adult, horned or polled, sheep and goats, is to place the device at the external occipital protuberance aimed so that the projectile tracks toward the base of the animal's chin (Gibson *et al* 2012; Sánchez-Barrera *et al* 2014; Collins *et al* 2017). While this technique is not explicitly ruled out in the guidelines for humane slaughter, there have been few reports of the efficacy of this technique in cattle.

Based on these studies, the age and weight of the animal, with the subsequent increases in thickness of bone and skin, appears to impart significant influence on the effectiveness of this technique. European guidelines for humane stunning of cattle consider NPCB unreliable for all ages and classes of cattle and suggest additional research into its application is required (Panel 2004). While USDA regulations do not explicitly prohibit the use of NPCB, there are unspecified suggestions that species, age and sex of the animal dictate the choice of device, ie older bulls, rams, and boars require penetrating devices (USDA FSIS 2013). Until an extensive investigation applying the independent factors of age, bodyweight, and sex on the biomechanics of anatomical location and type of device in a range of animals can be conducted, a reductive approach must be used to determine the effectiveness of these techniques in cattle of relevant production weight and age.

Therefore, the objective of this study was to compare the effectiveness of penetrating and non-penetrating captive bolt, utilising an occipital approach, in inducing loss of consciousness in calves weighing between 100–200 kg and aged 4–5 months. This weight and age range incorporates the typical characteristics of veal calves at the time of slaughter. Due to a lack of specific data in the scientific literature and regulatory guidelines regarding the effectiveness of captive bolt in veal calves, the overarching goal of this study was to provide a descriptive comparison of these two approaches. Our working hypothesis was that both techniques would effectively stun calves for a period of 5 min, whereupon a lethal solution of intravenous potassium chloride (KCl) was administered. Indicators of clinical effectiveness were assessed through brief neurological examination, assessment of respiratory and heart rate, rhythm and character, and recording of time to cessation of respiration, heart rate, and movement. The assessment of the effects on cardiac and brain function were carried out through electrocardiography (ECG) and electroencephalography (EEG) recordings. Finally, a post mortem evaluation was conducted to record the extent of damage produced in the target brain tissue of the cerebrum, cerebellum, and brainstem.

Materials and methods

Study animals

All activities described here were conducted under the approval of the University of Tennessee Institutional Animal Care and Use Committee (Protocol Number: 2469-0616). Twelve Holstein steer calves weighing a mean (± SD) of approximately 165 (± 38.02) kg (range: 102.73–205.00 kg) were acquired from two local dairy farms. In addition to the procedures described here, these animals were simultaneously enrolled in an additional study that contained scheduled euthanasia as a component of the experimental design. The contingents of the additional study dictated that the animals were individually housed and serially sacrificed at pre-determined times, two animals at each time-point, over the course of 14 days. Each calf was determined to be clinically healthy at the time of euthanasia and the activities of the additional experiment were not anticipated to influence the outcome of the current study. Upon enrolment in the euthanasia study, the calves were randomly allocated through use of a random number generator into one of two treatment groups; penetrating captive bolt (PCB; n = 6) and non-penetrating captive bolt (NPCB; n = 6). In addition, each calf's pre-determined scheduled euthanasia was randomised. During the course of the acclimation period one calf in the PCB group developed ruminal acidosis with acute septicaemia and subsequent pericarditis and was humanely euthanased outside the parameters described here with no euthanasia data recorded. That calf was therefore excluded from all analyses. An additional two calves in the NPCB group were excluded due to loss of contact with the electrodes during convulsive movements following impact. Due to loss of the electrodes the EEG and accelerometer data were excluded, however, the time to cessation of heart rate, respiratory rate, neurologic reflex, and post mortem data were maintained and reported.

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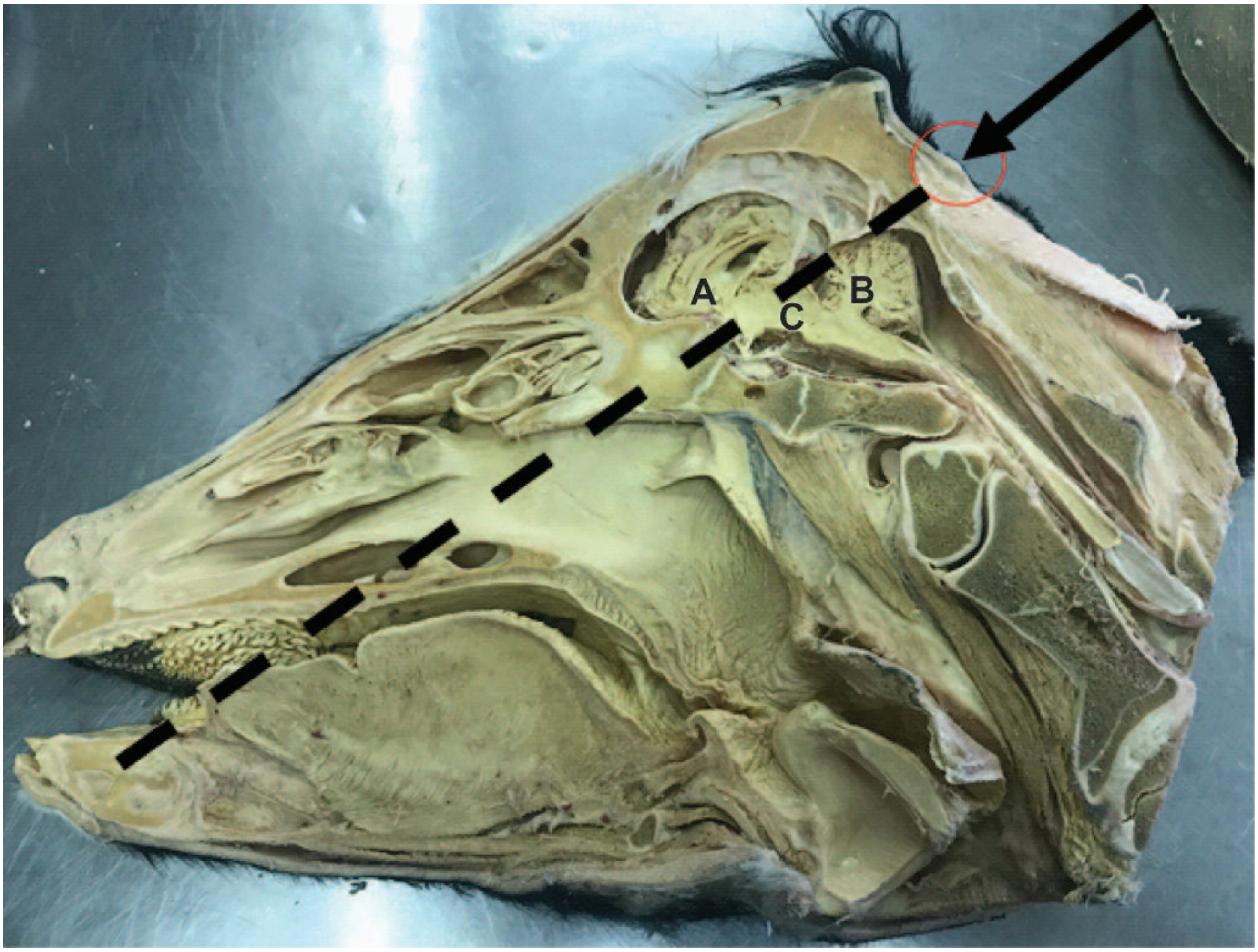
Figure 1



In the occipital approach, the captive-bolt device (black arrow) was placed on midline at the external occipital protuberance (red circle) and aligned so that the projected path of the bolt (black dashed line) would travel towards the intermandibular area or the tip of the tongue. The projected path of the bolt should disrupt either the cerebrum (A) or cerebellum (B) and the medulla oblongata (C).

Euthanasia procedure

At the time of euthanasia, each calf was sedated with xylazine hydrochloride (2 mg kg^{-1}, IM once, AnaSed® LA, xylazine hydrochloride 100 mg ml^{-1}, VetOne® MWI, Boise, ID, USA). Sedation was used to aid in the implementation of recording equipment and accurate shot placement. Calves became sternally recumbent within 5–10 min and were then outfitted for EEG and ECG. EEG and motion data were collected with the BioRadio device (Great Lakes Neuro Technologies, Cleveland, OH, USA) attached to a harness around the animal's neck. The EEG probes (gold cup electrodes) were placed around the shaved forehead of the calf with the reference channel placed in the centre of the forehead. Conductive electrode paste was used to interface the probes to the forehead of the animal, and tape was placed over the probes, supplemented with super glue on the edges, to limit sensor motion artefacts. Accelerometers were located inside the BioRadio device, where x, y, z-axis data were recorded to track animal motion/activity. All signals were sampled at 250 Hz with 16-bit resolution. Data were recorded prior to impact for up to 3 min and up to 8 min after the event. The time of the bolting events were noted manually and also confirmed with the distinctive bolting signature in the motion data. ECG data were monitored with a Cardell Veterinary Monitor 9403 (Midmark Co, Tampa, FL, USA) and leads were positioned in a base-apex pattern with electrodes positioned in the left jugular furrow, caudal to the left elbow, and above the withers.

The captive-bolt device (Cash Special captive bolt pistol, Accles and Shelvoke Ltd, Sutton Coldfield, UK) was used with a 0.25-calibre orange cartridge and either the non-penetrating attachment or the standard bolt (penetrating) according to the manufacturer's recommendation. For each technique, the location of impact was the external occipital protuberance (Figure 1). The muzzle of the device was placed flush, perpendicular to the surface of the skull and aimed rostrally toward the animal's intermandibular area. Each calf was restrained with a halter when needed in order to stabilise the head. The halter was required in eight calves,

Table 1 Mean (± SEM) time to loss of consciousness and cessation of heart rate, respiration, and convulsions as well as mean (± SEM) number of convulsions in calves following either non-penetrating captive bolt (NPCB) or penetrating captive bolt (PCB).

	Mean (± SEM) NPCB	Mean (± SEM) PCB
Loss of consciousness (s)	0 (± 0.0)	0 (± 0.0)
Cessation of heart rate (s)	300 (± 0.0)	273 (± 27.0)
Cessation of respiration (s)	0 (± 0.0)	60 (± 53.97)
Cessation of convulsions (s)	180 (± 60.24)	310.40 (± 79.74)
Number of convulsions	2.75 (± 1.03)	2 (± 0.837)

for which the sedation did not induce perfect stillness. The assessment of respiratory and heart rate and rhythm was performed manually via auscultation, nearly continuously from impact until cessation of heart rate, while safely avoiding involuntary movement. Other data points included; the timing of changes in corneal reflex and eye position, vocalisation events, the number and timing of involuntary convulsive movements, and the number and timing of attempts at righting reflex were monitored to assess consciousness. Loss of consciousness was considered when the animal displayed lateral recumbency, no vocalisation, fixed central eye position, lack of a corneal reflex and a loss of a co-ordinated righting response. A post-impact timeline of 5 min was chosen to represent the typical time-lapse encountered between stunning and exsanguination in an abattoir facility. After 5 min, a solution of KCl (330 mg kg^{-1} or approximately 500 ml administered to effect, IV once, 150 mg ml^{-1}, LectoMedical LLC, Decatur, AL, USA) was administered intravenously until cessation of heart rate. Post mortem analysis of the skull fractures and CNS tissue damage was performed by gross anatomical assessment of fresh sagittal sections of each calf's skull. Areas of disrupted tissue, cranial fractures, bone fragment displacements, and subarachnoid and *in situ* haemorrhage were characterised and recorded.

EEG analysis

EEG signals recorded from the BioRadio were imported to Matlab (Mathworks, Natic, MA, USA). All signals were band-pass filtered from 2 to 35 Hz and subsampled to 120 Hz before computing power parameters. The total power, P_{tot}, in the EEG signals was computed as described by Gibson *et al* (2007) where the root-mean-squared (RMS) values were computed over consecutive 1-s intervals, tapered by a Hanning window with a 50% overlap (Gibson *et al* 2007). A baseline RMS value was computed from a 1-min segment before the bolting event and used to normalise the P_{tot} values presented in this paper.

EEG data were qualitatively analysed in a manner modified from that previously described by Gibson *et al* (2007). Briefly, recordings were visually assessed and characterised into one of four categories; active EEG, transitional EEG, high amplitude-low frequency EEG, and isoelectric EEG. Active EEG represented normal cerebral activity and waveforms in sedated calves and were considered the baseline waveforms prior to captive bolt. Transitional EEG represented a reduction in waveform amplitudes less than 50% from the baseline amplitude with significant frequency changes. High amplitude-low frequency represented a waveform with a large variation in maximum and minimum amplitude with drastically reduced frequency. Isoelectric EEG represented wave forms of < 10% of the baseline waveform amplitude with minimal frequency, considered electrocerebral inactivity or cerebral death.

Accelerometer

Accelerometer data were also recorded from the BioRadio and imported to Matlab (Mathworks, Natic, MA, USA). The low end of the band-pass filter on the accelerometer data effectively removed the offset from the gravity and resulted in signals consistent with variations associated with forces from changing positions. This way relative activity could be observed over the experiment. The filtered accelerometer x, y, and z-axis data were squared and summed at each time sample and the square-root taken to obtain a magnitude of the acceleration vector. The acceleration magnitudes were then processed and normalised in the same manner as the EEG data. The processed accelerometer data, therefore, show the relative changes in force from motion relative to the bolting event.

Statistical analysis

Time to loss of consciousness, time to isoelectric EEG, and time to cessation of heart rate, respiration and convulsions were characterised descriptively and visualised using Kaplan-Meier survival graphs. Frequency counts of convulsions by treatment were likewise descriptively characterised. All analyses were carried out using SPSS Statistics v 25 (SPSS Inc, Chicago, IL, USA).

Results

An immediate and sustained loss of consciousness, including lack of a corneal reflex, fixed central eye position and lack of righting reflex and vocalisation was observed for all calves. One calf in the PCB group ceased heart rate at approximately 165 s following impact, while the remaining calves sustained a normal rate and rhythm until administration of KCl at the end of the 5-min observation period. Mean time to the cessation of heart rate and rhythm in calves was 300 (± 0.0) and 273 (± 27.0) s in NPCB and PCB, respectively (Table 1). Median time to cessation of heart rate for both treatments was 300 (± 0.0) s. Respiration immediately ceased regular rate and rhythm with infrequent apnoeic breaths in both treatment groups, except for one calf in the PCB treatment that continued sporadic breaths until the administration of KCl. The mean time to cessation of respiration was therefore numerically shorter for the NPCB treatment (0 [± 0.0]) than the PCB (60 [± 53.67] s). The median time to cessation of respiration was 0 s for both treatments.

Each of the calves displayed an immediate convulsive reflex following impact with the captive bolt, consistent with

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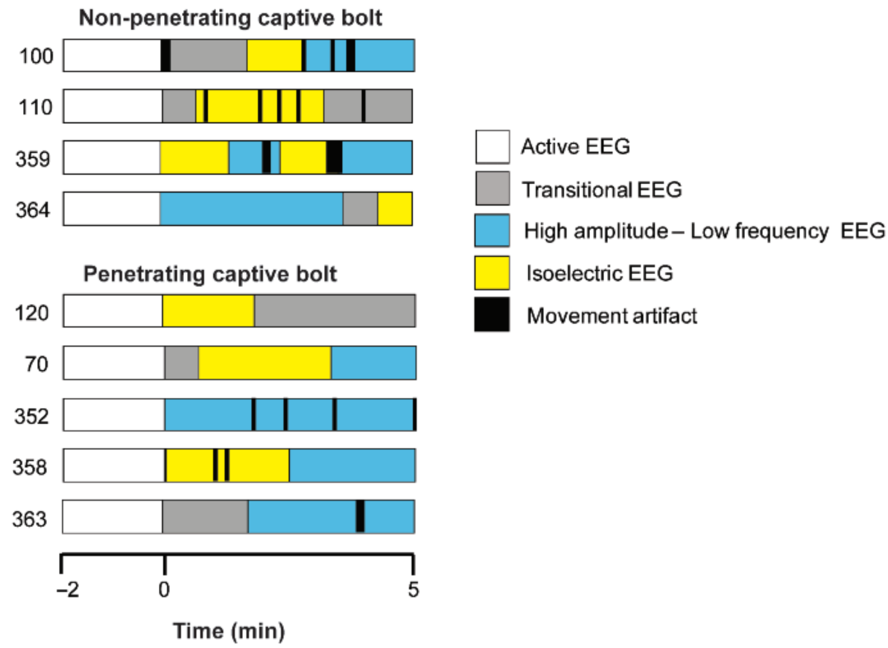
Figure 2



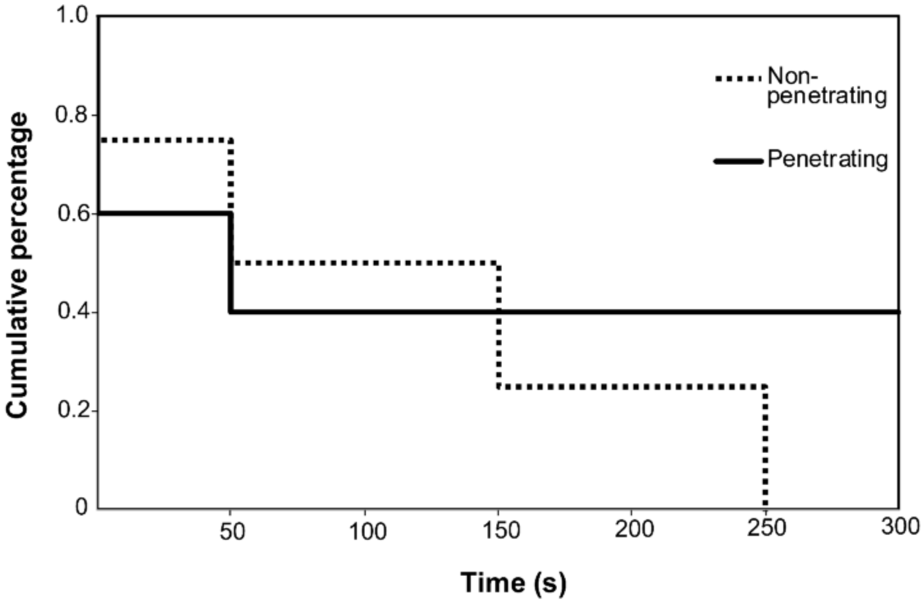
Each calf was outfitted with EEG probes and recorded for 2 min prior to impact with either non-penetrating captive bolt or penetrating captive bolt. All recordings were censored at 5 min corresponding to the administration of intravenous KCl. EEG recordings were visually assessed and characterised into one of four categories: active EEG represented normal cerebral activity and waveforms in sedated calves and were considered the baseline waveforms prior to captive bolt; transitional EEG represented a reduction in waveform amplitude less than 50% from the baseline amplitude with significant frequency changes; high amplitude-low frequency represented a waveform with a large variation in maximum and minimum amplitude with drastically reduced frequency; isoelectric EEG represented wave forms of < 10% of the baseline waveform amplitude with minimal frequency.

extensive damage to the frontal cortex. Three of four calves in the NPCB group and three of five calves in the PCB group for which data were obtained continued an inconstant series of sporadic, brief, and unco-ordinated convulsive movements of the limbs. The number of movements ranged from 1 to 5 and, in the case of one calf in the PCB group, continued movement up to 825 s, even after administration of KCl. However, at no time did any of the calves display controlled movements or attempts to right themselves from lateral recumbency. The mean time to cessation of convulsions for the NPCB and PCB treatment groups was 180 (± 60.24) and 310. 4 (± 79.74) s, respectively. The median time to cessation of convulsions was 225 s for the NPCB treatment and 240 s for the PCB treatment. The mean number of convulsions for each treatment group was 2.75 (± 1.03) and 2 (± 0.837) for NPCB and PCB, respectively.

The qualitative transformation of EEG total power (P_{tot}) estimate for each calf is displayed in Figure 2. NPCB immediately induced an isoelectric EEG recording in one of four calves for which data were obtained, while the remaining calves achieved isoelectric EEG recordings within 42, 163, and 249 s. None of these calves sustained isoelectric recordings throughout the observation period and fluctuated between high amplitude-low frequency, transitional, and isoelectric wave patterns. PCB induced an isoelectric EEG immediately following impact in two of five calves for which data were obtained and within 45 s of impact of an additional calf. Similar to calves in the NPCB group, these calves did not sustain an isoelectric wave pattern, but varied between isoelectric, transitional, and high amplitude-low frequency wave patterns. The remaining two calves never reached isoelectric EEG; one calf sustained the high amplitude-low frequency pattern while the other varied between transitional and high amplitude-low frequency patterns. The mean time to isoelectric wave patterns for NPCB and PCB treatments was 113.5 (± 56.87) and 69.0 (± 52.24) s, respectively. The median time to isoelectric wave patterns for NPCB and PCB treatments was 42 (± 81.5) and 45 (± 49.3) s, respectively. A Kaplan-Meier curve relating the cumulative onset of isoelectric EEG over time can be seen in Figure 3.

Post mortem analysis of the calves receiving NPCB revealed 6/6 calves exhibited mildly depressed fractures radiating outward from the occipital protuberance (Figure 4[A]). Fractures were identified in the occipital, parietal and frontal bones. In the calf with the most extensive damage, six independent fragments were identified and displaced outwardly from the site of the impact. Additionally, all NPCB calves demonstrated haemorrhage within the cranial cavity and ventrally around the brainstem. Although the brainstem was physically intact in each of these calves, extensive damage was induced in the caudal cerebrum and cerebellum. Both were characterised by loss of macroscopic tissue

Figure 3



The mean time to isoelectric wave patterns following treatment with NPCB or PCB treatments was 113.5 (± 56.87) and 69. 0 (± 52.24) s, respectively. The median time to isoelectric wave patterns for NPCB and PCB treatments was 42 (± 81.5) and 45 (± 49.3) s, respectively.

architecture and petechial haemorrhages. Four of five calves in the PCB treatment group displayed a linear tract of damaged tissue extending from the occipital protuberance toward the cranial brainstem and pons representing the projected path of the bolt. In the other calf (tag number 352), the path of the bolt was angled more rostrally and did not intersect the brainstem, but instead travelled further into the cerebellum and cerebrum. By comparison, the impact of the narrower penetrating bolt induced minimal fracturing of the adjacent bone and instead excised an approximately 1-cm diameter hole at the impact site. In 3/5 calves, the overlying fragment of bone from the site of impact was thrown ventrally into the base of the brainstem creating additional tissue damage (Figure 4[B]). PCB also consistently induced haemorrhage within the cranial cavity, surrounding the brainstem, and along the path of the bolt.

Discussion

This study details the comparative efficacy of both PCB and NPCB when using an occipital approach in calves up to 200 kg in bodyweight. The chief limitation of this study is the reduced number of animals included in the descriptive dataset. Therefore, if these techniques are utilised the operator needs to consider the lack of statistical evaluation compared to an industry-accepted technique. As mentioned previously, these calves were scheduled for sacrifice under the design of an additional study and, as a matter of convenience, the animals were evenly enrolled into the two treatments. Both techniques produced an immediate and sustained loss of consciousness that persisted throughout the 5-min observation period. All calves immediately collapsed into lateral recumbency and displayed loss of corneal reflexes, righting reflexes, and lack of vocalisation. Additionally, all but one calf (10/11) experienced an immediate cessation of respiration. Fricker and Riek (1981) and later Grandin (2002) used these signs as indicators of a successful stun. The 5-min observation period was chosen here to represent a time in which animals presented to an abattoir would typically be shackled, hoisted and exsanguinated following impact. In this study, we administered intravenous KCl as a secondary means of euthanasia rather than exsanguination, which may have induced less stimulus than that encountered in an abattoir. However, loss of consciousness entails an ensuing loss of peripheral sensation and awareness of the surrounding environment, therefore the application of these techniques should still be appropriate in an abattoir setting.

An immediate cessation in respiration is also considered necessary in effective stuns and in this study one calf (tag number 352, PCB treatment group) continued sporadic respirations throughout the observation period. In spite of this animal's rhythmic breathing pattern, all other indicators of a successful stun were present. Continued respirations in this calf, however, should be interpreted as an ineffective stun. Review of this calf's gross pathology data confirms this conclusion and indicates that there was a misalignment of the

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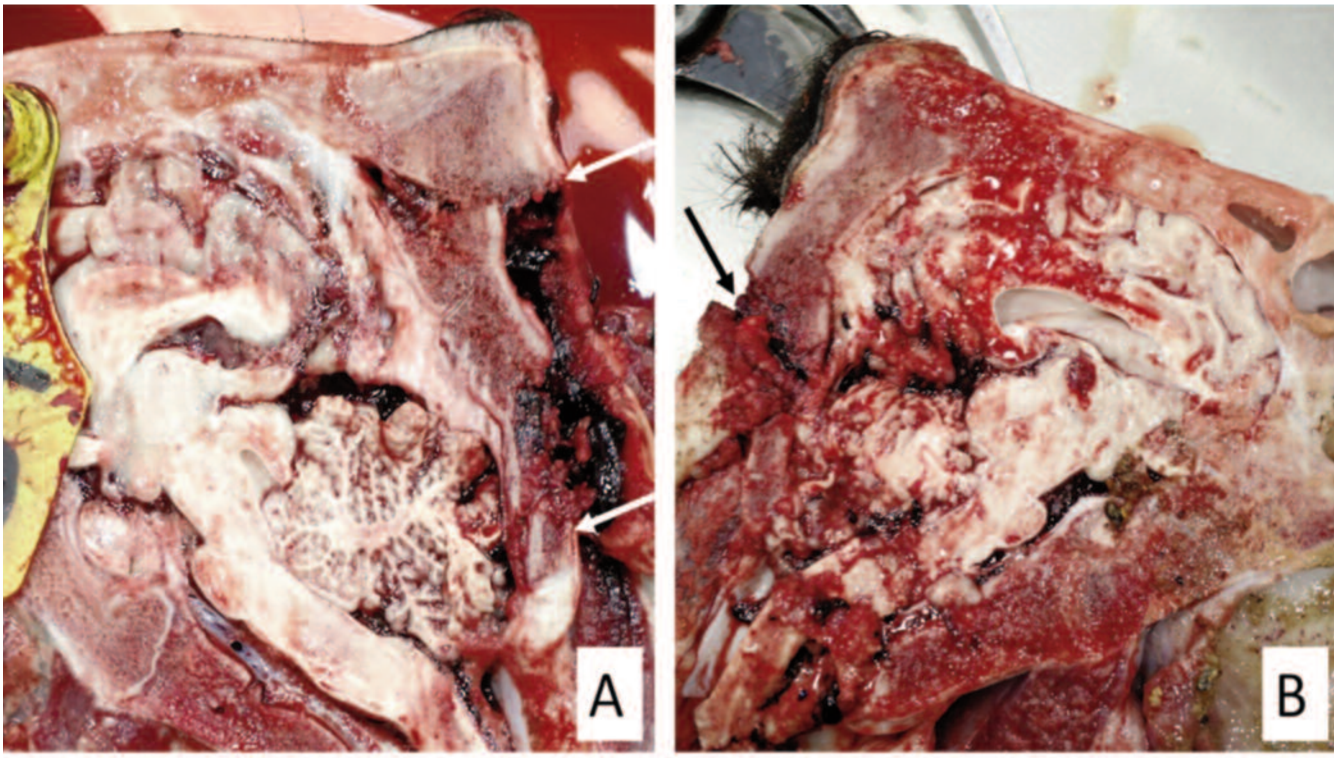
Figure 4



Representative post mortem images of heads in sagittal section following either non-penetrating captive bolt or penetrating captive bolt. Non-penetrating captive bolt applied to the external occipital protuberance (A). The white arrows indicate the extent of the depressed skull fracture. Note the haemorrhage located within and around the caudal cerebrum, cerebellum and brainstem. Penetrating captive bolt applied to the external occipital protuberance (B). The black arrow indicates the path of the bolt from the occipital protuberance toward the brainstem.

captive-bolt device where the cerebellum and cerebrum sustained massive tissue damage, but the brainstem was unharmed. This suggests an upward inflection of the device that produced a bolt path that travelled rostral to the brainstem, missing it. Most likely the alignment of the device was made too shallow so that the projected line of the bolt path intersected a point higher than the mandibular junction and more in line with the nasal planum or higher. Additionally, this calf never achieved an isoelectric EEG pattern, but instead sustained a pattern of high amplitude-low frequency morphology as well as consistent convulsive movement. Because the KCl was administered within 5 min of impact, it is not certain if this calf would have recovered consciousness, but this data does underscore the importance of accurate alignment of the device. The primary goal of PCB alignment is that the bolt should travel into the brainstem and at least one cerebral cortical hemisphere to directly disrupt tissues associated with rudimentary consciousness and respiration (Leary *et al* 2013). In comparison to the case above, if the alignment of the device is in a more vertical position, where the path passes directly into the brainstem but does not impact the cerebrum or cerebellum, this likely would result in a calf that collapses, with perhaps disrupted respiration, but a delayed loss of consciousness. Proper placement and alignment of the PCB device cannot be over-emphasised. NPCB, therefore, may provide a slight advantage, in that minor alignment mistakes can be compensated for, because the impact of the larger diameter bolt disperses the force through the entire target area and relies less on the direct disruption of those tissues. In all scenarios where the captive bolt is administered, continued respiration should be interpreted as a poor stun and should be re-administered, or other means of euthanasia used immediately.

The generation of rhythmic heart rate and cardiac contraction is controlled via spontaneous depolarisation of the sino-atrial node located within the right atrium of the heart. Extrinsic modulation of heart rate and cardiac output is applied through the cardiovascular control centre in the brainstem and circulating vasoactive hormones based on physiologic and/or behavioural factors. Shearer (2005) notes that disruption of the brainstem does not immediately stop heart contractions due to continued activity of the autonomous SA node. Consistent with this observation, calves in this study maintained a normal heart rate and rhythm (one calf ceased heart rate at approximately 165 s post-impact) until administration of KCl and the overwhelming chemical depolarisation of the SA node cells. Lambooji *et al* (2012) found an increase in heart rate following PCB for up to a mean of 200 s after captive bolt with or without incision of the carotid arteries, although they did not offer a possible physiologic mechanism. In this study, only the consistency and normal rhythm of the heart rate were recorded, not the change in beats per minute, so it is impossible to suggest if that pattern was also observed here. Nevertheless, the continuation of heart contractions after stunning has been noted elsewhere.

A convulsive reaction following captive bolt is an expected response, as the sudden impact of the bolt, then rupture and loss of neuronal membrane integrity, initiates action potentials that are transmitted through the spinal tracts and carried into the periphery by long neurons. The typical tonic-clonic nature of these movements is manifested first as rapid extension of all limbs, followed by kicking or paddling that may last from 15 to 45 s following impact. Martin *et al* (2018) compared the degree of post-stunning movement after impact with different bolt lengths and among different breeds of cattle. In that study, animals exhibited a range of 0–8 and 0–25 kicks in the forelimb and hind limb, respectively, after impact with a penetrating captive bolt. Interestingly, these authors also confirmed an empirical observation that Holstein cattle tend to exhibit more post-stun movement than other *Bos taurus* breeds. Terlouw *et al* (2015) showed that in adult cattle, post-impact movements occurring while hoisted lasted up to 3 min. In the current study, movement was not classified by hind limb or forelimb, however movements were minimal and continued in most animals throughout the time-period assessed. Ideally, captive bolt would render the animal entirely motionless and reduce the potential danger to workers handling stunned cattle, but the observations made here appear to be consistent with those of other captive-bolt studies.

The onset and maintenance of an isoeletric EEG morphology is the defined end-point for permanent insensibility. An isoelectric EEG is characterised by a severe reduction in amplitude and frequency of the electrical activity of the CNS, often referred to as theta and delta waves. In humans, isoelectric EEG wave patterns, along with evidence of irreversible neural damage, is interpreted as brain death. In this study, two of the nine calves for which data were obtained (both in the PCB treatment) never achieved an isoelectric pattern, yet immediately lost and maintained the loss of all other measures of consciousness. In one of the two calves, 352, the captive-bolt device was misaligned as has been mentioned, but there was no evidence to indicate a similar issue in the other calf. Other authors report loss of consciousness in the face of transitional, high-amplitude-low frequency and even normal EEG waveforms (Gibson *et al* 2009a,b; Meyer 2015). This suggests it may be incorrect to limit the association of loss of brain function with only isoelectric morphologies. The sudden change in power and frequency after impact from normal waveforms to the alternate patterns that were observed here, indicate an immediate alteration of brain function. Perhaps the onset of isoelectric activity can be correlated to degree and severity of tissue damage, however, post mortem necropsy findings revealed extensive trauma that was consistent within treatment groups. Other authors also report of the progressive deterioration of brain activity toward an isoelectric morphology and death, but an additional observation of the current study was that no animals that achieved isoelectric activity maintained that level of response throughout. In some cases, the calves resumed isoelectric activity, while others never regained a similar pattern. A potential explanation for this may be an inadequate post-impact time-period that did not allow for that progression to be observed. In previous studies, investigators have recorded EEG for up to 3 min after administration of the secondary euthanasia, while in this study EEG probes were removed at the time of secondary euthanasia. Another explanation for the dynamic EEG response may be that neither of these techniques induced sufficient trauma to permanently damage the CNS and that calves may have recovered sensibility if given a longer observation period. In either case, it's possible to state that both treatments induced a successful stun for at least 5 min, however caution is warranted in applying these results in circumstances where secondary euthanasia make take longer. Captive bolt as described here, as well as other methods, should never be considered a sole means of euthanasia. A secondary means of euthanasia is always necessary and should, whenever possible, be administered immediately following stunning.

Animal welfare implications and conclusion

There are limited reports in the literature providing efficacy of captive-bolt procedures in calves of typical size for veal slaughter. Ensuring the immediate and sustained loss of sensibility prior to slaughter is a necessary step in the harvesting of animals for human consumption as well as other circumstances of euthanasia. The goal of these procedures should be to remove or diminish the animals' distress and suffering. As new techniques are applied, each should be critically evaluated to ensure their validity and efficacy. The guidelines governing the use of penetrating and non-penetrating captive bolts in cattle currently provide limited assessment of use of these devices in younger animals, nor do they indicate the efficacy of an alternative occipital approach in applying them. Most likely this is the result of limited reports in the literature from which to discern information. Until a comprehensive body of work is produced that evaluates the efficacy of these techniques across multiple ages, sexes, and bodyweights of cattle, investigators must apply a reductive approach that examines these factors individually. Pursuant with that effort, the current study examined the effects of NPCB and PCB when applied to the occipital protuberance of 4–5 month old Holstein calves weighing up to 200 kg. These characteristics are relevant to the industry in that they are similar to the harvest age and bodyweight of conventionally raised veal calves. Based on our findings, both approaches successfully induced immediate loss of sensibility and therefore should be considered for further exploration as appropriate for this age group of cattle, if a secondary means of euthanasia can be administered within 5 min of impact.

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