Electroencephalographic assessment of concussive non-penetrative captive bolt stunning of turkeys

Running head: Captive bolt stunning of turkeys

Troy J. Gibson*, Carlos B. Rebelo, Timothy A. Gowers, Natalie M. Chancellor

Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, AL9 7TA, UK

* Correspondence to: Troy J. Gibson, Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, AL9 7TA, UK. E-mail: tgibson@rvc.ac.uk. Telephone: +44 (0) 1707 66 7078

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Keywords: Captive bolt stunning, non-penetrative, behaviour/brainstem reflexes, electroencephalogram, turkey

Abstract

1. The study examined electroencephalographic (EEG) and behavioural responses of turkeys (female ex-breeders, 29 weeks of age) stunned with three different concussive non-penetrative captive-bolt guns.
2. Thirty-one slaughter weight ex-breeding turkeys (mean 13.32 ± SD 0.65 kg) were stunned with the Cash Poultry Killer (CPK) (n=10), Turkey Euthanasia Device (TED) (n=10) and Zephyr EXL (n=11).
3. Mean peak kinetic energy was highest for the CPK compared to the TED and Zephyr EXL (75.9 ± 4.5, 28.4 ± 0.4 and 24.4 ± 0.7 J respectively).
4. Twenty-nine (94%) of the turkeys were rendered unconscious following captive bolt stunning, with total power of the EEG (Ptot) significantly reduced from baseline values (reductions of 67% CPK, 84% TED and 76% Zephyr EXL, $P<0.01$) and waveforms becoming isoelectric after periods of transitional EEG. However, two birds shot with the CPK and Zephyr EXL had periods of behavioural/reflexes (rhythmic respiration, nictitating membrane reflex, neck tension) and EEG activity (43-47 and 36-60+ s after the shot respectively) indicating incomplete concussion and return of consciousness. In one bird the shot was incorrectly positioned (Zephyr EXL), while the other appeared to be related to a defective cartridge (CPK).

5. In conclusion, all three captive bolt gun models were effective in producing unconsciousness in turkeys, provided they were positioned correctly and powerloads performed according to their specifications.

Keywords

Animal welfare, captive bolt, electroencephalogram (EEG), stunning, turkey

INTRODUCTION

The act of stunning is designed to render livestock and poultry unconscious prior to and during the act of slaughter (Gibson *et al.*, 2015a). There are a variety of stunning and slaughter methods for turkeys (*Meleagris gallopavo*), including electrical waterbath stunning, head-only electrical stunning, controlled atmospheric stunning (CAS), cervical neck dislocation and non-penetrative captive bolt. All stunning methods have their own strengths and weakness in terms of ease of use, efficiency, animal welfare, productivity, operating and capital costs, and operator safety (Erasmus *et al.*, 2010a, Gibson *et al.*, 2015a). For small to medium scale turkey poultry producers CAS systems can be financially prohibitive, with most producers instead relying on waterbath and head-only electrical stunning. There are many welfare concerns regarding waterbath stunning of poultry, due to the risks of pre-stun shocks, suboptimum stuns, stress and pain during inversion and suspension from shackles, variations in electrical current delivered to birds in multi-bird waterbath stunners and aspiration of waterbath water (EFSA, 2014, Hindle *et al.*, 2010). Meanwhile research on head-only constant voltage electrical stunning of end of lay hens (*Gallus g domesticus*) reported that for some birds the period of induced insensibility only lasted 9 seconds (8.5 – 31.5) seconds (Gibson *et al.*, 2016). This could result in some hens recovering from the stun prior to, or during the bleeding process, resulting in significant pain and distress. Furthermore, unpublished findings from researchers at University of Bristol have shown that the 130V used in commercially available constraint voltage stunners is insufficient to break down the initial high impedance to current flow in turkeys (pers. comm. Steve Wotton 2014).

Captive bolt stunning is widely used to render a range of species unconscious prior to the act of slaughter. Captive bolt guns that have been developed for poultry, are generally powered with either metal springs (Hillebrand *et al.*, 1996), elastic rubber tubes (Martin *et al.*, 2016), gun powder (Sparrey *et al.*, 2014) or pneumatically (Raj and O'Callaghan, 2001). Work by Raj and O'Callaghan (2001) found that when shooting with a pneumatically powered captive bolt, only the perpendicular position combined with 6 mm diameter bolt driven with an airline pressure of 827 kPa was effective in inducing rapid insensibility and unconsciousness in broilers. Gregory and Wotton (1990), reported that shooting broilers on the side of the head with a non-penetrative captive bolt (modified cash special) can be effective in abolishing or diminishing visual evoked potentials. Meanwhile, Erasmus *et al*., (2010a, 2010b) compared a recently developed commercially available pneumatic captive bolt stunner (Zephyr) with cervical neck dislocation (crushing and stretching methods) and blunt force trauma for on farm dispatch of turkeys. They reported that pneumatic stunning and blunt force trauma to the head were equally effective in inducing insensibility in turkeys. Finally, Martin *et al.* (2016), reported that stunning to kill with a penetrating captive bolt powered by elastic rubber tubes was less effective and reliable than manual and mechanical cervical neck dislocation. Recently several new captive bolt guns have been developed that can be used for poultry. The pneumatically powered Zephyr-EXL which is a more powerful version of the Zephyr examined by Erasmus *et al*., (2010a, 2010b) and studied for the dispatch of neonate piglets (Casey-Trott *et al.*, 2013; Grist *et al*., 2017), and the propane powered Turkey Euthanasia Device (TED), both of which were developed by Bock Industries Inc. (Sparrey *et al.*, 2014), have potential to replace other forms of stunning and improve welfare of poultry during the slaughter process. However, they have yet to be evaluated for their effectiveness in inducing rapid irrecoverable unconsciousness in turkeys.

The aim of this study was to assess the effectiveness of three different models of concussive non-penetrative captive-bolt guns (CPK, TED and Zephyr EXL) in inducing irrecoverable unconsciousness in slaughter weight turkeys. Stunning was assessed with behavioural and electroencephalographic (EEG) indices.

MATERIALS AND METHODS

Electroencephalographic and behavioural assessment of captive bolt stunning

All birds were sourced from a commercial turkey breeder. Birds were kept in accordance with normal husbandry practices. The turkeys had been previously used in an electrical stunning experiment, where they were allowed to recover consciousness. Only birds with normal EEG waveforms after full recovery were included in the captive bolt study. Captive bolt stunning was used as the dispatch method in accordance with the Home Office Project Licence under the provisions of the Animals (Scientific Procedures) Act 1986.

Thirty-one female ex-breeding turkeys (age 29 weeks, mean weight 13.32, range 12.09 – 14.33 kg) were randomly allocated into three stunning treatment groups. Birds were either shot with the .22 Cash Poultry Killer (CPK) (Accles & Shelvoke, Sutton Coldfield, UK) (n=10), Turkey euthanasia device (TED) (Bock Industries Inc. Philipsburg, PA, USA) (n=10) or Zephyr EXL (Bock Industries Inc. Philipsburg, PA, USA) (n=11) (table 1). All birds acted as their own controls. Thirty minutes prior to restraint and electrode placement a local anaesthetic (EMLA cream, lidocaine 2.5% and prilocaine 2.5%; AstraZenca UK Ltd, Cheshire, UK) was applied to the top of head to desensitise the skin. Prior to placement of the EEG recording electrodes and shooting, the birds were restrained in custom built cone, inclined 60° with the legs restrained in a padded clamp (Solutions for Research, Silsoe, Bedford, UK). The birds were restrained to minimise movement artefact during EEG recording.

TABLE 1

The CPK was fitted with the convex knocker head (25 mm diameter) and was powered with .22 brown 1 gr (nominal propellant charge 110 mg) black powder cartridges (Accles & Shelvoke, Sutton Coldfield, UK). While the TED with a circular knocker head consisting of two overlaid flat discs (proximal and distal discs, 10 and 20 mm respectively), was operated with a modified adaptor fitted that allowed 15 mm of bolt protrusion from the muzzle. This was powered by a propane fuel cell (Paslode, Illinois Tool Works Inc., Glenview, Illinois, USA), that was first primed with a priming shot, for every bird prior to stunning. The Zephyr EXL with a convex knocker head (25 mm diameter) was connected to a portable compressor (IM200-12L, Impax, NAP Brands Ltd, Warwick, UK) with a stable line pressure of 827 kPa (120 psi). Electroencephalogram electrodes were placed prior to recordings, with data collected from each bird 30 seconds prior to and 60 seconds after captive bolt shooting. Data was collected continuously throughout the recording period and included the captive bolt shot. All birds were stunned with the muzzle of the respective captive bolt guns placed on the surface of the top of the cranium at a perpendicular angle, with the head restrained by the free hand by holding onto the beak. The muzzle was positioned on midline on the skull, between the eyes and the ears. If birds showed any signs of incomplete concussion they were immediately reshot. Immediately after data collection (60-80 seconds post shot) all birds were bled with bilateral severance of the carotid arteries and jugular veins.

One channel of EEG was recorded from a three-electrode montage using three 24-gauge stainless steel subdermal needle electrodes (Neuroline Subdermal, Ambu Inc, Glen Burnie, MD, USA). The tips of each electrode were placed as follows: active (non-inverting) ≈6 mm right of midline, ≈3 mm rostral of bregma over the right optic lobe; reference (inverting), over the right rostral aspect of the forebrain ≈6 mm right of midline, ≈20 mm rostral of bregma; and ground electrode caudal to the back-of-the head, respectively. Electrodes were secured in position with superglue (RS Components, Corby, UK) and surgical tape (Durapore, 3M, Maplewood, MN, US). The electrode leads were further secured with a loose band of surgical tape around the neck.

Mean interelectrode impedance was 1.5 ± 0.1 (SEM) and ranged between 1.3 and 1.8 kΩ (MkIII Checktrode, UFI, Morro Bay, CA, USA). Electroencephalogram signals were amplified and filtered with an analogue filter (dual Bio Amp, ADInstruments Ltd., Sydney, Australia) with low and high pass filters of 100 and 0.1 Hz, respectively. The signals were digitalised (2 kHz) with a 4/20 PowerLab (ADInstruments Ltd, Sydney, Australia) digital to analogue converter and recorded on a Dell personal laptop 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 LabChart 8.1.5 (ADInstruments Ltd). All waveforms were digitally filtered with a pass band of 1 to 30 Hz and traces were inspected visually and compared to baseline using a modified version of the classification systems developed by Gibson *et al*., (2009b) and McKeegan *et al*., (2011, 2013). They were classified into one of four categories: Movement artefact; Normal EEG; Transitional EEG and Isoelectric EEG. Normal EEG represents activity which 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, or high amplitude and low frequency activity. Isoelectric EEG was classified as a trace with an amplitude of less than 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 analysed. Fast Fourier Transformation with a Welch window was applied to 1 second epochs, generating sequential power spectra with 1-Hz frequency bins. Subsequent analysis was performed using Microsoft Excel Mac 2016 (Microsoft Corporation, Redmond, USA). In addition to EEG analysis, behavioural/reflexes indices of brainstem function were recorded after the shot, these were: rhythmic breathing, nictitating membrane reflex and neck tension. Rhythmic breathing was assessed via observation and palpation of the posterior aspect of the abdominal cavity for signs of rhythmic air sack filling and examination of respiratory movement and noise from the beak. The nictitating membrane reflex was evoked with mechanical stimulation of the exposed corneal with the tip of a probe. Neck tension was examined with the raising of the neck, followed by the withdrawal of support with assessment of maintenance of muscle tone. Apnoea after stunning has been associated with damage to the medulla and reticular formation. The absence of the nictitating membrane reflex is associated with brainstem dysfunction, and the lack of neck tension relates to loss of CNS control of muscle tone (Gibson *et al.*, 2016, Terlouw *et al.*, 2016).

Mechanical performance of captive bolt guns

The peak velocity of the CPK, TED and Zephyr EXL were tested with a custom-built velocity meter (Solutions for Research, Silsoe, Bedford, UK), as previously described by (Gibson *et al.*, 2015a). Captive bolt guns were fired either 27 (Zephyr EXL) or 40 (CPK and TED) times into the meter using the same powerloads/airline pressures as described for the turkey stunning study. The TED was fired without an adaptor. Bolt weights were measured or provided by the manufacturer (TED and Zephyr EXL). Velocity was recorded and kinetic energy of the bolt calculated (kinetic energy = $[0.5 \text{ X } m] \text{ X } v^2$).

Statistical analysis

Electroencephalogram data for each turkey was calculated and displayed as percentage changes in the total power of the EEG power spectrum (Ptot) from pre-treatment values. Data contaminated by movement artefact was rejected from analysis. All data were analysed using Prism 7.0a (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 treatments in bird weights and EEG classifications was performed using a one-way ANOVA and the post hoc Tukey's multiple comparison test. Differences in peak velocity and kinetic energy was analysed with the Kruskal-Wallis test and post hoc comparisons made with Dunn's multiple comparisons test. Spectral data was analysed with a two-way ANOVA and with the post-hoc Tukey's multiple comparisons test. Mean EEG Ptot values are displayed ± standard error of the mean (SE), velocity and kinetic energy values as ± standard deviation (SD). The level of statistical significance for all tests was $P < 0.05$.

RESULTS

Electroencephalographic and behavioural assessment of captive bolt stunning

Immediately after non-penetrative captive-bolt stunning, respiration ceased in 94% (29/31) of birds. However, two birds showed signs of incomplete concussion. One turkey shot with the CPK (bird 10), presented rhythmic respiration and a positive nictitating membrane reflex in both eyes after the first shot. This shot was reported as sounding less loud than previous shots (soft shot). Meanwhile, another turkey shot with the Zephyr EXL (bird 23) presented rhythmic respiration and neck tension. This bird had to be reshot a further two times to ensure absence of breathing and reflexes. The position of this initial shot in this bird was 4 mm right of midline. No turkeys shot with the TED showed any signs of recovery or incomplete concussion (table 2).

TABLE 2

After shooting, birds initially showed slow uncoordinated tonic convulsions, these developed into more violent clonic convulsions (leg paddling and attempted wing-flapping). Anecdotally it was found that the convulsions were most sever in turkeys shot with the Zephyr EXL, followed by those from CPK stunned birds. However, convulsion severity was not consistently assessed. The skulls all had a circular depression in the shot position, this was often associated with fractures to the frontal bone. In most birds, there was bleeding immediately from the wound after the shot.

The pattern of changes in EEG activity following non-penetrative captive-bolt stunning, between and within captive bolt gun treatments groups, was not uniform (figures 1, 2 and 3). With all treatments, there was significant movement artefact in the EEG from the tonic and clonic convulsions. After shooting, the birds generally had a period of movement artefact that was followed by transitional EEG, with further bursts of movement artefact and transitional EEG before changing into isoelectric waveforms. The duration of the initial period of movement artefact varied between the treatments, birds shot with the Zephyr EXL (20.1 ± 13.2 seconds) and CPK (19.9 ± 19.2 seconds) had longer mean periods of movement activity compared to TED (8.9 ± 8.3 seconds) shot birds, however this difference was not significant. The two birds that had positive behavioural signs of consciousness also had EEG activity that was classified as normal compared to their baselines values. Bird 10 (CPK) had a brief period of normal like activity starting 43 seconds after the shot and lasting 4 seconds, before changing back to transitional activity (Figure 1). Meanwhile, bird 23 (Zephyr EXL) had normal EEG activity starting at 36 seconds after the shot and lasting beyond the recording period (>24 seconds) (Figure 3).

FIGURE 1

FIGURE 2

FIGURE 3

The mean time to the onset of the first period of transitional EEG following captive bolt stunning was 15.4 ± 9.6, 9.9 ± 8.3 and 20.1 ± 13.2 seconds for the CPK, TED and Zephyr EXL respectively. There were no significant differences in the time of offset or duration (CPK 17.1 ± 8.7; TED 6.9 ± 5.5; Zephyr EXL 18.4 ± 15.5 seconds) of transitional EEG between the three treatment groups, however the duration of transitional EEG for the TED compared to the Zephyr EXL was approaching significance ($P = 0.08$). There was no significant difference in the time of onset (CPK 35.3 ± 13.5; TED 29.3 ± 15.4; Zephyr EXL 37.2 ± 9.5 seconds) or duration (CPK 22.8 ± 13.7; TED 21.8 ± 15.4; Zephyr EXL 13.7 ± 6.3 seconds) of isoelectric EEG between the treatment groups.

After removal of the two incompletely concussed turkeys and exclusion of movement artefact contaminated epochs following captive bolt stunning, there was a significant decrease in Ptot for all captive bolt guns compared to pre-treatment values ($P<0.01$) (figure 4). There were no significant differences between treatments in Ptot values. In the first 30 seconds after stunning the mean percentage decrease from pre-treatment values was 67 ± 11 %, 84 ± 5 % and 76 ± 17 % for the CPK, TED and Zephyr EXL respectively.

FIGURE 4

Mechanical performance of captive bolt guns

There was a significant difference between the captive bolt guns in peak velocity and kinetic energy (Table 3). The TED had the highest mean peak velocity (30.4 ± 0.2 $m.s^{-1}$) compared to the CPK (29.1 ± 1.0 $m.s^{-1}$) and the Zephyr EXL (26.6 ± 0.4 $m.s^{-1}$) ($P < 0.0001$). However, the mean peak kinetic energy was significantly higher for the CPK compared to the TED and Zephyr EXL (75.9 ± 4.5, 28.4 ± 0.4 and 24.4 ± 0.7 J respectively) ($P < 0.0001$). Figure 5 is the velocity and kinetic energy profiles of the three models of captive bolt guns. Peak velocity and kinetic energy was recorded at 6, 26 and 18 mm from the end of the muzzle for the CPK, TED and Zephyr EXL respectively.

TABLE 3

FIGURE 5

DISCUSSION

The study examined changes in the EEG in turkeys shot with three different commercially available captive bolt guns. Stunning produced states of brain activity that were inconsistent with consciousness in 90% (n=1/10), 100% (n=10/10) and 91% (n=10/11) of bird's shot with the CPK, TED and Zephyr EXL respectively. However, two birds shot with the CPK and Zephyr EXL had periods of behavioural/brainstem reflexes and EEG activity that indicates that they were incompletely concussed and that consciousness may have returned. In the Zephyr EXL shot bird that recovered, the shot was 4 mm left of midline. It is likely in this bird that there was insufficient focal and diffuse damage to brain structures to induce complete insensibility. Work in mammalian species has shown the importance of shot position in inducing unconsciousness. Incorrect shot position, leading to insufficient trauma to structures of the brainstem, midbrain and hypothalamus has been associated with incomplete concussion in sheep (Gibson *et al.*, 2012) and alpacas (Gibson *et al.*, 2015b). Work by Erasmus *et al.* (2010b) reported substantial skull fractures, subcutaneous and subdural haemorrhage in turkeys shot with the Zephyr (lower power versions of the Zephyr EXL) in the recommended position. Those authors suggested that based on the level of damage combined with behavioural results from another of their experiments (Erasmus *et al.*, 2010a), that the Zephyr is effective and humane for inducing insensibility leading to death when birds are shot in the correct position.

In the bird that recovered after being shot with the CPK, there were behavioural and EEG signs of recovery, despite the shot being in the recommended position. The period of recovery appeared to be related to cartridge powerload as the actual shot was noted as being less loud compared to previous shots with the CPK and .22 1gr cartridges (recommend power load for the CPK and all poultry types). At the time the researchers described this as a 'soft shot'. During bench testing of the captive bolt guns it was found that the mean peak kinetic energy of the CPK (75.9 ± 4.5 J) was significantly higher than that of the TED (28.4 ± 0.4 J) or Zephyr EXL (24.4 ± 0.7 J). This was despite the TED having the highest values for peak velocity. The CPK showed the greatest variation in velocity between shots, potentially suggesting deviations in cartridge powerloads. In a separate study, it was reported that with the .22 Cash Special and 1.0 gr cartridge combination that there was a large significant variation in peak velocity, which was directly related to cartridge fill weight (Gibson *et al.*, 2015a). This finding has recently been confirmed by researchers at the University of Bristol (pers. comm. Steve Wotton 2016), who found significant variations in cartridge performance in terms of muzzle velocity for the CPK. Gregory *et al*., (2007) also reported similar findings for cattle with higher powered powerloads, but rather than assessing velocity they examined shot loudness with a decibel meter. They compared this to signs of incomplete concussion and found that shots ≤ 111 dB (4.5 gr cartridges) were associated with signs of a shallow depth of concussion in cattle (Gregory *et al.*, 2007). It has been suggested that one of the reasons for variability in cartridge performance, may relate to how the cartridges are filled and packed (Gibson *et al.*, 2015a). Cartridges with lower power loads require less propellant and more packing material. Ensuring the correct balance is important for maintaining performance, especially with lower powered powerloads, which contain very low propellant volumes.

The pattern of changes in spontaneous EEG activity following non-penetrative captive-bolt stunning was not uniform between individual turkeys and gun types. Despite this variation, the combination of the behavioural/brainstem reflexes and EEG data suggests that 29 of the turkeys were rendered unconscious following captive bolt stunning, with total power of the EEG significantly reduced and waveforms becoming isoelectric after periods of transitional EEG. Decreases in Ptot activity represents reduced functional activity of the EEG as it is progressing towards an isoelectric waveform. Similar reductions in Ptot activity and associated frequency bands have been previously associated with loss of consciousness in poultry and waterfowl species during stunning and slaughter (Beyssen *et al.*, 2004a, 2004b, Lines *et al.*, 2011, McKeegan *et al.*, 2011, Raj and O'Callaghan, 2004, Raj *et al.*, 2006). In the spontaneous EEG, there were variations between birds across treatments in the time point at which the EEG became isoelectric. The time of onset of transitional EEG generally related to the decrease in Ptot following stunning for successfully stunned birds with all three captive bolt models tested. Associated with these changes in brain activity was the immediate cessation of rhythmic respiration, nictitating membrane reflex and neck tension. Similar periods of transitional EEG and related high amplitude, low frequency activity following stunning and slaughter has been previously associated with unconsciousness in poultry during captive bolt stunning (Raj and O'Callaghan, 2001), whole house gas (McKeegan *et al.*, 2011) and gas-filled foam (McKeegan *et al.*, 2013) killing.

All animals initially displayed periods of very high amplitude and low frequency activity that was associated with movement artefact relating to convulsive activity. Some of this appeared like epileptic activity, however although EEG data collection was linked to video recordings it was not always possible to differentiate true epileptic waveforms from movement artefact in most birds. For this reason, very high amplitude low frequency activity (epileptic like) when not associated with movement was classified as transitional EEG, similar to the definition used during whole house gas killing of chickens (McKeegan *et al.*, 2011). In the study, the birds were restrained in an inclined cone with their legs further restrained to reduce movement. However, for most animals the head was only partly restrained by an operator during the convulsive stage. Even in studies where the head is fully restrained there are often significant periods where movement artefact impacts on data collection (Bager *et al.*, 1990, Gibson *et al.*, 2009a). The use of electrodes implanted on the surface of the brain can be used to reduce movement artefact (Bager *et al.*, 1990), however these were not used in the current study as they are more invasive (requiring induction and recovery from anaesthesia, surgery and post-surgery pain and complications) than the subdermal needle electrodes.

One potentially compromising factor of the study was that the turkeys had previously undergone the neurological insult of reversible head-only electrical stunning prior to captive bolt stunning. There is the possibility that this could have impacted on the electrophysiological changes in brain activity in response to the captive bolt. This was an unavoidable issue as the birds were involved in an electrical stunning experiment with captive bolt used as the final dispatch methods. To reduce the potential for complications, only the data from fully recovered birds with normal pre-treatment EEG waveforms that were undistinguishable from preelectrical stunned waveforms were included in the study. Furthermore, the behavioural changes in the turkeys in terms of tonic and clonic convulsions and the behaviour of the two birds that showed signs of incomplete concussion were comparable to those observed during commercial slaughter (T. J. Gibson, unpublished observation).

The study used turkeys of a similar age and live weight (13.32 kg) to that used in commercial slaughter (13.10 kg in June 2017) (Defra 2017). However, as the birds were all of the same sex, age and approximant weight there was little of the variation that is seen commercially between breeds, birds for different markets and farms. As with other species it is likely that the performance of the captive bolt guns tested in this study would decrease with older and heavier turkeys. However, this was not examined in the current study and could form the basis for future research.

In conclusion, the study found that stunning with non-penetrative captive bolt is effective in producing unconsciousness in turkeys. When shots failed, this was due to shot position or defective powerloads. This highlights the importance of marksmanship and consistency of powerloads. When used correctly captive bolt has significant advantages in terms of welfare over electrical and CAS stunning systems. However, the high operating costs, the increased labour requirements and lack of mechanisation, limits its practical use to just small scale producers or as a backup method for other systems.

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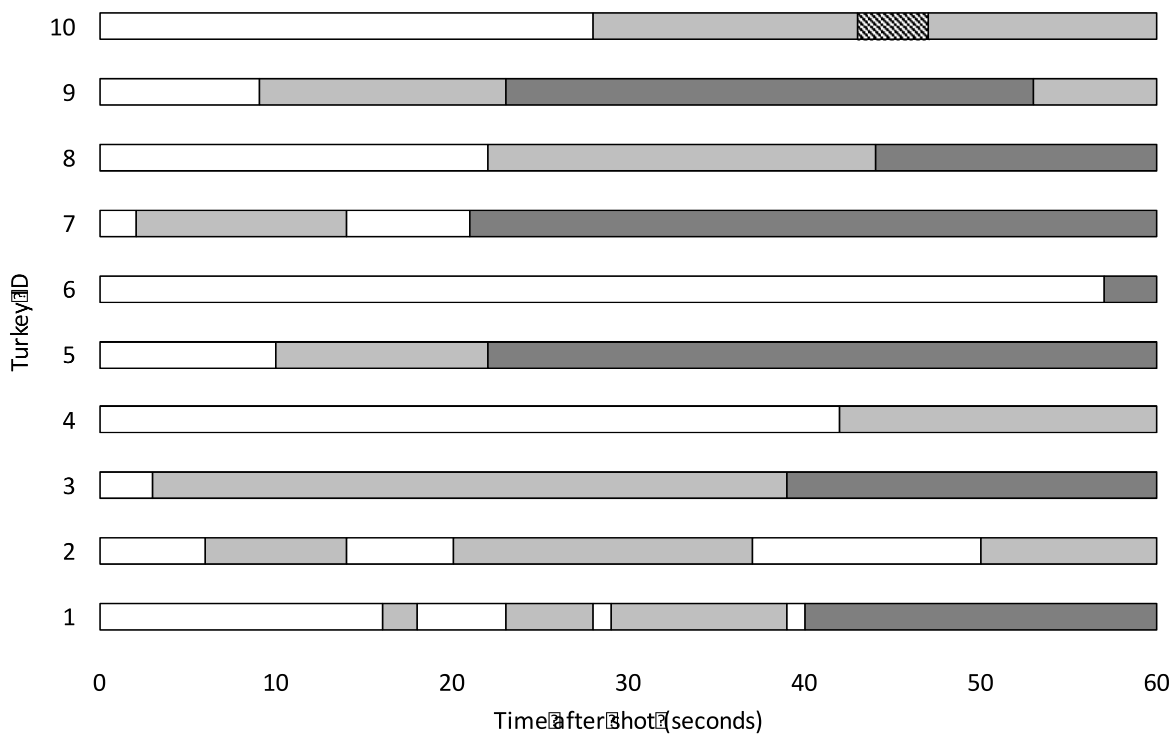

Figure 1. Characteristics of the EEG in individual turkeys in the 60 seconds after shooting with the **CPK non-penetrative captive bolt** (n=10). White bars represent movement artefact; grey transitional EEG; dark grey isoelectric EEG; and Cross hatched normal EEG activity.

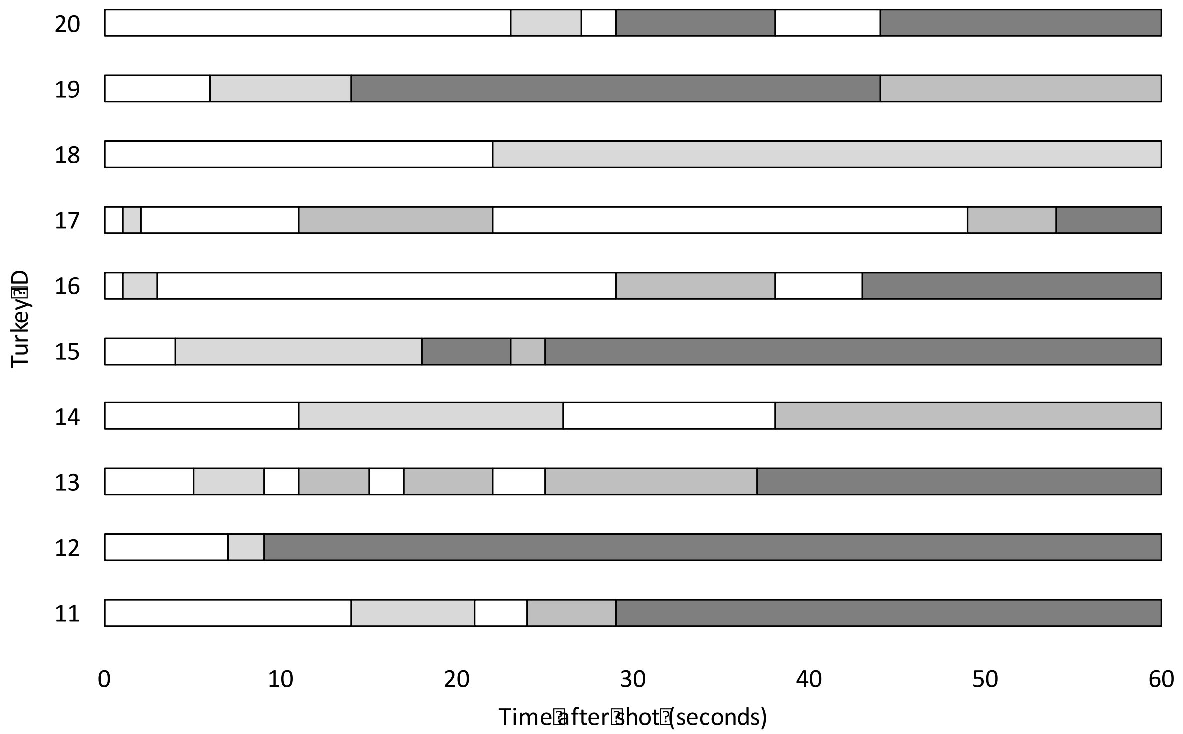

Figure 2. Characteristics of the EEG in individual turkeys in the 60 seconds after shooting with the **TED non-penetrative captive bolt** (n=10). White bars represent movement artefact; grey transitional EEG; and dark grey isoelectric EEG.

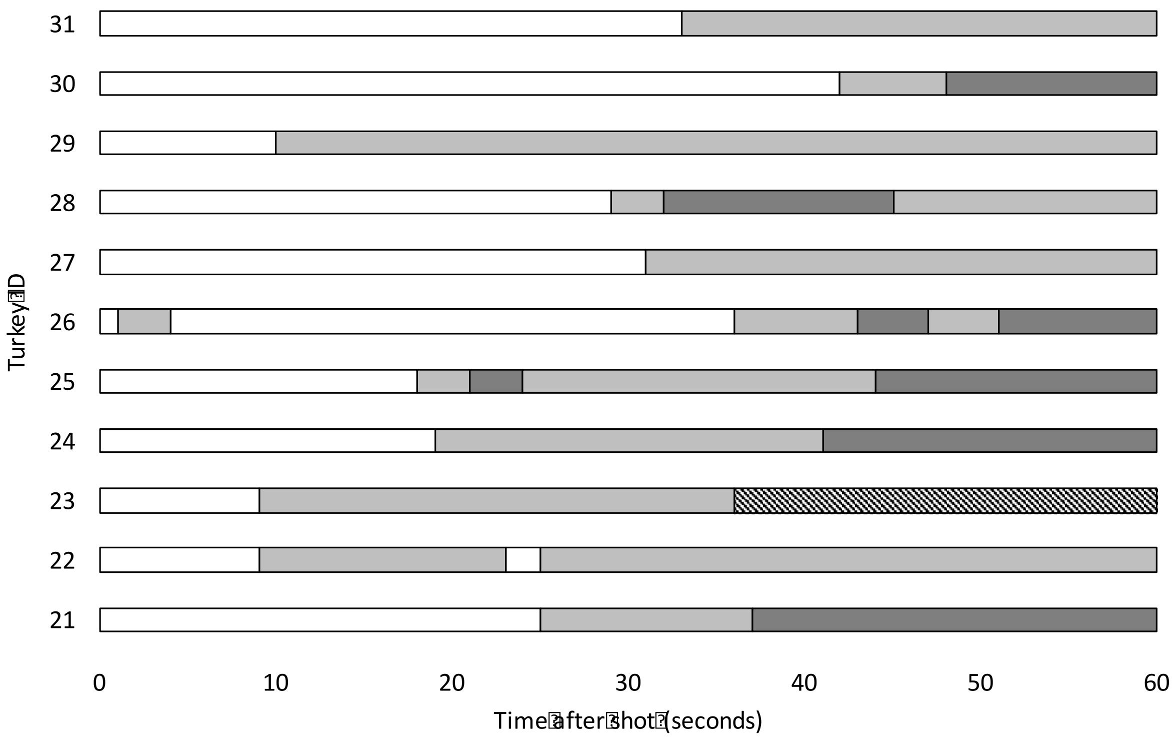

Figure 3. Characteristics of the EEG in individual turkeys in the 60 seconds after shooting with the **Zephyr EXL non-penetrative captive bolt** (n=11). White bars represent movement artefact; grey transitional EEG; dark grey isoelectric EEG; and Cross hatched normal EEG activity.

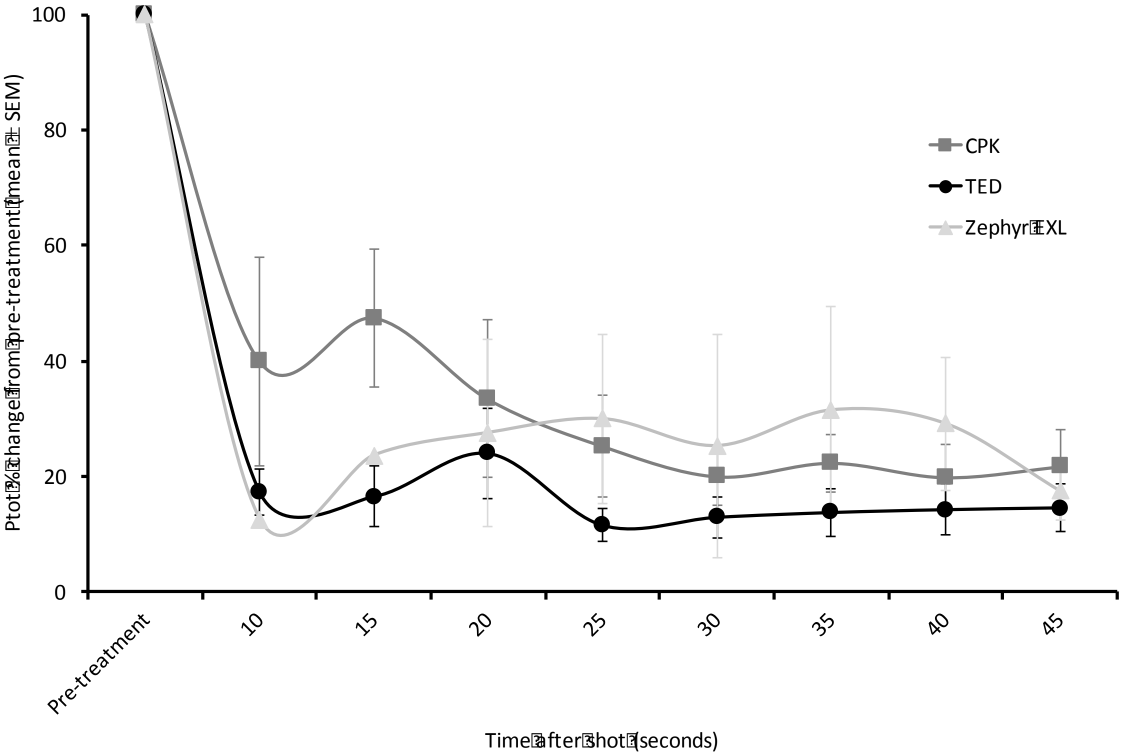

Figure 4. Mean (± SEM) changes in total power (Ptot) of the electroencephalogram (EEG) before and after effective non-penetrative captive bolt stunning of turkeys with the CPK (dark grey line) (n=9), TED (black line) (n=10) and Zephyr EXL (light grey line) (n=10). Note this excludes periods of movement artefact and the two turkeys incompletely concussed.

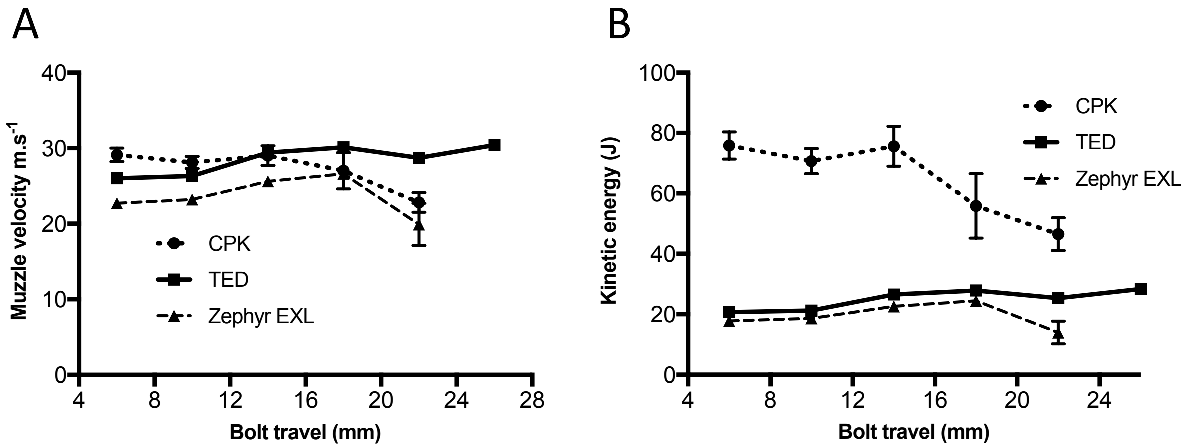

Figure 5. Mean (± SD) velocity (A) and kinetic energy (B) profiles for the CPK (dotted line), TED (black line) and Zephyr EXL (dashed line).

Table 1. Captive bolt guns tested, propellants, number of turkeys per treatment and the respective weights of the birds.

Gun type	Propellant type	Number	Weight (mean ± SD) kg	Weight range kg
CPK	Black powder .22 brown 1 gr cartridge*	10	13.24 ± 0.59	12.20 – 14.19
TED	Propane fuel cell	10	13.13 ± 0.65	12.09 – 14.33
Zephyr EXL	Compressed air 827 kPa	11	13.58 ± 0.67	12.48 – 14.31

* Nominal charge 110 mg

Table 2. Number and percentage of behavioural and cranial/spinal responses after captive bolt shooting with the CPK (n=10), TED (n=10) and Zephyr EXL (n=11).

Captive bolt type	Normal rhythmic breathing after shot	Positive nictitating membrane reflex	Presence of neck tension
CPK	1 (9%)	1 (9%)	0 (-)
TED	0 (-)	0 (-)	0 (-)
Zephyr EXL	1 (9%)	0 (-)	1 (9%)

Table 3. Comparison of bolt weight, velocity and kinetic energy of the CPK, TED and Zephyr EXL.

Captive bolt type	**Bolt weight (g)**	**Mean peak velocity ± SD ($m.s^{-1}$)**	**Velocity range ($m.s^{-1}$)**	**Mean peak kinetic energy ± SD (J)**
CPK	179	29.1 ± 0.9^{a}	19.3 - 30.9	75.9 ± 4.5^{a}
TED	61*	30.4 ± 0.2^{b}	25.4 - 30.9	28.4 ± 0.4^{b}
Zephyr EXL	69*	26.6 ± 0.4^{c}	14.2 - 27.7	24.4 ± 0.7^{c}

* Bolt weights provided by manufacturer
Means in a column with no common superscript letter differ significantly at $P < 0.05$.