Electroencephalographic evaluation of the effectiveness of blunt trauma to induce loss of consciousness for on-farm killing of chickens and turkeys

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ABSTRACT Euthanasia of small numbers of birds in case of injury or other illness directly on the farm may be necessary for welfare reasons. This should be done without transportation of the moribund animals in order to minimize pain and distress. Blood loss has to be avoided to minimize the risk of contaminating the environment. Cervical dislocation in combination with a blunt trauma may be an appropriate way to achieve this aim but the bird’s age and body weight may influence the practicability of this method in the field. In this study, we evaluated broilers, broiler breeders, and turkeys of different age groups and weights up to nearly 16 kg for the efficacy of blunt trauma to induce unconsciousness, allowing subsequent killing of the bird without pain. The effect of blunt trauma on the brain was determined by electroencephalography (EEG). Auditory evoked potentials (AEPs) were recorded for each animal. Convulsions or tonic seizures were observed in all investigated animals after blunt trauma, including strong wing movements, torticollis, and stretching of legs. The EEG results demonstrate that the blunt trauma induced by a single, sufficiently strong hit placed in the frontoparietal region of the head led to a reduction or loss of the AEP in all groups of birds. These results clearly indicate a loss of sensibility and induction of unconsciousness, which would allow painless killing of the birds immediately after the induction of the blunt trauma.

Key words: blunt trauma, poultry, electroencephalography, auditory evoked potential

2015 Poultry Science 94:147–155 http://dx.doi.org/10.3382/ps/peu038

INTRODUCTION

Stunning before slaughter is a binding requirement in European countries based on the Council Regulation 1099/2009 on the protection of animals at the time of killing (EU, 2009). Stunning for on-farm euthanasia may be necessary to allow killing of moribund or sick animals, which are unfit for transport and regular slaughter. Euthanasia requires that the animal dies with minimal pain and distress. Birds need to rapidly lose their consciousness followed by irreversible impairment of brain function and respiratory or cardiac arrest (AVMA, 2007). The effectiveness of a stunning method can be evaluated based on its efficacy to transfer the animal into a state of unconsciousness and insensitivity without anxiety, pain, suffering, or distress (Schutt-Abram et al., 1987), which would allow immediate killing for example by cervical dislocation using a Burdizzo-like forceps (Erasmus, 2010).

Unconsciousness in poultry can be achieved by a variety of methods, including electrical and CO₂ stunning, mainly used in the process of slaughter (Wenzlawowicz et al., 2000; McKeegan et al., 2007; Lines et al., 2011), or blunt trauma (Erasmus, 2010). Electrical and CO₂ stunning were extensively investigated regarding their effects on brain function, reflexes, convulsive readiness, breathing activity, and heart rate (Coenen et al., 2009; Prinz et al., 2010; Lines et al., 2011). Electrical stunning with higher frequencies may lead to defibrillation of the heart or cardiac arrest (EU, 2009; Lambooij et al., 2010) and, consecutively, inhibition of breathing activity due to hypoxia in the brain. CO₂ also induces hypoxia in the brain, and thus loss of breathing activity and heartbeat. Blunt trauma has not been sufficiently investigated in poultry, therefore, final conclusions about efficacy of inducing unconsciousness are lacking (Erasmus, 2010). However, due to the mechanically induced concussion of the brain it is expected that functions of the brain and the brain stem, including sensing of pain and distress, are interrupted (Shaw, 2002). The loss of consciousness can be explained by functional deafferentation of the cortex as a consequence of diffuse mechanically-induced depolarization and synchronized discharge of cortical neurons (Shaw, 2002). This leads to other physiological changes such as blood pressure increase and bradycardia.
Based on the absence of brain-stem reflexes, it has been previously suggested that blunt trauma induces immediate insensibility in turkeys and broilers (Erasmus et al., 2010). The size of the animal as well as the calcification of the skull, which may depend on the age of the bird, together with the force applied by the person conducting the blunt trauma, may determine if animals are unconscious after blunt trauma but still alive, or may subsequently die after the blunt trauma without additional application of a subsequent killing method.

Blunt trauma is an approved stunning method for poultry in Germany that has been applied for centuries. However, at the time of this study, it is only suggested in the context of slaughter, where death occurs by rapid blood loss after additionally severing major blood vessels in the neck area (based on German Regulations for Animal Protection and Slaughter; TierSchlV from 1997). For on-farm killing of sick and/or debilitated animals during the growth period, blunt trauma in combination with rapid blood loss is neither hygienic nor practicable without prior transportation of the animal. Hence, a method for pain- and bloodless sacrificing of moribund animals on the farm after an appropriate stunning still needs to be established (EFSA, 2004; Galvin et al., 2005).

Unconsciousness in poultry has been determined by a variety of methods including the measurement of reflexes. The loss of heart beat and breathing activity have also been included in the list of indicative parameters, but they are also a sign of immediate death (EFSA, 2004). Overall, breathing activity is difficult to evaluate due to the interfering convulsions in birds after blunt trauma (EFSA, 2004). Measurement of brain activity is a more precise way of evaluating unconsciousness. Electroencephalography is a reliable technique to determine brainstem function (Gregory and Wotton, 1990; McKeeegan et al., 2013a, b). An animal can be considered as unconscious and insensible if the previously evocable electrical activity, such as brain stem auditory evoked potentials (AEP) or flash visual-evoked potentials (VEP) disappear in the electroencephalogram (EEG) (Gregory and Wotton, 1986; Erasmus et al., 2010). The latter is indicative of the brain’s inability to receive and process external stimuli (EFSA, 2004). Previous studies in poultry and other farm animals have used visually-evoked responses to determine unconsciousness (EFSA, 2004), but head-blunt trauma does not allow the measurement of visual stimuli due to the possible damage to the orbital cavity and subsequently the eye balls during this process. However, measurement of auditory-evoked potentials is possible in the context of blunt trauma and known to be also well-suited to neurophysiologically monitor brainstem function and the subject’s level of unconsciousness (Shaw, 2002). Although AEPs had been applied in small pet birds, no tests have been conducted in poultry (Lucas et al., 2002).

The aim of our study was to develop a method to measure unconsciousness in poultry after head-blunt trauma. Auditory evoked potentials were used to determine brain function in combination with the evaluation of pupillary and corneal reflexes, spontaneous breathing, and convulsions (Prinz et al., 2010) before and after blunt trauma, respectively. Groups of broilers, broiler breeders, and turkeys of different weight and age were investigated for the efficacy of blunt trauma. Consecutively, all birds were sacrificed by cervical dislocation or internal decapitation and the lesions of the head were macroscopically evaluated.

MATERIALS AND METHODS

Animals

Groups of chickens and turkeys with 5 birds/group were transferred from commercial farms to the Clinic for Poultry and immediately used in the animal experiment, which had been approved by the Animal Welfare Committee of Lower Saxony (LAVES approval 33.9-42502-04-11/0403). Investigated groups consisted of broilers (group 1: 0.8–1.0 kg, group 2: 1.6–9.9 kg of body weight), broiler breeders (group 3: 1.4–1.7 kg, group 4: 3.8–4.4 kg of body weight), and turkeys (group 5: 4.9–5.5 kg, group 6: 14.2–15.7 kg of body weight).

Experimental Design

For the EEG measurements, turkeys were restrained with a blanket and broilers, as well as broiler breeders, with a cotton towel to control wing flapping. The head of the respective bird was left outside the blanket or towel without any additional fixation. A grounding electrode with rounded edges was placed in the animal’s cloaca. Before subcutaneous placement of EEG electrodes (0.4-mm cannula), the skin of the bird’s head was locally anesthetized with Xylocaine Pump Spray (10 mg/puff, AstraZeneca GmbH, Wedel, Germany). The negative electrode was placed directly behind the right ear and the positive one directly above the cranium’s basis on top of the cerebellum. The locations of the electrodes were tagged with a permanent marker to enable a quick repositioning after administration of blunt trauma. Each animal was evaluated for auditory evoked potentials (AEP) before stunning for 1 min with and 1 min without acoustic stimulus (Figure 1). Afterward, electrodes were removed and animals stunned by blunt trauma, which was placed on the frontoparietal region as indicated by the EFSA (EFSA, 2004) of the head using a 250-g scantling for turkeys (length 50 cm), a round metal stick of 290 g for broiler breeders (length, 40 cm), and a wooden handle of 90 g for broilers (length, 32 cm). The blunt trauma force correlated with the size of the birds; for larger animals, the force equalled the one needed to push a nail into a wooden piece with one strike. The smaller birds below 2 kg received a slightly milder blunt trauma to avoid smashing and
destruction of the head. The blunt trauma was delivered by a previously trained person and subsequent stunning was evaluated according to the “Signs of recognition of a successful mechanical stunning” (EFSA, 2004): Immediate collapse or severe wing flapping due to the destruction of the brain, apnea (absence of rhythmic thoracic breathing movements), immediate onset of tonic seizure (tetanus) lasting several seconds (“post-concussive convulsion”), loss of corneal reflex, gradual pupillary dilation, and delay or loss of the pupillary reflex. In rare cases (1 animal of a total of 30 investigated), a second stronger strike was applied if these signs were not immediately observed. Within seconds after the successful application of the blunt trauma, electrodes were repositioned and EEG measurements under auditory stimulation were repeated for each animal for at least 2 min. The disappearance of an auditory-evoked potential was interpreted as unconsciousness of the animal with inability to feel pain as well as to perceive and process external stimuli. After recording of the AEP for a maximum time of 2 min, the unconscious animals were killed with a modified Burdizzo clamp (turkeys) or by cervical dislocation (broilers). All birds were autopsied and investigated for pathological lesions, especially subcutaneous haemorrhages, macroscopically visible brain damage, and subdural hemorrhage.

**Setup for EEG Recording**

Acoustic stimuli (generated clicks, 1 click per s, monophasic, faradic stimulus) were generated with a stimulus generator (Hugo-Sachs Elektronik, March-Hugstetten, Germany, HSE Stimulator P, Type 201) connected to an amplifier and a broadband loudspeaker. The signals from the recording electrodes were amplified (10,000x, Differential-Amplifier, WPI World Precision Instrument DAM 50, Sarasota, USA), band-pass filtered (315-2000 Hz, Ithaco 4213 DL Instruments, Ithaca, USA) and then sent to an oscilloscope (Hitachi Digital VC 7504, Japan). The latter was used as an averager. Up to 32 individual EEG traces, time-locked to the onset of the stimulus pulse, were averaged per AEP.

**Statistical Analysis**

The recorded potentials were compared regarding the total area under the curve and the peak-to-peak (voltage minimum to maximum) amplitude. Because the data were not normally distributed, the Kruskal-Wallis test was applied to the area measurements to test for statistically significant differences. These data are presented as box-and-whisker-plots. Differences in peak-to-peak amplitudes were evaluated for each weight group with the one-way analysis of variance and subsequently the Tukey HSD all-pairwise comparisons test for normally distributed values. The AEP curve recorded prior to stunning was used as 100% reference value for the graphical presentation of the data. Reduced AEP amplitudes after stunning are given in percent of the respective reference value. Mean and standard deviations are reported in the Results section. $P < 0.05$ was considered statistically significant.

**RESULTS AND DISCUSSION**

Overall, the absence or significant reduction of evoked potentials, especially brain stem auditory and visual evoked potentials, has been used previously as an indicator for loss of brain responsiveness and therefore loss of consciousness in many species including poultry (EFSA, 2004; Cartner et al., 2007; AVMA, 2013).
However, upon reversion, the presence of evoked potentials and reflexes alone does not necessarily indicate consciousness, because visual evoked potentials can even be present in anesthetized animals, especially in poultry (Gregory and Wotton, 1988; EFSA, 2004). For the interpretation of a successful stun and unconsciousness, it may therefore be very important to use several indicators such as apnea, immediate onset of tonic seizures lasting several seconds, loss of reflexes, absence of response to a painful stimulus, and possibly others (EFSA, 2004). In this study, auditory evoked potentials were used to determine brain function in chickens and turkeys of different weight in combination with the evaluation of pupillary and corneal reflexes, spontaneous regular breathing, and convulsions before and after blunt trauma.

Overall, during our study none of the investigated animals showed vocalization after blunt trauma nor attempts to raise the head (data not shown). None of the 10 broilers from the weight groups 0.8 to 1.0 kg and 1.6 to 1.9 kg showed any AEP in response to acoustic stimuli post-stunning (Table 1, Figure 2). Overall, the post-stunning AEP was reduced in comparison to the stimulated birds before blunt trauma as determined by the measurement of the area underneath the EEG curve ($P = 0.0017$) and the peak-to-peak-amplitude EEG amplitudes ($P < 0.001$). The post-stunning AEP-values were comparable to those determined in conscious animals in the absence of acoustic stimuli (quiescent EEG; Figure 2). After blunt trauma, all broilers showed convulsions or tonic seizures for 30 to 115 s followed by respiratory arrest within 30 to 120 s. All 10 animals died within 2 min after blunt trauma (Table 1). Except for 1 animal, which showed pupillary reflexes after 50 s post-concussion for about 3 s, no other reflexes were observed in stunned broilers (Table 1).

All 10 broiler breeders showed convulsions or tonic seizures after blunt trauma (Table 1), which lasted for up to 68 s. Seven of 10 broiler breeders showed a quiescent EEG after concussion with an AEP-reduction being comparable to or even below the one observed in the respective bird without stimulus before stunning (Table 1, Figure 3). One broiler breeder of the lower weight group and 2 of the heavier animals showed an AEP with reduced peak-to-peak amplitude (amplitude of 81, 63, and 51% compared to the respective reference value set 100% for each animal). The overall reduction of the AEP in broilers with blunt trauma as determined by the comparison of the area underneath the EEG curve was significant ($P < 0.0001$) as well as the reduction in peak-to-peak amplitude ($P = 0.0001$ for both weight groups) in comparison with the conscious control measurements after acoustic stimuli was highly significant.

None of the broiler breeders with 1.4 to 1.7 kg body weight showed corneal or pupillary reflexes after blunt trauma, and 3 of these animals showed respiratory arrest and died during the EEG measurement (Table 1). The broiler breeder (bird number 1, Table 1), which had 19% AEP-reduction compared to 64% peak-to-peak amplitude reduction in this bird determined in a conscious state without stimulus (quiescent EEG), did not show any corneal or pupillary reflex during the experiment. But this bird showed irregular arrhythmic breathing with dropouts. Regular breathing activity was not observed in this animal after blunt trauma. The other two birds (broiler breeders number 6, 7) with a peak-to-peak amplitude reduction in the AEPs of 49 and 37% compared to the reduction in the conscious state without stimulus of 64 and 75% (quiescent EEG), respectively, showed remaining reflexes, in 1 case reduced or delayed cornea and pupillary reflexes. One of these birds showed respiratory arrest. One heavier broiler breeder (bird number 8) with loss of AEP also showed respiratory arrest despite the presence of corneal and pupillary reflexes after blunt trauma within the duration of the experiment. Two of the 5 heavier broiler breeders (broiler breeders number 9, 10) showed loss of corneal and pupillary reflexes, stopped rhythmic, regular breathing after blunt trauma and died within 2 min (Table 1).

The results of these investigations with broiler breeders indicate that presence of a corneal reflex does not necessarily imply consciousness. But when this reflex is absent, it is likely that the animal is unconscious (Anil and McKinstry, 1991; EFSA, 2004). The cause of the remaining pupil twitching and the detectable but delayed light response in 3 broiler breeders (Table 1) is unknown, but it may be connected to brain damage. Similar observations have been also made in the context of electrical waterbath stunning (Prinz et al., 2012). Experiences from studies with anesthetized birds for surgery demonstrate that it is not wanted to achieve complete loss of reflexes, such as corneal or palpebral reflexes (Mostachio et al., 2008), as otherwise the anesthesia is considered too deep and the bird may die (Korb, 1995). Reflexes, therefore, can be used as supportive measures to determine unconsciousness but should not be overinterpreted, as they are considered unreliable (Altman and Forbes, 2003).

All 10 turkeys lost their AEP (Table 1). As indicated in Figure 4, the reduction of the AEP in turkeys after blunt trauma was highly significant ($P < 0.0001$) as determined by the measurement of the area underneath the EEG curve as well as the peak-to-peak amplitude ($P = 0.0001$ and $P = 0.0004$ for the smaller and bigger weight group, respectively) compared to the AEP of conscious birds. All turkeys showed convulsions or tonic seizures up to 2 min after induction of blunt trauma (Table 1). All turkeys with body weights between 4.8 and 5.5 kg lost their corneal and pupillary reflexes, showed respiratory arrest, and died within 2 min after concussion (Table 1). Only 2 of the 5 heavier birds (14.2 and 15.0 kg) showed loss of corneal and pupillary reflexes. During convulsions, all investigated turkeys of the heavier weight group showed laryngeal

\[ P = 0.0004 \text{ for the smaller and big-} \]

\[ P = 0.0001 \text{ and } P = 0.0004 \]
Table 1. Effect of blunt trauma on the AEP amplitude, induction of convulsions, reflexes, breathing activity, and death

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[^1]: Respiratory arrest within 2 min after blunt trauma; birds without complete respiratory arrest showed gasping and irregular breathing.

[^2]: Reduction in peak-to-peak amplitude/area underneath the EEG curve.

[^3]: Death within 2 min after blunt trauma.

[^4]: This bird had immediately received a second strike due to insufficient convulsions after the first; AEP was determined after the second strike.

+ = observed parameter present; − = observed parameter absent.
Gasping, while rhythmic thoracic breathing activity was absent in all birds. After the termination of EEG measurements, these birds were killed using the Burdizzo-like forceps.

Normal breathing, characterized by rhythmic thoracic inspiration and expiration movements, differs from gasping or gagging, a rudimentary respiratory activity of the larynx occurring through the mouth (EFSA, 2006). In our study, gasping or gagging was observed frequently in the absence of AEPs, and therefore has to be judged as not being indicative for consciousness.

All chickens and turkeys investigated had subcutaneous haematomas in the frontoparietal region, some of the chickens and turkeys also showed bleeding in the brain tissue with severe lacerations and some broiler breeders head bone fractures (Table 1), which indicates a severe traumatic brain injury. There is no direct correlation between the observed pathological lesions and the effect on the AEP. In combination with the pathophysiology of concussion, which leads to a severe brain damage and functional disturbance of the central nervous system accompanied by immediate subarachnoid hemorrhage and excessive pressure on the brain followed by convulsions, the loss or reduction of the AEP precisely indicates traumatic unconsciousness (EFSA, 2004). Only 3 of a total of 30 investigated animals still showed AEP post-concussion, but with clear reduction in intensity of 19%, 37%, and 49% compared to the pre-trauma AEP measured with stimulus. Possibly factors such as the sex may influence the susceptibility of the birds to blunt trauma and subsequently the reduction of the AEP as it was shown previously after electrical stunning of chickens (Prinz et al., 2012). This point was not investigated in this study but may be of interest for future investigations.

Our study indicates that body weight may not be an appropriate parameter to predict the effect of blunt...
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Figure 3. Reduction of AEP after blunt trauma in broiler breeders. AEP was measured for each animal after an acoustic stimulus and without stimulus for 1 min. Subsequently, birds received a blunt trauma and AEP was determined after a stimulus for up to two minutes. (a) Determination of the area underneath the EEG curve ($n = 10$; body weight between 1.4 and 4.4 kg); (b) Measurement of percent reduction considering the minimum and maximum amplitude of the EEG curve ($n = 5$/weight group). Different superscript letters ($P < 0.01$, Kruskal-Wallis test) or '∗' indicate significant differences between different treatments. '∗∗∗' $P < 0.001$ based on the statistical analysis of peak-to-peak values by ANOVA.

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trauma on the AEP, because loss of the AEP was detected in all 10 turkeys up to 15.7 kg of body weight. The macroscopic lesions also did not differ between the groups of birds heavier than 1 kg including the heavier broilers, broiler parents, and turkeys. Overall, older broiler breeders have a more advanced ossification of the skull, which may influence the severity of the head trauma in comparison to younger birds. This aspect may be of interest for future investigations, which may subsequently help to determine the effect of age on the efficacy of blunt trauma. Reflexes persisted in some of the heavier broiler breeders with 4.0 kg or higher weight and also heavier turkeys with more than 15.5 kg of body weight, while they were lost in turkeys with a body weight of 4.9 to 5.5 kg.

In summary, the results of this study show that blunt trauma, if done with the appropriate tool and force, is an acceptable and reliable way to induce unconsciousness under on-farm conditions in chickens and turkeys of at least up to 16 kg, which allows painless killing of these animals by cervical dislocation within 2 min after the onset of blunt trauma. Characteristic post-concussive convulsions and tonic seizures were observed in all animals as it was also shown previously in other studies using comparable stunning procedures (Raj and O’Callaghan, 2001) and none of the investigated birds showed regular breathing activity after blunt trauma, therefore these parameters can be suggested as reliable indicators under field conditions for a successful stunning procedure.

ACKNOWLEDGMENT

The authors thank Anna Schwarz and Sabrina Techel, Clinic for Poultry, University of Veterinary Medicine Hanover, for their excellent support during the animal experiments and Günter Zengerling,
Figure 4. Reduction of AEP after blunt trauma in turkey. AEP was measured for each animal after an acoustic stimulus and without stimulus for 1 min. Subsequently, birds received a blunt trauma and AEP was determined after a stimulus for up to 2 min. (a) Determination of the area underneath the EEG curve ($n = 10$; body weight between 4.9 and 15.7 kg); (b) Measurement of percent reduction considering the minimum and maximum amplitude of the EEG curve ($n = 5$/weight group). Different superscript letters ($P < 0.01$, Kruskal-Wallis test) or ‘∗’ indicate significant differences between different treatments. ‘∗∗∗$P < 0.001$ based on the statistical analysis of peak-to-peak values by ANOVA.

Zentralverband der Deutschen Geflügelforschung e.V., Berlin, for fruitful discussions regarding the design of the experiment. Furthermore, we like to thank the Bundesverband der Geflügelzüchter e.V. for financial support of the study.

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