Development of Brain Damage as Measured by Brain Impedance Recordings, and Changes in Heart Rate, and Blood Pressure Induced by Different Stunning and Killing Methods

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ABSTRACT Poultry are electrically stunned before slaughter to induce unconsciousness and to immobilize the chickens for easier killing. From a welfare point of view, electrical stunning should induce immediate and lasting unconsciousness in the chicken. As an alternative to electroencephalography, which measures brain electrical activity, this study used brain impedance recordings, which measure brain metabolic activity, to determine the onset and development of brain damage. Fifty-six chickens were surgically equipped with brain electrodes and a canula in the wing artery and were subjected to one of seven stunning and killing methods: whole body electrical stunning; head-only electrical stunning at 50, 100 or 150 V; or an i.v. injection with MgCl₂. After 30 s, the chickens were exsanguinated. Brain impedance and blood pressure were measured. Extracellular volume was determined from the brain impedance data and heart rate from the blood pressure data. An immediate and progressive reduction in extracellular volume in all chickens was found only with whole body stunning at 150 V. This treatment also caused cardiac fibrillation or arrest in all chickens. With all other electrical stunning treatments, extracellular volume was immediately reduced in some but not all birds, and cardiac fibrillation or arrest was not often found. Ischemic conditions, caused by cessation of the circulation, stimulated this epileptic effect. A stunner setting of 150 V is therefore recommended to ensure immediate and lasting unconsciousness, which is a requirement for humane slaughter.

(Key words: extracellular volume, generalized epileptiform insult, ischemia, electrical stunning, unconsciousness)
tissue due to metabolic failure of the cells. A high voltage electrical stun that passes current through the whole body induces immediate and progressive decrease in extracellular volume, indicating immediate and lasting unconsciousness in broiler chickens (Savenije et al., 2000). Head-only stunning, with electrodes on both sides of the head, was studied as an alternative to whole-body stunning, assuming that a lower current could be used to induce unconsciousness if the current would be passed directly through the brain. Based on brain impedance recordings, inconclusive evidence was found regarding the immediate induction of unconsciousness by head-only stunning at 100 V.

To substantiate evidence for stunning efficiency, multiple parameters should be measured. Other central processes mentioned in the literature with respect to passing electric current through the brain are as follows: increase of extracellular (mostly excitotoxic) amino acid levels, increase of extracellular lactate levels, ionic shifts, rise in tissue temperature, and change in cerebral blood flow (Bode, 1992; Cook et al., 1995; Kawamata et al., 1995; Korf, 1996; Reilly, 1994). Unfortunately, it is very difficult to measure these central parameters in combination with brain impedance measurements and electrical stunning. In the periphery, an increase in systolic blood pressure and a reduction in heart rate and respiratory rate (Kuenzel and Walth, 1978) and reflex reactions (Gregory and Wotton, 1987) have been studied. Reflex reactions to stimuli on the head (e.g., cornea and comb pinch reflexes) are the results of central processing of the given stimuli, which can be measured as evoked responses. Heart function and respiratory rate reflect the level of oxygenation of the brain. With death of an animal, heart rate, respiratory rate, and blood pressure decline, indicating the onset of ischemic conditions in the brain. These parameters have not yet been measured together to evaluate poultry welfare at slaughter, and it could be of use to determine the state of the animal after stunning and exsanguination.

The purpose of this study was to evaluate the effectiveness of different methods of stunning and killing broiler chickens with respect to change in extracellular volume (ECV), blood pressure, and heart rate. For practical reasons, anesthetized chickens were used. We studied two positions of the stunning electrodes, whole body and head only, and anesthetized chickens were used. We studied two positions (ECV), blood pressure, and heart rate. For practical reasons, the purpose of this study was to evaluate the effectiveness of different methods of stunning and killing broiler chickens with respect to change in extracellular volume (ECV), blood pressure, and heart rate. For practical reasons, anesthetized chickens were used. We studied two positions of the stunning electrodes, whole body and head only, and three voltages, 50, 100, and 150 V. An injection with an overdose MgCl₂ was used to determine the effects of ischemia without interaction of electrical effects.

**Animals, Housing, and Surgery**

Commercially reared, 5-wk-old Cobb broiler chickens (n = 56) were purchased from a slaughter plant. Chickens from a commercial strain were chosen for practical relevance. The chickens were transported to the experimental animal facility of the Institute for Animal Science and Health and were housed under standard conditions (group housed on litter at 20°C; 23 h light:1 h darkness cycle; feed and water available ad libitum). The chickens were allowed 48 h of rest after transport. Approval for carrying out the experiment was obtained from the ethical committee of the Institute for Animal Science and Health.

Eight hours before surgery, feed was removed from the pen. Anesthesia was administered by an i.m. injection of 1 mL Ketamine, followed 15 min later by an i.v. injection of 0.3 to 0.8 mL Nembutal. The chickens were attached to a pair of silver electrodes in the striatum area of the brain as described by Ruis-Heutinck et al. (1998). For blood pressure measurements, an i.v. canula was inserted into the left or right wing artery (arteria ulnaris) and connected to a valve to prevent blood loss.

**Stunning and Killing Treatments**

Immediately after surgery and before the chickens recovered from anesthesia, the birds were hung by the legs from shackles. The brain electrodes were connected to an impedance-recording device and the canula valve was connected to the transducer of a digital electromanometer. All signals were registered on flat-bed recorders. Base impedance and blood pressure signals were recorded for 5 min. Just before stun, the brain impedance and blood pressure recording equipment were disconnected to prevent electrical damage. The chickens were randomly subjected to one of the following stunning and killing methods:

1. Whole-body electrical stunning for 4 s at 50 V, 100 V, or 150 V, 50 Hz (n = 8 for each voltage), followed 30 s later by exsanguination. With one stunning electrode on the comb and one on the cloaca, this method simulated the passage of electric current through the head and body of the conventional water bath stunning.
2. Head-only electrical stunning for 4 s at 50 V, 100 V, or 150 V, 50 Hz (n = 8 for each voltage), followed 30 s later by exsanguination. A pair of scissors-like tongs was used to connect the stunning electrodes to both sides of the head of the chicken. This method was studied as an alternative to whole-body stunning.
3. Induction of cardiac fibrillation by i.v. injection of an overdose MgCl₂ (2.5 mL of 300 mg/mL MgCl₂·6H₂O solution; n = 8), followed 30 s later by exsanguination. This group served as a control for determining the effects of circulatory arrest without the effects of electrical stunning.

During stun, the voltage and current between the stunning electrodes were registered on a flat-bed recorder. Immediately after stun, recording of the blood pressure and
brain impedance was started. Blood pressure was recorded for 75 s and brain impedance for 10 min. The experiment was carried out over 14 d, recording data from four chickens per day.

**Histological Analysis**

After recording was finished, the brain was dissected from the carcass and fixed in a 4% paraformaldehyde solution for at least 2 wk. The electrode position was then determined by comparing 40 µm thick brain slices with a stereotaxic atlas (Kuenzel and Masson, 1988), as described by Ruis-Heutinck et al. (1998).

**Presentation of Results and Statistics**

Changes in relative ECV were calculated at 1-min intervals from the brain impedance recordings according to Maxwell’s equation:

$$\frac{ECV_i}{ECV_B} = R_i$$

where ECV<sub>B</sub> = base ECV before stunning, which is defined as 100%; ECV<sub>i</sub> = ECV at i min after stunning; R<sub>B</sub> = recorded base tissue impedance before stunning; and R<sub>i</sub> = recorded brain tissue impedance at i min after stunning. ECV<sub>i</sub> thus calculated as the percentile deviation of the base ECV. The time needed to reach 50% of the decrease in ECV found at 10 min after stunning, t<sub>0–50%</sub>, was determined.

Blood pressure data (mm Hg) were read from the recorded graph as systolic and diastolic values, or every 0.2 s if systolic and diastolic pressures could not be distinguished. For the analysis of the blood pressure a running mean (period = 2) was calculated. From the blood pressure recordings mean heart rate values (beats per minute) were calculated as the number of systolic peaks every 5 s up to 30 s after stunning.

For the stunning and killing treatments, ECV at 0 and 10 min after stunning, t<sub>0–50%</sub>, heart rate, and blood pressure data were analyzed using regression analysis with the following model: \( y_i = SKM_i + e_i \), where SKM<sub>i</sub> = one of the seven stunning and killing methods, and e<sub>i</sub> = residual error. The delay time until the ECV differed significantly from the base level and other comparisons within one parameter over time were tested by means of Student’s t-test. Data are represented as means ± standard error. The Genstat statistical software package was used for all tests (Genstat 5, 1993).

**RESULTS**

**Electric Current Measurements**

Recorded voltages at 1 s in the stunning period showed some variance around the values set at the stunning apparatus. For whole-body stunning, settings were 52 ± 1, 100 ± 6, and 123 ± 25 V, and for head-only stunning settings were 55 ± 6, 112 ± 37, and 141 ± 10 V. Measured currents at 1 s in the stunning period for whole-body stunning were 42 ± 10, 97 ± 18, and 166 ± 28 mA and for head-only stunning were 86 ± 50, 122 ± 45, and 386 ± 129 mA. For all voltage settings, currents at 1 s were higher (\( P < 0.01 \)) with head-only stunning than with whole-body stunning.

**ECV Measurements**

Brain impedance was recorded for 10 min after stunning or injection in each chicken. ECV patterns for all treatment groups are shown in Figure 1. The ECV in the MgCl<sub>2</sub> injection and head-only stunning at 50 V treatment groups were not significantly decreased from base ECV immediately after stunning or injection. After head-only stunning at 50 V, the ECV differed (\( P < 0.05 \)) from base ECV from 3 min after stunning, and after MgCl<sub>2</sub> injection the ECV differed from base ECV from 1 min onward. The ECV in whole-body stunning treatment groups and the head-only stunning group at 150 V were significantly decreased from the base ECV (ECV 94.3 ± 4.7%; \( P < 0.001 \)) immediately after stunning.

In all treatment groups, ECV at 10 min after stunning or injection was significantly (\( P < 0.001 \)) decreased from base ECV. At 10 min, ECV levels did not differ among treatment groups (mean 53.9 ± 8.3%).

The t<sub>0–50%</sub> was the shortest (\( P < 0.01 \)) in whole-body stunning for the 150 V group (2.6 ± 0.5 min). The t<sub>0–50%</sub> of the MgCl<sub>2</sub> injection group, head-only group at 150 V, and whole-body group at 100 V were slightly longer (3.7 ± 0.9 min; \( P < 0.05 \)). The t<sub>0–50%</sub> did not differ among the other treatment groups (mean 4.4 ± 1.1 min).

Different patterns in ECV decrease were found between MgCl<sub>2</sub> injection and whole-body stunning at 150 V. MgCl<sub>2</sub> injection resulted in an initial delay in all chickens before decreasing, whereas whole-body stunning at 150 V resulted in an immediate decrease in all chickens in ECV, which continued to decrease while gradually leveling. Within all other treatment groups, ECV responses of individual chickens showed a more mixed pattern, which had little consistency with regard to decrease immediately after stunning and initial slope of the curve in relation to the applied voltage or the current passed between the stunning electrodes.

**Blood Pressure Measurements**

Mean arterial blood pressure was measured (mm Hg) from the recorded graphs at 1 s and at 5-s periods, up to 30 s after stunning. Blood pressure could not be recorded in one chicken in the head-only stunning at 100 V and 150 V groups and in two chickens in the whole-body stunning at 100 V group because of technical problems due to severe convulsions. Blood pressure responses per treatment group are shown in Figure 2. In the whole-body stunning at 150 V and MgCl<sub>2</sub>-injection groups, mean blood pressure was significantly (\( P < 0.05 \)) decreased from base blood pressure at 5 and 10 s after stunning or injection, respectively, and...
continued to decrease. In the other groups, mean blood pressure did not differ significantly over time. In most chickens, a similar response pattern was found, which consisted of an initial depression within 5 to 10 s and then transient increases that returned toward the blood pressure level of depression at 30 s. The variations in amplitude and timing, however, were too large to establish statistical significance. In all treatment groups except whole-body stunning at 150 V and MgCl₂ injection, pulse pressure was significantly ($P < 0.01$) increased from 5 s after stunning onward and did not return to the base pulse pressure within the recorded time.

Heart Rate Measurements

Heart rate was calculated in beats per minute from the blood pressure graph every 5 s up to 30 s after stunning or injection with MgCl₂ (Figure 3). Heart rate could not
FIGURE 3. Heart rate patterns for all treatment groups. Treatment groups are as follows: whole-body electrical stunning (WB) or head-only electrical stunning (HO) at 50, 100, or 150 V, or i.v. injection of a MgCl₂ solution. Points represent the mean heart rate over the previous 5-s period.

be determined in one chicken in 50 V stunning groups because of low signal resolution. Cardiac fibrillation or arrest was assumed when blood pressure declined rapidly and systolic and diastolic blood pressure could not be distinguished. Cardiac fibrillation or arrest was found in three chickens of the MgCl₂-injection group, in one chicken in either of the 100 V stunning groups, and in all chickens of the whole-body stunning group at 150 V. For statistical analysis, chickens with cardiac fibrillation or arrest were excluded. In all treatment groups, the mean heart rate at 5 s after stunning was significantly decreased ($P < 0.001$) from base heart rate. At 5 s the MgCl₂-injection group had a higher heart rate (258 bpm, $P < 0.05$) than the other treatment groups (mean 172 bpm). At 30 s after stunning, the mean heart rate within each treatment group remained significantly lower ($P < 0.001$) than the respective base heart rate. Heart rate did not differ significantly between 5 and 30 s in any electrical stunning groups, except the whole-body stunning at 50 V group. At 30 s, whole-body stunning at 50 V group had higher heart rates (266 bpm, $P < 0.05$) than the other treatment groups (mean: 190 bpm). Heart rates in the MgCl₂-injection group continued to decrease, resulting in a lower rate at 30 s (103 bpm, $P < 0.05$) than in the other treatment groups.

**Histological Analysis**

Histological examination of consecutive slices of the brain confirmed that all electrodes were positioned in the hyperstriatum ventrale or the neostriatum area of the brain. Thus, all brain impedance measurements were recorded from the correct areas of the brain.

**DISCUSSION**

**ECV**

The extreme treatments with regard to electrical impact on the body, i.e., whole-body stunning at 150 V, and MgCl₂ injection (no electrical impact), resulted in ECV profiles that were comparable with the results found by Savenije et al. (2000). Whole-body stunning at 150 V induced an immediate decrease in ECV after stunning, which continued to decrease over time. These results indicate an instantaneous and lasting severe brain dysfunction and concomitant unconsciousness. With MgCl₂ injection, the ECV decreased only after 1 min and not immediately after injection. This decrease in ECV was purely ischemic in nature. A mixture of a decrease or unchanged ECV immediately after stunning, and immediately starting decrease or an initial plateau in ECV, was found with some individuals in all other treatments. This mixture of patterns was found in whole-body stunned and head-only stunned groups and with low current (50 V stunning groups) and very high current (head-only stunning at 150 V group). Similar results have been reported by Savenije et al. (2000), but the results of this experiment were less bimodal in appearance, which supports the conclusion that the cause of the brain damage during stunning and bleeding is epileptic and ischemic in nature. It remains unclear, however, which criteria trigger an immediate increase in brain impedance or a delay or what determines the rate of increase in brain impedance in individual chickens. In cases in which an immediate and progressive decrease in ECV was not found, brain impedance recordings were not conclusive about the state of consciousness of the animal. Given the similar problem with mea-
suring brain electrical activity, from an animal welfare point of view it must be recommended that stunning settings are used at which we are sure unconsciousness is induced.

In all treatment groups, the ECV at 10 min was significantly reduced from base ECV, as would be expected after death of the birds. The amount of decrease in ECV was comparable with the study of Ruis-Heutinck et al. (1998). Exsanguination after 30 s also induced ischemia also in the brains of those chickens that were not already ischemic due to cardiac fibrillation or arrest. The actual amount of decrease in ECV, however, is not as important as the fact that, once decreased, the ECV remains below base level, which is essential to guarantee that welfare is not compromised. The lower t50–50% in treatment groups with chickens that had cardiac fibrillation indicated that an early induction of ischemia reduces the time needed to induce brain dysfunction and therefore is preferred for humane slaughter.

**Blood Pressure**

Basal blood pressure followed the same pattern described for telemetric recordings by Savory and Kostal (1997). Arterial blood pressure dropped sharply if cardiac fibrillation was induced. In the other treatment groups, arterial blood pressure did not differ significantly. The blood pressure response pattern observed, although not statistically significant in this experiment, with an initial decrease at 5 to 10 s after stunning followed by a transient increase has been described previously (Rhody and Kuenzel, 1981) in 120-V shocked, genetically seizure-prone Cornell White Leghorn chicks. They found a depression within 8 s and a transient increase up to 60 s after stunning. The pattern in normal chicks could not be shown with statistical significance in that study either. The initial depression is attributed to tonic convulsions. A sudden, deep inhalation, which is a similar chest movement in conscious chickens, causes a similar decrease in blood pressure (Bopelet, 1974). Vigorous muscle activity combined with a release of catecholamines and subsequent vasodilatation in skeletal muscles after head-only stunning would redirect more blood from the central vessels to the peripheral vessels and capillaries. However, the associated increase in arterial blood pressure found in lambs (Petersen et al., 1986) was not found in the broiler chickens in this study, despite severe clonic convulsions after head-only stunning at 50 and 100 V. It could be speculated that poultry have greater ability to reduce the effects of high blood pressure, possibly by exchange of water between blood and tissue fluid, as this mechanism works in reverse to compensate a decrease in blood pressure during hemorrhaging (Hillman and Lundvall, 1981). After the neck vein was cut, blood pressure declined rapidly, as described by Kuenzel and Walth (1978). This decline promotes rapid development of ischemia in the brain and onset of unconsciousness. Although not measured in this study, cardiac fibrillation does not negatively affect total bleed-out at slaughter, although it may require a longer time (Griffiths, 1983).

**Heart Rate**

Cardiac fibrillation or arrest occurred in all birds of the whole-body stunning at 150 V group, but this occurred only occasionally in the other treatment groups. Even the high currents measured with head-only stunning at 150 V were not sufficient to systematically induce cardiac fibrillation. Although it is expected that most current passed through the head, which causes mostly clonic convulsions, tonic convulsions occur with head-only stunning (Hillebrand et al., 1996). This effect may indicate a shunt through the body. The path of the current through the body remains unknown, but insufficient current passed through the heart to induce cardiac fibrillation or arrest. All electric stunning methods were capable of reducing the heart rate significantly for at least 30 s after stunning, as also found by Kuenzel and Walther (1981) and Rhody and Kuenzel (1978). Pulse pressure was increased during that time so the cardiac output might not have been reduced much. Injection with 2.5 mL of a 300 mg MgCl2/mL solution did not cause cardiac fibrillation or arrest in all chickens of that group, as suggested by Blomqvist and Wieloch (1985). The reason for this difference is unclear; however, cardiac function was reduced enough to cause a change in ECV by 1 min after injection and deteriorated over time. Only immediate heart fibrillation or arrest caused by 150 V (mean current: 166 mA) electrical whole-body stunning caused a more immediate decrease in heart rate, which is in accordance with results found by Gregory and Wotton (1987), who reported induction of cardiac fibrillation in 99% of the chickens at a minimum stunning current of 148 mA.

In conclusion, although it is clear that electric current can immediately reduce the ECV in broiler chickens and thereby induce immediate unconsciousness, this effect is not consistent for all birds. To ensure immediate and lasting unconsciousness, an immediate and progressive ECV decrease is required. Only with whole-body electrical stunning at 150 V was such a response in ECV consistently found in all chickens. The lack of circulation seems to play an essential role in the onset and development of brain damage. Only if the initial electrical effect is followed by ischemic conditions can immediate and lasting unconsciousness be guaranteed. The conditions require induction of cardiac fibrillation or arrest and therefore a high stunning current that is also applied over the heart. Gregory and Wotton (1987) reported a minimum current of 148 mA to ensure that cardiac arrest occurred in 99% of chickens. This experiment confirms that for humane slaughter, a minimum voltage of 150 V with whole-body stunning be used to ensure immediate unconsciousness by cardiac fibrillation or arrest and prevent any possibility of regaining consciousness.

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