CHAPTER 4

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 uses brain impedance recordings to determine the onset and development of brain damage, which measures brain metabolic activity. Fifty six chickens were surgically equipped with brain electrodes and a canula in the wing artery and subjected to one of seven stunning and killing methods: whole body electrical stunning, or 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 animals, and cardiac fibrillation or arrest was not often found. Ischemic conditions caused by a stop of the circulation stimulate 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
INTRODUCTION

Europe produces over 8.5 million tonnes of poultry meat per year. With the conventional method of slaughtering poultry, electrical waterbath stunning followed by cutting the neck veins, animal welfare and meat quality often require contradictory settings in the stunning apparatus. A low stunning current could lead to chickens suffering during death struggle. Poultry welfare during slaughter can be guaranteed by applying a stun-kill, which requires high current levels (Gregory and Wotton, 1987a). However, high current levels result in carcass and meat quality problems, such as hemorrhages and broken bones (Griffiths, 1985; Hillebrand et al., 1996). Therefore the industry is reluctant to use a high stunning current. As yet there has not been agreed upon a minimum current level for electrical stunning within the European Union.

From a welfare point of view a good electrical stun immediately renders the animal unconscious and insensitive to pain. The evaluation of stunning and slaughter systems is difficult because the onset and duration of unconsciousness can not be measured directly. As unconsciousness is a process of brain dysfunction, certain pathological states of the brain are associated with a state of unconsciousness. One of these states that is relevant with regard to electrical stunning is a general epileptiform insult. In the past epileptiform insults have been assessed by various means of looking at brain electrical activity (electroencephalogram, electrocorticogram, evoked potentials) (Gregory, 1987; Gregory and Wotton, 1987; Mohan Raj, 1998). However, the epileptic activity in the electroencephalogram and electrocorticogram of poultry may indicate not a general epileptiform insult, but a different epileptic condition the like of which in humans does not always cause unconsciousness (Gregory and Wotton, 1987).

An alternative approach for determining brain dysfunction is recording brain tissue impedance (Van Harreveld, 1972). This method was recently adapted for use with poultry (Ruis-Heutinck et al., 1998). Changes in brain impedance reflect changes in the extracellular volume (ECV) of the brain tissue due to metabolic failure of the cells. A high voltage electrical stun passing current through the whole body was shown to induce an 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 regarding the immediate induction of unconsciousness by head only stunning at 100 V was found.

To substantiate evidence for stunning efficiency multiple parameter should be measured. Other central processes mentioned in literature with respect to passing electric current through the brain are: 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 Walther, 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 the death of the animal, heart rate, respiratory rate and blood pressure drop, indicating the onset of ischemic conditions in the brain. Measuring these parameters together has not yet been done to evaluate poultry welfare at slaughter, and it can be of use in determining the state of the animal after stunning and exsanguination.

The purpose of this study was to evaluate the effectiveness of different ways 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. Two positions of the stunning electrodes, whole body and head only, and three voltages, 50, 100, and 150 V, were studied. An injection with an overdose MgCl₂ was performed to determine the effects of ischemia without interaction of electrical effects.

MATERIALS AND METHODS

Animals, housing and surgery

Commercially reared five weeks 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 housed under practical conditions (group housed on litter at 20°C, 23:1 h light:dark 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\textsuperscript{1}.

Eight hours before surgery, feed was removed from the pen. Anesthesia was administered by an i.m. injection of 1 ml Ketamine\textsuperscript{2}, followed 15 min later by

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an i.v. injection of 0.3 to 0.8 ml Nembutal\(^3\). The chickens were equipped with 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\(^4\) was inserted into the left or right wing artery (Arteria ulnaris) and connected to a valve\(^5\) to prevent blood loss.

**Stunning and killing treatments**

Immediately after surgery and before the animals recovered from anesthesia the chickens were hanged by the legs from shackles. The brain electrodes were connected to an impedance recording device\(^6\) and the canula valve was connected to the transducer of a digital electromanometer\(^7\). All signals were registered on flat bed recorders. Base impedance and blood pressure signals were recorded for 5 min. Just before stunning 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:

- Whole body electrical stunning for 4 s at 50V, 100V, 150V, 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 stunner.
- Head only electrical stunning for 4 s at 50V, 100V or 150V, 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 with both sides of the head of the chicken. This method was studied as an alternative to whole body stunning.
- Induction of cardiac fibrillation by i.v. injection of an overdose MgCl\(_2\) (2.5 ml of a 300 mg/ml MgCl\(_2\).6H\(_2\)O 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 stunning the voltage and current between the stunning electrodes were registered on a flat bed recorder. Immediately after stunning, recording of the blood pressure and brain impedance was started. Blood pressure was recorded for 75 s, brain impedance for 10 min. The experiment was carried out over 14 d, recording data from 4 chickens per day.

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Histological analysis

After recording was finished, the brain was dissected from the carcass and fixated in a 4% paraformaldehyde solution during 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 extracellular volume were calculated at 1 min intervals from the brain impedance recordings according to Maxwell's equation:

\[
\frac{ECV_i}{ECV_B} = \frac{R_B}{R_i}
\]

where \(ECV_B\) is the base ECV before stunning, which is defined as being 100%, \(ECV_i\) is the ECV at time \(i\) min after stunning, \(R_B\) is the recorded base tissue impedance before stunning, and \(R_i\) is the recorded brain tissue impedance at time \(i\) min after stunning. \(ECV_i\) is 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, \(t_{0-50}\%\) 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 times 0 and 10 min after stunning, \(t_{0-50}\%\), heart rate and blood pressure data were analyzed using regression analysis with the model: \(y_{ij} = \text{SKM}_i + e_{ij}\) where \(\text{SKM}_i\) is one of the seven stunning and killing methods, and \(e_{ij}\) the residual error. The delay time until the ECV differed significantly from the base level and other comparisons within one parameter in time were tested by means of Student’s t-test. Data are represented as means ± s.e. The Genstat statistical software package was used for all tests (Genstat 5, 1993).
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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 voltages were 52 ± 1, 100 ± 6 and 123 ± 25 V, and for head only stunning 55 ± 6, 112 ± 37 and 141 ± 10 V, respectively. 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 86 ± 50, 122 ± 45, and 386 ± 129 mA respectively. 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 fig. 1. The ECV in the MgCl\textsubscript{2} 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\textsubscript{2} injection the ECV differed from base ECV from 1 min onward. The ECV in the head only stunning at 100 V group was slightly decreased (ECV 98.3 ± 1.6%; P < 0.05) immediately after stunning. The ECV in all whole body stunning treatment groups and the head only stunning group at 150 V were seriously decreased from base ECV (ECV 94.3 ± 4.7%; P < 0.001) immediately after stunning.

In all treatment groups the 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 between treatment groups (mean 53.9 ± 8.3%).

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

Different patterns in ECV decrease were found between MgCl\textsubscript{2} injection and whole body stunning at 150 V. MgCl\textsubscript{2} injection resulted in all chickens an initial delay before decreasing, while whole body stunning at 150 V resulted in all chickens in an immediate decrease in ECV, which continued to decrease while gradually leveling. Within all other treatment groups the ECV responses of individual chickens showed a more mixed pattern, which had little consistency with regard to both the decrease immediately after stunning and the initial slope of the curve in relation to the applied voltage or the current passed between the stunning electrodes.
Figure 1. Extracellular volume over 10 minutes for all treatment groups. Treatment groups are whole body electrical stunning (WB) or head only electrical stunning (HO) at 50, 100, or 150 V, or intravenous injection of a MgCl$_2$ solution. Base ECV before stunning or injection was defined as 100%. Data are mean + s.e.

Blood pressure measurements
Mean arterial blood pressure was measured (mm Hg) from the recorded graphs at 1 s and in 5 s periods, up to 30 s after stunning. Blood pressure could not be recorded in 1 chicken in both the head only stunning at 100 V and 150 V groups, and in 2 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$_2$ 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 by transient increase which returned towards the blood pressure level of the depression at 30 s. The variation in amplitude and timing, however, was too large to establish statistical significance.
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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 period.

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. Heart rate could not be determined in 1 chicken in both 50 V stunning groups because of low signal resolution. Cardiac fibrillation or arrest was assumed when blood pressure dropped rapidly and systolic and diastolic blood pressure could not be distinguished. Cardiac fibrillation or arrest was found in 3 chickens of the MgCl₂ injection group, in 1 chicken in either of the 100 V stunning groups, and in all chickens of the whole body stunning at 150 V group. 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 was still significantly lower ($P < 0.001$) than their
Figure 3. Heart rate patterns for all treatment groups. Treatment groups are whole body electrical stunning (WB) or head only electrical stunning (HO) at 50, 100, or 150 V, or i.v. injection of a MgCl$_2$ solution. Points represent the mean heart rate over the previous 5 s period. Data are mean ± s.e.

respective base heart rate. Heart rate did not differ significantly between 5 and 30 s in all electrical stunning groups, except the whole body stunning at 50 V group. At 30 s the whole body stunning at 50 V a higher (266 bpm, $P < 0.05$) heart rate than the other treatment groups (mean: 190 bpm). The heart rate in the MgCl$_2$ injection group continued to decrease, resulting in a lower (103 bpm, $P < 0.05$) heart rate at 30 s rate than the other treatment groups.

**Histological analysis**

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

**DISCUSSION**
Extracellular volume

The extreme treatments with regard to electrical impact on the body, whole body stunning at 150 V and MgCl$_2$ 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 MgCl$_2$ injection the ECV decreased only after one minute, and not immediately after injection. This decrease in ECV is 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 were found with some individual animals in all other treatments. This mixture of patterns was found in both whole body stunned and head only stunned groups, and with both low (50 V stunning groups) and very high (head only stunning at 150 V group) currents. Similar results were reported by Savenije et al. (2000), but the results of this experiment were less bimodal in appearance. This supports the conclusion that the cause of the brain damage during stunning and bleeding is both epileptic and ischemic in nature. However, it remains unclear which are the criteria to trigger an immediate increase in brain impedance or a delay, nor what determines the rate of increase in brain impedance in individual chickens. In cases in which immediate and progressive decrease in ECV was not found, brain impedance recordings are not conclusive about the state of consciousness of the animal. Given the similar problem with measuring brain electrical activity, from an animal welfare point of view it must be recommended to use those stunner settings 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 the death of the animals. The amount of decrease in ECV was comparable with the study of Ruis-Heutinck et al. (1998). Exsanguination after 30 s 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 so much 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 $t_{50\%}$ 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 to be preferred under humane slaughter conditions.

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. A blood pressure response pattern as seen, though not statistically significant, in this experiment with an initial depression at 5
to 10 s after stunning followed by a transient increase has been described before by Rhody and Kuenzel (1981) in 120 V shocked genetically seizure prone Cornell White Leghorn chicks, who 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 the tonic convulsions. A sudden, deep inhalation, which is a similar chest movement in conscious chickens causes a similar drop 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 a higher ability to reduce the effects of high blood pressure, possible 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 dropped rapidly, like described by Kuenzel and Walther (1978). This promotes the rapid development of ischemia in the brain and the 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 bleed out time (Griffiths, 1983).

Heart rate

Cardiac fibrillation or arrest occurred in all animals of the whole body stunning at 150 V group, while 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 are known to occur with head only stunning (Hillebrand et al., 1996). This 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 (1978) and Rhody and Kuenzel (1981). Pulse pressure was increased during that time, so the cardiac output may not have been reduced much. Injection with 2.5 ml of a 300 mg/ml MgCl₂ 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 is unclear. However, cardiac function was enough reduced to cause a change in ECV already at 1 min after injection and deteriorated in 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. This 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, while 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 animals. To ensure immediate and lasting unconsciousness an immediate and progressive ECV decrease is required. Only with whole body electrical stunning at 150 V such a response in ECV was 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 immediate and lasting unconsciousness can be guaranteed. This requires the induction of cardiac fibrillation or arrest and therefore a high stunning current that is also applied over the heart. Gregory and Wotton (1987) found a minimum current of 148 mA to ensure cardiac arrest occurred in 99% of the chickens. This experiment confirms this and recommends for humane slaughter a minimum voltage of 150 V with whole body stunning to ensure the immediate induction of unconsciousness and prevent any possibility of regaining consciousness by cardiac fibrillation or arrest.

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REFERENCES

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