CHAPTER 2

Impedance recordings to determine change in extracellular volume in the brain following cardiac arrest in broiler chickens

L. F. M. Ruis-Heutink1*, B. Savenije1,2, F. Postema2, A. van Voorst1, E. Lambooj1, J. Korf2

Poultry Science 77: 1422 - 1427; 1998

1Institute for Animal Science and Health (ID-DLO)
P.O.Box 65, 8200 AB Lelystad, The Netherlands

2Department of Biological Psychiatry, University Psychiatric Clinic
P.O.Box 30001, 9700 RB Groningen, The Netherlands

*Present address: Research Station for Cattle, Sheep and Horse Husbandry (PR)
Runderweg 6, 8219 PK Lelystad, The Netherlands
Chapter 2

ABSTRACT

The present study describes a method to determine the onset and development of brain damage in broiler chickens. Exsanguination disrupts the brain metabolism and causes the brain to become ischemic. Energy requiring systems in the cell membrane fail, which results in an ionic shift over the membrane, accompanied by a water influx into the cell. This cellular edema decreases the extracellular volume of the brain tissue. In mammals this brain damage has been measured by recording brain impedance. We adapted this approach for use with poultry. Five to six-week-old commercial broilers were equipped with impedance recording electrodes in the striatum area of the brain. Cardiac arrest was induced by means of an intravenous injection of MgCl₂ and brain impedance was recorded for 30 min. The resulting curves showed a high similarity to those obtained in rats. No effects of 12 h antemortem feed deprivation on the size and rate of change in brain impedance could be found. Both in anesthetized and conscious birds, a change in brain impedance was found. We conclude that brain impedance can be used to determine the development of ischemic brain damage in broiler chickens.

Key words: ischemia - brain impedance - extracellular volume - brain damage - brain metabolism
INTRODUCTION

In the slaughter of poultry, death is mostly caused by bleeding of the animal (Schütt-Abraham et al., 1987). During bleeding, the brain becomes ischemic, thereby disrupting brain cell function and leading to irreversible brain failure. Brain processes occurring with stunning and bleeding are not well documented in poultry. Reflex reactions and brain electrical activity have not been shown to be parameters that can clearly indicate a state of unconsciousness, insensitivity to pain, and the development of brain damage (Gregory, 1987a). The epileptiform electroencephalogram of poultry is different than that of humans and other animals. Sensory evoked potentials can be used as a very conservative method to determine near death unconsciousness only (Newhook and Blackmore, 1982; Lopes da Silva, 1983; Gregory, 1987b; Gregory and Wotton, 1987; Bilgili, 1992; Peruche et al., 1995). If unconsciousness is a function of brain failure, then a method that can measure brain damage may valuable to assess the state of consciousness in poultry.

When the brain becomes ischemic (i.e., during bleeding) several energy-requiring processes are compromised. Failure of the membrane ion pumps results in a shift in ion concentrations ($Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$) over the cell membrane, concomitant with uptake of water from the extracellular space (Van Harreveld, 1972; Korf and Postema, 1988; De Boer et al., 1989). As total brain volume is a fixed value due to skull restriction, cellular edema therefore results in a decrease of extracellular volume (ECV). A decrease in ECV indicates loss of brain cell function, which will become irreversible if the lack of supply of glucose and oxygen persists. The size of the ECV can be estimated by recording the impedance of the brain tissue (Van Harreveld, 1972; Korf et al., 1988). With regard to passage of of electric current through brain, brain tissue consists of highly conductive (> 99.7%) extracellular fluid and of non-conductive (< 0.3%) cells. The impedance of brain tissue is therefore almost completely determined by the size of the extracellular volume (Aladjalova, 1964). Brain impedance ($R$) can be measured in a relatively simple way and the ECV can be calculated from it according to Maxwell's equation (Cole et al., 1969):

$$\frac{ECV_i}{ECV_0} = \frac{R_0}{R_i}$$

with time $0$ being before ischemia, time $i$ being at some time thereafter. $ECV_0$ was defined to be a reference value of 100%.
In rats a decrease in ECV of 50% within 15 min following cardiac arrest was found. (Korf and Postema, 1988; Korf et al., 1988). This change in ECV, as calculated from brain impedance recordings, was found to reflect ischemic brain cell damage. The present study was to determine whether this approach can also be applied for monitoring brain damage in poultry. In addition the effects of feed restriction and of anesthesia on changes in brain impedance were studied.

MATERIALS AND METHODS

Experimental animals
Male Ross broilers were reared on litter from 1 d to 5 wk of age at the animal facility of the Institute for Animal Science and Health (ID-DLO, Research Branch Beekbergen, The Netherlands). Room temperature was decreased gradually from 30 °C at the beginning to 20 °C at the end of the experiment. Food (13 MJ/kg metabolizable energy, 21% crude protein) and water were available for ad libitum consumption. A light/dark regime of 23:1 h was applied. Several days before surgery, the birds were housed individually in battery cages, but with visual contact to each other. Approval for carrying out the experiment was obtained from the ethical committee of the Institute for Animal Science and Health.

Surgery
The broilers were anesthetized by an i.m. injection of 2 mL Ketamine in the breast muscle, followed 15 min later by an i.v. injection of 0.3 to0.8 mL Nembutal (slowly administered) in the left wing vein. Surgery was started when corneal and comb pinch reflexes were absent and the comb had lost its red color. The chicken’s head was placed into a modified small animal stereotaxic instrument adjusted to the anatomy of the chicken (Gallus Domesticus) as described by Kuenzel and Masson (1988). The head feathers were removed and the skin was disinfected. A longitudinal incision in the skin was made and the periosteum was removed. Due to variations in head size and shape of broilers the coordinates based on the bregma point on the skull proved to be more accurate than those based on the intra-aural zero point of the stereotaxic instrument. The bregma point was used as a reference throughout this experiment. The electrodes were placed in the striatum of the left hemisphere at 8.6 mm anterior and respectively 5.0 and 7.0 mm lateral from the bregma point. Two small holes with a diameter of 0.5 mm were drilled in the skull, leaving the dura mater intact. After drilling the dura mater was carefully ruptured with an injection needle with a bent end.

1Institute for Animal Science and Health (ID-DLO), P.O.Box 65, 8200 AB Lelystad, The Netherlands
2AUV, P.O.Box 94, 5430 AB Cuijk, The Netherlands
3Sanofi Sante B.V., Maassluis, The Netherlands
electrodes were stereotaxically placed in the striatum at a depth of 5.0 mm in the brain tissue and fixed to the skull with dental cement. Finally, the skin was sutured. The baseline impedance signal was tested for 10 minutes.

**Impedance equipment**

The impedance in the brain was measured through a pair of Teflon-coated silver electrodes. The tip of each electrode was stripped, leaving an exposed end of 1 mm in length and 0.4 mm in diameter. The impedance was measured at a sinusoidal alternating current of 20 \( \mu \)A and a frequency of 20 kHz. The impedance signal was sent through coaxial cables to a locally manufactured recording device which was calibrated at 1 V and had a linear output between 0 and 10 k\( \Omega \).

**Experimental design**

Birds from a group of 10 animals were randomly assigned to either of two treatments. One treatment consisted of feed deprivation for 12 h prior to surgery. Birds receiving the other treatment were allowed access to feed throughout the experiment. Following surgery, the electrodes were connected to the recording device and impedance was measured until the signal was stable. Then the animals received an overdose of MgCl\(_2\) (2.5 mL of a 300 mg/mL MgCl\(_2\).6H\(_2\)O solution) administered i.v. in the right wing vein. Cardiac arrest occurred almost instantaneously (Blomqvist and Wieloch, 1985). Brain impedance was registered continuously during 30 min. After 30 min, the brain was dissected from the skull and stored in a 4%-paraformaldehyde solution for histological analysis at a later time.

Another group of 20 birds were equipped with brain electrodes and were then allowed to recover for 24 h. The next day, a random selection of 10 birds were anesthetized again before administrating an overdose of MgCl\(_2\). The other 10 animals received this injection while still conscious. Impedance recording and brain dissection were performed as described above.

**Histological examination**

The brains stored for at least 2 wk in a 4%-paraformaldehyde solution. Each brain was retrieved and stored overnight in a 20% glucose solution before histology. The next day they were rapidly frozen to approximately -20 °C. Then, starting at the frontal region, 40 \( \mu \)m thick slices were cut from the brain and stored in a phosphate buffer solution. The following day, the slices were stained using the Nissl staining procedure with Chresylviolet. Brain slices were compared with a stereotaxic atlas (Kuenzel and Masson, 1988) and the position of the tips of the electrodes was determined.

---

*Department of Biological Psychiatry, Electronics Section, University Psychiatric Clinic, P.O.Box 30001, 9700 RB Groningen, The Netherlands*
Chapter 2

Presentation of results and statistics

Brain impedance was recorded in kΩ and relative ECV values were calculated from these data as described by Pelligrino et al. (1981) using Maxwell's equation. The decrease in ECV over the first 10 min (\(\Delta ECV_{(0-10)}\)) postmortem was expressed as the percentile deviation of the base ECV, which was defined as 100%. All values are given as mean ± s.e. Treatment effects on \(\Delta ECV_{(0-10)}\) and on the time needed to reach 50% of the change in ECV over 10 min (\(t_{(0-50\% ECV)}\)) were studied by means of analysis of variance. Decrease of ECV over time was assessed with the Mann-Whitney U test. The Genstat 5 statistical programming language was used (Genstat 5, 1993).

RESULTS

The experiment started with 30 broiler chickens. Four birds died during surgery. In 12 birds, their behavior during the recovery period and severe convulsions after the induction of cardiac arrest damaged the connections to the electrodes. The data of two birds were also not sufficient. Therefore, data from only eight animals of the first group and four birds of the second group were analyzed.

The change in ECV over a period of 30 min in the striatum region of birds in the first group is depicted in figure 1. During the first 10 min, 85.4 ± 2.5% of the total decrease in ECV over 30 min was already established. The following two 10 min-periods accounted for 10.0% and 4.6%, respectively. The statistical analyses are therefore restricted to the first ten minute period postmortem.

Table 1. Weight, change in extracellular volume over 10 min (\(\Delta ECV_{(0-10)}\)), and time needed to reach 50% of that change (\(t_{(0-50\%)}\)).

<table>
<thead>
<tr>
<th>Item</th>
<th>Restrict Ed</th>
<th>Ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)1</td>
<td>1527 ± 35</td>
<td>1445 ± 32</td>
</tr>
<tr>
<td>(\Delta ECV_{(0-10)}) (%)</td>
<td>46.9 ± 4.3</td>
<td>45.0 ± 5.6</td>
</tr>
<tr>
<td>(t_{(0-50%)}) (s)</td>
<td>273 ± 23</td>
<td>274 ± 8</td>
</tr>
</tbody>
</table>

1Data are mean ± s.e. for each treatment group (n = 4).
A significant decrease in extracellular volume ($\Delta\text{ECV}_{(0-10)}$) was found (46.3 ± 3.2%; $P \leq 0.001$) at 10 minutes postmortem when analyzing the animals of the first group ($n = 8$). The decrease in ECV over time was analyzed on a minute to minute basis. The values obtained from 1 min postmortem onwards were significantly lower ($P \leq 0.05$) than those at the time cardiac arrest was induced. The average time needed to reach 50% of the decrease in ECV found over 10 min ($t_{(0-50\%\Delta\text{ECV})}$) was 274 ± 11 s. Data per group are shown in table 1. Food restriction did not seem to affect changes in ECV, but the small number of animals involved may have obscured existing differences.

![Figure 1](image_url)

**Figure 1.** Relative change in extracellular volume in the broiler brain following acute cardiac arrest at time $t = 0$ min. Base ECV is defined as 100%. Data are mean ± s.e. ($n = 8$).

Of the second group, one bird under anesthesia showed a decrease in ECV$_{(0-10)}$ of 37.3%. Cardiac arrest induced in three conscious birds resulted in changes in ECV$_{(0-10)}$ of 22.9%, 54.0% and 40.0%, respectively. The time needed to reach 50% of the decrease in ECV found over 10 minutes ($t_{(0-50\%\Delta\text{ECV})}$) was 360 seconds for the anesthetized broiler and 228, 348 and 378 seconds for the conscious animals.

From the histological examination it appeared that all electrodes were located either in the Hyperstriatum Ventrale or in the Neostriatum. The results of a typical histological analysis are shown in figure 2.
DISCUSSION

Brain impedance was used to determine change in ECV in the brain of commercial broiler chickens. This approach has shown its validity in rats, mice, guinea pigs, rabbits, and cats, but has so far not been studied in birds (Van Harreveld, 1972; Suga et al., 1990). Analogous to mammals, it is also assumed that also in broilers cardiac arrest leads to an immediate ischemia in the brain, resulting in cellular swelling and a decrease in ECV (Van Harreveld, 1972; Korf and Postema, 1988). As electrical conductivity of the brain tissue is solely determined by the size of the ECV (Aladjalova, 1964), brain impedance recording is therefore assumed to reflect cerebral ischemia in broiler chickens as well.

Brain impedance has been measured in the striatum. In birds, the striatum is a relatively large and rather homogeneous brain area, which is easy to reach stereotaxically. This is convenient for impedance measurements because small variations of the exact electrode position within this brain area will have little effect on the recordings. The ECV is inversely related to the recorded impedance. The precise value of the brain impedance depends on various factors. The length of the electrode tip and the distance between the electrodes in the brain could not be
Ischemia-induced change in brain impedance in broilers

assumed to be constant throughout the experiment. Using relative ECV values, taking the ECV corresponding to 100% as reference, circumvents this problem and also allows for easy comparison of effects on ECV between species (Van Harreveld, 1972).

This study shows that brain impedance recordings found in 5 to 6-wk-old chicks are similar to those found in other species. A decrease in extracellular volume over 10 min postmortem of 46% was found, which is about the same as has been recorded in rats (50%: Korf and Postema, 1988). The total decrease in ECV recorded over 30 minutes had already been established to be 85% within the first 10 min. Korf and Postema (1988) reported similar findings in rat striatum and hippocampus. J. Korf (1996, Department of Biological Psychiatry, University Psychiatric Clinic, P.O.Box 30001, 9700 RB Groningen, The Netherlands, personal communication) noted that after this initial stage other processes will start to play a role. Impedance recordings beyond 10 min postmortem could then no longer be considered to reflect exclusively ischemia-induced change in ECV.

Histological analysis proved that the stereotaxically determined location for electrode implantation was adequate. In the present study variation in head size and shape caused the bregma point on the skull to be a more accurate point of reference on the stereotaxic instrument than the intra-aural zero point. The rapid growth rate of commercial broiler chickens could contribute to this variation.

No effects of feed deprivation on the size and rate of change in ECV were found. Given the small number of experimental animals and the technical problems, conclusions must be drawn with care. In practice broilers don't receive feed for several hours before slaughter. Dijk et al. (1994) found that fasting decreases damaging effects of hypoxia-ischemia in rats, despite the excitotoxic effect of the increased glutamate levels found in the extracellular fluid. Thus, starvation could have resulted in a less pronounced reaction to ischemia than might otherwise have occurred.

Ischemia-induced cellular swelling is a measure of brain cell damage and loss of cell function. At this point in the process the animal is unconscious. Recording brain impedance is useful to monitor the onset and the development of brain damage and can provide a reference point for unconsciousness in the bird. Brain metabolic studies looking at the onset and development of brain damage may provide additional information to studies focussed on brain electrical activity. Also, information on neurotransmitter levels would add to our understanding of processes in the chicken brain during slaughter (Korf et al., 1988; Hirota et al., 1995).

This experiment shows ECV changes in the brain of birds that were either anesthetized or conscious at the time cardiac arrest was induced. So the proposed approach is suitable for unanesthetized animals that better reflects practical slaughter conditions. Electrical water bath stunning in poultry induces an epileptiform insult, which is different from that in mammals, often accompanied with cardiac fibrillation. Circulatory arrest and epilepsy cause increased extracellular
Chapter 2

Lactate levels and ischemia in rats (Kuhr et al., 1988; Lehmann and Hamberger, 1991; Swanson et al., 1995). The method of brain impedance recording to study brain failure as described here will be used for further studies looking at the effects of electrical stunning.

In conclusion, changes in brain impedance after cardiac arrest give similar results in broiler chickens as reported for other species. It may be a useful approach to study the onset and development of ischemia-induced brain damage in poultry. It allows the use of unanesthetized animals and will be studied further as a tool to evaluate electrical stunning methods at slaughter.

ACKNOWLEDGEMENTS

This research was funded by the European Commision (EU AIR3 Project CT 94-0885) and the Dutch Ministry of Agriculture. The authors wish to thank dr. B. Engel for help with the statistical analysis.

REFERENCES


