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## Short Communication

# A non-invasive technique for measuring the electroencephalogram of broiler chickens in a fast way: the 'chicken EEG clamp' (CHEC)

Eine nicht-invasive Technik zur schnellen Messung des Elektroencephalogramms bei Masthühnern: die 'chicken EEG clamp' (CHEC)

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### Introduction

According to the Council Directive 93/199/EC of the European Union, animals have to be stunned before slaughter. Stunning in an electrical water bath is a technique which is usually applied in commercial chicken slaughterhouses. The birds pass through an electrified water bath while hanging upside down on metal shackles which are connected to ground. A current flows through the body of the chickens when the bird's head touches the electrified water, producing stunning. A variety of electrical parameters are used to adjust the stunning setup in slaughterhouses, including waveform, frequency, voltage as well as stunning time. This leads to variations in stunning efficiency, with some setups causing cardiac arrest, while in others the chickens are able to recover (WILKINS et al., 1998). But, if the birds do not encounter cardiac arrest, they must remain in a state of unconsciousness until their death from bleeding (Council Directive 93/199/EC).

For animal welfare reasons it is important to assess the reliability of stunning procedures. It is generally known that testing of reflexes only is not a reliable estimate of consciousness in birds. The recording of brain waves by an electroencephalogram (EEG), as an indicator of vigilance and consciousness, has been recommended (RAJ and O'CALLAGHAN 2004). Commonly, electrodes are implanted into the chicken's skull, requiring surgery and adequate recovery time. This traditional method is costly and time consuming, and is therefore not suitable for large amounts of animals. D. Fletcher from the University of Georgia (personal communication) tried a method using plasters fitted with electrodes, which were applied to the chicken's head before entering the water bath. However, the method requires extensive handling of the birds before stunning, as the feathers on the chickens' head have to be plucked before application of the electrodes to ensure good contact, causing additional stress to the animals.

In order to appraise the reliability of the existing electrical stunning setups and to further improve stunning devices it is essential to record large numbers of birds within a short time. Therefore, a method is required, allowing fixing external EEG electrodes after electrical stunning without delay and with adequate accuracy. The method should also be applicable under the conditions of commercial slaughter plants.

### Materials and Methods

#### *Non-invasive EEG device*

The device is shown in Figure 1. It is fitted with pointed electrodes opposite to a grounded metal rail. The differential recording electrodes, insulated except the tips, are equipped with a pre-amplifier, transferring the EEG signals from an artefact sensitive high impedance circuitry to a low impedance circuitry, avoiding disturbance of the signal. The chicken's neck is placed into the rail, and the electrode tips are set on the head behind the chicken's comb, on both sides of the brain. Springs ensure tight and stable contact of the electrodes to the skin. To simulate conditions similar to water bath stunning, the bird's head is wetted with water before application of the electrodes in all validation experiments, perhaps even contributing to a good contact between skin and electrodes. The delay in recording of the EEG is minimised to a few seconds due to quick and standardised application of the device. After the electrodes have been fitted to the skin, a button on the device is pressed to discharge the filters in order to obtain a quick EEG signal. This method allows EEG recording within 5 seconds after the broilers had left the stunning bath.

#### *Validation of device*

The Animal Experimental Committee (DEC) of the Radboud University Nijmegen permitted consent for the validation experiment under number RU-DEC 2005-136. In order to evaluate the quality of the EEG signals recorded with the non-invasive EEG device, birds were measured in three conditions. In the first condition birds were measured under ketamine-xylazine anaesthesia (0.2 mg/kg ketamine and 0.5 mg/kg xylazine), in the second under carbon dioxide anaesthesia (50% of carbon dioxide and 50% of oxygen), while in the third condition locally anaesthetised

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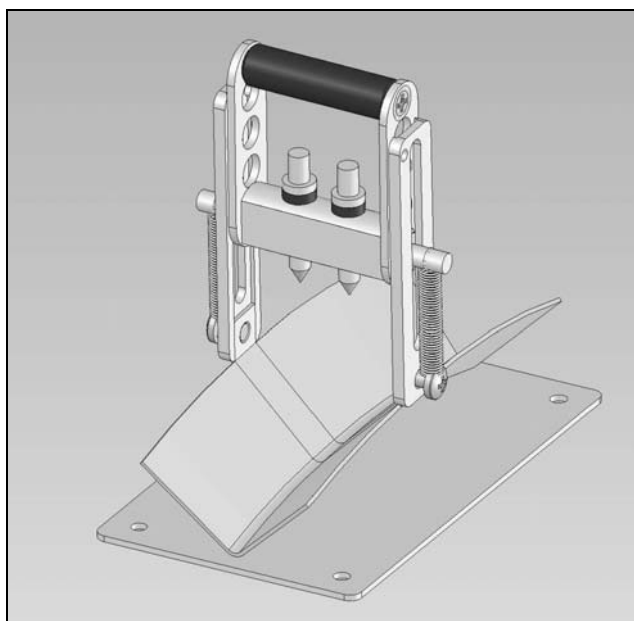


Figure 1. The basics of the non-invasive EEG device, the 'chicken EEG clamp' (CHEC), without cables and amplifiers. The bird's head is moved in the metal rail when the electrode rack is lifted. When the head is in a proper position, the electrode rack is moved down till the electrodes make tight and stable contact with the bird's head. See also the text. (Drawing by Norbert Hermesdorf).

*Schemazeichnung der Messeinheit zur nicht-invasiven Erfassung von EEGs bei Hühnern, die 'chicken EEG clamp' (CHEC), ohne Kabel und Verstärker. Der Kopf des Huhns wird in die Metallschiene herein geschoben, wenn die Klammer geöffnet ist. Sobald der Kopf in der richtigen Position ist, wird die Klammer geschlossen und die Elektroden fest in die Kopfhaut gedrückt. Siehe hierzu auch den Text. (Zeichnung von Norbert Hermesdorf)*

(with lidocaine) birds were registered. In all three conditions 10 commercially available, 5 week old broiler chickens (Ross) were used, both males and females. The average weight was 2.2 and 1.6 kg respectively. The device was fitted to the head of the birds as described above. The recordings included signals from 1-100 Hz, as well as from the 10-100 Hz band. The latter frequency trace is faster visible on the screen of the EEG monitor, due to filtering of the offset voltage. A 50 Hz notch filter prevented noise from the electrical net.

## Results and Discussion

In the first condition animals were intramuscularly injected with a mixture of ketamin and xylazine. When the birds showed adequate anaesthesia the EEG of animals was recorded. Representative EEG traces of two animals are shown in the upper panel of Figure 2. In the ketamine-xylazine condition the EEG shows a stereotyped rhythmical burst-like activity followed by a relatively iso-electric pause, in a 1 to 2 Hz periodicity. This EEG pattern is characteristic for a type of anaesthesia induced by xylazine and non-specific NMDA antagonists, such as ketamine. The burst-like activity is caused by neuronal spiking induced by the NMDA antagonists (SOLTESZ and DESCHENES, 1993). After recording the EEG, chickens were euthanized by an injection of an overdose of pentobarbital into the wing vein.



Figure 2. EEG records (1–100 Hz.) of three groups of broiler chickens registered by the chicken EEG clamp (CHEC). The two records in the upper panel are from two animals under ketamine-xylazine anaesthesia, the middle panel shows EEG records of two animals anaesthetised with carbon dioxide, while in the lower panel records are obtained from two locally anaesthetised broilers. See text for further explanations. EEG-Ableitungen (1-100 Hz) von drei Broilergruppen, gemessen mit der 'chicken EEG clamp' (CHEC). Die zwei Ableitungen im oberen Teil der Grafik stammen von zwei Tieren unter Ketamin-Xylazin-Anästhesie, die mittleren von zwei mit Kohlendioxid betäubten Tieren, und die unteren von zwei lokal anästhesierten Broilern. Weitere Einzelheiten im Text.

A carbon dioxide anaesthesia was induced in the second condition. Birds were placed in a box containing a mix of carbon dioxide and oxygen. This mix induces a fast anaesthesia, while animals show deep and slow breathings. The middle panel of Figure 2 displays the carbon dioxide EEG pattern registered by the chicken EEG clamp. The relatively high frequencies are typical for carbon dioxide anaesthesia (COENEN et al., 2000). The periodic variations in the wave-amplitude are smaller than compared to the wave variability in the ketamine-xylazine condition. The slow and deep breathings, occurring every five to eight seconds, are clearly shown in the EEG as movement artefacts superimposed on the low amplitude EEG. Animals were offered after the experiment by reducing the oxygen in the gas mixture.

Birds of the third group received a local anaesthesia with lidocaine under the skin of the entire skull. Animals were further restrained by immobilisation of wings and body. Their EEG was recorded and is presented in the lower panel of Figure 2. Signals are typical for wakeful birds, generally with small amplitude high frequency waves with a higher variability and some more movement artefacts than the anaesthesia wave patterns (COENEN et al., 2006). EEG traces are almost identical to those recorded with the traditional method by implanting permanent electrodes. Also spindling, so characteristic for the bird's EEG in particular over the frontal cortex, is sometimes clearly visible, for example in the lowest trace of Figure 2.

## Conclusions

Basically there are two strong arguments to consider the EEGs recorded with the non-invasive device, the 'chicken EEG clamp' (CHEC) as genuine EEGs and not as artefacts. The first argument is that the three conditions are associated with EEG patterns which can easily be distinguished from each other. In case the EEG is built up by artefacts not related to the brain state, traces should be more identical. The second argument is that the EEG characteristics of the three conditions are representative for the anaesthetic drug used in these conditions. Birds of the ketamine-xylazine condition show EEG traces that are typical for such anaesthesia, while that is also true for the carbon dioxide condition. One of the most powerful argument stems from the fact that clear spindling could be seen in the records of the birds, registered without complete anaesthesia. In conclusion, the experiment proved that the non-invasive technique for EEG recording allows quick recording of reliable and genuine EEG patterns. Hence, this method allows assessment under EEG guidance of the reliability of stunning in large numbers of birds under commercial slaughter house conditions.

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## Summary

A device was developed to measure in a fast way the electroencephalogram (EEG) of broiler chickens in a non-invasive way. The 'chicken EEG clamp' (CHEC) consists of a framework with two pointed electrodes, fitting as a clamp around the chicken's head. The EEG is recorded by the two active electrodes firmly contacting the skin overlying over the midst of the brain. The device is equipped with a pre-amplifier and is grounded. Validation of the CHEC was done in three groups of broilers: 1. chickens anaesthetised with ketamine-xylazine, 2. chickens anaesthetised with carbon dioxide in oxygen and 3. chickens locally anaesthetised with lidocaine applied under the skin of the brain.

It appeared that the EEG can be obtained almost immediately, after 5 to 10 seconds recordings are available. The EEG traces obtained with the CHEC device were considered as reliable for two reasons: EEG patterns of the three groups are different from each other, while the EEG characteristics of the groups are representative for the anaesthetic used. Even clear EEG spindling, a typical EEG pattern in wake chickens, could be seen in the locally anaesthetised animals. The experiment proved that reliable, external, non-invasive EEG recordings of broilers can be obtained in a fast way, allowing quick assessment of the EEG in large numbers of animals.

## Key words

Broiler, electroencephalogram, technique

## Zusammenfassung

### Eine nicht-invasive Technik zur schnellen Messung des Elektroencephalogramms bei Masthühnern: die 'chicken EEG clamp' (CHEC)

Im Rahmen des Projektes wurde eine Messeinrichtung zur nicht-invasiven Erfassung des Elektroencephalogramms (EEG) bei Broilern entwickelt. Die 'Chicken EEG clamp' (CHEC) besteht aus zwei in fixem Abstand installierten spitzen Elektroden in Form einer Klammer, die am Kopf des Tieres befestigt werden. Das EEG wird über die beiden Elektroden aufgezeichnet, die fest in die Kopfhaut über der Mitte des Gehirns gedrückt werden. Die Messeinrichtung besitzt einen Vorverstärker und ist geerdet. Die Validierung der Testeinrichtung erfolgte an drei verschiedenen Versuchstiergruppen: 1. Broiler anästhesiert mit Ketamin-Xylazin, 2. Broiler betäubt mit Kohlendioxid in Sauerstoff, 3. Broiler, die lokal mit Lidocain subkutan unter die Kopfhaut betäubt wurden.

Es zeigte sich, dass das EEG sehr schnell erfasst werden kann. Die ersten Aufzeichnungen waren nach 5 bis 10 Sekunden möglich. Die mit dem CHEC erhaltenen EEG-Ableitungen wurden aus zwei Gründen als vertrauenswürdig angesehen: 1. Die EEG-Ableitungen der drei Gruppen unterschieden sich in Abhängigkeit von der angewendeten Betäubungsart. 2. In den lokal anästhesierten Tieren konnten EEG-Spindeln beobachtet werden, die typisch für wache Hühner sind. Das Experiment belegt, dass eine zuverlässige, externe, nicht-invasive und schnelle Erfassung von EEGs bei Broilern möglich ist und so die schnelle Erfassung von EEGs in großen Tiergruppen erlaubt.

## Stichworte

Broiler, Elektroencephalogramm, Technik

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