Pain sensitivity in chicken embryos: English Translation

Innovate Animal Ag

Mar 31, 2023

The following is a summary of a research project commissioned by the Federal Agricultural Ministry in Germany regarding the perception of pain in chick embryos. The original text can be found here. It was translated to English using ChatGPT.

Contributors

Center for Preclinical Research, Klinikum rechts der Isar, Technical University of Munich

Department of Anesthesiology and Intensive Care Medicine, Klinikum rechts der Isar, Technical University of Munich

Reproductive Biotechnology, TUM School of Life Sciences, Technical University of Munich

Introduction

In Germany, the killing of male chicks has been legally prohibited since 2022 (Animal Welfare Act, 2022). Starting from 2024, it will be prohibited to kill male chicken embryos after sex determination in the egg on or after the 6th day of incubation. This developmental stage was chosen because previous studies suggest that chicken embryos may be capable of pain transmission from the 7th day of incubation (Embryonic Day, ED), and therefore, the ability for nociception (sensory reception of potentially damaging stimuli) cannot be ruled out in the egg (Bjørnstad et al., 2015; Krautwald-Junghanns et al., 2018). Based on this scientific background, the Federal Ministry of Food and Agriculture commissioned a study to determine the period during which chicken embryos develop the ability for nociception or the ability to perceive aversive sensory experiences as pain.

Methods

In the present study, Lohman Selected Leghorn "White Layer" chicken embryos were used from embryonic day (ED) 7 to ED19. All measurements were conducted in ovo (within the egg). In randomized order, a noxious (actually or potentially tissue-damaging) stimulus and a control stimulus were applied. A mechanical stimulus was used as the noxious stimulus for cardiovascular parameters and behavioral observations at the base of the beak. The response was compared to the control stimulus of touching the beak. As a second control group, lidocaine, a local anesthetic, was applied to the base of the beak before the mechanical stimulus at ED18. Noxious stimuli (heat and electrical) were used for electrophysiological parameters with a Peltier element and stimulation electrodes.

The reactions of chicken embryos were analyzed based on the following parameters:

- **Cardiovascular parameters:** Mean arterial blood pressure (MAP) and heart rate (HR) were recorded using a microcatheter inserted into an artery of the chorioallantoic membrane.
- **Behavior:** Movements were recorded and analyzed using a deep-learning software (DeepLabCut, DLC) and manual scoring.
- **Electroencephalogram (EEG):** Electrical brain activity was measured using electrodes placed in the hyperpallium and cerebellum.

Results

Cardiovascular parameters: After the mechanical stimulus, the mean arterial blood pressure (MAP) of chicken embryos on embryonic day (ED) 16 to 18 significantly increased. Similarly, a significant increase in heart rate (HR) after the mechanical stimulus was observed in ED17 and ED18 embryos. Injection of the local anesthetic lidocaine significantly reduced the MAP response to the mechanical stimulus in ED18 embryos. Individual embryos showed reactions in MAP and HR on ED15, but no significant differences between mechanical stimulus and touch were observed. No increase in MAP or HR was observed in embryos younger than ED15.

Behavior: The movements of the beak increased significantly after the mechanical stimulus in ED15 to ED18 embryos compared to touch. In the first 30 seconds after the mechanical stimulus, movements of the head, elbow, and metatarsus also significantly increased in ED18 embryos.

EEG: From ED7 to ED12, no physiological EEG of chicken embryos in the egg could be detected. From ED13, physiological brain activity could be reliably recorded. An

adequate EEG response to heat or electrical stimulus has not yet been recorded from ED13 onwards.

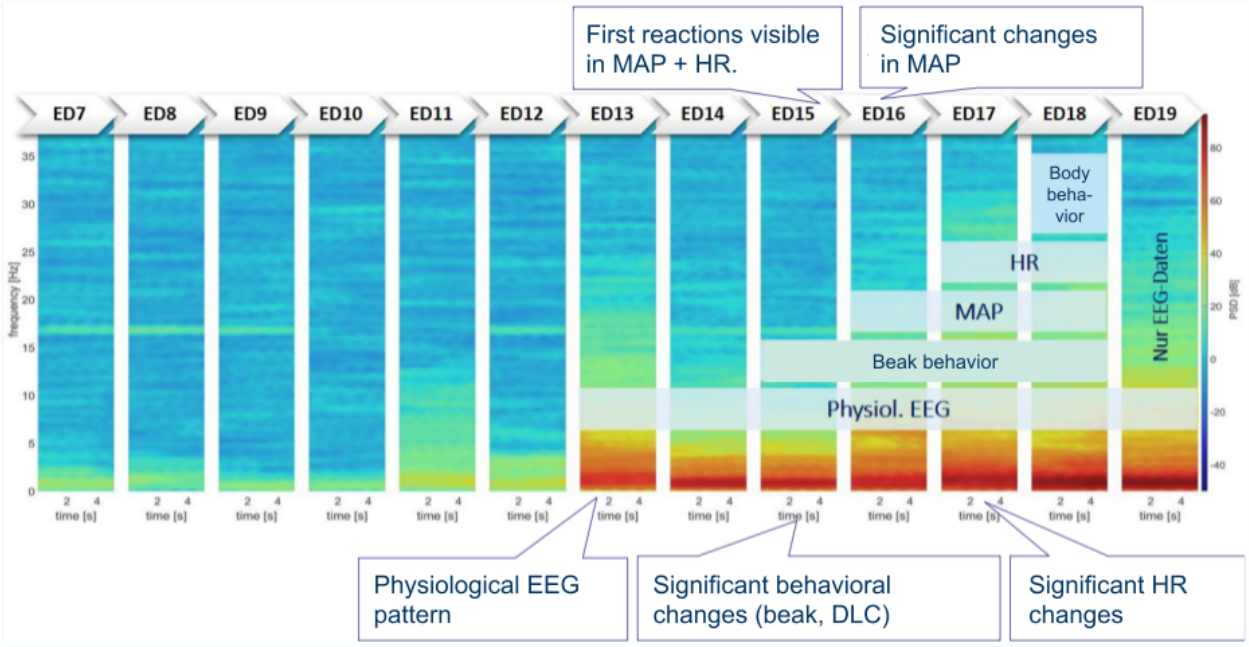

Fig. 1 Overview of significant responses to mechanical stimulation in the respective parameters (MAP, HR, behavior). Presentation based on the spectral analysis of a representative EEG for each incubation day, showing the onset of physiological EEG activity from ED13.

Summary

In summary, cardiovascular reactions to mechanical stimulation were significant from ED16 onwards, and in individual animals from ED15. A significant behavioral reaction to mechanical stimulation was also observed in embryos from ED15 to ED18. Based on the results, the ability for nociception (sensing of noxious stimuli) cannot be excluded at ED15 and can be assumed from ED16 onwards. Regarding EEG analysis, physiological neural activity (EEG) in the brain was measurable from ED13 onwards. This allows the conclusion that the ability for nociception, or the ability to perceive aversive sensory experiences as pain, is potentially present from this time point. On the other hand, it shows that processing of the pain stimulus in the brain is highly unlikely until ED12.