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Application of the wireless electroencephalogram to measure stress in White Pekin ducks

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Stress in poultry can produce many undesirable effects on bird health and production performance. The objective of this study was to develop and evaluate a potential measure to assess stress through analysis of brain activity using electroencephalography (EEG). In two experiments, White Pekin ducks were implanted with EEG transmitters and treated with potential stressors in a chamber or in their pens. Electrocardiograms and blood corticosterone levels were collected as standard measures of stress. EEG analysis showed an increase in the relative delta frequency and a decrease in the relative alpha frequency during the treatment period for shock ($P < 0.05$). EEG analysis of the second experiment showed no differences between time periods for all frequencies for all treatments. Based on these results, EEG is currently not a viable technique for the measurement of acute stress in commercial poultry.

1. Introduction

Stress is an important welfare concern in poultry and can produce many undesirable effects on bird health and production performance, including increased susceptibility to disease and decreased production efficiency (Horvath-Papp, 2008). Recent attempts to evaluate stress in poultry have moved away from qualitative measures, such as behavior observations, to quantitative measures, such as blood parameters. Stressful stimuli cause an increase in adrenal activity via the hypothalamic–pituitary–adrenal (HPA) axis (Klingbeil, 1985). This adrenal stress response leads to an increase in circulating corticosterone, the primary glucocorticoid in poultry (Sturkie, 1986; Singh et al., 2009). Corticosterone levels rise fairly quickly after stimuli and are considered a good indicator of stress in poultry (Davis et al., 2008; Rettenbacher et al., 2004). Subsequently, rising corticosterone triggers an increase in circulating heterophils and a decrease in circulating lymphocytes.

A potential additional quantitative assessment tool for animal welfare is the electroencephalogram (EEG), which represents the voltage recorded between electrodes applied to the scalp or implanted surgically. EEG in poultry has been used most commonly in connection with animal welfare studies to determine the time to unconsciousness and brain death during stunning for slaughter, euthanasia or depopulation (Alphin et al., 2010; Gerritzen et al., 2006; Raj, 1998). As in other species, EEG wave frequencies detectable in poultry include low-amplitude, high-frequency waves (alpha, 8–12 Hz; beta, 16–24 Hz) and high-amplitude, low-frequency waves (theta, 4–8 Hz; delta, 0.5–4 Hz) (Alphin et al., 2010; Benson et al., 2011, 2012). Several researchers have employed the use of high amplitude, low frequency (HALF) activity in the theta and delta waves for poultry to determine the point of unconsciousness (Raj et al., 1992; Raj, 1998; Gerritzen et al., 2004; Rankin, 2010, University of Delaware Master's Thesis; Benson et al., 2011, 2012; Caputo et al., 2012). Additionally, EEG has been used in an effort to document pain in poultry. Gentle and Hunter (1990) showed a high amplitude, low frequency EEG pattern during immobility after progressive removal of feathers in chickens. Gentle and Hunter (1990) concluded that feather removal was a painful experience that could be categorized as a welfare concern.

Methods of evaluating stress in poultry must be validated using experimental stress models. Several stimuli have been identified as capable of inducing a stress response in animals: electric shock, ammonia, and sound. Electric shock is an acute and measurable stimulus that has been used in several studies utilizing EEG. Ong et al. (1997) was able to make a correlation between EEG changes and behavioral changes of sheep in response to mild electric shock. Ong et al. (1997) showed that in the 4 s after the shock, there was an overall increase in absolute EEG power for delta 2 (2–3.9 Hz), theta 2 (6–7.9 Hz), alpha 1 (8–10.9 Hz), and alpha 2 (11–13.9 Hz). They concluded that EEG changes were a good measure of acute pain in sheep. McFarlane and Curtis (1989) conducted a seven-day shock study using Hubbard chicks. Throughout the trial, the intensity of the electric shock gradually increased and the length of each exposure was random. It was concluded that there was a significant

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increase in the heterophil to lymphocyte (H/L) ratio after the seven-day electric shock exposure; indicating a stress response was present. Ammonia is a common stressor present in production facilities and is a concern for poultry producers. The current acceptable level of ammonia in production houses is 25 parts per million (ppm); however, levels exceeding 50 ppm or even 100 ppm have been cited (Anderson et al., 1964; Saif, 2008). These levels for extended periods of time can have many undesirable effects such as keratoconjunctivitis, respiratory infections, and inhibited growth and performance (Saif, 2008). Jones et al. (2005) demonstrated that broilers chose to spend greater amounts of time in chambers with lower levels of ammonia. McFarlane and Curtis (1989) showed a significant increase in the H/L ratio of Hubbard chicks following an exposure of 125 ppm ammonia for seven days. In an EEG study in humans, van Toller et al. (1993) demonstrated that there is a rise in the alpha frequency during an exposure to ammonia. de Boer et al. (1988) demonstrated that rats subjected to a stressful auditory stimulus of white noise at 100 dBA for 10 min showed a significant increase in plasma corticosterone levels. Gross (1990) showed an increase in the H/L ratio of chickens after exposure to a stressful auditory stimulus of 104 dB. These and similar studies demonstrate that electric shock, ammonia and sound are appropriate stimuli to use as standards for evaluating the effectiveness of EEG as a measure of stress in poultry.

The objective of the current study is to evaluate the suitability of using EEG for determining quantitative trends in brain activity associated with stressors (electric shock, ammonia, sound) in poultry. It is the authors' hypothesis that EEG may have the capability not only to help researchers identify a stress response in the EEG brain waves, but also to help them discern between stressors based on patterns that may emerge in the brain waves.

2. Materials and methods

White Pekin ducks were chosen for this study because they are a meat-type floor-reared production bird of suitable temperament and availability. Relative frequency-based EEG analysis was used in both experiments. Electrocardiograms (ECGs) (Exp. 1) and blood corticosterone levels (Exp. 1 and 2) were collected as a standard measure of stress (de Boer et al., 1988; Harvey et al., 1980; Klingbeil, 1985). Table 1 summarizes the two experiments performed. Standard care and conditions followed the approved University of Delaware Agricultural Animal Care and Use Protocol.

2.1. Experiment 1

For each of the three successive replications, 25 straight-run ducks were obtained from a commercial hatchery (Metzer Farms, Salinas Valley, CA) and raised from 1 day of age. EEG transmitters (PhysioTel model F50-EEE, Data Sciences International St. Paul, MN) were surgically implanted in eight birds for each replication (total of 24 implanted birds), once birds reached the minimum size of 2000 g for surgery at approximately 5 weeks of age. The surgical procedure outlined in Savory and Kostal (1997) was followed. Ducks had food and water withheld for approximately 8 h and 2–6 h prior to surgery, respectively. Each duck was anesthetized using 5% isoflurane (IsoSol; Medco, Inc., St. Joseph, MO) at induction with 3% isoflurane for maintenance of anesthesia. Three leads were placed on the meninges covering the telencephalon through 0.9 mm holes that were drilled into the parietal bone; two holes on the right side of the midline and one on the left, using a high speed microdrill (model 18000 17, Fine Science Tools, Foster City, CA). Furthermore, two leads were implanted in the complexus muscle just below the base of the skull for electromyography (EMG) to measure muscle movement. The ducks were given 0.4 mg/kg carprofen and 10,000 units/kg penicillin injected subcutaneously immediately prior to the procedure. The birds were then provided 5 days for recovery.

Table 1
Summary of the two experiments performed using White Pekin ducks.

	Experiment 1	Experiment 2
Year	2012	2013
# of instrumented ducks	24	8
Treatments	Shock	Shock
	Sound	No shock
	Ammonia	Control
	Control	
Trial length	45 min	45 min
EEG	Yes	Yes
ECG	Yes	No
Corticosterone	Yes	Yes
Chamber	Yes	No

Signals from the wireless transmitter were recorded by four wireless telemetry receivers (model RMC-1, DSI) and brain activity was monitored and recorded by DSI Dataquest A.R.T. Acquisition software. The raw EEG files were analyzed in NeuroScore by adding labeled markers over 2 s epochs indicating specific time periods: pre-treatment, treatment and post-treatment. The markers were placed based on visual analysis of the EEG signal using the EMG signal as a reference to eliminate motion artifacts, which appear as high amplitude spikes in both the EEG and EMG channels. Due to artifacts, the number of epochs placed throughout each time period was variable based on each individual trial. Files that had a significant artifact or interference that greatly reduced the number of epochs for any one of the time periods were excluded. The mean EEG, mean EMG, alpha, beta, delta, theta, and sigma values and markers were exported on a 2 s epoch basis from NeuroScore to Excel (Microsoft Corp., Redmond, VA) and charted (Caputo et al., 2012). Relative frequencies were calculated for each epoch by dividing the power in each frequency band by the total EEG power within the 2-s window. A trial time of 45 min (2700 s) was broken into the following time periods: pre-treatment (first 1800 s), treatment (600 s), and post-treatment (final 300 s). The pre-treatment period was split into the first 10 min (600 s) (pre-treatment 1) and the last 10 min (600 s) (pre-treatment 2) for EEG analysis. For each trial, individual birds were placed in an observation chamber. Four treatments were used: control, electric shock, sound, and ammonia. Treatments were applied individually and only one type of treatment was used per trial based on a randomization performed in Excel (Microsoft Corp., Redmond, VA). All birds underwent one control trial of 45 min without any stimulus treatment prior to beginning the experiment to establish baseline EEG activity and stress levels. For the sound stimulus, an 88 dB alarm (SpectrAlert, System Sensor, St. Charles, IL) was applied for 12 s per min for the 10 min treatment period. For electric shock, a dog-training collar (SportDog Brand SD-400, Knoxville, TN) was positioned over the sternum of the duck and a single shock (~60 mA) was applied once (~1 s) per min throughout the 10 min treatment period. All birds wore the shock collar, regardless of treatment, to eliminate the differences due to physical restraint by the collar harness. For the environmental stimulus, approximately 50 ppm ammonia was continuously applied for the entire treatment period via heating ammonia hydroxide (NH_4OH) to produce ammonia gas (NH_3).

The observation chamber included two regions: a region for the bird (0.81 m × 0.56 m × 0.65 m) and a region for heating the ammonium hydroxide (0.81 m × 0.24 m × 0.65 m). The regions were separated from one another and the bird could not move between them. The ammonium hydroxide was heated on a hot plate in the ammonia region of the chamber to produce ammonia gas and a fan pulled the ammonia into the region with the bird. The post-treatment period served as the ventilation period. An internal control system was used to activate ventilation. Ammonia concentration in