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Avian reflex and electroencephalogram responses in different states of consciousness

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HIGHLIGHTS

- Defining states of clinical consciousness in animals is important.
- Validation of current approaches is required.
- We evaluate reflexes and EEG activity in four different states of consciousness.
- We provide a unique integration of reflex responses and EEG activity in two avian species.

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ABSTRACT

Defining states of clinical consciousness in animals is important in veterinary anaesthesia and in studies of euthanasia and welfare assessment at slaughter. The aim of this study was to validate readily observable reflex responses in relation to different conscious states, as confirmed by EEG analysis, in two species of birds under laboratory conditions (35-week-old layer hens (n = 12) and 11-week-old turkeys (n = 10)). We evaluated clinical reflexes and characterised electroencephalograph (EEG) activity (as a measure of brain function) using spectral analyses in four different clinical states of consciousness: conscious (fully awake), semi-conscious (sedated), unconscious-optimal (general anaesthesia), unconscious-sub optimal (deep hypnotic state), as well as assessment immediately following euthanasia. Jaw or neck muscle tone was the most reliable reflex measure distinguishing between conscious and unconscious states. Pupillary reflex was consistently observed until respiratory arrest. Nictitating membrane reflex persisted for a short time (<1 min) after respiratory arrest and brain death (isoelectric EEG). The results confirm that the nictitating membrane reflex is a conservative measure of death in poultry. Using spectral analyses of the EEG waveforms it was possible to readily distinguish between the different states of clinical consciousness. In all cases, when birds progressed from a conscious to unconscious state; total spectral power (PTOT) significantly increased, whereas median (F50) and spectral edge (F95) frequencies significantly decreased. This study demonstrates that EEG analysis can differentiate between clinical states (and loss of brain function at death) in birds and provides a unique integration of reflex responses and EEG activity.

1. Introduction

Defining states of clinical consciousness in animals is very important in many situations, such as in veterinary anaesthesia, euthanasia and welfare assessment at slaughter [3,8,12]. In situations where the routine recording of electroencephalographic (EEG) activity is not possible, there is a need to validate readily observable reflex responses in relation to different conscious states. This study aimed to achieve this in avian subjects by simultaneously measuring reflex responses and EEG activity. This approach has wide applicability for welfare assessment especially in poultry species for which assessment of the state of consciousness is highly relevant to issues such as humane slaughter which affects very large numbers of individuals.

In human and veterinary neurology, the clinical evaluation of brain function in conscious and unconscious individuals and the confirmation

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of death (brain death) are determined using a variety of methods. The assessment of the state of unconsciousness[1] or insensibility[2] is obtained either by observing clinical behavioural signs, namely the absence of cognitive responses and voluntary motor activities [2,3,13], the presence or absence of certain reflex reactions, or the measurement of autonomic changes and recording electroencephalographic (EEG) activity [1]. Many of these approaches are used to monitor the depth of anaesthesia to ensure that animals are maintained at an optimum level of unconsciousness. A depth of anaesthesia which is inadequate ('too light') may lead to awareness, whilst a depth which is excessive ('too deep') may compromise the animal's safety (e.g. lead to severe cardiovascular depression and/or respiratory failure). It is also possible to use sedatives to induce a state of semi-consciousness, closer to a sleep-like state with slowed cognitive function, distinct from anaesthesia where cognitive function is absent [23]. Sedation and anaesthesia provide experimental tools though which it is possible to consistently achieve a controlled semi-conscious and unconscious state through which comparisons of reflex reactions, behaviours and EEG responses with aware, conscious individuals can be made.

The definition and determination of death in humans, and to some extent animals, are a medical, legal and philosophical issue that also encompasses ethical, cultural and religious aspects and beliefs. A major
Q8 determinant of death in humans relates the concept of "brain death" [31]. The determination of brain death is defined by the "Harvard criteria", which requires the absence of all of the following: cerebral responsiveness, induced or spontaneous movement, spontaneous respiration, brain stem and deep tendon reflexes, through systematic clinical neurological examination [15].

Therefore in this context, the aim of this study was to identify and verify robust, readily observable reflexes and behaviours that are reliably associated with different states of consciousness and death in birds, since there are many situations in which measurement of EEG is impractical or impossible. To achieve this we carried out simultaneous reflex, behaviour and EEG assessments in two poultry species of significant commercial relevance (laying hens and turkeys).

2. Material and methods

2.1. Animals and husbandry

The birds used in the study were 12 thirty-four week old mature commercial laying hens (*Gallus gallus domesticus*; ISA Brown, ISA, Boxmeer, Netherlands) and 12 ten week old immature female turkeys (*Meleagris gallopavo*; BUT 6, Aviagen Turkeys Ltd, Tattenhall, UK) housed under standard conditions in a University of Glasgow, School of Veterinary Medicine Cochno Research Facility in 2 separate experiments one month apart. Birds were housed in a single room in adjacent individual pens (2 m x 1 m) furnished with deep wood shavings and had ad libitum access to water and appropriate standard commercial feed. All birds had visual and auditory contact throughout the experimental period. The experiments were performed with authorization of the UK Home Office and with the approval of the SRUC Animal Ethical Review committee.

2.2. Implantation of EEG electrodes

After a period of 7 day habituation in the pens, the birds underwent surgery to implant EEG electrodes under general anaesthesia in a surgical suite at the Cochno Farm Research Facility. Birds were transported a short distance by motor vehicle (<25 m) from the home pen to the surgical facility in animal carrier cages (L 0.9 × W 0.6 × H 0.7 m). At the surgical unit, the birds were weighed and then kept in a separate dark, warm and quiet holding area prior to surgery. DEXMEDETOMIDINE (Dexdomitor, Elanco Animal Health, Hampshire, UK) was used for premedication/sedation, combined in the same syringe with the opioid analgesic, BUTORPHANOL (Torbugesic, Zoetis UK Ltd, London, UK) 0.4 mg/kg, and administered intramuscularly (IM) into the pectoral muscle approximately 20 min prior to surgery. The dose of dexmedetomidine was initially set at 40 μg/kg for the hens (which underwent the implantation procedure as a group, prior to the turkeys), but – at this dose – it produced only minimal sedation, even in combination with the butorphanol; consequently, it was elected to increase the dose of dexmedetomidine to 80 μg/kg for implantation in the turkeys, and to utilise this dose for both groups during the recording phase of the experiment. Following administration of dexmedetomidine and butorphanol, the birds were returned to the holding area in the surgical unit until sedation became apparent. Anaesthesia was subsequently induced by face mask with SEVOFLURANE (SevoFlo, Abbott UK, Maidenhead, UK) at an 8% concentration vaporised in 100% oxygen. Once unconsciousness was achieved, the trachea was intubated with a suitably sized PVC uncuffed endotracheal tube (Smiths-Medical, Ashford, UK). The non-steroidal anti-inflammatory drug, CARPROFEN (Rimadyl, Zoetis UK Ltd, London, UK), was administered subcutaneously (SC) prior to the start of surgery, at a dose of 6 mg/kg in the hens and 4 mg/kg in the turkeys. General anaesthesia was maintained with sevoflurane at a vaporiser concentration (between 2 and 3% vaporised in 100% oxygen) until implantation of the Q9
electrode assembly was completed.

The EEG electrode implantation method has been described previously [20–22]. Briefly, birds were implanted with an electrode and socket assembly consisting of 0.35 mm diameter Teflon insulated silver electrode wires (World Precision Instruments, Stevenage, UK) connected to a socket (3 pin DIN cable jack, RS components, Corby, UK). The connection points in the assembly were sealed over with dental cement (Duralay, Dental Directory, Witham, UK) to minimise extraneous electrical noise and moisture ingress. During surgery, the 2 recording electrode wires were placed in contact with the surface of the dura through 1 mm holes drilled in the frontal bone of the cranium, one on each of the dorsal surfaces of the right and left telencephalon at their approximate rostro-caudal and medio-lateral midpoints. An indifferent electrode was placed between the skull and the overlying tissue under the comb. The implant assembly was secured to the skull with dental cement and the surrounding skin was closed with sutures (Prolene Blue, Ethicon, Johnson and Johnson Medical Ltd, Livingstone UK). Mean body weights for each species and dosing for implantation and reflex testing are shown in Table 1.

Physiological variables (heart rate, respiration rate, non-invasive (oscillometric) blood pressure, end-tidal CO_2 and sevoflurane) were monitored and recorded throughout the period of anaesthesia using a multiparameter monitor (Mindray Beneview T5, Mindray Medical International, Nanshan, China) and a separate oscillometric device (Cardell 4801, Midmark, Ohio, USA) and pulse oximeter (Nonin 9847 V, Nonin Medical Inc, Minnesota, USA). After initial recovery from anaesthesia, the birds were placed back into an animal transport cage in the recovery area and were closely monitored. After a minimum of 1 hour recovery time (able to stand and walk unaided) the birds were returned to their home pens. The birds were allowed to fully recover from implant surgery for a minimum of 5 days before undergoing any further experimental procedure. The implant sites were closely observed on a daily basis in all the birds for any potential issues with healing.

2.3. Experimental protocol

On the day of testing, birds were placed individually into animal carrier cages and transported to the surgical facility. After a period of rest after transportation, each bird was instrumented (see below) to collect data. In the study, we aimed to achieve a continuum of five different

[1] British Medical Journal definition in humans: unconsciousness is the lack of wakefulness and awareness of oneself and surroundings and lack of responsiveness to environmental stimuli.

[2] Both are often used interchangeably, although the latter tends to refer to stunning and slaughter in animals.

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