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SPINDLES IN THE EEG OF CHICKENS: GENUINE EEG FEATURES OR EYEBALL ARTIFACTS?

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INTRODUCTION

The EEG of birds distinguishes between waking and sleep, just as in mammals. Similarly to mammals, sleep in birds is characterised by high amplitude slow waves, while waking is associated with small voltage high frequency waves ^{1,2}. In the EEG of mammals, such as rats and humans, spindles are common phenomena, occurring particularly in light slow wave sleep ³. However, although the avian EEG has been thoroughly studied in the past, most sleep studies indicate that birds do not show the presence of spindle activity in the EEG ^{1, 2, 4}. Nevertheless, in a recent study of Hunter et al. ⁵, spindle activity in the bird's EEG was found. We also recorded clear spindling in the EEG of birds under several states of vigilance. The present paper reports in more detail about this spindle activity. In a search of the literature on spindling, an early paper of Paulson ⁶ appeared in which it was shown that removal of the eyeballs leads to a total elimination of all spindle activity. In Paulson's paper the hypothesis was formulated that spindles in the chicken EEG should be regarded as artefacts, produced by the movements of the eyes. This hypothesis is discussed.

METHODS

Eleven one-month old chickens (*Gallus domesticus*) weighing between 1200 and 1400g (males and females) were used. They were kept on an intermittent light-dark schedule and had access to standard chicken food and water. Birds underwent surgery to implant EEG recording electrodes under general ketamine-xylazine anaesthesia. The EEG implant consisted of three Teflon coated silver wires soldered to a DIN plug. Two electrodes were placed on the dura through holes drilled in the skull, on the dorsal surfaces of the right and left telencephalon at the approximate rostro-caudal and medio-lateral midpoint. The ground electrode was placed between the skull and the overlying tissue. The plug was placed on the skull and fixed with dental cement. The chickens were allowed to recover for one week before any recordings were made. Animals were connected to a recording cable and placed in a chamber measuring 50 x 50cm. EEG signals with a bandwidth between 1 and 100 Hz were recorded and digitised using a sample rate of 1024 Hz. EEG recordings were made for one hour in the light and for one hour in the dark period, in order to facilitate the occurrence of all vigilance states. The behaviour of the animals was visually observed and coded manually; a dimmed red bulb allowed for observations in the dark period.

RESULTS AND DISCUSSION

Based on visual inspection of the EEG and behavioural observations, the state of vigilance was classified into three categories, wakefulness, drowsiness and sleep. A slow wave, high amplitude EEG coinciding with behavioural observations of immobile behaviour, closed eyes, and 'beak pointing down' posture was coded as sleep. A fast wave, small amplitude EEG signal accompanied by active standing or sitting behaviour or sitting passively with eyes open and head movements was coded as wakefulness. Drowsiness was described as an intermediate state or a transitory mixing of fast and slow waves in the EEG, accompanied by a motionless sitting posture and signs of sleepiness, such as closing the eyes or dropping the head^{1, 2, 4}.

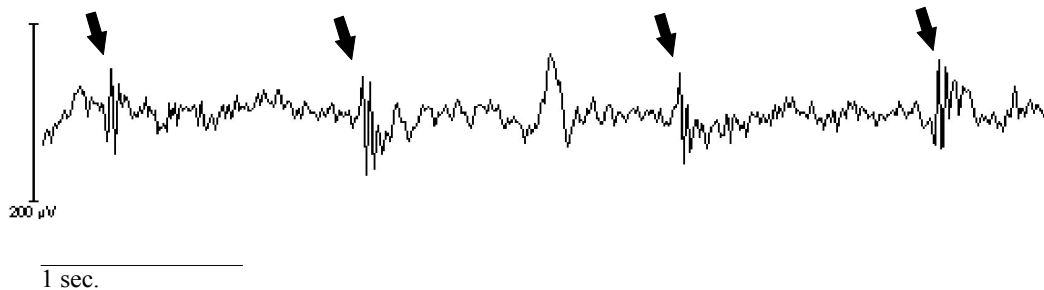


Fig. 1. A representative EEG from the waking state of a chicken, with discharges of spindles (arrows).

Representative segments of each vigilance category per animal, completely free of artefacts, were selected. Spindles were seen in every animal and mostly occurred in regular discharges of two, three or four single spindles. A representative piece of a raw EEG showing typical spindle discharges is presented in Fig. 1, while in Fig. 2 spindles belonging to the three vigilance states are shown. The number of spindles within these selected segments was counted. A spindle is identified as follows: 1. the spindle must have a sinusoidal shape and must be composed of at least one and a half sinusoid; 2. the maximal amplitude of the slope composing the spindle should be at least twice the average amplitude of the base-line EEG; 3. the frequency of the event, as obtained with an FFT, should be between 20 and 32 Hz, the common spindle frequency range.

A sub-sample of ten spindle discharges out of each vigilance category was selected randomly for further inspection. This was done for each subject and spindles were identified by visual inspection of the EEG. The number of spindles appearing in each spindle discharge was counted. The amplitude, defined as the difference between the highest and lowest voltage within the entire spindle in μV , and the visually determined duration in seconds as well as the frequency in Hz obtained with an FFT, were calculated for each spindle. Results were averaged for each category over subjects and the data are presented in Table 1.

Table 1. Characteristics of spindles of different vigilance states (means and SEMs).

	<i>spindles in discharge</i>	<i>Amplitude (μV)</i>	<i>Duration (sec)</i>	<i>frequency (Hz)</i>
wake	2.83 \pm 0.22	100 \pm 3.7	0.12 \pm 0.01	28 \pm 0.6
drowsy	3.18 \pm 0.28	117 \pm 5.6	0.15 \pm 0.01	26 \pm 0.5
sleep	3.47 \pm 0.44	130 \pm 8.0	0.16 \pm 0.02	25 \pm 0.7

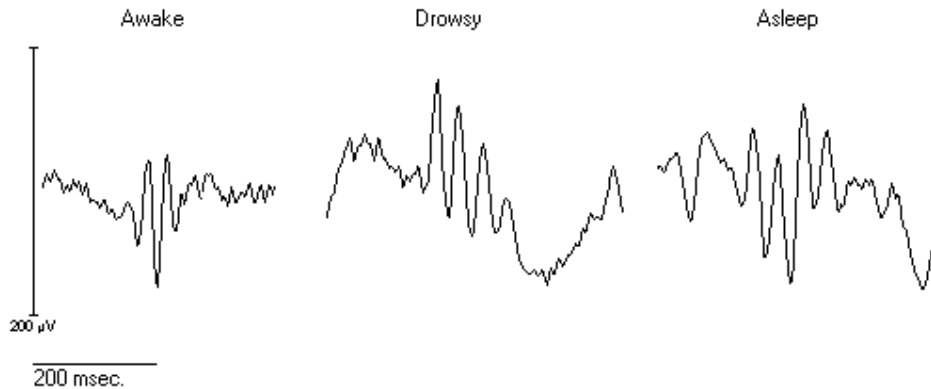


Figure 2. Examples of spindles belonging to the vigilance states ‘wakefulness’, ‘drowsiness’ and ‘sleep’.

Repeated measures ANOVA yielded the following significant effects: 1. numbers of spindles in each spindle discharge ($F_{2,20}=3.94$, $p<0.05$) were significantly ($p<0.05$) higher for drowsiness than for wakefulness and higher for sleep than for wakefulness; 2. spindle amplitudes ($F_{2,20}= 9.19$, $p<0.05$) were significantly ($p<0.05$) higher in the drowsy than in the awake state and higher in the sleep than the awake state; 3. the duration ($F_{2,20}= 5.42$, $p<0.05$) of spindles were significantly ($p<0.05$) longer in the drowsy and sleep state compared to the awake state and also longer in the sleep state than in the drowsy state and, finally, 4. spindle frequency ($F_{2,20}= 26.99$, $p<0.05$) was significantly higher in the awake state than in the drowsy state and also higher in the drowsy state than in the sleep state.

Spindle activity was clearly present in the EEG of all birds and appeared both during wakefulness and sleep, as well as during drowsiness. The presence of spindles in the avian EEG has not been noted by several studies^{1, 2, 4}, and it is intriguing why these studies have not described EEG spindling. A possibility could be that the electrode position is critical. Avian sleep studies have usually used an electrode placement in the ‘Wulst’, an enlarged structure at the upper region of the telencephalon, which is unique to birds. Our electrodes were also placed in this area, but more frontally than in most other studies. Hunter et al.⁵, in a paper on the development of the chicken brain, noted identical spindle-like activity in young, wake chickens, both in frontal, but also in posterior regions. This makes it unlikely that the electrode position is very critical for spindling. Nevertheless, it seems that the more frontal the recording is, the clearer spindling is. Furthermore, the compact EEG recordings together with the fact that authors may not directly have been interested in spindling, could account for them not describing these transients.

Hunter et al.⁵ propose in a rather cryptic remark, which was not explained further, that this rhythmical activity could be associated with saccadic eye movements. In a further search of available literature on this topic, we found an early paper of Paulson⁶ in which clear spindle

activity is shown. Paulson did several experiments investigating the origin of the spindles and came to the conclusion that spindles are presumably artefacts due to saccadic eye movements. Spindle-like activity was most clear when recorded with peri-orbital electrodes but could be seen as far posterior as the occipital region. The main evidence for the view of Paulson⁶ that spindles are artefacts of saccadic eye movements, was that removal of all orbital contents eliminated spindling activity completely. Studies have shown that spontaneous eye saccades are present in birds and contain oscillatory eye movements, while each saccade is accompanied by 25 to 30 Hz sinusoidal oscillations⁷.

If Paulson's conclusion is correct these spindles could form a model for studying eye movements in birds. In particular the differences between spindles occurring in the several vigilance states, and thus in saccadic eye movements, across these states of vigilance, are interesting. Moreover, the question regarding the function of saccadic eye movements during sleep arises. If these spindles are indeed movement artefacts (confirmation of which requires additional evidence), this has implications for the interpretation of other clear EEG transients. As this study has shown, the source of these potential artefacts is difficult to ascertain and it may be difficult to exclude the possibility that such transients arise from a previously undescribed movement, somewhere near the brain.

CONCLUSION

The main finding of this paper is the occurrence of short lasting spindles in the frontal EEG of chickens and their modulation across the different vigilance states of wakefulness, drowsiness and sleep. The density of spindles is highest while the subjects are awake, and decreases towards drowsiness to reach a minimum during sleep. Compared to wakefulness, sleep spindles are slower, longer, and have increased amplitude. Evidence exists that these spindles are the electrical correlates of saccadic eye movements as suggested by Paulson⁶, who demonstrated that similar spindles could be artefacts associated with ocular movements. Further evidence is required to strengthen this conclusion and to explain the modulation of the spindle activity across the several states of vigilance.

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