



ORIGINAL ARTICLE

# Sleep in ostrich chicks (*Struthio camelus*)

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## Abstract

It has been reported that adult ostriches displayed the longest episodes of rapid eye movement (REM) sleep (up to 5 min) and more REM sleep (24% of the nighttime) than any other bird species. If the mammalian ontogenetic trend exists in the ostrich, then the amounts of REM and the duration of sleep episodes in young ostriches may be greater than those reported in adults. We investigated sleep in 1.5–3.5 month old ostrich chicks. Recordings were conducted during nighttime (20:00–08:00), the main sleep period in ostriches, which are diurnal. The polygrams were scored in 4-s epochs for waking, non-rapid eye movement (NREM) sleep and REM sleep, as in other bird studies. REM sleep in ostrich chicks occurred during both cortical EEG activation and during slow waves, as was described in adult ostriches. The chicks spent  $69.3\% \pm 1.5\%$  of the night in NREM sleep. REM sleep occupied  $14.1\% \pm 1.8\%$  of the night or  $16.8\% \pm 2.0\%$  of nighttime sleep. Episodes of REM sleep lasted on average  $10 \pm 1$  s and ranged between 4 and 40 s. Therefore, the total amount and duration of REM sleep episodes in ostrich chicks were substantially smaller than reported in adult ostriches while the amounts of NREM sleep did not greatly differ. The developmental profile of REM sleep ontogenesis in the ostrich may be remarkably different from what has been reported in all studied mammals and birds.

## Statement of Significance

In altricial mammals and in humans, the amount of time spent in REM sleep is highest in young animals and gradually decreases to adult values as the animal's age. While precocial mammalian species do not greatly change REM sleep amount with age, no mammalian species has been reported to increase the amount of REM sleep with age. A smaller amount of REM sleep and shorter duration of REM sleep in the ostrich chicks when compared to adults, as we report in this study may be a unique feature of the sleep ontogenesis in this species—different from what has so far been observed in all studied mammals and birds and previously hypothesized to indicate a developmental function for REM sleep.

**Key words:** ostrich; Palaeognathae; Neognathae; birds; mammals; sleep phylogeny; sleep ontogenesis; NREM sleep; REM sleep

## Introduction

An electrophysiological study of adult ostriches reported a very unusual pattern of sleep. It has features of both slow wave

sleep (SWS) and rapid eye movement (REM) sleep [1]. In adult ostriches, REMs, head jerks and drops, eye closure and reduced muscle tone characteristic of REM sleep occurred both during

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activated EEG characteristic of waking and REM sleep, and during EEG slow waves characteristic of SWS. This pattern is similar to what we have described in the adult platypus and echidna [2, 3]. It was reported that in adult ostriches this state occupied 24.0% of the night on average and 26.3% of 24-h total sleep time (TST) [1]. These values are the highest that have ever been reported in birds [4–6]. The reported duration of episodes of REM sleep in adult ostriches averaged 27 s and REM episodes could last up to 5 min. Both are the longest reported values, with REM episode duration typically less than 10 s in studied birds [7–12].

While the vast majority of modern birds belong to the Neognathae group, the ostrich is a member of the Palaeognathae, considered the most basal group of living birds. The ancestors of ostriches separated from the main line of evolution of birds which produced the modern Neognathae birds more than 110 m.y.a. [13]. A “heterogeneous” or “mixed” sleep state in adult ostriches has been considered a form of REM sleep. This is because it has the key feature of REM (or paradoxical) sleep, which is REMs. Based on the reported data, it was proposed that this sleep state may be unique to two basal groups of the lineages of birds and mammals, paleognaths and monotremes. The presence of the heterogeneous sleep state in both groups also suggests an early evolution of this form of REM sleep, having a mixture of cortical activation and high voltage EEG activity with REMs and muscle tone suppression [1, 3]. However, a recent study of sleep in the tinamou, another member of the “ancient” Paleognathae bird group, concluded that the pattern of sleep in the species does not differ from that in all other species of “modern” Neognathae birds [14]. Thus, it was suggested that ecological and behavioral adaptations rather than just evolutionary history are needed to explain the presence of the heterogeneous sleep in the ostrich.

In altricial mammals and in humans, the amount of time spent in REM sleep is highest in young animals and gradually decreases to adult values as animals grow older [15–19]. Precocial mammalian species, such as the guinea pig do not greatly change REM sleep amount with age [16]. Although data are limited, a similar age-related decline in REM sleep has been reported in birds [20–22]. This declining trend is produced by a decrease of the number and/or duration of episodes of REM sleep. However, no mammalian species has been reported to increase the amount of REM sleep with age. If the mammalian ontogenetic trend exists in the ostriches, then the amounts of REM and the duration of sleep episodes in young ostriches may be higher and longer, respectively, than the already high amounts reported in adults. In addition, it is of great interest to determine if the heterogeneous sleep state is present in ostrich chicks and how it relates to other features of REM sleep such as postures, level of cortical activation and muscle tone. Therefore, the aim of this study was to examine the pattern of sleep in ostrich chicks. A surprising finding of our study is that the total amount and duration of episodes of REM in ostrich chicks was considerably smaller than that which has been reported in adult ostriches.

## Methods

### Experimental birds

Five 2–4-week-old ostrich chicks (*Struthio camelus*) were purchased from an ostrich farm and then transported to the Utrish

Marine Station of the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (44.70493445°N, 37.47129142°E). The birds were housed together at the station from 2 to 5 weeks before they were implanted for polygraphic sleep studies. All procedures were reviewed and approved by the Committee for Bioethics of the Severtsov Institute.

### Surgery (electrode implantation)

After premedication with zoletil (Virbac, France; 5 mg/kg, i.m.) each bird was anesthetized with isoflurane, 1%–3% and then implanted for electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) recording. EEG was recorded epidurally from symmetrical derivations of the right and left hemispheres from two pairs of stainless-steel screws (1 mm in diameter). The electrodes were positioned above the hyperpallium 2–3 mm lateral to the midline. The rostral and caudal pairs were 10 mm apart from each other. Another one or two pair of screws was implanted into the supraorbital bone above one or two eyes to record EOG. Four Teflon-coated multi-stranded stainless-steel wires (0.3 mm in diameter) were inserted into the nuchal muscles to record EMG. A pair of longer wires was also inserted subcutaneously in two birds (ostrich 1 and 2) to record the EMG of the intercostal musculature to measure respiratory rate. An additional screw implanted into the frontal part of the skull served as a reference electrode for monopolar recording. The electrode leads were soldered to a micro-connector and attached to the skull with acrylic cement. After surgery, the implanted ostriches were returned to the enclosure and stayed with other birds for at least 5 days to allow full recovery before the experiments started. During this time, they were given antibiotics (Baytril, Bayer, Germany) and analgesics (Rimadyl, Pfizer Animal Health, USA). All birds displayed normal behavior and activity levels within 2–5 h after the end of surgery.

### Housing conditions, polygraphic recording

The experiments were conducted in July–September. During the daylight hours (08:00–19:00) ostriches stayed together in a 4.5 × 3 m outdoor enclosure. They were given a turkey pelleted food and grass (*The Portulaca oleracea*, *Urtica dioica*, *Trifolium pratense*) three to four times per day depending on their age, based on the recommendations of the breeder. Water was available at all times. At 19:00 (the beginning of twilight) ostriches were moved to an adjacent indoor shelter (1.8 × 2.7 m) equipped with an infrared heat lamp where they stayed until the morning. The light in the shelter was off between 20:00 and 08:00. The birds were weighed weekly. During the first 1–2 months, the birds gained between 1 and 2 kg per month. At the time of recording, the chicks were 5–14 weeks old (Table 1). The weight of four younger birds ranged from 3.0 to 4.8 kg (5–9 weeks old). The oldest chick weighed 8 kg (14 weeks old). It was determined that ostrich 1 and 2 were males and ostriches 3–5 were females. For the entire period of study, daytime ambient temperature in the outdoor enclosure ranged between 18 and 38°C. Nighttime indoor air temperature ranged between 18 and 25°C.

Video recording and observations showed that ostriches were alert and active during daytime. The majority of the time (>95% of the total recording time [TRT]) they walked around

**Table 1.** The main characteristics of sleep and wake states in ostrich chicks

| Parameter  | Ostrich |      |      |      |      | Mean ( $\pm$ SEM, $n = 5$ ) |
|--|---------|------|------|------|------|-----------------------------|
|  | 1       | 2    | 3    | 4    | 5    |                             |
| Age (weeks)  | 8       | 9    | 14   | 6    | 5    |                             |
| Sleep latency (min)  | 10      | 8    | 14   | 20   | 12   | 13 $\pm$ 5                  |
| Amount of sleep and wake states (% of TRT)                 |         |      |      |      |      |                             |
| AW   | 3.9     | 0.9  | 2.3  | 3.0  | 1.4  | 2.3 $\pm$ 0.5               |
| QW   | 13.4    | 10.5 | 16.2 | 12.9 | 15.5 | 13.7 $\pm$ 1.0              |
| NREM sleep   | 71.8    | 71.0 | 71.5 | 68.2 | 63.9 | 69.3 $\pm$ 1.5              |
| REM sleep  | 10.6    | 16.9 | 9.3  | 15.0 | 18.5 | 14.1 $\pm$ 1.8              |
| UN   | 0.3     | 0.7  | 0.7  | 0.9  | 0.7  | 0.6 $\pm$ 0.1               |
| Amount of REM and NREM sleep (hours per 12-h night period) |         |      |      |      |      |                             |
| NREM sleep   | 8.6     | 8.5  | 8.6  | 8.1  | 7.7  | 8.3 $\pm$ 0.1               |
| REM sleep  | 1.3     | 2.0  | 1.1  | 1.8  | 2.2  | 1.7 $\pm$ 0.2               |
| Amount of REM and NREM sleep (% of TST)                    |         |      |      |      |      |                             |
| SWS/TST  | 87.1    | 80.8 | 88.5 | 81.9 | 77.5 | 83.2 $\pm$ 2.0              |
| REM/TST  | 12.9    | 19.2 | 11.5 | 18.1 | 22.5 | 16.8 $\pm$ 2.0              |
| Episodes of NREM sleep                                     |         |      |      |      |      |                             |
| Mean duration (s)  | 32      | 31   | 33   | 30   | 21   | 29 $\pm$ 2                  |
| Number (per night)   | 926     | 1001 | 923  | 966  | 1300 | 1,023 $\pm$ 71              |
| Maximal duration (s)                                       | 380     | 180  | 240  | 136  | 172  | 222 $\pm$ 43                |
| Episodes of REM sleep                                      |         |      |      |      |      |                             |
| Mean duration (s)  | 10      | 10   | 8    | 11   | 9    | 10 $\pm$ 1                  |
| Number (per night)   | 450     | 730  | 469  | 586  | 884  | 624 $\pm$ 82                |
| Maximal duration (s)                                       | 36      | 28   | 24   | 40   | 40   | 34 $\pm$ 3                  |

Amount of sleep and wake state is presented as % of nighttime (20:00–08:00). UN, unidentified; SEM, standard error.

the enclosure pecking the ground. If they stopped or sat down, they continued to display features of activity such as looking around, preening and “dust bathing” (Supplementary Table S1). When in the shelter with light off between 20:00 and 08:00 the ostriches rested and slept most of the time until sunrise. The chicks were very active and restless if they were separated from other birds during daytime. However, if the ostriches were separated around 20:00 and the light went off, they became quiet quickly and remained inactive for the rest of the night. For polygraphic recording two ostriches were placed in two adjacent recording chambers 10 min before 20:00. The chambers measured 0.6  $\times$  0.6  $\times$  0.8 m and were made from welded wire mesh forming a grid with 13  $\times$  13 mm openings. These were positioned in a sound attenuated, temperature, and light controlled laboratory room. The dimensions of the chamber allowed the birds to turn around, assume the sleep postures and stay fully extended with the head up. The light was turned off at 20:00 and the recording continued for 12-h until 08:00. The air temperature in the room was measured at the beginning and at the end of the recording period. It ranged between 25–26°C and 26–27°C, respectively. Recording was conducted from one or two birds simultaneously, using a cable connection, for three consecutive nights in each bird. When the light was turned off, both birds became quiet within the first 1–20 min (Table 1) and then spent most of the night resting/sleeping.

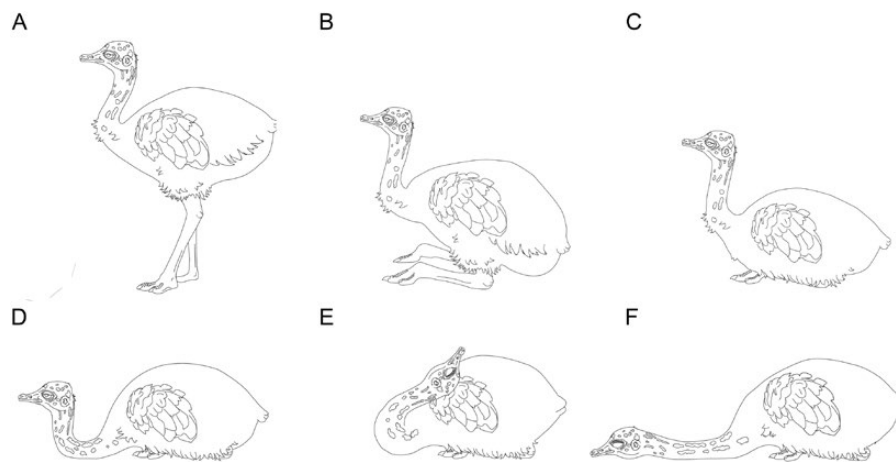
The EEG of the two cortical hemispheres, EMG, EOG signals was acquired at 200 Hz for on-line viewing and off-line analysis using a 16-channel amplifier (3500, AM System, USA), and Power 1440+ ADC convertor and Spike 2.0 v. 5.24 software (CED, Great Britain). The behavior of ostriches was continuously video recorded with several high-resolution remote control cameras fitted with infrared lights. The eyes were carefully monitored

allowing us to reliably confirm the identification of REM sleep, along with validating our EOG recordings.

### Data analysis

We present the data collected during the last of the three-recorded nights in each of the five ostriches. The polygrams were scored visually in 4-s epochs as active waking (AW), quiet waking (QW), SWS or non-rapid eye movement (NREM) sleep, and REM sleep, as was done in other bird studies including in adult ostriches [1] and in the tinamou [14].

Waking was subdivided into two substages. AW was scored when the birds were moving in the recording chamber, grooming or displaying other features of activity such as looking around while standing or sitting, pecking, or vocalizing. EEG during AW was contaminated with high amplitude artifacts, so behavior was the main criteria for scoring AW. QW was scored when the ostriches were standing, sitting or lying as shown in Figure 1 with occasional changes in body position. The EEG at this time was low voltage compared to the EEG during SWS, except for periods with movement artifacts. NREM sleep was scored when high voltage EEG activity in the range of 1–6 Hz was recorded in at least one of the cortical hemispheres in the absence of gross movements. During NREM sleep, the eyes could be closed or open. REM sleep was scored when repeated eye movements and head jerks were recorded simultaneously while both eyes were closed in behaviorally quiescent birds, irrespective of the EEG pattern. A small portion (on average 0.6% of TRT) of epochs was marked as unidentified. They included mostly epochs with some polygraphic signs of REM sleep (such as REMs or head jerks) without clear confirmation of those features on the video records.



**Figure 1.** Sleep postures in ostrich chicks. Top row (A–C): standing and two sitting positions. Bottom row (D and E): lying with the neck resting on the ground and the head held above the ground, with the head and neck on the back, and with the neck fully extended along the ground and the head rested on the ground.

A 50% criteria was used to score AW, QW, and NREM in 4-s epochs (each epoch was assigned the state which occupied 2 s or longer). Considering the short duration of REM sleep episodes and that the intensity and duration of phasic events were not always reflected in the recorded electrical activity, an epoch was scored as REM sleep if electrically recorded REMs or characteristic head jerks and drops of any duration occurred during at least 1 s of the epoch. Thus, this approach might lead to a slight overestimation of the amount of REM sleep compared to the 50% criteria.

The EEG power between 0.8 and 16 Hz was analyzed in the left hemisphere in artifact-free epochs by fast Fourier transformation using Spike 2 software (the Hamming window). Slow wave activity (SWA, power in the range of 0.8–4.0 Hz) was measured during QW, NREM, and REM sleep separately at 1-h intervals across the 12-h recording period. The average 1-h SWA values were calculated for all artifact-free NREM sleep epochs in each hour. The duration of episodes of NREM and REM sleep (also called bouts) was calculated by adding the number of consecutive epochs scored as a given state. A single epoch of another state terminated each episode. The duration of states and episodes was calculated across the entire 12-h recording period and in 1-h consecutive intervals. Reported values are means  $\pm$  SEM for all five ostriches, for the entire night or 1-h interval period. All statistical analyses were performed using Sigma Plot 11.0 Software.

## Results

### Behaviors, postures, active, and quiet wakefulness

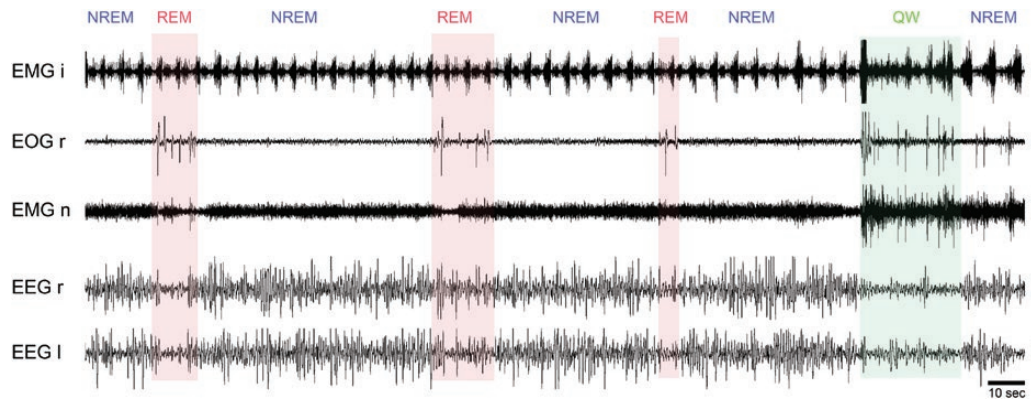
During the night, ostriches spent most of the time either sitting with their neck held up or lying with the neck resting on the ground. While in the lying position they held the head above the ground, rested the head on their back or laid the head on the ground (Figure 1). Ostriches spent on average  $18.8\% \pm 6.2\%$  of TRT (20:00–08:00) while in the sitting position and  $77.9\% \pm 6.7\%$  of TRT in the lying positions. Walking and standing accounted for only  $3.3\% \pm 1.5\%$  of TRT (Supplementary Table S2). AW occupied  $2.3\% \pm 0.5\%$  of TRT and QW  $13.7\% \pm 1.0\%$  of TRT (Table 1). In QW, EEG ranged between low voltage cortical activation and intermediate voltage lower frequency (1–6 Hz) activity. High voltage EOG waves produced high voltage EEG artifacts. Their amplitude varied among the birds.

During QW, the EMG and EOG activity was generally greater than during NREM sleep (Figure 2). When resting and sleeping the ostrich chicks took different positions in the chamber in relation to the adjacent chamber with a companion bird (Supplementary Figure S1).

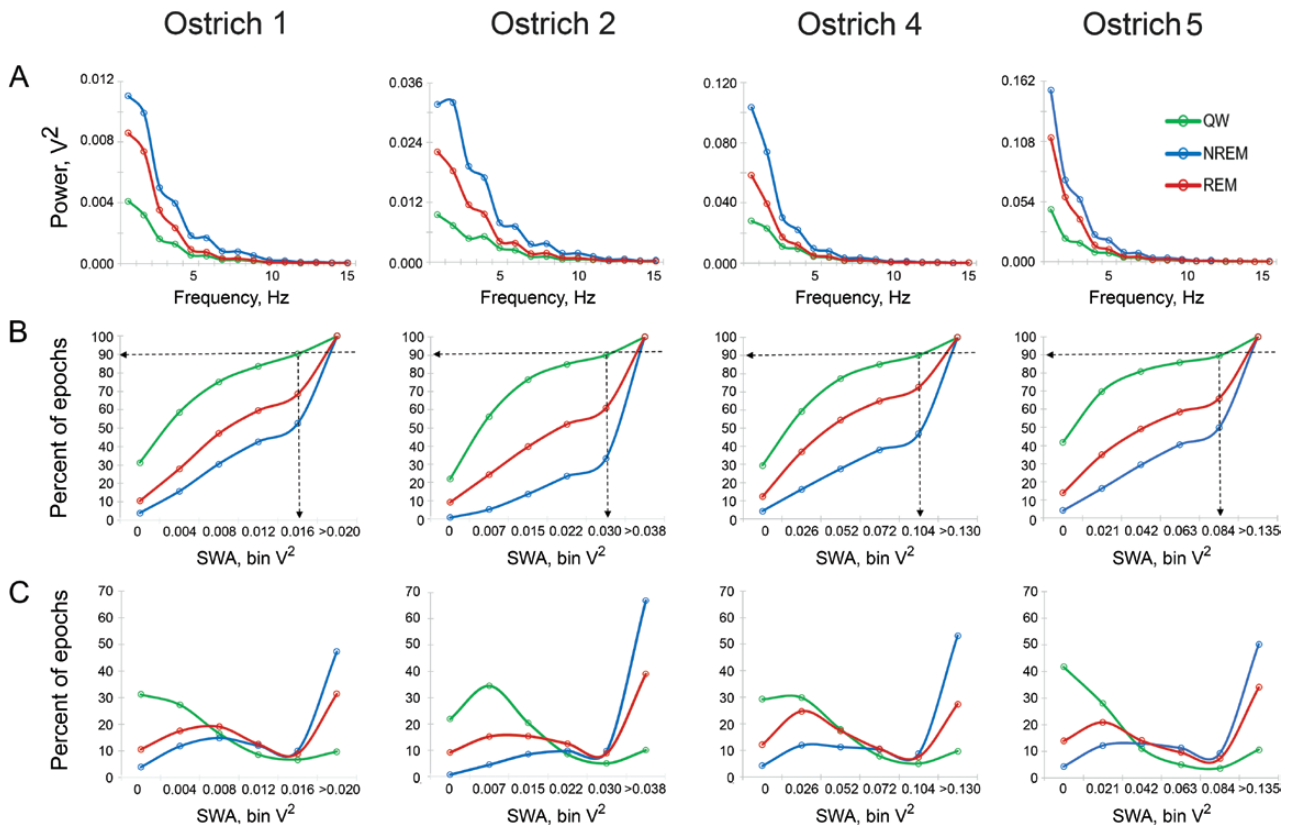
### NREM sleep

NREM sleep occurred in ostriches in all postures shown in Figure 1. The EEG comprised intermediate and high voltage slow waves, which most often developed synchronously in symmetrical cortical recordings (Figure 2). The EEG power during NREM sleep calculated in the range of 0.8–16 Hz for artifact-free epochs was maximal at the lowest frequencies (<4.0 Hz) and then progressively decreased in amplitude at higher frequencies (Figure 3, A). The transition from waking to NREM sleep occurred within 8–40 s (2–10 epochs) after the cessation of movement (Figure 2).

The neck muscle tone during NREM sleep was variable depending on the posture and position of the head. Minor postural changes did not necessarily interrupt EEG slow waves (e.g. changing the position of the head while sleeping sitting with the head held above the ground or lying with the head on the ground). The EEG activity during this time was indistinguishable from that during NREM without motion. During NREM sleep, activity in the EOG was low and it could be absent for minutes if the eyes were closed (Figure 2). Single deflections in the EEG were often linked to a brief closure of the open eye(s), suggesting EOG artifacts in the EEG. The state of left and right eye was determined in three ostriches (O1, O2, and O3) for a total of 634 and 365 min of NREM sleep, respectively (Supplementary Figure S2). The left eye was closed on average 87% of the time and the right was closed 91% of the time. The eyes were open the remaining time (on average 13% and 9%, respectively). In two ostriches (O1 and O2), the state of both eyes could be determined during 47 and 171 min of NREM sleep. In O1, both eyes were closed 68% of the time and they were open 23% of the time. In O2, both eyes were closed 87% of the time while they were open less than 4% of the time. The remaining 9% of the time the eyes were in asymmetrical state (one was open and the other was closed). Thus, during NREM sleep the ostriches' eyes appear to be closed for most, but not all of the time.



**Figure 2.** Representative episodes of waking, NREM and REM sleep in an ostrich chick. EMG i, EMG of intercostal muscles. EOG r, electrooculogram of the right eye. EMG n, electromyogram of the neck muscles. EEG, electroencephalogram of the right (r) and left (l) cortical hemispheres. This recording shows 3 episodes of REM sleep lasting 4, 5, and 2 epochs (16, 20, and 8 s, respectively). The episodes of REM sleep alternated with NREM sleep with high voltage slow waves in the EEG. All three episodes of REM sleep were characterized by REMs as shown in the EOG. The neck muscle tone during the first two episodes was reduced compared to NREM sleep. The EEG of both hemispheres during the episodes of REM sleep ranged from low voltage fast activity similar to that during QW (or cortical activation; e.g. episode 1), intermediate voltage slow waves (episode 3) and high voltage slow wave activity, almost indistinguishable from that during NREM sleep (episode 2). The respiratory rate as shown by EMG was regular during NREM sleep with the majority of respiratory pauses ranging between 7 and 9 s. The breathing rate was not generally affected by episodes of REM sleep. During this recording, the chick was lying on the ground with its head resting on the cage floor as shown in Figure 1F. Vertical bars on the right of the EEG, EOG, and EMG traces correspond 100  $\mu$ V. More polygrams of sleep are presented in Supplementary Figures S3–S11.



**Figure 3.** The power of EEG during sleep and waking in ostrich chicks. The data are presented for four ostriches (O1, O2, O4, and O5). QW, quiet waking; NREM, NREM sleep; REM, REM sleep. The EEG in ostrich 3 was contaminated with EOG artifacts during REM sleep due to eye movements and was not analyzed for spectral power. The value below X-axes marks the left margin of each bin interval. The top row (A). Power of the EEG in the range of 0.8 and 16 Hz during QW, NREM, and REM sleep. The width of the first bin is 1.2 Hz (0.8–2.0 Hz) and the width of each remaining bins is 1.0 Hz. The values are means for each bin for all artifact free epochs of QW, NREM, and REM sleep in each ostrich chick ( $n$  ranged between 120 in QW and 7,580 in NREM sleep). Note that during REM sleep the mean power in each frequency bin between 0.8 and 6–9 Hz was intermediate between the power during QW and NREM sleep. The middle row (B). Number of epochs of QW, NREM, and REM sleep with each amount of slow wave activity (SWA, power in the range of 0.8–4.0 Hz) as percent of all artifact-free epochs of the corresponding state. The absolute power varied substantially between the birds. To allow comparison between states and birds, the SWA is presented in volts<sup>2</sup> ( $V^2$ ) in six bins. The width of each bin was normalized in such a way that the first five bins included 90% of all QW epochs (C). In other words, the first five bins include and represent “the majority of QW” in each ostrich and the sixth bin includes all remaining epochs with the SWA greater that (“90% of QW”) value. Thus, the number of REM sleep epochs within the first five bins of each graph is an estimate the degree of cortical activation in REM sleep in each bird when compared to that during QW. The bottom row (C). The cumulative number of epochs with different SWA during QW, NREM, and REM sleep in four ostriches. Note that the first five bins included 90% of QW epochs, that is “the majority of QW.”

Interhemispheric EEG asymmetry during NREM sleep was recorded occasionally in ostrich chicks (Supplementary Figure S3). The majority of episodes with slow wave EEG asymmetry lasted less than 20 s. Since the state of two eyes could not be reliably determined during all episodes, a quantitative analysis of the correlation between the eye states and EEG asymmetry was not possible.

## REM sleep

REM sleep occurred with ostrich chicks both in the sitting position and in the lying position. REM sleep was characterized by REMs, head jerks and bilateral eye closure. The amplitude of EEG during episodes of REM sleep ranged between low voltage, as recorded during QW (cortical arousal) and high voltage, as recorded during NREM sleep. In the majority of cases, periods of REMs were accompanied by a decrease in the amplitude of slow waves but the duration of decrease was shorter than the period of REMs. Usually episodes of REM sleep occurred in a series and alternated with episodes of NREM sleep without awakening. Representative episodes of REM sleep are shown in Figure 2 and Supplementary Figures S4–S11.

During REM sleep the average power of EEG between 0.8 and 6.0 Hz was intermediate between QW and NREM sleep (Figure 3, A). The histogram of the distribution of REM sleep epochs based on SWA (0.8–4.0 Hz) also had a profile intermediate between QW and NREM sleep (Figure 3, B and C). On average in  $67.0\% \pm 2.4\%$  of REM sleep epochs SWA was in the same range as in the majority (90%) of QW epochs.

Most often, the first REMs preceded cortical activation and appeared during EEG slow waves (Supplementary Figures S4–S6) or cortical activation and REMs emerged synchronously (Supplementary Figures S6 and S7). Less often, cortical activation preceded REMs (Supplementary Figure S8). The end of REM sleep was either marked by behavioral arousal (as indicated by postural changes, increased muscle tone and opening of the eyes, see Supplementary Figure S9) or could gradually transition to NREM sleep, so eye movements (REMs) were seen in parallel with EEG slow waves during the transition and then disappeared (e.g. Supplementary Figures S8, S10, and S11).

The neck muscle tone during REM sleep was variable depending on the bird's posture. Generally, it was smaller or similar than that during the preceding NREM sleep episode. Evident hypotonia or distinct atonia were often seen in the lying position with the head on the ground. If seen, the decrease of muscle tone usually developed after the appearance of eye movement or in parallel with them, rarely ahead of phasic events (Figure 2 and Supplementary Figures S4–S11). When in the sitting position, ostriches dropped the head repeatedly but continued to maintain it above the ground throughout the entire episode. The amplitude of EMG during this time was variable (Supplementary Figure S5).

## Breathing and respiration rate during NREM and REM sleep

Based on the measurements conducted in two ostriches with EMG electrodes implanted in the intercostal muscles, breathing pauses in these birds while they were sleeping with the heads lying on the ground, ranged between 5 and 10 s. The mean breathing pauses during NREM sleep and REM sleep were 7–8 s. Neither means or range of variation of the breathing pause differed significantly between the sleep states (Supplementary Table S3 and Figures S4, S5, S7, S10, and S11, Figure 2).

## Total amount, duration of episodes and timing of NREM sleep

The ostrich chicks spent  $69.3\% \pm 1.5\%$  of the night (TRT) in NREM sleep,  $83.2\% \pm 2.0\%$  of TST. Average episode duration was  $29 \pm 2$  s. The longest of the uninterrupted episodes lasted 380 s (Table 1). On average  $70\% \pm 10\%$  of all NREM sleep episodes (63%–84% in different birds) were shorter than 30 s and  $93\% \pm 3\%$  of all episodes (89%–98%) were shorter than 1 min (Figure 4, A).

The time spent in NREM was fairly stable for the 12-h recording period (Figure 5, A). It did not change across the night (one-way ANOVA,  $F_{11,44} = 0.906$ ,  $p = 0.542$ , factor "hour of night"). The SWA did not change noticeably during the first half of the recording period but it slowly increased reaching maximum in hours 06 and 07 in all birds (Figure 5, B). The increase over the night was statistically significant ( $F_{11,44} = 4.600$ ;  $p < 0.001$ ). The difference

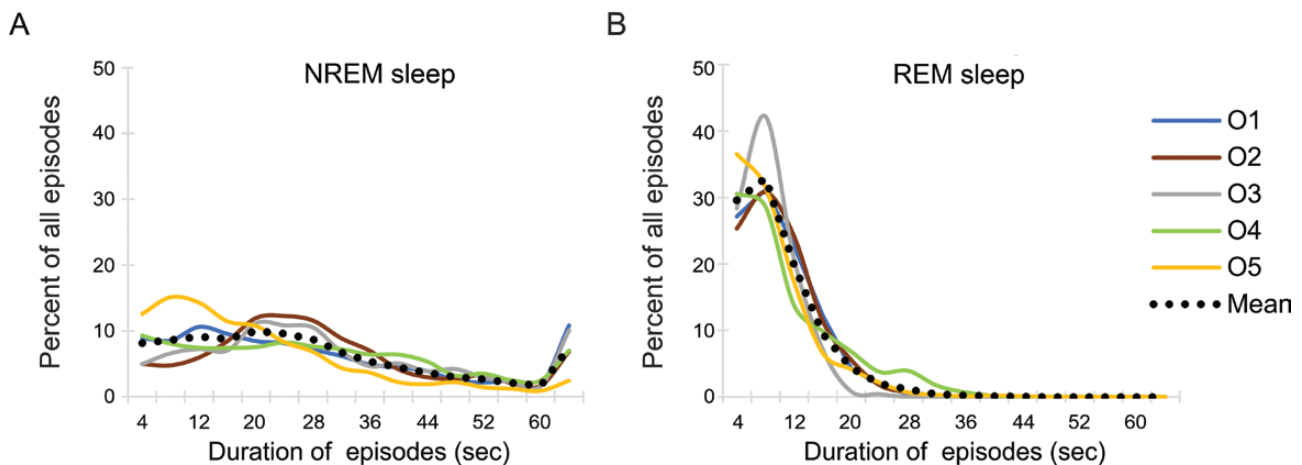
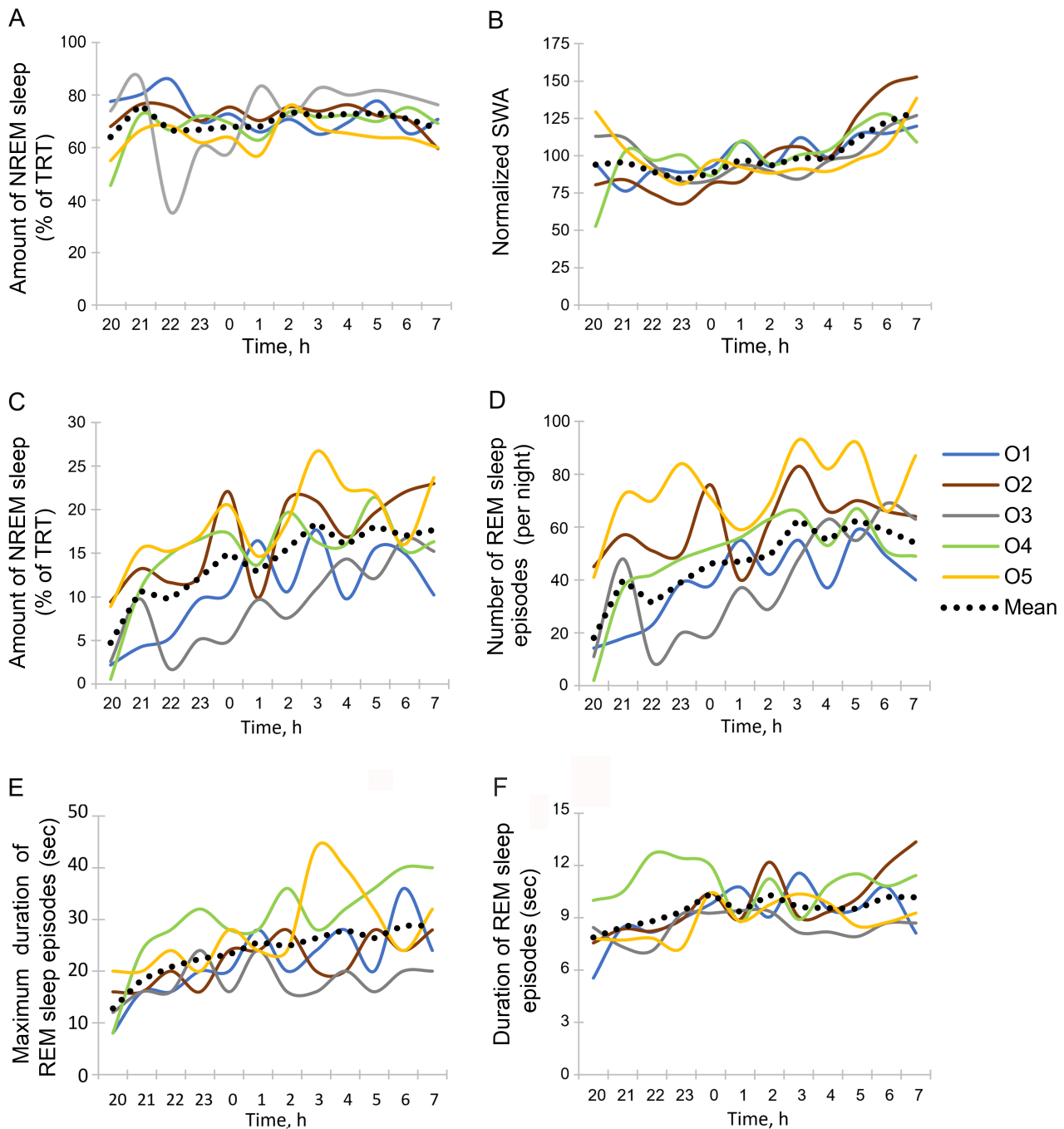


Figure 4. Histograms of distribution of episodes of sleep in ostrich chicks. (A) NREM sleep. (B) REM sleep. The graphs show profiles for individual ostriches (color lines, O1–5) and the mean profiles (black dotted plotted). The value below X-axes marks the right margin of each bin interval.



**Figure 5.** The time-course of the parameters of NREM and REM sleep across the nighttime recording in ostrich chicks. The graphs show profiles of 1-h mean data for individual ostriches (color lines, O1–5) and the mean profiles (black dotted plotted). (A) The amount of NREM sleep as percent of TRT. (B) Normalized SWA (power in the range of 0.8–4.0 Hz) as percent of the average SWA across the entire period (all 1-h intervals). (C) The amount of REM sleep as percent of TRT. (D) Number of episodes of REM sleep per night. (E) Maximum duration of REM sleep episodes in seconds. (F) Mean duration of episodes of REM sleep in seconds.

was significant between the amount of SWA in the beginning of night (hours of 20–23 and 0) and the last two morning hours (6 and 7;  $p < 0.05$ , Tukey's post hoc test). Thus, the depth of NREM in the studied ostriches as measured by SWA increased in the morning hours.

#### Total amount, duration of episodes and timing of REM sleep

The total amount of REM sleep in ostrich chicks accounted for  $14.1\% \pm 1.8\%$  of the night (TRT),  $16.8\% \pm 2.0\%$  of TST (Table 1). The

time spent in REM sleep was substantially more variable (coefficient of variation is 28.1%) across the studied ostriches than in NREM sleep (4.8%, almost a 6-fold difference). Episodes of REM sleep ranged between 4 and 40 s and were  $10 \pm 1$  s on average. The mean maximum duration was  $34 \pm 3$  s. In each bird, approximately 1/3 of all REM sleep lasted 4 s (1 epoch) and another 1/3 lasted 8 s (2 epochs). On average  $96\% \pm 4\%$  of all episodes were 20 s or shorter (90%–100% in different birds; Figure 4, B).

In contrast to NREM sleep, the time spent in REM sleep noticeably increased across the night in all ostriches (Figure 5, C). In 3 out of 5 birds, it increased progressively over most of the

night reaching its maximum between 05:00 and 08:00. In two other birds, the amount of REM sleep steadily increased until 03:00 and then it varied within 10% below the maximal values until the end of the night. There was a significant effect of time on amount of REM sleep ( $F_{11,44} = 8.006$ ;  $p < 0.001$ ). Similar to the SWA, the difference was significant between the amount of REM sleep in the beginning of night (hours of 20–23) and in the last morning hours (5, 6, and 7;  $p < 0.05$ , Tukey's post hoc test). In addition to that, the amount of REM sleep during the first recording hour (20) was significantly smaller when compared to all hours (the exception was hour 22).

The increase in REM sleep over the night in ostriches was primarily due to an increase in the number of REM episodes. The difference between the number of episodes was significant between the two evening hours (20 and 22) and several morning and night hours (3, 5, and 7;  $p < 0.05$ ; following one-way ANOVA,  $F_{11,44} = 6.633$ ;  $p < 0.001$ ; [Figure 5, D](#)). The number of REM sleep episodes during the first recording hour (20) was also significantly smaller when compared to all hours after the midnight (hours 0–7). The increase of the maximum duration of REM sleep episodes over the recording period was also significant ( $F_{11,44} = 4.076$ ;  $p < 0.011$ ; [Figure 5, E](#)). The difference between means was significant between the first hour (20) and all hours starting from midnight (hours 0–7). The mean duration of REM sleep did not change significantly across the night ( $p > 0.05$ ; [Figure 5, F](#)).

### Sleep parameters and age

The highest amount of REM sleep was recorded in the youngest chick (ostrich 5, 18.5% of TRT, 5 weeks old) and the smallest amount of REM sleep was recorded in the oldest bird (ostrich 3, 9.3% of TRT, age 14 weeks old; almost a two-fold difference; [Table 1](#)). The same was also for the maximal duration of REM sleep episodes (40 and 24 s, respectively). However, among the REM sleep parameters tested only the maximal duration of REM sleep statistically significantly decreased with the post hatched age of the studied ostrich chicks ( $R_{xy}$ , Pearson coefficient of correlation is  $-0.935$ ;  $p = 0.024$ ). No significant correlation was found between the age and parameters of NREM in the ostrich chicks studied ([Supplementary Table S4](#)).

### NREM and REM sleep in different postures

Almost all NREM sleep occurred in ostriches in the lying or sitting positions accounting for  $83.4\% \pm 6.0\%$  and  $16.2\% \pm 5.9\%$  of total amount of NREM sleep, respectively (the ratio of means was 5 to 1, [Supplementary Table S2](#)). Those values were close the total duration of both behaviors per night ( $77.9\% \pm 6.7\%$  and  $18.8\% \pm 6.2\%$  of TRT, respectively; the ratio was 4:1). Four out of 5 ostriches had episodes of NREM sleep in the standing position but the total amount averaged only  $0.4\% \pm 0.1\%$  of NREM sleep time.

In all ostriches, the majority of REM sleep occurred in the lying positions (74%–99% of total REM sleep; [Supplementary Table S2](#)). Therefore, even considering that ostriches, overall, spent more time while lying than sitting, REM sleep was more correlated with the lying positions compared to the sitting posture (on average  $91.5\% \pm 4.6\%$  and  $8.5\% \pm 4.6\%$  of all REM sleep, respectively; ratio almost 11:1 vs. 4:1).

REM sleep in ostriches occurred while they were sitting and lying with different positions of the head ([Figure 1](#)). All five ostriches displayed a comparable number of REM sleep episodes when lying with the head held above the ground or with the neck stretched out and head resting on the ground. Two of the birds displayed either only a few REM sleep episodes while sitting or no episodes while lying with the head resting on the back ([Figure 6, A](#)). The longest episodes of REM in all individuals were recorded while lying with the head resting on the ground ([Figure 6, B](#)). A large disproportion between the sample sizes did not allow comparison the duration of REM sleep episodes in all postures but only for the two lying positions. While the maximum duration of REM sleep episodes with the head on the ground was significantly greater than when the head was held above the ground ( $p < 0.001$ ,  $df = 4$ , paired T-test, [Figure 6, B](#)), the mean duration of episodes of REM sleep did not differ between these two positions ( $p > 0.05$ , [Figure 6, C](#)).

## Discussion

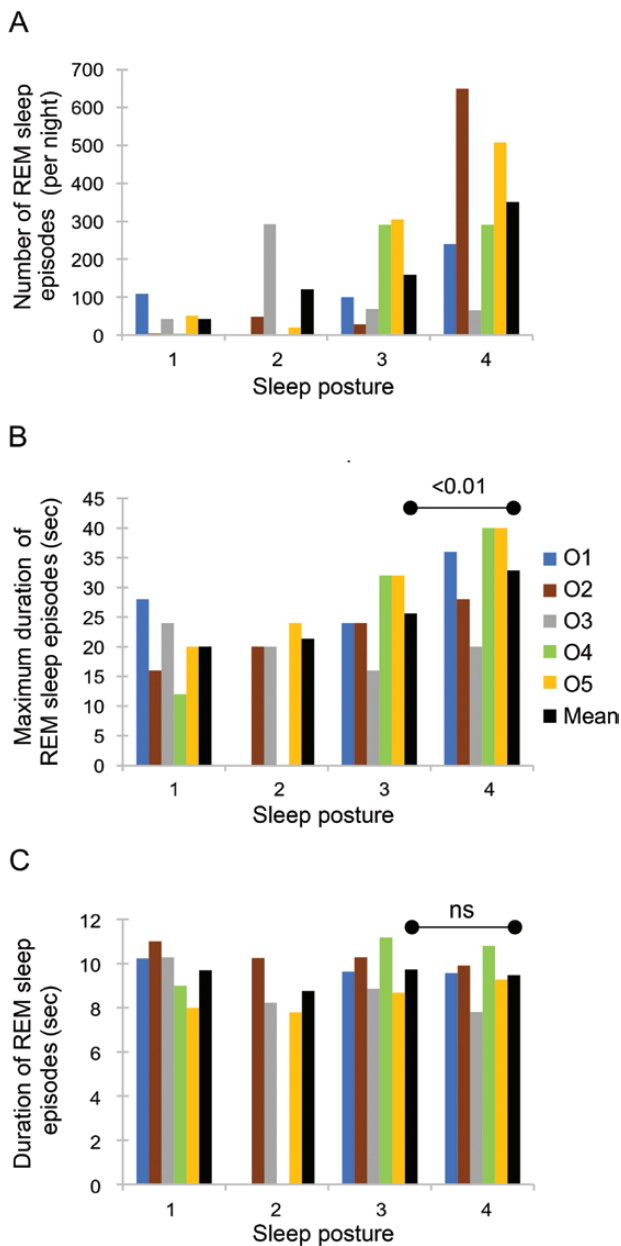
There are two main findings of this study. First, REM sleep in ostrich chicks occurred both during cortical activation of EEG and during EEG slow waves, as was described in adult ostriches [1]. The second main and unexpected finding is that total duration of REM sleep, as well as the duration of episodes of REM sleep, in chicks were significantly shorter than in adult ostriches.

### “Heterogeneous” or “mixed” sleep in ostriches

Episodes of REM sleep in ostrich chicks have features of SWS and REM sleep and resemble the “heterogeneous” or “mixed” state of sleep reported in adult ostriches [1] and in the platypus [3]. Spectral analysis showed that the level of cortical activation during REM sleep in chicks was intermediate between that in QW and NREM sleep. More precisely, in approximately 70% of the epochs of REM sleep, the level of cortical activation as measured by SWA was similar to that in 90% of QW epochs. This means that REM sleep in ostrich chicks, in most cases, featured a decrease in the amplitude of SWA, that is, cortical activation as in waking. In contrast, ostrich chicks also displayed episodes of REM sleep in which the amplitude of the SWA did not decrease or decreased slightly compared to the amplitude in the preceding NREM sleep (~1/3 of all REM sleep epochs). The prior report on sleep in adult ostriches did not characterize the degree of cortical activation during REM sleep.

All three key characteristics of REM sleep/PS as described in placental mammals (cortical activation, REMs and muscle tone reduction) were present in ostrich chicks while their expression and timing varied across the episodes. For instance, the amplitude of EEG slow waves could drop at REMs onset, decline progressively and then increase, or remain at the same level during the entire period of eye movements and muscle jerks, as in the prior period of NREM sleep. EEG activation could precede REMs but those episodes were rare. In mammals, cortical activation, REMs and muscle tone reduction occur synchronously [23]. In some mammals, PGO-spikes or waves can be early predictors for the onset of REM sleep. They are most pronounced in the cat first appearing in the pons, geniculate nucleus, and occipital cortex during SWS before the cortical arousal and muscle tone reduction which mark REM sleep [24, 25]. However, episodes of





**Figure 6.** Parameters of episodes of REM sleep recorded in different postures in ostrich chicks. The graphs show data for individual birds (color bars) and overall mean values (black bars). (A) The number of episodes per night. (B and C) Maximum and mean duration of episodes in seconds. The X axes are sleep postures: (1) sitting (Figure 1C), (2) lying with the head on the back (Figure 1E), (3) lying with the head held above the ground (Figure 1D), and (4) lying with the head on the ground (Figure 1F). The level of significance of difference between two means:  $<0.05 - p < 0.05$  (the paired T-test,  $n = 5$ ,  $df = 4$ ). ns, non-significant ( $p > 0.05$ ).

sleep with PGO-waves in the cat were scored as transitional between SWS and REM because eye movement bursts only occur after complete desynchrony and muscle atonia. REM sleep with “a mixture of high voltage and fast wave EEG activity” was described in the ferret (“REM-2”) [26, 27] while in the armadillo REM sleep with low EMG activity was recorded both during “activated” and “deactivated EEG pattern” [28]. Both were similar in many respects with “REM-M” and “REM-H” (REM sleep with moderate- and high-voltage EEG) in the platypus [3]. Similar to other studied birds, the decrease in muscle tone was also not strictly tied to cortical activation or REMs in ostrich chicks. The

timing and extent varied and largely depended on the posture and the position of the head. Thus, it is REMs that ultimately mark REM sleep in ostriches while cortical activation and muscle tone reductions are variable in their extent and timing. It would be interesting to quantify and compare the timing and extent of the three key polygraphic features of REM sleep across other animals in order to establish similarities and differences between the “mixed” stages of sleep in different species of mammals and birds.

### Sleep ontogenesis

The magnitude of the changes of different sleep features in the postnatal period has been hypothesized to depend on the level of maturity of animals at birth. In altricial mammals and in humans, the amount of REM sleep is maximal at birth, decreasing gradually to adult levels [15–19, 29]. In precocial mammals, the amount of REM sleep is relatively stable across the early period of postnatal development while adults still display less REM than young animals [16, 19, 30]. The postnatal ontogeny of sleep has so far been tracked in only a few avian species [12, 22]. The data suggest that the general trend of the changes is similar to that in mammals.

Ostriches are precocial. They are born with both eyes open and a well-developed down cover. They can leave their nest and follow adult birds within 2–3 days after hatching as other precocial birds do [31, 32]. The total amount of REM sleep in 1.5–3.5-month old ostrich chicks accounted for on average 14% of the night and almost 17% of TST. Both values are among the highest in the studied species of birds [4–6]. Polygraphic recordings from fetal lambs revealed that they have a differentiated sleep–wake cycle with REM sleep occupying 50% of TRT at 115–120 days of gestation (0.8 of full term). This amount is greater than in newborn lambs [33]. Thus, our results raise the possibility that REM sleep in the ostrich is even more predominant “in ovo.”

We found obvious similarities between the features of sleep in adult ostriches [1] and in their chicks at the postpartum age of 1.5–3.5 months, such as the pattern and timing of EEG during episodes of REM sleep, the sleep posture with the head held high above the ground and diurnality. The majority of episodes of Interhemispheric EEG asymmetry during SWS in the chicks were also short and sporadic as reported in adult ostriches [1]. We have also found an indication that the amount of REM sleep, number of REM sleep episodes and the maximal duration of REM sleep may decline within 1.5–3.5 months of post hatch life in ostrich chicks. More studies need to be done in younger ostrich chicks to establish how the pattern of sleep changes during the first weeks and months of their lives.

It was shown that in humans, in some mammals (most of them were rodents) and birds the amounts of SWS/NREM sleep and SWA were highest in the beginning of the main sleep period and then progressively declined to the end of the sleep period [14, 34–37]. The amounts of NREM sleep in ostrich chicks did not change over the night while SWA increased in the end of the night, opposite to the pattern in humans and in mammals. In the pigeon and tinamou SWA changed little over the dark time period [14, 38]. In contrast to NREM, the REM sleep percentage in ostrich chicks increased over the night reaching its maximum in the morning. This profile exactly mirrors the situation in humans, rodents, and in most birds. The daily profile of REM sleep parameters appears to be more consistent and conservative across

mammals and birds, including the ostrich chicks, than that of SWS/NREM sleep.

When in a group, adult ostriches do not rest or eat at the same time [32], presumably an example of herd (collective) vigilance [39]. It has also been reported that in adult ostriches both eyes are open during SWS but are closed during REM sleep [1]. Sleeping with eyes open may allow ostriches to process visual information during sleep and to respond faster to threat. Periods of SWS with both eyes open appear to be widespread among birds and mammals [14, 22, 38, 40–42]. This can be seen as an alternate strategy to having unihemispheric sleep and unilateral eye closure, as reported in mallard ducks [43] and in marine mammals (cetaceans and some pinnipeds) [18, 44]. In contrast to adult ostriches, our data indicate that 1.5–3.5 month old ostrich chicks had NREM sleep with both eyes closed more often than with both eyes opened. The closure of both eyes and the predominance of sleep in the lying position in ostrich chicks causes them to be less vigilant and responsive than adults [32, 45]. Thus, the behavioral strategy of maintaining individual vigilance during sleep is not fully developed in ostrich chicks during at least in the first 3 months of their age.

Sleep occurred in ostrich chicks in different positions including with the neck stretched out and head resting on the ground. Three-six-week old greater rhea chicks also displayed sleep while resting with their necks touching the ground. However, they rarely rested the head on the floor as the ostrich chicks in our study. Instead, while in this position the greater rhea chicks usually rested the head on the back [46]. In captivity, adult ostriches and emus also sleep in a prone position with the head on the ground [32, 47]. However, no sleep in such position was reported in the Lesku et al. study [1] when the adult birds were held in the barn or after they had been released in the open range. It is obviously that sleeping with the head held above the ground allows a faster reaction and escape in case of emergency. All of these suggest that the prevalence of postures of sleep in the ostrich (and likely in other Palaeognathae birds) depends both on the postnatal age (degree of maturity) and the level of bird vigilance.

### Quantitative differences between chicks and adults

The main difference between sleep reported in adult ostriches [1] and in the chicks we report on here, is the amount of REM sleep and duration of REM sleep episodes. We found that 1.5–3.5 month old ostrich chicks displayed substantially less of the amount of REM sleep reported in the adults during the night which is the main sleep period in the species (14.1% vs. 24.0% of TRT, respectively; a 1.7-fold difference). The proportion of REM sleep in chicks during the night was also substantially smaller than in adults (16.8% vs. 26.3% of TST, a 1.6-fold difference). In addition, the mean duration of REM sleep episodes in young birds was 10.0 vs. 27.0 s in the adults (a 2.7-fold difference). The maximum duration of REM sleep episodes was 40 s vs. 5 min in the adults (an 8-fold difference) and the mean maximum was 29 s vs. 2.3 min in the adults (a 4.6-fold difference). The difference between the amounts of night NREM sleep in adults and ostriches was unsubstantial (62.2% vs. 69.3% of TRT). In contrast, all prior studies have shown maximal REM sleep in newborn humans and in a wide range of the youngest mammals, decreasing considerably with age [12, 15–17, 30]. While the developmental

data are not abundant, similar age-related mammalian-like declines in REM sleep have been reported in birds [22]. Thus, in all studied animals the total amount of REM sleep and duration of REM sleep episodes either decreased or showed no signs of change, but no increases were reported across ontogeny. What can explain the huge differences in the amount of REM sleep and the duration of REM sleep episodes between the chicks and adult ostriches?

### Recording conditions

The report of REM amounts in adult ostriches was obtained in a group of freely moving birds housed in a large, outdoor enclosure. Our study in ostrich chicks was conducted in laboratory conditions using conventional cable recording techniques. Therefore, we should first consider whether the differences in the recording conditions and data analysis between our and Lesku et al. [1] studies might account for the remarkable difference in the amount of REM sleep and duration of episodes between adults and chicks.

In our study, the ostrich chicks were recorded in experimental chambers located in a laboratory. Their movement distances were restricted when compared with the adult ostriches [1]. At the same time, all our chicks were adapted to the recording chambers, in which they could move and turn around. They ate and gained weight during the entire period of the study. To minimize the impact of isolation, the two chambers were placed together. When inside two chicks often rested side by side touching each other through the wire wall and even disturbing each other as they did while sleeping in the barn. They stayed calm in the recording chambers during the night. It should also be noted that ostriches (chicks and adults) are diurnal. The same appears to be true for other species of paleognaths when in laboratory conditions, in farms and in the wild (e.g. greater rheas and Australian emus) [32, 46]. The sample size in our study and in Lesku et al. study was comparable (5 chicks and 6 adults) with one undisturbed recording period used for analysis in each study. We also used the last of three recording nights in a series for the data analysis to eliminate the possibility of a “first night effect.”

A few studies have shown that laboratory conditions do impact some parameters of sleep in animals. Two studies indicated that animals (sloths and elephants) spent less time asleep while in the wild (including REM sleep) compared to captive/laboratory conditions [48, 49]. Humans in natural conditions sleep less than humans in industrial societies [50]. Thus, while the recording conditions could affect sleep parameters in ostrich chicks when compared to their sleep in a group, it seems unlikely the amount of nighttime sleep and REM sleep in ostrich chicks was greatly reduced due to our conditions.

It is well known that sleep amount is affected by the environmental temperature. It is maximal in the animal's thermoneutral zone and it decreases above and below the zone [51–53]. Though REM sleep is more sensitive to ambient temperature changes, SWS is also decreased at low temperature. In the rat, the amount of REM sleep was maximal at 29°C and doubled the amount recorded at 23°C [52].

Ratites are capable of maintaining constant body temperature across a substantial range of air temperatures, including ostriches from 14 to 52°C, emus from –5 to 45°C and kiwis from

5 to 30°C [54, 55]. This makes them competent homeotherms over the range of environmental temperatures they usually encounter in their natural habitat. The thermoneutral zone has been established for the emu and kiwi, and it extends to 10°C at the low edge and 35°C at the high edge [56]. Cloacal and deep body temperatures of adult ostriches are typically about 39°C and range from 37.9 to 43.3°C which is lower than in many other birds [57]. Body temperatures in ostrich chicks at 2–15 days post-hatching measured at a variety of ambient temperatures (ranged from 13° to 36°C) were  $38.9 \pm 0.2^\circ\text{C}$  (ranged from 36.1 to 41.9°C) as in adults [58]. This indicates that ostrich chicks are efficient at thermoregulation from an early age. When air temperature exceeded 30°C, the chicks showed typical cooling behavior, such as raising or lowering the wings away from the body to expose thinly feathered areas on their sides, shade seeking, and panting. When exposed to lower temperatures, the chicks shivered, huddled with other chicks, or lay down [32, 58]. During our recording, air temperature in the experimental room ranged from 25 to 27°C. At these temperatures, we did not see any evidence of shivering in ostrich chicks, which would indicate a response to cool environment or panting and raising their wings due to overheating [32, 58]. At the same time, we did observe cooling behavior as described above in chicks while outdoor at air temperatures above 30°C.

Two ostriches in the neighboring recording chambers were indeed seen to lay down occasionally touching each other through the metallic net. However, they also slept comparable amounts of time staying (sitting or lying) at a distance from each other without physical contact. Thus, both thermoregulatory huddling as an effective means of retaining heat and filial huddling as a social aggregation phenomenon were not displayed by all chicks under the conditions of our study. It is also reasonable to suggest that if temperature were to critically impact sleep in ostrich chicks, the effect should be greater in younger and smaller individuals. However, there was a significant negative correlation between the age of chicks and maximal duration of REM sleep episode as shown in [Supplementary Table S4](#). The total amount of REM also showed a decreasing trend with the ostrich chick age. In addition as reported by Lesku et al., air temperature during recording in adult ostriches in the natural conditions was on average 15°C. It was described as unexceptional for that place and season [1]. This temperature was almost half that during recording in our ostrich chicks. Under the conditions of recording in adult ostriches the environmental temperature was also much closer to the lower edge of the species thermoneutral zone than during recording in the chicks. Therefore, if the air temperature had a critical impact on the parameters of REM sleep leading to a reduction of the amount and duration of episodes of REM sleep in both (the Lesku et al. and our) studies, then the impact on ostrich chicks could not be greater than on adults. This would also imply that in the natural environment ostrich chicks have even less REM sleep since air temperature at night appears to be lower than it was in our study. In the end, we cannot fully exclude the impact of temperature conditions on REM sleep parameters in ostrich chicks we report in our study. This may be a subject of future studies. However, the temperature alone cannot be held responsible for such a remarkable difference between the duration of episodes and amounts of REM sleep in ostrich chicks and adults.

## Video monitoring and scoring criteria

An important difference between the recording conditions in our study of sleep in the ostrich chicks and the study in adult ostriches is that we continuously video recorded the behavior of chicks. We used remote control infrared cameras with a zoom lens to obtain close-up footage of the bird's head during sleep. While this did not always allow for the video recording of the state of both eyes, we were able to record videos of the head with at least one eye visible almost all the time. In the study of sleep in adult ostriches, video recording was conducted only at the first stage when the birds were in a smaller enclosure [1]. The reported data on sleep in adult ostriches were processed based on polygrams and 3D-movement accelerometry.

The closure of both eyes is linked to REM sleep in adult ostriches [1], as well as in other bird species [14]. In ostrich chicks during NREM sleep, both eyes were often closed, but sometimes one eye (or both eyes) was open, as we report. In this case, they always closed the eyes before the start of REMs, sometimes just a few seconds before REMs. Generally, EOG allowed us to identify REMs in the ostrich chicks. However, the amplitude and pattern of EOG activity did not unambiguously correspond to the direction, amplitude and speed of eye movement, or to the state of the eye. In addition, during episodes of REM sleep in a sitting position, head jerks and drops could lead to artifacts in EMG and EOG. Therefore, in our study, all episodes scored as REM sleep were also visually inspected for the presence of REMs and eye closure, allowing to identify REM sleep and exclude periods where episodes of QW might have been scored as REM sleep.

During episodes of REM sleep, the intensity and frequency of REMs in ostrich chicks varied. In longer episodes, this may look like the alternating of instances of phasic and tonic periods as described in the cat [59]. In chicks, the episodes without REMs within an episode of REM sleep usually lasted only seconds. If a period without obvious features of REM sleep (such as REMs and head jerks with both eye closed) lasted 4 s or longer, then this 4 s epoch no longer met the formal criteria of REM sleep. Such epochs were scored based on the pattern of the EEG, either QW or NREM sleep, or in rare cases as unidentified ([Supplementary Figure S11](#)). However, we cannot fully exclude that such epochs were epochs of tonic REM sleep. When we rescored REM sleep by allowing one 4-s interruption without breaking the continuity of the episode, we found that the maximum duration of REM sleep increased from 40 to 52 s (a 30% increase), the mean maximum duration increased from 34 to 40 s (a 18% increase). At the same time, the mean total amount of REM sleep changed very little (14.1% vs. 14.5% of TRT, respectively; a 3% only). Thus, the length of epochs affects primarily duration of REM sleep episodes and to a lesser extent the total amount of REM sleep. Therefore, if we adjust the criteria allowing 4-s interruption of the phasic events, which is obviously would then be a different method than that used to study and characterize sleep in adult ostriches [1], the longest recorded episode of REM sleep in the ostrich chicks would be still 1/6 of that recorded in the adults. We can assume that the above differences in experimental conditions could affect the estimate of REM sleep parameters in adult ostriches and chicks, primarily the maximum duration of REM sleep. At the same time, this still cannot explain the large differences between chicks and adult ostriches in the parameters of REM sleep.

## REM sleep in different postures

One may expect that repeated head jerks and drops should cause disruption to the REM sleep in short episodes if during sleep the head is held above the ground as most birds do. However, we have not found striking differences between the duration of REM sleep episodes that occurred with the head rested on the ground and the head held above the ground in the ostrich chicks. While the longest episodes of REM sleep were recorded in the positions with the head rested on the ground, the overall number and proportion of such episodes were very small. More important, in both lying positions REM sleep episode was 10 s on average.

It has been recently discovered that the tinamou, another Palaeognathae bird species, does not display an ostrich-like mixed REM sleep state [14]. The tinamou is diurnal as the ostrich. The duration of REM sleep episodes (on average 6 s) and the total amount of REM sleep (11% of the night) in the tinamou was lower than in adult ostriches [1] and comparable to values for Neognathae bird species [4–6]. As the authors note, the absence of an ostrich-like mixed sleep state in the tinamou does not support the hypothesis that this type of sleep is characteristic of all Palaeognathae birds. It has been proposed then that a fully integrated brainstem and forebrain REM sleep state is not compatible with the posture of sleep in adult ostriches, which is sleeping with long neck held fully extended and perpendicular to the ground [14]. However, we have found that the ostrich chicks displayed REM sleep with EEG slow waves (the mixed sleep) state both while sitting with the head held above the ground (as adult ostriches) and in a lying position with the neck stretched out and resting on the ground. Thus, our data do not support the hypothesis that sleeping with the head held up prevents ostriches from having cortical activation during REM sleep. The adult and chick ostriches have a mixed sleep state resembling that seen in the platypus. This suggests, as Lesku and colleagues noted [1], that some portion of Palaeognathae birds, for reasons not yet understood, have sleep similar to that of the monotreme mammals [2, 3] whereas others have evolved a sleep pattern more similar to that of placental mammals.

## Long episodes of REM in adult ostriches and risk of predation

Mammalian species sleeping in riskier environments display less REM sleep [19, 39, 60]. While no significant relationship was found between the amounts of REM sleep and sleep exposure in birds [6], there are examples indicating that birds also adjust their sleep pattern depending on the environmental circumstances. Thus, in the pigeon, the time spent in REM sleep and intensity of SWA declined when birds slept on low-perches where birds were more vulnerable to predation from ground mammals compared to when they slept on elevated perches [61]. The survival of ostriches certainly depends on early detections of predators. They sleep with both eyes open during NREM sleep but they close both eyes in REM sleep. Staying in REM sleep with both eyes closed would decrease their ability to respond to predators and decrease their chances of survival. It is not clear how adult ostrich would benefit from having long episodes of REM sleep (up to 5 min) as reported.

## Concluding Remarks

The smaller amount of REM sleep and shorter duration of REM sleep in the ostrich chicks when compared to adult ostriches may be a feature of ostrich sleep ontogenesis. If this is correct, then it would indicate a unique profile of the sleep ontogenesis in the ostrich—different from what has so far been observed in all studied mammals and birds. While the differences in experimental conditions (primarily ambient temperature and separation from other individuals) and sleep scoring could affect the estimate of the maximum duration of REM sleep episodes and the total amount of REM sleep in ostrich chicks and adult birds, this still cannot explain the large differences between the parameters of REM sleep among them. Future studies may provide an insight into the evolutionary and ecological determinants of the developmental change and the of role experimental conditions in REM sleep pattern in the ostrich.

## Supplementary material

Supplementary material is available at *SLEEP* online.

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## Author contributions

O.I.L. and J.M.S. designed research and wrote the article. O.I.L. and A.S.K. performed research. O.I.L. analyzed data.

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