Supplementary Material

Sleep in ostrich chicks (Struthio camelus)

Oleg I. Lyamin [a,b]*, Anton S. Kibalnikov [c] and Jerome M. Siegel [a]*

[a]Department of Psychiatry, University of California Los Angeles, Los Angeles, CA, Greater Los Angeles Healthcare System, North Hills, United States of America; and [b]A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia; and [c] Votvete Veterinary Center, Rostov-on-Don, Russia

*Corresponding authors: Oleg Lyamin, Center for Sleep Research, 16111 Plummer St, North Hills, CA 92343, USA. Email: olyamin@ucla.edu; and Jerome Siegel, Center for Sleep Research, 16111 Plummer St, North Hills, CA 92343, USA. Email: jsiegel@ucla.edu

Table S1. Behavior of ostrich chicks during daytime in an outdoor enclosure as percent of the total recording time (TRT).

Ostrich	3	4	5		
Age (weeks)	2	3	3	Mean	S.E.M.
Walking	90.9	92.2	92.2	91.8	0.4
Eating	6.1	7.8	7.8	7.2	0.6
Standing, sitting, lying	3.0	0.0	0.0	1.0	1.0
Total	100.0	100.0	100.0		
Ostrich	3	4	5		
Age (weeks)	2	3	3	Mean	S.E.M.
Walking	85.9	88.8	90.9	88.5	1.4
Eating	8.8	8.8	8.8	8.8	0.0
Standing, sitting, lying	5.3	2.4	0.3	2.7	1.4
Total	100.0	100.0	100.0		
Ostrich	3	4	5		
Age (weeks)	6	7	7	Mean	S.E.M.
Walking	93.3	93.0	94.1	93.5	0.3
Eating	4.8	4.8	4.7	4.8	0.1
Standing, sitting, lying	1.9	2.2	1.2	1.7	0.3
Total	100.0	100.0	100.0		

The table presents the data collected during 3 daytime periods in 3 ostrich chicks (3-5) when they were 2-3 and 6-7 weeks old (2 and 1 recording periods, respectively). Usually ostriches stayed outdoors between 08:00 and 19:00 (the first and the last recordings). However, during some days the outdoor periods were shorter due to the weather conditions (the second recording, 08:00-17:00). The behavior was scored in 1-min epochs. The minute was assigned to the behavior which occupied more than 30 seconds of each minute.

Video recordings in the outdoor enclosure showed that the majority of the time (94.6-100% of TRT) the chicks were walking along the enclosure and pecking the ground (“walking”) or eating food from the food trays (“eating”). The remaining time they spent while in the standing, sitting or lying positions (0.0 – 5.3%. of TRT). Those episodes were brief. They had features of active behaviors such monitoring the environment, preening, pecking the ground or “dust bathing”.

Table S2. The amounts of different behaviors, NREM sleep, REM sleep and QW. The duration of behaviors is presented as percent of TRT. The duration of sleep and QW is presented as percent of the total amount of the corresponding state over the entire recording period. The data are means ± SEM. R L/R is a ratio of the amount of times spent in the lying / sitting position during the recording period or the amounts of NREM sleep, REM sleep and QW recorded in the lying / sitting position.

Amounts of different behaviors (% of TRT)					
	Ostrich	walking	standing	lying	sitting
	1	0.8	4.0	59.5	35.7
	2	0.1	0.4	94.4	5.1
	3	0.1	3.6	65.3	31.0
	4	2.5	1.9	88.7	6.9
	5	1.2	1.9	81.7	15.2
	Mean	0.9	2.4	77.9	18.8
	S.E.M.	0.4	0.7	6.7	6.2
	R L/S			4.1	1
NREM sleep (% of total amount of NREM sleep)					
	Ostrich	walking	standing	lying	sitting
	1	0.0%	0.6%	65.3%	34.1%
	2	0.0%	0.0%	97.7%	2.3%
	3	0.0%	0.8%	73.9%	25.3%
	4	0.0%	0.2%	93.0%	6.8%
	5	0.0%	0.2%	87.3%	12.5%
	Mean	0.0%	0.4%	83.4%	16.2%
	S.E.M.	0.0%	0.1%	6.0%	5.9%
	R L/S			5.2	1
REM sleep (% of total amount of REM sleep)					
	Ostrich	walking	standing	lying	sitting
	1	0.0%	0.0%	74.6%	25.4%
	2	0.0%	0.0%	99.4%	0.6%
	3	0.0%	0.0%	89.1%	10.9%
	4	0.0%	0.0%	99.4%	0.6%
	5	0.0%	0.0%	94.8%	5.2%
	Mean	0.0%	0.0%	91.5%	8.5%
	S.E.M.	0.0%	0.0%	4.6%	4.6%
	R L/S			10.7	1
QW (% of total amount of QW)					
		walking	standing	lying	sitting
	1	0.0%	7.2%	32.8%	60.0%
	2	0.0%	1.6%	71.9%	26.5%
	3	0.0%	7.5%	22.0%	70.5%

	4	0.0%	11.0%	72.8%	16.2%
	5	0.0%	10.8%	49.8%	39.4%
	Mean	0.0%	7.6%	49.9%	42.5%
	S.E.M.	0.0%	1.7%	10.2%	10.1%
	R L/S			1.2	1

Table S3. Characteristics of breathing rate during NREM and REM sleep in two ostrich chicks sleeping while lying with their heads resting on the ground.

Ostrich	1		2	
Breathing pause (sec)	NREM	REM	NREM	REM
Mean	7.2	7.2	7.8	7.5
SEM	0.09	0.16	0.08	0.15
N	86	29	105	35
Minimum	5.6	5.8	6.9	5.3
Maximum	10.2	9.4	9.0	10.5
Dispersion (S2)	0.70	0.78	0.62	0.79
F statistic (S2 NREM/ S2 REM)		1.114		1.273
		(p>0.05)		(p>0.05)

The data presented in Table S3 were collected in ostriches 1 and 2 between 06:00 and 07:00 while both birds were in the lying position with their heads on the ground. They included a total of 17 and 21 min of NREM and REM sleep in this posture. F statistic is a ratio of the dispersion (S2) during REM sleep to the dispersion during REM sleep. P is the level of significance of the differences.

Table S4. Pearson correlation between NREM and REM sleep parameters and age in ostrich chicks.

		Amount of sleep (% TST)		Episodes of NREM sleep			Episodes of NREM sleep		
Ostrich	Age (weeks)	SWS (%TST)	REM sleep (%TST)	Mean duration (sec)	Number (per night)	Maximal duration (sec)	Mean duration (sec)	Number (per night)	Maximal duration (sec)
1	9	71.8%	10.6%	32.0	926	380	9.8	450	36
2	9	71.0%	16.9%	30.7	1001	180	10.0	730	28
3	14	71.5%	9.3%	32.9	923	240	8.5	469	24
4	6	68.2%	15.0%	30.2	966	136	11.0	586	40
5	5	63.9%	18.5%	21.0	1300	172	9.0	884	40
	R_{xy}	0.757	-0.775	0.718	-0.637	0.368	-0.533	-0.636	-0.925
	R^2	0.573	0.601	0.516	0.406	0.135	0.284	0.404	0.856
	P	0.139	0.124	0.172	0.248	0.542	0.355	0.249	* 0.024

The table presents the data on correlation between the listed parameters of NREM and REM sleep with the age of the 5 studied ostrich chicks. Rxy – Pearson coefficient of correlation. R2 – square of Rxy. P – significance of the tested correlation between the sleep parameter and age.* -$p<0.05$.

Figures

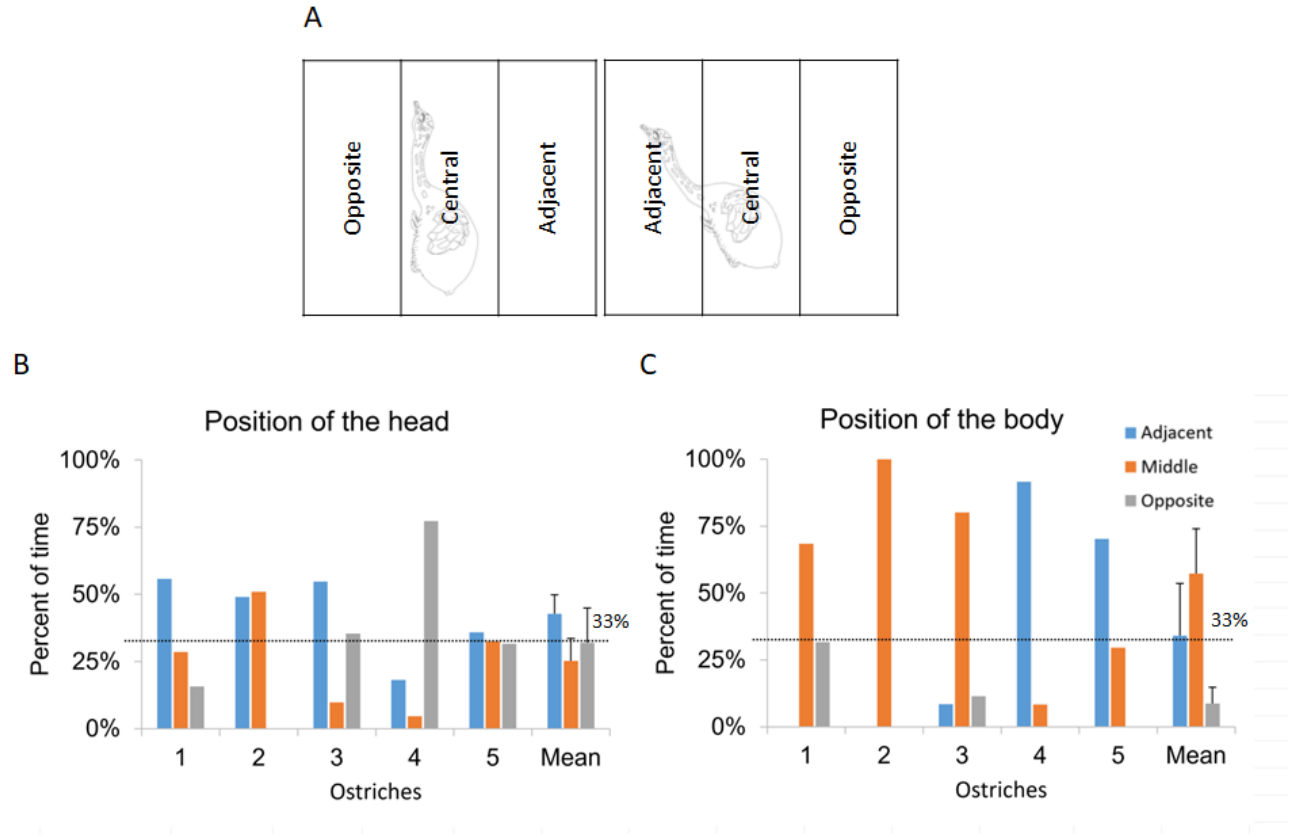

Figure S1. Position of the recorded ostrich (in the left chamber) relative to the adjacent chamber with the second companion ostrich. A. Schematic drawing of 2 recording chambers and 3 zones in the chambers used to characterize the positions of the ostriches during recording. B and C. The amount of time the recorded ostriches spent with different positions of the head and body during the recording nights presented as percent of recording time. The graphs also present the overall mean ±S.E.M. The dotted line is drawn to mark the 33% value (the probability for one out of 3 randomly occurring events).

For each of the 4-sec epochs when the recorded ostrich was sitting or lying, the position of the head and the body (the caudal part) were coded in relation to the 2nd chamber as “adjacent”, “central” or “opposite”. Thus, in the left ostrich both the position of the head and body is “central”. In the right ostrich the position of the head is “adjacent” while the position of the body is “central”. The total number of epochs used to characterize the position of the ostriches ranged from 8797 (in ostrich 1) to 10741 (in ostrich 2). The position of the 2nd (companion) ostrich was not analyzed.

As follows from figure B, during recording ostriches positioned the head in different zones of the chamber. While two ostriches (O1 and O3) appeared to prefer directing the head toward the 2nd ostrich (the head was in the “adjacent” zone), the 3rd ostrich (O4) primarily directed its head away from the neighboring bird (the head was in the “opposite” zone). The 4th ostrich (O2) appeared to avoid the “opposite” zone while the 5th (O5) did not show a preference for a zone. As follows from figure C, only 2 out of 5 ostriches (O4 and O5) appeared to prefer positioning the body in the

proximity to the other chick (in the “adjacent” zone) while the 3 other birds stayed largely in the middle part of the chamber (in the “center” zone).

For the entire group of ostriches, the difference between the amounts of time spent with different positions of the head was not significant (repeated measures ANOVA, factor “zone”, $F_{4,2}=0.537$, $p=0.604$). The same was true for the position of the body ($F_{4,2}=1.693$, $p=0.244$).

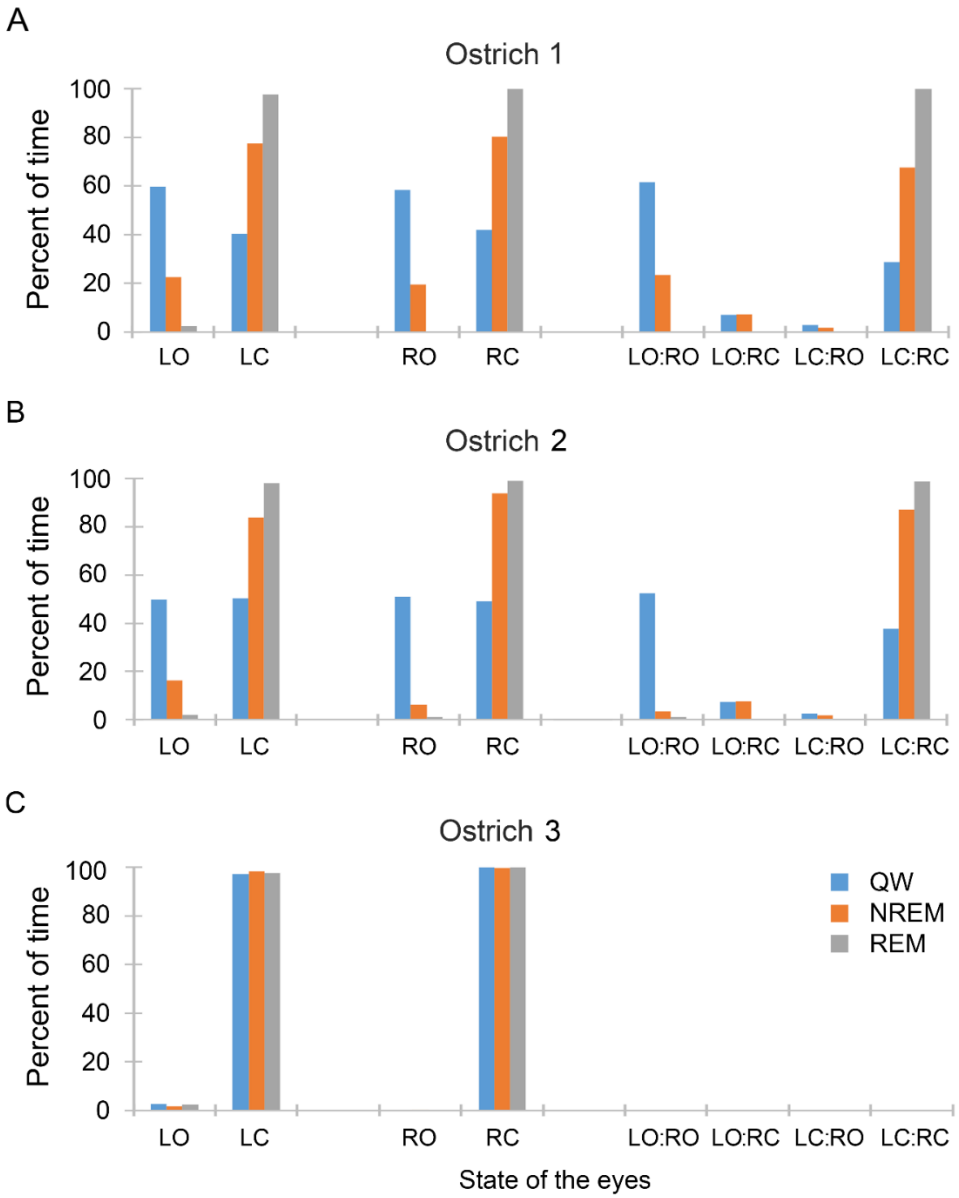

Figure S2. State of the eyes in ostrich chicks during QW, NREM and REM sleep. The state of the left (L), right (R) or both eyes (L:R) was scored as open (O) or closed (C) during QW, NREM and REM sleep. It is presented as the percent of the time each eye or both eyes were visible during the particular state. Three ostriches are marked as O1, O2 and O3.

In O1 the state of the left eye was determined during the epochs which included 15 min of QW, 112 min of NREM sleep and 16 min of REM sleep and the state of the right eye for the epochs which included 16 min of QW, 85 min of NREM sleep and 8 min of REM sleep. The state of both eyes was determined during 10 min of QW, 47 min of NREM sleep and 5 min of REM sleep.

In O2 the state of the left eye was determined during the epochs which included 53 min of QW, 434 min of NREM sleep and 108 min of REM sleep and the state of the right eye for the epochs which included 40 min of QW, 218 min of NREM sleep and 44 min of REM sleep. The state of both eyes was determined during 29 min of QW, 171 min of NREM sleep and 36 min of REM sleep.

In O3 the state of the left eye was determined during the epochs which included 7 min of QW, 88 min of NREM sleep and 17 min of REM sleep and the state of the right eye for the epochs which included 24 min of QW, 62 min of NREM sleep and 7 min of REM sleep. The state of both eyes was determined only during < 6 min of NREM sleep and < 1 min of REM sleep. These data were not included in the figure.

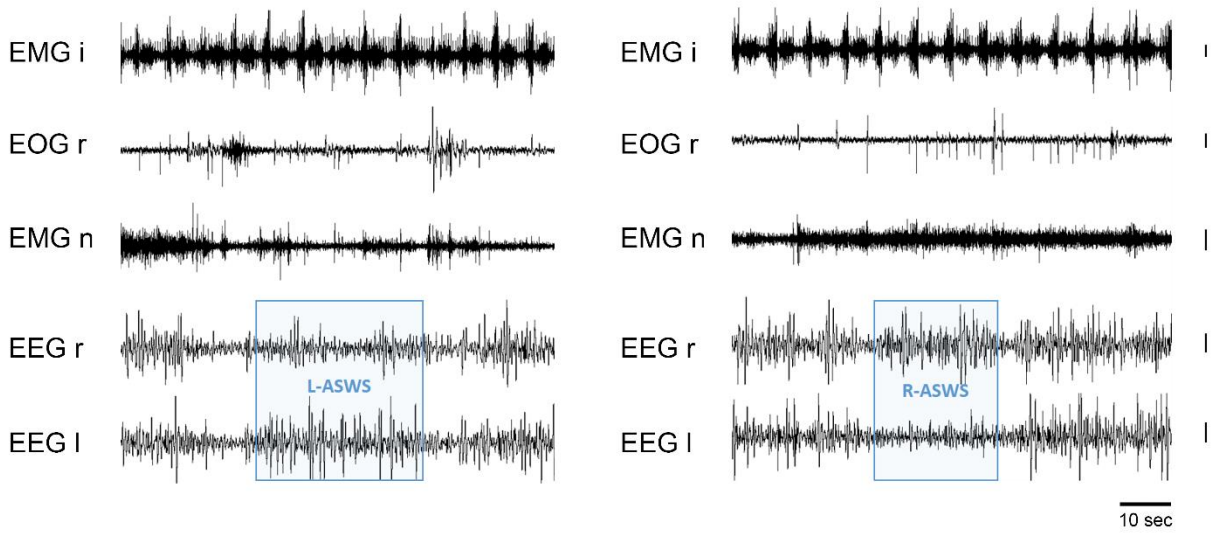

Figure S3. Two episodes of slow wave asymmetry in the EEG between the right and left cortical hemispheres in an ostrich chick. EEG, electroencephalogram of the left (l) and right (r) cortical hemispheres. EMG n, electromyogram of the neck muscles. EMG i, EMG of intercostal muscles. EOG, electrooculogram of the right eye. The duration of both records is 90 sec. The left polygram shows a 35-sec episode of EEG asymmetry with higher voltage slow waves in the left hemisphere compared to the right hemisphere (high voltage left asymmetrical SWS / NREM sleep, L-ASWS). The ostrich lay holding the head above the ground. The right polygram shows a 25-sec episode of EEG asymmetry with a higher voltage slow waves in the right hemisphere (high voltage right asymmetrical NREM sleep, R-ASWS). Only left eye was visible during both recordings. It was open during the episode of L-ASWS and it was closed during the episode of R-ASWS. The ostrich was lying with the head resting on the back (Figure 1E). Vertical bars on the right of the EEG, EOG and EMG traces correspond 100 μV.

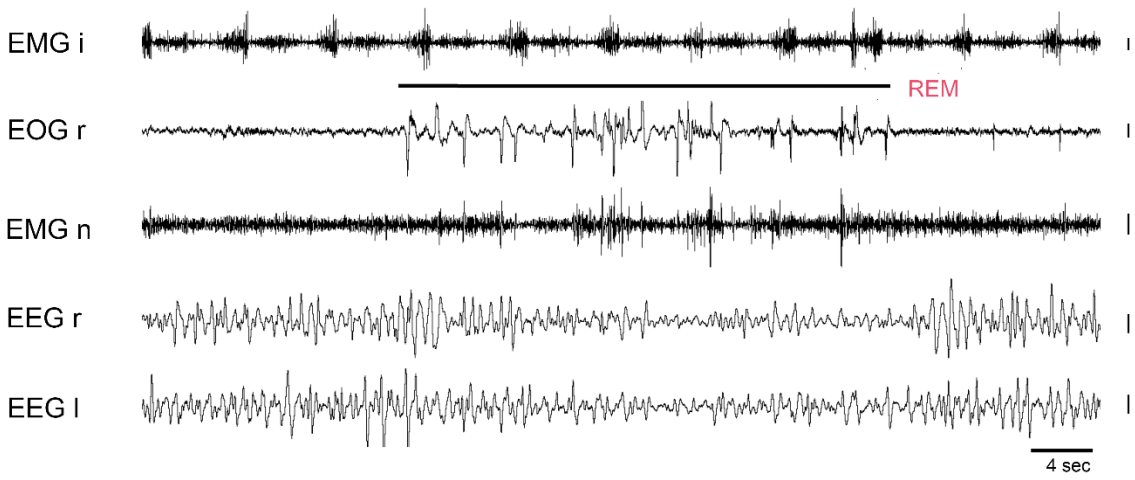

Figure S4. A 30-sec long episode of REM with cortical activation which started to develop 7 sec after the first REMs. The muscle tone was interrupted increasing during intensive head jerks / twitches. The ostrich was lying with the neck and head on the ground.

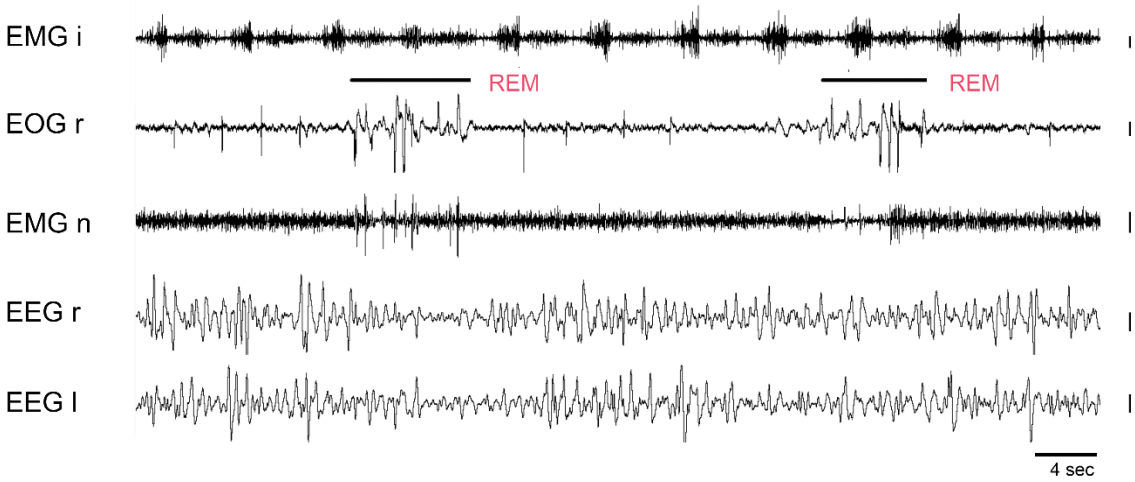

Figure S5. Two episodes of REM sleep (8 and 10 sec) while an ostrich chick was sitting. During the 1st episode of REM sleep the amplitude of EEG of both hemispheres decreased featuring cortical activation. During the 2nd episode EEG remained intermediate voltage as during NREM sleep.

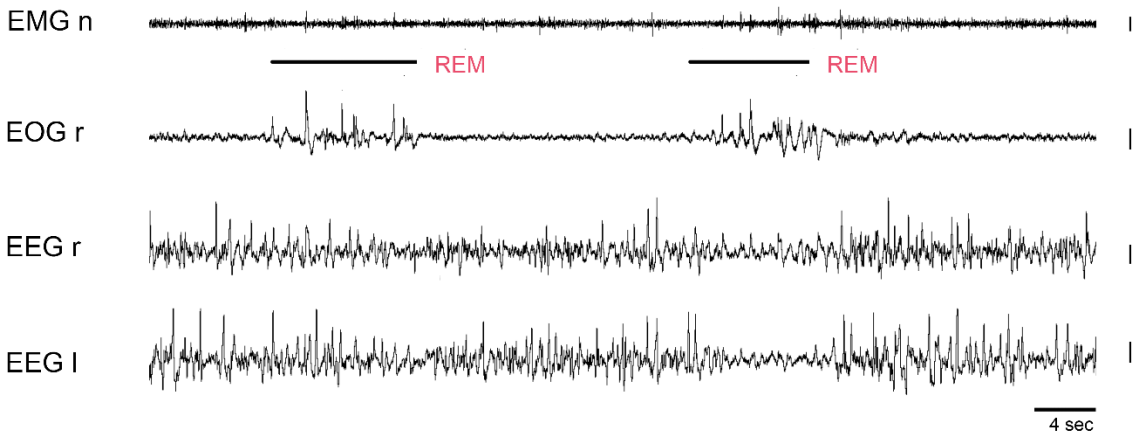

Figure S6. Two episodes of REM sleep (both lasted 12 sec) while an ostrich chick was lying with the head resining on the cage floor. In the 1st episode REMs appeared during EEG slow waves. In the 2nd episode REMs and cortical activation emerged synchronously.

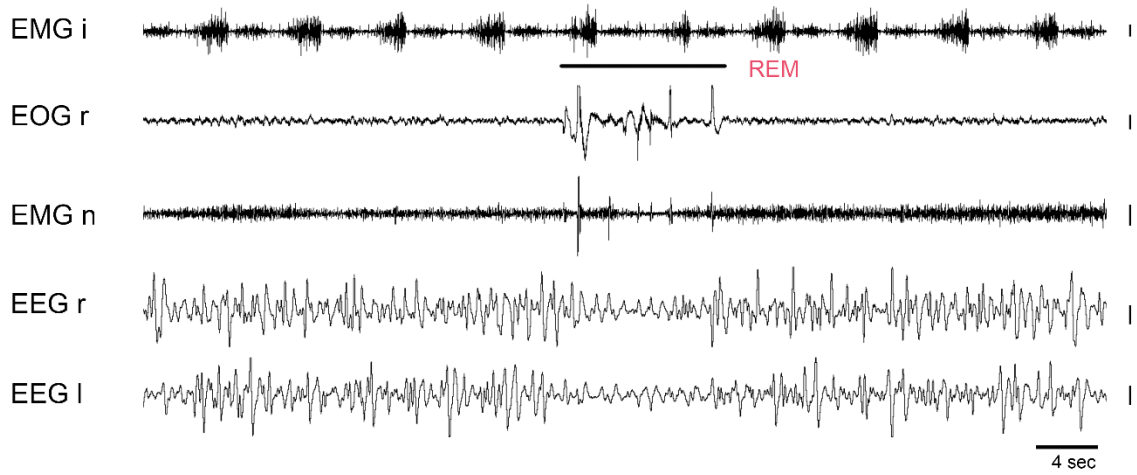

Figure S7 A 10-sec long episode of REM sleep while an ostrich chick was lying with the neck and head on the ground. Cortical activation developed simultaneously with the first REMs. The muscle tone was interrupted and slightly decreased. Head jerks are reflected in the EMG.

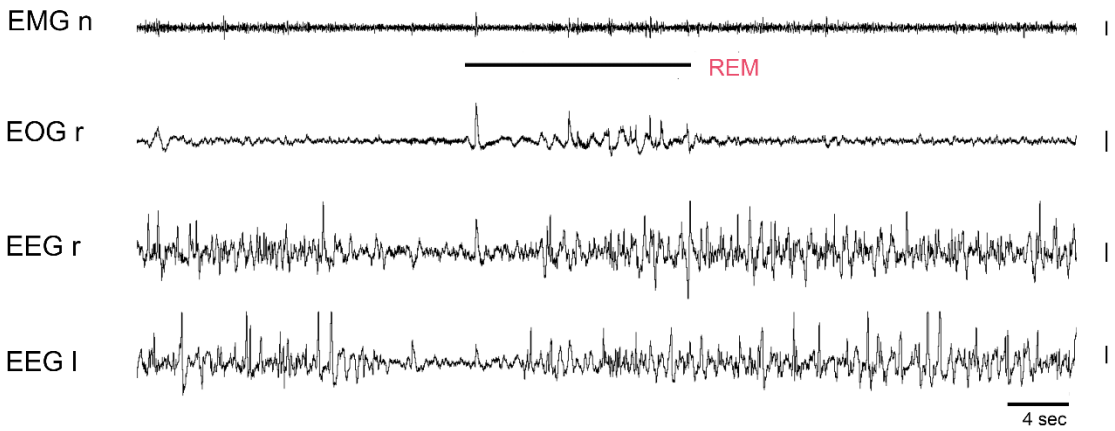

Figure S8. An episodes of REMs (16 sec long) during EEG slow waves. Cortical activation appeared 8 seconds before REMs. The ostrich was lying with the neck and head on the ground.

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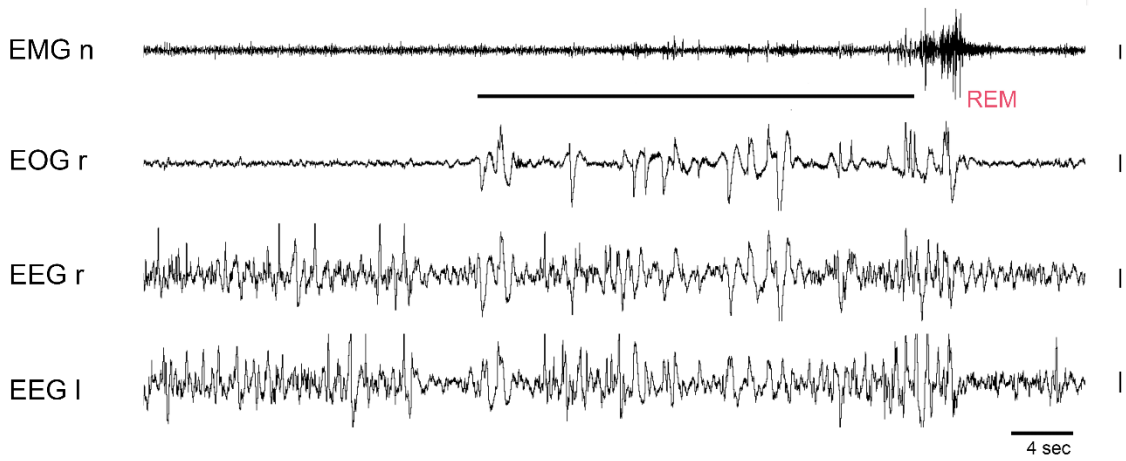

Figure S9. An episodes of REM sleep (30 sec long) occurred during EEG slow waves and was ended by arousal. The ostrich was lying with the neck and head on the ground.

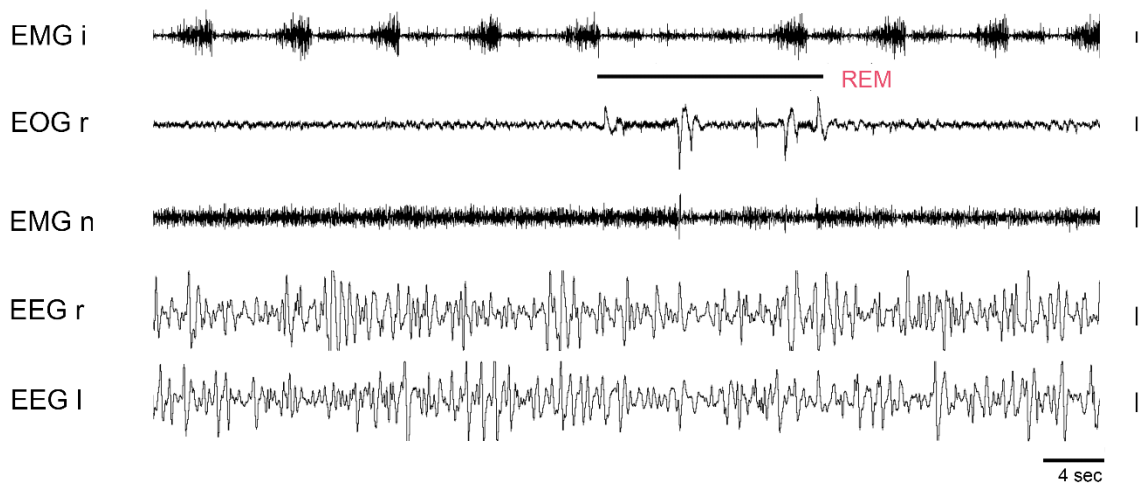

Figure S10. A 14-sec long episode of REM sleep with SWA. The amplitude of EEG slow waves was modulated during the episode (decreased after the first REMs and then increased). The muscle tone decreased 5 sec after the first REMs. This is one of the rare cases when the breathing pause during REM sleep was substantially longer than during NREM sleep. The ostrich was lying with the neck and head on the ground.

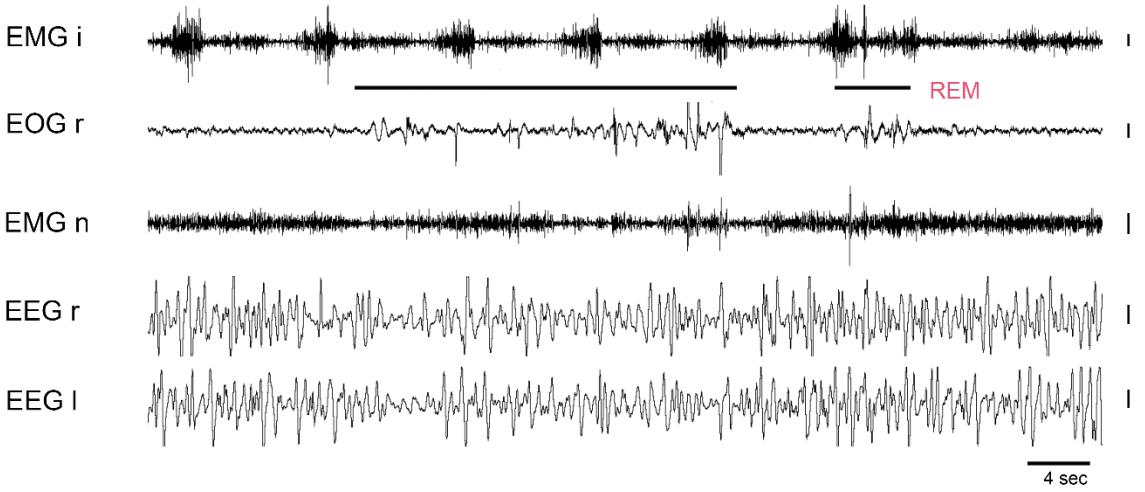

Figure S11. Two episodes of REM interrupted by a 7-sec episode of NREM sleep. During the 1st episode of REM sleep (24 sec long) the amplitude of EEG ranged between low voltage cortical activation and high voltage slow waves. The 1st episode was interrupted by short periods of SWA without REMs (scored as NREM sleep). During the 2nd episode of REM sleep SWA was as during high voltage NREM sleep. Both episodes were marked by REMs. The ostrich was lying with the neck and head on the ground.