

Sleep in the lesser mouse-deer (*Tragulus kanchil*)

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Supplementary Material

Tables

Table S1. The amounts of REM sleep in the mouse-deer in consecutive recording days shown as percent of 24-h.

	Mouse-deer				Mean±SEM (n=4)
Scoring and days	1	2	3	4	
Preliminary scoring of REM sleep in days 1-4					
Day 1	0.3	0.6	0.5	0.5	0.5±0.1
Day 2	1.2	0.9	1.1	1.2	1.1±0.1
Day 3	1.1	1.9	1.4	1.3	1.4±0.2
Day 4	1.0	-	-	-	-
Final scoring of REM sleep in day 3					
Day 3	1.2	2.4	1.3	1.8	1.7±0.3

The Table presents the results of preliminary scoring of REM sleep during all recording days in 1-min epoch and the results of final scoring during the 3rd day in 20-sec epochs in each mouse-deer. An epoch was scored as REM sleep if it met the criteria for this stage (described in the main text) during at least 50% of each minute (preliminary scoring) or 20-sec epoch (final scoring).

Table S2. State of the eyes during quiet wakefulness and NREM sleep in the lesser mouse-deer.

	Mouse-deer				Mean±SEM, (n=4)
Parameter	1	2	3	4	
NREM sleep					
Number of 20-sec epochs of NREM sleep with					
one or both eyes are open or partly open	638	1785	761	1005	
one or both eyes are closed	17	4	3	0	
Sum	655	1789	764	1005	
Total number of NREM sleep epochs	2298	2434	1882	1985	
The amount of NREM sleep with (percent of time at least one eye was visible)					
one or both eyes are open or partly open	97.4	99.8	99.6	100.0	99.2±0.6
one or both eyes are closed	2.6	0.2	0.4	0.0	0.8±0.6
Sum	100.0	100.0	100.0	100.0	
The amount of time at least one eye was visible (percent of NREM sleep)	28.5	73.5	40.6	50.6	48.3±9.5
Quiet wakefulness					
Number of epochs of QW with					
one or both eyes open or partly open	111	593	441	248	
one or both eyes are closed	0	4	2	0	
Sum	111	597	443	248	
Total number of QW epochs	813	1201	1355	1488	
The amount of QW sleep with (percent of time at least one eye was visible)					
one or both eyes open or partly open	100.0	99.3	99.5	100.0	99.7±0.2
one or both eyes are closed	0.0	0.7	0.5	0.0	0.3±0.2
Sum	100	100	100	100	
The amount of time at least one eye was visible (percent of QW)	13.7	49.7	32.7	16.7	28.2±8.3

During REM sleep the eyes could be closed or open. The eye lids often closed and opened in parallel with REMs, and it was difficult to characterizes the state of eyes as open or closed for the majority of time. During REM sleep the eyes were also visible in a smaller number of epochs.

Table S3. Duration and number of NREM and REM sleep episodes in the lesser mouse-deer.

Parameter	Mouse-deer				Mean±SEM (n=4)
	1	2	3	4	
Episodes of NREM sleep					
Mean duration (min)	3.2±0.3	1.9±0.1	1.8±0.1	2.0±0.1	2.2±0.3
Maximal duration (min)	27.7	19.0	12.7	16.0	18.8±3.2
Number (per 24-h)	235	425	344	334	334.5±38.9
Percent of episodes which lasted					
20 sec	23.0	27.1	25.9	22.5	24.6±1.1
≤1 min	39.1	58.6	55.8	50.0	50.9±4.3
≤5 min	79.1	91.1	92.7	93.1	89.0±3.3
>5 min	20.9	8.9	7.3	6.9	11.0±3.3
Episodes of REM sleep					
Mean duration (min)	1.3±0.2	2.2±0.5	2.1±0.5	2.4±0.6	2.0±0.2
Maximal duration (min)	2.7	8.0	4.3	5.3	5.1±1.1
Number (per 24-h)	13	16	9	11	12.3±1.5
Percent of episodes					
20 sec	7.7	6.3	11.1	9.1	8.5±1.0
≤1 min	53.8	37.5	44.4	45.5	45.3±3.3
≤3 min	100.0	75.0	66.7	63.6	76.3±8.2
>3 min	0.0	25.0	33.3	36.4	23.7±8.2

Table S4. Amount of time spent in different sleep and wake states, and in the house and enclosure during daytime (06-18) and nighttime (18-06) in the lesser mouse-deer.

Parameter	Mouse-deer										P
	1		2		3		4		Mean \pm SEM (n=4)		
	Time of day	18-06	06-18	18-06	06-18	18-06	06-18	18-06	06-18	18-06	
Amount of sleep and wake states (of 12-h)											
Active wakefulness	28.8	19.0	17.8	6.5	27.5	13.5	27.1	5.4	25.3 \pm 2.5	11.1 \pm 3.2	0.0125
Quiet wakefulness	20.3	17.5	24.6	31.3	32.5	30.4	42.9	26.9	30.1 \pm 5.0	26.5 \pm 3.2	0.5012
Drowsiness	1.2	4.2	1.1	0.3	3.1	3.0	0.1	0.5	1.4 \pm 0.6	2.0 \pm 1.0	0.5200
NREM sleep	48.4	58.3	53.2	60.3	36.8	50.6	28.6	64.8	41.8 \pm 5.6	58.5 \pm 3.0	0.0862
REM1	0.4	0.6	1.6	0.2	0.0	0.7	0.2	0.8	0.6 \pm 0.4	0.6 \pm 0.1	0.9665
REM2	0.9	0.5	1.6	1.4	0.0	2.0	1.0	1.6	0.9 \pm 0.3	1.4 \pm 0.3	0.4486
REM sleep	1.3	1.0	3.3	1.6	0.0	2.6	1.3	2.4	1.5 \pm 0.7	1.9 \pm 0.4	0.0863
REM/TST	2.6	1.7	5.8	2.6	0.1	4.9	4.2	3.6	3.2 \pm 1.2	3.2 \pm 0.7	0.6547
Rumination (of 12-h)	31.4	24.4	14.8	9.4	19.5	11.0	11.3	13.4	19.2 \pm 4.4	14.5 \pm 3.4	0.1386
Amount of time (of 12-h) spent											
in the house	0.0	5.4	3.5	90.3	0.0	86.4	16.1	83.4	4.9 \pm 3.8	66.3 \pm 20.4	0.0495
outside the house	100.0	94.6	96.5	9.7	100.0	13.6	83.9	16.6	95.1 \pm 3.8	33.7 \pm 20.4	0.0495
Episodes of REM sleep											
Number (per 12-h)	8	5	14	2	1	8	3	8	6.5 \pm 2.9	5.8 \pm 1.4	0.8734
Mean duration (min)	1.2	1.5	1.7	5.8	0.3	2.3	3.0	2.1	1.5 \pm 0.6	2.9 \pm 1.0	0.2917
SEM	0.3	0.2	0.4	2.2		0.5	1.3	0.7	0.6 \pm 0.3	0.9 \pm 0.4	
Minimal duration (min)	0.3	1.0	0.3	3.7	0.3	0.7	0.7	0.3	0.4 \pm 0.1	1.4 \pm 0.8	
Maximal duration (min)	2.7	2.3	6.0	8.0	0.3	4.3	5.0	5.3	3.5 \pm 1.3	5.0 \pm 1.2	0.2187

P - difference between the daytime and nighttime values (the paired T-test; the data passed the test for normality).

Figures

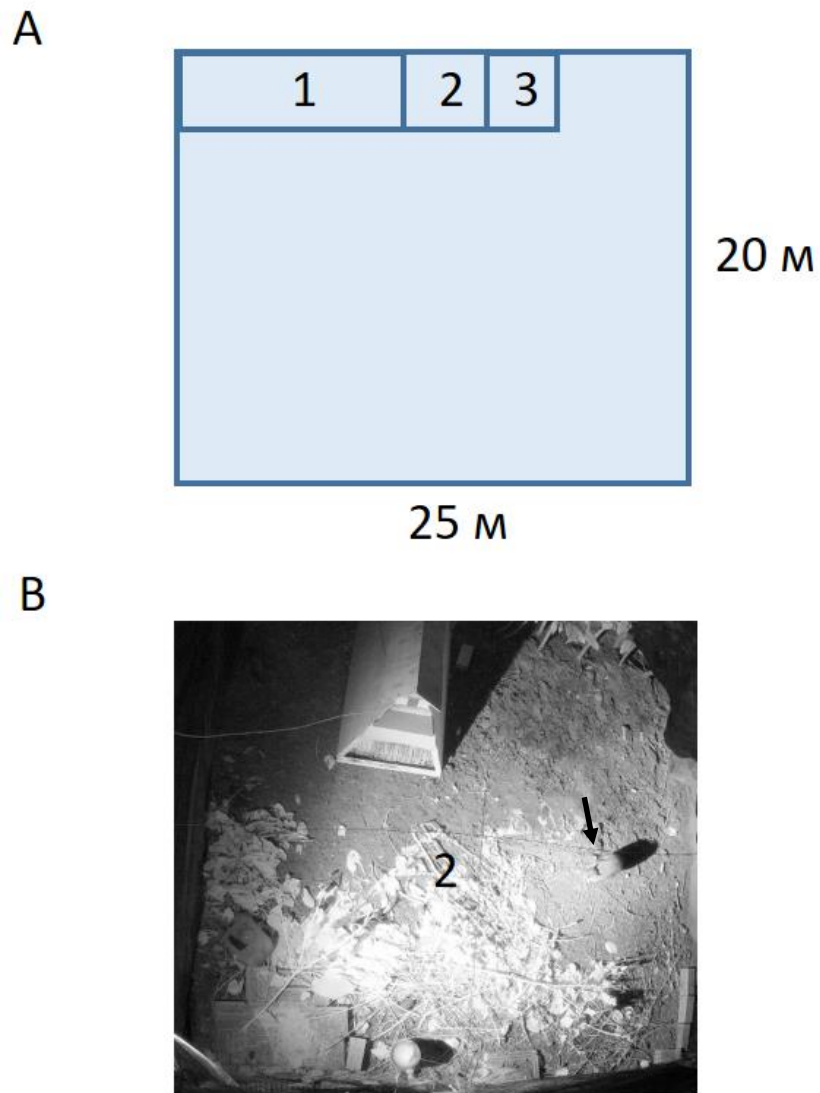


Figure S1. A layout and photo of the experimental area. (A). A layout of 3 enclosures within a large (25 x 20 m) fenced holding area including one with 9 adult females with 2 calves (12x3.5 m, N1), the experimental enclosure for 24-h recording (3.5x 3.5 m, N2) and an enclosure for temporary holding of implanted animals (3x3.5 m, N3). (B). A view of the experimental enclosure at night via a camera installed above the enclosure. A mouse-deer (marked by the arrow) is resting in the open part of the enclosure. The area was lit with IR-illuminators. There was a shelter (house) in the enclosure. The back side of the enclosure had shrubs.

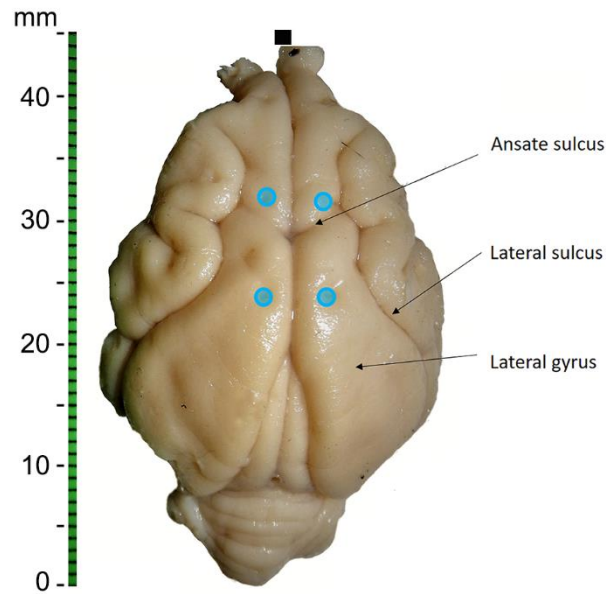


Figure S2. A dorsal view of the brain of the lesser mouse-deer (mouse-deer 1) with a reconstruction of the position of cortical EEG electrodes shown by blue markers. In 3 other animals the frontal electrodes were also positioned anterior to the ansate sulcus and the parietal electrodes were posterior to the ansate sulcus as in mouse-deer 1. In ungulates the somatosensory and motor cortical areas are usually located anterior to the ansate sulcus and the visual cortex is located in the lateral gyrus [25]. The reference electrode was located approximately 14 mm rostral to the ansate sulcus (black rectangular) in the frontal part of the skull above the nasal passages.

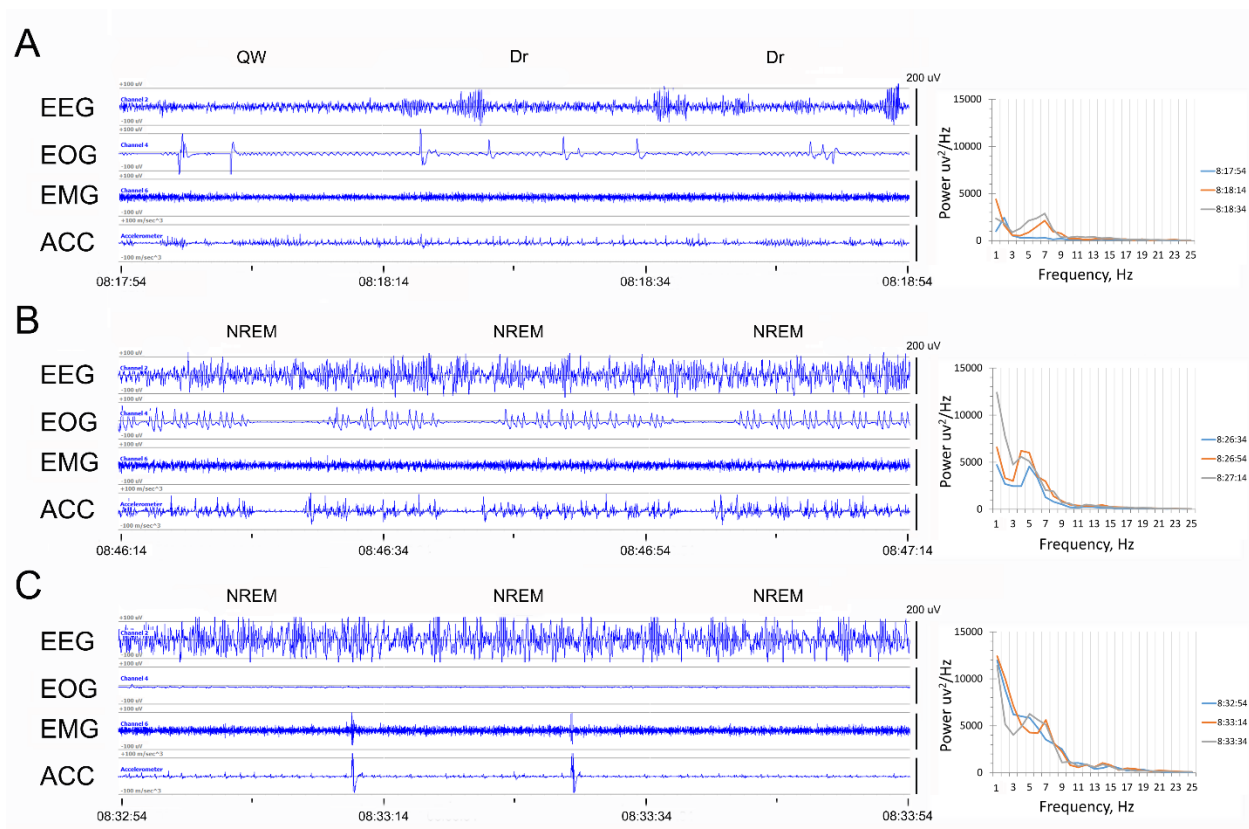


Figure S3. Representative polygrams of QW, Dr and NREM sleep with and without rumination in mouse-deer 3. Duration of each polygram is 60 sec or three 20 sec epochs. EEG, electroencephalogram of the left cortical hemispheres. EOG, electrooculogram of the left eye. EMG, electromyogram of the neck muscles. ACC – total body acceleration. Composition of spectral power in the range of 0.8-25 Hz is calculated for 3 epochs of each polygram. The width of the first bin in the spectral power diagrams is 1.2 Hz (0.8-2.0 Hz) and the width of each remaining bin is 1 Hz. The values below X-axes mark the left margin of each bin interval. (A). One epochs of QW and two epochs of Dr which included bursts of rhythmic activity in the EEG in the range of 6-8 Hz. (B). Three epochs of NREM sleep with rumination characterized by rhythmic movement related artifacts in the ACC and EOG (2-3 per sec) signals. (C). Three epochs of NREM sleep without rumination. Note that rumination did not interfere with the EEG activity and the composition of EEG power.

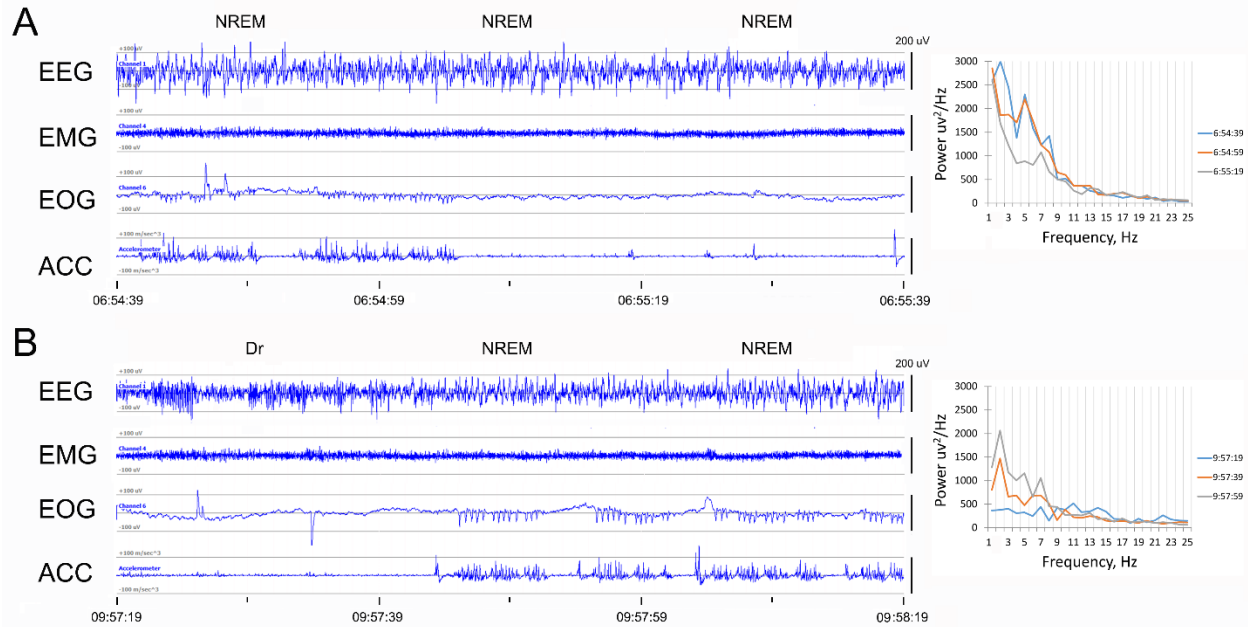


Figure S4. Representative polygrams of NREM sleep with and without rumination in mouse-deer 1. Note that rumination (recorded in the 1st and during a part of the 2nd epochs in A, and during the 2-3rd epochs in B) interfered with the EEG activity as indicated by prominent peaks of the EEG power in the range of 2-3 Hz and also the timing of the peaks in the EEG and EOG. The first epoch in B also includes bursting activity in the EEG in the range of 7-8 Hz. For other details see Figure S3 legend.

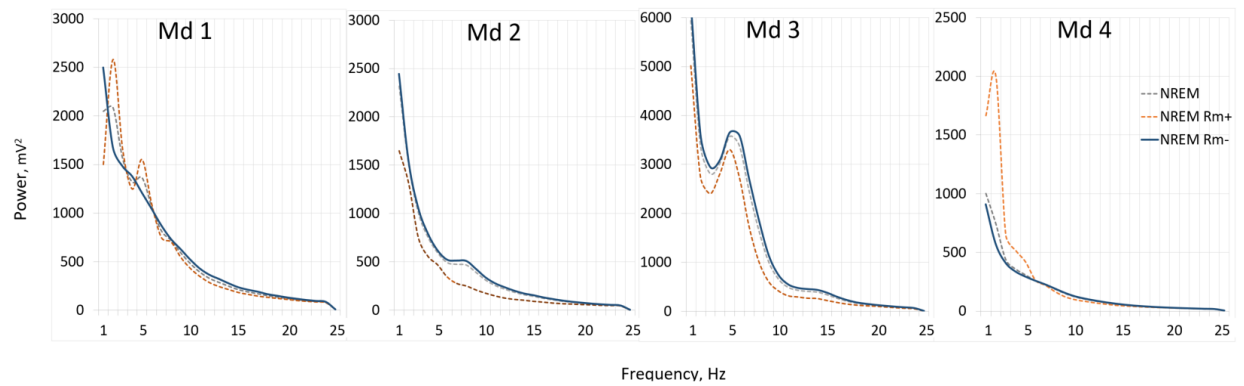


Figure S5. Spectral power of EEG during NREM sleep in the lesser mouse-deer. The data are presented for 4 mouse deer (Md 1- Md 4). NREM, all artefact free epochs of NREM or SWS sleep. NREM Rm+, NREM sleep with rumination. NREM Rm-, NREM sleep without rumination. The width of the first bin is 1.2 Hz (0.8-2.0) and the width of each remaining bin is 1 Hz. The values below X-axes mark the left margin of each bin interval. In 2 animals (Md 1 and Md 4) Rm affected the spectral composition of EEG as marked by prominent peaks in the range of 2-3 Hz. In one of these animals (Md 1), the EEG during Rm also had a peak between 5 and 6 Hz. Rm did not cause a clear change in the composition of the EEG power in the other two animals (Md 2 and Md 3).

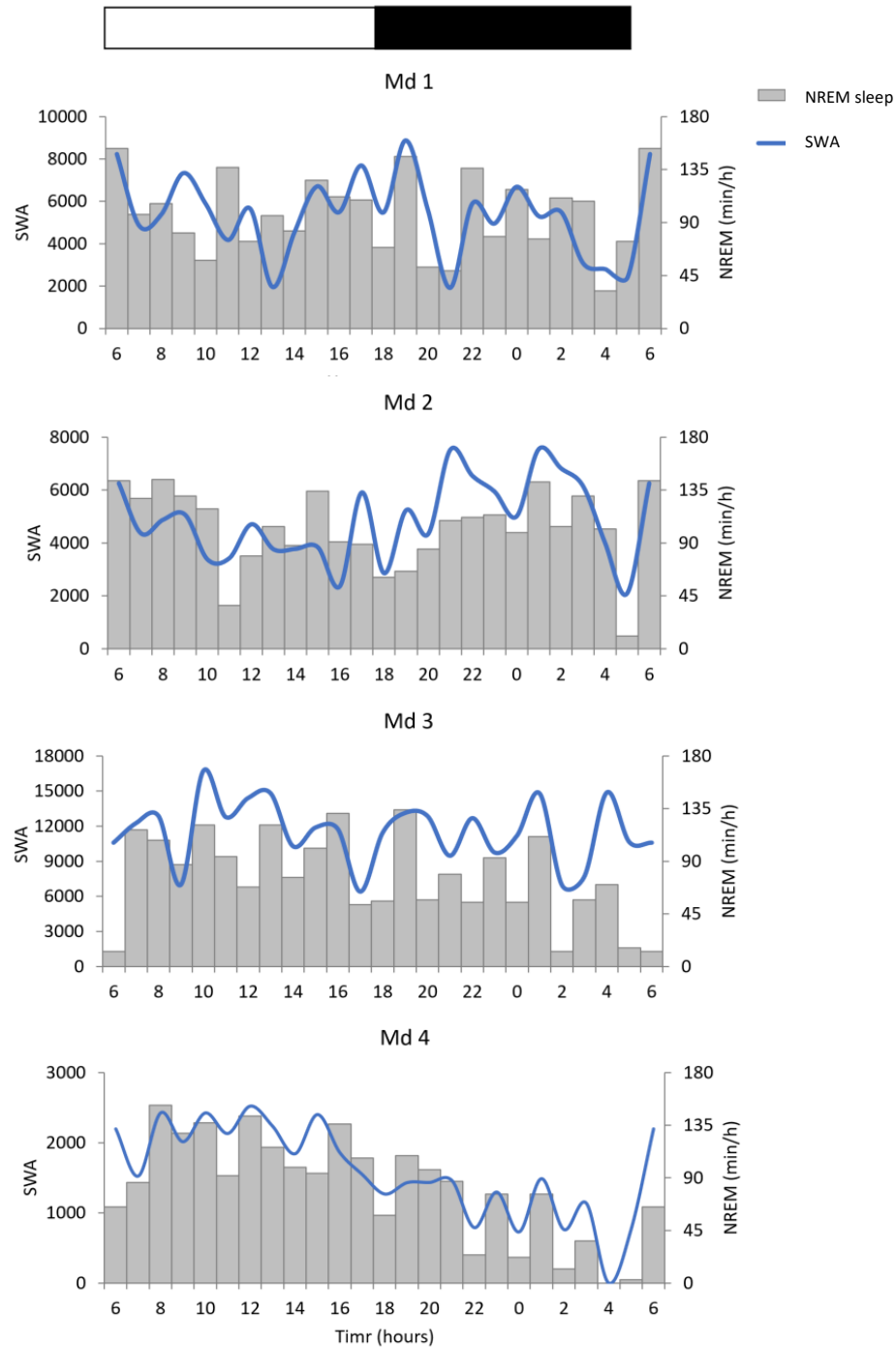


Figure S6. The time-course of slow wave activity (SWA) and the amount of NREM sleep over the 24-h period in the lesser mouse-deer. The graphs show 1-h means without rumination (power in the range of 1.2-4.0 Hz, $\mu V^2/1-h$) and the amounts of NREM sleep in 4 mouse-deer (Md 1 – Md 4).