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ORIGINAL ARTICLE

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

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Abstract

The mouse-deer or chevrotains are the smallest of the ungulates and ruminants. They are characterized by a number of traits which are considered plesiomorphic for the Artiodactyla order. The objective of this study was to examine sleep in the lesser mouse-deer (*Tragulus kanchil*), which is the smallest in this group (body mass < 2.2 kg). Electroencephalogram, nuchal electromyogram, electrooculogram, and body acceleration were recorded in four adult mouse-deer females using a telemetry system in Bu Gia Map National Park in Vietnam. The mouse-deer spent on average 49.7 ± 3.0% of 24 h in non-rapid eye movement (NREM) sleep. REM sleep occupied 1.7 ± 0.3% of 24 h or 3.2 ± 0.5% of total sleep time. The average duration of REM sleep episodes was 2.0 ± 0.2 min, the average maximum was 5.1 ± 1.1 min, and the longest episodes lasted 8 min. NREM sleep occurred in sternal recumbency with the head held above the ground while 64.7 ± 6.4% of REM sleep occurred with the head resting on the ground. The eyes were open throughout most of the NREM sleep period. The mouse-deer displayed polyphasic sleep and crepuscular peaks in activity (04:00–06:00 and 18:00–19:00). The largest amounts of NREM occurred in the morning (06:00–09:00) and the smallest before dusk (at 04:00–06:00). REM sleep occurred throughout most of the daylight hours (08:00–16:00) and in the first half of the night (19:00–02:00). We suggest that the pattern and timing of sleep in the lesser mouse-deer is adapted to the survival of a small herbivorous animal, subject to predation, living in high environmental temperatures in the tropical forest undergrowth.

Statement of Significance

Comparative sleep studies are an approach for understanding the environmental and evolutionary determinants of sleep. The lesser mouse-deer is the smallest of ungulates. This species retains morphophysiological features which are ancestral for even-toed ungulates. We have found that total sleep time in the mouse-deer is the largest among all studied ungulates, whereas the amount of rapid eye movement sleep is low as in the majority of ungulates. Distinct features of the mouse-deer are polyphasic sleep and crepuscular peaks of activity. The prevalence of non-rapid eye movement (NREM) sleep with both eyes open in the mouse-deer suggests that visual processing may be possible during NREM sleep. The pattern of sleep in the mouse-deer appears to be adapted to the environmental conditions of the tropical forest.

Key words: sleep; NREM sleep; slow wave sleep; REM sleep; activity cycle; environmental temperature; predation; evolution; the lesser-mouse deer; *Tragulus kanchil*; Tragulidae; Artiodactyla; ungulate

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Introduction

The first electroencephalogram (EEG) studies of sleep in ungulates were conducted on farm animals using direct cable connections. It was found that they sleep in either a recumbent or standing posture. The total amount of slow wave sleep (SWS or non-rapid eye movement, NREM sleep) and especially paradoxical sleep (PS or rapid eye movement sleep, REM sleep) is relatively small when compared with most other mammalian species. REM sleep is characterized by muscle atonia or well-demarcated hypotonia, EEG activation, as well as REMs, [1–3]. Drowsiness state (Dr) was scored in the majority of species, although the criteria for this stage were not consistent. Differences between species were seen largely in the duration of the sleep stages. For example, horses, cows, and sheeps displayed small amounts of SWS within 9%–14% of 24 h while pigs [1, 2] and ponies [3] almost twofold greater (>25% of 24 h). In farm artiodactyls rumination (Rm) occurred both in quiet waking (QW) and SWS and never in REM sleep [1, 2].

The first electrophysiological study of sleep in a wild ungulate was performed in the Siberian musk deer (*Moschus moschiferus*) in laboratory conditions [4]. Sleep occurred in recumbency with the head held above the ground (also called sternal recumbency) or with the head resting on the ground. Dr was characterized by bursts of rhythmic activity in the range of 3–5 Hz. The frequency of sleep spindles was 12–15 Hz. In total, wakefulness accounted for, on average, 47.0% of 24 h, Dr—10.2%, SWS—39.6%, and REM sleep—3.1% of 24 h. During SWS the eyes could be open or closed. Episodes of SWS and REM sleep were recorded at different times of the day. As in farm animals, Rm occurred during Dr and SWS but never in REM sleep.

Two recent studies examined the pattern of sleep in two species of wild African ungulates, including the Arabian oryx and the wildebeest [5, 6]. The animals were instrumented with portable data loggers and were recorded in spacious open ranges in their natural habitats. It has been reported that in the oryx total sleep time (TST) was 6.7 h in winter and 3.8 h in summer. REM sleep accounted for a very small percentage of 24 h, which is only 0.4% of 24 h in summer and 1.2% of 24 h in winter. TST in wildebeest was 4.3 h and the amount of REM sleep was same as in the winter oryx. It was suggested that a dramatic reduction of TST and REM sleep in the summer oryx might be adaptive, due to the animals needing to allocate more time for active foraging and grazing in the summer. A reduction of REM in the summer was suggested to be adaptive by preventing brain overheating.

Tragulids, chevrotains, or mouse-deer, are even-toed ungulates (the order Artiodactyla) which belong to the family Tragulidae. They are the smallest ungulates and ruminants in the world. They inhabit the tropical forest in South Asia and Africa [7, 8]. Tragulids are less advanced than other living ungulates in terms of possession of several morphological, physiological, and behavioral characteristics which are considered plesiomorphic (archaic) for artiodactyls (e.g. the lack of a true omasum and scent glans, retention of upper canines, a gall-bladder and appendix, and rudimentary social behavior). Tragulids emerged 40–50 m.y.a. and have not changed much in their skeletal attributes since then, leading them to being referred to as “living fossils” [9, 10]. Six extant species of the family Tragulidae are recognized. The common name lesser mouse-deer is often applied to two species: *Tragulus kanchil* (inhabits South-East Asia including Vietnam) and *Tragulus javanicus* (also called the Javan mouse-deer which is found in Java only). Morphometric differences between the species are small. The weight of adult animals of both species rarely exceeds 2 kg and their body length is less than 50 cm [7, 8, 11]. All mouse-deer species are solitary animals [7, 8, 12, 13]. The lesser and Javan mouse-deer are characterized as more active during the day [14], diurnal to cathemeral, [8] or crepuscular [15].

To summarize, our knowledge of sleep in ungulates is limited and incomplete. The mouse-deer differ in their morphophysiological features and evolutionary history and ecology from the other animals of the order Artiodactyla. Because of their small size and because sleep duration in ungulates has been shown to correlate with body mass, the sleep parameters of mouse deer can test our existing understanding of the determinants of sleep parameters. The aim of this study was to examine sleep in the lesser mouse-deer in a naturalistic environment.

Methods

Experimental animals

The experiments were conducted on four lesser mouse-deer (*T. kanchil*, Figure 1A) in Bu Gia Map National Park in Vietnam (12.088947525525246°N, 107.15679645354659°E). The animals were adult females (Md 1–Md 4). Their weight ranged from 1.7 to 2.2 kg and length from 42 to 48 cm (from the tip of the nose to the base of the tail). All procedures were reviewed and approved by the Committee for Bioethics of the Severtsov Institute of Ecology and Evolution (Moscow, Russia) and the Joint Russian-Vietnamese Tropical Research and Technological Center (Hanoi, Vietnam).

Housing conditions

The mouse-deer (20 adult females and 2 adult males) were caught and then housed in a spacious park enclosure (approximately 25 × 20 m) for at least 2 years before this study. Some females gave birth to young during this time. There were a total of 12 shelters (“houses”) in the enclosure. The inner dimensions of each house were 1.1 m long, 0.75 m wide, and 0.5 m high at the center. There was also a hole in the ground with the inner dimensions greater than in the house. When disturbed, the animals rushed to hide inside the houses or in the hole. Six months before the experiments started, a group of nine females was separated from the main group and placed to a smaller 12 × 3.5 m portion of the main enclosure (Supplementary Material, Figure S1). All animals used in this study were then selected from this group of females.

Electrode implantation

The mouse-deer were premedicated with Zoletil (Virbac, France; 15–18 mg/kg, i.m.), which caused immobility within 1–2 min. Then they were injected with antibiotics (Baytril, Bayer, Germany, 2 mg/kg i.p.) and analgesics (Rimadyl, Pfizer Animal Health, USA; 2 mg/kg i.p.). During the next 10 min, the animals were taken to the laboratory where they were injected with Domitor (Orion Pharma, Finland; 0.1 mg/kg i.m.) which produced anesthesia within 15 min. All animals were also injected with lidocaine at least 10 min before the incision (0.5 mL, 0.1%, subcutaneously above the dorsal part of the skull). During the

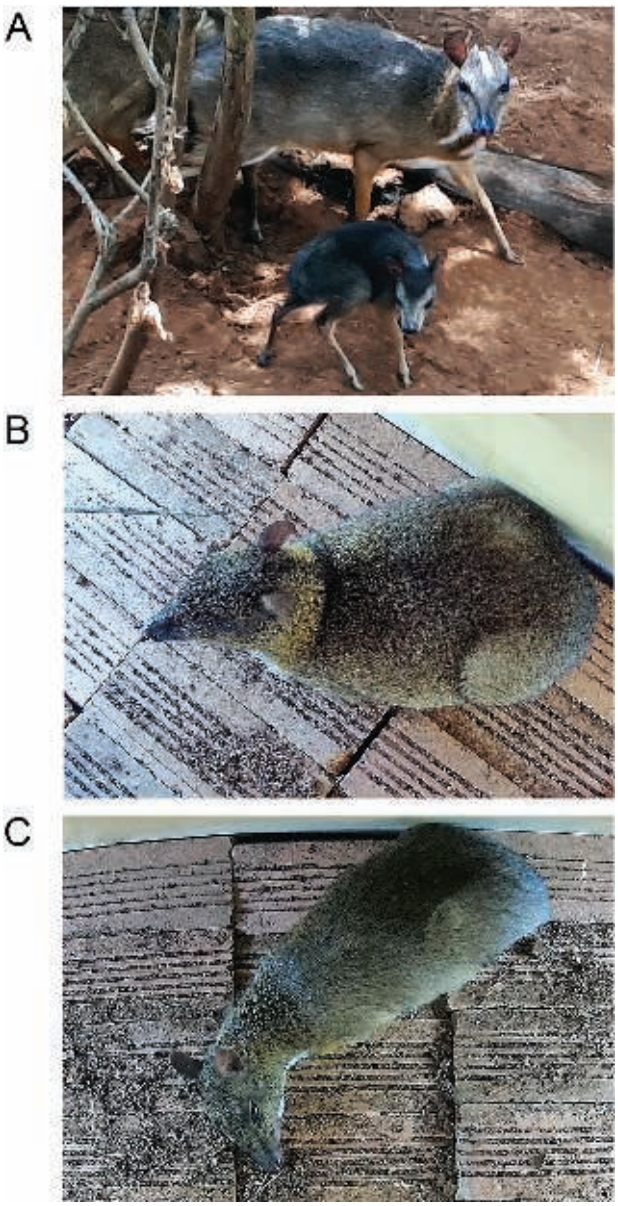

Figure 1. The lesser mouse-deer. (A). An adult female of the mouse-deer with a 1-wk old calf. (B). A mouse-deer is resting in a shelter in the most typical posture with the head held above the ground (sternal recumbency). (C). A mouse-deer is resting while lying with the head placed on the ground. Note that the left eye facing the camera is open on B and C.

surgery, which lasted 2 h on average, the depth of anesthesia and state of the animal were monitored based on the animal's reflexes, heart and breathing rates, and rectal temperature. The surgical level of anesthesia lasted 1–2 h and could be prolonged by additional administrations of Domitor for 10–15 min (one-third of the initial dose).

Two pairs of stainless-steel screws (1 mm in diameter) were implanted symmetrically over the frontal and parietal part of the skull 3 mm lateral to the sagittal line, to record EEG. The frontal and parietal electrodes were located 10 mm apart from each other. They were positioned rostral and caudal to the ansate sulcus, respectively (Supplementary Figure S2). Another pair of screws was implanted into the supraorbital bone above one or two eyes to record electrooculogram (EOG). Four Teflon-coated multi-stranded stainless-steel wires (0.3 mm in diameter) were inserted into the nuchal muscles to record electromyogram (EMG). An additional screw was implanted into the frontal part of the skull (above the nasal passages), serving as a reference electrode. The electrode leads were soldered to a micro-connector and attached to the skull with acrylic cement. All animals displayed normal behavior and activity levels within several hours after the end of surgery. The implanted animals were allowed at least 4 days to fully recover in the experimental enclosure (3.5 × 3.5 m) or a separate enclosure (3.0 × 3.5 m; Supplementary Figure S1) before the experiments started. During the first 3 days, they were given antibiotics and analgesics daily.

Polygraphic recording

Three to five days after the surgery a micro-plug on the mouse-deer head was connected to a radio-transmitter attached to a collar on the deer's neck. After that, the recording continued for 4 days in one animal (Md 1) and for 3 days in the three other animals (Md 2–Md 4). The recordings were performed using a telemetry system (http://biorecorder.com/en/br8v1.html) which allowed for the recording of up to eight parameters and total acceleration within 10–12 m of the receiver. All recordings were bipolar. The initial configuration was set to record EEG from the two symmetrical pairs of cortical frontal and parietal electrodes, EOG from two electrodes in each orbit and EMG from two pairs of wires. The reference electrode was used only as the ground point. The raw data were digitized and stored. The bandwidth was 0.1–100 Hz at an acquisition rate of 250 Hz. To maximize the duration of continuous recording from the battery, in each animal we registered one EEG signal (left frontal-parietal) at an acquisition rate of 100 Hz, one EOG (left eye) at 100 Hz, one EMG at 250 Hz, and acceleration at 25 Hz. This configuration allowed continuous recording of the four to five listed parameters between 3 and 4 days while minimizing the weight of the collar with a transmitter and a battery to <90 g (approximately 5% of the body mass). In three animals the EEG from two symmetrical cortical derivations and EOG from two eyes were also recorded for 2–3 h. All acquired parameters were continuously monitored using EDF browser software (Moem 0.8, a universal viewer of edf/bdf files; https://www.teuniz.net/edfbrowser/index.html).

During experiments, the mouse-deer were housed individually in a 3.5 × 3.5 m enclosure (Supplementary Figure S1). Tall cashew nut trees grew along the enclosure fence. The treetops shaded most of the enclosure. About one-fifth of the enclosure along one side was covered with shrubs characteristic of the area and the rest of the space had no vegetation. A small house similar to the shelters in the main area (0.9 × 0.7 × 0.7 m) was positioned in the experimental enclosure. The behavior of animals was continuously video recorded with two high-resolution remote-control 4 MP IP-cameras with optical zoom and fitted with infrared lights. The first camera was installed above the enclosure and the second camera on the roof of the house. The cameras allowed us to monitor the animals while in the enclosure and in the house, including close-up footage of the head and often the eyes to determine the characteristics of sleep-waking states. The animals were given fresh leaves twice a day between 06:00 and 07:00 and 15:00 and 16:00 by the park staff. Ripe fruit also fell into the enclosure from the cashew nut trees. Water was available at all times. Over the entire period of

recording (March 8–26) the sunrise time in the area shifted from 06:03 to 05:54 and sunset between 18:03 and 18:04. The length of the day increased from 12:00 to 12:09. During the recording period, daytime (06:00–18:00) ambient temperature ranged between 29.0 and 35.0°C. The mean daytime temperature was 31.3 ± 0.2°C, mean maximum temperature was 34.8 ± 0.3°C, and mean minimum temperature was 30.0 ± 0.4°C. Nighttime (18:00–06:00) ambient temperature ranged between 26.5 and 29.5°C. The mean nighttime temperature was 27.8 ± 0.3°C, mean maximum temperature was 28.6 ± 0.3°C, and mean minimum temperature was 26.9 ± 0.2°C (https://www.timeanddate.com/weather/@12.09079,107.14775/historic?month=3&year=2020).

Data analysis

REM sleep was scored in 1-min epochs in all mouse-deer during all days. In each animal, the amount of REM sleep in a series was the smallest on the first day (Supplementary Table S1). In three animals (Md 2–Md 4), the daily amounts of REM sleep increased over the three recording days. In the fourth animal (Md 1), the amount of REM sleep was the greatest on the second recording day. However, the difference between the second and fourth days was not remarkable. Thus, we chose to score the third day in each mouse-deer which was also the last in a series in three out of four animals. Then, all polygrams of the third recording day were scored visually in 20-sec epochs based on the EEG of the left hemisphere, EOG of the left eye, EMG, and acceleration using EDF browser.

A total of four main stages were scored, including active waking (AW), quiet waking (QW), NREM sleep (or SWS), and REM sleep, and two transitional stages, including Dr and transitional from NREM to REM sleep (tREM) in 20-sec epochs. A 50-% criterion was used to assign the dominant state to each epoch except for Dr. AW included walking around the enclosure and eating or drinking. QW was scored when the mouse-deer were standing (including grooming and leaking) or resting on the ground in a recumbent position and while EEG was generally low voltage with occasional high voltage deflections synchronous with movements. Dr was scored when there were bursts of rhythmic activity in the EEG with the frequency of 5–14 Hz and the amplitude at least twofold higher than low voltage waking EEG. The epoch was scored as Dr if one burst 5 sec or longer or 2 bursts 3 sec or longer occurred per 20-sec epoch during typical QW low voltage EEG. The scoring criteria for Dr were similar to that used in the previous musk deer study [4].

NREM sleep was scored when intermediate to high voltage EEG in the range of 1–6 Hz was recorded in a cortical hemisphere in the absence of gross movements in recumbency or a standing position. If bursting activity was intermixed with EEG slow waves and was present during at least 50% of the epoch time, the epoch was scored as NREM sleep. REM sleep was scored when REMs were recorded in recumbent positions (Figure 1, B and C). REMs were accompanied by cortical arousal, muscle tone reduction, and occasional head and ear jerks. A small portion (on average 0.5 ± 0.3% of 24 h) of epochs were marked as unidentified. They included mostly epochs with Rm when high voltage artifacts in the EEG and EOG masked physiological activity. Those epochs represented either QW or NREM sleep. Rm was recognized based on the acceleration signal and characteristic artifacts in the EEG and EOG. Epochs with Rm were scored based on a 50-% criterion. Additional details are provided in the Results section.

In each animal, the EEG power between 0.8 and 25 Hz in the left hemisphere was computed in artifact-free epochs by fast Fourier transformation using EDF browser software. The width of the first bin was 1.2 Hz (0.8–2.0 Hz) and the width of each remaining bin was 1 Hz. Slow wave activity (SWA, power in the range of 0.8–4.0 Hz) was first calculated during NREM sleep artifact-free epochs and then averaged at 1-h intervals across the 24-h period.

Eye state was scored in all mouse-deer during QW and NREM sleep when at least one eye was visible. The most reliable assessment of eye opening was at night by detection of the white spot (IR-light reflected from the tapetum) in the area of the orbit. The glow was present when the eye was partly or fully open. In the daytime, the state of the eye was determined by an estimate of the gap between the eyelids. The eye was considered open if 2–3 mm gap (and the eyeball) was clearly visible. Thus, whenever the state of the eye was scored as open the eye could be fully or partly open. Both the epochs in which the state of the eye (or eyes) was scored as open and a lesser degree as closed could include episodes with minimal eye opening.

The duration of episodes of NREM sleep, REM sleep, and Rm was calculated by adding the number of consecutive epochs scored as a given state. A single epoch of another state terminated each episode. Reported values are means ± S.E.M. for all four mouse-deer, for the entire period (1 h, 12 h, or 24 h). All statistical analyses were performed using Sigma Plot 11.0 Software. Data were assessed for statistical significance using the paired *t*-test or one-way repeated measures ANOVA followed by Tukey's post hoc multiple-comparison tests after they had passed the test for normality. In only one case the data failed to pass the test and the Kruskal-Wallis H test was applied to estimate the significance (detailed in the Results section).

Results

Wakefulness

Polygraphic features of AW and QW states in the mouse-deer (Figure 2) were within the ranges of those of the majority of studied mammals (e.g. Refs. [16, 17]]), including farm animals [1–3] and three studied wild species of the order Artiodactyla [4–6]. During AW, EEG, EOG, and EMG were contaminated with high voltage artifacts. Some of the mouse-deer behaviors (such as walking, feeding, and grooming) could be identified based on the pattern of accelerometer signal. The behavior of animals (video footage) was the main criteria for scoring AW. During QW tonic and phasic components of EMG were variable, reflecting the level of animal's activity and postures. The EOG activity was high voltage during movements. When the animals were inactive, the amplitude and power of the EEG were low. The mouse-deer were awake on average 46.2 ± 3.0% of 24 h or 11 h per day. AW and QW constituted 40% and 60% of total wakefulness, respectively (Table 1). On average 26.1 ± 3.2% of AW (4.5 ± 0.2% of 24 h) accounted for eating or drinking while the rest was walking (73.9 ± 3.2% of AW or 13.6 ± 2.4% of 24 h). On average 14.4 ± 1.9% of QW (9.5%–18.1% in different animals) was accompanied by Rm, which represented episodes of regular jaw movements with a frequency of 1–2 per second.

Bursting activity in EEG and drowsiness

Bursts of rhythmic activity in EEG were most often recorded during transitions from QW to NREM sleep and during awakening

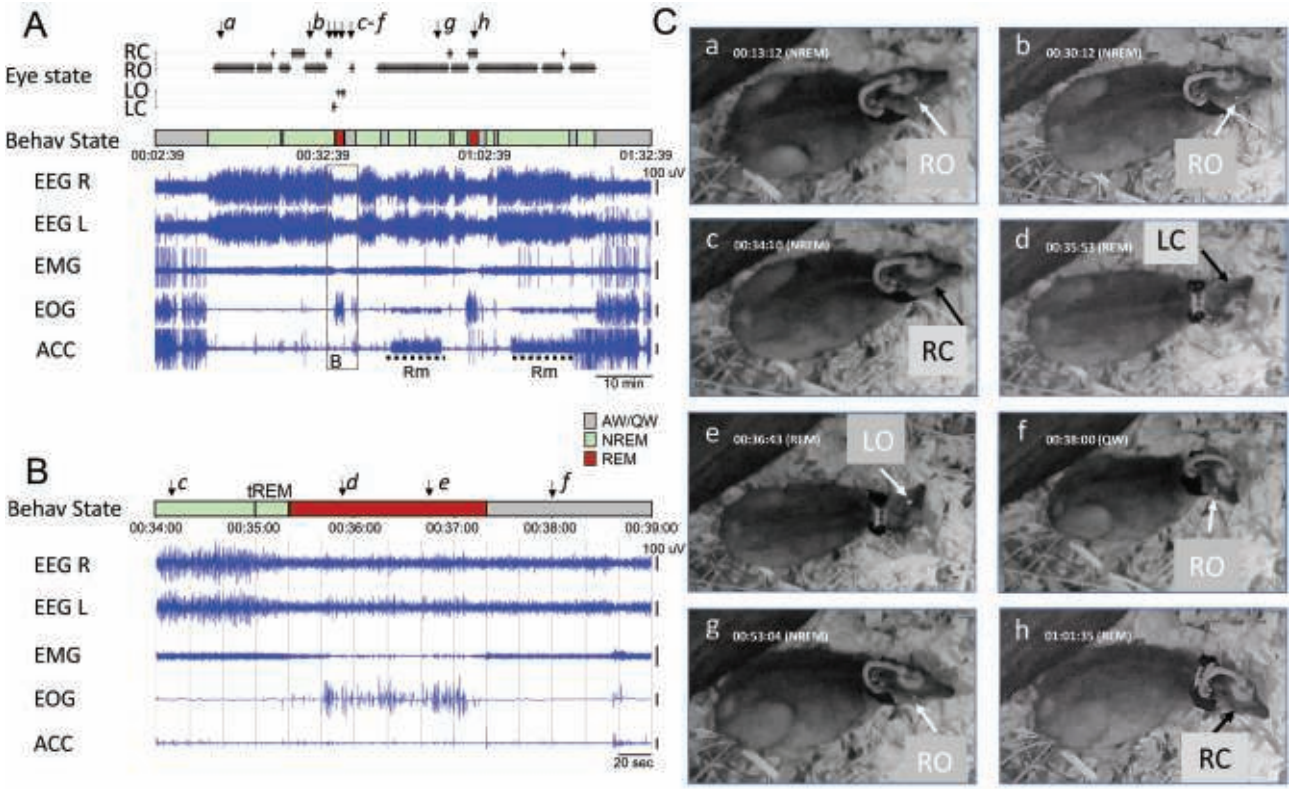

Figure 2. Representative polygrams of waking, NREM and REM sleep and the state of eyes in a mouse-deer. EEG, electroencephalogram of the right (R) and left (L) cortical hemispheres; EMG, electromyogram of the neck muscles; EOG, electrooculogram of the left eye; ACC total acceleration. The color panel marks behavioral states (AW and QW, NREM sleep, REM sleep, and tREM).

(A) A representative 90-min polygram and sleep wake-states in mouse-deer 1. The top panel shows the state of right (R) and left (L) eyes scored in 20-sec epochs. O and C denote the open or closed state of the eye, respectively. Arrows and letters (a–h) mark the time when the photographs (the right panel, C) were taken. The right eye was visible during most of this time. The left eye was visible only during the first episode of REM sleep when the animal rested the head on the ground and tuned it to the right side. During NREM sleep the eye directed to the camera was predominantly open. A 5-min episode marked with a dotted line (denoted with B) is enlarged on panel B. During REM sleep the visible eye was either closed or open. Episodes of rumination (Rm) are marked by dotted lines. Calibration—100 uV and 10 min.
(B) A 5-min episode (marked on A with B) illustrates transition from NREM to REM sleep and then to awakening. The first of six epochs of the REM sleep episode was scored as REM1 and the 2–6th epochs were scored as REM2. Calibration—100 uV and 20 sec.
(C) Photographs show postures and the state of eyes (a–h) in the same mouse-deer. The time of each photograph and the behavioral state are on the photos. The eyes glowed in the night reflecting light from IR-source. The white arrows point to the open eye as indicated by a white spot in the area of the orbit. The black arrows point to the orbit where no glowing is seen suggesting the eye was closed. During QW and NREM sleep the visible eye was predominantly open (photos a, b, f, g). The right eye was closed during the last 2 min of NREM sleep before REM sleep started (c). The left eye was closed during the first 40 sec of the REM sleep episode (d) and then it was open during the last 2 min of the REM sleep episode (e). The right eye was closed during the second REM sleep episode (h).

(Supplementary Figures S3 and S4). In different animals, the frequency ranged between 5 and 14 Hz (Figure 3). The amplitude of the EEG during bursting could exceed the amplitude of desynchronized activity by fourfold. The duration of bursts varied substantially. During QW, bursts were often recorded between head movements but no association was noticed between bursting and eye state. Bursts were also recorded during NREM sleep while their duration was shorter (3–4 sec) than during QW. Dr was scored when bursting activity in the EEG occupied most of a 20-sec epoch. This state was pronounced in two animals (Md 1 and Md 3) accounting for 2.7% and 3.0% of 24 h, respectively. In two other animals (Md 2 and Md 4), Dr accounted for less than 1% of 24 h (Table 1).

NREM sleep

While in the enclosure, the mouse-deer exhibited sleep predominantly in the open space and rarely in the bushes. NREM sleep occurred while lying on the sternum with front and hind legs tucked under the body and holding the head above the ground facing forward (sternal recumbency; Figure 1B). NREM sleep in the main posture accounted for on average 96.0 ± 2.2% of all NREM sleep (90.2%–99.4% in different animals). Less often, NREM sleep occurred while standing (0.5%–9.8% of all NREM sleep; on average 3.7 ± 2.2%). In two animals on a few occasions, NREM sleep was recorded when they briefly rested the head on the ground (0.1% and 0.9% of all NREM sleep).

NREM sleep was characterized by intermediate and high voltage EEG slow waves and occasional bursts of rhythmic activity (Figures 2–3 and S3–S4). The EEG power during NREM sleep was maximal at low frequencies. In two animals, there were peaks of EEG power in the range of 5–7 and 7–10 Hz (Md 3 and Md 2). The maxima of these peaks coincided with the maxima during Dr and at a lesser degree in QW. To maximize the duration of uninterrupted recording, we limited the number of the transmitted parameters and recorded EEG mostly from one cortical hemisphere. However, whenever we recorded from two symmetrical cortical derivations, slow waves in the left and right EEG developed synchronously (Figure 2).

The neck muscle tone during NREM sleep was similar to or less than that in QW without head movements, which briefly interrupted SWA, leading to cortical arousal, bursts of rhythmic activity, or a decrease in SWA activity. During NREM sleep, the ears of the mouse-deer moved from time to time. Such movements were not accompanied by a decrease in the amplitude of SWA.

During NREM sleep, the EOG was virtually flat indicating no eye movement except for when the mouse-deer moved their heads (Figure 2). The majority of the time only one eye was visible. Whenever both eyes were visible they were in a symmetrical state. The state of eyes (one or both) was determined on average in 48.3 ± 9.5% epochs of NREM sleep and 28.2 ± 8.3% epochs of QW (Supplementary Table S2). During NREM sleep, the eyes (one or both) were fully or partially open on average 99.2 ± 0.6% and during QW—99.7 ± 0.2% of the time when the state of eyes was identified. Therefore, both during QW and NREM sleep the mouse-deer virtually did not close their eyes.

Table 1. Total amount of sleep and wakefulness states in the lesser-mouse deer

Parameter	Mouse-deer 1	2	3	4	Mean ± SEM (n = 4)
Amount of sleep and wake states (% of 24 h)					
Wakefulness	42.6	39.7	51.8	50.5	46.2 ± 3.0
Total sleep time	54.5	59.0	45.2	47.9	51.7 ± 3.1
Active wakefulness	23.8	12.0	20.4	16.1	18.1 ± 2.6
Quiet wakefulness	18.8	27.7	31.4	34.4	28.1 ± 3.4
Drowsiness	2.7	0.7	3.0	0.3	1.7 ± 0.7
NREM sleep	53.2	56.2	43.6	45.9	49.7 ± 3.0
REM1	0.5	0.9	0.3	0.5	0.6 ± 0.1
REM2	0.7	1.5	1.0	1.3	1.1 ± 0.2
REM sleep	1.2	2.4	1.3	1.8	1.7 ± 0.3
tREM	0.1	0.4	0.3	0.2	0.3 ± 0.1
Un	0.2	0.6	0.0	1.3	0.5 ± 0.3
REM sleep (% of TST)	2.0	4.1	2.8	3.8	3.2 ± 0.5
Rumination (% of 24 h)	27.9	12.4	15.3	12.3	17.0 ± 3.7

REM1 and REM2 are two substages of REM sleep.
tREM, transitional to REM sleep; Un, unidentified; SEM, standard error. Total sleep time includes NREM sleep, REM sleep (both REM1 and REM2), and tREM.

Rm accompanied on average 24.3 ± 7.4% of NREM sleep (11.6%–45.2% in different animals). In two animals (Md 1 and Md 4), Rm affected the spectral composition and amplitude of EEG. In both cases, during Rm, the EEG power profile had prominent peaks in the range of 2–3 Hz, which were absent during NREM sleep without Rm (Supplementary Figure S5). The frequency of jaw movements during Rm was also 2–3 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).

Panting was recorded during NREM sleep in all mouse-deer. It consisted of episodes of shallow repeated breathing with an instantaneous rate of at least 3 per second as indicated by the nostrils and body movements, and characteristic activity on an accelerometer channel. Episodes of panting lasted between several seconds to several minutes. Panting was recorded mostly during daytime and excluded Rm.

REM sleep

REM sleep in the mouse-deer occurred in two positions: in sternal recumbency (as during most of NREM sleep) or lying with the head resting on the ground (Figure 1, B and C). The main features of REM sleep were REMs, a decreased muscle tone, and cortical arousal (Figure 2). In all animals, a peak with a maximum frequency of 6–7 Hz was also present in the spectrogram (Figure 3). REMs could be accompanied by movements of the ears. The eyes always closed before the onset of REM sleep. While REM sleep progressed, the eyes could remain closed or wide open for the entire 20-sec epoch, or blinks could occur in parallel with REMs. Opening of the eyes during REM sleep occurred during instances of atonia with the animal head resting on the ground. In agreement with previous studies in farm ruminants and in the musk deer [1–4], Rm in the mouse-deer was not recorded during REM sleep.

REM sleep was subdivided into two substages (Figure 2B). During REM1, the muscle tone was either sustained and comparable with that during with NREM sleep, or it progressively

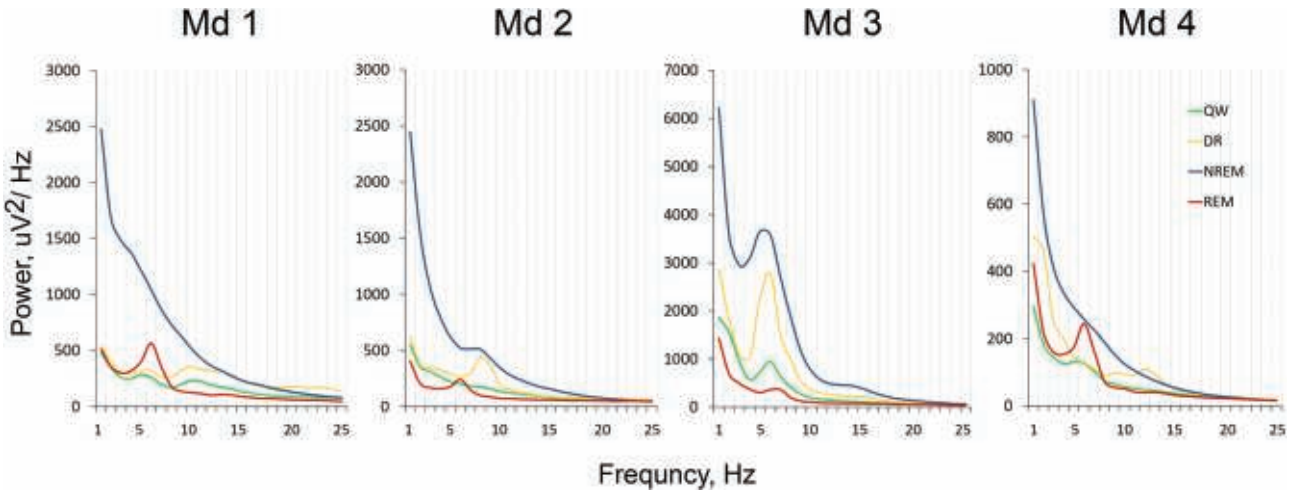

Figure 3. Spectral power of EEG during sleep and waking in the lesser mouse-deer. The data are presented for 4 mouse-deer (Md 1–Md 4). QW, quiet wakefulness; Dr, drowsiness; NREM, NREM sleep without rumination; REM, REM sleep. In all animals the power was calculated in the frontal-parietal derivations of the left hemisphere. 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. Epochs of QW, Dr, and NREM sleep with rumination were not used to plot these graphs (see Supplementary Figures S3–S5).

decreased while the head swayed forward toward the ground. REMs usually occupied less than 50% of the epoch. REM2 was characterized by muscle atonia and REMs for at least 50% of the epoch time. Most REM2 epochs featured almost continuous eye movements. REM2 epochs can be also characterized as a phasic and REM1 as a tonic stage.

Sometimes single deflections in the EOG appeared during EEG slow waves or brief arousal. The amplitude of such peaks was lower than in REM1 or REM2. No evidence of eye movements was seen on video at that time and the muscle tone was usually similar to that during NREM sleep. Such epochs with partial features of REM sleep were scored as transitional to REM (tREM; Figure 2B).

Episodes of REM sleep usually started after an extended period of high voltage NREM sleep. In the majority of cases, REM1 preceded REM2. In a few cases REM2 occurred quickly featuring a head drop and muscle atonia, REMs, and cortical activation. tREM episodes did not precede every REM sleep episode.

Total amount, duration of episodes of NREM sleep, REM sleep, and rumination

The mouse-deer spent on average 49.7 ± 3.0% of 24 h or almost 12 h per day in NREM sleep (Table 1). The longest uninterrupted episode of NREM sleep episodes lasted almost 28 min and the average maximum was 18.8 ± 3.2 min. On average, 11.0 ± 3.0% of all episodes were longer than 5 min while 50.9 ± 4.3% were shorter than 1 min. Almost 25% of all episodes lasted one 20-sec epoch (Supplementary Table S3).

The amount of REM sleep (both REM1 and REM2) in different mouse-deer accounted for between 1.2% and 2.4% of 24 h, on average 1.7 ± 0.3% of 24 h or 24 min per day. REM sleep represented only 3.2 ± 0.5% of TST, one of the smallest ratios in mammals reported thus far (Table 1). Overall 35.3 ± 6.4% of REM sleep (REM1 and REM2 sleep) occurred in sternal recumbency (with the head held above the ground) and 64.7 ± 6.4% while lying with the head on the ground. The majority of REM1 sleep (82.0 ± 2.2%) was associated with sternal recumbency while the majority of REM2 sleep (87.9 ± 4.9%) occurred when the head rested on the ground.

A total of 12.3 ± 1.5 episodes of REM sleep per day were recorded (Supplementary Table S3). The average duration of REM sleep episodes was 2.0 ± 0.2 min. The longest uninterrupted episode of REM lasted 8.0 min and the average maximum was 5.1 ± 1.1 min. Three out of four animals had episodes of REM sleep longer than 3 min. Those episodes represented one-third of all REM sleep episodes. At the same time, among all animals, 45.3 ± 3.3% of REM sleep episodes were shorter than 1 min and 8.5 ± 1.0% lasted one 20-sec epoch.

The mouse-deer spent between 12.3% and 27.9% of the 24-h ruminating, on average 17.0 ± 3.7% (Table 1). Periods of Rm (a series of episodes of Rm without interruptions longer than 10 sec) were 4.9 ± 1.5 min on average and ranged between 20 sec and 25 min. On average 68.3 ± 9.1% of all Rm was recorded during NREM sleep and 26.8 ± 7.1% in QW. The remaining amount of Rm (about 8%) was associated with either NREM sleep or QW (the sleep state of those epochs was not identified due to artifacts). The ratio of the amounts NREM sleep with Rm to QW with Rm (2.5) was greater than the ratio of the amounts of NREM sleep to QW (1.8).

Distribution of sleep and wake states across the 24-h period

Three out of four mouse-deer spent most of the daytime in the house (77%–90% of the time) and most of the nighttime outside the house (84%–97% of the time). The fourth animal briefly entered the house only four times during daytime, when it was disturbed by a noise (Supplementary Table S4).

Under the conditions of this study, the amount of AW in the mouse-deer was significantly greater during nighttime than during daytime ($p = 0.012$, the paired t-test; Supplementary Table S4 and Figure 4). All animals displayed more NREM sleep during daytime than during nighttime. However, the difference between the means did not rich the level of significance ($p = 0.086$). The amounts of time spent in REM sleep, the number and duration of REM sleep episodes, and the amounts QW did not differ between the daytime and nighttime periods ($p > 0.05$).

When evaluated in 1-h intervals, the average amount of AW had two peaks: between 04:00 and 06:00 (60% of 1 h which is threefold greater than the average daily value) and between 18:00 and 19:00 (36% of 1 h which is almost twofold greater than the daily average value; Figure 5B). Average hourly activity was low in the afternoon (12:00–17:00), in the morning (07:00–09:00), and after sunset (18:00–19:00). The repeated measures ANOVA revealed a statistically significant effect of time on the amount of AW ($F3,69 = 3.303$, $p < 0.001$). The difference was significant between the amounts of AW at 05:00–06:00 (the daily maximum) and almost a half of the remaining hours (10 out of 23 h except for hours of 0, 2–4, 6, 12,17, 18, and 20–21; $p < 0.05$, Tukey's post hoc test). In contrast to AW, the time spent in QW did not change across the 24-h period ($F3,69 = 0.990$; $p = 0.489$; Figure 5C).

The mouse-deer exhibited NREM sleep during most of the 24-h period. The largest individual amounts of NREM sleep were recorded in the morning between 06:00 and 09:00 after the period of the smallest amounts between 04:00 and 06:00 (Figure 5D). The average daily 1-h sleep value was maximal at 08:00 (on average 71% of 1 h). After that, it progressively declined reaching the daily minimum at 05:00 (15% of 1 h). However, only 2 out of 4 animals exhibited a declining trend (Supplementary Figure S6). Overall, there was a significant effect of time of day on the amount of NREM sleep across the 24-h period ($F3,69 = 2.144$, $p = 0.008$). The difference was significant between hour 5 (the daily minimum; on average 15% of 1 h) and hours 8 (the daily maximum; 71%, $p = 0.008$), 16 (67%, $p = 0.025$) and 15 (65%, $p = 0.039$). During NREM sleep, the timing of individual SWA varied over the 24-h period (Supplementary Figure S6). Overall, hourly SWA values were not affected by the time ($F3,63 = 1.003$, $p = 0.473$; Figure 5E).

REM sleep was recorded during most of the light hours (08:00–16:00) and in the first half of the night (19:00–02:00) when the animals had the majority of their NREM sleep (Figure 5F). None of the five longest episodes of REM sleep, which lasted between 4 and 8 min, were recorded between 09:00 and 16:00. The amount of time spent in REM sleep was generally not affected by the time of day ($F3,69 = 1.248$; $p = 0.238$, The Kruskal-Wallis H test).

Discussion

During our experiments, the mouse-deer were in a naturalistic-like condition, ambient temperature, noise, and light/dark cycle. The polygraphic recording was conducted

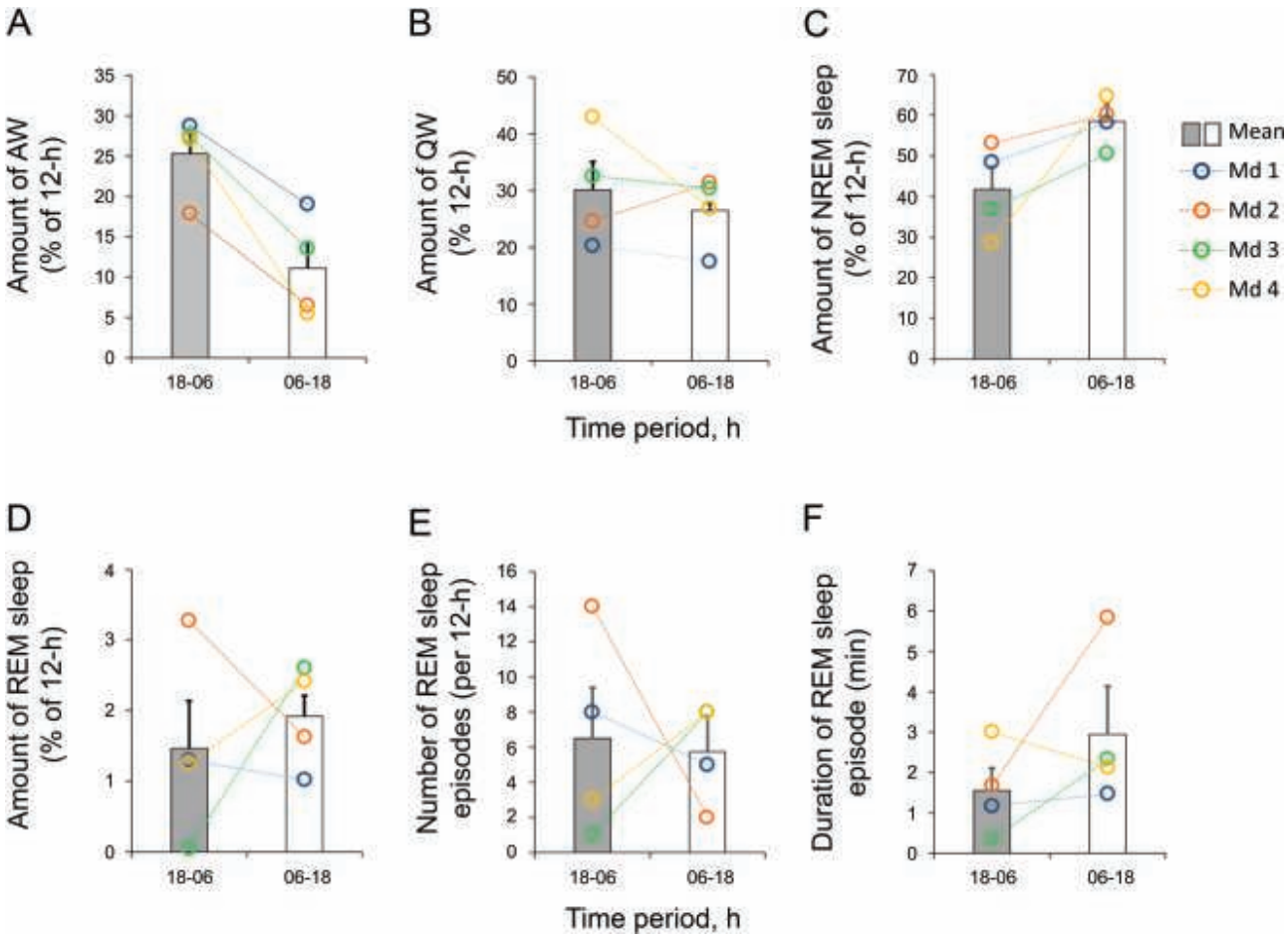

Figure 4. Parameters of wake and sleep states during the dark (18:00–06:00) and light (06:00–18:00) periods in the lesser mouse-deer. The graphs show the parameter values for four lesser-mouse deer (color lines, Md 1–Md 4) and the means (gray and white bars). (A–D). Amounts of AW, QW, NREM sleep, and REM sleep. (E). Number of REM sleep episodes. (F). Duration of REM sleep episodes. The difference between the night and day time values was significant only for AW ($p < 0.05$, the paired *t*-test, Supplementary Table S4).

using a telemetry system. The electropolygraphic data has been supplemented by a high-quality video of the animal's behavior which revealed a number of interesting features of the animals' behavior. Even though our recording conditions were lacking all the complexities of the wild and all studied mouse-deer were females, we believe that the data collected in this study extends our knowledge on sleep in ungulates and allows us to test several hypotheses suggested by prior studies and publications.

Sleep postures

In ungulates, NREM sleep has most frequently been recorded in recumbency and REM sleep was seen in lateral recumbency with the head resting on the ground [1–4]. A characteristic posture and phasic events facilitate identification of this state in ungulates in behavioral studies, for example, in the elephant and giraffe [18, 19]. This led to a suggestion that elephants in the wild can go without REM sleep for several days since they displayed only standing rest/sleep for periods of several days [20]. However, horses appear to be able to have REM sleep while standing while the episodes were shorter when compared to those in lateral recumbency. This was accompanied by a reduced neck muscle tone and head drops [21]. If not awakened after that, the horse could then collapse and fall on its knees, which led to an awakening. The majority of NREM sleep in the lesser mouse-deer was recorded in sternal recumbency with the head held above the ground. Although most of the REM sleep was recorded with the head resting on the ground, about one-third of the total REM sleep also occurred in sternal recumbency. Thus, lateral recumbency is not an absolute condition for REM sleep for all ungulate species.

Drowsiness state

Dr was scored as a separate state in most prior studies of sleep in ungulates [1–4]. Bursts of rhythmic activity in the EEG with frequency between 3–5 Hz and 14 Hz were often emphasized as one of the features of this stage. However, in several studies, Dr was described as a mixture of both low voltage and high voltage slow waves. Therefore, the criteria for scoring episodes of Dr were not always consistent in these studies. In the lesser mouse-deer, Dr accounted for a very small amount of time, less than in other studied species (e.g. 10% of 24 h in the musk deer [4]; between 8% and 31% of 24 h in farm animals [1]). In two mouse-deer, based on the animals' behavior at the time of bursting and the frequency of EEG, this activity had features of the alpha-like or somato-sensory rhythms recorded in other

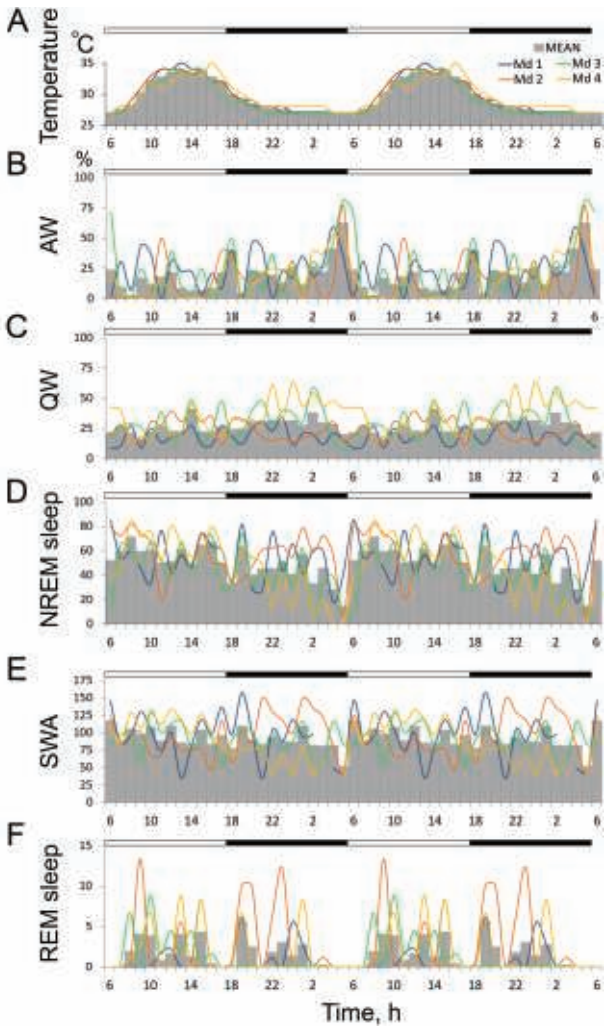

Figure 5. The time-course of sleep and wake states, and slow wave activity over the 24-h period in the lesser-mouse deer. (A) Ambient temperature. (B–D, F). Amounts of AW, QW, NREM sleep, and REM sleep as percent of 1 h. (E) SWA (EEG power in the range of 0.8–4.0 Hz) in 1-h intervals in artifact free epochs of NREM sleep without rumination as percent of the mean SWA for all NREM sleep epochs without rumination. The data are for individual animals (color lines, Md 1–Md 4) and means for all animals (gray columns). The data is double plotted.

mammalian species. Those rhythms were recorded from either visual or motor cortex when the animals (cat, dog, monkey) were immobile but behaviorally awake. Both activities were suppressed by movements [22–24]. In two other mouse-deer, shorter bursts were recorded in the EEG both during transition between QW and NREM sleep, and in high voltage NREM sleep. They met the criteria for sleep spindles. The exact localization of the motor, somato-sensory, and visual cortex in the lesser-mouse deer is not known. However, based on the data for other ungulates [25], the frontal EEG electrodes in the mouse-deer were located anterior to the ansate sulcus (where somatosensory and motor cortical areas are usually located) and the parietal electrodes in the anterior part of the lateral gyrus (where the anterior part of the visual cortex is located). Thus, the variation in the expression of bursting activity in the EEG of the mouse-deer in our study was probably due to variation in the location of EEG electrodes in relation to the sensory and motor cortical areas.

Sleeping with the eyes open

Monitoring the surroundings during sleep may allow the animals to detect predators. Some marine mammals and birds can sleep with one eye open at a time. Presumably with the aid of unihemispheric sleep/wakefulness, they maintain positions with the open eye facing the direction where danger or socially meaningful information is expected to occur [26–28]. It has been reported that some farm animals (e.g. the goat, sheep, and horse) and the musk deer may have SWS with their eyes open while in other (pigs and ponies) the eyes were usually closed in SWS [1–4]. The eyes were closed in behaviorally sleeping elephants and giraffes [18, 19]. SWS with two open eyes has been also reported in several avian species [29] and rabbits [30].

Most wild ungulates are colonial animals with complex social behaviors. During the day, African ungulates select an open habitat, which allows good visibility of approaching predators. They rely on coordinated action and increased vigilant behavior [31, 32]. Thus, a recent study has reported that wild giraffes select areas with minimal trees or bushes for rest at night [33]. While several individuals were vigilant (standing and feeding), the others were observed resting in a sitting position, including in the posture which is characteristic of REM sleep [19].

Mouse-deer are solitary prey animals. They often use burrows or hollow trees for resting or hiding [8]. It has been suggested that mouse-deer primarily use acoustic and olfactory cues for information regarding predators. This sensory reliance is considered one of the archaic features of this group [8, 13, 14, 34]. Interestingly, we recorded only rare instances when the mouse-deer rested or slept in the bushes including during the light hours. Both during daytime and nighttime, they usually chose a position for sleep on the open forest floor. Similar behaviors were recorded in the mouse-deer in more naturalistic conditions [14]. We have also found that the mouse-deer had a substantial portion of NREM sleep with their eyes open both while sleeping in the house and in the open forest floor.

In our prior work in marine mammals (cetaceans, fur seals, and sea lions), we showed that unilateral eye opening was linked to unilateral EEG activation or unihemispheric sleep/wakefulness. Moreover, in fur seals, both eyes were usually closed during bilateral (both low and high voltage) SWS and during REM sleep [26, 27]. These data support the hypotheses that USWS serves a sentinel function [26, 28]. As we have found during this study, whenever both eyes in the mouse-deer were visible during NREM sleep, the state of the eyes was symmetrical. Also based on visual observations, EEG slow waves appear to developed synchronously in both cortical hemispheres in the mouse-deer. There is no indication at this time that during NREM sleep the mouse-deer may exhibit interhemispheric EEG asymmetry to the extent comparable to that in marine mammals [26, 27] or birds [28]. Therefore, in contrast to the situation in marine mammals, the mouse-deer have most of their NREM sleep with both eyes fully or partially open, suggesting that visual processing may occur in these animals during bilateral EEG slow waves.

Comparative aspects of the pattern and timing of sleep in ungulates

The family Tragulidae (mouse-deer) is among the three known basal groups of the Artiodactyla. All three groups originated 40–50 m.y.a. but only the tragulids are extant. Paleontological data suggest that modern Tragulidae have common features with the

ancestral forms. Five other families of the Artiodactyla originated approximately 20–30 m.y.e. after the tragulids [9, 10]. Prior studies attempted to link some features of sleep in mammals with their evolutionary history, specifically the degree of differentiation of REM sleep. For instance, REM sleep in the armadillo and the ferret is less differentiated than in other placental mammals. It has some similarity with "H-sleep" (REM sleep with high voltage EEG) as described in the platypus, in which sleep has features of both REM and NREM sleep [35–37]. Both the armadillo and ferret have morphophysiological features which are considered archaic for placental mammals, similar to some characteristics of the mouse-deer. Another example is less differentiated REM sleep in ostriches [29, 38] which are also a basal group of birds. However, despite the phylogenetic status of the mouse-deer, features of their REM sleep do not differ substantially from that of other placental mammals or other ungulates such as the Arabian oryx and wildebeest [5, 6] which originated after the tragulids. REM sleep is well-differentiated in the mouse-deer as indicated by cortical activation, EEG theta-activity, muscle atonia, and almost continuous REMs. This discussion should not be considered as a definitive attempt to address the issue of whether phylogenetic relatedness between species explains some of the variations in sleep amounts or sleep patterns as it was examined in other publications [39].

TST and amounts of NREM sleep. Diet type was found to correlate with TST. Carnivores generally sleep more than herbivores and body mass significantly and inversely correlates with TST in herbivores [17, 39–41]. Malungo et al. [6] have reported that TST in two wild African artiodactyls (the Arabian oryx and wildebeest) is close to what would be predicted based on their body mass in comparison to other herbivores. We can further extend the comparison and include our new data for the lesser-mouse deer and the data for the Siberian musk-deer [4]. These two species are the smallest of the studied ungulates with the body mass less than 10 kg while in the prior studies the correlation was tested for the ungulates larger than 30 kg. For all studied species, the TST fall reinforces the hypothesis that sleep time in herbivores is inversely correlated with body mass (Figure 6). Following the prior discussion of this correlation, we note that the analysis and comparison did not account for phylogenetic relationships between species [6, 17, 20, 39–41]. The strength of this correlation also differs among the studies, with the highest correlations between sleep time and body mass seen when a logarithmic scale was used for body mass calculations, reflecting the several orders of magnitude in the variation in weight across animals and the much more limited range of possible sleep durations. Several factors may be responsible for the negative correlation between TST and body mass in herbivores and likely in ungulates. This topic has been extensively reviewed in other publications [17, 40, 41].

In our recording conditions, the lesser mouse-deer exhibited the largest amounts of TST and NREM sleep which have been recorded among ungulates and ruminants. There are several considerations that would favor a high level of TST and NREM sleep in the mouse-deer. First, it was suggested that foraging time in herbivores tends to increase with body mass [42]. For instance, virtually all studies on wild elephants have reported that feeding (eating, grazing) was the dominant daytime behavior accounting for between 40% and 90% of the daytime hours (e.g. Refs. [43, 44]). The difference between the amounts was usually related to the differences between the areas, between the wet and the dry season and between the animals' sex and age. Regarding smaller ungulates, there is information that zebras may spend 53%–58% of their daytime hours; grazing [45], wildebeest—40% [46], and impala—31% [47]. Captive musk-deer, which are among the smallest of ungulates (8–12 kg), spent between 18% and 20% of the 24-h period feeding [48]. In our experimental conditions, the mouse-deer were active between 12% and 24% of 24 h. The majority of this time they spent eating or looking for food, which is comparable to the values in the musk-deer. In contrast to many other ungulates, the tragulids are not obligate herbivores. In the wild along with shoots and leaves they eat fallen fruit, mushrooms, and even insects [7, 8]. The limited amount of vegetation in the undergrowth of tropical forests is compensated by a smaller amount of food needed which is also easier to digest. Therefore, a smaller amount of time needed for the mouse-deer for foraging may reduce daily activity and increase TST. These data do not contradict a hypothesis of trade-offs between foraging and sleeping time [40, 41].

A second consideration is that the lesser mouse-deer inhabits the tropical rain forest. It is not well adapted to cold or heat, with an optimal temperature range of 26.6–29.0°C [49]. When the ambient temperature is above 30°C, the animals become hyperthermic and the oxygen uptake and evaporative heat loss increases. During our recordings mean daylight ambient temperature was 31.3 ± 0.2°C and the mean maximum was 34.8 ± 0.3°C. Every day between 09:00 and 16:00 the temperature was greater than 30°C which is the upper limit of the thermoneutral zone in the mouse-deer. Tragulids do not appear to possess the carotid rete blood vessel system that has been found in all other studied artiodactyls [50, 51]. The rete is considered to allow selective brain cooling facilitating adjustment potential to hot and dry conditions [52]. We also observed that within 5–10 min after being caught, the rectal temperature of the mouse-deer was often above 40°C. A substantial amount of daytime QW and NREM was accompanied by panting. Even considering that the mouse-deer probably inhabits one of the most comfortable climates in the

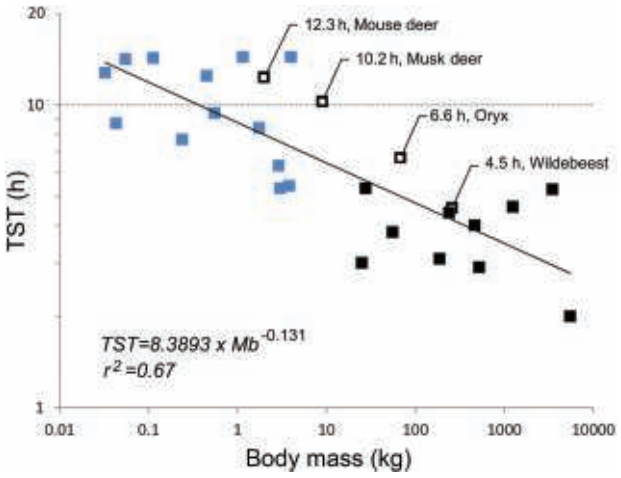

Figure 6. The correlation between TST and body mass in herbivores based on the plot presented in Malungo et al. [6] including the regression and R^2. The data (both EEG and behavioral studies) used in this plot is derived mainly from Siegel [17], with data for African ungulates from Gravett et al. [20], Davimes et al. [5], and Malungo et al. [6]. The data for the Siberian musk deer is from Sokolov et al. [4]. The data for ungulates are marked in black and for other herbivores in blue. The open rectangles mark polysomnographic studies in wild ungulates.

world living on the forest floor in the shade of the sun, high activity in the daytime will be followed by hyperthermia and the need to spend energy on cooling via panting. A great number of behavioral studies on wild African ungulates reported that the animals usually substantially reduced their activity during the daytime hours when environmental temperature is high (e.g. Refs. [45, 46]). The Arabian oryx also exhibits most of its sleep during the hottest hours [5].

Third, the risk of predation is considered to be a major factor affecting the sleep behavior of prey species [53]. The mouse-deer are prey species and wild cats are their main predators [7, 8]. The mouse-deer has a limited ability (if any) to fight and relies primarily on hiding and fleeing. Low activity, sleep, and hiding in such conditions could contribute to survival. Thus, in all of these cases sleep may serve as a state of adaptive inactivity filling spare time, conserving energy, and avoiding unnecessary activity during unfavorable hours [54].

REM sleep. The amounts of REM sleep in ungulates is smaller than in other mammalian groups. If we compare the amounts among all ungulates, then in 3 out of 4 wild species it will be smaller (on average 1.2% both in the winter oryx and in the wildebeest, 1.7% in the mouse-deer, and 3.1% in the musk deer) than in all five domesticated ungulates (2.4% in the sheep, 3.1% in the horse, 3.2% in the cow, 7.0% in the pony, and 7.3% in the pig). Ponies of differing ages and pigs (piglets) are the record holders in ungulates, although the amount of REM sleep in the horse is only a half of that in the pony [1–6]. Based on behavioral criteria, the amounts of REM sleep in the largest of living ungulates is also low (on average, less than 1% of 24 h in the giraffe and undetermined in elephants [18–20]). While it is not yet clear what factors determine variation in the amount of REM sleep in different species of ungulates, domestication may be one of the issues leading to an increased amount of daily time spent in REM sleep [6, 53]. It is possible that domestication may cause a decrease of vigilance during sleep when compared to wild ungulates and an increase of the total amount and the ratio of REM sleep in TST.

REM sleep is characterized by impaired thermoregulation [55, 56]. In addition, the onset of REM sleep may be accompanied by an increase in brain temperature [57–60], which prompted the suggestion that danger of overheating of the brain in the Arabian oryx may be a reason for the low amount of REM sleep and the shorter duration of REM sleep episodes in the summer in comparison to the winter [5]. While our data indicate that the duration of REM sleep episodes in the mouse-deer did not differ between the daytime and nighttime periods, longer episodes of REM sleep (4–8 min) were not recorded in the afternoon. Therefore, the relatively small amount of REM sleep that we have recorded in the mouse-deer seems to be in good agreement with the conclusion that the thermoregulatory abilities of the mouse-deer are limited [49].

The arousal threshold during REM sleep was shown to be higher or comparable with that during high voltage SWS or NREM sleep [61–63]. Reduced responsiveness to external stimuli along with muscle atonia may increase the vulnerability to predation. There is evidence that mammals and birds suppress REM sleep in riskier situations, such as fur seals sleeping in water (which may face killer whale or shark attacks) compared to when they sleep on land [27, 60] or pigeons sleeping on low-perches (may be vulnerable to predation from ground mammals) compared to when they slept on elevated perches [64]. This idea is supported by a significant negative correlation between amounts of REM sleep and the risk of predation both in mammals and birds [17, 39–41, 53]. It seems that shorter duration of REM sleep could not only reduce the possible adverse effects of brain overheating but also could shorten a period of reduced responsiveness to danger.

Circadian activity/timing of sleep. Among the three electrophysiologically studied species of wild ungulates, the wildebeest and Arabian oryx (in the winter) displayed predominantly nocturnal sleep patterns. In the summer, the oryx exhibited predominantly diurnal sleep [5, 6]. The musk deer did not show a preference for sleeping at any particular time of day [4]. However, the animals were recorded under laboratory conditions. In prior behavioral studies the lesser mouse-deer were reported to be diurnal [14], diurnal to cathemeral [8, 65], or crepuscular [15]. In our experimental settings, 3 out of 4 studied mouse-deer preferred to stay in their shelters during the day and moved to the open forest floor during the night as in naturalistic conditions [8, 14]. Based on the formal criteria, the mouse-deer in our study were nocturnal (since they were more active at night than during the day) and had a crepuscular activity pattern (since they displayed the main activity peak in the morning and at a smaller degree at dusk). The lesser mouse-deer also exhibited polyphasic sleep similar to that of others studied ungulates. They go to sleep at different times of the day but displayed more NREM sleep during daytime than during nighttime. The same behavioral pattern appears to be characteristic for the lesser mouse-deer in the forest of Cambodia [15].

Two factors may determine the timing of sleep in the mouse-deer. They are optimal foraging time and the risk of predation. Large artiodactyls experiencing hot environmental temperatures decrease their activity during daytime [5, 45, 46, 66, 67]. The ambient temperature in the habitat of the mouse-deer is not extremely hot but it exceeds the upper limit of the species optimal temperature zone for most of the light phase [49]. We have found that the mouse-deer reduced activity during the hottest time (09:00–16:00), and spent most of the time in NREM sleep avoiding longer episodes of REM sleep. They also increased their activity during the hours with lower temperature conditions, that is the end of the night and close to twilight hours. Thus, the mouse-deer appeared to respond to adverse temperature conditions by optimizing both the pattern of sleep and the timing of daily activity as other ungulates do.

In tropical forests, many carnivores can hunt on their prey at different times of the day [7, 8, 34, 68]. Wild cats are among the main predators of mouse-deer. Some cats are mostly nocturnal (such as the clouded leopard) while others are mostly diurnal (such as the Asiatic golden cat) or completely diurnal (the marbled cat) [7, 8, 69, 70]. However, wild cats also display crepuscular activity peaks [71] and often adjust their activity to the pattern of their prey species [72]. A strictly nocturnal or strictly diurnal activity pattern would also be maladaptive for the mouse-deer under the conditions when predators may be active at different times of the day. Therefore, the crepuscular activity peaks, polyphasic sleep, and the flexibility to adjust (cathemerality) if environmental conditions change may serve to reduce predation of the mouse-deer.

The timing of SWA

In humans and other mammals, the amounts of SWS/NREM sleep and SWA are highest in the beginning of the main sleep

period and then progressively decline to the end of the sleep period [73, 74]. The amounts of NREM in the mouse-deer were greatest in the morning following the period of highest activity which is some indication of homeostatic regulation. During the rest of the 24-h period, the average hourly SWA decreased in parallel with the amount of NREM sleep. However, the decrement of average SWA was rather small and the timing of SWA in different animals was not consistent. Thus, it is not clear if the timing of SWA during the 24-h period in the lesser mouse-deer is similar to that in other mammalian species.

Concluding remarks

Our data suggest that the pattern of sleep in the lesser mouse-deer, including polyphasic sleep, the largest TST among all studied ungulates, and a low amount of REM sleep, predominantly sternal recumbency, open eyes while asleep and crepuscular peaks of activity appear to be associated with environmental factors in the tropical forest including hot but not extreme daytime ambient temperatures, a number of potential predators which can be active at different times of the day, and the mouse-deer diet with different types of vegetation and fruit.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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Authors' Contributions

OIL, JMS, and VVR designed research and wrote the article. OIL and EAN conducted research. OIL analyzed data.

References

1. Ruckebusch Y. The relevance of drowsiness in the circadian cycle of farm animals. *Anim Behav.* 1972;**20**(4):637–643.
2. Bell FR, *et al.* The electroencephalogram of sheep and goats with special reference to rumination. *Physiol Behav.* 1973;**11**(4):503–514.
3. Dallaire A, *et al.* Sleep and wakefulness in the housed pony under different dietary conditions. *Can J Comp Med.* 1974;**38**(1):65–71.
4. Sokolov VE, *et al.* Electrophysiological study of sleep in the musk deer. *Proc Acad Sci USSR.* 1988;**302**(4):1005–1009.
5. Davimes JG, *et al.* Seasonal variations in sleep of free-ranging Arabian oryx (*Oryx leucoryx*) under natural hyperarid conditions. *Sleep*. 2018;**41**(5). doi:10.1093/sleep/zsy038.
6. Malungo IB, *et al.* Sleep in two free-roaming blue wildebeest (*Connochaetes taurinus*), with observations on the agreement of polysomnographic and actigraphic techniques. *IBRO Neurosci Rep.* 2021;**10**:142–152.
7. Kuznetsov GV. *Mammals of Vietnam*. Moscow, Russia: KMK Press; 2006.
8. Meijaard E. Tragulidae. In: Wilson DE, Mittermeier RA, eds. *Handbook of the Mammals of the World. Volume 2: Hoofed Mammals*. Madrid, Spain: Lynx Edicions; 2011:320–335.
9. Janis C. Tragulids as living fossils. In: Eldredge N, and Stanley SM, eds. *Living Fossils*. New York, NY: Springer-Verlag; 1984:87–94
10. Hackmann TJ, *et al.* Ruminant ecology and evolution: perspectives useful to ruminant livestock research and production. *J Dairy Sci*. 2010;**93**:1320–1334.
11. Timmins R, *et al. Tragulus kanchil. The IUCN Red List of Threatened Species*; 2015:e.T136297A61978576. https://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T136297A61978576.en.
12. Matsubayashi H, *et al.* Social system of the lesser mouse-deer (*Tragulus javanicus*). *Mammal Study*. 2006;**31**:111–114.
13. Prikhod'ko VI, *et al.* Behavior and phylogenetic relations among artiodactyla families (Artiodactiyla, Mammalia). *Biol Bull Rev.* 2011;**1**:345–357.
14. Matsubayashi H, *et al.* Activity and habitat use of lesser mouse-deer (*Tragulus javanicus*). *J Mamm.* 2003;**84**:234–242.
15. Gray TN. Monitoring tropical forest ungulates using camera-trap data. *J Zool*. 2018;**305**(3):173–139.
16. Zepelin H, *et al.* Mammalian sleep. In: Kryger MK, Roth T, Dement WC, eds. *Principles and Practice of Sleep Medicine*. 4th ed. Philadelphia, PA: Elsevier Saunders; 2005:91–100.
17. Siegel JM. Clues to the functions of mammalian sleep. *Nature.* 2005;**437**(7063):1264–1271.
18. Tobler I. Behavioral sleep in the Asian elephant in captivity. *Sleep.* 1992;**15**(1):1–12. doi:10.1093/sleep/15.1.1.
19. Tobler I, *et al.* Behavioural sleep in the giraffe (*Giraffa camelopardalis*) in a zoological garden. *J Sleep Res.* 1996;**5**(1):21–32.
20. Gravett N, *et al.* Inactivity/sleep in two wild free-roaming African elephant matriarchs – Does large body size make elephants the shortest mammalian sleepers? *PLoS One*. 2017;**12**(3):e0171903.
21. Williams DC, *et al.* Qualitative and quantitative characteristics of the electroencephalogram in normal horses during spontaneous drowsiness and sleep. *J Vet Intern Med*. 2008;**22**(3):630–638.
22. Lopes da Silva F, *et al.* Organization of thalamic and cortical alpha rhythm: spectra and coherences. *Electroencephalogr Clin Neurophysiol.* 1973;**35**:627–639.
23. Rougeul-Buser A, *et al.* Rhythms in the alpha band in cats and their behavioural correlates. *Int J Psychophysiol.* 1997;**26**(1–3):191–203.

24. Sterman MB. Sensorimotor EEG operant conditioning: experimental and clinical effects. *Pavlov J Biol Sci*. 1977;**12**(2):63–92.
25. Adrian ED. Afferent area in the brain of ungulates. *Brain*. 1943,**66**:89–103.
26. Lyamin OI, *et al*. Cetacean sleep: an unusual form of mammalian sleep. *Neurosci Biobehav Rev*. 2008;**32**(8):1451–1484.
27. Lyamin OI, *et al*. Sleep in the northern fur seal. *Curr Opin Neurobiol*. 2017;**44**:144–151.
28. Rattenborg NC, *et al*. Facultative control of avian unihemispheric sleep under the risk of predation. *Behav Brain Res*. 1999;**105**(2):163–72.
29. Lesku JA, *et al*. Ostriches sleep like platypuses. *PLoS One*. 2011;**6**(8):e23203.
30. Pigarev IN, *et al*. Visually triggered K-complexes: a study in New Zealand rabbits. *Exp Brain Res*. 2011;**210**:131–142.
31. Underwood R. Vigilance behaviour in grazing African antelopes. *Behaviour*. 1982;**79**:81–107.
32. Creel S, *et al*. Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community. *Behav Ecol*. 2004;**25**(4):773–784.
33. Burger, AL, *et al*. Nightly selection of resting sites and group behavior reveal antipredator strategies in giraffe. *Ecol Evol*. 2020; **10**:2917–2927.
34. Rozhnov VV. *Mediated Communication by Scent Mark in Social Behaviour of the Mammals*. Moscow, Russia: KMK Scientific Press; 2011.
35. Prudom AE, *et al*. Electrographic correlates of sleep behavior in a primitive mammal, the Armadillo *Dasypus novemcinctus*. *Physiol Behav*. 1973;**10**(2):275–82.
36. Jha SK, *et al*. Sleep and sleep regulation in the ferret (*Mustela putorius furo*). *Behav Brain Res*. 2006;**172**(1):106–13. doi:10.1016/j.bbr.2006.05.001.
37. Siegel JM, *et al*. Sleep in the platypus. *Neuroscience*. 1999;**91**(1):391–400.
38. Lyamin OI, *et al*. Sleep in ostrich chicks (*Struthio camelus*). *Sleep*. 2021;**44**(5). doi:10.1093/sleep/zsaa259.
39. Lesku JA, *et al*. History and future of comparative analyses in sleep research. *Neurosci Biobehav Rev*. 2009;**33**(7):1024–36.
40. Allison T, *et al*. Sleep in mammals: ecological and constitutional correlates. *Science*. 1976;**194**:732–734;
41. Capellini I, *et al*. Phylogenetic analysis of the ecology and evolution of mammalian sleep. *Evolution*. 2008;**62**:1764–1776.
42. Owen-Smith RN. *Megaherbivores: The Influence of Very Large Body Size on Ecology*. Cambridge, UK: Cambridge University Press; 1988.
43. Leggett K. Diurnal activities of the desert-dwelling elephants in northwestern Namibia. *Pachyderm*. 2009;**45**:20–33.
44. Lukacs DE, *et al*. Diurnal and nocturnal activity time budgets of Asian elephants (*Elephas maximus*) in a zoological park. *Anim Behav Cogn* 2016;**3**(2):63–77.
45. Regassa R. Diurnal activity pattern of Burchell's zebra (*Equus burchelli*, Gray, 1824) in Yabello Wildlife Sanctuary, Southern Ethiopia. *Int J Curr Res Biosci Plant Biol*. 2014;**1**:70–78.
46. Vrahimis S, *et al*. Daily activity of black wildebeest in a semi-arid environment. *Afr J Ecol*. 1993;**31**:328–336.
47. Kurauwone MV, *et al*. Activity budgets of impala (*Aepyceros melampus*) in closed environments: the Mukuvisi Woodland Experience, Zimbabwe. *Int J Biodivers*. 2013;**2013**:8.
48. Xue C, *et al*. Activity rhythm and behavioral time budgets of the captive forest musk deer (*Moschus berezovskii*)in spring. *Acta Theriol Sin*. 2008;**28**(2):194–200.
49. Whittow GC, *et al*. Temperature regulation in the smallest ungulate, the lesser mouse-deer (*Tragulus javanicus*). *Comp Biochem Physiol Part A Physiol*. 1977;**56**(1):23–26.
50. Fukuta K, *et al*. Absence of carotid rete mirabile in small tropical ruminants: implications for the evolution of the arterial system in artiodactyls. *J Anat*. 2007;**210**(1):112–116.
51. O'Brien HD. Cranial arterial pattern of the Sri Lankan spotted chevrotain, *Moschiola memmina*, and comparative basicranial osteology of the Tragulidae. *PeerJ*. 2015;**3**:e1451; DOI 10.7717/peerj.1451
52. Mitchell G, *et al*. The carotid rete and artiodactyl success. *Biol Lett*. 2008;**4**(4):415–418.
53. Lima SL, *et al*. Sleeping under the risk of predation. *Anim Behav*. 2005;**70**(4):723–736.
54. Siegel JM. Sleep viewed as a state of adaptive inactivity. *Nat Rev Neurosci*. 2009;**10**(10):747–753.
55. Parmeggiani PL. Thermoregulation and sleep. *Front Biosci*. 2003;**8**:s557–s567.
56. Szymusiak R, *et al*. Maximal REM sleep time defines a narrower thermoneutral zone than does minimal metabolic rate. *Physiol Behav*. 1981;**26**(4):687–690.
57. Kawamura H, *et al*. Elevation in brain temperature during paradoxical sleep. *Science*. 1965;**150**: 912–913.
58. Kovalzon VM. Brain temperature variations during natural sleep and arousal in white rats. *Physiol Behav*. 1973;**10**(4):667–670.
59. Ungurean G, *et al*. Comparative perspectives that challenge brain warming as the primary function of REM Sleep. *iScience*. 2020;**23**(11):101696.
60. Lyamin OI, *et al*. Fur seals suppress REM sleep for very long periods without subsequent rebound. *Curr Biol*. 2018;**28**(12):2000–2005.
61. Twyver VH, *et al*. Arousal threshold in the rat determined by "meaningful" stimuli. *Behav Biol*. 1972;**7**(2):205–215.
62. Grahnstedt S, *et al*. Awakening thresholds for electrical brain stimulation in five sleep-waking stages in the cat. *Electroencephalogr Clin Neurophysiol*. 1980;**48**(2):222–229.
63. Ermis U, *et al*. Arousal thresholds during human tonic and phasic REM sleep. *J Sleep Res*. 2010;**19**(3):400–406.
64. Tisdale RK, *et al*. The low-down on sleeping down low: pigeons shift to lighter forms of sleep when sleeping near the ground. *J Exp Biol*. 2018;**221**:jeb182634.
65. Kuznetsov GV, *et al*. Food consumption, digestibility and certain forms of feeding behavior inthe Javan chevrotain *Tragulus javanicus* under captiveconditions. In: Sokolov VE, Kuznetsov GV, eds. *Materials of Zoological Research in Vietnam (1987–1990)*. Moscow, Russia: Institut Evolutsionnoi Morfologii iEkologii Zhivotnykh A.N. Severtsova; 1992:39–46.
66. Sargeant G, *et al*. Thermoregulation by mule deer (*Odocoileus hemionus*) in arid rangelands of southcentral Washington. *J Mammal*. 1994;**75**:536–544.
67. Maloney SK, *et al*. Alteration in diel activity patterns as a thermoregulatory strategy in black wildebeest (*Connochaetes gnou*). *J Comp Physiol A*. 2005;**191**:1055–1064.
68. Rozhnov VV, *et al*. Ecological and ethological observations on the arboreal Viverrid species of Vietnam. In: Sokolov VE, Kuznetsov GV, eds. *Zoological Studies in Vietnam*. Moscow, Russia: Nauka; 1992:132–148.
69. Grassman LI, *et al*. Ecology of three sympatric felids in a mixed evergreen forest in North-central Thailand. *J Mammal*. 2005;**86**:29–38.
70. Kamler JF, *et al*. Diet, prey selection, and activity of Asian golden cats and leopard cats in northern Laos. *J Mammal*. 2020;**101**(5):1267–1278.
71. Mukherjee S, *et al*. Activity patterns of the small and medium felid (Mammalia: Carnivora: Felidae) guild in northeastern India. *J Threat Taxa*. 2019;**11**(4):13432–13447.

72. Foster VC, *et al*. Jaguar and puma activity patterns and predator-prey interactions in four Brazilian biomes. *Biotropica*. 2013;**45**:373–379.
73. Achermann P, *et al*. Sleep homeostasis and models of sleep regulation. In: Kryger MK, Roth T, Dement WC, eds. *Principles and Practice of Sleep Medicine*, 6th ed. New York, NY: Academic Press/Elsevier; 2017:377–387.
74. Tobler I. Phylogeny of sleep regulation. In: Kryger MK, Roth T, Dement WC, eds. *Principles and Practice of Sleep Medicine*. 4th ed. Philadelphia, PA: Elsevier Saunders; 2005:77–90.