Reindeer in the Arctic reduce sleep need during rumination

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Summary

Timing and quantity of sleep depend on a circadian (~24-hour) rhythm and a specific sleep requirement[1]. Sleep curtailment results in a homeostatic rebound of more and deeper sleep, the latter reflected in increased electroencephalographic (EEG) slow-wave activity (SWA) during non-rapid eye movement (NREM) sleep[2]. Circadian rhythms are synchronized by the light-dark cycle, but persist under constant conditions[3–5]. Strikingly, arctic reindeer behavior is arrhythmic during the solstices[6]. Moreover, the Arctic's extreme seasonal environmental changes cause large variations in overall activity and food intake[7]. We hypothesized that the maintenance of optimal functioning under these extremely fluctuating conditions would not only require adaptations in daily activity patterns, but also in the homeostatic regulation of sleep. We studied sleep using non-invasive EEG in four Eurasian tundra reindeer (*Rangifer tarandus tarandus*) in Tromsø, Norway (69°N) during the fall equinox and both solstices. As expected, sleep-wake rhythms paralleled daily activity distribution and sleep deprivation resulted in a homeostatic rebound in all seasons. Yet, these sleep rebounds were smaller in summer and fall than in winter. Surprisingly, SWA decreased not only during NREM sleep but also during rumination. Quantitative modeling revealed that sleep pressure decayed at similar rates during the two behavioral states. Finally, reindeer spent less time in NREM sleep the more they ruminated. These results suggest that they can sleep during rumination. The ability to reduce sleep need during rumination – undisturbed phases for both sleep recovery and digestion – might allow for near-constant feeding in the arctic summer.

Keywords

Arctic, reindeer, caribou, NREM sleep, rumination, slow-wave activity, sleep homeostasis, circadian rhythms

Results

Reindeer sleep can be assessed by electroencephalography

Reindeer (*Rangifer tarandus*) in the Arctic appear to have a weak or even absent circadian clock driving daily behavioral rhythms[8,9]. Daily activity patterns and the secretion of melatonin do not display 24-hour rhythms under constant light or darkness, but instead follow the environmental light conditions[10]. It is, to our knowledge, unknown if the absence of 24-hour rhythmicity under constant light conditions in reindeer also applies to sleep-wake rhythms.

Therefore, we collected electroencephalographic (EEG) data in four adult, female reindeer (*R. t. tarandus*) in winter, summer, and fall (Figure 1AB). Recordings took place at the animal facility of UiT The Arctic University of Norway (Tromsø). Lighting conditions followed civil twilight, except for winter when we covered the stable windows to simulate constant darkness. Recordings lasted for four days in each season, including two short sleep deprivations – one at midday and one at midnight (each > 2 h wake) – and a 24-hour baseline recording (5am – 5am, Figure S1). We measured brain and muscle electrophysiology by a non-invasive method, assuring minimal disturbance of the animals' physiology and well-being. Data quality was high with a good signal to noise ratio, allowing for the identification of the classical vigilance states wake, rapid eye movement (REM) sleep, and non-REM (NREM) sleep together with rumination (Figure 1AC, Table S1). During the 24-hour baseline recording, reindeer spent on average 5.4 ± 0.8 h in NREM sleep, 0.9 ± 0.2 h in REM sleep, and 2.9 ± 1.5 h ruminating (mean ± SD). Total time as well as the number and duration of episodes spent in each vigilance state did not significantly differ across seasons (Table S2). While direct transitions from REM sleep to rumination never occurred during the 24-hour recordings, all other combinations of transitions could be observed (Table S3).

Reindeer behavioral rhythms follow the seasonally changing light-dark conditions

To identify 24-hour behavioral rhythms, we compared the amount of time spent in inactive states identified from the EEG signal (NREM sleep, REM sleep, and rumination) between the first and second half (two 12-hour segments) of the 24-hour baseline recording. The 12-hour segments were shifted in 1-hour steps across the circularized 24-hour baseline recording to detect 24-hour rhythms not aligned with the fall light-dark cycle. Time spent in inactive states was contrasted between the two 12-hour segments for each of the 24 segment combinations. The distribution of all inactive states showed no significant 24-hour rhythm in winter and summer under constant lighting conditions. In fall, animals spent more time in inactive states during the dark than the light phase (Figure 2).

Reindeers' homeostatic increase in sleep pressure is less pronounced in summer and fall than in winter

Having observed consistent sleep quantity across seasons, our next objective was to assess sleep quality. The homeostatic sleep process (Process S) is driven by sleep-wake history, promoting sleep with increasing time awake[2]. These homeostatic changes in sleep pressure can be determined by the quantification of slow waves (high amplitude waves of ~0.5 – 4.5 Hz) in the EEG signal during NREM sleep. To assess sleep homeostasis in reindeer, we performed a two-hour sleep deprivation at midday and midnight (Figure S1) and calculated slow-wave activity (SWA) during NREM sleep before and after sleep deprivation. To investigate the increase of sleep pressure across prolonged waking, we compared SWA during the two hours before and after sleep deprivation and found that two hours of waking were successful in raising SWA above prior levels in all three seasons. However, we observed a marked seasonal modulation in this response. In winter, the increase was larger than in fall and summer, suggesting a larger build-up of sleep pressure during the same time spent awake (Figure 3A). To assess if sleep pressure decreases across sleep, we investigated the course of SWA across the six hours after sleep deprivation and found that SWA had decreased to baseline levels two to four hours after sleep deprivation (Figure 3B). There was no seasonal modulation for the decrease of SWA.

Sleep pressure decreases across rumination similarly to NREM sleep in reindeer

As reported above, reindeer spent only about 23% of their time in NREM sleep independent of season. Especially given the observation that already a two-hour sleep deprivation significantly increased sleep pressure, phases of NREM sleep might not be enough for sleep recovery. Thus, the question arises whether reindeer developed strategies in addition to NREM sleep to prevent the accumulation of sleep pressure. Rumination could be a candidate as this behavior shares some features with NREM sleep such as behavioral quiescence. We therefore compared these two behavioral states both on a behavioral and on the EEG level to assess whether sleep recovery in reindeer could take place during rumination. Rumination in reindeer was characterized by behavioral quiescence and body positions similar to those of NREM sleep (quiet sitting or standing). Another behavioral definition of sleep is the increase of the arousal threshold[1,11]. Thus, in an exploratory analysis, we assessed the reactions (none, directed, or undirected) of reindeer to regularly occurring noises produced when neighboring animals changed posture (sitting down or standing up). Subsequently, we compared the ratio of none, directed, and undirected reactions among vigilance states. Reactions differed most between NREM sleep and wake states, while they were intermediate for rumination (Figure S2). Directed reactions showed the largest difference. They were very common when reindeer were awake (45 %), rare when in NREM sleep (5 %) and intermediate when ruminating (25 %).

Thus, based on the behavioral reaction to a disturbance, reindeer may not always be awake when ruminating. The assumption that during rumination animals can assume a "sleep-like" state has further been supported by electroencephalography. Brain oscillations characteristic of NREM sleep – such as slow waves and sleep spindles – have been observed during rumination in both domestic ruminants[12–16] and one wild ruminant, the Lesser mouse-deer (*Tragulus kanchil*)[17]. In reindeer, we detected slow waves and sleep spindles in the rumination EEG using established algorithms[18,19]. Additionally, we found a temporal coupling between slow waves and sleep spindles that resembled the slow-wave spindle coupling specific to NREM sleep (Figure S3).

To our knowledge, the quantitative differences between sleep recovery during rumination and NREM sleep and its effect on sleep homeostasis have never been systematically investigated. To this end we used sleep pressure dissipation to assess the functional relevance of NREM sleep characteristics during rumination in reindeer. Within rest periods (not interrupted by waking bouts longer than 5 min) SWA during NREM sleep was significantly lower after rumination than before rumination (Figure 4A). This SWA decrease across rumination was positively correlated with rumination duration (Figure 4B). Furthermore, when calculating SWA from the rumination EEG, SWA displayed an initial build-up and then a continuous dissipation during long (> 30 min) rumination episodes (Figure 4C). When omitting the low frequencies most affected by chewing (2 – 4.5 Hz instead of 1 – 4.5 Hz), the decay in SWA was preserved (data not shown). These SWA time courses resembled those observed during long NREM sleep episodes (Figure 4D). These analyses included all rumination and NREM sleep episodes of sufficient length independent of the preceding vigilance state.

To quantitatively assess if the decline of SWA across rumination indicates sleep pressure dissipation during rumination, we modeled SWA dynamics during NREM sleep episodes based on the homeostatic Process S, as defined by the two-process model of sleep regulation[20]. We modeled Process S to the increase and decrease of SWA according to the distribution of wake, REM sleep, NREM sleep, and rumination (Figure S4A). Then, for each recording, we fitted two models to the dynamics of SWA during NREM sleep: the first assumes that rumination decreases sleep pressure as NREM sleep does, the second that rumination increases sleep pressure as when awake. A direct comparison of these two alternative models resulted in a significant preference for the first model, i.e., sleep pressure decreases during rumination (Δ BIC weighted mean ± SD = -129 ± 41.2, Figure 4E). The estimated parameters of the preferred model did not significantly differ between seasons for Process S dynamics, but the time constants for the synchronization and desynchronization of SWA to Process S showed differences between seasons. They describe how fast SWA reaches the levels that are associated with Process S within a given NREM sleep episode. While the synchronization of SWA to Process S was faster in fall, the desynchronization of SWA from Process S was faster in summer than in the other seasons (Figure S4BC).

Total rumination duration and total NREM sleep duration within 24 hours were negatively correlated (Figure 4F), indicating that reindeer spend less time in NREM sleep the more they have been ruminating.

Discussion

The present study on reindeer sleep regulation across the year was the first to employ EEG in reindeer. The experimental setup of this study does not reflect field conditions. As animals were held in a stable during the experiment with food provided ad libitum and regulated ambient temperature, they were not challenged as in the wild. Nevertheless, as animals were well adapted to the experimental setup and because voluntary food intake of reindeer changes across the year even when held in a captive environment with food provided ad libitum[7], we are convinced that our findings can be related to seasonal changes in reindeer physiology.

Our results are in line with previously reported reindeer activity data suggesting the lack of 24-hour rhythms under constant light conditions during the arctic winter and summer and rhythmic behavior during the equinoxes[6]. This further supports the assumption that the reindeer circadian system may be weak or absent[9,10] when solely considering behavioral rhythms. While activity rhythms based on EEG recordings, i.e., sleep-wake rhythms, precisely reflected activity rhythms as assessed by actigraphy, total daily sleep time did not mirror seasonal differences in overall activity. Despite higher activity levels during the summer months[6], we showed that reindeer do not decrease their total sleep time. Regardless of environmental conditions, reindeer appear to require a similar amount of sleep across the year. These results are opposed to findings in various other animals that demonstrate pronounced flexibility in total sleep time in response to environmental conditions when metabolic demands are competing with sleep requirements[21–25]. Another wild ungulate, the Arabian oryx (*Oryx leucoryx*) native to steppe and desert areas in the Arabian Peninsula, decreases sleep time in summer to almost half of its winter sleep time[25]. Increased time spent foraging and grazing during summer due to decreased food availability and quality might be the cause of this compromise on time spent sleeping. Reindeer also experience a massive increase in food intake during summer, peaking in August[7]. Nevertheless, we did not find a compromise on sleep time in reindeer, which speaks for an essential adaptation and tight regulation of sleep. Constant sleep quantity across seasons further implies other strategies to cope with limited sleep time during the arctic summer when food intake is high.

In this context, we showed that the homeostatic increase in sleep depth after prolonged wake periods is less pronounced in summer and fall compared to winter. This observation could be interpreted as a slower accumulation of sleep pressure during wake periods in summer and fall, possibly related to increased food intake[26] and/or repetitive behaviors[27], which both have been

related to a slower homeostatic sleep pressure build-up. Data of the four reindeer investigated in this study, however, did not reveal seasonal differences in the time constants for Process S and thus do not provide evidence for a slower accumulation of sleep pressure during waking. Alternatively – consistent with the dynamics of the exponential saturating function describing the relationship between waking and SWA – the smaller sleep rebounds in summer and fall might be the result of overall elevated sleep pressure, i.e., a reduced capacity for a further increase in sleep pressure. To compare sleep pressure across seasons, absolute SWA levels are not suited, as physiological differences are likely masked by differences arising from technical inconsistencies. Instead, results from the modeling of the homeostatic Process S can be considered: The faster synchronization of SWA to the levels of Process S in fall compared to other seasons may indicate higher sleep pressure in fall because the build-up of SWA within a NREM sleep episode is proportional to the level of Process S[28]. Reindeer may experience highest sleep pressure in fall due to the uneven distribution of daily sleep time in the fall season. This seasonal sleep pattern may lead to the accumulation of sleep debt during the light phase when reindeer sleep less. During summer, on the other hand, it appears that other mechanisms come into play, as sleep is distributed evenly across the 24-hour day, presumably keeping sleep pressure on a more constant level. Another finding of the modeling of process S is the faster desynchronization of SWA from Process S in summer as compared to winter and fall. This acceleration of "waking up" might be related to the alerting effects of light[29]. Furthermore, studies in both humans[30,31] and rats[32–35] suggest that light suppresses the build-up of SWA. In reindeer, constant exposure to light during the summer experiment could therefore explain the less pronounced increase in SWA after sleep deprivation.

Our data demonstrated that phases of rumination contribute to sleep recovery and that reindeer spend less time in NREM sleep the more they ruminate, likely because they sleep during rumination. This strategy allows reindeer to cover sleep and digestive needs simultaneously. This is of special importance in reindeer, as a large part of their yearly food intake occurs during the summer months when food is abundant[7]. As rumination is crucial to increase the access to nutrients by reducing particle size of the ingested food[36–38], total time spent ruminating likely optimizes energy efficiency. Thus, spending enough time ruminating seems to be crucial to gaining weight in summer and fall in anticipation of winter. Accordingly, the observation that sleep pressure decreases during rumination suggests that reindeer do not have to compromise on sleep recovery when they spend more time ruminating and less time in NREM sleep.

Previous sleep EEG studies in wild ruminants showed that rumination co-occurs with NREM sleep in approximately 70 % of total rumination time in the Lesser mouse-deer[17], a study in dromedary camels (*Camelus dromedarius)* considered rumination as an independent state[39], and studies in the Arabian oryx[25] and the Blue wildebeest (*Connochaetes taurinus*)[40] have not reported data on rumination at all. Further investigation is needed to elucidate the involvement of rumination in sleep recovery in other ruminants. This study is, to our knowledge, the first to

show that phases of rumination not only share EEG characteristics with NREM sleep, but that they also contribute to the dissipation of sleep pressure similarly to NREM sleep. In conclusion, this study suggests that reindeer sleep regulation contributes to the optimization of energy intake in the highly seasonal arctic environment. Seasonal adaptations to sleep-wake behavior together with a year-round intact homeostatic regulation of sleep may support reindeer by keeping sleep pressure in an optimal range. Furthermore, as reindeer seem to be able to reduce sleep need during rumination, they can cover both sleep restorative and digestive requirements simultaneously. This ability likely supports near-constant feeding in summer when food is abundant in anticipation for the long and food sparse arctic winter.

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Author contributions

Conceptualization, S.A.B., G.C.W., R.H., S.A.M. and M.F.; Methodology, M.F., S.A.M., M.J., P.F., S.A.B., G.C.W. and R.H.; Investigation, M.F., S.A.M., M.J., M.A.S., S.A.B., G.C.W. and R.H.; Writing – Original Draft, M.F.; Writing – Review & Editing, S.A.M., M.J., P.F., S.A.B., M.A.S, G.C.W. and R.H; Funding Acquisition, G.C.W., M.A.S., S.A.B., R.H and M.F.; Resources, R.H., G.C.W., M.A.S., S.A.B. and P.F.; Supervision, R.H., G.C.W, M.A.S. and S.A.B.

Declaration of interests

The authors declare no competing interests.

STAR Methods

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources can be directed to the lead contact, Reto Huber (reto.huber@kispi.uzh.ch) and to Gabriela Wagner (gabriela.wagner@nibio.no).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- EEG raw data have been deposited at Zenodo (10.5281/zenodo.10125593) and are publicly available as of the date of publication. Pre-processed data as well as video data reported in this paper will be shared by the lead contact upon request. DOIs are listed in the key resources table.
- All original code has been deposited at Zenodo (10.5281/zenodo.10182325) and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL DETAILS

Animals

Four female, adult (3 years at study start, 4 years at study end) reindeer (*Rangifer tarandus tarandus*) held at the animal facility of UiT The Arctic University of Norway (Tromsø) were included in the study. For EEG data acquisition, animals were brought from an outdoor enclosure into a stable. The ambient temperature in the experimental stable was kept at approximately 10°C. In addition to natural light from a big stable window, programmable ceiling lamps provided light following civil twilight. Animals were kept in individual stalls and secured with a rope attached to a collar with room to eat, sit, and lie down. The reindeer had been trained previously for research purposes, accepted the presence of humans in the stable well, and had in a pilot study already been adapted to the non-invasive EEG method.

All procedures were approved by the Norwegian Food Safety Authority (Mattilsynet, ID 13982).

METHOD DETAILS

Experimental setup

Measurements were conducted in the months of July (constant light), September (8.75 h darkness), and December. In December, stable windows were covered with aluminum foil to simulate constant darkness (outside conditions: 4.4 hours of civil twilight). No data were collected in spring due to restrictions related to the COVID19 pandemic.

For each of the three experiments animals spent about one week in the stable with an adaptation day, two preparation days (i.e., shaving, mounting bandage harnesses and gluing electrodes, etc., see below), and four EEG acquisition days (Figure S1). Data acquisition included a 46-hours adaptation period (incl. 2-3 hours disturbance for feeding and readjusting the EEG setup after 20 hours) followed by a 17-hour period with two 2-hour sleep deprivation phases (see below: sleep deprivations) and ended with a 28-hours recording period with only one very short disturbance (approx. 25 min after approx. 11 hours) for feeding. Within these 28 hours, a baseline was defined that started and ended at 5 am, the time at which the light was turned on during the fall measurements. Directly after termination of the EEG data acquisition period, animals were released into their outdoor enclosure.

The animals had *ad libitum* access to a concentrate feed developed for reindeer (Felleskjøpet Agri, Norway). In addition, they also received willow (*Salix sp.*) and birch twigs (with fresh leaves in summer and fall and dry twigs in winter) and a mixture of lichens (mainly *Cladonia stellaris* and *C. rangiferina*) year-round. During the preparation days and the first day of EEG data acquisition, reindeer received fresh food in the morning between 9 am and 11 am. During the period of sleep restrictions, they received fresh food at the beginning of each of the two short sleep deprivations (at 12 am and 12 pm respectively) and during the baseline recording period at approximately 4 pm. The exact timepoint to enter the stable around 4 pm during the baseline recording was adjusted according to behaviors observed on the video to avoid waking animals up. Before feed distribution, old and new feed were weighed to estimate food intake. In winter dry twigs were offered together with pelleted feed. The twigs were weighed separately. In summer and fall only feed pellets were weighed as twigs with fresh leaves were given separately.

Sleep deprivations

During each experiment animals were kept awake for two hours from 12 – 2 pm (midday sleep deprivation) and from 12 – 2 am (midnight sleep deprivation) by distributing fresh food at the beginning of the 2-hours period encouraging animals to eat and by the continuous presence of two investigators in the stable (talking, walking around, petting the animals, etc.). Animals were continuously observed by the investigators to ensure that they were awake during the whole two hours.

Video recordings

Video was recorded continuously by one (winter) to two (summer and fall) surveillance cameras (Milesight 5MP Weatherproof Mini Dome Network Camera, MS-C5375-PB) mounted to the stable wall. Data were transferred to a computer situated outside the stable through an ethernet connection and stored locally. Video quality remained high during dark periods due to the camera's night vision (infra-red light) functionality. All animals were visible on the video recordings, and it was possible to distinguish different behaviors, body positions, eye closures, and chewing. The video recordings served two essential purposes. During the experiment they were used for monitoring the animals' well-being and activities without causing any disturbance. Subsequently, video data were used in the process of establishing sleep scoring rules for reindeer. In the final scoring process video data were utilized to confirm instances of rumination and REM sleep when making a decision based solely on EEG data was not feasible (see Table S1).

EEG data acquisition

Two days prior to the experiment patches of hair located at the desired electrode positions were shaved and the remaining hair was removed with hair removal cream. All procedures were performed without anesthesia and did not cause visible signs of stress in the animals. Two surface electrodes (Grass Gold Cup Electrodes) for non-invasive measurement of brain and muscle activity were placed on the animals' forehead over frontal brain regions and two electrodes over the neck or ear muscles. The common reference and the ground electrode were placed between the antlers above the sagittal crest. Electrodes were glued on the skin with collodion and filled with an electrolyte gel. To ensure good contact, the skin was cleaned with a peeling cream and alcohol before attaching the electrodes. Impedances were checked with a mobile app and kept as low as possible by re-filling the electrodes with electrolyte gel and adding collodion glue. Small, lightweight, portable recording devices (Avatar EEG, electrical Geodesics – EGI, discontinued) were used to record EEG with a sampling rate of 500 Hz. Devices were attached to the reindeer's back with a single use harness made of self-adhesive elastic bandages. Recordings lasted for approx. 91 hours (for details see above). Winter EEG data of one reindeer were excluded due to bad data quality resulting in a sample size of $n = 3$

for winter EEG data. Data of another reindeer during the adaptation period were also of bad quality. This first part of the recording was therefore excluded, resulting in a recording length of 43 hours. In summer no data had to be excluded (n = 4) and in fall (n = 4) one recording was only 72 hours long due to missing data during the adaptation period.

Sleep and rumination scoring

For sleep and rumination scoring EEG data were bandpass filtered from 0.5 to 40 Hz and down sampled to 128 Hz. For an additional bipolar EEG signal, the signal of one EEG channel was subtracted from the other. EMG data were also re-referenced to a bipolar referencing and filtered between 20 and 40 Hz for the visualization of muscle activity. Non-rapid (NREM) and rapid eye movement (REM) sleep stages as well as rumination were visually identified in 4-second epochs from those signals (2 EEG, 1 bipolar EEG, 1 EMG). In case of uncertainty, the behavior of the animal was checked from the video recordings. This was sometimes necessary for REM sleep and rarely for rumination. Consequently, NREM sleep was always defined based on the EEG whereas rumination and REM sleep were defined based on the video in case of unclarity of the EEG signal. The criteria applied for scoring are summarized in Table S1.

Pre-processing of NREM sleep and rumination EEG data

Filtered (0.5 – 40 Hz) and down sampled (128 Hz) EEG data were used for the following procedures. Artifacts during NREM sleep were identified by visual inspection during scoring and by semiautomatically identifying artifacts on a 4-second epochs resolution based on power thresholds in the 0.75 – 4.5 Hz and 20 – 30 Hz bands. Additionally, the same semiautomatic procedure was used for the identification of artifacts during rumination. Note that the included rumination epochs still contained the typical regular chewing artifacts. Spectral power was calculated for each 4-second epoch by means of a fast Fourier transform routine (Hanning window, frequency resolution of 0.25 Hz). Spectral power data together with scoring and artifact information were temporally aligned across all reindeer and seasons. To calculate SWA during NREM sleep, power values were averaged over 1 – 4.5 Hz for all artifact-free NREM sleep epochs. Subsequently, normalized SWA values were calculated by dividing each value by the mean SWA of the baseline recording. This way, absolute differences between reindeer and seasons that might have arisen from differences in the recording devices and the placement of electrodes, were accounted for. Finally, normalized SWA was averaged over the time windows of interest. To calculate SWA during rumination, the same frequency range (1 – 4.5 Hz) and procedure for normalization (all values divided by mean baseline SWA during ruination) were used.

QUANTIFICATION AND STATISTICAL ANALYSIS

Episodes

For the comparison of episode number and duration, the following criteria were applied: NREM sleep episodes were defined as consecutive NREM sleep epochs with interruptions of less than one minute of rumination or wake and less than 30 seconds of REM sleep. Rumination episodes were terminated by interruptions of at least one minute of NREM sleep or wake and by at least 30 seconds of REM sleep. For REM sleep episodes, interruptions of 30 seconds or more terminated the episode. The minimum episode duration was one minute for NREM sleep and rumination and 30 seconds for REM sleep.

Behavioral rhythms

To quantify 24-hour sleep-wake rhythms, sleep scoring data of the 24-hour baseline recordings were used in a circular way. Each 24-hour baseline recording was split into two 12-hour segments. These segments were shifted in 1-hour steps resulting in 24 segment combinations. For each combination, total sleep time (incl. NREM sleep, REM sleep, and rumination) was calculated and compared between the two 12-hour segments for each season separately using paired t-tests. P-values were adjusted using the Benjamini–Hochberg (step-up) procedure to control for the false discovery rate (FDR).

Sleep Deprivation

To assess the effects of prolonged waking (short sleep deprivation) on sleep depth, SWA during NREM sleep was averaged over 2-hour time windows including two hours before sleep deprivation and six hours after sleep deprivation. The sleep deprivation time window was defined separately for each animal and time point as starting after the last sleep episode (either NREM sleep, REM sleep, or rumination) and ending before the first sleep episode. To assess the effect of time window and the interaction with season, linear mixed models were computed with time window (time from sleep deprivation) and season as fixed effects and time point (midday and midnight sleep restriction) and animal as random effects (random intercept). For the SWA change after sleep deprivation (including three time windows), post-hoc pairwise comparisons were performed with Tukey correction.

Slow-wave activity across rumination

To investigate the decrease in SWA across rumination episodes, inactivity episodes of all reindeer and seasons were pooled (winter: 2 x 91 h, 1 x 43 h; summer: 4 x 91 h; fall: 3 x 91 h, 1 x 72 h). Inactivity episodes included NREM sleep, REM sleep, and rumination and were terminated by five or more minutes of continuous wake. Only inactivity episodes containing at

least 5 min of rumination were considered. SWA was then averaged over the NREM sleep epochs prior to rumination and over the NREM sleep epochs following rumination (at least one minute of NREM sleep each). Only NREM sleep epochs within 15 min before and after rumination were included for the calculation of SWA. A paired t-test was used for the comparison of SWA from before to after rumination. Pearson correlation was used to correlate this SWA change with rumination duration.

For the visualization of SWA changes within long rumination and NREM sleep episodes, episodes that contained at least 30 min of NREM sleep or rumination, respectively, were included. For rumination, 1 min or longer breaks by other vigilance states terminated an episode. For NREM sleep, interruptions of up to 5 min were permitted to obtain NREM sleep episodes of sufficient length. Subsequently, all artifact-free epochs of the included episodes were aligned and for each corresponding epoch, mean SWA over all episodes was calculated.

Relationship between rumination and NREM sleep durations

For the relationship between total NREM sleep and total rumination time, linear mixed models were calculated with animal and season as random effects (random intercept). All analyses were performed in Matlab R2020b and R 4.2.1 (packages: "lme4", "lmerRest", "emmeans").

Modeling of Process S and SWA during NREM sleep

Process S was modeled based on the distribution of wake, NREM sleep, REM sleep, and rumination (4 seconds resolution) for all 11 recordings (Winter: 2 x 91 h, 1 x 43 h; Summer: 4 x 91 h; fall: 3 x 91 h, 1 x 72 h). Across wake and REM sleep, an exponential saturating increase and during NREM sleep, an exponential decrease was assumed according to these formulas:

Increases Process S: $S[t+1] = U - (U - S[t]) \times e^{(-dt_W/\tau_W)}$

Decreases Process S: $S[t+1] = L + (S[t] - L) \times e^{(-dt_N/\tau_N)}$

U: Upper asymptote, L: Lower asymptote, dt_W: Time spent in wake and REM sleep (and rumination if model assumes rumination increases Process S), dt_N: Time spent in NREM sleep (and rumination if model assumes rumination decreases Process S), τ_W: Time constant for increase Process S, τ_N: Time constant for decrease Process S

Process S is generally predicted from SWA, yet SWA measures could not directly and accurately be fitted to Process S dynamics. As in humans, it takes a few minutes for SWA in reindeer to reflect Process S levels, and the high sleep fragmentation in reindeer made NREM sleep episodes of more than 10 min rare and, thus, difficult to estimate the level of Process S. Therefore, both SWA dynamics and Process S were modeled together. The SWA dynamics

were fitted to SWA measured in reindeer and depend on Process S according to the following formulas:

Increase SWA: $SWA[t+1] = -e^{(-dt_N/\tau_{Synchro})} \times (S[t] - SWA[t]) + S[t+1]$

Decrease SWA: $SWA[t+1] = L + (SWA[t] - L) \times e^{(-dt_W/\tau_{Desynchro})}$

$\tau_{Synchro}$: time constant for SWA to reach the level of Process S during a NREM sleep episode, $\tau_{Desynchro}$: Time constant for SWA to decrease during a wake or REM sleep episode. dt_W: Time spent in wake and REM sleep (and rumination if model assumes rumination increases Process S), dt_N: Time spent in NREM sleep (and rumination if model assumes rumination decreases Process S)

The parameters of the model were estimated by minimizing the Residual Sum of Squares (RSS) between the modeled SWA and the measured SWA. R packages "optimx" and the algorithm "nlminb" were used to minimize the RSS. The upper asymptote of Process S (U) was fixed to 5.13 [relative SWA level] and the lower asymptote (L) to 0.09 [relative SWA level] across all recordings. These values were derived from SWA data by calculating the mean across recordings of the 1st (lower asymptote) and 99th (upper asymptote) percentiles of relative SWA.

Initial values used for optimization were defined as:

$\tau_W = 10$; $\tau_N = 3$; $\tau_{Desynchro} = 0.005$; $\tau_{Synchro} = 0.02$; Initial value Process S = 2

Lower and upper bounds of parameters, respectively, were defined as:

Initiation of Process S: 0.1 – 5 [relative SWA level]
Process S time constants: 0.5 – 30 [hour]
SWA time constants: 0.00005 – 24 [hour]

To assess the involvement of rumination in sleep homeostasis, two different models were fitted for each recording separately: 1) Parameters for rumination fixed, same as NREM sleep, and 2) Parameters for rumination fixed, same as wake. To quantify the fit of each model by taking the model complexity into account (i.e., how many parameters the model includes), the Bayesian Information Criterion (BIC) was calculated, assuming a normal distribution of the residuals. To compare the resulting BICs between the two models, a weighted (by the amount of rumination in the data) paired t-test (R package "weights") was performed. Parameters that resulted from the optimization of model 1 are reported in Table S4. Only in one of the 11 recordings one parameter (time constant for wake) reached the upper limit. This limit was chosen as the highest reasonable value in regard to previous studies that have reported parameters from the modeling of Process S in humans and rats[32,41].

Arousal threshold analysis

For a comparison of the arousal threshold across different vigilance states, a characteristic noise that was regularly produced by the reindeer themselves during the whole experiment was utilized. All four reindeer were kept in one stable in evenly distanced stalls. Reindeer were placed in every second stall. Each stall was equipped with a rubber mat for increased comfort when reindeer were laying down. When standing, however, only the front feet were on the mat while the hind legs were behind the mat. Whenever a reindeer lay down or stood up, it caused a noise due to the hoofs scratching on the metal floor. These noises were used as an external stimulus to access the reactions of neighboring reindeer. In total, 48 hours (5 am – 5 am) of the summer recordings were analyzed, including the two short sleep deprivations (12 pm – 2 pm and 12 am – 2 am) and the 24-hour baseline recording as defined previously. Using summer recordings allowed for the most accurate analysis of video data due to constant light and no data loss. In a first step, both video recordings (one video per two reindeer) were manually inspected until one reindeer stood up or lay down. The exact timepoint, the reaction of the neighboring reindeer (one or two reindeer of interest), and the most likely vigilance state of the reindeer of interest (based on the video) were noted. An event was not considered in the following cases: 1) If a standing-up was caused by a disturbance that resulted in a simultaneous reaction in all reindeer, 2) If the reindeer of interest was engaged in eating, drinking, or grooming. The exact timepoint was defined as the moment of the first reaction of the reindeer of interest. In case of no reaction, the timepoint that caused the loudest noise during the process of the body position change was chosen.

Reactions were separated into three categories:

0: No reaction (no movement of head or opening of eyes visible)

1: Undirected reaction (movement of head, but not directed to the noise-causing reindeer, and/or clear opening of eyes)

2: Directed reaction (looking at the noise-causing reindeer, i.e., a clear turn of the head towards the noise-causing reindeer)

In a second step, all registered timepoints were converted to find the same timepoints in the EEG recordings. The vigilance state derived from the EEG recording was noted for each event. For an optimal synchronization of the video and EEG data (EEG time was usually slightly ahead of video time), the most prominent movements caused by the change of the head position during NREM sleep were matched with artifacts in the EEG signal. Accordingly, the shift between EEG and video time was estimated for each reindeer (range 35 – 60 sec). From the resulting EEG timepoints the following rules were applied for a consistent definition of the current vigilance state: The vigilance state was defined as NREM sleep if there was a NREM sleep episode at the estimated timepoint, if a NREM sleep episode ended within 30 sec before the estimated timepoint, or if a NREM sleep episode started and ended within 30 sec after the

estimated timepoint. The same procedure was applied for rumination and REM sleep. Otherwise, the vigilance state was defined as wake. Due to a very low number of events (n = 9, pooled sample), reactions during REM sleep were excluded for all analyses.

Slow-wave detection, spindle detection and cross-frequency coupling analysis

Electroencephalographic data of one recording from summer (43 hours, including two short sleep deprivations and a baseline recording) with a visible spindle peak in the power spectrum (Figure S3) was used for analyses. Data were filtered between 0.1 and 45 Hz and down sampled to 256 Hz.

For slow wave detection, a method similar to that described by Leach and colleagues[19] was applied. In short, data were filtered between 0.5 – 4 Hz, slow waves were detected by their negative peaks and included when the frequency was 0.5 – 4 Hz. Slow wave duration was defined as the time from the negative zero-crossing before the negative peak to the negative zero-crossing after the negative peak. Sleep spindles were detected by an algorithm described by Ferrarelli and colleagues[18]. Briefly, data were filtered between 12 and 15 Hz and a sleep spindle was detected if the signal amplitude exceeded an upper threshold of 5 times the mean signal amplitude. A lower threshold of two times the mean signal defined the beginning and end of a sleep spindle. Spindle density was defined as the number of sleep spindles per minute of NREM sleep.

Cross-frequency coupling analysis was performed for all epochs with a spindle frequency higher than 12 spindles/min. The coupling between phase in the delta range (0.75 – 4 Hz) and power in higher frequencies (4 – 40 Hz) was calculated on a 20-second epoch resolution with 4 second overlap using the Matlab toolbox fieldtrip[42]. This computation was performed similarly as described by Trot and colleagues[43] and Krugliakova and colleagues[44]. Briefly, a modulation index (MI) was calculated that deviates from zero if the observed amplitude distribution of higher frequencies over 18 phase bins of the delta range deviated from a uniform distribution. As the MI depends on the signal-to-noise ratio, i.e., power differences, a Z-transformed MI was calculated (zMI) by repeatedly (n = 200) shuffling the delta phase time series in relation to the fast frequency amplitude timeseries and using the resulting null distribution for transformation of the MI. Reported zMI values represent the mean over all included epochs for rumination and NREM sleep separately.

References

1. Campbell, S.S., and Tobler, I. (1984). Animal sleep: a review of sleep duration across phylogeny. Neurosci Biobehav Rev *8*, 269–300.

2. Achermann, P., and Borbély, A.A. (2017). Sleep homeostasis and models of sleep regulation. In Principles and Practice of Sleep Medicine, M. H. Kryger, T. Roth, and W. C. Dement, eds. (Philadelphia: Elsevier), pp. 377–387.

3. Welsh, D.K., Takahashi, J.S., and Kay, S.A. (2010). Suprachiasmatic nucleus: cell autonomy and network properties. Annu Rev Physiol *72*, 551–577. 10.1146/annurev-physiol-021909-135919.

4. Czeisler, C.A., Duffy, J.F., Shanahan, T.L., Brown, E.N., Mitchell, J.F., Rimmer, D.W., Ronda, J.M., Silva, E.J., Allan, J.S., Emens, J.S., et al. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. Science *284*, 2177–2181. 10.1126/science.284.5423.2177.

5. Edgar, D.M., Kilduff, T.S., Martin, C.E., and Dement, W.C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. Physiol. Behav. *50*, 373–378. 10.1016/0031-9384(91)90080-8.

6. van Oort, B.E.H., Tyler, N.J.C., Gerkema, M.P., Folkow, L., Blix, A.S., and Stokkan, K.-A. (2005). Circadian organization in reindeer. Nature *438*, 1095–1096. 10.1038/4381095a.

7. Larsen, T.S., Nilsson, N.O., and Blix, A.S. (1985). Seasonal changes in lipogenesis and lipolysis in isolated adipocytes from Svalbard and Norwegian reindeer. Acta Physiol. Scand. *123*, 97–104. 10.1111/j.1748-1716.1985.tb07566.x.

8. van Oort, B.E.H., Tyler, N.J.C., Gerkema, M.P., Folkow, L., and Stokkan, K.-A. (2007). Where clocks are redundant: weak circadian mechanisms in reindeer living under polar photic conditions. Naturwissenschaften *94*, 183–194. 10.1007/s00114-006-0174-2.

9. Lu, W., Meng, Q.-J., Tyler, N.J.C., Stokkan, K.-A., and Loudon, A.S.I. (2010). A circadian clock is not required in an arctic mammal. Curr. Biol. *20*, 533–537. 10.1016/j.cub.2010.01.042.

10. Stokkan, K.-A., van Oort, B.E.H., Tyler, N.J.C., and Loudon, A.S.I. (2007). Adaptations for life in the Arctic: evidence that melatonin rhythms in reindeer are not driven by a circadian oscillator but remain acutely sensitive to environmental photoperiod. J. Pineal Res. *43*, 289–293. 10.1111/j.1600-079X.2007.00476.x.

11. Neckelmann, D., and Ursin, R. (1993). Sleep stages and EEG power spectrum in relation to acoustical stimulus arousal threshold in the rat. Sleep *16*, 467–477. 10.1093/SLEEP/16.5.467.

12. Klefot, J.M., Murphy, J.L., Donohue, K.D., O'Hara, B.F., Lhamon, M.E., and Bewley, J.M. (2016). Development of a noninvasive system for monitoring dairy cattle sleep. J. Dairy Sci. *99*, 8477–8485. 10.3168/JDS.2015-10695.

13. Bell, F.R., and Itabisashi, T. (1973). The electroencephalogram of sheep and goats with special reference to rumination. Physiol. Behav. *11*, 503–514. 10.1016/0031-9384(73)90037-1.

14. Perentos, N., Martins, A.Q., Watson, T.C., Bartsch, U., Mitchell, N.L., Palmer, D.N., Jones, M.W., and Jennifer Morton, A. (2015). Translational neurophysiology in sheep:

Measuring sleep and neurological dysfunction in CLN5 Batten disease affected sheep. Brain *138*, 862–874. 10.1093/brain/awv026.

15. Ruckebusch, Y. (1972). The relevance of drowsiness in the circadian cycle of farm animals. Anim. Behav. *20*, 637–643. 10.1016/S0003-3472(72)80136-2.

16. Perentos, N., Martins, A.Q., Cumming, R.J.M., Mitchell, N.L., Palmer, D.N., Sawiak, S.J., and Morton, A.J. (2016). An EEG investigation of sleep homeostasis in healthy and CLN5 batten disease affected sheep. J. Neurosci. *36*, 8238–8249. 10.1523/jneurosci.4295-15.2016.

17. Lyamin, O.I., Siegel, J.M., Nazarenko, E.A., and Rozhnov, V. V (2022). Sleep in the lesser mouse-deer (Tragulus kanchil). Sleep *45*. 10.1093/sleep/zsab199.

18. Ferrarelli, F., Huber, R., Peterson, M.J., Massimini, M., Murphy, M., Riedner, B.A., Watson, A., Bria, P., and Tononi, G. (2007). Reduced sleep spindle activity in schizophrenia patients. Am J Psychiatry *164*, 483–492. 10.1176/ajp.2007.164.3.483.

19. Leach, S., Chung, K.Y., Tüshaus, L., Huber, R., and Karlen, W. (2020). A protocol for comparing dry and wet EEG electrodes during sleep. Front. Neurosci. *14*. 10.3389/fnins.2020.00586.

20. Borbély, A.A. (1982). A two process model of sleep regulation. Hum Neurobiol *1*, 195–204.

21. Northeast, R.C., Vyazovskiy, V. V., and Bechtold, D.A. (2020). Eat, sleep, repeat: the role of the circadian system in balancing sleep–wake control with metabolic need. Curr. Opin. Physiol. *15*, 183–191. 10.1016/j.cophys.2020.02.003.

22. Siegel, J.M. (2009). Sleep viewed as a state of adaptive inactivity. Nat. Rev. Neurosci. *10*, 747–753. 10.1038/nrn2697.

23. Rattenborg, N.C., Voirin, B., Cruz, S.M., Tisdale, R., Dell'Omo, G., Lipp, H.P., Wikelski, M., and Vyssotski, A.L. (2016). Evidence that birds sleep in mid-flight. Nat. Commun. *7*. 10.1038/ncomms12468.

24. Lesku, J.A., Rattenborg, N.C., Valcu, M., Vyssotski, A.L., Kuhn, S., Kuemmeth, F., Heidrich, W., and Kempenaers, B. (2012). Adaptive sleep loss in polygynous pectoral sandpipers. Science *337*, 1654–1658. 10.1126/science.1220939.

25. Davimes, J.G., Alagaili, A.N., Bhagwandin, A., Bertelsen, M.F., Mohammed, O.B., Bennett, N.C., Manger, P.R., and Gravett, N. (2018). Seasonal variations in sleep of free-ranging Arabian oryx (Oryx leucoryx) under natural hyperarid conditions. Sleep *41*. 10.1093/sleep/zsy038.

26. Panagiotou, M., Meijer, J.H., and Deboer, T. (2018). Chronic high-caloric diet modifies sleep homeostasis in mice. Eur. J. Neurosci. *47*, 1339–1352. 10.1111/EJN.13932.

27. Milinski, L., Fisher, S.P., Cui, N., McKillop, L.E., Blanco-Duque, C., Ang, G., Yamagata, T., Bannerman, D.M., and Vyazovskiy, V. V. (2021). Waking experience modulates sleep need in mice. BMC Biol. *19*. 10.1186/S12915-021-00982-W.

28. Achermann, P., Dijk, D.J., Brunner, D.P., and Borbély, A.A. (1993). A model of human sleep homeostasis based on EEG slow-wave activity: Quantitative comparison of data and simulations. Brain Res. Bull. *31*, 97–113. 10.1016/0361-9230(93)90016-5.

29. Cajochen, C. (2007). Alerting effects of light. Sleep Med. Rev. *11*, 453–464. 10.1016/j.smrv.2007.07.009.

30. Cajochen, C., Dijk, D.-J., and Borbéiy, A.A. (1992). Dynamics of EEG slow-wave activity and core body temperature in human sleep after exposure to bright light. Sleep *15*, 337–343.

31. Chellappa, S.L., Steiner, R., Oelhafen, P., Lang, D., Götz, T., Krebs, J., and Cajochen, C. (2013). Acute exposure to evening blue-enriched light impacts on human sleep. J. Sleep Res. *22*, 573–580. 10.1111/JSR.12050.

32. Franken, P., Tobler, I., and Borbély, A.A. (1991). Sleep homeostasis in the rat: Simulation of the time course of EEG slow-wave activity. Neurosci. Lett. *130*, 141–144. 10.1016/0304-3940(91)90382-4.

33. Vyazovskiy, V. V., Achermann, P., and Tobler, I. (2007). Sleep homeostasis in the rat in the light and dark period. Brain Res. Bull. *74*, 37–44. 10.1016/j.brainresbull.2007.05.001.

34. Trachsel, L., Tobler, I., and Borbély, A.A. (1986). Sleep regulation in rats: effects of sleep deprivation, light, and circadian phase. Am J Physiol *251*, 1037–1044. 10.1152/ajpregu.1986.251.6.R1037.

35. Alföldi, P., Tobler, I., and Borbély, A.A. (1990). The effect of light on sleep and the EEG of young rats. J. Physiol. *417*, 398–403.

36. Soriani, N., Panella, G., and Calamari, L. (2013). Rumination time during the summer season and its relationships with metabolic conditions and milk production. J. Dairy Sci. *96*, 5082–5094. 10.3168/jds.2013-6620.

37. Perez-Barberia, F.J., and Gordon, I.J. (1998). Factors affecting food comminution during chewing in ruminants: a review. Biol. ofthe Ltnnean Sociep *63*, 233–256. https://doi.org/10.1006/bijl.1997.0183.

38. Trudell-Moore, J., and White, R. (1983). Physical breakdown of food during eating and rumination in reindeer. Acta Zool. Fenn. *175*, 47–49.

39. Allali, K. El, Beniaich, Y., Farsi, H., Mhani, M.E.M., Jabal, M.S., Piro, M., Achaâban, M.R., Ouassat, M., Challet, E., Besson, M., et al. (2022). Sleep pattern in the dromedary camel: a behavioral and polysomnography study. Sleep *45*. 10.1093/sleep/zsac101.

40. Malungo, I.B., Gravett, N., Bhagwandin, A., Davimes, J.G., and Manger, P.R. (2021). Sleep in two free-roaming blue wildebeest (Connochaetes taurinus), with observations on the agreement of polysomnographic and actigraphic techniques. IBRO Neurosci. Reports *10*, 142–152. 10.1016/j.ibneur.2021.02.005.

41. Rusterholz, T., Dürr, R., and Achermann, P. (2010). Inter-individual differences in the dynamics of sleep homeostasis. Sleep *33*, 491–498. 10.1093/sleep/33.4.491.

42. Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J.M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput. Intell. Neurosci. 10.1155/2011/156869.

43. Tort, A.B.L., Komorowski, R., Eichenbaum, H., and Kopell, N. (2010). Measuring phase-amplitude coupling between neuronal oscillations of different frequencies. J. Neurophysiol. *104*, 1195. 10.1152/JN.00106.2010.

44. Krugliakova, E., Volk, C., Jaramillo, V., Sousouri, G., and Huber, R. (2020). Changes in cross-frequency coupling following closed-loop auditory stimulation in non-rapid eye movement sleep. Sci. Rep. *10*. 10.1038/s41598-020-67392-w.

Figures

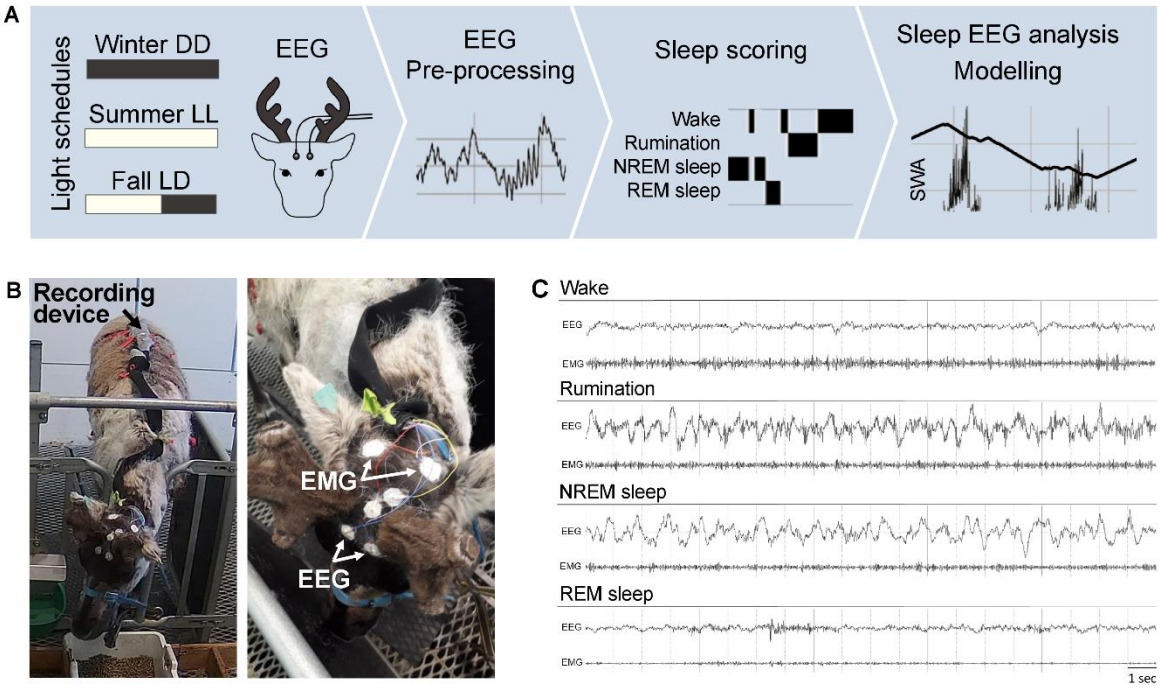

Figure 1. Reindeer sleep assessed by EEG. A) Sleep was measured using non-invasive EEG in four female reindeer. Recordings lasted for approx. 91 hours (incl. adaptation, two short sleep deprivations, and a baseline recording) and were repeated in winter (constant darkness DD, Dec. 18-21), summer (constant light LL, July 2-5), and fall (light-dark LD, Sept. 16-19). Phases of waking, rumination, NREM sleep, and REM sleep were manually identified (sleep scoring). Homeostatic changes in sleep pressure were modeled (Process S, upper curve) using the standard EEG marker slow-wave activity (SWA). B) Brain (EEG) and muscle (EMG) activity were measured using surface electrodes and a small recording device that was secured on the reindeer's back. C) Representative examples of EEG and EMG signals during waking, rumination, NREM sleep and REM sleep. See also Figure S1 and Tables S1, S2 and S3.

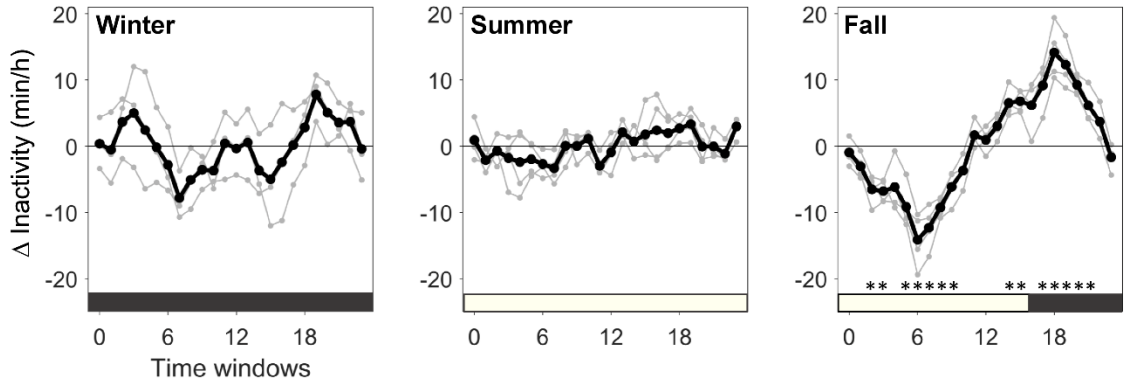

Figure 2. Distribution of time spent in inactive states across 24 hours in reindeer. The difference between total time spent in inactive states (NREM sleep, REM sleep, and rumination) during a 12-hour time window and its opposite 12-hour window within the 24-hour baseline recording (Δ inactivity). Time windows are shifted in 1-hour steps. The x-axis shows the mid-point of each 12-hour time-window relative to fall light onset. Grey curves represent the time course for each reindeer (winter: $n = 3$, summer and fall: $n = 4$). Black curves represent the mean. Asterisks indicate time windows with significantly different total sleep durations (paired t-tests, FDR-corrected, $p < 0.05$). See also Figure S1.

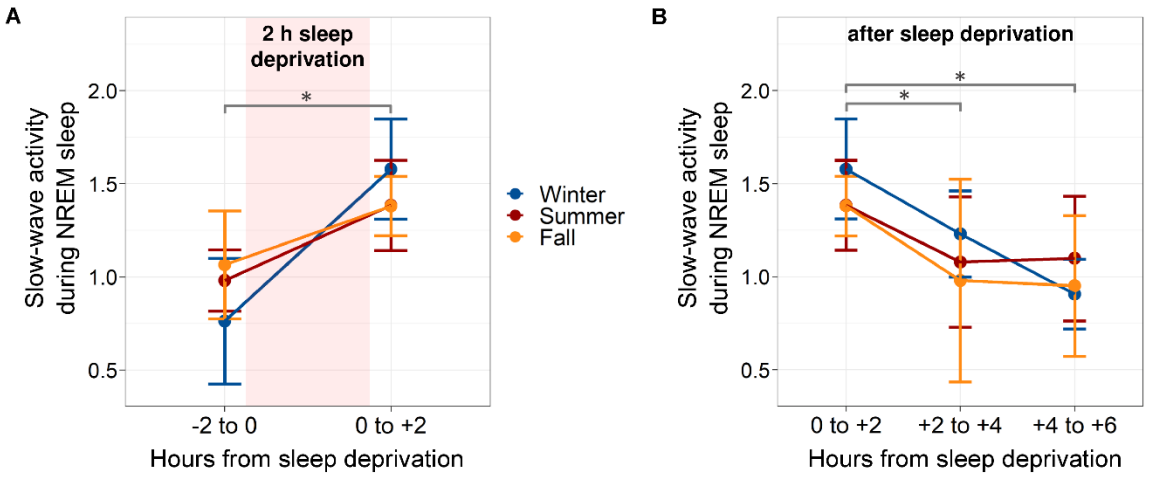

Figure 3. Homeostatic sleep regulation in reindeer. Slow-wave activity (SWA, EEG power 1 – 4.5 Hz) during NREM sleep prior to and after a short sleep deprivation (starting at time 0) in 2-hour intervals. SWA was normalized with the mean SWA across the 24-hour baseline recording. Blue, red, and orange curves show mean ± SD SWA across reindeer (winter: $n = 3$, summer and fall: $n = 4$) per season pooled across time points (midday and midnight). Statistics result from linear mixed models (reindeer and time point as random effects). Significant differences in SWA between time windows (hours from sleep deprivation) are indicated with asterisks ($p < 0.05$, Tukey pairwise comparison). A) Across sleep deprivation, a significant effect of time window ($F(36,1) = 49.00$, $p < 0.001$) and a significant interaction with season ($F(36,2) = 4.06$, $p = 0.03$) indicates a homeostatic increase of SWA across prolonged waking that is less pronounced in summer and fall than in winter. B) After sleep deprivation, a significant effect of time window ($F(56,2) = 14.24$, $p < 0.001$) indicates a homeostatic decrease of SWA across NREM sleep following a 2 h sleep deprivation. See also Figure S1.

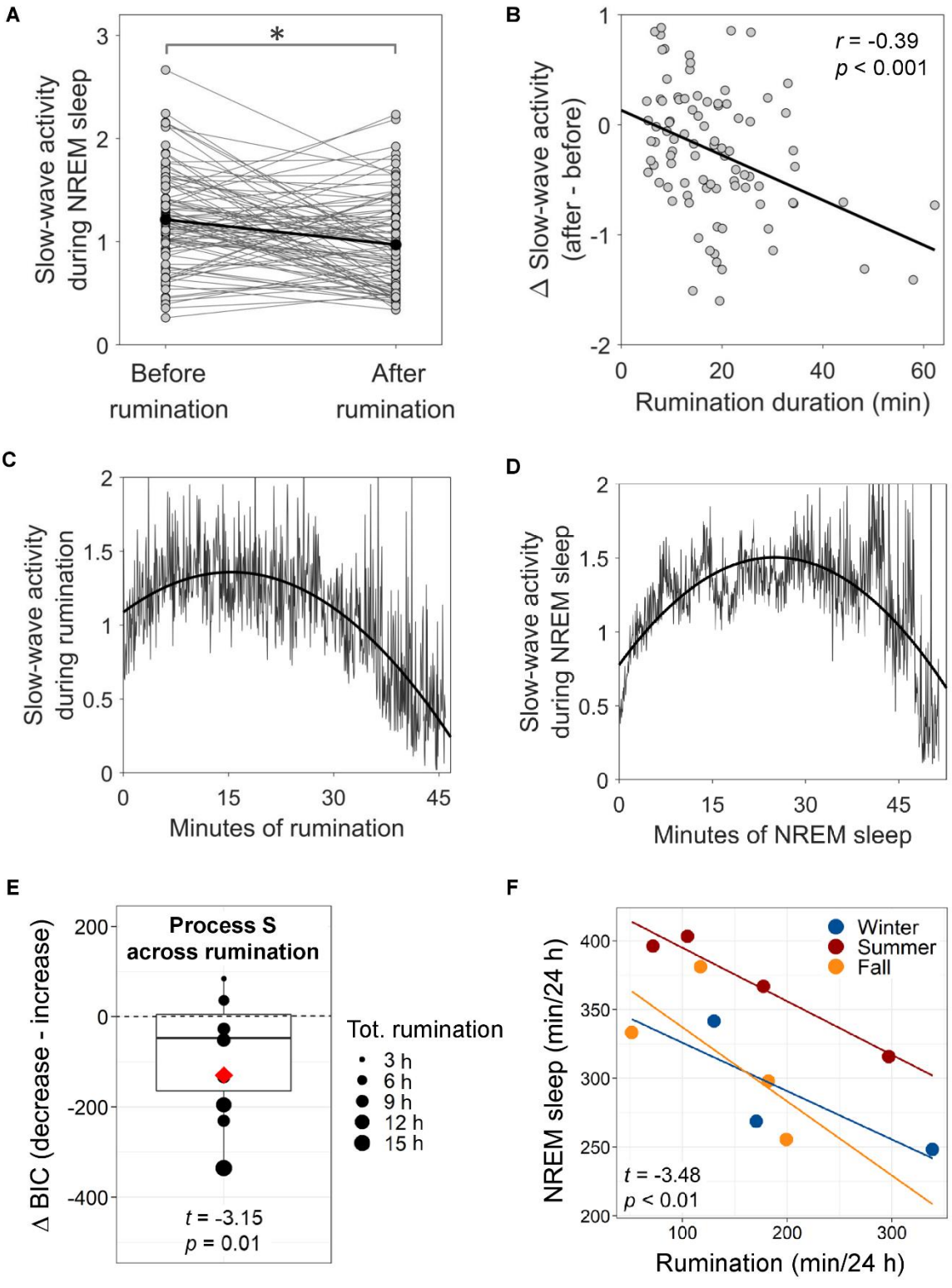
A
Slow-wave activity during NREM sleep
*
Before rumination
After rumination
B
Δ Slow-wave activity (after - before)
r = -0.39
p < 0.001
Rumination duration (min)
C
Slow-wave activity during rumination
Minutes of rumination
D
Slow-wave activity during NREM sleep
Minutes of NREM sleep
E
Process S across rumination
Δ BIC (decrease - increase)
t = -3.15
p = 0.01
Tot. rumination
3 h
6 h
9 h
12 h
15 h
F
NREM sleep (min/24 h)
Winter
Summer
Fall
t = -3.48
p < 0.01
Rumination (min/24 h)

Figure 4. Slow-wave activity (sleep pressure) dissipation across rumination and relationship between rumination and NREM sleep in reindeer. SWA was normalized with the mean SWA across the 24-hour baseline recording. A) Change in SWA during NREM sleep from before rumination to after rumination within rest periods (< 5 min disruptions by wake) that contain both rumination (> 5 min) and NREM sleep ($t = 3.97$, $p < 0.001$, $n = 90$, animals and seasons pooled, paired t-test). B) Association between the SWA change across rumination (Δ slow-wave activity) and the duration of rumination. Pearson correlation revealed a significant relationship between rumination duration and the SWA change across rumination. C) Slow-wave activity calculated from the rumination EEG. Averaged (mean for each 4-second epoch) course of SWA during long rumination episodes (> 30 min, $n = 15$). Black curve represents fitted quadratic curve. D) Averaged (mean for each 4-second epoch) course of SWA during long NREM sleep episodes (> 30 min, $n = 57$, up to 5 min interruptions included). Black curve represents fitted quadratic curve. E) Process S (homeostatic changes in sleep pressure) modeled based on SWA during NREM sleep. Black dots represent the difference in the Bayesian Information Criterion (BIC, how well the model fits SWA data) between two models for each recording. Red dots represent the mean Δ BIC across all data points weighted with the amount of rumination that occurred during each recording (total rumination). Significantly more negative Δ BIC values indicate a better fit for the model assuming rumination to decrease Process S like NREM sleep as compared to the model assuming rumination to increase Process S like wake (weighted t-test). F) Correlation of total rumination with total NREM sleep duration during the 24-hour baseline recording (linear mixed model with season and individual as random effects). See also Figures S2, S3, S4 and Table S4.