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Original Article

ORIGINAL ARTICLE

Seasonal variation in sleep homeostasis in migratory geese: a rebound of NREM sleep following sleep deprivation in summer but not in winter

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Abstract

Sleep is a behavioral and physiological state that is thought to serve important functions. Many animals go through phases in the annual cycle where sleep time might be limited, for example, during the migration and breeding phases. This leads to the question whether there are seasonal changes in sleep homeostasis. Using electroencephalogram (EEG) data loggers, we measured sleep in summer and winter in 13 barnacle geese (*Branta leucopsis*) under semi-natural conditions. During both seasons, we examined the homeostatic regulation of sleep by depriving the birds of sleep for 4 and 8 h after sunset. In winter, barnacle geese showed a clear diurnal rhythm in sleep and wakefulness. In summer, this rhythm was less pronounced, with sleep being spread out over the 24-h cycle. On average, the geese slept 1.5 h less per day in summer compared with winter. In both seasons, the amount of NREM sleep was additionally affected by the lunar cycle, with 2 h NREM sleep less during full moon compared to new moon. During summer, the geese responded to 4 and 8 h of sleep deprivation with a compensatory increase in NREM sleep time. In winter, this homeostatic response was absent. Overall, sleep deprivation only resulted in minor changes in the spectral composition of the sleep EEG. In conclusion, barnacle geese display season-dependent homeostatic regulation of sleep. These results demonstrate that sleep homeostasis is not a rigid phenomenon and suggest that some species may tolerate sleep loss under certain conditions or during certain periods of the year.

Statement of Significance

This study in barnacle geese under semi-natural conditions demonstrates high flexibility in sleep–wake regulation and sleep homeostasis. Independent of season, the geese spent 2 h less in NREM sleep during full moon compared with new moon. Moreover, the birds spent on average 1.5 h per day less in NREM sleep during summer compared to winter. Also, in winter the birds displayed a clear day–night rhythm with mostly nocturnal sleep, whereas in summer sleep was spread out over the 24-h cycle. Interestingly, the geese displayed a homeostatic NREM sleep rebound after sleep deprivation in summer but not in winter. These findings suggest that sleep homeostasis is not a rigid phenomenon and some species may tolerate sleep loss during certain phases of the year.

Key words: sleep homeostasis; sleep deprivation; sleep rebound; seasonality; photoperiod; ambient temperature; moon

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Introduction

Sleep is a wide-spread phenomenon that is thought to occur in all animals, suggesting that sleep has basic functions [1–3]. A common theory is that sleep serves as a recovery process from prior wakefulness, which is supported by findings that sleep is homeostatically regulated and that the build-up of sleep pressure occurs during the waking phase [4, 5]. However, most knowledge on sleep regulation comes from studies in mammals kept under constant laboratory conditions. It has been argued that we can learn more about the regulation of sleep by studying non-model organisms and by studying sleep under the natural conditions where it evolved [6–8].

Birds are an interesting group of animals to study sleep with great potential for insightful comparisons with mammals because they have rather similar EEG sleep stages, that is, rapid-eye-movement (REM) sleep and non-REM (NREM) sleep. Moreover, just as in mammals, studies have suggested that in birds NREM sleep is homeostatically regulated in response to prior wakefulness [9–11]. In response to sleep deprivation, these studies showed a (partial) sleep rebound, that is, an increase in sleep time and/or sleep intensity as reflected in EEG spectral power.

On the other hand, studies in birds have also produced findings that deviate from the common view based on studies in mammals. For example, the proportion of REM sleep relative to total sleep time in birds averages 8%, which is less than the average of 18% in mammals [12, 13]. Moreover, studies on sleep in birds under semi-natural and natural conditions have reported substantial intraspecific variation and changes in sleep in response to environmental factors that do not occur under constant laboratory conditions. For example, earlier studies showed that wild songbirds rest 5 h more in winter compared with summer [14, 15]. This was recently confirmed by an EEG study of European starlings (*Sturnus vulgaris*) housed in groups outside, which sleep 5 h less per day during summer than in winter [16]. Great frigatebirds (*Fregata minor*) in the wild sleep less than an hour per day during continuous 6-day foraging flights compared to 12 h of sleep once back on land [17]. Also, some male pectoral sandpipers (*Calidris melanotos*) largely go without sleep during their 3-week breeding phase in Arctic Alaska and males that slept the least sired the most offspring [18]. Such studies put into question the view that sleep is a tightly controlled homeostatic process and also question the view that reduced performance is an inescapable outcome of sleep loss. Yet, too little data are available on sleep homeostasis under more natural conditions, that is, few studies have performed detailed assessments of sleep homeostatic responses to experimental sleep deprivation in a natural habitat and across different seasons.

Today's modern technology allows researchers to measure sleep in freely moving animals [7, 19, 20]. In the current study, we applied this technology to measure sleep and sleep homeostasis in barnacle geese (*Branta leucopsis*), group housed in a spacious outdoor confinement. To determine sleep homeostatic responses and potential seasonal variation herein, the birds were exposed to experimental sleep deprivation for 4 and 8 h during both winter and summer.

Methods

Animals and housing

Thirteen adult barnacle geese were studied (eight males and five females). The birds were initially kept on a large outdoor meadow (length × width = 68 m × 60 m) with a water pond (25 m × 15 m) at the animal facilities of GELIFES at the University of Groningen. The geese were habituated to the presence of humans. Flight feathers were clipped to prevent them from flying away. One week prior to EEG measurements, animals were transferred in groups of five to separate outdoor aviaries (length × width = 5 m × 4 m). In these aviaries, the birds remained exposed to natural day light and ambient temperatures. Throughout the entire study period, food and water were present ad libitum (food item numbers 615220 and 384020; Kasper Faunafood, Woerden, The Netherlands).

Surgery

All animals underwent surgeries for implantation of EEG and EMG electrodes. Surgeries were done under isoflurane anesthesia (1.5%–2%). Before the surgery, meloxicam was injected subcutaneously as an analgesium (0.17 mL; 0.5 mg/kg) and diazepam was injected subcutaneously to reduce stress 10 min prior to surgery (0.68 mL; 2 mg/kg). After carefully exposing the crania, five holes (0.5 mm in diameter) were drilled through the skull. Electrodes were inserted through the holes to the level of the dura mater: two frontal EEG electrodes, one per hemisphere covering the hyperpallium (4 mm lateral of the midline) and three electrodes in a left-to-right line over the caudal part of the telencephalon that consists of one EEG reference electrode (4 mm lateral of the midline), one caudal electro-myogram (EMG) reference electrode (on the midline), and one ground electrode (4 mm lateral of the midline). All cortical electrodes consisted of rounded gold-plated pins (0.5 mm diameter, BKL Electronic 10120538, Lüdenscheid, Germany). Two flexible wires were placed on the neck muscle to record an EMG (PlasticsOne, Ranoke, VA, USA). All electrodes were soldered to a connector (BKL Electronic 10120302, Lüdenscheid, Germany) and the connector was fixed to the skull with Paladur dental acrylic (Heraeus Kulzer, Hanau, Germany), a 0.6 mm screw served as an anchor point for the implant. After the surgery, the animals could recover for at least 2 weeks before moving them to the recording aviaries.

Sleep recordings

At the start of the experiments, a datalogger was attached to the implant on the head (Neurologger 2A; Evolocus, Tarrytown, NY, USA). The datalogger recorded EEG and EMG signals as well as head movements through an on-board accelerometer (LIS302DLH; STMicro-electronics Geneva, Switzerland). The data were recorded with a sample frequency of 100 Hz and stored on the datalogger memory chip. The loggers ran for approximately 15 days on a 3.6 V battery (LS 14250; Saft, Levallois-Perret, France).

Sleep–wake patterns for individual geese were recorded for periods up to 15 days in winter (February; five males and four females) and/or summer (June; four males and four females). Some individuals, but not all, were recorded in both seasons (see Table S1 for a detailed overview of the recording dates and individuals). In both seasons, the birds were subjected to a sleep deprivation of either 4 h (4SD) or 8 h (8SD) starting at sunset. While we had no data on sleep–wake patterns in geese, based on behavioral observations we assumed that the dark phase was the most important sleep phase. We therefore started the onset of sleep deprivation at sunset to cover as much as possible the main sleep phase for both seasons. Consequently, the 4 and 8 h sleep

deprivation ended at different circadian times. We could have chosen to align the end of sleep deprivation with sunrise, but this would still have been a different circadian time for winter and summer. The loggers were attached to the EEG implant of the geese before noon to have an undisturbed baseline night recording. The first sleep deprivation took place during the next night. The geese in the two aviaries received a different duration of sleep deprivation (i.e. 4 or 8 h), in a cross-over design. After a week, the sleep deprivation was repeated and the two groups were reversed. Birds undergoing the 4SD or 8SD treatment were kept awake by means of "mild stimulation" [21]. Standing and walking in the aviary by the experimenter was largely sufficient to keep the geese awake but, whenever the birds showed signs of eye-closure and inactivity, they were gently touched on the back. In between the recording sessions conducted in February and June, the geese were returned to the larger outdoor meadow.

Data analyses

All recordings were scored with an automated EEG scoring program using machine learning algorithms with input from a human scorer who was unaware of the season of the recordings

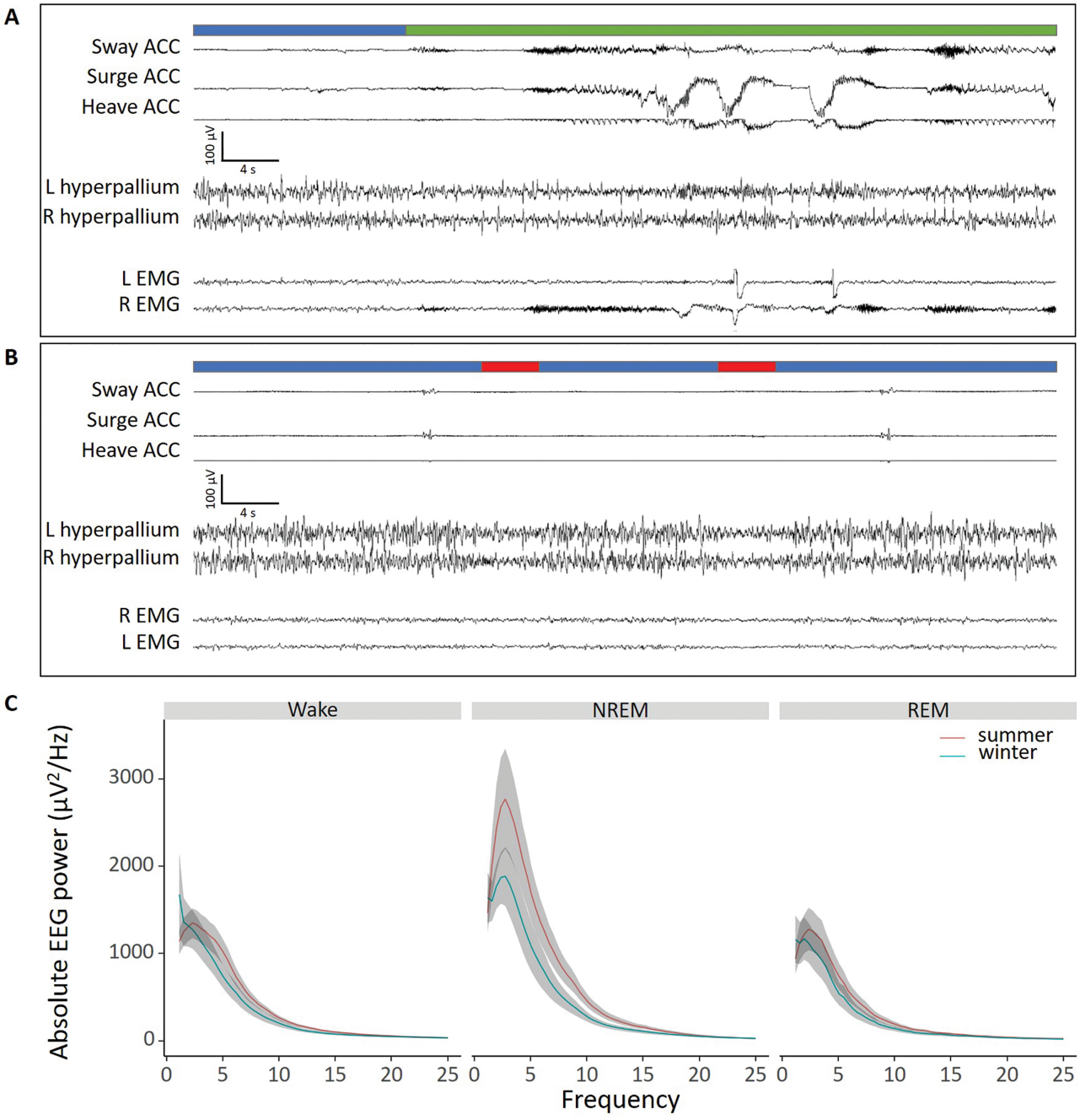

Figure 1. Representative examples of EEG, EMG and accelerometer traces. Two representative electrophysiological output of a 60 s sleep of one individual recording (panels A and B). The traces shown consist of three accelerometer channels (Sway, Surge, and Heave), two EEG channels over the hyperpallium in both hemispheres, and two EMG channels. Based on the channels we scored for wakefulness (green), NREM sleep (blue), and REM sleep (red). The averaged EEG power of these stages of geese recorded in winter (blue) and summer (red) are shown in panel C.

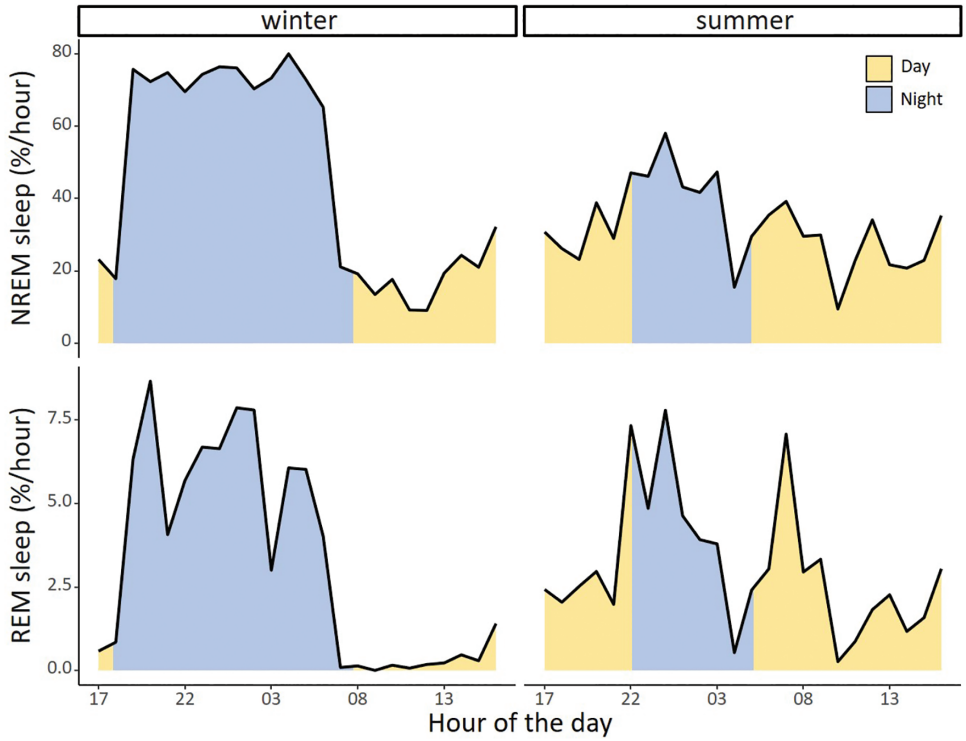

Figure 2. Daily distribution of NREM and REM sleep in winter and summer. Daily patterns of NREM sleep (top) and REM sleep (bottom) in winter (left) and summer (right). The plots in winter and summer are averages of all animals recorded on one baseline day before the start of the first sleep deprivation experiment started. Average night length, excluding twilight was in winter 13.41 ± 0.08 h and in summer 7.04 ± 0.00 h (indicated in blue).

and treatment of the birds (Somnivore Pty. Ltd., Parkville, VIC, Australia). The program used all electrophysiological (EEG + EMG) and accelerometer channels to determine the sleep–wake state every 4 s. The recordings were scored for NREM sleep, REM sleep, and wakefulness according the criteria presented in Figure 1. Wakefulness was characterized by relatively low-amplitude, high-frequency EEG activity, coinciding with elevated EMG signals and high accelerometer output. An epoch was scored as NREM sleep when the EEG signal showed an amplitude twice that of wakefulness, a low EMG signal, and no movements in the accelerometer output. REM sleep was scored when an epoch showed low-amplitude, high-frequency waves similar to wakefulness but with very low EMG signals and a lack of accelerometer output or sometimes with signs of head drops. The automated scoring program was validated with various animal species including pigeons [22]. For avian EEG recordings, the program was shown to have an accuracy for wakefulness of 0.96 ± 0.006; NREM sleep 0.97 ± 0.01; REM sleep 0.86 ± 0.02 as compared with human scorers. We did an additional validation for our own geese based on 4 of the 24-h baseline winter-recordings. The correlations between the program and the human scorer were 0.98 ± 0.01 for wakefulness, 0.97 ± 0.01 for NREM sleep and 0.84 ± 0.04 for REM sleep.

To obtain clean EEG traces for spectral analysis, a second round of scoring was performed to filter out artifacts that did not affect stage classification but might affect spectral qualities. Epochs were scored as artifacts when the EEG contained peaks of at least twice the amplitude for a given vigilance state that coincided with movements seen in the accelerometer channels. The amount of artifacts scored was for wake: 55.8% ± 3.5%, NREM sleep: 12.6% ± 2.8%, and REM sleep: 7.0% ± 2.0%. A fast Fourier transformation (FFT) was performed over the artifact-free NREM sleep EEG epochs of the left hyperpallium derivation. The FFT yielded 50 bands with a bin width of ~0.39 Hz. To account for inter-individual differences in EEG signal strength, the power for every epoch in every frequency bin was normalized to the average power of the 24-h baseline day in the same frequency bin. Since a NREM sleep deficit may be compensated for by deeper sleep as reflected in an increased EEG spectral power, we calculated the spectral power for grouped frequencies bands that are commonly used in sleep literature (Delta: 1.56–3.91 Hz; Theta: 3.91–8.20 Hz; Alpha: 8.20–14.06 Hz; Beta: 14.06–30.08 Hz; Gamma: 30.08–50 Hz). To get a complete picture of how sleep was recovered after sleep deprivation, we calculated the cumulative NREM sleep EEG power by calculating the product of NREM sleep time and EEG power.

We acquired data on ambient temperature from a nearby weather station in Eelde, the Netherlands (53°08′07.7″N 6°34′12.0″E) to be able to relate the sleep results to ambient temperature. These data were only available as daily averages. We acquired data on the solar and lunar cycle specifically for our study site from https://www.timeanddate.com. Because most of our recordings took place around days with new or full moon, we assigned them to one of these two categories (new moon = surface illumination < 30%; full moon = surface illumination > 64%). There were no recordings when the surface of the moon was illuminated between 30% and 64%. We did not a priori control for moon phase during our sleep deprivation experiments but included a post hoc analysis to address this issue.

Statistics

We first analyzed our data for differences in sleep between winter and summer with a linear mixed effect model where we

took bird ID as a random effect using the lme4 package [23, 24]. In a second analysis, we assessed if these differences could be explained and/or modulated by specific environmental factors that we had data on, that is, ambient temperature and moon phase. In this analysis, we included season, ambient temperature, and moon phase as possible predictors. Through model selection we ended up with a minimum adequate model (MAM), which we used to predict the model estimates that are presented in the figures. Statistical differences between groups was tested with a posthoc Tukey HSD test using the lsmeans package [25]. Differences were considered statistically significant when $p < 0.05$. Data and text in figures are expressed as mean ± SEM

Results

Baseline sleep in winter and summer

Figure 1 shows two representative baseline recordings of 60 s including the spectral composition for the three vigilant states (wakefulness, NREM sleep, and REM sleep). Across all baseline recordings, that is, all the recording days excluding the sleep deprivation and subsequent recovery days, barnacle geese had on average 57.6% ± 1.7% of wakefulness, 37.4% ± 1.4% of NREM sleep, and 5.0% ± 0.7% of REM sleep per 24 h. The amount of REM sleep made up 11.5% ± 0.8% of total sleep time (TST).

Both NREM and REM sleep were more present during the night hours than during the daytime hours but this was much more so in winter than in summer (Figure 2). In winter, the geese had a clear daily rhythm in sleep with most of their sleep during night time (nighttime: 55.6% ± 2.2% NREM sleep, 8.1% ± 1.0% REM sleep; daytime: 16.8% ± 1.0% NREM sleep, 1.6% ± 0.5% REM sleep). In summer, sleep was more distributed across the 24-h cycle (nighttime: 40.4% ± 1.9% NREM sleep, 6.6% ± 0.7% REM sleep; daytime: 29.1% ± 1.8% NREM sleep, 3.3% ± 0.4% REM sleep). The day–night ratio for NREM sleep was 3.6 ± 0.3 in winter and 1.4 ± 0.1 in summer ($p < 0.001$). The day–night ratio for REM sleep was 11.3 ± 2.9 in winter and 3.2 ± 1.3 in summer ($p = 0.02$). Overall, the geese had 1.5 h more NREM sleep per 24-h day in winter than in summer (9.31 ± 0.35 vs 7.78 ± 0.61 h, respectively; lmer model with Tukey HSD posthoc test, $p = 0.0001$, Figure 3A). Also, the average bout length for NREM sleep was significantly longer in winter compared with summer (Figure 3D, lmer model with Tukey posthoc test, $p = 0.046$). Interestingly, there were no significant differences in REM sleep between winter and summer,

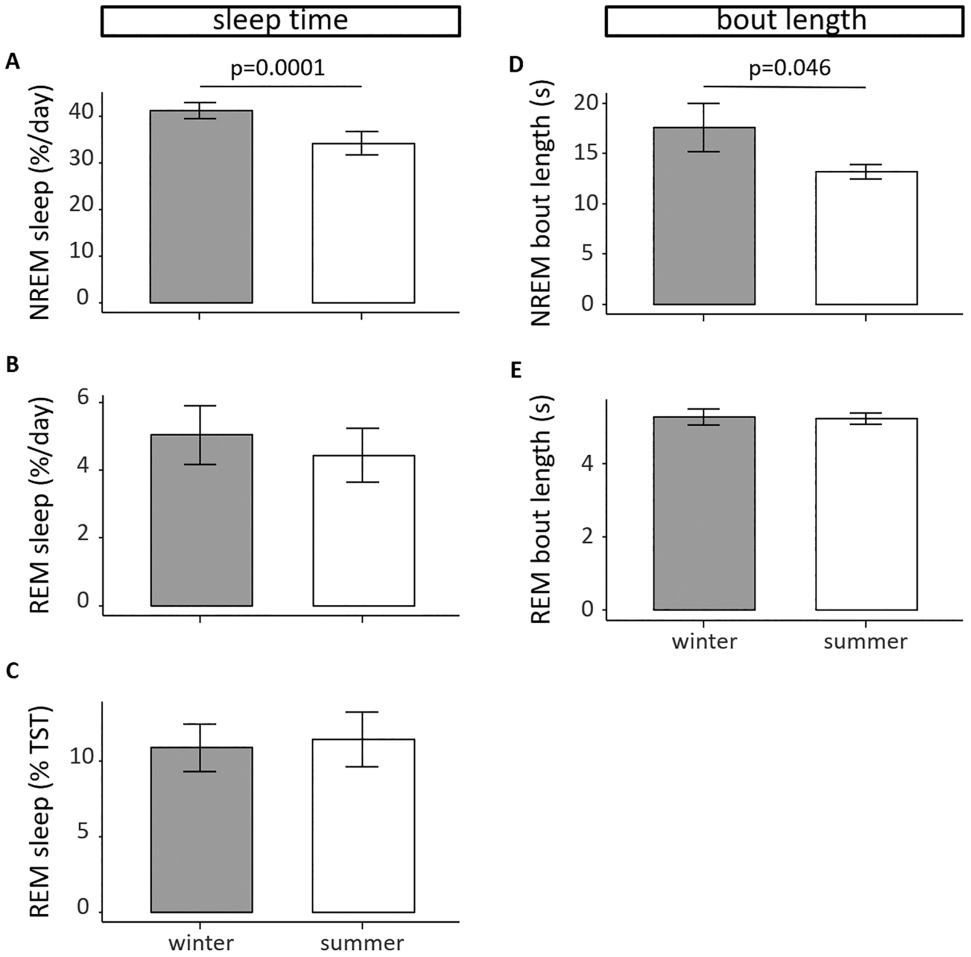

Figure 3. Seasonal changes in sleep time and sleep bout length. Daily averages of NREM sleep in winter (gray bar, $n = 8$) and summer (white bar, $n = 8$) averaged over all recordings. In summer, barnacle geese sleep an estimated amount of 7.13% less (panel A, lmer model and Tukey HSD posthoc test, $p = 0.0001$). In contrast, the daily average of REM sleep does not change between seasons (panel B). At the same time, the proportion of daily averaged REM sleep to TST does not change between seasons (panel C). The bout length of NREM sleep is significantly higher in winter compared with summer (panel D, lmer model and Tukey HSD posthoc test, $p = 0.046$). There is no difference in the bout length of REM sleep (panel E). Data shown are group averages ± SEM.

neither for the absolute amount of REM sleep per 24-h day nor for the amount of REM sleep as a proportion of TST (Figure 3B and C). Also the bout length for REM sleep did not differ between seasons (Figure 3E). There were no sex differences in NREM and REM sleep time, neither in winter nor in summer (Figure S1).

Response to sleep deprivation

The geese were subjected to a sleep deprivation of 4 and 8 h by means of mild stimulation. Noteworthy, the actual amount of sleep that was lost during these experiments was less than 4 and 8 h, first of all because the birds did not spend 100% of these hours asleep under baseline conditions, and second because the sleep deprivation itself was not 100% effective. In general, it did not appear to be difficult to keep the birds awake and the nearby presence or approach of the experimenters was most often sufficient to keep the birds moving around. Still, most birds managed to accrue some sleep in the course of the sleep deprivation. Also, even though the experimenters and procedure were the same, the efficacy of the sleep deprivation was somewhat higher in winter compared with summer, as seen in the last 4 h of the 8SD in summer where the amount of NREM sleep did not differ from baseline. Nonetheless, for all sleep deprivation sessions the geese suffered a net sleep loss compared to the baseline condition (Figure 4). On the baseline days in winter the birds slept 2.54 ± 0.16 h and 4.51 ± 0.41 h during the first 4 and 8 h after sunset, respectively. In summer, this was 2.10 ± 0.21 h and 3.72 ± 0.41 h, respectively. The actual amounts of NREM sleep that were lost were: 2.14 ± 0.09 h and 3.41 ± 0.58 h during 4 and 8 h sleep deprivation in winter; 1.37 ± 0.25 h and 2.00 ± 0.42 h during 4 and 8 h sleep deprivation in summer. Also, the actual amounts of REM sleep that were lost were: 0.17 ± 0.06 h and 0.50 ± 0.17 h during 4 and 8 h sleep deprivation in winter; 0.27 ± 0.06 h and 0.61 ± 0.18 h during 4 and 8 h sleep deprivation in summer.

In winter, the NREM sleep time that was lost during the sleep deprivations were not compensated, neither during the remainder of the experimental day nor during the next day (lmer model, $p < 0.05$, Figure 5A–D). Interestingly, during summer the NREM sleep that was lost during sleep deprivation was recovered by the end of the day (Figure 5E–H).

Since a NREM sleep deficit may be compensated for by deeper sleep as reflected in an increased EEG spectral power, we plotted the normalized EEG power for the different frequency bands relative to the mean of the baseline day (Figure 6). While there were small differences in NREM sleep EEG power after sleep deprivation compared with baseline, there was no consistent pattern or direction for these difference. For the delta band in winter after 8SD, there was a slight significant increase in EEG power (lmer model, posthoc Tukey HSD test $p < 0.05$). To produce a complete picture of the homeostatic NREM sleep response to sleep deprivation incorporating both sleep time and EEG spectral power, we calculated the cumulative spectral power, that is, the product of NREM sleep time and spectral power (Figure 7). In winter, sleep deprivation was not followed by significant changes in cumulative power in any of the frequency bands. At the end of the experimental day, cumulative NREM sleep EEG power was

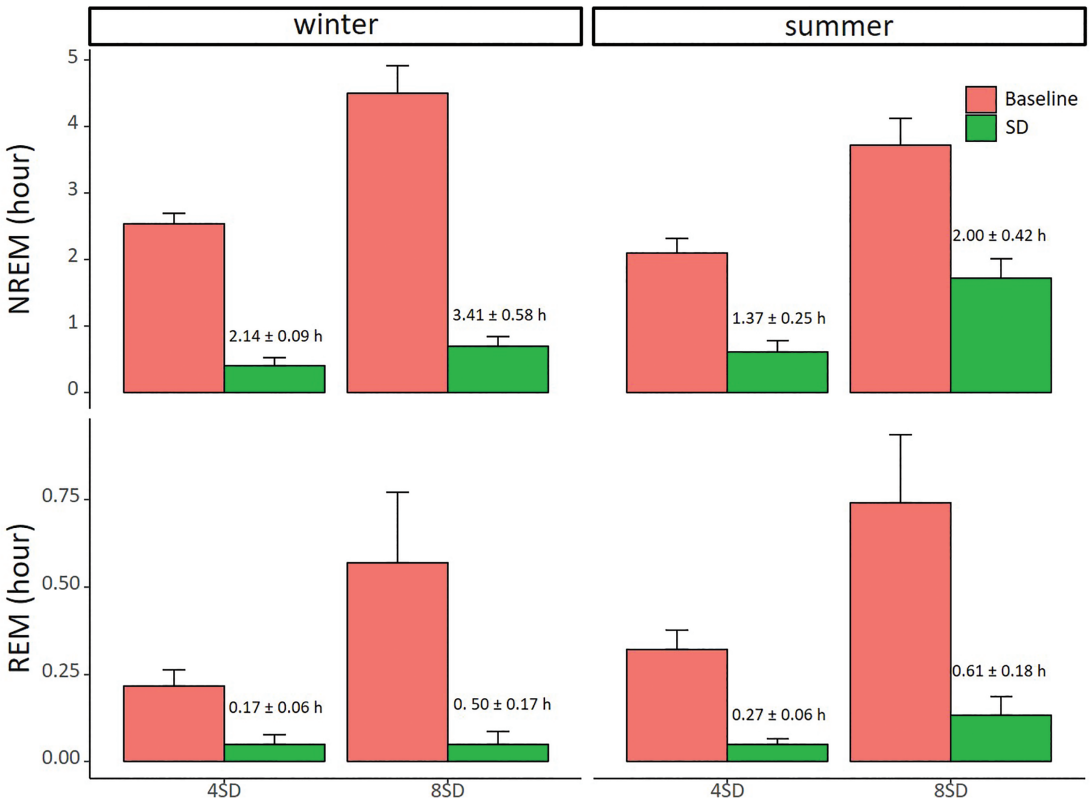

Figure 4. Sleep deprivation efficacy. Sleep deprivation efficacy (4 and 8 h) in winter (left panels, 4SD: $n = 8$; 8SD $n = 6$) and summer (right panels, 4SD: $n = 7$; 8SD $n = 8$) for NREM sleep (top panels) and REM sleep (bottom panels). Compared with baseline (red bars), the sleep deprivation (green bars) caused a significant reduction of both NREM and REM sleep (lmer model, $p < 0.0001$). The numbers on the green bars indicate the net sleep loss during the hours of sleep deprivation compared with the baseline. Data shown are group averages ± SEM.

significantly lower in all frequency bands than at the end of the baseline day (lmer model, $p < 0.05$, Figure 7). Also, there was no sign of delayed recovery, since cumulative power on the second recovery day did not differ from cumulative power during baseline for any of the frequency bands. In summer, however, both 4 and 8 h sleep deprivation were followed by increased power accumulation in all frequency bands. At the end of the experimental day, the accumulated NREM sleep EEG power was no longer different from baseline for any of the frequency bands (Figure 7).

Moreover, we calculated the differences in cumulative spectral power between the baseline day and experimental day at two time points, that is, at the end of the sleep deprivation (hour 4 or 8) and at the end of the day (hour 24). In winter, the difference in cumulative power at the end of the day (hour 24) was similar to the difference immediately after sleep deprivation indicating that none of the NREM sleep power lost was recovered. In summer, the difference between these time points significantly decreased (lmer model, 4SD: $p < 0.0001$; 8SD: $p < 0.0025$, Figure 8). Thus, the sleep that is lost in summer is significantly compensated in cumulative EEG power.

The overall patterns of REM sleep both during baseline and after sleep deprivation were more variable and not clearly consistent within or between seasons (Figure 9). Nonetheless, the loss of REM sleep during sleep deprivation was partly recovered in both winter and summer by an increase in the number of REM sleep epochs except for the 8SD group in summer (lmer model, $p = 0.017$, Figure 9H).

In addition to sleep time and/or EEG power, sleep homeostasis and sleep drive might be reflected in the degree of sleep consolidation. To assess possible changes in sleep consolidation, we did an analysis on sleep bout length for a 2-h window immediately following sleep deprivation and compared this to sleep bout length during the same baseline window. The analysis

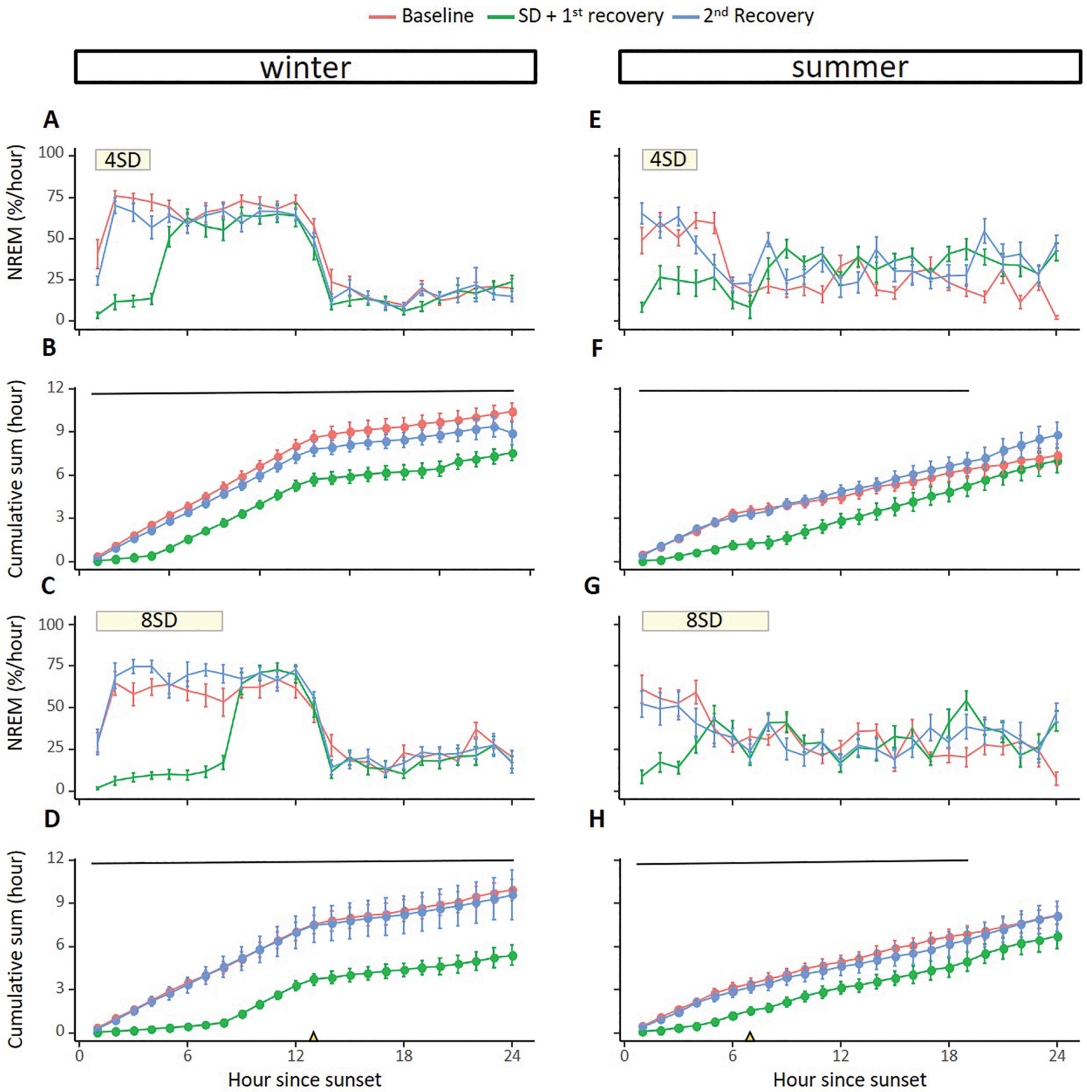

Figure 5. Effect of sleep deprivation on NREM sleep time. Effects of sleep deprivation in winter (left panels, 4SD: $n = 8$; 8SD $n = 6$) or summer (right panels, 4SD: $n = 7$; 8SD $n = 8$) on NREM sleep patterns and cumulative NREM sleep time. Sleep deprivation started after sunset and lasted for either 4 h (panels A, B and E, F) or 8 h (panels C, D and G, H). The yellow triangles on the x-axes denote sunrise. The period of sleep deprivation is indicated by the yellow horizontal bar at the top of the panels (panels A, E and C, G). Every panel shows NREM sleep for the baseline day (red line), the day with sleep deprivation and subsequent recovery time during the remainder of the day (green line), and a second recovery day (blue line). Significant differences between the baseline day and the SD + first recovery day are indicated by the horizontal lines ($p < 0.005$). Data shown are group averages ± SEM.

shows that there are no significant differences in average NREM sleep bout length and REM sleep bout length between baseline and sleep deprivation, neither during winter nor during summer (Figure S2).

Correlations with ambient temperature and moon phase

Since most of our recordings took place around new or full moon, they were assigned to a category new moon (surface illumination < 31%) or full moon (surface illumination > 64%). The amount of NREM sleep per 24 h was on average 2.0 h lower during full moon compared to new moon, independent of season (lmer model, $p < 0.001$, Figure 10). Moreover, independent of moon phase, daily NREM sleep time was correlated with ambient temperature, with the highest amount of NREM sleep around 0°C (lmer model, $p < 0.001$, Figure 11). In contrast, moon phase and ambient temperature had no significant correlation with the amount of REM sleep. Since moon phase affected the overall amount of NREM sleep, we performed an additional analysis to assess whether moon phase modulated the NREM sleep rebound after sleep deprivation. This analysis shows that the (lack of) homeostatic sleep rebound in winter is independent of moon phase. Also, in summer there was no consistent effect of moon phase on recovery from sleep deprivation for both NREM sleep time and spectral power (Supplemental Figures S3–S5).

Discussion

The barnacle geese in the present study displayed pronounced seasonal differences in both the overall amount and distribution of sleep as well as the homeostatic response to sleep deprivation. Overall, the geese spent about 1.5 h per day more in NREM sleep in winter than in summer (9.3 vs 7.8 h, respectively) while the amount of REM sleep per day did not differ significantly between the seasons (1.2 vs 1.0 h, respectively). In winter the geese had a clear day–night rhythm in sleep with most of their sleep during nighttime. In summer, sleep was more spread out over the 24-h cycle. Independent of season, full moon caused a decrease of 2.0 h in NREM sleep time relative to new moon (7.5 vs 9.5 h, respectively). We assessed sleep homeostatic responses by subjecting the birds to a 4 and 8 h sleep deprivation starting at sunset. In summer, the geese fully compensated for the sleep deprivation-induced loss of NREM sleep by subsequently increasing sleep time and by a limited increase in EEG spectral power in all frequency bands, a presumed measure of sleep intensity. In stark contrast, sleep deprivation in winter did not elicit a compensatory NREM sleep response. REM sleep overall was more variable, but there was at least partial recovery after 4 h sleep deprivation in both winter and summer.

The geese in our study were subjected to sleep deprivation by a procedure that consisted of experimenters moving around in the aviaries and, when necessary, approaching the birds. Although it was intended to keep the geese awake for the full 4

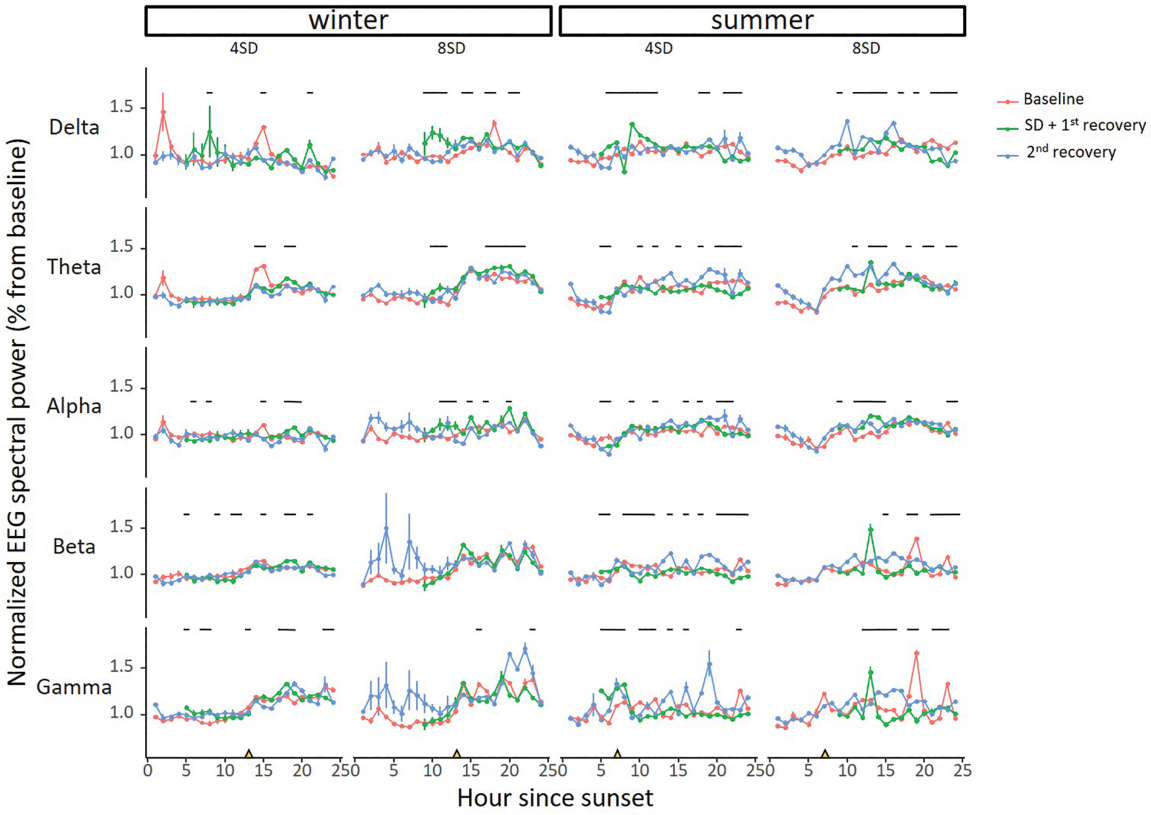

Figure 6. Changes in NREM sleep EEG power following sleep deprivation. Relative spectral NREM sleep EEG power during the baseline, SD and recovery day in winter (left panels, 4SD: $n = 8$; 8SD $n = 6$) and summer (right panels, 4SD: $n = 7$; 8SD $n = 8$) for the analyzed frequency bands (Delta: 1.56–3.91 Hz; Theta: 3.91–8.20 Hz; Alpha: 8.20–14.06 Hz; Beta: 14.06–30.08 Hz; Gamma: 30.08–50 Hz). The yellow triangles on the x-axes denote sunrise. There does not seem to be a clear pattern in the homeostatic response in EEG spectral power after both sleep deprivations in both seasons. The lines indicate significant differences between the baseline and the SD + first recovery day (lmer model, $p < 0.05$). Data shown are group averages ± SEM.

and 8 h sleep deprivation windows, the birds occasionally had brief sleep episodes that went unnoticed, perhaps because they were sleeping while standing in a group among conspecifics. For unknown reasons, this was true particularly for the 8 h sleep deprivation in summer. Yet, despite the fact that sleep deprivation in summer was less efficient, the birds thereafter displayed a full compensation, whereas after the more successful sleep deprivation in winter they did not show any compensation at all, neither by increasing sleep time nor by increasing cumulative EEG spectral power.

Overall, the changes in EEG spectral power after sleep deprivation were fairly limited in the geese, both in winter and summer. This is in contrast to mammals that show predictably increases in EEG spectral power after sleep deprivation, particularly in the 1–4 Hz delta range or slow-wave range, which have become a generally accepted read out of sleep homeostasis [26–29]. A number of studies on birds have shown that sleep deprivation is followed by an increase in EEG spectral power over a broader frequency range, but with the largest increase usually occurring in the delta band [9–11, 30]. Interestingly, this increase in the delta power after 24 h of sleep deprivation is lacking in pigeons [31]. In the geese, there were minor significant increases in EEG delta power after both 4 and 8 h sleep deprivation compared to baseline but at other hours of the recovery phase delta power dropped below baseline. Together, the accumulation of NREM sleep EEG spectral power after sleep deprivation in winter did not amount to any compensation of the sleep that was lost, neither in the EEG delta band nor in the other frequency bands. Also, in summer there was a small increase in EEG delta power at a few time points after 4 h sleep deprivation, but not so much after 8 h sleep deprivation. Hence, also in summer an increase in EEG spectral power was not a major indicator and contributor of the sleep homeostatic response. However, during summer the geese did increase NREM sleep time after sleep deprivation, which in itself was sufficient to compensate for the sleep that was lost. Thus, the geese in our study mainly compensated sleep loss in summer by increasing sleep time instead of increasing EEG power.

One might argue that the lack of compensation for loss of NREM sleep in winter was perhaps related to the fact that the birds in winter had a higher amount of baseline sleep. The reasoning could be that winter birds sleep more than they actually need and a few hours of lost sleep would therefore not immediately require a homeostatic rebound as long as they had their core sleep [32]. However, this is not likely a full explanation for the complete lack of sleep rebound in winter. First, the amount of NREM sleep during winter full moon and summer new moon were similar, yet there was a clear sleep rebound in summer but not in winter. Second, the 8 h sleep deprivation in winter resulted in a net 3.4 h reduction of NREM sleep, which is more

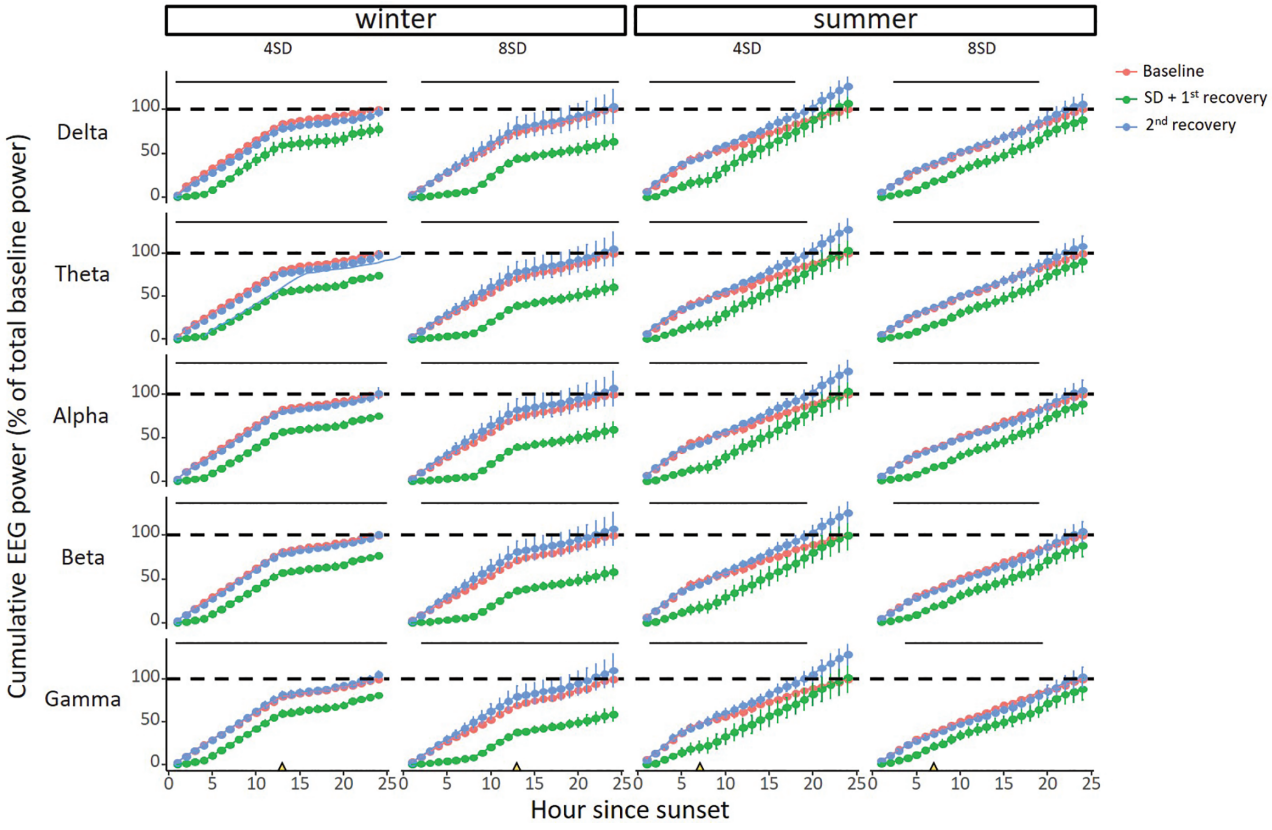

Figure 7. Seasonal differences in cumulative NREM sleep EEG power after sleep deprivation. Cumulative analysis of NREM sleep EEG power in the analyzed frequency bands (rows) during baseline day (red), sleep deprivation day + first recovery day (green) and second recovery day (blue) for all sleep deprivations in both seasons (columns). The yellow triangles on the x-axes denote sunrise. In winter, barnacle geese do not compensate for the induced sleep loss compared to baseline (dotted line) (4SD: $n = 8$; 8SD $n = 6$). In contrary, in summer, barnacle geese fully compensate for the induced sleep loss (4SD: $n = 7$; 8SD $n = 8$). This pattern is present in all the analyzed frequency bands (Delta: 1.56–3.91 Hz; Theta: 3.91–8.20 Hz; Alpha: 8.20–14.06 Hz; Beta: 14.06–30.08 Hz; Gamma: 30.08–50 Hz). Significant differences between the baseline day and the SD + first recovery day are indicated by the horizontal lines (lmer model, $p < 0.005$). Data shown are group averages ± SEM.

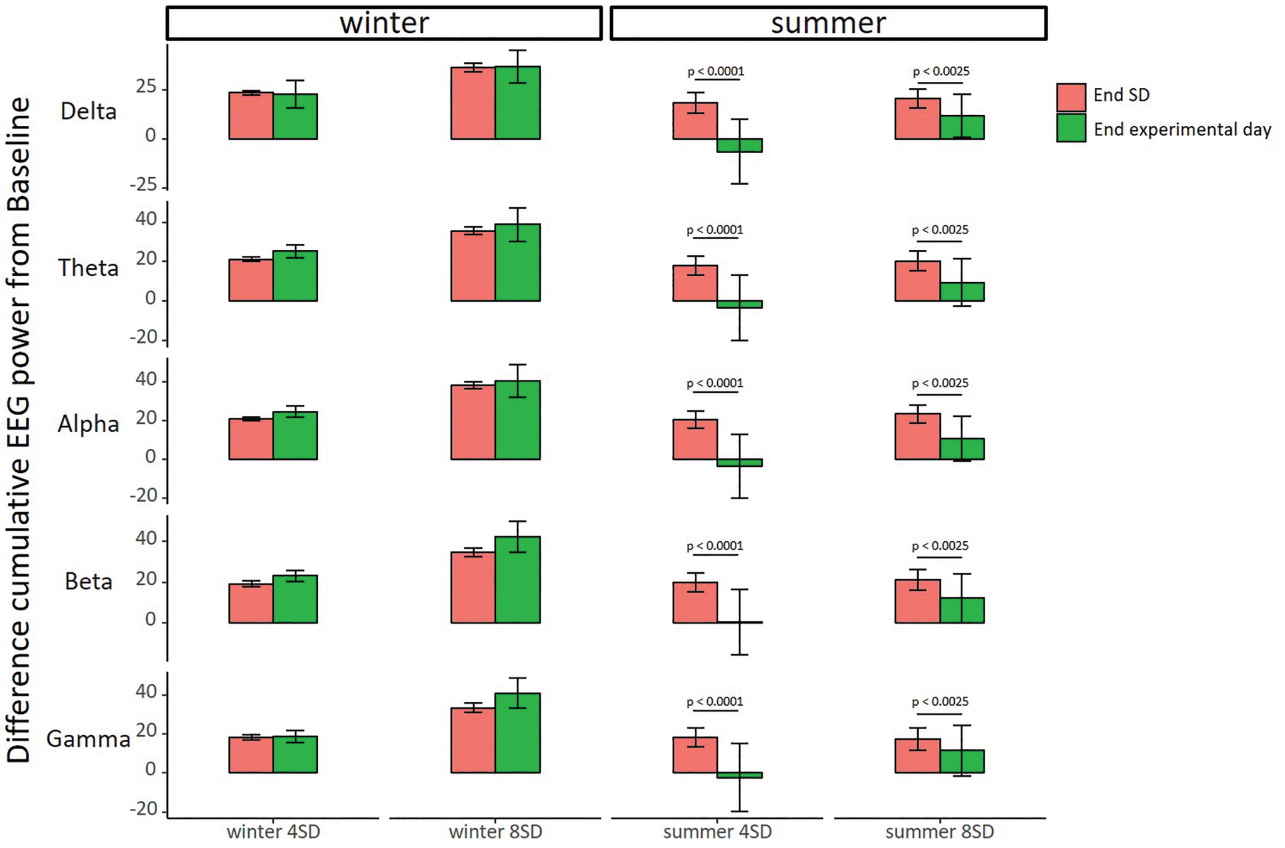

Figure 8. Seasonal differences in compensation for sleep loss. Difference between the cumulative NREM sleep EEG power between the baseline day and the first recovery day after sleep deprivation (red bars) and after the entire experimental day (green bars). In winter, independent of frequency band, the cumulative EEG power that has been lost due to sleep deprivation remains the same at the end of the experimental day (4SD: $n = 8$; 8SD $n = 6$). In contrast, in summer, this difference decreased significantly after both sleep deprivations independent of frequency band (4SD: $n = 7$; 8SD $n = 8$) (lmer model, 4SD: $p < 0.0001$; 8SD: $p < 0.0025$). Data shown are group averages ± SEM.

than the 1.4 h reduction after 4 h sleep deprivation in summer, even if one adds the 1.5 h lower amount of baseline NREM sleep in summer. Yet, in summer the birds displayed a clear rebound after sleep deprivation that fully compensated for the sleep loss while in winter there was not even a partial compensation. Hence, there must be other reasons why geese in winter did not make up for the sleep that was lost. Perhaps seasonal changes in physiological state and waking behavior result in a slower build-up rate of the homeostatic need for sleep. It remains to be established what these seasonal changes in physiology and behavior could be and why birds would have normal sleep homeostasis during summer but not in winter. Recent research supports the notion of seasonal changes in physiology in geese, for example, heart rate and body temperature changes in relation to photoperiod [33]. Clearly, these findings demonstrate the importance of more detailed studies in non-mammalian species under more natural conditions.

In contrast to NREM sleep, there was no seasonal difference in the REM sleep response to sleep deprivation. The sleep deprivation-induced loss of REM sleep was at least partly recovered in both winter and summer, except for the REM sleep loss after 8 h sleep deprivation in summer. One explanation for the latter might be that the baseline levels for that recording session were somewhat higher compared to the other days. At this point we can only speculate as to why these baseline levels might differ but it is important to note that overall the amount of REM sleep was more variable and perhaps less tightly regulated than NREM sleep. Across different bird species REM sleep can vary from mammalian-like amounts in zebra finches (*Taeniopygia guttata*, about 25% of TST [34];) and white-crowned sparrows (*Zonotrichia leucophrys*, about 16% of TST; [35]) to nearly no REM sleep in starlings (about 2% of TST; [10, 36]). With 11.5% REM sleep relative to TST, our barnacle geese are close to the average of 8% REM sleep per TST reported for birds in general [12]. An increase of REM sleep after sleep deprivation have been reported for pigeons [9, 31, 37] but this response is lacking in starlings [10]. It remains unclear what is causing this variation in REM sleep among bird species but it cannot be explained by taxonomy since most of the above-mentioned species belong to the group of songbirds.

Seasonal modulation of sleep in the barnacle geese was not only reflected in overall amount of NREM sleep time and NREM sleep homeostatic responses, but also in the distribution of sleep across the day. While in winter sleep predominantly occurred during night time, in summer both NREM sleep and REM sleep were much more scattered across the 24-h cycle. This attenuated circadian organization may relate to the fact that barnacle geese in the wild show long-distance migration to breeding areas in the High Arctic where sunlight is constantly present most of their stay [38]. Under such near-constant light conditions strong daily rhythmicity in activity and sleep might be unnecessary or even disadvantageous. It is not unusual for animals to become arrhythmic in the High Arctic under constant lighting conditions. This has been shown in other bird

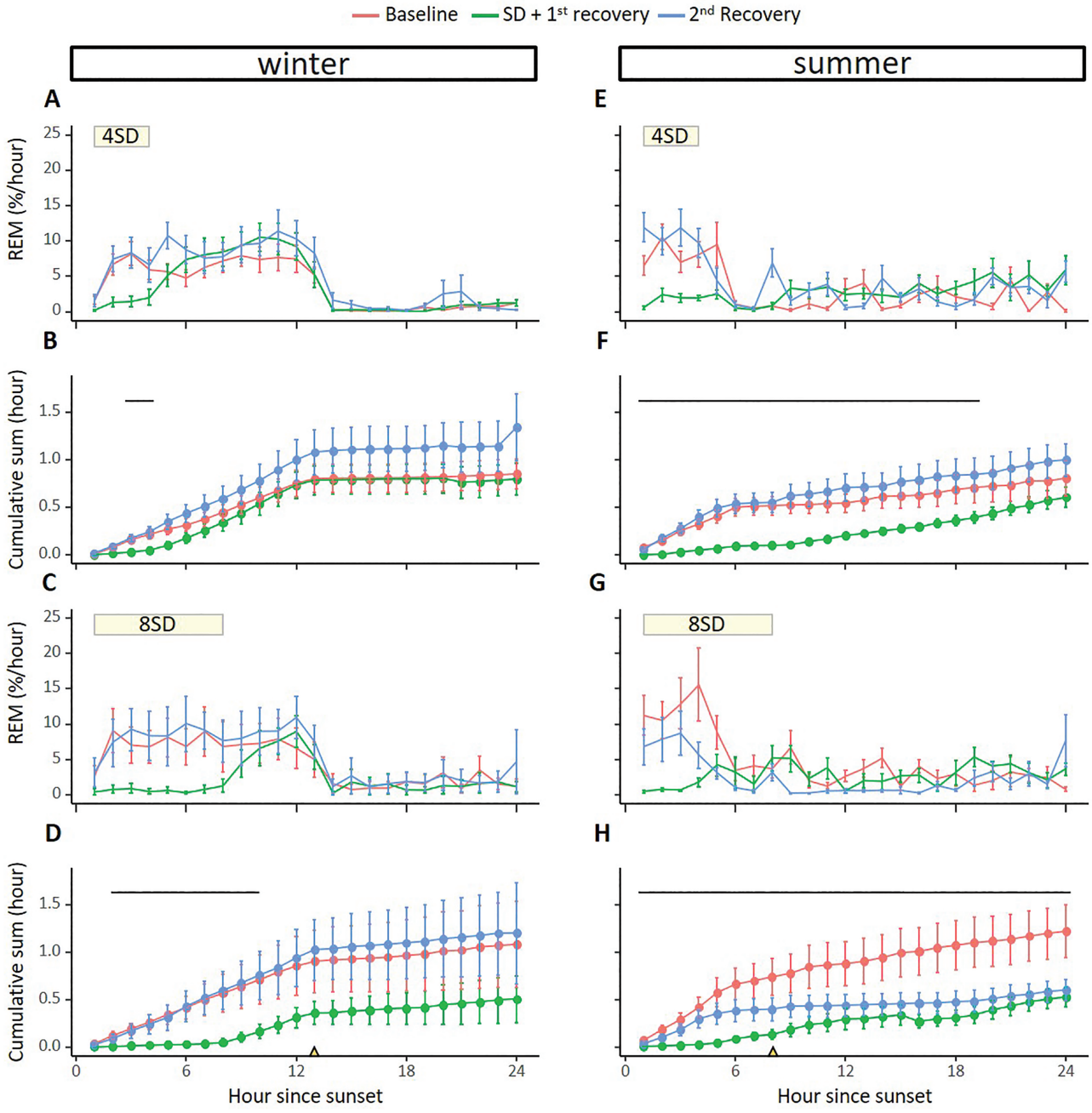

Figure 9. Effect of sleep deprivation on REM sleep time. Effects of sleep deprivation in winter (left panels; 4SD: $n = 8$; 8SD $n = 6$) or summer (right panels; 4SD: $n = 7$; 8SD $n = 8$) on REM sleep patterns and cumulative REM sleep time. Sleep deprivation started after sunset and lasted for either 4 h (panels A, B and E, F) or 8 h (panels C, D and G, H). The yellow triangles on the x-axes denote sunrise. The period of sleep deprivation is indicated by the yellow horizontal bar at the top of the panels (panels A, E and C, G). Every panel shows REM sleep for the baseline day (red line), the day with sleep deprivation and subsequent recovery time during the remainder of the day (green line), and a second recovery day (blue line). Significant differences between the baseline day and the SD + first recovery day are indicated by the horizontal lines (lmer model, $p < 0.005$). Data shown are group averages ± SEM.

species (e.g. ptarmigan, *Lagopus muta* [39]) as well as mammals (e.g. reindeer, *Rangifer rangifer* [40]). It was proposed that this seasonal arrhythmia might result from a weaker circadian clock that would allow animals to feed at any time of day [41]. Interestingly, other species under seasonal constant light conditions maintain clear 24-h rhythmicity, which includes insectivorous bird species with circadian activity that coincides with the activity peaks of insects [42]. The herbivorous barnacle goose in this study may be one of those species that could profit from seasonal arrhythmia to facilitate foraging around the clock.

In addition to the option of feeding around the clock, another reason for arrhythmia might be the need for a constant level of vigilance during the summer season, when geese are breeding and molting. During the breeding phase, the birds have the constant care of the nest or the young, and during the molting phase the birds themselves are flightless and potentially more vulnerable to predation [43, 44]. Therefore, it might be beneficial for the birds to attenuate their circadian rhythmicity to allow for vigilant and active behavior at any time of day. Together, these findings support the notion that the biological

clock can be adapted to species-specific behavior under constant arctic light conditions [45].

In our restricted recording periods in winter and summer we had a fair amount of variation in ambient temperature, even independent of season. The results show that the amount of NREM sleep was at least partly dependent on ambient temperature, with the highest amount of NREM sleep occurring around a temperature of 0°C. Perhaps this is the optimal temperature for geese to sleep because it might be near the thermoneutral zone. While the exact thermoneutral zone of the barnacle goose is not known, an averaged result of 33 sea bird species showed a lower critical temperature in summer of 5.4 ± 2.2°C [46].

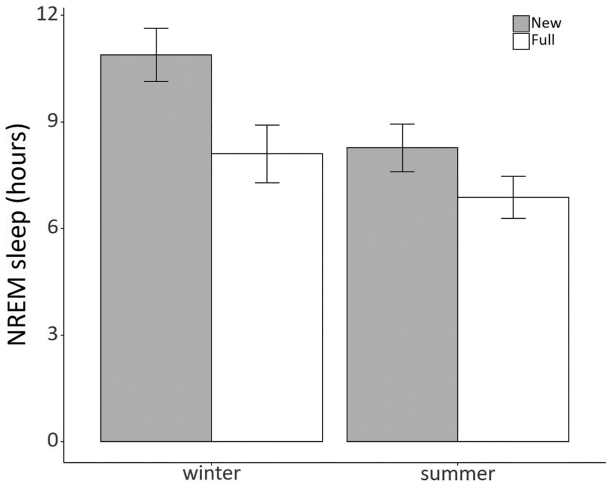

Figure 10. NREM sleep time correlates with moon phase. Daily averages of NREM sleep in winter and summer during nights with a new moon (gray) and full moon (white). Nights with full moon significantly decreases the amount of NREM sleep by 2 h independent of season (lmer model, $p < 0.001$). Data shown are group averages ± SEM.

Interestingly, the amount of REM sleep in the barnacle geese was not significantly related to ambient temperature whereas in mammals REM sleep in particular appears to be sensitive to environmental temperature [47–49]. Several studies in mammals have shown decreases and even complete disappearance of REM sleep at lower ambient temperatures close 0°C [48–51]. A partial explanation for the difference with our geese might be that these studies were done in relatively small mammalian species with a high thermoneutral zone compared to our larger geese with a much lower thermoneutral zone.

Another relevant environmental factor that affects sleep in geese independent of season is moon phase. Indeed, the daily amount of NREM sleep was 2.0 h lower during full moon than during new moon. This finding appears to be in agreement with earlier reports showing that barnacle geese are more active and have an increased heart rate and body temperature during nights with a full moon [52]. Also, our own recent work in starlings showed that this songbird on average sleeps 2 h less on nights with a full moon [16]. Little is known on how the moon affects sleep time in birds. There might be a direct effect of light of the moon that decreases NREM sleep time. This idea is supported by the findings that low level ambient light during the night can influence sleep time in birds [53–56]. However, the moon might affect sleep time in birds through other mechanisms than light, for example, through magnetism. The moon's

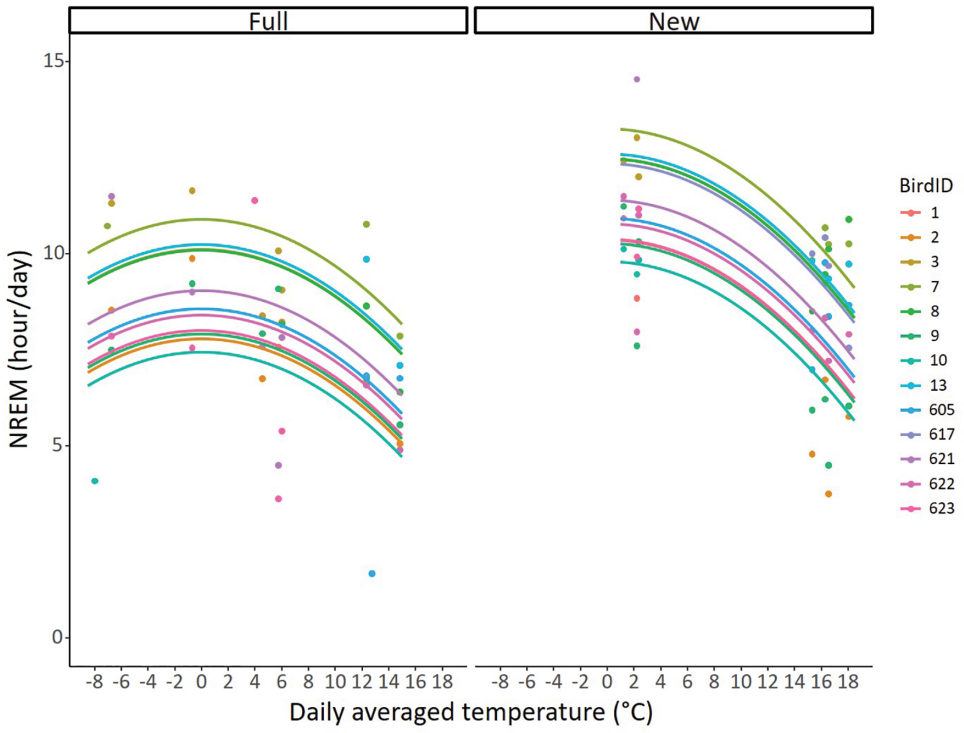

Figure 11. NREM sleep time correlates with ambient temperature. NREM sleep during nights with full moon (left panels) and new moon (right panels) as a function of ambient temperature plotted separately for every individual bird. Independently of moon phase, NREM sleep significantly decreases when ambient temperature increases (lmer model, $p < 0.001$). The amount of NREM sleep is significantly higher during the days where there was a new moon during the night compared to the full moon (lmer model, $p < 0.001$).

gravitational pull can affect the magnetic field of the planet [57]. It is known that some migratory birds can observe the magnetic field and it is proposed that the main magneto-sensory molecule is cryptochrome [58–60].

In conclusion, this study demonstrates that sleep regulation in migratory bird species is strongly affected by environmental factors and season. Importantly, the results demonstrate that sleep homeostasis is not a rigid phenomenon and suggest that some species may not immediately compensate for sleep loss under certain conditions or during certain periods of the year.

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