Article

Current Biology

Sleep Time in the European Starling Is Strongly Affected by Night Length and Moon Phase

Highlights

- Starlings display strong phenotypical variation in sleep-wake regulation
- Full moon reduces sleep time in starlings with about 2 h
- Starlings sleep 5 h less per day during summer compared to winter
- Starlings have more mid-day naps and higher sleep pressure in summer

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In Brief

Sleep regulation in the starling is highly flexible and sensitive to environmental factors. Sleep time is 5 h less during summer than during winter, which is best explained by night length. Additionally, the birds sleep around 2 h less during full-moon nights. van Hasselt et al. show the importance of conducting research with a more natural approach

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Sleep Time in the European Starling Is Strongly Affected by Night Length and Moon Phase

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SUMMARY

Sleep is considered to be of crucial importance for performance and health, yet much of what we know about sleep is based on studies in a few mammalian model species under strictly controlled laboratory conditions. Data on sleep in different species under more natural conditions may yield new insights in the regulation and functions of sleep. We therefore performed a study with miniature electroencephalogram (EEG) data loggers in starlings under semi-natural conditions, group housed in a large outdoor enclosure with natural temperature and light. The birds showed a striking 5-h difference in the daily amount of non-rapid-eye-movement (NREM) sleep between winter and summer. This variation in the amount of NREM sleep was best explained by night length. Most sleep occurred during the night, but when summer nights became short, the animals displayed mid-day naps. The decay of NREM sleep spectral power in the slow-wave range (1.1–4.3 Hz) was steeper in the short nights than in the longer nights, which suggests that birds in summer have higher sleep pressure. Additionally, sleep was affected by moon phase, with 2 h of NREM sleep less during full moon. The starlings displayed very little rapid-eye-movement (REM) sleep, adding up to 1.3% of total sleep time. In conclusion, this study demonstrates a pronounced phenotypical flexibility in sleep in starlings under semi-natural conditions and shows that environmental factors have a major impact on the organization of sleep and wakefulness.

INTRODUCTION

Most animal species spend a large part of their life asleep, suggesting that sleep serves important functions [1–3]. It is commonly accepted that sleep is homeostatically regulated and that the need for sleep builds up during wakefulness [4–6]. However, much of what we know about sleep is based on a handful of mammalian species under tightly controlled laboratory conditions, particularly nocturnal rodents. Given that sleep appears to be a wide-spread phenomenon that may have evolved early in evolution, we can perhaps learn about the regulation and functions of sleep by (1) studying sleep in other non-mammalian, non-model species and (2) by studying sleep under the natural conditions where it evolved [7, 8].

With respect to the first point, birds are of particular interest because they share key sleep features with mammals, including the presence of rapid-eye-movement (REM) sleep and non-rapid-eye-movement (NREM) sleep [2, 9]. At the same time, there are interesting differences in sleep between birds and mammals. For example, the proportion of REM sleep per total sleep time is on average 18% in mammals [10], although in birds, this is on average about 8% [11].

The notion that studying sleep under more natural conditions may be an important approach is supported by findings of pronounced differences in sleep timing and duration between conspecifics in the wild and captivity, suggesting that ecological factors can have a strong influence on sleep [12–14]. Environmental challenges and natural behaviors, such as breeding or migration, are often absent in studies under controlled laboratory conditions.

A recent study reported exceptional sleeplessness in wild pectoral sandpipers (*Calidris melanotos*) breeding under constant light during the Arctic summer. Some of the male birds showed a near complete reduction of sleep time for over 3 weeks yet were able to maintain high waking performance [15]. The males that are awake the most are more attractive to the females and thus sire the most offspring. Also, several types of birds engage in long, non-stop flights during migration and foraging that seemingly leave little time for sleep [16]. Recently, it has been shown that frigatebirds (*Fregata minor*) sleep during foraging flights where they stay airborne for 6 consecutive days. Although sleeping on the wing is possible, they only sleep 2.89% of the time during the flight compared to 53.28% of the time on land [17]. Furthermore, sleep and activity recordings in captive white-crowned sparrows (*Zonotrichia leucophrys*) have shown an enormous flexibility in activity throughout the annual cycle, where they stay active close to 24 h a day during the entire breeding season and migratory phase [18, 19]. Such findings challenge the common view based on studies in mammals that decreased performance and health is an inescapable outcome of sleep loss and beg for follow-up studies.

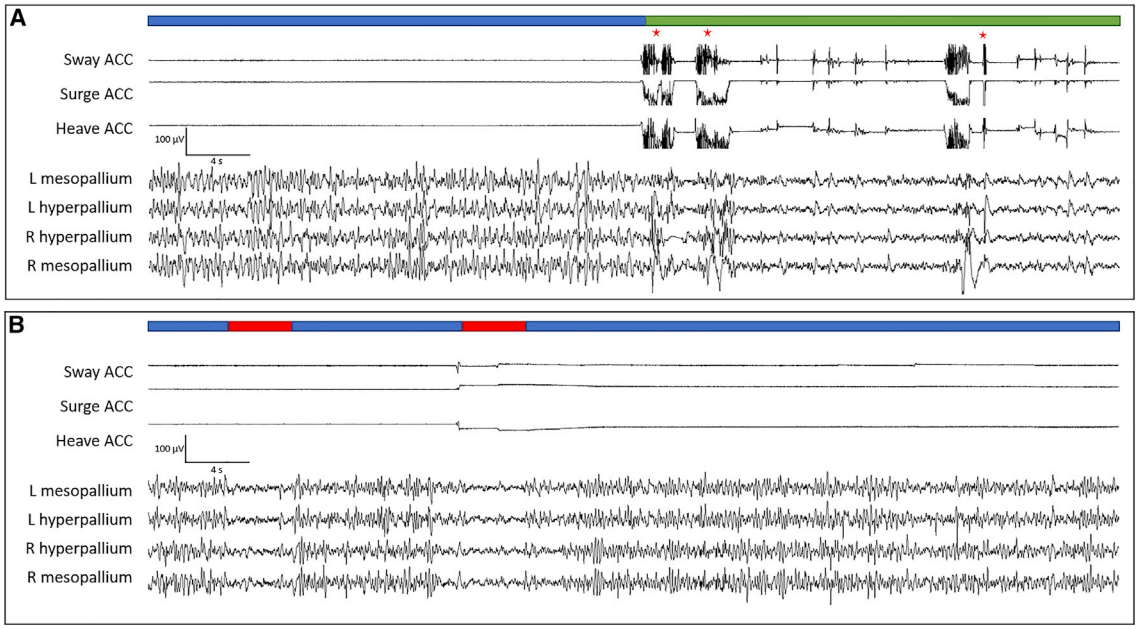

Figure 1. Two Representative Examples of the EEG and Accelerometer Traces of a Starling

A 60-s representation of the EEG channels (L+R mesopallium and L+R hyperpallium) and accelerometer channels (sway, surge, and heave). All recordings are scored for wakefulness (green bar), NREM sleep (blue bar), and REM sleep (red bar). The spectral analysis was done without epochs containing movement artifacts (red asterisk).

Although the advancements in data loggers technology now allow for recordings of sleep in animals under natural conditions, seasonal changes in electroencephalogram (EEG)-defined sleep have not been examined in freely moving birds due to the challenges of recapturing individual animals across multiple seasons. Therefore, in the present study, we measured sleep across the year in captive birds living under semi-natural outdoor conditions to address the question how birds cope with the large variation in environmental conditions over the year, particularly changes in light and temperature. We chose to do this in the European starling (*Sturnus vulgaris*) because we previously reported EEG recordings in this species under controlled indoor conditions that serve as a basis for comparison with the current outdoor measurements [20].

RESULTS

Strong Seasonal Variation in Sleep Duration

The outdoor experiment yielded 40 high-quality, 24-h recordings. Representative examples of wakefulness, NREM, and REM sleep are shown in Figure 1. Across all recordings, the starlings spent on average 57.1% ± 3.6% of the 24-h day-night cycle awake, 42.3% ± 3.7% in NREM sleep, and 0.6% ± 0.2% in REM sleep. Because REM sleep occurred very little, wakefulness and NREM sleep are mathematically opposite. Hence, wakefulness was not included in further analysis and graphs.

The starlings displayed strong variation in the daily amount of NREM sleep across the year, with the highest amount of sleep during winter and the lowest amount during summer (see Figures 2A and 2B). The daily amount of NREM sleep displays an annual rhythm with an estimated 5.12 h more sleep during winter than during summer (linear mixed effect [lmer] model; $p < 0.001$; $r^2_{\text{marginal [m]}} = 0.50$; $r^2_{\text{conditional [c]}} = 0.55$; Figure 2B).

The fraction of REM sleep relative to total sleep time (TST) displayed some seasonal variation (Figure 2D), with a significantly higher proportion of REM sleep in winter compared with summer (lmer model; $p = 0.0027$; $r^2_m = 0.18$; $r^2_c = 0.62$). In other words, winter was not only associated with the highest overall amount of NREM sleep but also with the highest fraction of REM sleep.

In addition to the seasonal changes in daily NREM and REM sleep time, NREM and REM bout lengths also varied across the year (see Figures 2B–2E). Bout length was longer during winter compared with summer for both NREM ($p < 0.001$; $r^2_m = 0.24$; $r^2_c = 0.58$; Figure 2C) and REM sleep ($p = 0.04$; $r^2_m = 0.11$; $r^2_c = 0.11$; Figure 2E).

Night Length and Moon Phase Strongly Influence Sleep

Most of the sleep the starlings displayed occurred during the night time. A large part of the variation in sleep time across the year could be explained by night length (lmer model; $p < 0.001$; $r^2_m = 0.51$; $r^2_c = 0.54$; with a repeatability of 0.11; Figure 3A). With every hour increase of night length, NREM sleep increased with 0.49 ± 0.08 h (Figure 3A). Also, the proportion of REM sleep of TST significantly increased when night length increased (lmer model; $p = 0.003$; $r^2_m = 0.19$; $r^2_c = 0.62$; Figure 3B). When night length was longer than 9.8 h (i.e., in winter), the total amount of NREM sleep was less than the duration of the night (right gray-shaded area in Figure 3C). When night length was shorter than 9.8 h (i.e., in summer), the total amount of sleep exceeded the duration of the night (left gray-shaded area in Figure 3C). In the latter case, the birds seem to increase the amount of sleep particularly around the middle of the day (Figure 2A).

We anticipated that some variation in sleep time across the year might be related to variation in ambient temperature. However, in a model with night length and temperature together, variation in

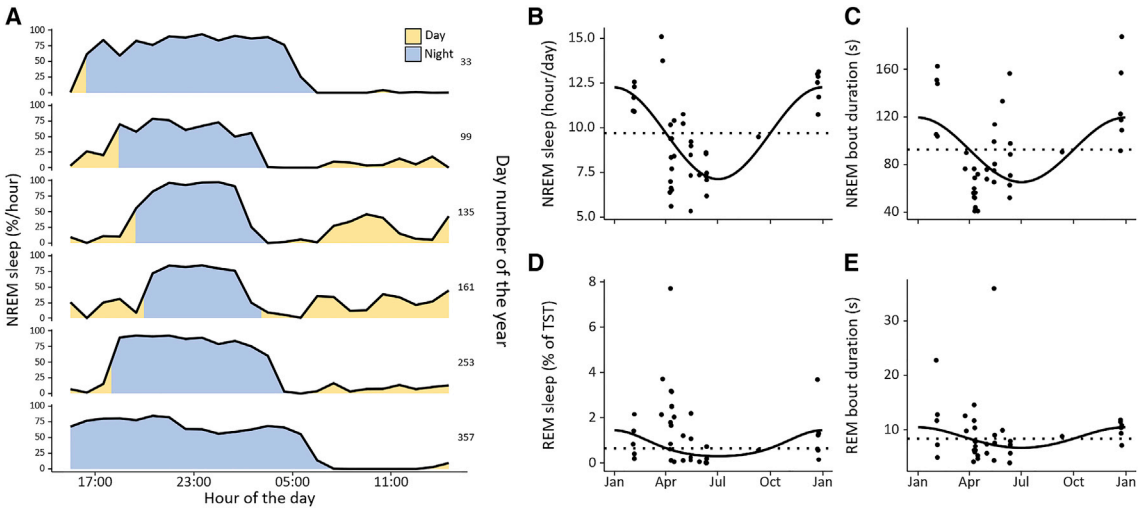

Figure 2. Strong Seasonal Modulation of Sleep

(A) Representative recordings from multiple individuals showing the distribution of NREM sleep over the day and night (secondary y axis indicates day number). The hours spent in NREM sleep at night get shorter until day number 161 (colors under the curve indicate the natural light dark cycle; blue, night; yellow, day).

(B) Daily number of NREM sleep hours across the year. The same pattern is visible as shown in (A). The hours spent in NREM sleep follow a sine curve (lmer model; $p < 0.001$; $r^2_m = 0.50$; $r^2_c = 0.55$) with an amplitude of 2.56 h. Starlings sleep 5.12 h less during summer compared with winter.

(C) NREM sleep bout length shows an identical pattern across seasons with longer bout length during winter compared with summer ($p < 0.001$; $r^2_m = 0.24$; $r^2_c = 0.58$).

(D) Log-transformed REM sleep as a percentage of total sleep time (TST) plotted over the year on a linear scale. The fraction of TST that consists of REM sleep can be explained according to a sine wave ($p = 0.024$; $r^2_m = 0.18$; $r^2_c = 0.62$).

(E) Log-transformed REM sleep bout length shows an identical pattern across seasons with longer bout length during winter compared with summer ($p = 0.04$; $r^2_m = 0.11$; $r^2_c = 0.11$).

sleep time was significantly explained by night length, but not temperature ($p = 0.27$). This lack of effect of ambient temperature in our dataset might in part be due to the fact that fluctuations in temperature across the year were strongly correlated with night length.

Another potentially relevant environmental factor that might affect sleep is moonlight. Based on lunar cycle data from https://www.timeanddate.com, we calculated the percentage of the moon surface that was illuminated as a proxy of the amount of moonlight. In a model together with night length, this percentage of moon surface illuminated was a significant predictor for the amount of NREM sleep (lmer model; $p = 0.006$; $r^2_m = 0.59$; $r^2_c = 0.63$). Independent of night length, the starlings slept 0.0253 ± 0.009 h less for every percent increase in the illumination of the moon's surface. On average, the daily amount of NREM sleep was about 2 h less on days with a full moon as compared to days with a new moon (Figure 3D).

The results so far were based on an analysis of total daily sleep time. We did a further and more detailed analysis on how environmental factors influenced sleep during different phases of the day, specifically "night," "twilight," and "day time." We used nautical twilight to define the twilight period per recording. The time spent in NREM sleep during the night was significantly increased when night length increased (lmer model; $p < 0.001$; $r^2_m = 0.90$; $r^2_c = 0.95$; Figure 4A). In contrast, the time spent in NREM sleep during twilight and daytime was significantly decreased when night length increased (lmer model; twilight: $p < 0.001$, $r^2_m = 0.45$, $r^2_c = 0.45$; day: $p = 0.005$, $r^2_m = 0.21$, $r^2_c = 0.36$; Figure 4A).

If the effect of moon phase on NREM sleep time was mediated by light, one might expect this effect to occur mainly during the night. We therefore did an additional and more detailed analysis for effects of moon phase on NREM sleep time residuals from Figure 4A for different sections of the 24-h cycle, i.e., night time, twilight, and day time. There was a significant relationship between moon surface illumination and the amount of NREM sleep during the night (lm model; $p < 0.020$; $r^2 = 0.14$; Figure 4B). Moon illumination had no significant relationship with NREM sleep time during twilight or daytime (Figure 4B). This supports the hypothesis that the effect of moon phase was mediated by moon light.

Pressure for Sleep Is Greater during Summer

The finding of increased NREM sleep during the daytime in summer might indicate that the nights at this time of year are too short to dissipate all sleep pressure that is built up during the daytime waking phase. Together with the finding that the overall amount of NREM sleep is much lower during summer, one might argue that birds during summer live with higher sleep debt and sleep pressure than they do during winter. To address this issue, we analyzed EEG spectral power as an indicator of sleep debt and sleep intensity. Based on fast Fourier transformation of all artifact-free NREM sleep EEG data, we averaged the spectral power between 1.17 and 4.30 Hz, a frequency range that is known to reflect sleep debt in starlings [20]. These power values cannot be compared directly because of interindividual differences in EEG signal strength, but instead we performed linear regression across the power values for the first 3 h of the night, excluding twilight, for all recordings and used the slope of this line as an indicator of the decay in sleep pressure (Figure 5A). This slope of power decay during NREM sleep significantly depended on the

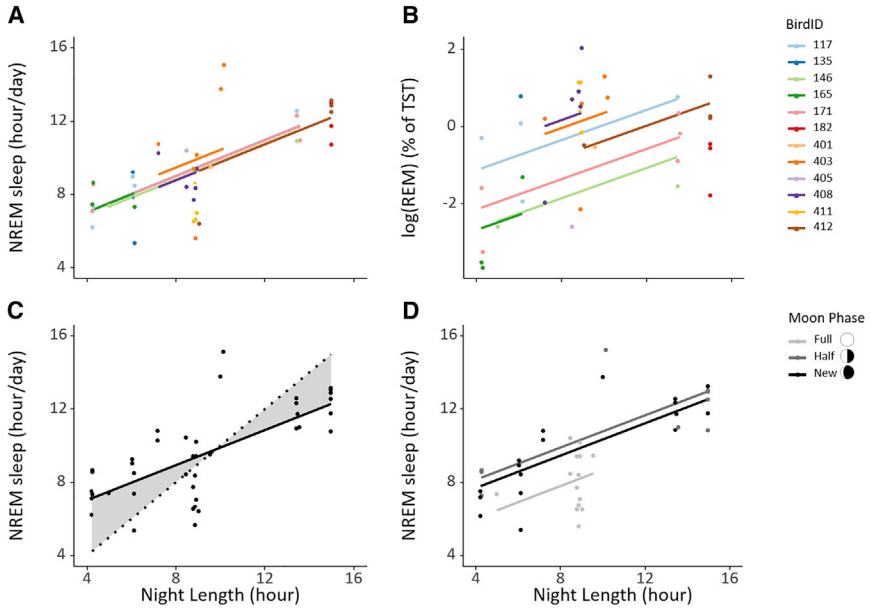

Figure 3. Night Length and Moon Phase Strongly Influence Sleep

(A) NREM sleep in relation to night length plotted for every individual. The model predicts that, for every hour the night gets longer, the starlings sleep an additional 0.49 ± 0.08 h ($p < 0.001$). Model results are $r^2_m = 0.51$ and $r^2_c = 0.54$ with a repeatability of 0.11.

(B) Log-transformed REM sleep as a percentage of TST in relation to night length ($p = 0.003$; $r^2_m = 0.19$; $r^2_c = 0.62$). Individual repeatability was 0.51.

(C) NREM sleep in relation to night length with a dotted black line that represents x = y, i.e., when night length is equal to the hours spent in NREM sleep. When the night length is below 9.78 h, there is an overshoot in NREM sleep during the day (left gray area). Alternatively, when the night length is longer than 9.78 h, there seems to be an undershoot (right gray area).

(D) Hours spent in NREM sleep in relation to night length and moon phase. For graphical presentation of the model, we divided moon surface illumination into three categories: full; half; and new moon, with full moon = 67%–100% illumination, half-moon = 33%–67% illumination, and new moon = 0%–33% illumination. Moon phase has a significant effect on the amount of NREM sleep independent of night length ($p < 0.001$). Full moon phase results in significantly less hours of NREM sleep compared with half-moon phase ($p = 0.015$) and new moon ($p = 0.02$). No significant differences were observed between full and half-moon ($p = 0.79$).

duration of the night (lmer model; $p = 0.008$; $r^2_m = 0.18$; $r^2_c = 0.41$; Figure 5B). The slope was more negative, i.e., the power decay is steeper when the night was shorter. This supports the idea of higher sleep pressure at the beginning of the short summer nights.

DISCUSSION

The starlings investigated under semi-natural conditions displayed a striking 5-h variation in the daily amount of NREM sleep across the seasons, with ca. 12.5 h of NREM sleep/day during winter and 7.5 h NREM sleep/day during summer. Much of the variation in the daily amount of NREM sleep could be explained by night length, with most sleep occurring during winter, when nights are longest. In summer, when nights are shorter, the birds increased the amount of daytime sleep by including mid-day naps. In addition to night length, moon phase was associated with variation in sleep time: during full moon nights, the amount of NREM sleep was approximately 2 h less than during half-moon or new moon. Full moon thus had a sleep-depriving effect. Overall, the birds displayed a minimal amount of REM sleep, which on average made up less than 1.3% of total sleep time. Yet this small amount of REM sleep was still subject to seasonal modulation that paralleled the seasonal variation in NREM sleep: REM sleep as a fraction of total sleep time was longest during long winter nights.

During the summer, the total daily amount of sleep was 5 h less than during the winter. As a consequence, the birds may be living under higher sleep pressure during summer than winter, a suggestion that is supported by the steeper decline in EEG power during the first hours of the night during summer as compared to the winter. The slope of the power decline is thought to reflect the dissipation of sleep debt [21], also in starlings [20]. A steeper decline in power during sleep on summer nights might indicate deeper sleep at this time of year. Despite this indication of deeper sleep, the birds may not have been able to offset all their need for sleep during the short summer. When the duration of the night became shorter than 9.8 h, the birds displayed more sleep during the mid-day. This finding suggests that, even with deeper sleep at night, starlings may need around 10 h to compensate for the NREM sleep pressure that builds up during the preceding waking phase; otherwise, an overshoot of NREM sleep occurs during the day.

The seasonal variation in NREM sleep time observed in starlings is in agreement with earlier research on seasonal changes in resting behavior in wild songbirds [22, 23]. In addition to not being EEG based, these studies only recorded sleep behavior in the nest/roosting box. Consequently, napping in a bush in the daytime could have occurred. Despite these limitations, they found similar seasonal changes in resting behavior, showing close to 5 h more rest in the winter.

A limited number of studies in mammalian species have reported seasonal variation in sleep time as well. An EEG-based study of three free-ranging Arabian oryx (*Oryx leucoryx*) showed substantial seasonal variation in sleep time related to ambient temperature rather than photoperiod [24]. An actigraphy study of humans in pre-industrial societies in Africa and Latin America showed close to 1 h more sleep in winter than in summer [25]. Overall, data on seasonal variation in sleep, particularly under natural conditions, are limited.

Much of the additional daytime sleep during summer occurred in the middle of the day rather than in the morning as an extension of the main sleep phase. This might be due to a strong circadian drive for wakefulness early in the morning. Such an early morning drive for wakefulness could be related to, for example, a need to search for food. This early morning drive for

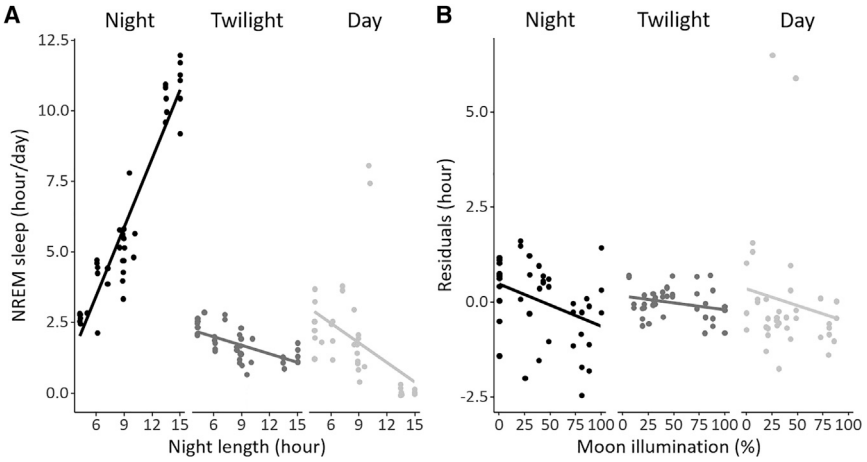

Figure 4. Variation of NREM Sleep during Different Phases of the Day Is Partly Explained by Moonlight

(A) NREM sleep time in relation to night length during different light phases (night, twilight, and day). During the night, the number of hours spent in NREM sleep increases significantly when night length increases (lmer model; $p < 0.001$; $r^2_m = 0.90$; $r^2_c = 0.95$). Furthermore, during the day and twilight, NREM sleep decreases when the night lengthens (lmer model; twilight: $p < 0.001$, $r^2_m = 0.45$, $r^2_c = 0.45$; day: $p = 0.005$, $r^2_m = 0.21$, $r^2_c = 0.36$).

(B) Residuals of the model shown in (A) plotted against moon illumination. During the night, moon illumination had a significant effect on the residuals (lm model; $p = 0.020$; $r^2 = 0.14$). Moon illumination had no significant effect on the residuals during twilight and day.

wakefulness is supported by our earlier study on the effects of sleep deprivation in starlings under controlled conditions [20]. When birds were sleep deprived at night, they showed a compensatory increase in sleep time not in the early morning immediately following sleep deprivation but later during the day.

In our analysis, night length explained a large part of the seasonal variation in sleep time, but it remains to be determined whether this is a direct effect of night length or a consequence of some correlated seasonal change in physiological state and behavior, for example, reproduction. Even though, in our semi-natural setting, the starlings were not breeding, one might argue that their lower amount of sleep in summer may have partly resulted from restlessness associated with an underlying physiological reproductive state. Importantly, such seasonal changes in behavior and physiology are regulated by photoperiod, but in that case, the changes in sleep would be an indirect or secondary consequence of night length/day length rather than a direct effect on sleep itself. Alternatively, it is possible that the seasonal change in night length and associated light exposure affects sleep directly. A direct effect of light on sleep is supported by our finding of a strong reduction in night-time sleep time during full moon.

An intriguing finding was that moon phase strongly affects sleep time. Our analysis of a relationship between sleep duration and moon phase has the limitation that we did not have detailed information on the actual visibility of the moon and potential reductions herein due to cloud coverage. Nevertheless, the finding that starlings sleep significantly less during full moon nights can be explained by a direct sleep-depriving effect of light. This is supported by our analysis showing that there was only an effect of moon phase on night-time sleep, but not on sleep during twilight and day time. To some extent, a possible influence of moonlight on sleep is also supported by other studies in birds showing that low-level artificial white light can suppress sleep [26] and increase night-time activity [27–29]. Furthermore, our findings of reduced sleep, i.e., increased wakefulness during full moon nights, are in agreement with recent studies in barnacle geese showing that heart rate and body temperature increase when there is full moon and this effect is most pronounced when it coincides with perigee (the closest point of the moon to the Earth) [30].

In general, there is little information available on how moonlight affects sleep and EEG activity. There have been a few EEG studies on the relationship between moon phase and sleep in humans, but those were done under indoor conditions, where moonlight itself was not visible [31–33]. The effect of moon phase on human sleep EEG activity remains inconclusive, as literature found that moon phase was able to decrease EEG spectral power in the delta range (1–4 Hz) [31, 32] or that moon phase has no effect on sleep EEG at all [33]. Studies based on recording of rest and activity with motion sensors in human pre-industrial societies have reported contradicting results on the relationship between moon phase and sleep, with either a decrease or an increase in total sleep time with increasing moonlight [34, 35]. Perhaps the moon has effects on sleep through mechanisms other than light, for example, through changes in the magnetic field of the Earth caused by the moon. Birds can detect the magnetic field and use it to orientate and navigate during migration [36]. It is known that the moon's gravitational pull on the planet affects the Earth's magnetism with daily variation [37]. Birds might be able to detect such changes in magnetism. It is proposed that migratory birds have a magneto-sensitive receptor in the retina and that cryptochrome is the main magneto sensory molecule [38, 39]. Therefore, it cannot be ruled out that the changes in sleep time in relation to moon phase are changes sensed in the Earth's magnetism in addition to a direct effect of moon light.

Our finding of a minimal amount of REM sleep in the starling is in agreement with our previous reported study of sleep in the starling under controlled indoor conditions [20] and with an earlier study in the same species [40]. The assessment of REM sleep is more difficult in birds than in mammals because REM sleep episodes are short and rarely associated with a clear drop in muscle tone, as measured by electromyography (EMG). In our study, the scoring of REM sleep was based on EEG in combination with head movements (i.e., accelerometry recordings with the head-mounted EEG logger) instead of EMG. REM sleep is characterized by periods of EEG activation without head movements or with signs of head dropping. In birds, such head drops often occur even without clear changes in the neck EMG signal [41]. Consequently, accelerometry is more reliable than EMG. Our approach has been successfully used for scoring sleep in other bird studies [17, 41, 42].

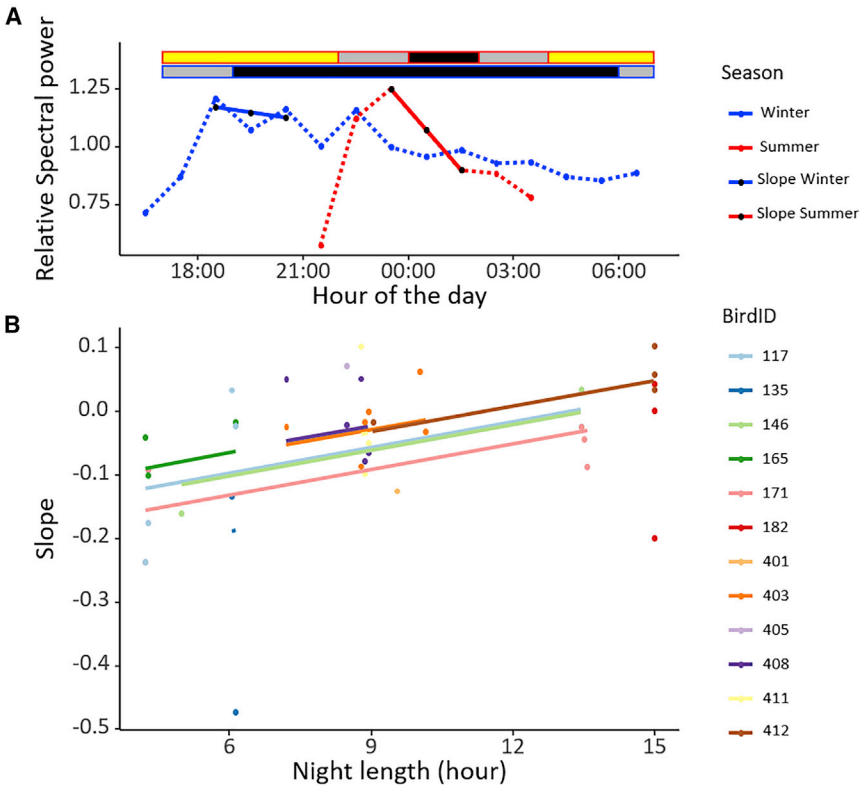

Figure 5. Decay in EEG Spectral Power Is Steeper during Short Nights

(A) Two relative spectral power lines of the same individual recorded in winter and summer (dashed blue and red lines for, respectively, winter and summer). The bars on top indicate the light-dark cycle; gray, nautical twilight; black, night; yellow, day. The first and third points during the night phase were used to calculate the slope using a regression model. The solid lines with black dots represent the calculated slopes as shown for all 40 recordings in (B).

(B) The slope of relative EEG spectral power (1.17–4.30 Hz) during the first 3 h of the night in relation to the night length in hours. The slope becomes significantly more positive with increasing night length with a rate of 0.02 ± 0.005 power units per hour ($p = 0.008$; $r^2_m = 0.18$; $r^2_c = 0.41$).

Nonetheless, we cannot fully exclude that some of the REM episodes in the starlings may have been microarousals without head movements. However, if that were the case, this would mean that we overestimated the amount of REM sleep. In other words, the very low amount of REM sleep would be even lower.

We had anticipated that starlings in the current study under semi-natural and social housing conditions might have more REM sleep, but this clearly was not the case. Yet despite the small overall amount of REM sleep, there was seasonal variation in the amount of REM sleep with a higher proportion of REM in winter as compared to summer. This suggests that, even with this minimal amount of REM sleep, there was some degree of regulation.

Overall, the amount of REM sleep is highly variable among bird species, ranging from less than 5% of total sleep time in starlings [20, 40], rooks [43], parakeets [44], and turtle doves [45] to more mammalian-like numbers in white-crowned sparrows [19] and zebra finches [46] with 16% and 25% of total sleep time, respectively. Even within the same orders, there are substantial differences in the amount of REM sleep, for example, in songbird species (e.g., the starling and white-crowned sparrow). Therefore, a simple taxonomic explanation for this variation of REM sleep does not apply on the reported values of REM sleep in the different bird species. Data on more species will be required before we can begin to understand the interspecific variation in the amount of REM sleep. Clearly, the current results provide important fuel to the discussion on what REM sleep is, how it is regulated, and what its functions might be. It also cautions against broad generalizations based on studies in laboratory rats and mice.

In summary, our data demonstrate that sleep-wake regulation of the European starlings under semi-natural conditions shows a striking variation across the annual cycle. Starlings change their sleep architecture according to night length and the lunar phase. These findings emphasize the importance of conducting sleep experiments in different species with a more natural approach to learn about sleep regulation in the real world.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS

P.M., S.V., N.C.R., and M.R. conceived of the project and designed the experiment. A.L.V. supplied the necessary hardware and software to conduct the experiment. S.J.v.H. and M.R. executed and analyzed the experiments. The manuscript was written by S.J.v.H. and P.M. and reviewed by S.V. and N.C.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Sturnus Vulgaris	Max Planck Institute for Ornithology, Seewiesen Oudehaske, the Netherlands (52°58'19.2"N 5°51'38.0"E)	N/A
Software and Algorithms		
Downloader v1.28	Evolocus	http://www.evolocus.com
RemLogic v3.4.0.2361	Embla Systems	https://neuro.natus.com/products-services/embla-remlogic-software
R studio 3.4.1		https://www.rstudio.com
lme4		http://lme4.r-forge.r-project.org/
multicomp		https://CRAN.R-project.org/package=multcomp

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources, raw images and recordings should be directed to, and will be fulfilled by, the Lead Contact, Peter Meerlo (p.meerlo@rug.nl). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

For this study, we used 12 adult starlings of both sexes (7 males and 5 females). Five of them were wild caught animals obtained from the Max Planck Institute for Ornithology in Seewiesen, Germany. The other seven were caught in the area of Oudehaske, the Netherlands (52°58'19.2"N 5°51'38.0"E). The birds were group-housed in an outdoor aviary (length = 500 cm, width = 400 cm, height = 230 cm) at the University of Groningen, the Netherlands (53°14'35.4"N 6°32′15.7"E). The aviary contained two horizontal ropes of 2.5 m as cage enrichment and for the birds to perch. Three water bowls were available and universal bird food was present *ad libitum* (food item number 6659; Kasper Faunafood, Woerden, the Netherlands). All procedures were approved by the national Central Authority for Scientific Procedures on Animals (CCD) and the Institutional Animal Welfare Body (IvD, University of Groningen, the Netherlands).

METHOD DETAILS

Surgery

Animals were surgically implanted with a 7-channel implant for EEG recordings as previously described [20]. The surgeries were performed under isoflurane anesthesia (1.5%–2% vaporized in 1 l/min air). The top of the skull was carefully exposed and 7 holes with a diameter of 0.5 mm diameter were drilled to the level of the dura at precise locations for insertion of the electrodes. Four EEG electrodes were arranged in a left-to-right line over the rostral part of the telencephalon (two electrodes per hemisphere, 2 and 6 mm lateral from the midline). The medial electrodes were over the hyperpallium and the lateral electrodes were over the mesopallium. Two reference electrodes (one per hemisphere) were placed caudally close to the cerebellum (4 mm lateral of the midline). Lastly, a ground electrode was placed medially on the right hemisphere (6 mm from the midline). All electrodes were made from gold-plated pins with rounded tips (0.5 mm diameter BKL Electronic 10120538, Lüdenscheid, Germany). Each electrode was placed on the dura mater and glued in position using cyano-acrylic adhesive and wired to a 7-channel connector (BKL Electronic 10120302, Lüdenscheid, Germany) that was mounted on the head and secured with Paladur dental acrylic (Heraeus Kulzer, Hanau, Germany). The connector was covered with a lightweight protective plug (BKL Electronic 10120602, Lüdenscheid, Germany). After two weeks of recovery, we started training the animals with dummy loggers to accustom them to carrying the logger weight. Dummy weight was increased in 3 steps (1.5 g, 2.5 g, 3.5 g), with each step lasting 3 days. The last dummy weight represents the final logger weight which is less than 5% of their total body weight. After one week of habituation to the final dummy weight, the measurements started.

Data collection

To record and store EEG data, we used miniature dataloggers (Neurologger 2A; Evolocus, Tarrytown, NY, USA). The neurologgers contained a three-axis accelerometer board that allowed for a detailed assessment of head movements (LIS302DLH; STMicroelectronics, Geneva, Switzerland). The loggers were powered by two ZA13, 1.45 V batteries (Ansmann ZA13, Assamstadt, Germany) which enabled recordings to run continuously for about three-and-a-half days. The loggers were attached to the head connector at noon so the handling of the birds would not affect night time sleep during the first recording night. EEG data was stored on a memory chip in the Neurologger at a rate of 200 Hz. The recordings lasted three days each, and took place at 20 different days over the year, covering all seasons. Because we had a particular interest in photoperiod, we included recordings around the longest day / shortest night of the year (June 21 in the Northern hemisphere) and the shortest day / longest night of the year (December 21 in the Northern hemisphere). In our text and figures we refer to these periods as summer and winter, even though formally defined winter and summer come after the shortest and longest day, respectively. We decided to use the term winter and summer because they have the same intuitive meaning for readers all over the globe.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analysis

At the end of every three-day recording, the EEG and accelerometry data were retrieved from the EEG loggers and transferred to the EEG-scoring program RemLogic (Natus Medical, Pleasanton, California). With the EEG loggers and batteries that we used in this study, every recording could run for a maximum of three days. However, in a few cases birds lost the logger before the end of the 3-day period or the logger malfunctioned due to, for example, low battery power. We divided all multi-day recordings in successful and complete 24 h parts resulting in a total of 40 recordings of 24 h. Individual birds were recorded at multiple time points across different seasons. All recordings were manually scored on the same EEG derivation (left mesopallium) with an epoch duration of 4 s by the same individual. All 4 s epochs were scored as either wakefulness, NREM sleep, or REM sleep according to criteria described in Figure 1. Wakefulness was scored when the signal was characterized by low amplitude and high-frequency EEG activity and often accompanied by high accelerometer movements. NREM sleep was scored when at least half of the 4 s epoch had an amplitude twice that of alert wakefulness, low-frequency EEG and lacking accelerometer movements. REM sleep was characterized by periods of EEG activation (> 2 s) without noticeable head movement in the accelerometer signal (Figure 1B, first REM epoch) or sometimes with signs of head dropping visible in the accelerometry data indicative of reduced muscle tone (Figure 1B, second REM epoch). Our measurements of head movements (or lack of head movements) served the same purpose as EMG recordings in mammals and has been used successfully for scoring REM sleep in other bird studies [17, 41, 42]. Based on the 4 s scoring, we subsequently calculated the amounts of NREM sleep and REM sleep per hour for all recording days.

A fast Fourier transformation was applied for all 4 s epochs of artifact free NREM sleep EEG data from the same frontal electrodes to calculate spectral power density for different frequency bins. This transformation yielded 256 frequency bands with a bandwidth of ~0.39 Hz. All EEG recordings were visually inspected and EEG artifacts were labeled and excluded from the analysis. Most artifacts arose from movements during wakefulness and were easily recognized in the accelerometer data. To compensate for interindividual differences in EEG signal strength, the spectral power values for each frequency band in each 4 s epoch of NREM sleep were normalized by expressing them relative to the power in the same frequency band averaged for all nighttime NREM sleep.

To be able to relate variation in sleep data to variation in environmental factors, we acquired light-dark cycle data and lunar cycle data from https://www.timeanddate.com. Ambient temperature and weather data were retrieved from a nearby weather station in Eelde, the Netherlands (53°08'07.7"N 6°34'12.0"E).

Statistics

All data were analyzed in R [47]. For analyzing the data, linear mixed effect (lmer) models were used with bird identity as a random effect using the lme4 package [48]. For all computed linear mixed effect models, marginal (r^2_m) and conditional (r^2_c) R^2 values were calculated [49]. For comparisons between moon phases the multcomp package was used, from this package, the Tukey HSD test was used as a posthoc test [50]. REM sleep was not normally distributed and therefore log-transformed. Further analysis of REM sleep and acquired p values are based on log-transformed REM sleep.

DATA AND CODE AVAILABILITY

Requests upon further information on raw data and R-scripts should be directed and will be fulfilled by the Lead Contact, Peter Meerlo (p.meerlo@rug.nl).