A comparison of continuous and intermittent EEG recordings in geese: How much data are needed to reliably estimate sleep–wake patterns?

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Abstract
Recent technological advancements allow researchers to measure electrophysiological parameters of animals, such as sleep, in remote locations by using miniature dataloggers. Yet, continuous recording of sleep might be constrained by the memory and battery capacity of the recording devices. These limitations can be alleviated by recording intermittently instead of continuously, distributing the limited recording capacity over a longer period. We assessed how reduced sampling of sleep recordings affected measurement precision of NREM sleep, REM sleep, and Wake. We analysed a dataset on sleep in barnacle geese that we resampled following 12 different recording schemes, with data collected for 1 min per 5 min up to 1 min per 60 min in steps of 5 min. Recording 1 min in 5 min still yielded precise estimates of hourly sleep–wake values (correlations of 0.9) while potentially extending the total recording period by a factor of 5. The correlation strength gradually decreased to 0.5 when recording 1 min per 60 min. For hourly values of Wake and NREM sleep, the correlation strength in winter was higher compared with summer, reflecting more fragmented sleep in summer. Interestingly for hourly values of REM sleep, correlations were unaffected by season. Estimates of total 24 h sleep–wake values were similar for all intermittent recording schedules compared to the continuous recording. These data indicate that there is a large safe range in which researchers can periodically record sleep. Increasing the sample size while maintaining precision can substantially increase the statistical power, and is therefore recommended whenever the total recording time is limited.

KEYWORD
birds, EEG, geese, scheduled recordings, sleep,
1 | INTRODUCTION

Recent technological advancements and the development of miniaturised dataloggers allow researchers to measure sleep in freely moving animals, even under semi-natural and natural conditions (Massot et al., 2019; Rattenborg et al., 2017; Vyssotski et al., 2006). Such studies can provide important insights in the natural sleep patterns and the influence of environmental conditions on sleep in different species. For example, starlings (Sturnus vulgaris) show a strong reduction in sleep time between seasons with on average 5 h less sleep in summer compared with winter (van Hasselt et al., 2020). Besides photoperiod, the moon phase has also been reported to have a strong sleep-suppressing effect on birds, with on average a 2 h reduction in both starlings and barnacle geese (Branta leucopsis) (van Hasselt et al., 2021). Also, studies in birds have shown that pectoral sandpipers (Calidris melanotos) forgo large amounts of sleep for about 14 days during the mating season (Lesku et al., 2012) and great frigatebirds (Fregata minor) have the capability of sleeping during foraging flights although they sleep much less in flight compared with on land (2.89% vs 53.28%, respectively) (Rattenborg et al., 2016). Together, these findings on sleep in birds indicate that there is a large amount of flexibility in the regulation of sleep.

Studies of long duration in freely moving animals are generally constrained by the battery life and memory capacity of the dataloggers, especially in smaller species where minimizing the size and weight of the loggers is an important issue (Bridge et al., 2011). One possible solution for this limitation is programmable dataloggers that allow for intermittent recordings over longer periods of time as opposed to continuous recordings of shorter duration. However, in the field of sleep research continuous recordings are a gold standard that few deviate from (Kurtz et al., 2009). An important question therefore is can intermittent recordings reliably reflect the true sleep–wake patterns of animals.

To address these questions, we re-analysed a dataset on sleep–wake patterns in barnacle geese that was based on continuous EEG recordings during winter and summer under semi-natural conditions (van Hasselt et al., 2021), and compared the original continuous recordings with different intermittent recording schedules selected from the same data set.

2 | METHODS

2.1 | Animals and housing

Thirteen barnacle geese (Branta leucopsis) (8 males and 5 females) were used in this study. The birds were kept on a fenced meadow (68 m x 60 m) with a pond (25 m x 15 m) at the facilities of our institute in Groningen when they were not used for the experiment. The geese were fully habituated to the presence of humans. Flight feathers were clipped to prevent them from flying away. One week prior to the start of sleep recordings, the animals were transferred in groups of 5 into two separate outdoor aviaries (5 m x 4 m). In these aviaries the geese remained exposed to outdoor light, ambient temperatures, and weather conditions. Water and food were present ad libitum (food item numbers 615220 and 384020; Kasper Faunafood). 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).

2.2 | Surgery

The surgical procedures for implantation of EEG and EMG electrodes were done as described earlier (van Hasselt et al., 2021). Prior to the surgery, the animals received meloxicam as an analgesic (0.5 mg/kg, 0.17 mL subcutaneously) and diazepam to reduce stress (2 mg/kg; 0.68 mL subcutaneously). The surgeries were performed under isoflurane anaesthesia (1.5–2%). Five holes were drilled (0.5 mm in diameter) after carefully exposing the crania and the EEG electrodes were inserted to the level of the dura mater. We inserted two frontal electrodes, one per hemisphere covering the hyperpallium (4 mm lateral of the midline). Three more electrodes were inserted 83 mm caudally from the frontal electrodes: an EEG reference electrode (4 mm left lateral of the midline), an electromyogram (EMG) reference electrode (on the midline) and a ground electrode (4 mm right lateral of the midline). The electrodes consisted of gold-plated, round-tipped pins (0.5 mm diameter, BKL Electronic 10120538). For measuring EMG, two flexible wires were inserted subcutaneously on the neck muscle (PlasticsOne). All electrodes were soldered to a connector (BKL Electronic 10120302) that was fixed to the skull using Paladur dental cement ( Heraeus Kulzer). A 0.6 mm screw was drilled into the skull to serve as an anchor point for the dental cement-covered implant. The animals could recover for at least 2 weeks after the surgery before moving them to the recording aviaries.

2.3 | Sleep recordings

All animals were equipped with a small datalogger (Neurologger 2A; Evolocus) that recorded and stored the EEG and EMG signals, as well as head movements by an on-board accelerometer (LIS302DLH; STMicroelectronics). The data were recorded with a sample frequency of 100 Hz and stored on an on-board memory chip. The device could record continuously for approximately 15 days on a 3.6 V battery (LS 14250; Saft).

Sleep–wake patterns in the geese were recorded in winter (February) and summer (June). In winter: n = 9, total of 14 recordings (1 or 2 per individual), average duration 135 ± 22 h. In summer: n = 8, total of 8 recordings, average duration of 238 ± 18 h. During these recording periods, the birds were subjected to two different durations of sleep deprivations (4 and 8 h starting at sunset), which we reported earlier (van Hasselt et al., 2021). The analysis of the data in this paper excludes the sleep deprivation day and subsequent recovery day. All other days were considered as baseline days.

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between the recording sessions in winter and summer, the geese were returned to the larger outdoor meadow.

2.4 | Data analyses

All recordings were scored with an automated scoring program using machine learning algorithms with input from a human scorer who was unaware of the animal’s identity and the time of recording (Somnivore Pty Ltd; Allocca et al., 2019). The program used all available data channels (i.e., EEG, EMG, accelerator) to determine the vigilance state on a 4 s epoch basis. The recordings were scored for wakefulness (W), rapid-eye-movement (REM) sleep, and non-rapid-eye-movement (NREM) sleep. An epoch was scored as wakefulness when the EEG signal showed low amplitude and high frequency activity together with high EMG and accelerometer activity. REM sleep was scored when the EEG signal was similar to that of wakefulness but EMG activity was low, and the accelerometer showed either no head movements or signs of head drops. An epoch was scored as NREM sleep when the EEG amplitude was at least twice that of wakefulness, the EMG signal was low, and the accelerometer showed no activity. The automated scoring program has been validated with various species and for pigeons yielded an accuracy for wake of 0.96 ± 0.006; NREM 0.97 ± 0.01; REM 0.86 ± 0.02 as compared with a human scorer (Allocca et al., 2019). We have also done an additional validation in our geese based on four of the 24 h winter recordings, which yielded correlations between program and human scorer of 0.98 ± 0.01 for Wake, 0.97 ± 0.01 for NREM sleep, and 0.84 ± 0.04 for REM sleep (van Hasselt et al., 2021).

To assess how well sleep–wake patterns based on intermittent recordings correlate with the patterns based on continuous recordings, we created 12 datasets with different intermittent recording schemes, all derived from the original continuous dataset. Specifically, we selected epochs to simulate EEG that was recorded for the first minute of every 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 min where the first minute is the same among all datasets. Since the vigilance states were scored on a 4 s basis, all three vigilant states could be present in the sampled minute of the intermittent recordings.

For each of these data sets we then estimated hourly values of wakefulness, NREM sleep, and REM sleep, based on the percentages of these different vigilance states during the 24 h recordings, intervals in those hours. These estimated hourly values of wakefulness, NREM sleep and REM sleep of the simulated intermittent recording data sets were then correlated with the true hourly values of these vigilance states during the original continuous recordings. Furthermore, we analysed the first day of the winter and summer recordings to assess differences in the 24 h patterns and averaged 24 h values of sleep and wakefulness based on the continuous and intermittent recordings.

2.5 | Statistics

To assess differences in sleep patterns based on continuous and intermittent recordings, data were analysed in R according to a linear mixed effect model by taking animal ID as a random effect using the lme4 package (Bates et al., 2015; R Development Core Team 3.0.1., 2013). The correlations between the hourly percentages of each vigilance state in the original recording and the simulated intermittent recordings were calculated using the Pearson’s correlation coefficients. From the lme4 package the BootMer function was used for bootstrapping to make model predictions by running 10,000 simulations to acquire more reliable prediction estimates with 95% confidence intervals (CI) (Buckland et al., 1998; Morris, 2002). Statistical differences between groups were tested with a post-hoc Tukey HSD test using the lsmeans package (Lenth, 2016). Data and text in figures are expressed as mean ± SEM.

3 | RESULTS

Figure 1 shows the patterns of Wake, NREM sleep, and REM sleep during the first day of the winter and summer recordings for the complete dataset as well as the 12 intermittent datasets. The 24 h sleep–wake patterns based on the complete and intermittent datasets were not significantly different from each other until the intermittent recordings had an interval that was larger than 1 min every 50 min in winter and 1 min every 40 min in summer, with the exception for 1 min per 55 min in summer (p < 0.05, post hoc test after linear mixed model). Interestingly, these differences were only found for wakefulness and NREM sleep, not for REM sleep (Figure 1).

When the hourly values of the continuous recordings for each individual were correlated with the hourly values of the simulated intermittent recordings, the Pearson correlation coefficients gradually decreased when the interval between the intermittent recording episodes increased. In other words, correlations were generally strongest for the simulated intermittent schemes of 1 min recording per 5 min and weakest for the intermittent schemes of 1 min recording per 60 min (an example of all correlations for one individual is shown in Figure 2). Moreover, the match between full and intermittent recordings was highest when hourly values are between the 0–20 and 80–100% range.

For all individuals together, this gradual decrease in correlation coefficient based on hourly values was significant for each of the three vigilance states (p < 0.001; linear mixed model; Figure 3). More specifically, the correlation strength for Wake decreased from 0.95 (CI = 0.95, 0.99) to 0.68 (CI = 0.65, 0.71) in winter and from 0.97 (CI = 0.93, 1.0) to 0.49 (CI = 0.45, 0.53) in summer. The correlation strength for NREM sleep decreased from 0.94 (CI = 0.9, 0.98) to 0.67 (CI = 0.63, 0.7) in winter and from 0.96 (CI = 0.91, 1.0) to 0.45 (CI = 0.41, 0.49) in summer. The correlation strength for REM sleep decreased from 0.86 (CI = 0.82, 0.9) to 0.48 (CI = 0.45, 0.52) in winter and from 0.89 (CI = 0.85, 0.94) to 0.48 (CI = 0.43, 0.52) in summer. Importantly, the correlation coefficient was significantly higher in winter compared with summer for Wake and NREM sleep (p < 0.001; linear mixed model; Figure 3).

The 24 h estimations of Wake, NREM sleep, and REM sleep from the intermittent recordings are similar to the daily averages of these
vigilant states from the continuous recording (Figure 4). The precision of intermittent recordings remained high. The variation around the 24 h values was more or less similar for all intermittent recording schedules. Also, the seasonal differences in Wake and NREM sleep time that has been reported for this species is statistically present for all intermittent recordings (p < 0.001 for both states, post hoc test after linear mixed model). Data are presented as mean ± SEM. Panel inlay denotes intermittent recording rate: 1/40: 1 min per 40 min; 1/45: 1 min per 45 min; 1/50: 1 min per 50 min; 1/55: 1 min per 55 min; 1/60: 1 min per 60 min

FIGURE 1  Averaged patterns of Wake, NREM sleep, and REM sleep for the first 24 h day of the recordings in winter (panel a) and summer (panel b), plotted for the complete data set (red) and the intermittent dataset (blue). In winter there are significant differences between the datasets from an intermittent recording of 1 min per 50 min. In summer, statistical differences are present from an intermittent dataset of 1 min per 40 min (significant differences are indicated by dots above the line graphs, p < 0.05, post hoc test after linear mixed model). Data are presented as mean ± SEM. Panel inlay denotes intermittent recording rate: 1/40: 1 min per 40 min; 1/45: 1 min per 45 min; 1/50: 1 min per 50 min; 1/55: 1 min per 55 min; 1/60: 1 min per 60 min

4 | DISCUSSION

Our analysis indicates that intermittent EEG recordings accurately estimate sleep–wake patterns. In fact, estimated 24 h averages of sleep–wake data did not differ among intermittent recordings and between the true values of the continuous recording. This indicates that an intermittent recording scheme up to 1 min per 60 min reliably predicts 24 h sleep–wake values compared with a continuously recorded dataset. When we compared the first 24 h sleep–wake patterns based on continuous EEG recordings with the patterns derived from simulated intermittent recordings, there were no significant differences until intermittent recordings of 1 min per 50 min in winter and 1 min per 40 min in summer.

Also, the hourly values of sleep and wakefulness based on continuous recordings correlated significantly with estimated hourly values based on intermittent recordings; as expected, the correlation coefficient steadily decreased when the interval between recording episodes increased, but was still at 0.5 when recording only 1 min out of 60 min. Interestingly, this decrease in the strength of the correlation in our geese depended on season and was stronger in summer than in winter but only for NREM sleep and wakefulness. In other words, the intermittent recordings better predicted true sleep–wake patterns in winter than in summer on an hourly resolution. A likely explanation for this finding is that the geese had a much more pronounced daily sleep–wake rhythm in winter with most sleep
occurring during the night-time and little day-time sleep (van Hasselt et al., 2021). The large variation in hourly values of NREM sleep and wakefulness across the 24 h cycle in winter would produce stronger correlation coefficients compared with the summer condition when sleep was more spread out over the 24 h day and hourly values of sleep and wakefulness varied less. This loss of day–night rhythmicity in sleep is a phenomenon that is observed in more migratory bird species that breed in summer under constant light conditions of the High Arctic (Stokkan et al., 1986).

An intriguing finding was that, in contrast to wakefulness and NREM sleep, the decrease in correlation strength for REM sleep was not dependent on season. Moreover, the estimated 24 h REM sleep pattern based on intermittent recordings did not significantly differ from the pattern based on continuous recordings, not even with the most restricted protocol of 1 min recording per hour. Since REM sleep loses circadian rhythmicity in parallel with NREM sleep in barnacle geese, one might expect to observe a similar season-dependent effect (van Hasselt et al., 2021). A reason for this not to be the case may lay in the fact that the overall amount and the range of hourly values of REM sleep was much smaller than for NREM sleep and waking.

Studies that reported the reliability of intermittent EEG recordings in comatose patients showed an agreement between intermittent and continuous EEG registrations higher than 95% (Alvarez et al., 2013). Estimation of REM sleep values per night in pigeons (Columba livia) appeared to yield a high accuracy by scoring the first epoch per minute up to 1 epoch every 5th minute (Lesku et al., 2011). Importantly, while intermittent sampling may decrease precision over the recording period, it can increase the value of the data set by recording for longer. From a statistical perspective,
perspective, it is more informative to measure, for example, half of the time of two days than to measure one day continuously. So intermittent sampling allows what is in general a better solution to the trade-off between the duration and precision of the recording. One potential disadvantage of this method is that one cannot accurately estimate bout length. This is particularly true for species that have sleep episodes that are longer than the duration of the sampling period.

In conclusion, the results of this analysis suggest that intermittent EEG recordings can be a reliable approach for assessing sleep–wake patterns under conditions that preclude continuous recordings, for example, in case of long-duration recordings with data-loggers limited by battery life or data storage capacity. The strength of the explanatory value of intermittent recordings depends on the resolution of the analysis. Furthermore, seasonality and other environmental factors may influence the reliability of intermittent EEG recordings and optimal species-specific recording schedule should be determined.

ACKNOWLEDGEMENTS
This study was supported by an Adaptive Life Program scholarship from the Groningen Institute for Evolutionary Life Sciences, an Ubbo Emmius scholarship provided by the Faculty of Science and Engineering at the University of Groningen, and a grant from the Dutch Research Council (OCENW.KLEIN.240). NCR was supported by the Max Planck Society.
CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
SJHV, PM, NCR, and TP conceived of the project and designed the experiment. SJHV executed and analysed the experiments. The manuscript was written by SJHV and PM and reviewed by SV, NCR, and TP.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author.

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How to cite this article: van Hasselt, S. J., Verhulst, S., Piersma, T., Rattenborg, N. C., & Meerlo, P. (2022). A comparison of continuous and intermittent EEG recordings in geese: How much data are needed to reliably estimate sleep–wake patterns? Journal of Sleep Research, 31, e13525. https://doi.org/10.1111/jsr.13525