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ORIGINAL ARTICLE

Homeostatic regulation of NREM sleep, but not REM sleep, in Australian magpies

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Abstract

Study Objectives: We explore non-rapid eye movement (NREM) and rapid eye movement (REM) sleep homeostasis in Australian magpies (*Cracticus tibicen tyrannica*). We predicted that magpies would recover lost sleep by spending more time in NREM and REM sleep, and by engaging in more intense NREM sleep as indicated by increased slow-wave activity (SWA).

Methods: Continuous 72-h recordings of EEG, EMG, and tri-axial accelerometry, along with EEG spectral analyses, were performed on wild-caught Australian magpies housed in indoor aviaries. Australian magpies were subjected to two protocols of night-time sleep deprivation: full 12-h night (n = 8) and first 6-h half of the night (n = 5), which were preceded by a 36-h baseline recording and followed by a 24-h recovery period.

Results: Australian magpies recovered from lost NREM sleep by sleeping more, with increased NREM sleep consolidation, and increased SWA during recovery sleep. Following 12-h of night-time sleep loss, magpies also showed reduced SWA the following night after napping more during the recovery day. Surprisingly, the magpies did not recover any lost REM sleep.

Conclusions: Only NREM sleep is homeostatically regulated in Australian magpies with the level of SWA reflecting prior sleep/wake history. The significance of emerging patterns on the apparent absence of REM sleep homeostasis, now observed in multiple species, remains unclear.

Statement of Significance

We investigated non-rapid eye movement (NREM) and rapid eye movement (REM) sleep homeostasis in wild-caught Australian magpies. Magpies exposed to all-night and half-night sleep deprivations recovered lost NREM sleep by sleeping more, with increased NREM sleep consolidation, and increased slow-wave activity (SWA) during recovery sleep. After 12 hours of night-time sleep deprivation, magpies also showed reduced SWA on the subsequent night after napping more during the recovery day. Interestingly, we did not detect any REM sleep rebound following sleep deprivation. Overall, our results indicate that NREM sleep is homeostatically regulated in Australian magpies reflecting prior sleep/wake history. However, the importance of emerging patterns on the absence of REM sleep homeostasis, which has now been observed in several species, remains unclear.

Key words: birds; EEG; sleep regulation; slow-wave activity; spectral analysis; SWA

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Introduction

Sleep appears to be an evolutionarily conserved behavior, as it has been observed in all animals studied to date [1–3]. This ubiquity across Animalia is not surprising given that sleep serves many critical physiological functions, including those related to energy homeostasis [4, 5], and brain development [6, 7] and maintenance [8–11]. Moreover, sleep functions lead to important behavioral consequences, such as learning [12, 13], and more broadly, maintaining high waking neurobehavioral performance [9, 14, 15]. These physiological functions and behavioral consequences are thought to be adaptive given the persistence of sleep under dangerous situations [16–18], and when animals would otherwise benefit from uninterrupted wakefulness [19, 20].

The homeostatic regulation of sleep also suggests that there are non-trivial sleep functions. Birds and mammals exhibit two types of sleep, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. During REM sleep, the electroencephalogram (EEG) is characterized by low-amplitude, high-frequency activity resembling the brain waves associated with wakefulness; conversely, during NREM sleep, the EEG is characterized by high-amplitude, low-frequency (≤4 Hz) activity [21–23]. When birds and mammals lose sleep, they recover from lost REM and NREM sleep by sleeping more, and, for NREM sleep, sleeping more intensely [24–26]. The intensity of NREM sleep can be quantified by the incidence and/or amplitude of EEG slow-waves, called slow-wave activity (SWA; typically 0.5–4.5 Hz power density). In mammals, the level of SWA predicts the intensity of stimuli needed for arousal to wakefulness [27], but this has yet to be shown in birds. In animals that consolidate wakefulness, SWA is highest at the beginning of the sleep bout and declines with time spent asleep. Moreover, the level of SWA increases further following sleep loss, and decreases following daytime naps [28]; the former having been demonstrated in a variety of mammals [26, 29–32] and birds [19, 20, 24, 33–36]. Although a dose-dependent increase in SWA following extended wakefulness has long been recognized in mammals [29], similar dose-dependent dynamics have yet to be demonstrated in birds. Nonetheless, in mammals and birds, the level of SWA is thought to reflect homeostatically regulated processes tied to the function of NREM sleep [15, 37–39].

The regulation and function of REM sleep remains less consistent and less clear. REM sleep in birds and mammals is thought to be involved in the maturation of the central nervous system, in part because the amount of REM sleep is highest in these young animals [6, 40, 41], and, in mammals, REM sleep-related skeletomuscular twitching is important for the development of sensorimotor maps [42–44]. REM sleep is homeostatically regulated in mammals and birds in that these animals typically engage in more REM sleep following sleep loss; REM sleep does not appear to have an intensity dimension [25, 26]. In many mammals, both total sleep deprivation and selective REM sleep deprivation elicit a rebound in the amount of REM sleep (e.g., cat [45]; cow [46]; dog [47]; rat [48]; human [49]; mouse [31]). Conversely, tree shrews (*Tupaia belangeri*) recovered only a modest amount of REM sleep after deprivation [50]. Furthermore, fur seals (*Callorhinus ursinus*) that suppressed REM sleep while in water showed little or no REM sleep rebound when they returned to sleep on land [51]. In birds, evidence for REM sleep homeostasis appears less consistent. Although pigeons (*Columba livia*) recover [24, 52, 53] and barnacle geese (*Branta leucopsis*) partially recover from lost REM sleep [35], REM sleep homeostasis has not been observed in sleep-disturbed white-crowned sparrows (*Zonotrichia leucophrys gambelii*) [33], European starlings (*Sturnus vulgaris*) [36] or Australian magpies (*Cracticus tibicen tyrannica*), the latter having had sleep disrupted by artificial light at night [34] and urban noise [54].

Here, we sought to further explore NREM and REM sleep homeostasis in Australian magpies using a more direct method of sleep deprivation than exposure to light at night [34] or urban noise [54] might have allowed. We suppressed sleep by actively stimulating the birds whenever signs of restfulness were present. In addition to this more direct approach for inducing wakefulness, we used two durations of night-time sleep deprivation (12 h and 6 h) to provide a better understanding of the dependence of sleep architecture on prior sleep/wake history in magpies. We tested the hypothesis that, like some other birds and mammals, sleep is homeostatically regulated in Australian magpies. We predicted that magpies would recover lost sleep by spending more time in NREM and REM sleep, and by engaging in more SWA-rich NREM sleep. Furthermore, we also predicted that this rebound would be larger after 12 h of sleep loss relative to the 6 h protocol.

Materials and Methods

Animals and housing

In January 2019, we caught 12 adult Australian magpies, equally sexed (based on plumage), in the city of Melbourne, Australia, using walk-in traps baited with grated cheese. All magpies were non-breeding and non-paired individuals without a territory (Connelly, pers. obs.). When unpaired, Australian magpies often form mixed flocks containing both sexes. These flocks are fluid, with some individuals remaining in these flocks for years, while others remain for only a few days. From personal observations (F.C.), birds do not reproduce while in these flocks; individuals that have emigrated from mixed flocks have been observed breeding, but only when paired in a defined territory. Following capture, each bird was banded with numbered metal and plastic leg bands for individual identification. Immediately after banding, birds were transported to an indoor aviary facility at La Trobe University, Melbourne. Here, the magpies were housed individually in aviaries (1.8 m high x 1.8 m deep x 0.9 m wide) in two rooms, with 6 birds per room (3 males and 3 females). Magpies are social birds, such that we allowed them to both see and hear one another throughout their time in the animal house. Each aviary contained two wooden rectangular perches (15 cm wide), one 1.3 m above the floor at the back of the aviary and the other 0.45 m above the floor at the front of the aviary; aviaries also contained a wooden cylindrical perch 0.45 m above the floor at the front. Additionally, all aviaries were equipped with two infrared video cameras to record animal behavior and eye state (when visible); one positioned at the highest perch where the magpies usually slept, and the other was mounted on the aviary door focussing on the lower perches and the aviary floor. Both rooms were temperature controlled (22 ± 5 C) and insulated from all external light. Room lighting (153 ± 18 lux; room mean from measurements in front of each aviary) was kept on a 12:12 light:dark cycle with lights-off at 1800 h. A light imitating the intensity of moonlight (average ~0.1 lux

at the level of the highest perch) was present in each room allowing the magpies to move safely in their aviary at night.

Magpies were maintained on a diet of 55 g minced meat combined with an insectivore mix (Wombaroo Food Products, Australia) and calcium powder fed daily at 0900 ± 1 h. Clean water was provided daily in a large bowl, so the magpies could drink and bathe. Aviary floors were covered in woodchips. As enrichment, 15–20 live mealworms were scattered throughout the woodchips each day giving the magpies an opportunity to forage on the ground, as they would do in the wild.

All procedures were carried out with permission from the Department of Environment, Land, Water, and Planning (permit number: 10008264), La Trobe University Animal Ethics Committee (AEC18034), and the Australian Bird and Bat Banding Scheme (#1405).

Electrode implantation and recording

To reveal changes in sleep architecture in response to sleep loss, three gold-plated, round-tipped pins were placed on the right hemisphere on the dura over the (1) hyperpallium, (2) mesopallium, and (3) either the nidopallium caudolaterale (NCL) or *area parahippocampalis* (APH), using standard stereotaxic techniques to record the EEG (see Aulsebrook et al.[34] for details). The hyperpallium could be seen through the cranium as a pink oval. The hyperpallial electrode was situated at the anterior end of this oval and 2 mm lateral of the midline, the electrode for the mesopallium was 7 mm lateral of the midline (or 5 mm lateral of the hyperpallium), and the electrode over the NCL/APH was placed 9 mm posterior to the mesopallial electrode (in the absence of a brain atlas for Australian magpies or histological analysis, we could not resolve whether the posterior electrode was seated over the NCL or APH). A bare stainless steel wire (AS633 electrode wire, Cooner Wire) was laid over the neck muscle to record the electromyogram (EMG). All electrodes were referenced to the cerebellum; a ground electrode was positioned over the left hyperpallia. Electrode wires were pre-soldered to a small connector (6.0 mm wide) attached to the top of the head with dental acrylic, forming a "head plug" to which the EEG/EMG datalogger would later connect. Each magpie was given a minimum of two weeks of post-operative recovery before experiments began. To record the EEG and EMG, we connected the datalogger (Neurologger 2A) powered by two zinc air batteries (ZA675 1.4V, Renata, Switzerland) to the head plug. Dataloggers continuously recorded the EEG, EMG, and three-dimensional head acceleration through an in-built tri-axial accelerometer at a sampling rate of 100 Hz. Each datalogger with batteries (6 g) was wrapped in kinetic thread seal tape for protection from moisture and physical damage. Magpies showed no overt behavioral changes when fitted with a datalogger. Nevertheless, following attachment of the datalogger, all magpies were given at least 24 h of habituation to the datalogger before baseline recordings commenced.

Ten of the 12 magpies were successfully equipped with EEG/EMG electrodes. One of the two other birds developed a pronounced cardiac arrhythmia whenever under isoflurane anesthetic, such that we could not perform surgery safely on this bird; the other bird aspirated under anesthesia and died. For the 12-h sleep deprivation, we obtained data from eight of the ten birds, but only seven birds included also the recovery day and night. For the 6-h sleep deprivation, we obtained complete data from five birds. Incomplete data was due to logger failure, or loggers falling off during the experiments.

Experimental design

Magpies underwent a full or half-night of sleep deprivation. Whenever behavioral signs of sleep were present (restfulness), we stimulated the magpie by moving towards the aviary, making a noise, tapping the aviary, or, towards the end of the deprivation, gently touching the birds (similar to Martinez-Gonzalez et al.[24]). Magpies underwent two experimental sleep deprivation protocols: 12-h and 6-h of sleep deprivation (in that order). During the treatment night of the 12-h protocol, the birds were deprived of a full night of sleep (i.e., 1800–0600 h); during the treatment night of the 6-h protocol, the birds were deprived of the first one-half of the night of sleep (i.e., 1800–0000 h). Both protocols consisted of a continuous 72-h recording, where each treatment night was preceded by an undisturbed baseline day and night and a pre-treatment day, and was followed by an undisturbed recovery day and night (Figure 1). This research was conducted as part of a larger study investigating the effects of extended wakefulness on avian cognition; as such, birds were tested on a cognitive task between 0600–0900 h on the pre-treatment day and recovery day. Results from this cognitive work will be published in a forthcoming manuscript.

Sleep staging

To analyze sleep, we used the supervised machine-learning algorithm Somnivore™ to score wakefulness, NREM sleep, and REM sleep in 4-s epochs [55]. In Somnivore, all available channels (3 EEG, 1 EMG, and a 3-dimensional accelerometer) were used when scoring signals, and scoring was manually checked for errors and artifacts. Briefly, NREM sleep was characterized as high-amplitude, low-frequency EEG waves with stable, moderate muscle tone and without head movements. This was quite unlike wakefulness in which head movements were frequent and large, supported by high and variable muscle tone, and an EEG that showed either low-amplitude, high-frequency activity, or high-amplitude, high-frequency artifacts. REM sleep resembled alert wakefulness in the EEG, but with moderate or low muscle tone, a head that was still (when the head was resting on the back) or drooping (when the head was facing forward), and the animal could wobble, as seen in video recordings. Somnivore has been validated for use on Australian magpies [54] and other birds and mammals [34, 55].

Spectral power density analysis

We performed fast Fourier transforms on Hamming-windowed, artifact-free epochs of NREM sleep in 0.39 Hz bins from 0.78 to 25 Hz using RemLogic v. 3.4.4 (Embla Systems, Pleasanton, USA)

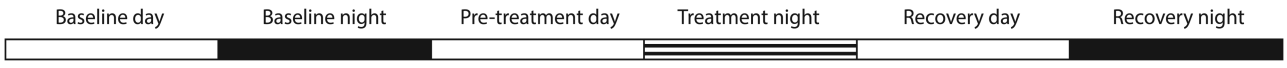

Figure 1. Experimental designs for the two sleep deprivation protocols, along with nomenclature of the various 12-h days and 12-h nights. The treatment night (black-and-white horizontal lines) consisted of either a full-night, or half-night, of sleep loss beginning at lights-off.

to visualize spectral changes in sleep, including SWA (< 4 Hz) and faster frequencies [39]. Epochs that contained a mixture of NREM sleep and another state (i.e., where one state was less than 4 s) were excluded from the spectral analysis [39]. Spectral power density was calculated for each quarter of the baseline, treatment, and recovery nights, and expressed as a percentage of the 12-h baseline night mean per frequency bin. We also calculated spectral power density for each 12-h day (baseline, pre-treatment, and recovery) and similarly expressed these as a percentage of the 12-h baseline night mean per 0.39 Hz bin. In addition, we calculated NREM sleep SWA (mean 1.17–4.30 Hz power density) for the *area parahippocampalis*, and slow-wave energy (SWE, or cumulative SWA). SWE was used to determine whether increased NREM sleep intensity fully compensated for lost NREM sleep. We used two-tailed paired t-tests when comparing aspects of sleep between the different conditions. All statistical analyses were conducted in R version 3.5.2 (R Development Core Team 2018).

Release into the wild

Importantly, after the study was complete, all magpies except the individual that died under anesthesia were released back into city parks in July 2019. A week before their release, the magpies were put under isoflurane anesthesia and the head plug was removed with residual dental acrylic being greatly reduced. Over the first year following release, nine of the eleven birds were seen alive in the wild. Five birds were known to have established territories and were observed regularly by F.C. throughout 2020. The most recent observation was made in April 2021 and included the sighting of an individual feeding a juvenile. Such serendipitous sightings suggest no long-lasting effect of captivity or surgery.

Results

Baseline sleep and efficacy of sleep deprivation

A detailed report on sleep architecture in laboratory-housed magpies can be found in Connelly et al. [54]. Briefly, under baseline conditions, magpies slept predominantly at night, with a mid-day nap (Figure 2). During the baseline before the 12-h sleep deprivation, the magpies spent 46.6 ± 1.1% of the 24-h day asleep, with 5.6 ± 1.6% occurring during the light phase and 87.6 ± 0.9% during the dark phase. Likewise, during the baseline before the 6-h sleep deprivation, the magpies spent 47.6 ± 1.8% of the 24-h day asleep, with 6.5 ± 2.7% during the day and 88.6 ± 2.0% during the night.

The baseline nights before the 12-h and 6-h sleep deprivations consisted of: 78.7 ± 1.4% and 81.2 ± 2.0% NREM sleep, and 8.9 ± 0.9% and 7.4 ± 0.4% REM sleep, respectively; the rest of the night,

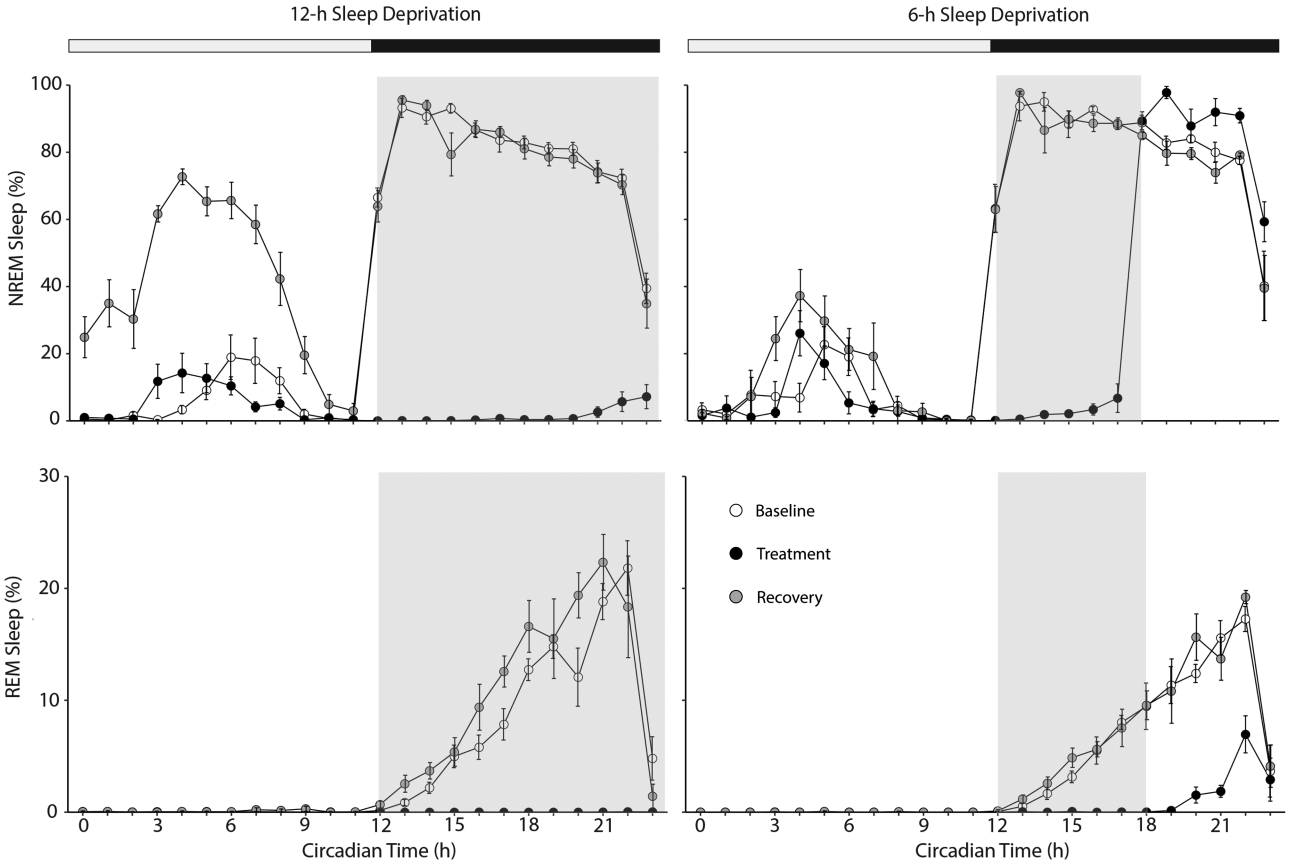

Figure 2. Effect of 12-h sleep deprivation (left column) and 6-h sleep deprivation (right column) on time spent in NREM sleep (top row) and REM sleep (bottom row). The percent time (mean ± SEM) spent in each state is plotted for each hour for each of the three 24-h periods. The first 24-h period served as an undisturbed baseline (open circles). The second 24-h period is divided in two halves: a 12-h pre-treatment day and a 12-h treatment night (black circles). The third 24-h period is divided into a 12-h recovery day and a 12-h recovery night (grey circles). The grey shaded area indicates the timing and duration of the sleep deprivation *per se*. The horizontal white and black bar at the top of the plot indicates day and night, respectively. Time on the x-axes is expressed as circadian time representing the 12:12 light:dark cycle, with "lights on" at 0 and "lights off" at 12.

the birds were awake. During the 12-h and 6-h baseline nights, REM sleep made up 10.2 ± 1.0% of total sleep time (n = 8) and 8.4 ± 0.5% of total sleep time (n = 5), respectively. The low 8.4% REM sleep value, observed in the 6-h baseline night, is notably lower than the 14.4 ± 1.2% that has been reported for these same birds (see Connelly et al. [54], where n = 8). Our low REM sleep value reported here did not arise from inter-scorer variability, because sleep was scored by one person across all studies. Instead, the difference appears to be due to a lower sample size on the 6-h baseline night (n = 5, compared to n = 8). Nonetheless, similar to other birds, including magpies, the amount of NREM sleep decreased, and the amount of REM sleep increased, across the baseline nights (Figure 2).

On the treatment night, sleep was substantially reduced (Figure 2). During the 12-h and 6-h periods of sleep deprivation, NREM sleep was reduced to: (1) 1.5 ± 0.8% on the treatment night during the 12-h sleep deprivation protocol, and (2) 2.5 ± 1.3% during the first half of the treatment night during the 6-h sleep deprivation protocol. REM sleep was virtually eliminated during the deprivation protocols (12-h baseline night: 8.9 ± 0.9%; 12-h treatment night: 0.0 ± 0.0%; first half of the baseline night: 3.2 ± 0.3%; first half of the treatment night: 0.0 ± 0.0%).

Recovery sleep architecture

12-h sleep deprivation. After the 12-h sleep deprivation night, the amount of NREM sleep significantly increased during the day (baseline day: 5.6 ± 1.6%; recovery day: 40.3 ± 5.3%, t = 13.455, df = 6, p < 0.001; Figure 2). In contrast, the amount of daytime REM sleep did not increase significantly following sleep deprivation (baseline day: 0.0 ± 0.0%; recovery day: 0.1 ± 0.1%, t = 1.199, df = 6, p = 0.276). During the recovery night, the amount of NREM and REM sleep was similar to baseline values (NREM sleep baseline night: 78.7 ± 1.4%; recovery night: 76.9 ± 1.7%, t = –0.904, df = 6, p = 0.401; REM sleep baseline night: 8.9 ± 0.9%; recovery night, 10.7 ± 1.3%, t = 1.374, df = 6, p = 0.219). To affirm this non-significant REM sleep result, we conducted a linear mixed-effects model on the baseline and recovery nights and found no significant difference between nights (F = 2.761, df = 1,176, p = 0.098). Moreover, we conducted pairwise post-hoc Tukey tests that compared each hour of the baseline night to that during the recovery night and again found no significant difference in REM sleep amounts (all p between 0.156 and 0.999).

6-h sleep deprivation. During the second half of the treatment night, when sleep was allowed to occur unhindered, the amount of NREM sleep was significantly higher than baseline levels (second half of baseline night: 75.6 ± 2.5%; second half of treatment night: 86.2 ± 1.5%, t = 6.225, df = 4, p = 0.003). However, NREM sleep during the second half of treatment night was not significantly different compared to the first half of baseline night (first half of baseline night: 86.9 ± 2.0%; t = –0.281, df = 4, p = 0.793). Although the birds engaged in some REM sleep during the second half of the treatment night, the amount of REM sleep was consistently lower than baseline levels (second half of baseline night: 11.6 ± 0.6%; second half of treatment night: 2.2 ± 0.7%, t = –13.168, df = 4, p < 0.001; first half of baseline night: 3.2 ± 0.3%; t = –1.348, df = 4, p = 0.249).

During the following (recovery) day, the amount of NREM and REM sleep was not significantly different from baseline (NREM sleep 12-h baseline day: 6.5 ± 2.7%; recovery day: 12.4 ± 3.2%, t = 1.908, df = 4, p = 0.129; REM sleep 12-h baseline day: 0.0 ± 0.0%; recovery day: 0.0 ± 0.0%, t = 1.372, df = 4, p = 0.242). During the recovery night, there was a modest decrease in the amount of NREM sleep (baseline night: 81.2 ± 2.0%; recovery night: 79.3 ± 2.1%, t = –2.872, df = 4, p = 0.045), but REM sleep did not differ from baseline levels (baseline night: 7.4 ± 0.4%; recovery night: 7.9 ± 0.8%, t = 0.741, df = 4, p = 0.500).

Duration and number of sleep episodes

12-h sleep deprivation. NREM sleep episodes were shorter and fewer during the treatment night, reflecting sleep fragmentation, and longer and more abundant during the recovery day, reflecting sleep consolidation (Table 1). REM sleep did not occur during the baseline or recovery days. During the subsequent recovery night, the duration and number of NREM and REM sleep episodes were not different from baseline.

6-h sleep deprivation. Note that for each 12-h night of the entire 6-h sleep deprivation protocol, the duration and number of sleep bouts were analyzed in 6-h time bins owing to the half-night nature of this treatment. Thus, in Table 2, B1 and B2 correspond to the first, and second, half of the baseline night, respectively, and so on for the treatment (T) and recovery (R) nights.

In addition to reducing the amount of sleep during the first half of the treatment night (Figure 2), the sleep deprivation (T1) also resulted in significantly shorter NREM sleep episodes, compared to the first half of the baseline night (B1) (Table 2). During the second half of the treatment night, when the birds were free to sleep undisturbed, NREM and REM sleep bouts were fewer compared to baseline, but NREM sleep episodes were longer; the latter was an effect that persisted across the 12-h recovery day. REM sleep did not occur during the recovery (or baseline) day. NREM and REM sleep bout duration and number were not significantly different between the baseline and recovery nights.

Spectral power density

We explored NREM sleep spectral power density from 0.78 to 25 Hz in 0.39 Hz frequency bins for the hyperpallium, mesopallium, and *area parahippocampalis* (APH, or nidopallium caudolaterale), across the baseline, treatment, and recovery nights for the 12-h (Figure 3) and 6-h (Figure 4) protocols divided into quarterly (3-h) time bins, and during days (Figure 5) quantified as a single 12-h bin owing to little sleep occurring during the day. We also calculated NREM sleep SWA (mean 1.17–4.30 Hz power density) for the *area parahippocampalis* (Figure 6A, B) along with slow-wave energy (SWE, or cumulative SWA; Figure 6C, D). General patterns are highlighted below.

12-h sleep deprivation

Baseline night. In all brain areas recorded, low-frequency power density (<*c.* 3 Hz) decreased across the baseline night, being highest in the first quarter of the night (Q1), lower in the second (Q2), and lowest in either the third (Q3) or fourth (Q4) (Figure 3; Figure 6A). Power in frequencies (<*c.* 18 Hz) were generally highest during Q1. During Q4, we identified (1) an increase in *c.* 3-8 Hz power density observed in all baseline (and recovery) nights, and in all brain regions, and (2) an increase in faster frequencies (above *c.* 20 Hz).

Table 1. Effects of 12-h sleep deprivation on the mean number and duration of bouts of NREM and REM sleep in Australian magpies

		Day 1 Baseline (B)	Day 2 Pre-Treatment (Pre-T)	Day 3 Recovery (R)	P B–Pre-T	P B–R	P Pre-T–R
NREM	no. bouts	195.1 ± 38.9	221.5 ± 26.5	543.7 ± 68.4	$t = 1.58$ $p = 0.16$	$t = 4.20$ **$p < 0.01$**	$t = 4.29$ **$p < 0.01$**
	bout duration (s)	10.8 ± 1.5	9.6 ± 0.9	35.1 ± 4.7	$t = -1.18$ $p = 0.28$	$t = 7.74$ **$p < 0.01$**	$t = 7.06$ **$p < 0.01$**
		Night 1 Baseline (B)	Night 2 Treatment (T)	Night 3 Recovery (R)	P B–T	P B–R	
NREM	no. bouts	778.0 ± 37.2	99.8 ± 44.6	888.4 ± 55.2	$t = -10.11$ **$p < 0.01$**	$t = 0.01$ $p = 0.99$	
	bout duration (s)	44.6 ± 2.6	5.1 ± 0.5	38.7 ± 3.6	$t = -15.46$ **$p < 0.01$**	$t = -1.63$ $p = 0.15$	
REM	no. bouts	574.0 ± 34.5	-	685.6 ± 72.9	-	$t = 1.66$ $p = 0.15$	
	bout duration (s)	6.6 ± 0.3	-	6.5 ± 0.3	-	$t = -0.95$ $p = 0.38$	

P-values are from paired *t*-tests that compared across days (top) and nights (bottom). Means ± SEM for each 12-h period; $n = 8$ for all comparisons, but $n = 7$ for recovery day and night. Daytime REM sleep contained zero values and was unable to be calculated. Shaded comparisons denote significance.

Table 2. Effects of 6-h sleep deprivation on the mean number and duration of bouts of NREM and REM sleep in Australian magpies

		Day 1 Baseline (B)		Day 2 Pre-Treatment (Pre-T)		Day 3 Recovery (R)		P B–Pre-T	P B–R	P Pre-T–R	
NREM	no. bouts	250.4 ± 103.9		216.2 ± 99.2		301.6 ± 74.9		$t = -0.49$ $p = 0.65$	$t = 0.58$ $p = 0.59$	$t = 1.54$ $p = 0.20$	
	bout duration (s)	11.6 ± 0.8		12.6 ± 1.0		17.1 ± 1.3		$t = 0.83$ $p = 0.45$	$t = 3.24$ **$p = 0.03$**	$t = 2.89$ **$p = 0.04$**	
		Night 1 B1	Night 1 B2	Night 2 T1	Night 2 T2	Night 3 R1	Night 3 R2	P B1–T1	P B2–T2	P B1–R1	P B2–R2
NREM	no. bouts	228.8 ± 29.6	517.4 ± 34.7	84.4 ± 46.5	244.4 ± 38.2	251.8 ± 35.4	569.8 ± 10.7	$t = -2.51$ $p = 0.06$	$t = -10.98$ **$p < 0.01$**	$t = 0.97$ $p = 0.39$	$t = 1.62$ $p = 0.18$
	bout duration (s)	91.6 ± 16.9	31.6 ± 2.3	6.2 ± 0.6	84.1 ± 12.5	81.5 ± 13.9	27.5 ± 1.5	$t = -5.15$ **$p < 0.01$**	$t = 4.85$ **$p < 0.01$**	$t = -1.07$ $p = 0.34$	$t = -2.41$ $p = 0.07$
REM	no. bouts	120.8 ± 9.1	402.4 ± 14.9	-	89.2 ± 25.2	147.8 ± 15.2	421.2 ± 36.1	-	$t = -19.12$ **$p < 0.01$**	$t = 1.50$ $p = 0.21$	$t = 0.46$ $p = 0.67$
	bout duration (s)	5.6 ± 0.1	6.2 ± 0.2	-	5.3 ± 0.3	5.3 ± 0.1	6.2 ± 0.1	-	$t = -1.94$ $p = 0.12$	$t = -2.13$ $p = 0.10$	$t = 0.12$ $p = 0.91$

P-values are from paired *t*-tests that compared across days (top) and nights (bottom). Means ± SEM for each daytime 12-h period, and each night-time 6-h period; $n = 5$. Daytime REM sleep contained zero values and therefore was unable to be calculated. B1 and B2 correspond to the first, and second, half of the baseline night, respectively, and so on for the treatment (T) and recovery (R) nights. Shaded comparisons denote significance.

Recovery night. Spectral power density during the recovery night was broadly similar to that observed during the baseline night (Figure 3; Figure 6A). Notable exceptions were significantly *lower* low-frequency (<*c.* 7 Hz in the APH and < 2 Hz in the hyperpallium) during Q1 of the recovery night relative to Q1 of the baseline night, likely arising from reduced sleep pressure following an increase in daytime napping (Figure 2). *Circa* 12–16 Hz power density was higher in the APH during Q1 and Q2; *c.* 10–15 Hz power was higher in the hyperpallium and mesopallium during Q2 and Q4.

Daytime. Following 12-h of sleep deprivation, low-frequency (*c.* < 3 Hz) and *c.* 8–12 Hz power density were higher during the recovery day in the hyperpallium and mesopallium relative to baseline (Figure 5). The prominent hump in *c.* 3-8 Hz activity is enigmatic.

Slow-wave energy. By the end of Q3 on the recovery day, SWE was not significantly different to that observed at the end of the baseline night ($t = 1.902$, $df = 6$, $p = 0.106$), suggesting that the magpies had recovered lost SWA by the third quarter of recovery day (Figure 6C).

6-h sleep deprivation

Baseline night. The patterns identified in the baseline night of the 12-h protocol were also observed during the 6-h protocol baseline night, even if those patterns did not always reach statistical significance (Figure 4; Figure 6B). Power density was broadly highest in Q1, and decreased across the night. Here, this pattern was clearest in the APH. Faster frequencies were again higher towards the end of the night, and the hump in *c.* 3–8 Hz power density was again present during Q4.

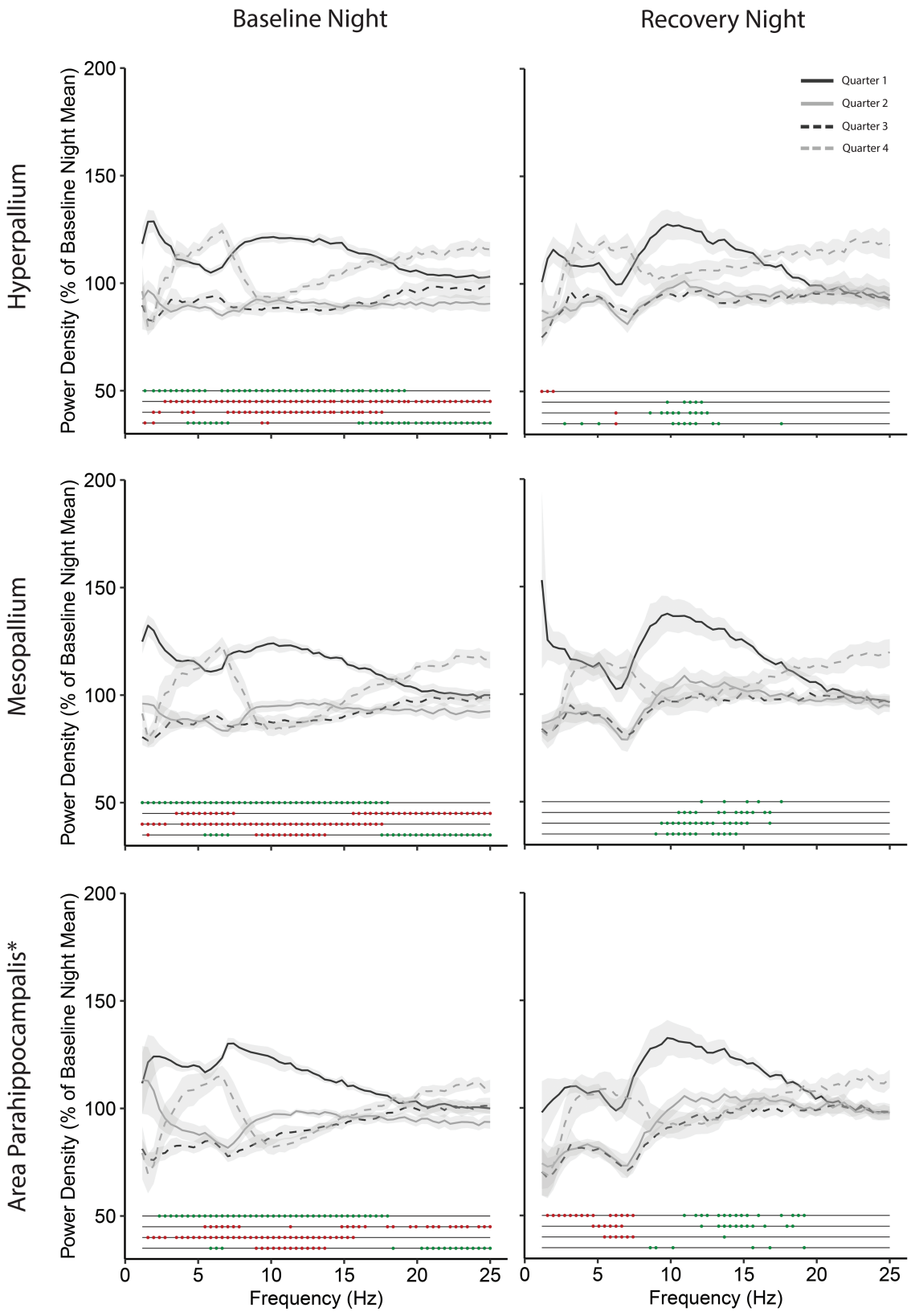

Figure 3. NREM sleep EEG spectral power density (0.78–25 Hz) during the 12-h sleep deprivation protocol. Each column shows a 12-h night and each row shows a brain region. The power density for each quarter (Q1, solid black line; Q2, solid grey line; Q3, dashed black line; Q4, dashed grey line) of each night is expressed as a percent of the entire baseline night NREM sleep mean for each frequency bin. Shaded areas represent error bars. For each plot, significance is indicated by colored circles on the lines at the bottom of each plot; green and red denote positive and negative change relative to either the 100% baseline mean or the corresponding quarter of the baseline night (as specified next), respectively. For the baseline (B) night plots, the top line compares BQ1 to 100% baseline mean, and so on. For the recovery (R) night plots, the top line compares RQ1 to BQ1, the second line compares RQ2 to BQ2, and so on.

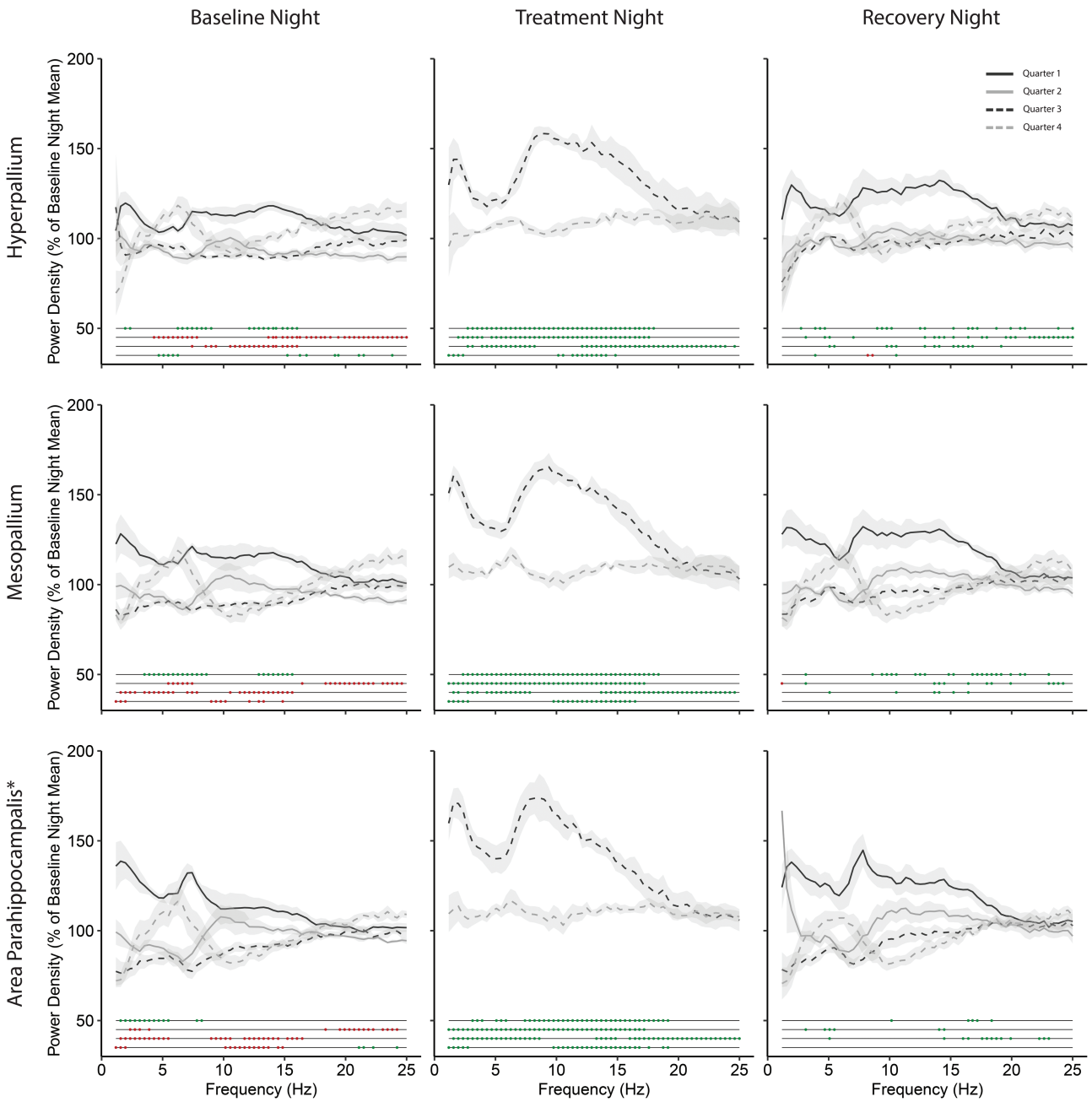

Figure 4. NREM sleep EEG spectral power density (0.78–25 Hz) during the 6-h sleep deprivation protocol. Each column shows a 12-h night and each row shows a brain region. The power density for each quarter (Q1, solid black line; Q2, solid grey line; Q3, dashed black line; Q4, dashed grey line) of each night is expressed as a percent of the entire baseline night NREM sleep mean for each frequency bin. Shaded areas represent error bars. For the baseline night (B), values for each quarter and frequency bin were compared to the entire 100% baseline mean. For the treatment night (T), only Q3 and Q4 are plotted since the Q1 and Q2 occurred during the actual 6-h sleep deprivation; values for Q3 were compared to Q1 and Q3 of B, and values for Q4 were compared to Q2 and Q4 of B. For the recovery night (R), values for each quarter and frequency bin were compared to the corresponding quarter of the baseline night. For each plot, significance is indicated by colored circles on the lines at the bottom of each plot; green and red denote positive and negative change relative to either the 100% baseline mean or the corresponding quarter of the baseline night (as specified next), respectively. For the baseline night plots, the top line compares BQ1 to 100% baseline mean, and so on. For the treatment night plots, the top line compares TQ3 to BQ1, the second line compares TQ3 to BQ3, the third line compares TQ4 to BQ2, and the fourth line compares TQ4 to BQ4. For the recovery night plots, the top line compares RQ1 to BQ1, the second line compares RQ2 to BQ2, and so on.

Treatment night. Spectral power density for only Q3 and Q4 of the treatment night is shown, as there was insufficient sleep during Q1 and Q2 (i.e., during the sleep deprivation) for a meaningful spectral analysis (Figure 4; Figure 6B). Following an extended period of wakefulness during the first half of the treatment night, power density was highest across a broad range of frequencies (*c.* 1–18 Hz) in Q3 when compared to either Q1 or Q3 of the baseline night. This increase persisted in Q4 for frequencies less than 3 Hz and *c.* 10–15 Hz, relative to both Q2 and Q4 of the baseline night.

Recovery night. During the recovery night, power density largely resembled that observed during the baseline night (Figure 4; Figure 6B).

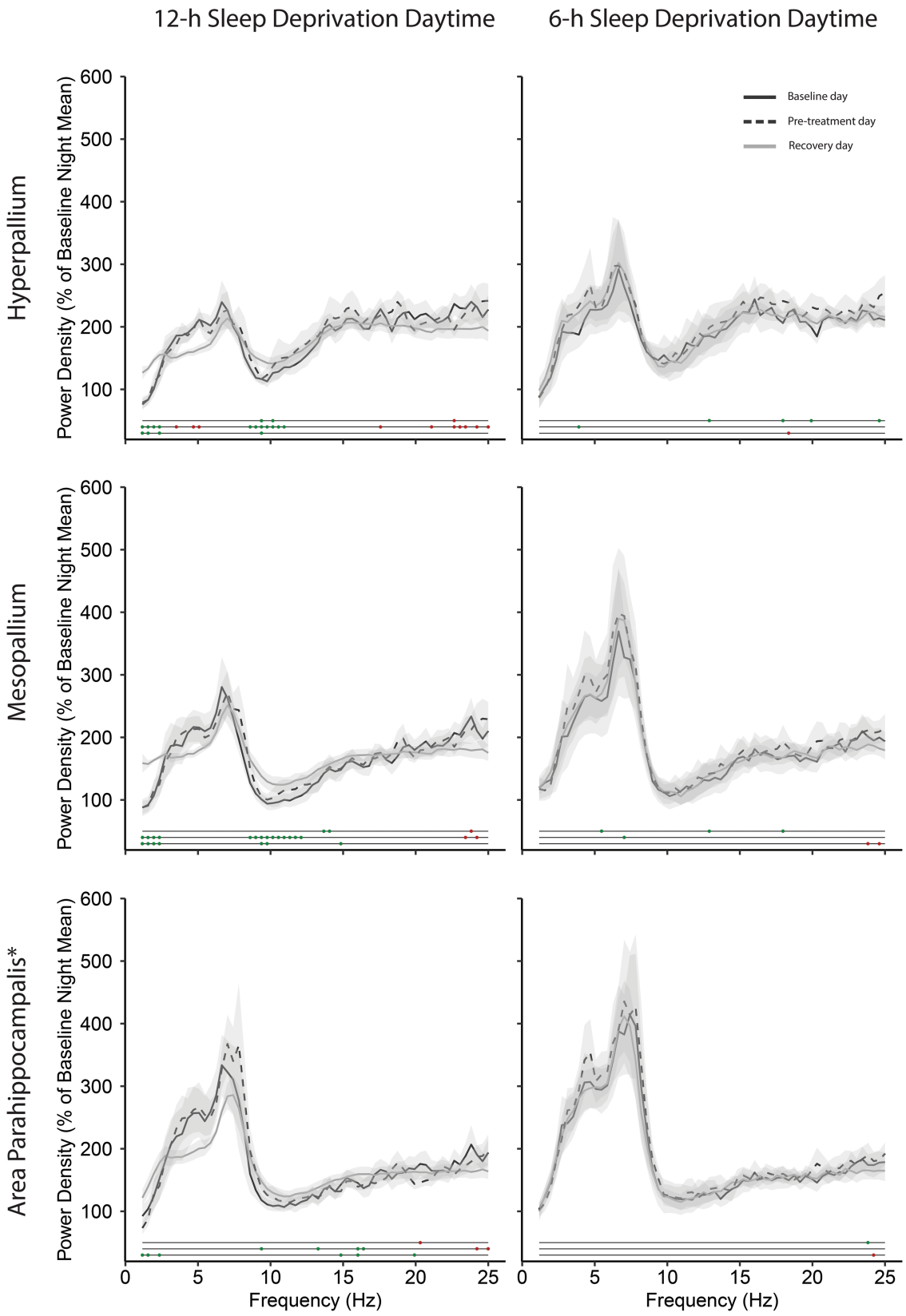

Figure 5. Daytime NREM sleep EEG spectral power density (0.78–25 Hz) during the 12-h and 6-h sleep deprivation protocols for each brain region. The power density for each full 12-h day (baseline day, solid black line; pre-treatment day, dashed black line; recovery day, solid grey line) is expressed as a percent of the entire baseline night NREM sleep mean for each frequency bin. Shaded areas represent error bars. For each plot, significance between days is indicated by colored circles on the lines at the bottom of each plot; green and red denote positive and negative change relative to the baseline day, respectively. The top line compares the baseline day with the pre-treatment day, the second line compares the baseline and recovery days, and the third line compares the pre-treatment day with the recovery day.

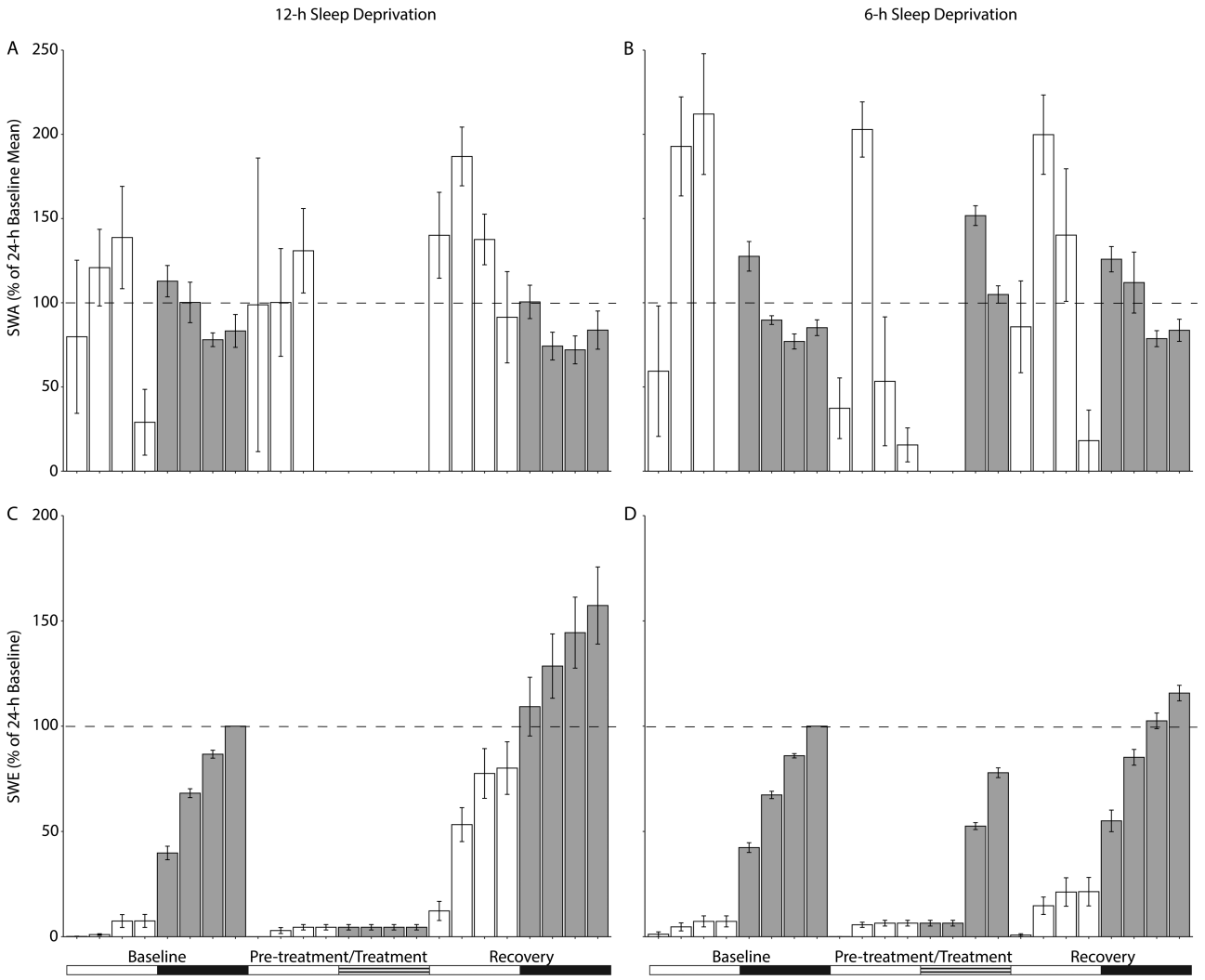

Figure 6. Effect of 12-h sleep deprivation (left column) and 6-h sleep deprivation (right column) on NREM sleep slow-wave activity (SWA; top row) and slow-wave energy (SWE; bottom row). The horizontal white and black bar at the bottom of the figure indicates day and night, respectively. Each day and night are broken up into quarters (3-h means) which are represented by each column with error bars showing standard error. For the SWA plots (panels A and B), each quarter is calculated as the mean power density between 1.17–4.30 Hz for that quarter. For the SWE plots (panels C and D), each quarter represents the accumulation of SWA up until and including that specific quarter. SWA for each quarter is expressed as a percent of the entire 24-h baseline NREM sleep SWA mean. SWE for each quarter is expressed as a percent of the entire 24-h baseline NREM sleep SWA sum.

Daytime. Spectral power density was not different between the three days (Figure 5). Similar to that observed during the 12-h recovery day, following 6-h of sleep loss, a large hump in 3-8 Hz activity was present during all days in all brain regions examined.

Slow-wave energy. Magpies did not appear to have recovered lost SWA by the end of the treatment night ($t = -9.540$, $df = 4$, $p < 0.001$) nor by the end of the recovery day ($t = -11.628$, $df = 4$, $p < 0.001$). Not until Q3 of the recovery night did the magpies seem to fully recover lost SWA ($t = 0.681$, $df = 4$, $p = 0.533$; Figure 6D).

Discussion

Australian magpies are diurnal, in accordance with previous studies [34, 54]. When we prevented the birds from falling asleep for either the first half of the night, or the entire night, we found evidence that magpies recovered lost NREM sleep, but failed to recover lost REM sleep. Taken together with previous studies of sleep disruption on Australian magpies [34, 54], these results question the robustness of REM sleep homeostasis in this species. Because a detailed description of sleep in undisturbed Australian magpies has been presented previously [54], here we focus on the consequences of our two sleep deprivation protocols on subsequent sleep architecture and spectral power density during NREM sleep.

12-h sleep deprivation

NREM and REM sleep were suppressed during the all-night sleep deprivation, and remnant NREM sleep was heavily fragmented. On the following day, when the birds were allowed to sleep undisturbed, magpies showed a rebound of NREM sleep, with more and longer NREM sleep episodes. Moreover, low-frequency power density (<2.34 Hz) was highest during the recovery day. Collectively, increased NREM sleep with greater continuity and more SWA reflects NREM sleep homeostasis. On the recovery night, the amount

of NREM sleep resembled that observed on the baseline night. Interestingly, however, power below 2 Hz in the hyperpallium, and below 7 Hz in the *area parahippocampalis*, was *lower* during the first quarter of the recovery night relative to baseline. This reduction in NREM sleep SWA likely arose from reduced sleep pressure owing to an increase in daytime naps [26]. In contrast to the increases seen in the amount, continuity, and intensity of NREM sleep, REM sleep showed no rebound during the recovery day or night.

6-h sleep deprivation

We effectively reduced sleep during the first half of the treatment night with remaining NREM sleep being fragmented into shorter episodes; REM sleep was virtually eliminated. When magpies were allowed to sleep undisturbed during the second half of the treatment night, they had more NREM sleep relative to the second, but not first, half of the baseline night. Increased NREM sleep was achieved through longer and fewer NREM sleep bouts, at least when compared against the second half of the baseline night. Relative to the *first* half of the baseline night, NREM sleep episodes remained shorter, and more numerous, perhaps a lingering effect of stress induced during the deprivation procedure. Power density, including SWA, was broadly higher during the second half of the treatment night relative to both the same circadian timepoint on the baseline night, and the first half of the baseline night. In contrast to the increase in NREM sleep, REM sleep remained low. Indeed, the amount of REM sleep never reached or exceeded baseline levels. During the recovery day and night, most architectural and spectral features of sleep had returned to baseline levels.

Taken together, these results reveal a homeostatic response to extended periods of wakefulness in Australian magpies, but only for NREM sleep. Magpies increased the amount, continuity, and intensity of NREM sleep at night following 6-h of sleep loss; and they compensated for 12-h of sleep loss by sleeping during the day. Interestingly, following these daytime naps, magpies had reduced SWA at night. Thus, as supported by our analysis of SWE, SWA increases *and decreases* following night-time wakefulness and daytime naps, respectively, as in humans [56, 57]. Even under baseline conditions, SWA is highest in magpies at the start of the night, and decreases with time spent asleep. This repeatable decline resembles the pattern seen in mammals and other birds that consolidate wakefulness to one part of the 24-h day. In some animals, the decline is not specific only to low-frequency activity. In starlings, 1–25 Hz power density decreases across the entire night [36]. Similar to starlings, 1–18 Hz activity in magpies was higher following 6-h of sleep loss. Conversely, animals that take frequent naps, such as guinea pigs (*Cavia porcellus*) [58] and pigeons [24, 34, 52, 59], do not show a clear decline in SWA during undisturbed sleep.

Other spectral aspects of magpie sleep were similar to that reported in other birds and mammals. For instance, during the baseline nights, fast (*c.* 18–25 Hz) activity was highest towards the end of the night. A similar increase in higher frequencies has been observed in pigeons and starlings [24, 36, 52], and in Syrian hamsters (*Mesocricetus auratus*) [30], Djungarian hamsters (*Phodopus sungorus*) [60], and rats [61], but not in rabbits [62]. The increase in fast brain waves nearest the start of the day is thought to be modulated by circadian changes in brain temperature to prepare the animal for wakefulness, rather than being under sleep homeostatic control [63].

We are unable to explain the hump in 3–8 Hz activity during NREM sleep at the end of the night. This waveform was found repeatedly in all brain regions examined, and during all baseline and recovery nights. An exaggerated form can also be seen during each day. A similar, albeit less pronounced, pattern was observed in pigeons [24] and starlings [36]. This bandwidth overlaps that of theta waves exhibited by the mammalian hippocampus during REM sleep. However, a hippocampal theta rhythm has not been detected in birds [64, 65], and the waveform was observed during NREM sleep. Ultimately, the origin and significance of this hump is unclear; higher density EEG recordings may provide new insight. Furthermore, from the spectral power data, there appears to be a bimodal distribution of power between lower (*c.* 2 Hz) and higher (*c.* 16 Hz) activity; such patterns appear also in other bird species [24, 33] and may reflect an idiosyncrasy of avian NREM sleep.

In neither of our two sleep deprivations did we find evidence for a rebound of REM sleep. In mammals [26] and pigeons, [24, 52, 53] the amount of REM sleep increases following sleep loss. In contrast, recent studies on starlings [36] and Australian magpies [34, 54] did not find evidence for REM sleep homeostasis; and in tree shrews only a small fraction of lost REM sleep was recovered [50]. Why Australian magpies did not recover from lost REM sleep is unclear. Further studies investigating the effects of sleep deprivation, of various durations, might provide insight into the answer. It is noteworthy that we recorded brain activity for 24 and 36 hours after the end of the 12-h and 6-h sleep deprivation, respectively. Whether the magpies delayed their REM sleep rebound to after this period is unknown. However, Connelly et al.[54] disrupted sleep in magpies using auditory stimulation for 24 hours and recorded sleep for a subsequent 48 hours and still found no evidence for increased REM sleep.

Conclusion

We show that Australian magpies recover from lost NREM sleep by sleeping more and with increased NREM sleep consolidation and NREM sleep SWA during recovery sleep. Furthermore, magpies showed reduced SWA at night after napping more during the day. Thus, NREM sleep is homeostatically regulated in these birds with the level of SWA reflecting prior sleep/wake history. Surprisingly, despite multiple durations of sleep loss, with recovery occurring both at night and during the day, we failed to observe evidence for REM sleep homeostasis. This result builds upon previous findings in magpies [34, 54], starlings [36], white-crowned sparrows [33], fur seals [51], and tree shrews [50] that showed either no or weak rebound of REM sleep. The significance of these emerging patterns for the regulation and function of REM sleep is unclear.

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References

1. Nath RD, *et al*. The Jellyfish *Cassiopea* exhibits a sleep-like state. *Curr Biol*. 2017;**27**(19):2984–2990.e3.
2. Kanaya HJ, *et al*. A sleep-like state in *Hydra* unravels conserved sleep mechanisms during the evolutionary development of the central nervous system. *Sci Adv*. 2020;**6**(41):eabb9415.
3. Ungurean G, *et al*. Evolution and plasticity of sleep. *Curr Opin Physiol*. 2020;**15**:111–119.
4. Siegel JM. Sleep viewed as a state of adaptive inactivity. *Nat Rev Neurosci*. 2009;**10**(10):747–753.
5. Schmidt MH, *et al*. State-dependent metabolic partitioning and energy conservation: a theoretical framework for understanding the function of sleep. *PLoS One*. 2017;**12**(10):e0185746.
6. Scriba MF, *et al*. Linking melanism to brain development: expression of a melanism-related gene in barn owl feather follicles covaries with sleep ontogeny. *Front Zool*. 2013;**10**(1):42.
7. Kayser MS, *et al*. A critical period of sleep for development of courtship circuitry and behavior in *Drosophila*. *Science*. 2014;**344**(6181):269–274.
8. Xie L, *et al*. Sleep drives metabolite clearance from the adult brain. *Science*. 2013;**342**(6156):373–377.
9. Tononi G, *et al*. Sleep and synaptic down-selection. In: Buzsáki G, Christen Y, eds. *Research and Perspectives in Neurosciences: Micro-, Meso- and Macro-Dynamics of the Brain*. Switzerland: Springer International Publishing; 2016: 99–106.
10. Fultz NE, *et al*. Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. *Science*. 2019;**366**(6465):628–631.
11. Zada D, *et al*. Sleep increases chromosome dynamics to enable reduction of accumulating DNA damage in single neurons. *Nat Commun*. 2019;**10**(1):895.
12. Huber R, *et al*. Local sleep and learning. *Nature*. 2004;**430**(6995):78–81.
13. Derégnaucourt S, *et al*. How sleep affects the developmental learning of bird song. *Nature*. 2005;**433**(7027):710–716.
14. Weaver TE, *et al*. Self-efficacy in sleep apnea: instrument development and patient perceptions of obstructive sleep apnea risk, treatment benefit, and volition to use continuous positive airway pressure. *Sleep*. 2003;**26**(6):727–732. doi: 10.1093/sleep/26.6.727.
15. Diekelmann S, *et al*. The memory function of sleep. *Nat Rev Neurosci*. 2010;**11**(2):114–126.
16. Lima SL, *et al*. Sleeping under the risk of predation. *Anim Behav*. 2005;**70**(4):723–736.
17. Tefft BC. Acute sleep deprivation and culpable motor vehicle crash involvement. *Sleep*. 2018;**41**(10).doi: 10.1093/sleep/zsy144.
18. Ferretti A, *et al*. Sleeping unsafely tucked in to conserve energy in a nocturnal migratory songbird. *Curr Biol*. 2019;**29**(16):2766–2772.e4.
19. Lesku JA, *et al*. Adaptive sleep loss in polygynous pectoral sandpipers. *Science*. 2012;**337**(6102):1654–1658.
20. Rattenborg NC, *et al*. Evidence that birds sleep in mid-flight. *Nat Commun*. 2016;**7**:12468.
21. Steriade M. Grouping of brain rhythms in corticothalamic systems. *Neuroscience*. 2006;**137**(4):1087–1106.
22. Vyazovskiy VV, *et al*. Cortical firing and sleep homeostasis. *Neuron*. 2009;**63**(6):865–878.
23. Lesku JA, *et al*. Avian sleep. *Curr Biol*. 2014;**24**(1):R12–R14.
24. Martinez-Gonzalez D, *et al*. Increased EEG spectral power density during sleep following short-term sleep deprivation in pigeons (*Columba livia*): evidence for avian sleep homeostasis. *J Sleep Res*. 2008;**17**(2):140–153.
25. Rattenborg NC, *et al*. Avian sleep homeostasis: convergent evolution of complex brains, cognition and sleep functions in mammals and birds. *Neurosci Biobehav Rev*. 2009;**33**(3):253–270.
26. Tobler I. Phylogeny of sleep regulation. In: Dement WC, Roth T, Kryger MH, eds. *Principles and Practice of Sleep Medicine*. 5th ed. Philadelphia, PA: Saunders; 2011: 112–125.
27. Neckelmann D, *et al*. Sleep stages and EEG power spectrum in relation to acoustical stimulus arousal threshold in the rat. *Sleep*. 1993;**16**(5):467–477. doi: 10.1093/sleep/16.5.467.
28. Borbély AA. From slow waves to sleep homeostasis: new perspectives. *Arch Ital Biol*. 2001;**139**(1/2):53–61.
29. Tobler I, *et al*. Sleep EEG in the rat as a function of prior waking. *Electroencephalogr Clin Neurophysiol*. 1986;**64**(1):74–76.
30. Tobler I, *et al*. Sleep and EEG spectra in the Syrian hamster (*Mesocricetus auratus*) under baseline conditions and following sleep deprivation. *J Comp Physiol A*. 1987;**161**(3):449–459.
31. Huber R, *et al*. Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. *Brain Res*. 2000;**857**(1–2):8–19.
32. Tobler I, *et al*. Sleep in the blind mole rat *Spalax ehrenbergi*. *Sleep*. 2001;**24**(2):147–154. doi: 10.1093/sleep/24.2.147.
33. Jones SG, *et al*. Homeostatic regulation of sleep in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *BMC Neurosci*. 2008;**9**:47.
34. Aulsebrook AE, *et al*. White and amber light at night disrupt sleep physiology in birds. *Curr Biol*. 2020;**30**(18):3657–3663.e5.
35. van Hasselt SJ, *et al*. Seasonal variation in sleep homeostasis in migratory geese: a rebound of NREM sleep following sleep deprivation in summer but not in winter. *Sleep*. 2020;**44**(4). doi: 10.1093/sleep/zsaa244.
36. van Hasselt SJ, *et al*. The European starling (*Sturnus vulgaris*) shows signs of NREM sleep homeostasis but has very little REM sleep and no REM sleep homeostasis. *Sleep*. 2020;**43**(6). doi: 10.1093/sleep/zsz311.
37. Tononi G, *et al*. Sleep function and synaptic homeostasis. *Sleep Med Rev*. 2006;**10**(1):49–62.
38. Tononi G, *et al*. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron*. 2014;**81**(1):12–34.
39. Lesku JA, *et al*. Local sleep homeostasis in the avian brain: convergence of sleep function in mammals and birds? *Proc Biol Sci*. 2011;**278**(1717):2419–2428.
40. Jouvet-Mounier D, *et al*. Ontogenesis of the states of sleep in rat, cat, and guinea pig during the first postnatal month. *Dev Psychobiol*. 1969;**2**(4):206–239.
41. Blumberg MS, *et al*. What is REM sleep? *Curr Biol*. 2020;**30**(1):R38–R49.
42. Blumberg MS, *et al*. Spatiotemporal structure of REM sleep twitching reveals developmental origins of motor synergies. *Curr Biol*. 2013;**23**(21):2100–2109.
43. Blumberg MS, *et al*. Twitching in sensorimotor development from sleeping rats to robots. *Curr Biol*. 2013;**23**(12):R532–R537.
44. Blumberg MS. Developing sensorimotor systems in our sleep. *Curr Dir Psychol Sci*. 2015;**24**(1):32–37.
45. Kiyono S, *et al*. Effects of sleep deprivation upon the paradoxical phase of sleep in cats. *Electroencephalogr Clin Neurophysiol*. 1965;**19**:34–40.

46. Ruckebusch Y. Sleep deprivation in cattle. *Brain Res*. 1974;**78**(3):495–499.
47. Takahashi Y, *et al*. Temporal distributions of delta wave sleep and rem sleep during recovery sleep after 12-h forced wakefulness in dogs; similarity to human sleep. *Neurosci Lett*. 1978;**10**(3):329–334.
48. Borbély AA, *et al*. Sleep-deprivation: effects on sleep and EEG in the rat. *J Comp Physiol*. 1979;**133**(1):71–87.
49. Endo T, *et al*. Selective REM sleep deprivation in humans: effects on sleep and sleep EEG. *Am J Physiol*. 1998;**274**(4):R1186–R1194.
50. Coolen A, *et al*. Telemetric study of sleep architecture and sleep homeostasis in the day-active tree shrew *Tupaia belangeri*. *Sleep*. 2012;**35**(6):879–888. doi: 10.5665/sleep.1894.
51. Lyamin OI, *et al*. Fur seals suppress REM sleep for very long periods without subsequent rebound. *Curr Biol*. 2018;**28**(12):2000–2005.e2.
52. Tobler I, *et al*. Sleep and EEG spectra in the pigeon (*Columba livia*) under baseline conditions and after sleep deprivation. *J Comp Physiol A*. 1988;**163**(6):729–738.
53. Newman SM, *et al*. Sleep deprivation in the pigeon using the Disk-Over-Water method. *Physiol Behav*. 2008;**93**(1–2):50–58.
54. Connelly F, *et al*. Urban noise restricts, fragments, and lightens sleep in Australian magpies. *Environ Pollut*. 2020;**267**:115484.
55. Allocca G, *et al*. Validation of 'Somnivore', a machine learning algorithm for automated scoring and analysis of polysomnography data. *Front Neurosci*. 2019;**13**:207.
56. Feinberg I, *et al*. Homeostatic changes during post-nap sleep maintain baseline levels of delta EEG. *Electroencephalogr Clin Neurophysiol*. 1985;**61**(2):134–137.
57. Achermann P, *et al*. Simulation of human sleep: ultradian dynamics of electroencephalographic slow-wave activity. *J Biol Rhythms*. 1990;**5**(2):141–157.
58. Tobler I, *et al*. Vigilance states, EEG spectra, and cortical temperature in the guinea pig. *Am J Physiol*. 1993;**264**(6 Pt 2):R1125–R1132.
59. Tisdale RK, *et al*. The low-down on sleeping down low: pigeons shift to lighter forms of sleep when sleeping near the ground. *J Exp Biol*. 2018;**221**(19):jeb182634.
60. Deboer T, *et al*. Sleep and cortical temperature in the Djungarian hamster under baseline conditions and after sleep deprivation. *J Comp Physiol A*. 1994;**174**(2):145–155.
61. Trachsel L, *et al*. Electroencephalogram analysis of non-rapid eye movement sleep in rats. *Am J Physiol*. 1988;**255**(1 Pt 2):R27–R37.
62. Tobler I, *et al*. Sleep and EEG spectra in the rabbit under baseline conditions and following sleep deprivation. *Physiol Behav*. 1990;**48**(1):121–129.
63. Deboer T. Brain temperature dependent changes in the electroencephalogram power spectrum of humans and animals. *J Sleep Res*. 1998;**7**(4):254–262.
64. Rattenborg NC, *et al*. Hippocampal memory consolidation during sleep: a comparison of mammals and birds. *Biol Rev Camb Philos Soc*. 2011;**86**(3):658–691.
65. van der Meij J, *et al*. Evolution of sleep in relation to memory—a birds' brain view. *Curr Opin Behav Sci*. 2020;**33**:78–85.