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Local sleep homeostasis in the avian brain: convergence of sleep function in mammals and birds?

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The function of the brain activity that defines slow wave sleep (SWS) and rapid eye movement (REM) sleep in mammals is unknown. During SWS, the level of electroencephalogram slow wave activity (SWA or 0.5–4.5 Hz power density) increases and decreases as a function of prior time spent awake and asleep, respectively. Such dynamics occur in response to waking brain use, as SWA increases locally in brain regions used more extensively during prior wakefulness. Thus, SWA is thought to reflect homeostatically regulated processes potentially tied to maintaining optimal brain functioning. Interestingly, birds also engage in SWS and REM sleep, a similarity that arose via convergent evolution, as sleeping reptiles and amphibians do not show similar brain activity. Although birds deprived of sleep show global increases in SWA during subsequent sleep, it is unclear whether avian sleep is likewise regulated locally. Here, we provide, to our knowledge, the first electrophysiological evidence for local sleep homeostasis in the avian brain. After staying awake watching David Attenborough's *The Life of Birds* with only one eye, SWA and the slope of slow waves (a purported marker of synaptic strength) increased only in the hyperpallium—a primary visual processing region—neurologically connected to the stimulated eye. Asymmetries were specific to the hyperpallium, as the non-visual mesopallium showed a symmetric increase in SWA and wave slope. Thus, hypotheses for the function of mammalian SWS that rely on local sleep homeostasis may apply also to birds.

Keywords: potentiation; slow wave activity; synaptic downscaling; synaptic strength

1. INTRODUCTION

The function of the brain activity that defines slow wave sleep (SWS) and rapid eye movement (REM) sleep in humans and other mammals is an unanswered question in neuroscience [1,2]. At the neuronal level, SWS is seen as a slow (approx. 1 Hz) oscillation of membrane potentials between a depolarized up-state with action potentials and a hyperpolarized down-state without [3,4]. The oscillation is synchronized among neurons by cortico-cortical connectivity [5,6] and manifested in the electroencephalogram (EEG) as high-amplitude low-frequency waves, typically quantified as 0.5–4.5 Hz power density or slow wave activity (SWA; [7]). The level of SWA increases and decreases as a function of prior time spent awake and asleep, respectively [8,9]. Additionally, SWA increases locally in the brain in response to local brain use during wakefulness [10–17]. Thus, SWA is thought to reflect homeostatically regulated processes potentially tied to maintaining optimal brain functioning [18–24].

Birds are the only animals, outside of mammals, known to engage in unequivocal SWS and REM sleep [25–27], a similarity that may have arisen via convergent evolution, as sleeping reptiles and amphibians do not show similar brain activity [28]. Although it has recently been shown that sleep-deprived birds show a global increase in SWA during subsequent sleep [29,30], it is unclear whether this effect reflects brain use *per se* or is mediated by central brain regions involved in the 'whole-brain' regulation of sleep ([31]; see also [32,33]). Below, we demonstrate a local increase in SWA following waking brain use in pigeons—to our knowledge, the first electrophysiological evidence for local sleep homeostasis in the avian brain.

2. MATERIAL AND METHODS

Seven adult homing pigeons (*Columba livia*, three males, four females, genetically sexed) were housed individually in wooden enclosures (79 cm length × 60 cm width × 60 cm height). A mesh-covered window on the front door allowed ventilation. Lights were mounted on the outside of the back wall, which was composed of white, translucent Plexiglas (400–500 lux at head level in the centre of the box). An inverted ceramic dish in the centre of the box served as a perch. Each cage was equipped with four video cameras,

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one in each corner, and a ceiling-mounted infrared illuminator (940 nm) for night recordings. A flatscreen computer monitor (41 cm length × 34 cm width) was mounted on one side. Birds were maintained on a 12 L : 12 D photoperiod with lights on at 08.00 h. Pigeons were returned to the breeding aviary at the end of the study.

(a) *Implanting EEG electrodes*

To record the EEG, pigeons were implanted with electrodes symmetrically placed over each hemisphere. Briefly, a stereotax-mounted pigeon was anaesthetized with isoflurane (1.5–2.0% vapourized in 1.0 LPM O_2). Eight holes (0.5 mm diameter) were drilled through the cranium to the level of the dura; holes were arranged as two rows of four. The anterior (A) row was positioned at A +13.0 mm while the posterior row was positioned at A +9.25 mm [34]. Within a row, holes were drilled 2.0 and 6.0 mm lateral (L 2.0 and L 6.0) of the midline overlying each hemisphere. The medial electrodes (L 2.0) of both rows were positioned over the hyperpallium apicale while the electrodes placed more laterally (L 6.0) were seated over the mesopallium. The mesopallium is a non-visual region that has been implicated in higher cognitive processes, such as motor-learning and innovation [35,36]. Conversely, the hyperpallium is a primary visual processing region comparable to the primary visual (striate) cortex in mammals [37]. In pigeons, each hyperpallium receives visual input primarily from the contralateral eye [38]. As a result, visual stimuli presented to only one eye cause EEG activation in the contralateral hemisphere [39]. An additional hole was drilled over the cerebellum for the reference electrode, and another was drilled along the midline 2.0 mm anterior of the anterior row for the ground. All electrodes were gold-plated, round-tipped pins (0.5 mm diameter), glued in place using cyanoacrylic adhesive. Electrode wires terminated at a connector fixed on the head with Paladur dental acrylic (Heraeus Kulzer, www.heraeus-kulzer.com). After a post-operative recovery period of at least two weeks, the feathers around the left and right eye were clipped and a Velcro ring (2.5 cm diameter) was glued around the eyes using a non-toxic water-soluble skin glue. The bird's headplug was then connected to the recording cable, which in turn attached to a ceiling-mounted commutator (Plastics One, Inc., www.plastics1.com). Baseline recordings commenced after at least one week of habituation to these recording conditions.

(b) *Experimental design*

Baseline EEG and video recordings were obtained for one 12 h night starting at lights off (20.00 h) following an undisturbed day. At 12.00 h the next day, a Velcro-ringed cardboard cap was attached to the eye-ring around the left eye; bandage tape around the margin further reduced the input of ambient light to the eye. This method of monocular occlusion is frequently used in studies of brain lateralization [40]. Given that the avian brain is functionally lateralized [40], it is conceivable that the two hemispheres would respond differently to sleep loss [13,16,41]. However, we chose to cap only the left eye (chosen randomly) in the present study, because SWA increases symmetrically between the hemispheres in pigeons subjected to enforced wakefulness without unilateral visual stimulation [30]. After the eye-cap was secured, the computer monitor began to show moving, non-repetitive video of wild birds (David Attenborough's *The Life of Birds*, BBC Video) continuously, without audio, for the next 8 h until lights off. Although the pigeons usually oriented their uncapped eye towards the monitor (figure 1), occasionally they had to be re-positioned by the experimenter standing in front of the open cage door. During this period, the birds were also gently stimulated (by the experimenter) to stay awake whenever EEG-signs of sleep (i.e. slow waves) appeared in the hyperpallium or mesopallium of either hemisphere. Because pigeons housed in the laboratory spend around 50 per cent of the last 8 h of the day asleep [30], the pigeons were deprived of 4 h of sleep. This sleep deprivation procedure has been shown to effectively reduce sleep to less than 10 per cent of baseline values, and residual sleep is heavily fragmented [30]. At lights off, the monitor was turned off, the eye-cap removed and the pigeons were allowed to sleep undisturbed for the next 12 h (i.e. 'recovery' sleep). Recordings concluded at lights on the following day.

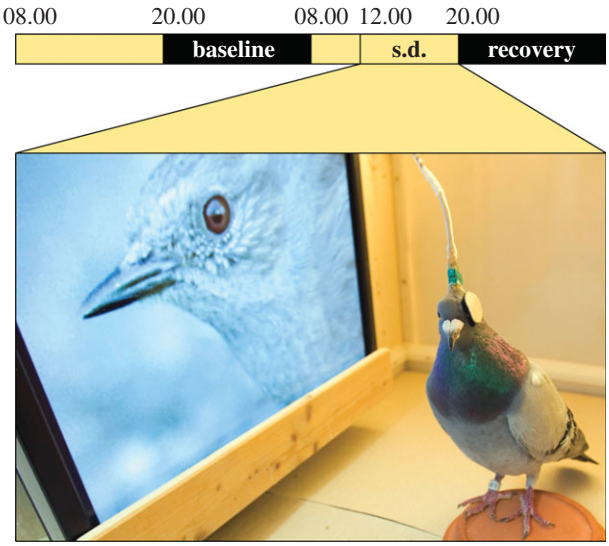

Figure 1. Experimental design: a 12 h baseline night, 8 h period of bihemispheric sleep deprivation with unilateral visual stimulation (s.d.) and a 12 h recovery night. Photograph shows the experimental environment during the treatment (Copyright: Axel Griesch).

(c) *Processing EEG signals*

Eight unipolar EEG derivations were referenced to the cerebellum and digitally recorded at 200 Hz using commercially available amplifiers (Embla A10) and visualized in real-time with Somnologica Science v. 3.3.1 (Embla, www.embla.com). The low-cut finite impulse response (FIR) filter was set at 0.5 Hz (−6 dB at 0.5 Hz and 0 dB at 0.8 Hz) and the high-cut anti-aliasing FIR filter was set at 100 Hz (−20 dB at 100 Hz and 0 dB at 80 Hz). The Embla A10 system automatically applies the anti-aliasing filter after first sampling the data at 2000 Hz; the data is then down-sampled to 200 Hz. For sleep scoring and data analysis, we created four bipolar EEG derivations for the left and right hyperpallium (A +13.0–+9.25, L 2.0) and left and right mesopallium (A +13.0–+9.25, L 6.0).

(d) *Sleep scoring*

Twelve hour baseline and recovery night EEG recordings were scored for SWS and REM sleep using 4 s epochs with the aid of video recordings. An epoch was scored as SWS when the majority of the epoch showed slow (≤4 Hz)

waves with amplitudes at least twice that of alert wakefulness. The appearance of slow waves was usually associated with behavioural signs of sleep onset (e.g. immobility and closure of at least one eye). All epochs were assessed for signs of SWS (i.e. 10 800 possible epochs of SWS per night). An epoch was scored as REM sleep when at least 50 per cent of the epoch showed high-frequency, low-amplitude EEG activity similar to that observed during alert wakefulness, but occurring with bilateral eye closure and behavioural signs of reduced muscle tone (e.g. drooping of the head). REM sleep was sampled at an interval of once per minute (i.e. 720 possible epochs of REM sleep per night), as simulations demonstrated that this sampling interval yielded REM sleep values similar to continuous scoring (see the electronic supplementary material for details).

(e) *Spectral analyses*

Fast Fourier transforms were performed on epochs of SWS and expressed as power density in 0.39 Hz bins from 0.78 to 25.00 Hz using Somnologica Science v. 3.3.1. Epochs containing artefacts and transitional epochs (i.e. $> 2 < 4$ s of SWS) were excluded from all spectral analyses. Spectral power density was calculated for each quarter of the baseline and recovery nights and expressed as a percentage of the 12 h baseline night SWS mean per frequency bin.

(f) *Wave slopes*

The slope of slow waves during SWS has been used as a measure of synaptic strength [7,42–46], because steeper slopes are thought to reflect more synchronous alternations between up and down states of the slow oscillation resulting from increased synaptic strength [4,43]. Accordingly, slow waves are steeper (synapses are stronger) following extended periods of wakefulness in mammals [42,44–46]. To quantify potential changes in wave slope in response to unilateral visual stimulation during enforced wakefulness, we used the filter settings and definitions of Vyazovskiy *et al.* [46]. EEG signals were band-pass filtered using the Matlab (The Math Works, Inc., www.mathworks.com) function 'band-pass' from the Filter Design toolbox (function parameters: band-pass: 0.5–4.0 Hz, band-stop: < 0.1 Hz, > 10 Hz, rippel in band-pass 3 dB, attenuation in band-stop 20 dB). This filter exploits a Chebyshev Type II filter design (Matlab, function 'design' with parameter 'Cheby2'). The filter was applied twice, left to right and right to left (function 'filter' from the Filter Design toolbox), in order to maintain zero phase shift of the transform. The up slope (change in amplitude per duration from one negative peak to the next positive peak) and the down slope (slope from the positive peak to the next negative peak) were calculated from these band-pass filtered EEG signals. These calculations were repeated for each wave in an epoch and expressed as an epoch mean. Mean up and down slopes were calculated for the first quarter of the baseline and recovery nights, and expressed as a percentage of the all-night baseline mean.

(g) *Eye state*

Pigeons can keep one eye open during SWS, a behaviour associated with lower SWA in the contralateral hemisphere [47]. To determine if any asymmetries in the level of SWA on the recovery night were owing to changes in unilateral eye opening, we examined instantaneous bilateral eye state at the start of each minute for epochs scored as SWS during the first quarter of the baseline and recovery nights, when the treatment effect is expected to be greatest.

(h) *Statistical analyses*

We conducted one, two or three-way repeated measures analysis of variance (rmANOVA) with factors variously as 'night' (baseline, recovery), 'brain region' (left, right hemisphere), 'quarter of night' and 'frequency'. Significant rmANOVAs were followed up by paired *t*-tests to determine the level(s) at which significance ($\alpha = 0.050$) was reached. Data from all seven birds was used in all comparisons, except those involving the visually deprived hyperpallium on the recovery night where data from one bird was omitted owing to a technical problem. Wave slope and eye state analyses were conducted using paired *t*-tests. Statistical analyses were performed in Systat 10 (©SPSS, Inc., www.systat.com).

3. RESULTS

(a) *Amount of SWS and REM sleep*

The amount of SWS and REM sleep on the baseline and recovery nights was similar to those observed in a previous study on sleep regulation in pigeons [30]. Specifically, the percentage of SWS decreased across the baseline ($F_{3,18} = 6.514$, $p = 0.004$) and recovery ($F_{3,18} = 5.390$, $p = 0.008$) nights, but did not differ between the two nights ($F_{1,42} = 1.732$, $p = 0.195$; baseline night mean $\pm$ s.e. $= 77.56 \pm 1.69\%$, recovery night $= 76.08 \pm 1.95\%$). Conversely, the amount of REM sleep increased across the baseline ($F_{3,18} = 13.023$, $p < 0.001$) and recovery ($F_{3,18} = 7.049$, $p = 0.003$) nights, and there was significantly more REM sleep on the recovery night ($F_{1,42} = 6.789$, $p = 0.013$; baseline night mean $\pm$ s.e. $= 11.69 \pm 1.33\%$, recovery night $= 13.83 \pm 0.49\%$), largely owing to more REM sleep in the last quarter of the recovery night ($p = 0.012$), reflecting REM sleep homeostasis [9,48,49].

(b) *Spectral power density*

(i) *Stimulated hyperpallium*

Spectral power density differed between the baseline and recovery nights in the stimulated hyperpallium ($F_{1,3397} = 464.226$, $p < 0.001$). Specifically, 1.17–13.28 Hz power density was significantly higher during the first quarter of the recovery night (figure 2). Power in the 0.78–4.69 Hz bandwidth (i.e. that which most closely approximates SWA as typically defined in mammals) decreased across the recovery night ($F_{3,261} = 11.897$, $p < 0.001$; figure 2). Despite this decline, ≤ 2.73 Hz activity remained significantly higher than baseline throughout most of the recovery night (figure 2). Power density of *ca* 7.81–14.06 Hz was higher across the entire recovery night (figure 2).

(ii) *Visually deprived hyperpallium*

Unlike the stimulated hyperpallium where the most predictive factor in the rmANOVA was 'night' (baseline or recovery), the strongest determinant of power density for the visually deprived hyperpallium was 'quarter of night' ($F_{3,3149} = 191.381$, $p < 0.001$). Accordingly, power density was not significantly different from baseline at most frequency bins across the recovery night, although in the second, third and fourth quarters 21.09–25.00 Hz activity was reduced relative to baseline (figure 2).

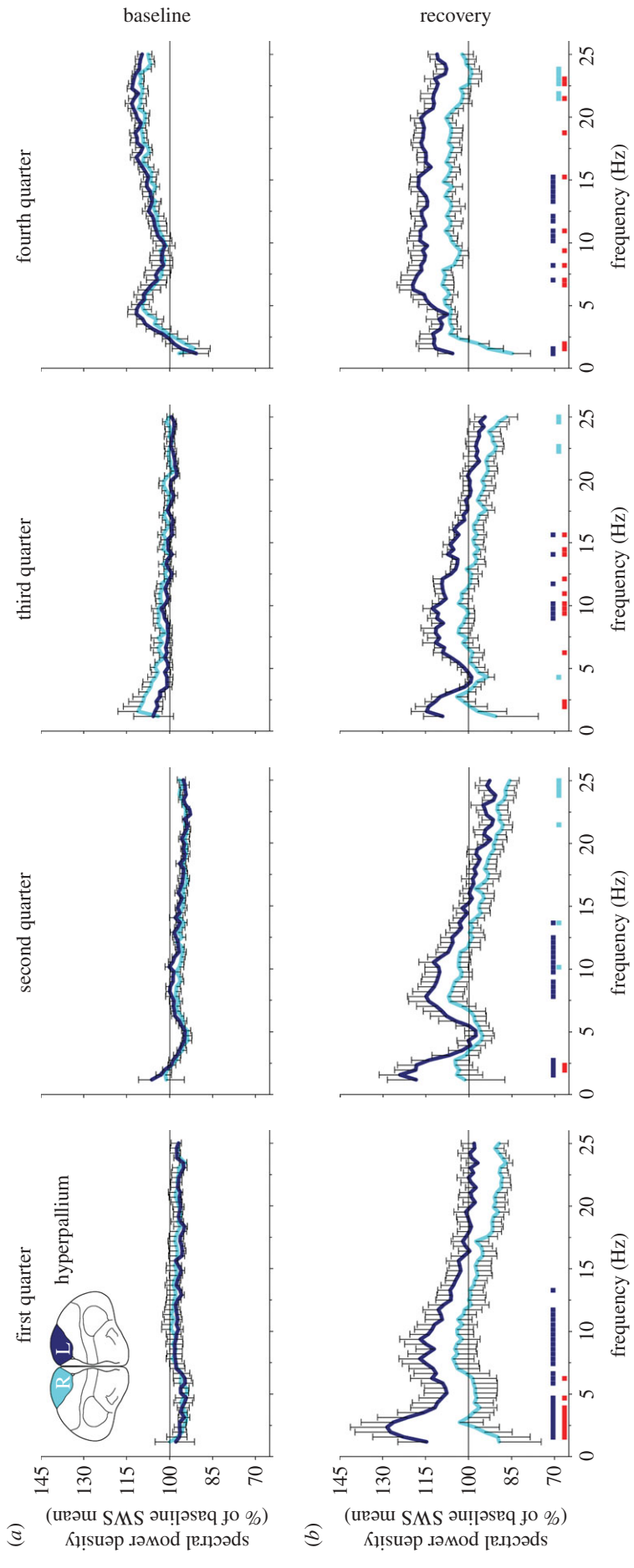

Figure 2. Spectral power density (0.78–25.00 Hz) across the four quarters of the (*a*) baseline and (*b*) recovery nights for the stimulated (dark blue) and visually deprived (light blue) hyperpallia. Data are presented as mean ± s.e. Coloured squares at the bottom of each recovery night plot reflect a significant pairwise comparison between the baseline and recovery night of the stimulated (dark blue) and visually deprived (light blue) hyperpallia; red squares denote a significant asymmetry between the left (L) and right (R) hyperpallia during recovery sleep. Note the broad symmetry across the baseline night, and the decreasing low-frequency (less than 5 Hz) asymmetry across the recovery night. Inset: frontal view of a transverse section through the cerebrum of a pigeon highlighting the hyperpallium.

(iii) *Inter-hyperpallium*

Across the baseline night, power density was not significantly asymmetric between the left and right hyperpallia ($F_{1,3397} < 0.001$, $p = 0.996$; figure 2). In contrast to this symmetry, the left and right hyperpallia showed a significant asymmetry during the recovery night ($F_{1,3149} = 344.964$, $p < 0.001$). Specifically, there was a significant asymmetry in low-frequency (1.17–4.69 Hz) power density between the left and right hyperpallia, with the stimulated hyperpallium showing greater power (figure 2). The magnitude of this low-frequency asymmetry attenuated across the recovery night (figure 2). In the last half of the recovery night, power in some faster frequencies (greater than 6.25 Hz) was also significantly asymmetrical (figure 2).

(iv) *Mesopallium contralateral to the stimulated eye*

Spectral power density in the mesopallium contralateral to the stimulated eye differed between the baseline and recovery nights ($F_{1,3397} = 380.673$, $p < 0.001$). Low-frequency (1.95–2.73 Hz) power density was significantly elevated during the first quarter of recovery sleep. Greater than *ca* 14.06 Hz activity was reduced across the recovery night (figure 3).

(v) *Mesopallium contralateral to the deprived eye*

Spectral power density likewise differed between the baseline and recovery nights in the mesopallium contralateral to the deprived eye ($F_{1,3397} = 13.661$, $p < 0.001$). The mean increase in low-frequency (1.17–3.13 Hz) activity was not significant ($p < 0.100$) during the first quarter of the recovery night, but was significant for frequencies $\leq$1.56 Hz during the second quarter (figure 3). Power density of higher frequencies (*ca* 6.25–10.55 Hz) was also significantly elevated during recovery sleep (figure 3).

(vi) *Inter-mesopallium*

Power density was not significantly different between the left and right mesopallia during baseline sleep ($F_{1,3397} = 0.142$, $p = 0.707$; figure 3). During the recovery night, however, despite a symmetric increase in less than 3 Hz power density (figure 3), the mesopallium contralateral to the stimulated eye responded differently to treatment than the mesopallium contralateral to the deprived eye ($F_{1,3397} = 215.905$, $p < 0.001$). The mesopallium contralateral to the stimulated eye showed lower 6.25–17.58 Hz power density during the first quarter; this asymmetry was no longer detected in the fourth quarter (figure 3).

(c) *Wave slopes*

(i) *Hyperpallium*

The hyperpallium showed no significant asymmetry for the up ($p = 0.275$) or down ($p = 0.197$) slopes during the first quarter of the baseline night (figure 4). During recovery sleep, however, the slope of slow waves in the stimulated hyperpallium was significantly steeper relative to baseline (up slope: $p = 0.027$, down slope: $p = 0.020$), but in the visually deprived hyperpallium, the slope of slow waves was not significantly different from baseline (up slope: $p = 0.549$, down slope: $p = 0.271$; figure 4). Consequently, there was a significant asymmetry in wave slope between the stimulated and visually deprived hyperpallia in both the up ($p = 0.006$) and down ($p = 0.017$) slopes, with waves in the stimulated hyperpallium showing steeper slopes (figure 4).

Does this asymmetry in wave slope arise simply because of asymmetries in other wave parameters? That is, waves in the stimulated hyperpallium could be steeper because waves of greater SWA, higher amplitude or shorter period might be constrained to rise and fall more quickly [7,42,46]. To address this question, we matched slope and SWA data for the stimulated and visually deprived hyperpallia. It is clear from figure 2 that the stimulated hyperpallium exhibited high SWA values not found in the visually deprived hyperpallium. Hence, we excluded the highest 20 per cent of these SWA values, effectively removing the once-significant asymmetry in SWA between the left and right hyperpallia ($p = 0.666$). Despite now showing no significant inter-hyperpallial asymmetry in SWA, a significant asymmetry persisted in the up slope ($p = 0.022$) with steeper slopes still found in the stimulated hyperpallium, although this asymmetry was no longer significant for the down slope ($p = 0.112$). Next, the inter-hyperpallial asymmetry in wave slope was associated with a similar asymmetry in slow wave amplitude ($p = 0.004$) that arose because waves in the stimulated hyperpallium were of higher amplitude relative to baseline ($p = 0.049$) and waves in the visually deprived hyperpallium were not ($p = 0.444$). By excluding the highest 20 per cent of amplitude values in the stimulated hyperpallium, we removed this asymmetry ($p = 0.446$); however, the resulting asymmetry in wave slope was only marginally significant for the up slope ($p = 0.069$) and non-significant for the down slope ($p = 0.524$). Finally, we calculated slow wave period in the stimulated and visually deprived hyperpallia during recovery sleep to see if an asymmetry in period could explain the asymmetry in slope; however, no asymmetry in period was identified ($p = 0.858$).

(ii) *Mesopallium*

Wave slopes were not significantly asymmetric between the left and right mesopallia during the first quarter of the baseline night (up slope: $p = 0.884$, down slope: $p = 0.811$; figure 4). The up ($p = 0.026$) and down ($p = 0.056$) slopes increased during recovery sleep in the mesopallium contralateral to the stimulated eye, but the mean increase in slope in the mesopallium contralateral to the deprived eye was not significantly higher than baseline for the up ($p = 0.143$) or down ($p = 0.136$) slope (figure 4). Nevertheless, wave slope was not significantly asymmetric between the left and right mesopallia during the first quarter of the recovery night (up slope: $p = 0.845$, down slope: $p = 0.898$; figure 4).

(d) *Eye state*

The proportion of SWS spent with unilateral eye opening did not differ significantly between the first quarter of the baseline and recovery nights (left eye closed/right eye open baseline mean $\pm$ s.e. = 17.99 $\pm$ 5.22%, recovery 26.32 $\pm$ 7.53%, $p = 0.238$; left eye open/right eye closed baseline 13.87 $\pm$ 6.23%, recovery 1.17 $\pm$ 0.62%, $p = 0.088$). The occurrence of bilateral eye closure during SWS likewise did not differ between the baseline and recovery nights (baseline 43.93 $\pm$ 11.02%, recovery 51.43 $\pm$ 9.83%, $p = 0.455$). Because only an increase in

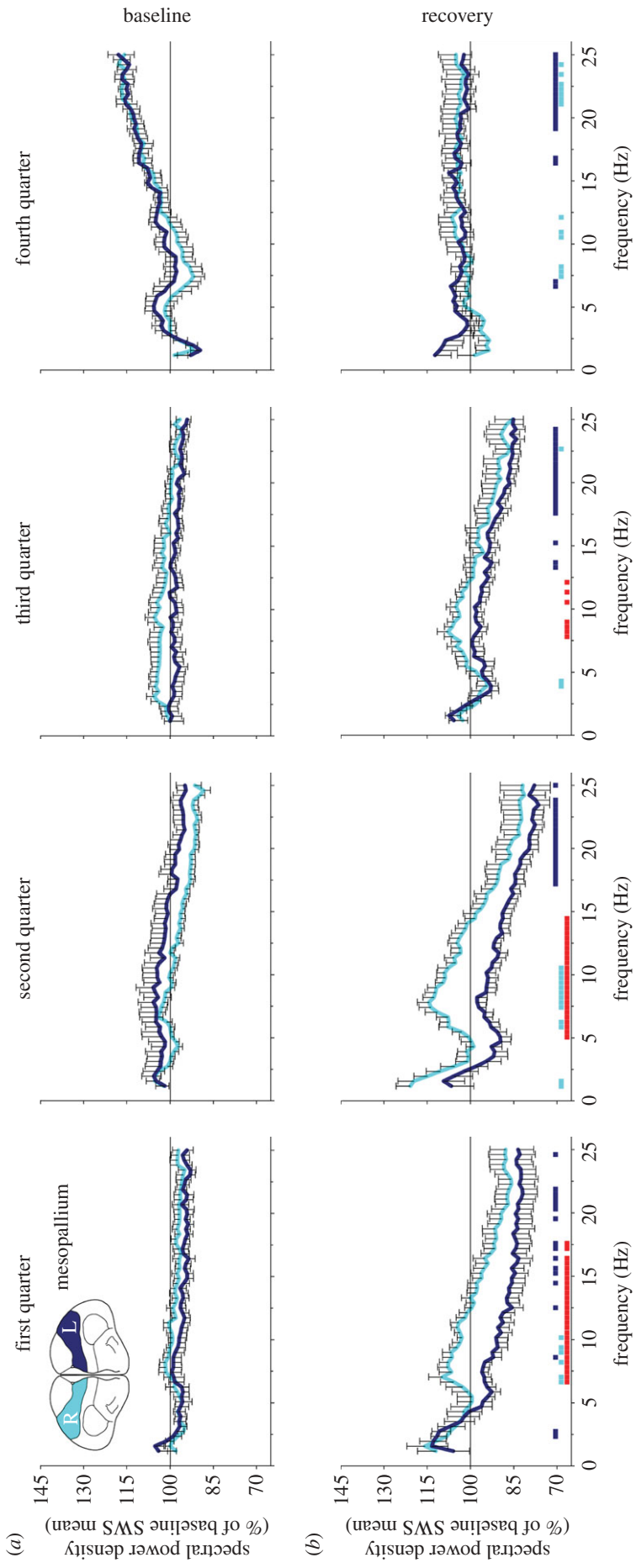

Figure 3. Spectral power density (0.78–25.00 Hz) across the four quarters of the (*a*) baseline and (*b*) recovery nights for the mesopallium contralateral to the stimulated eye (dark blue) and the visually deprived eye (light blue). Data are presented as mean ± s.e. Coloured squares at the bottom of each recovery night plot reflect a significant pairwise comparison between the baseline and recovery night of the mesopallium contralateral to the stimulated eye (dark blue) and deprived eye (light blue); red squares denote a significant asymmetry between the left (L) and right (R) mesopallia during recovery sleep. Note the broad symmetry across the baseline night, and the less than 5 Hz symmetry across the recovery night. Inset: frontal view of a transverse section through the cerebrum of a pigeon highlighting the mesopallium.

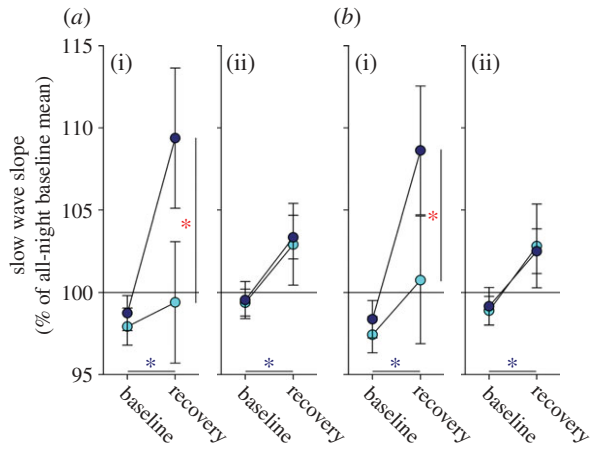

Figure 4. (*a*) Up and (*b*) down slopes of slow waves in the (i) hyper- and (ii) mesopallium contralateral to the stimulated eye (dark blue) and deprived eye (light blue) during the first quarter of the baseline and recovery night. Data are presented as mean ± s.e. Significant changes in slope between the baseline and recovery nights are marked with an asterisk (contralateral to the stimulated eye in dark blue, contralateral to the deprived eye non-significant); significant asymmetries between the left and right hemisphere for a given region are denoted by a red asterisk. Note the asymmetry between the stimulated and visually deprived hyperpallia during recovery sleep, with the stimulated hyperpallium showing steeper slopes, and the symmetric mean increase in the mesopallium.

the proportion of SWS spent with left eye open/right eye closed could influence the inter-hyperpallial asymmetry in SWA in the manner observed, this asymmetry in SWA was not owing to changes in eye state. Moreover, this suggests that eye state alone may not be a good indicator of local sleep homeostasis, at least in pigeons under these conditions [50].

4. DISCUSSION

In this study, we provide, to our knowledge, the first electrophysiological evidence for local sleep homeostasis in the avian brain. Specifically, following unilateral visual stimulation during enforced wakefulness, SWA during SWS on the recovery night was asymmetric in the hyperpallium—a primary visual processing region—with the greatest SWA observed in the hyperpallium contralateral to the stimulated eye. This inter-hyperpallial asymmetry appears to reflect a specific response to visual stimulation rather than a hemisphere-wide response, because the non-visual mesopallium showed a symmetric increase in SWA. This local effect is similar to those described in mammals [10–17]. Although many factors contribute to the local level of SWA [21], recent studies suggest that increased SWA reflects synaptic potentiation (or strengthening) accrued during prior wakefulness in a use-dependent manner (e.g. [51]). Accordingly, the SWA-related patterns identified here are mirrored by similar patterns in the slope of SWS-related slow waves, a potential marker of synaptic strength [7,42–46]. The stimulated hyperpallium showed steeper slopes than those in the visually deprived hyperpallium, independent of the level of SWA or wave period, while the mesopallium showed a symmetric increase in slope. However, the slope asymmetry in the hyperpallium was only marginally significant once the asymmetry in wave amplitude was taken into account, a finding that should be revisited with a larger sample size. In addition, other, more direct measures of synaptic potentiation (e.g. [52]) may be needed to confirm whether local increases in SWA truly reflect local potentiation in birds.

The lack of increased SWA or wave slope in the visually deprived hyperpallium during recovery sleep may reflect the absence of a net change in synaptic strength. Indeed, in mammals, SWA can decrease locally in response to disuse alone, resulting from local synaptic depression [53]. Along these lines, in an earlier study by our group using the same sleep deprivation protocol, but without unilateral visual stimulation, SWA increased symmetrically in both hemispheres [30], indicating that the reduction of visual input to the hyperpallium contralateral to the capped eye in the present study caused a reduction in SWA. SWA in mammals can also increase locally from baseline levels in response to time awake in the absence of sensory stimulation [16]. Specifically, rats subjected to enforced wakefulness and unilateral whisker removal showed increased SWA during subsequent sleep in both the left and right barrel cortex (albeit to a lesser extent in the region contralateral to the cut whiskers; [16]). Thus, the lack of change in SWA and wave slope in the visually deprived hyperpallium could reflect the competing effects of decreased visual input and increased time awake, weakening and strengthening synapses, respectively. Overall, it appears that, as in mammals, SWA increases *and decreases* locally following use and disuse, respectively, during prior wakefulness in birds.

The response of frequencies faster than the SWA bandwidth was consistent with previous studies of sleep regulation depending on the brain region considered. For instance, in the stimulated hyperpallium on the recovery night, SWS power density increased out to (at least) 14 Hz, including a dip in activity around 5–6 Hz. Such patterns are not uncommon following the sleep loss in mammals [54] and birds [29,30]. However, the reason for the increase in higher frequencies is unknown. It has been proposed that such a response may reflect frequency-independent increases in neuronal synchrony owing to a strengthening of synapses during prior wakefulness [24], but the activity in the mesopallium seemingly argues against this idea. While the low frequencies of the SWA bandwidth increased symmetrically in the mesopallium during recovery sleep, faster frequencies (6–18 Hz) were asymmetric between the left and right mesopallia, with the mesopallium contralateral to the stimulated eye showing lower activity. Although the functional significance of this asymmetry remains unclear, three points are noteworthy. First, it is highly reproducible, being present in all birds. Second, the asymmetry is caused by two divergent patterns: an increase in 6–11 Hz activity in the mesopallium contralateral to the deprived eye and a decrease in 12–18 Hz activity in the mesopallium contralateral to the stimulated eye. Third, there is no clear relationship between the magnitude of the low-frequency asymmetry in the hyperpallium and the magnitude of the high-frequency asymmetry in the mesopallium, suggesting that the two phenomena are unrelated. Future studies employing methods for measuring pallial activity with higher spatial resolution (e.g. high-density depth local field potentials or functional magnetic resonance imaging) may help elucidate the

source and function of this interesting phenomenon in pigeons, as well as the increase in frequencies faster than SWA in mammals.

Several non-mutually exclusive factors could account for our finding of local sleep homeostasis in birds. Sleep-regulatory substances, such as tumour-necrosis factor α and interleukin-1β, produced in response to waking neuronal activity, increase SWA during subsequent sleep in mammals [21] and might increase the level of SWA in birds as well. Local brain metabolism may also play a role in the use-dependent nature of SWA, as brain regions used more extensively during prior wakefulness can deplete their local stores of glycogen, increasing extracellular adenosine, which in turn may increase SWA [20,55,56]. In addition, synaptic potentiation accrued during prior waking brain use may increase neuronal synchrony, and thereby SWA, locally during subsequent sleep [24].

All of these factors may account for our results, but the latter 'synaptic homeostasis hypothesis' [24] is particularly appealing, because it provides an explicit mechanism for both the increase in SWA resulting from brain use, and the decrease in SWA with time asleep. Evidence for synaptic homeostasis in mammals and birds is as follows. In response to brain stimulation during wakefulness, synapses strengthen in mammals, based on: (i) molecular markers of potentiation [57,58], (ii) the frequency and amplitude of miniature excitatory postsynaptic potentials [52], (iii) the slope and amplitude of cortical-evoked responses [58], (iv) the slope of SWS-related slow waves [7,42–46], (v) the synchrony of transitions between up- and down-states among neurons [4], and (vi) a large-scale computer model of the thalamocortical system [59]. Synapses also appear to strengthen in a use-dependent manner in awake birds (this study), at least to the extent that slow wave slope reflects synaptic strength. Increased connectivity results in more synchronous slow oscillations during subsequent sleep ([4]; see also [43]), as evidenced by a local increase in SWA in mammals [10–17,51] and birds (this study). In mammals, such plastic changes are facilitated (in part) by the potentiating and synaptogenic action of brain-derived neurotrophic factor (BDNF) expressed during wakefulness [57,60], which increases the level of SWA during subsequent sleep [61,62]. Although it is unknown whether a similar relationship exists between BDNF and SWA in birds, the expression of other genes involved in long-term potentiation is elevated during wakefulness when compared with sleep in the forebrain of white-crowned sparrows (*Zonotrichia leucophrys gambelii*; [63]).

Under the synaptic homeostasis hypothesis, the synchrony of the slow oscillation is not only thought to reflect potentiation, but is also hypothesized to be the mechanism by which SWS reduces synaptic strength [24]. Stimulation at a frequency similar to the slow oscillation induces long-term depression [64] and so may the SWS-related burst firing of action potentials [59,65]. The neuromodulatory milieu during SWS is also conducive to depression [66] and genes involved in this process are preferentially expressed during sleep in mammals [57] and birds [63]. Regardless of the specific mechanism(s) by which SWS facilitates downscaling, several lines of evidence suggest that synapses weaken during SWS [4,7,42–46,52,58,59]. In addition to maintaining synaptic weights at an optimum level, synaptic homeostasis can also account for some of the enhancements in performance on various cognitive tasks observed in mammals post-sleep [11,12,14,15], perhaps by increasing the signal-to-noise ratio of relevant circuits [59,67]. Although recent evidence suggests that sleep plays a role in imprinting [68], auditory discrimination [69] and song learning [70–74] in birds, the role (if any) of synaptic downscaling in these processes is unknown.

If the interpretation above is correct, then slow oscillation-mediated synaptic downscaling may be a unique feature of mammalian and avian sleep. Although downscaling also occurs in sleeping *Drosophila melanogaster* [75,76], pointing to an 'ancient' origin for this sleep function, fruitflies appear to lack the mammal (or bird)-like slow oscillation during sleep [77,78], suggesting that *Drosophila* have a downscaling mechanism unrelated to synchronous low-frequency neuronal activity. Understanding the reason for the different mechanism may provide insight into whether slow oscillation-mediated downscaling serves an additional function not found in flies, or a more efficient means for downscaling in relatively complex brains, such as those of mammals and birds [28,37,48]; however, further study is needed to resolve this important issue in the evolution of sleep.

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