Report

Current Biology

White and Amber Light at Night Disrupt Sleep Physiology in Birds

Highlights

- Birds exposed to urban intensities of light at night have disrupted sleep
- In pigeons, the impacts of white and amber light at night are very similar
- In Australian magpies, sleep is more disrupted by white than amber light
- The impacts of different types of lighting on sleep may be species specific

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In Brief

Aulsebrook, Connelly et al. show that birds exposed to urban intensities of artificial light at night sleep less, sleep less intensely, and have more fragmented sleep. For pigeons, white (blue-rich) and amber (blue-reduced) lighting have similar impacts. However, sleep in Australian magpies is more disrupted by white than amber light.

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White and Amber Light at Night Disrupt Sleep Physiology in Birds

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SUMMARY

Artificial light at night can disrupt sleep in humans [1–4] and other animals [5–10]. A key mechanism for light to affect sleep is via non-visual photoreceptors that are most sensitive to short-wavelength (blue) light [11]. To minimize effects of artificial light on sleep, many electronic devices shift from white (blue-rich) to amber (blue-reduced) light in the evening. Switching outdoor lighting from white to amber might also benefit wildlife [12]. However, whether these two colors of light affect sleep similarly in different animals remains poorly understood. Here we show, by measuring brain activity, that both white and amber lighting disrupt sleep in birds but that the magnitude of these effects differs between species. When experimentally exposed to light at night at intensities typical of urban areas, domestic pigeons (*Columba livia*) and wild-caught Australian magpies (*Cracticus tibicen tyrannica*) slept less, favored non-rapid eye movement (NREM) sleep over REM sleep, slept less intensely, and had more fragmented sleep compared to when lights were switched off. In pigeons, these disruptive effects on sleep were similar for white and amber lighting. For magpies, however, amber light had less impact on sleep. Our results demonstrate that amber lighting can minimize sleep disruption in some birds but that this benefit may not be universal.

RESULTS

We examined the effects of light at night on sleep physiology through three experiments. We first determined whether exposure to white light throughout the night affected sleep and subsequent sleep recovery in pigeons (experiment 1). We then tested whether amber light was less disruptive for night-time sleep in pigeons compared to white light (experiment 2). Finally, we explored whether our pigeon results could be generalized to another urban bird by conducting a similar experiment on Australian magpies (experiment 3). To further elucidate whether effects on sleep were primarily visual (i.e., sleep was only disrupted during light exposure) or due to a more sustained physiological effect (i.e., continued disruption after lights were switched off), magpies were exposed to light at night during only the first third of the night.

In each experiment, we used a miniature data logger to record brain activity (by means of electroencephalography), muscle tone (electromyography), and head movements (tri-axial accelerometry) [13, 14]. These recordings allowed us to estimate durations of non-rapid eye movement (NREM) and REM sleep, as well as the continuity (or fragmentation) and intensity of sleep. In birds and mammals, sleep intensity is indicated by increased incidence and/or amplitude of slow waves in the electroencephalogram during NREM sleep (“slow-wave activity” [SWA]) [15].

White Light at Night Disrupts All Aspects of Sleep in Pigeons

To explore potential effects of white light at night on sleep in pigeons (n = 9), we analyzed sleep over 3 consecutive nights (experiment 1). During the first night, lights were switched off (baseline night: approximately [approx.] 0.05 lux); throughout the second night, pigeons were exposed to white light, similar in intensity to streetlighting (treatment night: approx. 18 lux; 4,190 K); and during the third night, lights were switched off again (recovery night: approx. 0.05 lux). We compared sleep architecture (amount, composition, intensity, and continuity) across the 3 12-h nights and the amount and intensity of sleep across the subsequent 3 12-h days (Table S1).

During the treatment night, all aspects of sleep architecture were disrupted (Figures 1 and 2; Table S2) and the percentage of sleep allocated to REM sleep was reduced, relative to baseline. The prolonged reduction in NREM sleep SWA was partially, but not consistently, due to increased eye opening (STAR Methods).

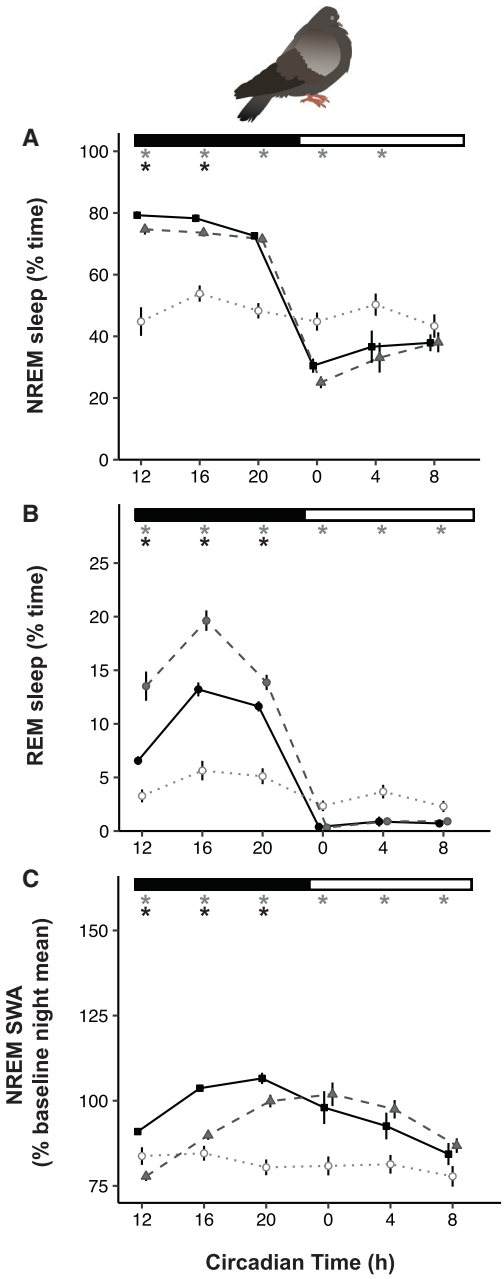

Figure 1. White Light at Night Reduces Sleep and Sleep Intensity in Pigeons

Changes in the amount of non-rapid eye movement (NREM) sleep (A), REM sleep (B), and NREM-sleep-related slow-wave activity (SWA) (C) in pigeons exposed to 12 h of white light at night (experiment 1). Plots show 4-h means (±SE) across a 24-h period for baseline (black squares, solid line), light treatment (white circles, dotted line), and recovery (gray triangles, dashed line). In (C), SWA (0.78–3.91 Hz power density) is expressed as a percentage of the entire baseline night mean. For all panels, time of day is represented as circadian time; lights were switched on/off at 0 and 12 h, respectively. The black horizontal bar at the top of each plot indicates nighttime; the white bar reflects daytime. Post hoc significant differences between the baseline and light treatment (gray asterisks) or recovery (black asterisks) are indicated at the top of each plot ($p < 0.05$; also see Table S2). Illustration is by Juliane Gaviraghi Mussoi.

Pigeons Recover Only Some Sleep after White Light Exposure

Following the treatment night in experiment 1, pigeons recovered some lost NREM and REM sleep by sleeping more the following day (Figure 1; Table S2). However, pigeons did not recover lost NREM sleep by increasing daytime sleep intensity.

During the recovery night, pigeons increased REM sleep (in absolute and relative measures) and had longer bouts of REM sleep (Figures 1 and 2; Table S2). In contrast, NREM sleep during most of the recovery night was significantly, albeit modestly, reduced, and bouts of NREM sleep were again shorter for most of the night. Furthermore, SWA continued to be lower across the recovery night. Compared to the baseline night, the SWA "curve" was also delayed, with SWA peaking early the next morning instead of during the final one-third of the night.

By the second day after the light treatment (post-recovery day, i.e., 24 h after the end of the treatment night), pigeon sleep was indistinguishable from the baseline day (Figures 1 and 2; Table S2). Surprisingly, SWA never significantly exceeded baseline values, indicating that pigeons did not recover lost NREM sleep by increasing sleep intensity. In total, birds lost 3.3 h of NREM sleep when exposed to light at night but recovered only 1.4 h during the post-treatment day and none during the subsequent night.

Pigeon Sleep Is Equally Disrupted by White and Amber Lighting

To test whether amber lighting was less disruptive for sleep in pigeons (n = 8) than white lighting, we analyzed sleep across two consecutive nights (experiment 2). During the first night, lights were switched off (baseline night: <0.02 lux). The following night, birds were exposed to either white (18.89 ± 0.67 lux; 4,190 K) or amber (17.83 ± 0.63 lux; 2,140 K) light throughout the night. After 4–6 days, we repeated the procedure with the lighting treatments reversed (birds initially exposed to white light were exposed to amber light and vice versa). The effects of white light at night were broadly consistent with the previous experiment (experiment 1), confirming that our main findings were robust. We found no evidence for a color-specific effect of light on any aspect of sleep (Table S1). Pigeons slept less, slept less intensely, and had more fragmented sleep compared with the preceding baseline night, irrespective of whether light at night was white or amber (Figures 3 and 4; Table S3).

Magpie Sleep Is More Disrupted under White Light Than Amber Light

To further explore the effects of white and amber light on avian sleep, we measured the effects of light exposure on sleep in magpies using a modified protocol (experiment 3; n = 8). Instead of exposing magpies to light throughout the night, we exposed them to light only during the first one-third of the night, which allowed us to examine sleep recovery during the same night. On the first night of this experiment, lights were switched off (baseline night: approx. 0.10 lux). The following night (treatment night), magpies were exposed to either white (9.63 ± 0.36 lux; 4,700 K) or amber light (9.63 ± 0.31 lux; 2,190 K) for the first 4 h of the night, followed by darkness for the remaining 8 h of the night (lights off; approx. 0.1 lux). After a further 48 h without light at night (recovery nights), this protocol was repeated using the

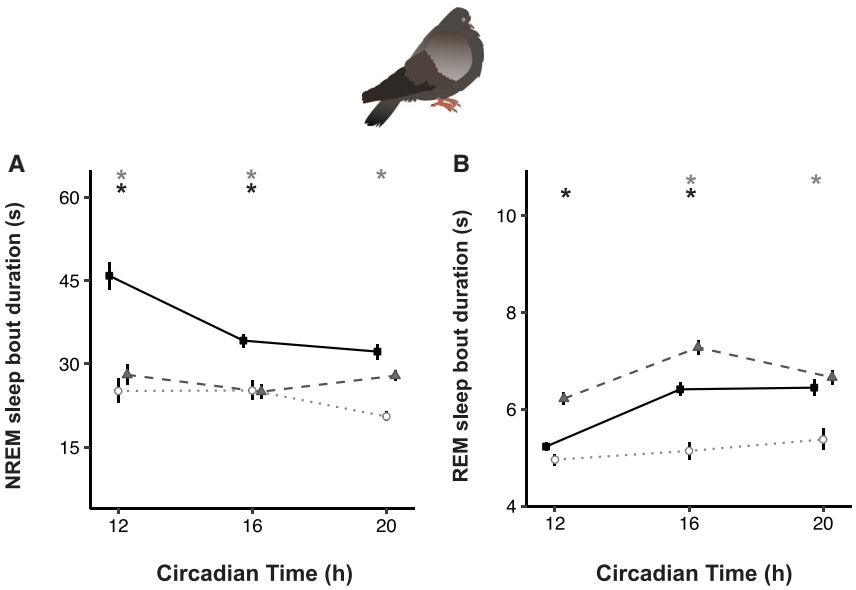

Figure 2. White Light at Night Fragments Sleep in Pigeons

Changes in mean bout duration of NREM sleep (A) and REM sleep (B) at night in pigeons exposed to 12 h of white light at night (experiment 1). Plots show 4-h means (±SE) across a 12-h period for baseline (black squares, solid line), light treatment (white circles, dotted line), and recovery (gray triangles, dashed line). Time of night is expressed as circadian time; lights were switched off at 12 h. Significant differences between the baseline and light treatment (gray asterisks) or recovery (black asterisks) are indicated at the top of each plot ($p < 0.05$; also see Table S2). Illustration is by Juliane Gaviraghi Mussoi.

other lighting treatment. Briefly, we found significant differences between nights (baseline, treatment, and recovery) in all aspects of sleep and a significant difference between days (baseline, post-treatment, and post-recovery) in NREM sleep (Table S4). Unlike pigeons, however, almost all aspects of sleep in magpies were significantly influenced by light color (white or amber; Figures 3 and 4; Table S4).

During the 4-h exposure to white and amber light, magpies had less NREM and REM sleep than during the equivalent first 4 h of the baseline night (Figure 3; Tables S5 and S6). However, magpies had half as much NREM sleep under white light compared with amber light. The loss of NREM sleep under white light in magpies was far greater than that experienced by pigeons during the first 4 h of their white light treatment (experiment 2). On average, magpies lost 76% of NREM sleep under white light, whereas pigeons lost only 44% over the equivalent time period. Under amber light, the amount of NREM sleep was more similar between the two species: magpies lost 48% of NREM sleep relative to baseline, whereas pigeons lost 37%. Magpies showed no difference in the amount of REM sleep between the two light treatments (Table S7). During exposure to both light colors, magpies had very little REM sleep; three magpies had no REM sleep under white light, and one had no REM sleep under amber light. Sleep composition also shifted under white light, but not amber light. Specifically, the percentage of sleep allocated to REM sleep during the first third of the baseline night was small (3.7% ± 0.4%) but was significantly smaller during the white light treatment night (1.5% ± 0.7%; Table S5).

In magpies, the effects of early-night light exposure on the intensity and fragmentation of sleep also depended on light color. During the 4-h exposure to white light, sleep intensity was reduced relative to baseline (Figure 4). In contrast, during the amber light exposure, sleep intensity was more variable but was no different on average to baseline. NREM sleep was more fragmented (shorter bouts) during both light exposures but was relatively more fragmented under white light than amber light. Similar to pigeons (during the first 4 h of light exposure in experiments 1 and 2), there was no difference in REM sleep bout duration for either light color, compared with the first 4 h of the baseline night.

Magpies Rapidly Recover NREM Sleep after Exposure to Light at Night

During the remaining 8 h of the treatment night, after lights had been switched off, magpies showed a rebound in NREM sleep, but not in REM sleep (experiment 3). In the 4 h immediately following exposure to white or amber light, magpies had more NREM sleep, which was also more intense and less fragmented (longer bouts; Figures 3 and 4). Unlike pigeons, REM sleep in magpies showed no such rebound, with the amount of REM sleep remaining lower than baseline levels throughout the first 4 h of darkness. Accordingly, the percentage of REM sleep (out of total sleep) was also reduced during this period. Bouts of REM sleep were also shorter in the first 4 h after the white light exposure, but not following exposure to amber light, compared with baseline. During the final 4 h of the night, magpies continued to have increased NREM sleep, but all other characteristics of sleep were indistinguishable from baseline (Tables S5 and S6).

During the subsequent (post-treatment) day and recovery night, magpie sleep was very similar to baseline and did not differ according to preceding light color (Figures 3 and 4; Tables S5–S7). The only exception was that magpies had slightly more NREM sleep during the middle of the post-treatment day after early-night exposure to white light, but not amber light. Despite losing 36 and 19 min of REM sleep during the nights of 4-h white and amber light exposures, respectively, magpies showed no recovery of REM sleep during the subsequent night or day.

DISCUSSION

Our experiments demonstrate that exposure to artificial light at night (comparable in intensity to street lighting) disrupts the amount, composition, continuity, and intensity of avian sleep. However, in magpies, sleep was less disrupted by amber light than white light and amber lighting had no effect on NREM sleep

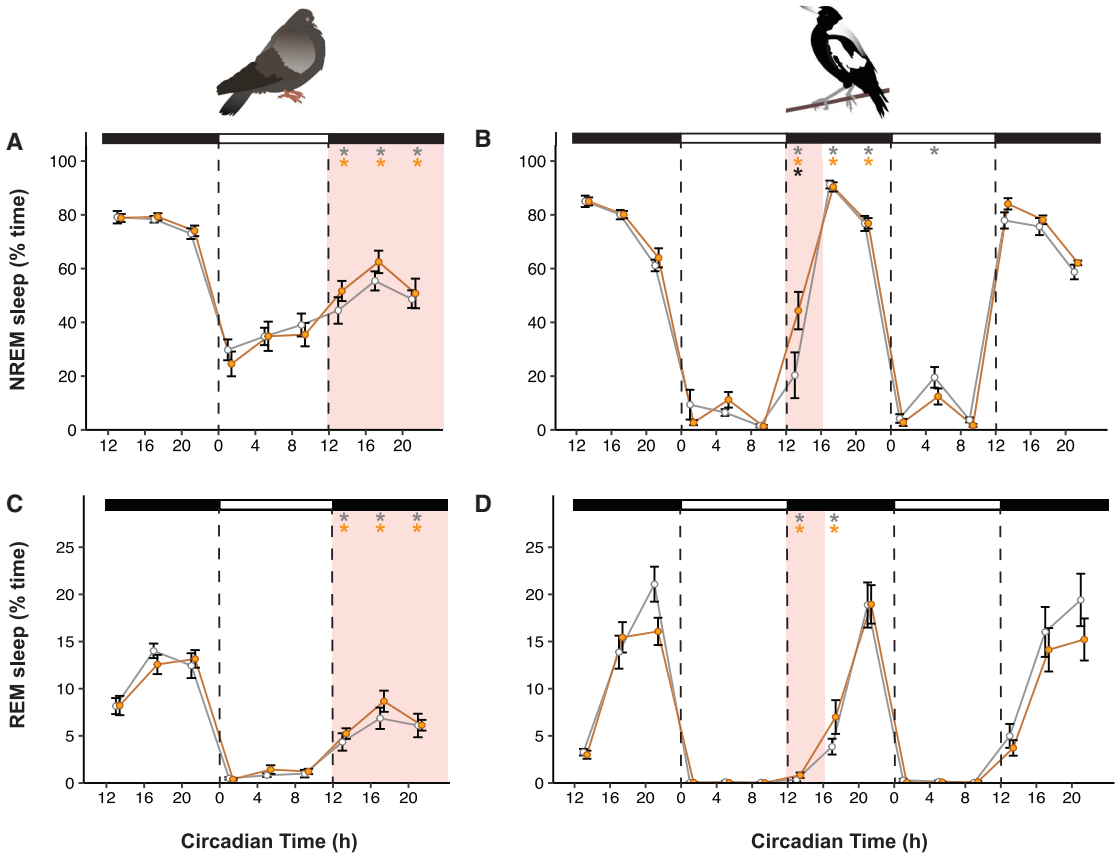

Figure 3. Pigeon Sleep Is Reduced Equally by White and Amber Light, whereas Magpie Sleep Is Affected More by White Light

Changes to the amount of NREM (A and B) and REM sleep (C and D) in response to white and amber light exposure in pigeons (12-h exposure; left) and magpies (4-h exposure; right; experiments 2 and 3). Plots show 4-h means (±SE) for the white light (white circles) and amber light (orange circles) treatments. Time of day is expressed as circadian time; lights were switched on/off at 0 and 12 h, respectively. The black horizontal bar along the top of each plot indicates nighttime; the white bar reflects daytime. The first 24 h of each recording functioned as the baseline. Red shading shows the timing and duration of the light exposure. Post hoc significant differences between the white light treatment and prior baseline (gray asterisks), amber light treatment and baseline (orange asterisks), and the white and amber light treatments (black asterisks) are indicated at the top of each plot ($p < 0.05$; also see Tables S3 and S5–S7). Illustrations are by Juliane Gaviraghi Mussoi.

intensity. In contrast, the effects of white and amber light on sleep in pigeons were similar. This divergence is unlikely to be explained by differences in light intensity or exposure duration: magpies were exposed to (slightly) less intense light at night for a shorter duration yet were more affected by the white light than pigeons. Instead, these results suggest that the relative impacts of white and amber light on sleep in birds may be species specific.

Our findings indicate that previous studies on humans, which concluded that blue-reduced light at night has less impact on sleep than blue-rich light [16–18], are applicable to some, but not all, avian species. There are at least three mutually non-exclusive explanations for this result. First, light that visually resembles natural light cues (including daylight, blue-rich twilight, or moonlight) may elicit stronger behavioral responses in some species than in others, depending on their ecology (e.g., higher risk of predation at particular times of day). In our study, the experience of captivity also may have influenced responses to light (wild-caught magpies might have perceived a greater level of threat under white light than the captive-raised pigeons). Second, varying effects of white and amber lighting may relate to inter-specific differences in visual sensitivity [19]. Unfortunately, there are limited data available to compare visual sensitivity between our two species. Third, light at night may elicit species-specific physiological responses [20]. In mice, the effects of light on circadian rhythms are mediated by cone-related pathways, perhaps providing another avenue by which species evolve different responses to lighting cues [21]. Mouland and colleagues [21] further suggested that amber lighting has a stronger effect on mammalian circadian rhythms than blue-rich light. We found no support for this idea in our two avian species.

Our study cannot identify the mechanisms by which artificial light at night affects avian sleep, but our results offer some insights. Following exposure to white light, pigeons continued to have lower NREM sleep intensity for the next 24 h. In addition, during the night after the white light exposure, the pigeons' usual increase in SWA appeared delayed. This reduction and shift in sleep intensity could indicate a physiological disruption of the homeostatic and circadian regulation of sleep, perhaps mediated by melatonin [22]. Unlike pigeons, magpies recovered NREM sleep immediately after early-night exposure to both white and amber light. One possible interpretation is that the shorter (4-h) exposure to light directly inhibited sleep in magpies,

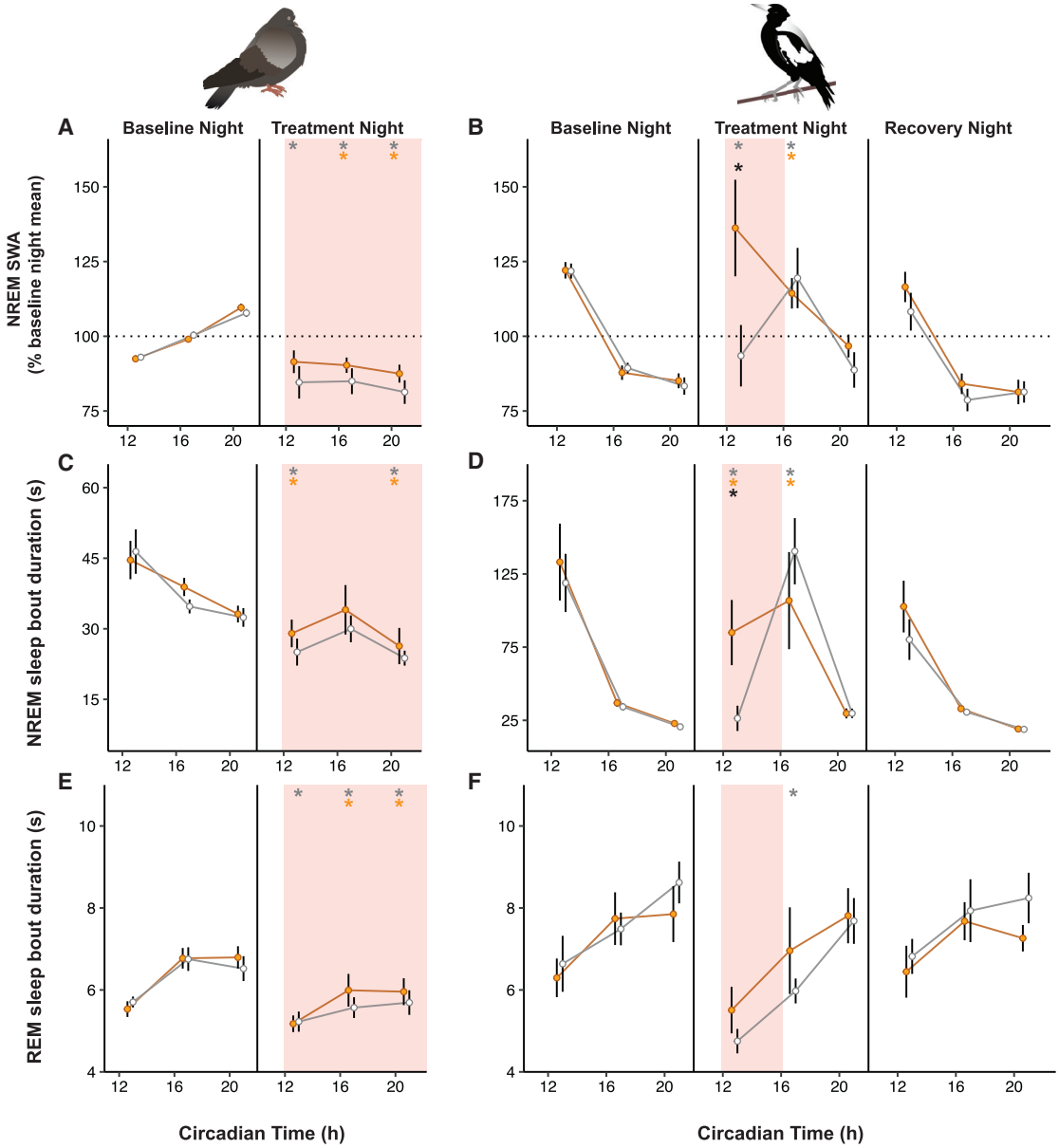

Figure 4. White and Amber Light Reduce Sleep Intensity and Bout Duration Equally in Pigeons, whereas White Light Has Greater Impact on Magpies

Changes in NREM-sleep-related SWA (A and B) and mean bout durations of NREM sleep (C and D) and REM sleep (E and F) at night in response to white and amber light exposure (treatment night) in pigeons (12-h exposure; left) and magpies (4-h exposure; right; experiments 2 and 3). Lights were off during the baseline and recovery nights. Plots show 4-h means (±SE) for the white light (white circles) and amber light (orange circles) treatments. SWA (0.78–3.91 Hz power density) is expressed as a percentage of the entire baseline night mean (the 100% dashed line). Time of day is expressed as circadian time; lights were switched off at 12 h. Red shading shows the timing and duration of the light exposure. Post hoc significant differences between the white light treatment and prior baseline (gray asterisks), amber light treatment and baseline (orange asterisks), and the white and amber light treatments (black asterisks) are indicated at the top of each plot ($p < 0.05$; also see Tables S3 and S5–S7). Illustrations are by Juliane Gaviraghi Mussoi.

so magpies could recover NREM sleep as soon as lights were switched off. This would suggest that both white and amber light had a primarily visual effect on the magpies rather than a sustained physiological effect.

In pigeons, the absence of a compensatory increase in NREM sleep intensity following sleep loss differs from previous studies [23, 24]. However, we did find a rebound in REM sleep following sleep loss, reflecting REM sleep homeostasis [23, 25, 26]. In contrast, magpies did show recovery of NREM sleep after early-night light exposure (i.e., increased NREM sleep amount, intensity, and continuity) but no rebound in REM sleep. Furthermore, REM sleep remained reduced immediately after the light exposure. Because magpies spent 90% of the time immediately after the light exposure in NREM sleep, they may have

physiologically prioritized recovery of NREM sleep at the expense of REM sleep. In any case, the absence of a robust REM sleep rebound in magpies is inconsistent with prior research [23, 25, 26], although a similar result has recently been reported in starlings (*Sturnus vulgaris*) [27] and northern fur seals (*Callorhinus ursinus*) [28]. It remains unclear why REM sleep homeostasis is observed in some situations, but not others.

Our experiments demonstrate that realistic intensities of urban light at night can disrupt sleep in birds. Importantly, many of these effects—including the distinct effects of white and amber light on sleep in magpies—would have been impossible to detect by measuring only the total amount of sleep or sleep behavior, highlighting the necessity of electrophysiologically based measures of sleep [29]. It should be noted that we investigated the effects of short-term light exposure in a context where birds could not avoid light at night. Thus, we were unable to determine whether birds habituate to light at night or would otherwise avoid intensely lit areas if afforded the opportunity [30, 31]. Nevertheless, the less-disruptive effects of amber lighting on sleep in magpies, but not pigeons, within a very similar experimental environment emphasize the need for research beyond single "model" bird species (see also [32] for a similar argument in reptiles). It is therefore imperative we study sleep across a richer diversity of species to better understand how artificial light at night, and other anthropogenic changes, impact wildlife.

STAR★METHODS

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

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2020.06.085.

A video abstract is available at https://doi.org/10.1016/j.cub.2020.06.085#mmc3.

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AUTHOR CONTRIBUTIONS

A.E.A., F.C., J.A.L., R.D.J., R.A.M., and T.M.J. conceived and designed the experiments. A.E.A., F.C., and R.D.J. collected and analyzed the data with the assistance of J.A.L., M.L.H., T.M.J., and those mentioned in the acknowledgments. J.A.L. conducted surgeries to implant electrodes with the assistance of A.E.A., F.C., and R.D.J. A.L.V. supplied the hardware and software for recording sleep physiology. A.E.A. and F.C. wrote the manuscript with input from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Analyzed data	This paper; Mendeley Data	Mendeley Data: https://doi.org/10.17632/tzjzkyrrvh.1
Experimental Models: Organisms/Strains		
Pigeon (domestic)	Local breeders	N/A
Australian magpie (wild)	Wild-caught	N/A
Software and Algorithms		
Machine-learning algorithm Somnivore	[33]	https://doi.org/10.3389/fnins.2019.00207
RemLogic v. 3.4.4	Embla Systems, United States	https://neuro.natus.com/products-services/embla-remlogic-software
R version 3.6.0	[34]	https://www.R-project.org/; RRID: SCR_001905
lme4	[35]	https://cran.r-project.org/package=lme4; RRID: SCR_015654
lmerTest	[36]	https://CRAN.R-project.org/package=lmerTest; RRID: SCR_015656
emmeans	[37]	https://CRAN.R-project.org/package=emmeans; RRID: SCR_018734
Other		
Daytime lighting sensor	UPRTek, Taiwan	MK350 LED Meter
Night-time (dim) lighting sensor	Skye Instruments, United Kingdom	SKL 30046473 light meter with sensor SKL 310L 46472
Neurologger 2A	[13]	http://www.vyssotski.ch/neurologger2
White LED streetlights	Ruud Lighting, United States	IP66 LEDway SLM Streetlight
White LED work lights	Iron Horse Industries LLC, Unites States	IronHorse 50W LED Worklight
Amber filters (770 Burnt Yellow filter)	Lee Filters, United Kingdom	http://www.leefilters.com/lighting/colour-details.html#770
White diffusion filters (416 Three Quarter White Diffusion filters)	Lee Filters, United Kingdom	http://www.leefilters.com/lighting/colour-details.html#416&filter=tf

RESOURCE AVAILABILITY

Lead Contact

Requests for further information and resources should be directed to, and will be fulfilled by, the Lead Contact, Anne Aulsebrook (aulsebrooka@gmail.com).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

Analyzed data and R-scripts generated during this study have been deposited to Mendeley Data: https://doi.org/10.17632/tzjzkyrrvh.1. The raw datasets have not been deposited in a public repository because the files are exceedingly large, but are available from the corresponding authors on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Domestic Pigeons

In August 2016, 10 adult domestic pigeons (*Columba livia*; six males and four females, genetically sexed) were sourced from local breeders. We ultimately obtained data from nine pigeons (*Experiment 1*: five males and four females; *Experiment 2*: four males and four females; see Experimental design). Pigeons were healthy and their mean weight was 356 g (range: 285 - 540 g).

Pigeons were transported to an indoor aviary facility at La Trobe University (Bundoora, Melbourne, Australia), where they were housed individually in standard wire mesh aviaries (1.8 high × 0.9 wide × 1.8 m deep). Each aviary had a wooden-dowel perch located near the top of the enclosure. To record and monitor pigeon behavior (including eye state), video surveillance cameras with an infra-red illuminator were positioned on either side of the perch. Water, shell grit, and food pellets were provided *ad libitum* and replaced each morning. Pigeons were acclimated to a 12:12 light/dark cycle for at least two weeks prior to any surgical or experimental procedures.

When investigating effects of white light at night on sleep and sleep recovery (*Experiment 1*), pigeons were housed in a single, large room and experienced fluctuating temperatures that varied with outdoor temperature (mean ± SD during the day: 18.6 ± 2.1°C, range 12.5 - 23.5°C; night 16.0 ± 2.2°C, range 11.0 - 21.0°C). Light during the light phase (0720 - 1920 AEST) was provided by ceiling lamps and white LED work lights positioned above each aviary (mean light intensity at perch height ± SE: 3695 ± 103 lux, measured using daytime lighting sensor). During the dark phase (1920 - 0720 AEST), a small amount of light entered the facility from external sources (approx. 0.05 lux), mimicking naturally 'dark' conditions. When comparing effects of white and amber light on night-time sleep (*Experiment 2*) pigeons were moved to temperature-controlled rooms (21°C), due to increasing ambient temperatures. Four birds were kept in each of the first two rooms; two birds in a third room were not used. Light during the light phase (0700 - 1900 AEST) was provided by the same lights as *Experiment 1.* Light intensity during the dark phase (1900 - 0700 AEST) was < 0.02 lux.

Pigeons were not involved in any other research procedures prior to this study. All methods were approved by the La Trobe University Animal Ethics Committee (AEC16-30.4).

Australian Magpies

In January 2019, 12 wild adult Australian magpies (*Cracticus tibicen tyrannica*; six males and six females, sexed based on plumage) were captured in Melbourne, Victoria (Australia), using a walk-in trap baited with grated cheese. We ultimately obtained data from eight magpies (four males and four females; see Experimental design). Magpies were healthy and their mean weight was 345 g (range: 302 - 408 g).

Magpies were transported to an indoor aviary facility at La Trobe University, where they were individually housed in identical aviaries to those used for the pigeons. Each aviary contained one high and two low perches. The birds typically slept on the high perch. To record and monitor magpie behavior, one video camera with infrared capabilities was positioned at one end of the high perch, while a second camera was mounted on the aviary door to monitor the lower perches and the floor of the aviary. Water and food were provided *ad libitum.* Magpies were fed a mixture of minced meat and an insectivore mix (55 g; Wombaroo Food Products, Australia), which was replaced each morning. Aviary floors were covered in woodchips, and to provide enrichment, 15 - 20 mealworms were scattered throughout the woodchips each day. Magpies were acclimated to a 12:12 light/dark cycle for at least two weeks prior to surgical and experimental procedures.

Throughout the study (*Experiment 3*), magpies were housed in two experimental rooms with similar configurations (three males and three females in each room). Rooms were temperature controlled (22°C) and insulated from all external light. During the light phase (0600 - 1800 AEST), light was provided by ceiling lamps (mean light intensity at high perch ± SE: 153 ± 18 lux). During the dark phase (1800 h - 0600 h), a night light was used to mimic the intensity of moonlight (approx. 0.1 lux at the high perch), which also allowed magpies to move around safely at night.

Prior to this study, all magpies had been part of research investigating the effects of urban noise pollution on sleep and cognition (unpublished data) and short-term sleep deprivation; seven weeks and one week prior to the studies presented herein, respectively. All methods were approved by the La Trobe University Animal Ethics Committee (AEC18034). Birds were captured and released with permission from the Department of Environment, Land, Water and Planning (permit number: 10008264) and the Australian Bird and Bat Banding Scheme (ABBBS number 1405).

METHOD DETAILS

Experimental design

We used a repeated-measures design to investigate whether exposure to white and/or amber light at night affected avian sleep.

To investigate potential impacts of white light at night on sleep and sleep homeostasis in pigeons (*Experiment 1;* October - November 2016), sleep was analyzed across three consecutive nights: one night of baseline (lights off; approx. 0.05 lux, measured using night-time lighting sensor), one night of light treatment (white lights on; 18.08 ± 0.79 lux, 4190 K), and one night of recovery (lights off; approx. 0.05 lux). Light intensity during the daytime was the same for all treatments (approx. 3700 lux). We retrieved the sleep data loggers (see Recording Sleep) on the night after the recovery night, immediately after lights-out. As some initial recordings were unsuccessful, the experiment was repeated over four blocks, with at least five nights between light exposures. Data were successfully collected from nine birds (five males and four females). Data collection from the remaining male was unsuccessful owing to a damaged connector (see Recording Sleep).

Room temperature did not differ substantially between baseline, light treatment and recovery nights. We found no significant difference in temperature between the baseline (mean ± SD: 16.5 ± 2.2°C) and light treatment nights (16.6 ± 2.2°C; Welch Two Sample t test: $t = 0.37$, $df = 142$, $p = 0.705$). During the recovery nights, the average temperature was slightly cooler (14.9 ± 1.7°C), compared with baseline ($t = 4.76$, $df = 135$, $p < 0.001$) and light treatment nights ($t = 5.15$, $df = 134$, $p < 0.001$). However, this difference was small

(< 2°C) and unlikely to have had a substantial influence on night-time sleep. Furthermore, the results from *Experiment 1* were almost entirely consistent with the results of *Experiment 2,* when pigeons were kept in temperature-controlled rooms (see below).

To assess whether blue-reduced night-time lighting was less disruptive for sleep in pigeons (*Experiment 2;* January - February 2017), we conducted additional recordings to compare the effects of exposure to white (blue-rich; 18.89 ± 0.67 lux, 4190 K) and amber (blue-reduced; 17.83 ± 0.63 lux, 2140 K) light at night. Daytime light intensities were the same for all treatments and consistent with *Experiment 1* (approx. 3700 lux). Sleep was analyzed across two consecutive nights: baseline (< 0.02 lux) and light treatment (see above). Loggers were retrieved in the morning after the light at night treatment, which meant we did not record recovery sleep. On the light treatment night, birds in one room were exposed to white light, while birds in the other room were exposed to amber light. The procedure was then repeated with the lighting treatments reversed, such that birds initially exposed to white light were exposed to amber light and vice versa (with 4 - 6 days between light treatments). Light intensity did not differ between rooms for either light treatment (paired t test; white: $t = -0.66$, $df = 3$, $p = 0.556$; amber: $t = -1.60$, $df = 3$, $p = 0.207$). As with the previous experiment, this experiment was repeated over four blocks (i.e., two exposures to each light color), as some initial recordings were unsuccessful. Data were collected successfully from eight birds (four males and four females), with light treatments balanced between rooms and dates.

To investigate the effects of early-night white and amber lighting on magpie sleep (*Experiment 3;* June - July 2019) we analyzed sleep over an 8-day period. The first 24-h period (starting at lights-out) functioned as the first baseline. The magpies were then subjected to an early-night lighting treatment. This treatment consisted of 4 h of white light (9.6 lux, 4700 K; Figure S1) or amber light (9.6 lux, 2190 K) at the beginning of the night (1800 - 2200 h), followed by 8 h of darkness (lights off; approx. 0.1 lux), after which the night ended. The birds then had 48 h of recovery, under conditions identical to the initial baseline. This procedure (baseline, treatment, recovery) was then repeated with the other light treatment color. Four birds were exposed to the white treatment first, and four birds were exposed to the reverse (amber then white). Data were successfully collected from eight magpies. Six of these magpies (four males and two females) were recorded under both light treatments; two magpies (females) lost their loggers before completing the second light treatment, meaning that we obtained complete data from these birds from only one light treatment (white and amber, respectively).

Artificial light at night

For pigeons, artificial light at night was provided by white LED streetlights angled toward the ceiling to simulate ambient lighting. Amber light was produced by covering lights with amber filters that produced a warmer color temperature (2140 K; see Key Resources Table) and almost completely suppressed emission of blue wavelengths (Figure S1). As these filters also reduced light intensity, additional amber lights were used during the amber light treatment, to produce similar night-time light intensities for white and amber light treatments.

For magpies, artificial light at night was provided by a single white LED work light in the center of each room, which was projected upward. Filters were fitted to each light to produce the desired wavelengths and light intensity (Figure S1). For white lighting, the lights were fitted with five white diffusion filters (see Key Resources Table). For amber lighting, the lights were fitted with the same amber filter as for the pigeon experiments, in addition to two white diffusion filters, to match the intensity of the white lighting.

The light intensities used for our experiments were based on measurements recorded near street lights in urban parks, where birds have been observed roosting and nesting (wild magpie nests in Melbourne, Australia: range: 0.06 - 19.7 lux). While lux is based on human spectral sensitivities and thus does not necessarily represent what a bird perceives, human perceptions of illumination are typically given precedence when designing and planning lighting [38]. We therefore chose to measure light intensity in lux so that results from this research can be more easily transferred to real-world management contexts.

Recording sleep

To record sleep, we implanted birds with electroencephalogram (EEG) and electromyogram (EMG) electrodes [24]. Briefly, birds were anesthetized with isoflurane (induction at 4%–5%, maintenance at 1%–3%, vaporized in 100% oxygen) and mounted in a stereotax over a heating pad. The EEG electrodes consisted of medical grade electrode wire (AS633 electrode wire, Cooner Wire, United States) soldered to gold-plated round-tipped pins (0.5 mm diameter). Electrode pins were positioned on the dura (membrane overlying the brain) by first drilling holes (0.5 mm diameter) through the cranium. In pigeons, two electrodes were placed over the left hyperpallia and two over the right hyperpallia, and a fifth electrode was placed over the left hemisphere for the ground. The hyperpallium is visible through the pigeon skull as a pink oval. In magpies, four electrodes were placed over the right hemisphere, over each of four brain areas (hyperpallium, mesopallium, nidopallium caudolaterale (NCL), and cerebellum; the latter used as the reference), and a fifth electrode was placed over the left hemisphere for the ground. The electrode over the NCL may actually have been located on the *area parahippocampalis* - a thin region of the hippocampal complex overlying the NCL. This arrangement of electrodes in magpies was selected to meet the needs of a separate research project, investigating effects of sleep deprivation on different brain areas. Electrode position in magpies was estimated based on (i) our experience with other birds [24, 39–41], (ii) a brain atlas for a corvid (jungle crow, *Corvus macrorhynchos*) [42], a similarly sized passerine, and (iii) prior examination of the brain of a dead Australian magpie and pied currawong (*Strepera graculina*; belonging to the magpie family, Artamidae).

The EMG electrodes for both pigeons and magpies consisted of electrode wire laid upon the nuchal (neck) muscle. All seven wires had been previously soldered to a small connector (to which the data logger would later connect), which was then fixed to the top of the bird's head using dental acrylic (Paladur dental acrylic, Kulzer, Germany).

We gave the birds at least two weeks of post-operative recovery in their aviary prior to recording data. During the final nine days of their post-operative recovery, pigeons wore a dummy data logger on their head that matched the weight, shape, and size of a real data logger (25 × 15 × 15 mm; 6.0 g). This dummy logger habituated the pigeons to wearing a data logger. Pigeons also wore this mock logger between experimental recordings. We did not give magpies a mock logger, as they were less accustomed to being handled than pigeons; we therefore minimized handling to minimize potential stress. Nonetheless, after connecting a real data logger, all birds were given at least 24 h to habituate before commencing data collection.

To record the EEG and EMG, we captured birds by hand and connected an EEG/EMG data logger (Neurologger 2A) [13] powered by two zinc air batteries (ZA675 1.4V, Renata, Switzerland). The logger also included an inbuilt tri-axial accelerometer that measured accelerations of the head. The combined weight of the logger and batteries was approximately 6 g. The logger was configured to continuously record the EEG, EMG, and head acceleration at 100 Hz. We wrapped the logger and batteries in kinetic thread seal tape to protect them from moisture and physical damage.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analyzing sleep

To assess the effects of artificial light at night on sleep, we used the supervised machine-learning algorithm Somnivore [33] to score wakefulness, non-rapid eye movement (NREM) and REM sleep in 4-s epochs, such that each 24-h day had 21600 scored epochs. Somnivore has been validated previously for use with pigeons [33] and also shows high agreement with magpie manual scoring (92.5 ± 2.4%; unpublished data). A single scorer (pigeons: AEA, magpies: RDJ) first 'trained' Somnivore for each sleep recording by scoring a minimum of 150 epochs of each state, dispersed over the 24-h day. Wakefulness was characterized by EEG activation (fast, low-amplitude waves of brain activity) accompanied by head movements (seen from the accelerometer). NREM sleep was identified by slow, high amplitude waves in the EEG, accompanied by quiescent behavior. REM sleep was characterized by similar EEG activation to wakefulness, but without movement, or only very slow head movements (e.g., head drooping forward indicative of relaxed muscle tone), and a decrease, or no sharp increase, in the EMG from preceding NREM sleep. Epochs containing multiple states were scored as the state that formed the majority of the epoch. When these 150 epochs of each state had been scored, the machine-learning function of Somnivore was used to score all remaining epochs. The resulting scores were visually scanned to ensure that there were no systematic errors in the automated scoring (e.g., lower amplitude NREM sleep being classified as wake or REM sleep).

To calculate slow wave activity (SWA), we performed fast Fourier transforms on epochs in 0.39 Hz bins using RemLogic v. 3.4.4 (Embla Systems, Pleasanton, United States). SWA was calculated for each third (4 h) of the day and night, then expressed as a percentage of the mean SWA during NREM sleep across the entire baseline night.

Analyzing eye state

During NREM sleep, pigeons can keep one or both eyes open, which is associated with lower SWA in the hemisphere contralateral to the open eye [43]. To determine whether potential differences in SWA between light treatments could be explained by eye opening, we used a similar protocol to Lesku et al. [24] and examined instantaneous eye state every 2 min during NREM sleep, for the first and last quarter of each night, using video recordings. For both experiments, one pigeon was excluded from the analysis for the first quarter of the night because its eyes could very rarely be seen during one or more nights (i.e., < 10 instances when at least one eye was visible in the video).

Although increased eye openings during NREM sleep can partly explain reduced sleep intensity under white light (Experiment 1), there was very little association between SWA and eye openings during subsequent recovery, or during Experiment 2. In Experiment 1, pigeons in NREM sleep during the treatment night spent more time with at least one eye open at the first (baseline: mean ± SE = 25.4 ± 9.3%; light treatment: 52.3 ± 5.9%; $t = 3.27$, $df = 7$, $p = 0.014$) and final quarter of the night (baseline: 28.9 ± 6.8%; light treatment: 48.4 ± 6.0%; $t = 2.51$, $df = 8$, $p = 0.036$), compared with the baseline night. Pigeons in NREM sleep also spent marginally more time with at least one eye open in the first quarter of the recovery night (53.2 ± 14.1%; $t = 2.31$, $df = 7$, $p = 0.054$) but not the final quarter (33.0 ± 8.7%; $t = 0.83$, $df = 8$, $p = 0.433$), compared with the baseline night. In Experiment 2, time spent with at least one eye open during NREM sleep did not differ between the baseline and light treatment nights, for the first quarter of the white light treatment (baseline: 54.3 ± 12.3%; light treatment: 53.9 ± 8.3%; $t = -0.03$, $df = 6$, $p = 0.978$), final quarter of the white light treatment (baseline: 36.1 ± 9.4%; light treatment: 45.6 ± 9.9%; $t = -0.38$, $df = 7$, $p = 0.376$), first quarter of the amber light treatment (baseline: 64.1 ± 9.9%; light treatment: 56.6 ± 6.9%; $t = -0.81$, $df = 6$, $p = 0.448$), or final quarter of the amber light treatment (baseline: 50.4 ± 9.0%; light treatment: 43.6 ± 11.2%; $t = -1.15$, $df = 7$, $p = 0.288$). Consequently, eye opening alone cannot account for reduced sleep intensity during, or after, exposure to light at night in pigeons.

As magpies typically sleep with their eyes hidden by their feathers, we were unable to examine whether decreased NREM sleep intensity was associated with increased eye openings in these birds. In any case, sleeping with obscured eyes would (if anything) reduce the amount of light reaching the eyes, potentially reducing effects of light at night on magpie sleep. The influence of sleeping posture on sleep architecture would be worth exploring in future research.

Statistical analysis

We conducted all analyses in the statistical environment R version 3.6.0 [34]. We used linear mixed effects models to investigate effects of light at night on the amount of each state (wakefulness, NREM, and REM sleep), percentage of total sleep composed of REM

sleep, and SWA during NREM sleep, as well as mean duration of NREM and REM sleep bouts at night (calculated as the overall mean of the mean bout length for each bird). Daytime and night-time data were modeled separately for each experiment, with *day/night*, time of night (*third*), and an interaction term between *day/night* and *third* as categorical fixed effects. For experiments that compared light colors (white and amber), we also included *light color* as a fixed effect, as well as a three-way interaction term between *day/night*, *third*, and *light color*. *Bird identity* was included as a random effect in all models to account for repeated-measures. Analyses indicated very little effect of light at night on the post-treatment day in magpies, and no effect on the recovery night or subsequent recovery day; we therefore chose to exclude the recovery day from figures.

Models were fitted using the package *lme4* [35]. We used the package *lmerTest* to calculate degrees of freedom (Satterthwaite's method) and p values [36]. Dependent variables were transformed [log (x+1)] to meet assumptions for model residuals, assessed by visually inspecting model residuals. For models of the percentage of night-time NREM sleep, variance in model residuals decreased with the mean; we therefore modeled the log transformation of the inverse [log (100-%NREM)], then inversed output values for interpretation. We used a type 3 analysis of variance (ANOVA) to test for overall effects of fixed factors or interactions in the models (Tables S1 and S4). Where there was evidence for an overall effect, we conducted post hoc comparisons using the *emmeans* package (Figures 1, 2, 3, and 4; Tables S2, S3, and S5–S7) [37]. For post hoc comparisons, we focused exclusively on two types of comparisons: (1) comparisons with baseline, where each period of the day/night was compared with the equivalent period (matched by circadian time) of the preceding baseline day/night, and (2) comparisons between equivalent periods of the white and amber light treatments (for *Experiments 2* and *3*). To control for the false discovery rate in these post hoc analyses, we adjusted p values across each experiment using a Benjamini-Hochberg correction [44, 45]. There were no significant differences between the white and amber baseline periods for *Experiments 2* or 3 (Tables S3 and S7). We also analyzed all comparisons using paired Wilcoxon signed rank tests and results were qualitatively the same (values not shown), indicating that our results are robust to the type of analysis used. To test for differences in eye opening during NREM sleep in pigeons, we used paired t tests (see 'Analyzing eye state').