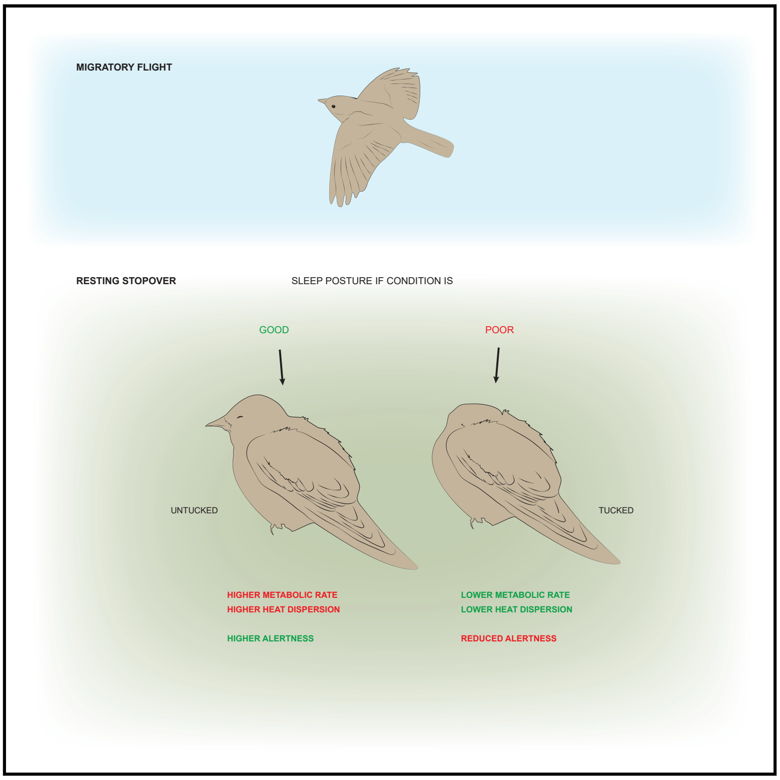
Report

Current Biology

Sleeping Unsafely Tucked in to Conserve Energy in a Nocturnal Migratory Songbird

Graphical Abstract



Highlights

- In migratory warblers, sleep pattern and posture reflect condition
- Birds that sleep with the head tucked under the feathers reduce energy consumption
- Birds that sleep tucked react slower to noise of potential predators
- Migratory warblers adjust energy saving and predation risk depending on condition

Authors

Andrea Ferretti, Niels C. Rattenborg, Thomas Ruf, Scott R. McWilliams, Massimiliano Cardinale, Leonida Fusani

Correspondence

andrea.ferretti1989@gmail.com

In Brief

Ferretti et al. show that migratory warblers sleep mostly with the head tucked under the feathers when they are in poor condition. This posture is associated with a lower energy consumption and may allow a faster recovery compared to sleeping untucked, but it exposes birds to a higher risk of predation.

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Report

Sleeping Unsafely Tucked in to Conserve Energy in a Nocturnal Migratory Songbird

Andrea Ferretti,[1,2,7,*] Niels C. Rattenborg,[3] Thomas Ruf,[4] Scott R. McWilliams,[5] Massimiliano Cardinale,[6] and Leonida Fusani[1,2]
[1]University of Vienna, Department of Cognitive Biology, Althanstr. 14, 1090 Vienna, Austria
[2]University of Veterinary Medicine, Vienna, Department of Interdisciplinary Life Sciences, Konrad Lorenz Institute of Ethology, Savoyenstr. 1, Vienna 1160, Austria
[3]Max Planck Institute for Ornithology, Avian Sleep Group, Haus 5, Seewiesen 82319, Germany
[4]University of Veterinary Medicine Vienna, Department of Interdisciplinary Life Sciences, Research Institute of Wildlife Ecology, Savoyenstr. 1, Vienna 1160, Austria
[5]University of Rhode Island, Department of Natural Resources Science, 102 Coastal Institute, Kingston, RI02881, USA
[6]Swedish University of Agricultural Sciences, Department of Aquatic Resources, Marine Research Institute, Turistgatan 5, Lysekil 45330, Sweden
[7]Lead Contact
*Correspondence: andrea.ferretti1989@gmail.com
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SUMMARY

Each spring and fall, millions of normally diurnal birds switch to migrating at night. Most of these are small songbirds (passerine) migrating long distances that need to alternate their migratory flights with refueling stopovers [1, 2], which can account for up to 80% of the total migratory period [3]. After a long nocturnal flight, these birds face the contrasting needs to recover sleep and refill depleted energy stores, all while vulnerable to predation [4, 5]. Here, we investigated how garden warblers at a Mediterranean stopover site modulate their sleep behavior in relation to their metabolic state. At night, garden warblers in poor metabolic condition sleep more and exhibit less migratory restlessness than birds in good condition do. In addition, rather than sleeping with their head facing forward, birds in poor condition prefer to sleep with their head turned and tucked in their feathers. We further show that sleep with the head tucked is associated with lower respiratory and metabolic rates and reduced heat loss mediated by hiding the head—the body part with the highest heat dissipation—under the feathers. However, the benefit of conserving energy while sleeping with the head tucked was countered by reduced anti-predator vigilance. Birds presented with a sound simulating the approach of a predator responded more slowly when the head was tucked than when it was untucked. Consequently, our study demonstrates that through changing their sleep position and intensity, migrating songbirds can negotiate a previously unknown trade-off between sleep-mediated energy conservation and anti-predatory vigilance.

RESULTS AND DISCUSSION

The highest mortality in migratory birds occurs during migration [4, 5]. Most songbirds are diurnal but perform their migratory flights at night, when the risk of predation is reduced and weather conditions are more favorable [6, 7]. Small passerine birds that migrate from their African wintering quarters to breeding areas in Europe cannot complete their journey in one leg because they cannot carry sufficient energy stores for the entire distance [1, 2]. These species thus make stopovers during which they rest and forage to restore their energy reserves. Particularly in spring, birds need to reach their breeding sites as early as environmental conditions allow because their reproductive success is strongly linked to timing of arrival [8]. Therefore, each stay at a stopover site should be as short as is required to recover from the preceding flight and to rebuild depleted energy stores according to the specific migratory strategy [9, 10].

Among the major constraints in reducing stopover duration is the need to rest and sleep. Sleep deprivation may lead to serious consequences such as deficits in cognitive performance and memory consolidation [11–16] and in the worst cases might lead to death [17–20]. Some bird species show an extended reduction of sleep when experiencing challenging situations, such as during endurance-foraging flights [21], breeding [22], or migratory periods [23]. Sleep deprivation, experienced during periods of nocturnal restlessness, did not affect cognitive abilities of captive passerine migrants with food provided *ad libitum* [23], yet how wild migrants balance sleep and foraging needs *en route* remains unknown. One possibility is that they sleep on the wing at night [24]. However, in the only study to demonstrate sleep in flight, great frigatebirds (*Fregata minor*) slept during soaring, but not flapping, flight [21]. As most passerine species flap their wings almost continuously during their flights [25], sleeping in flight seems to be unlikely in these species. Indeed, field observations suggested that migratory songbirds need to stop over and sleep even when they have enough energy stores to continue their migration [26, 27].

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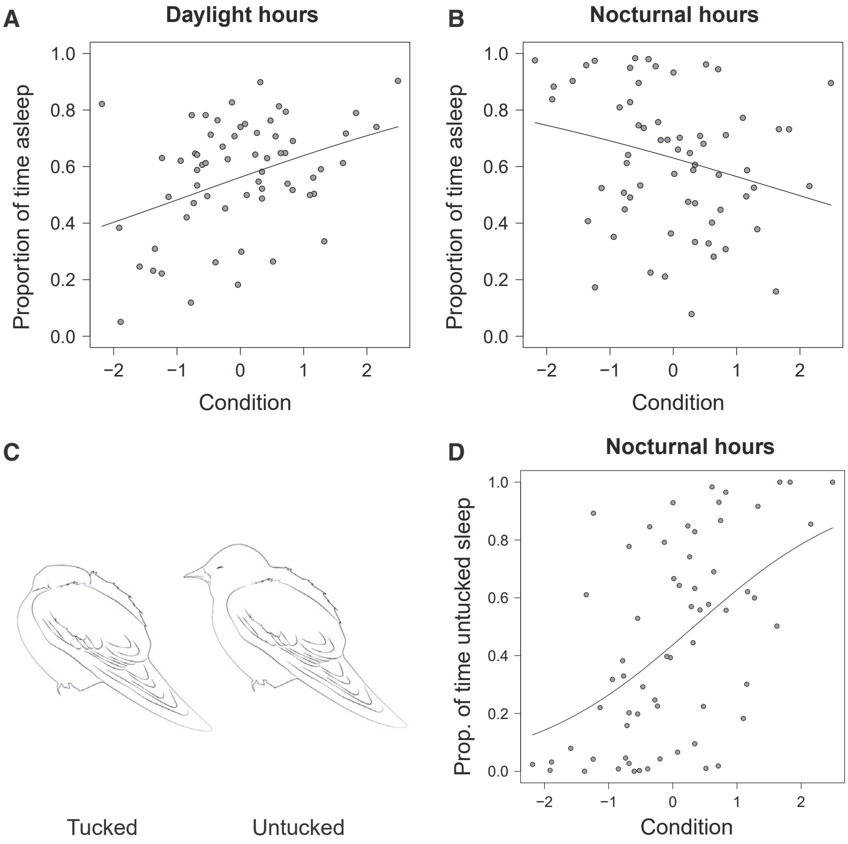

Figure 1. The Diurnal Pattern of Sleep and the Posture Displayed during Sleep Are Correlated with Condition in Migrating Garden Warblers at a Stopover Site

(A) The relative duration of sleep during daytime was positively correlated with an index of body condition calculated from body mass and extent of fat stores and muscle mass (beta regression model: Pseudo R^2 = 0.159, p = 0.001).

(B) The relative duration of sleep during the night was negatively correlated with body condition (beta regression model: Pseudo R^2 = 0.082, p = 0.028), as expected in migratory birds in good condition that show migratory restlessness at night.

(C) Most birds show two sleep postures: the untucked posture, where the neck is retracted and the head rests on the breast pointing forward, and a tucked posture, where the neck is turned backward and the head is tucked in the scapular feathers.

(D) Birds in better conditions spent considerably more time sleeping in the untucked position during the night, whereas temperature (mean = 21.7°C; SD = 1.29) did not affect the postural choice (beta regression model: Pseudo R^2 = 0.416; condition: $p < 0.001$; temperature: p = 0.06). During the day, most birds slept in the untucked position independently of condition (beta regression model: Pseudo R^2 = 0.001; condition, p = 0.996).

We investigated sleep patterns of migrating wild garden warblers on the island of Ponza, a major stopover site in the Mediterranean. During spring migration, thousands of passerine birds arrive to the island after crossing more than 500 km of open sea. We caught birds with mist nets, collected a blood sample, and recorded standard morphological and physiological variables [28], and then we placed them in customized cages and recorded their behavior with infrared cameras until the following morning. An index of body condition was extracted using principal component analysis so that animals with larger body mass, fat stores, and muscle mass had a higher condition index. We found that birds in better condition spent a larger proportion of time sleeping during the day hours (Figure 1A). At night, the pattern was inverted (Figure 1B), as expected from previous studies showing that migratory garden warblers in better conditions express intense nocturnal restlessness [29], a good proxy of migratory disposition [30, 31].

Despite the lack of a sleep posture preference during daylight hours, we found differences in the posture adopted by the birds during nocturnal sleep. Birds in better condition slept mainly in the “untucked” posture (Figure 1D), where the neck is retracted and the head is pulled toward the body facing forward, whereas birds in poorer condition slept mainly in the “tucked” posture, where the neck is turned backward and the head tucked in the scapular feathers [32] (Figure 1C). Because the tucked posture is usually displayed by birds at colder temperatures [33–36], this posture might conserve energy and thereby allow lean birds to preserve their already poor physiological state.

These results show that garden warblers adjust their sleep posture according to their physiological condition and suggest that the choice to sleep with the head tucked or untucked is related to energy management. To verify this hypothesis, we placed garden warblers in a respirometry chamber to record their oxygen consumption, a common measure of metabolic rate. An infrared-sensitive camera recorded the behavior of the birds during the respirometry measurements (Figure 2A). The average ambient temperature during the experiment was 22.6°C (SD = 0.695; max = 24.5°C; min = 21.2°C). As expected, we found that the metabolic rate varied with activity, with the highest metabolic rate in active birds, intermediate in inactive but awake birds, and lowest in sleeping birds (Figure 2B), consistent with the known reduction in metabolic rate below the basal metabolic rate during slow-wave sleep [37, 38], one of the two type of sleep in birds and mammals [39]. Among sleeping birds, moreover, we found that the metabolic rate was lower in birds with their head tucked than in birds with their head untucked (Figure 2B). In addition, we found that another indicator of metabolic rate, respiratory rate, was reduced in birds sleeping tucked compared to those untucked (Figure 2C). The association between posture and metabolic rate was not influenced by ambient temperature (Figure 2D), suggesting that the choice between these two postures is linked to energy conservation strategies.

Field observations on a number of species have suggested that the tucked posture is a strategy to reduce heat dispersion from the bill and the eye region [40]. This seems to be also the case for garden warblers. To further corroborate this result, we placed birds in a modified cage where one of the side walls was replaced by an infrared-transparent polymer, and we

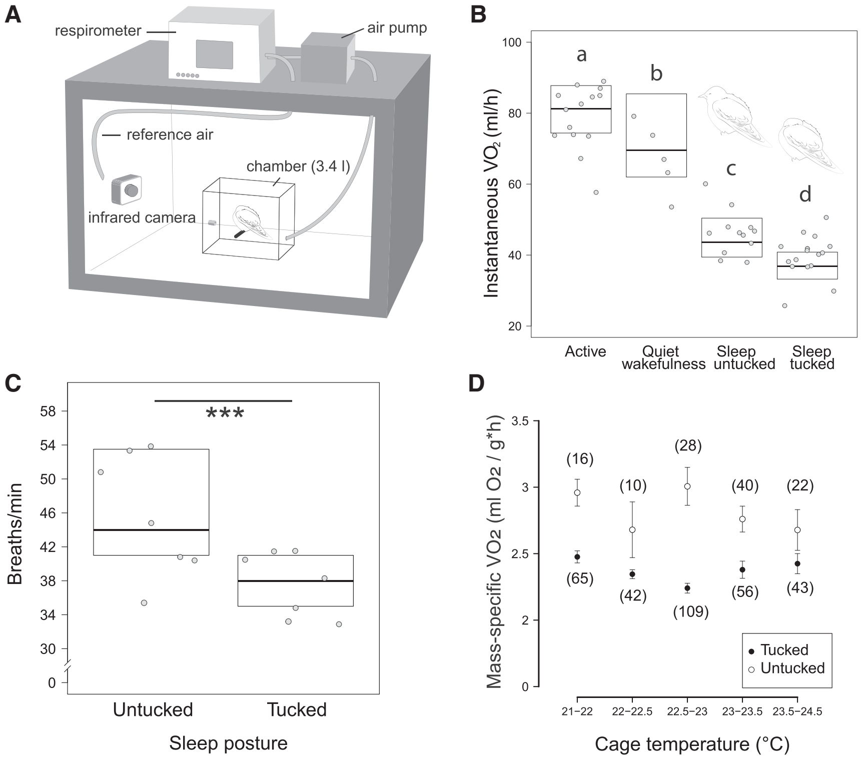

Figure 2. The Metabolic Rate Is Lower in Garden Warblers Sleeping with the Head Tucked Compared to Those Sleeping Untucked

(A) Schematic drawing of the experimental setup showing the respirometry chamber placed in the sound-proof box. An infrared-sensitive camera placed outside the chamber recorded the behavior of the birds throughout the experiments.

(B) When the instantaneous rate of consumed oxygen was compared between different behavioral states, we found a clear reduction in birds that were quiet but awake (quiet wakefulness: 33 blocks from 5 individuals) compared with restless (active: 188 blocks from 14 individuals) birds and in sleeping birds compared with quiet awake birds. The lowest oxygen consumption was found when birds were in the tucked position (sleep untucked: 116 blocks from 12 individuals; sleep tucked: 315 blocks from 16 individuals). Different letters (a–d) indicate states that differed significantly from each other (pairwise post hoc test; active vs. quiet wakefulness: $p < 0.001$; active vs. untucked: $p < 0.001$; active vs. tucked: $p < 0.001$; quiet wakefulness vs. untucked: $p < 0.001$; quiet wakefulness vs. tucked: $p < 0.001$; untucked vs. tucked: $p < 0.001$). Boxplots show the median and inter-quartiles calculated on the entire sample, and dots show the individual mean values for each behavioral state. A plot of all measurements is shown in Figure S1.

(C) The number of breaths per minute was higher in untucked compared to tucked birds (LMMs; estimate = 38.341; SE = 2.134; t value = 13.007). Boxplots show the median and inter-quartiles calculated on the entire sample, and dots show the individual mean values for each sleep posture.

(D) Plot of mass-specific metabolic rate versus ambient temperature. For better visualization, circles show the mean ± SE mass-specific metabolic rate for each temperature interval. Open circles show values for untucked birds, closed circles for tucked birds. Slopes of the regression lines calculated from the individual data points were not significantly different (LMMs; untucked:Ta: estimate = −0.908, SE = 0.894, t = −1.016; tucked:Ta: estimate = −1.202,SE = 0.893, t = −1.346).

recorded their surface temperature using a thermovision camera (Figure 3A). When we compared average heat dissipation from different areas of birds sleeping in the untucked posture (Figure 3B), we found that the heat dissipation from, and average surface temperature of, the body and the bill were significantly lower compared to the head, including the eye region (Figure 3C). Thus, in garden warblers perched at night, the greatest heat dissipation occurs through the eye region, and a significant reduction of heat loss can be achieved by hiding this area under the feathers (Video S1). The tucked posture therefore reduces the overall conductance of the bird's surface and would permit further energy savings at low ambient temperatures.

These experiments demonstrated that the tucked posture allows garden warblers to minimize heat loss and likely contributed to the reduction in energy expenditure. The fact that birds in good condition preferred to sleep untucked, however, indicated that the choice of keeping the head tucked has costs. One of the highest risks that migrants encounter at stopover sites is predation [41]. Garden warblers—like most other songbirds—do not migrate in social groups and do not appear to benefit from group vigilance against predators [42–44]. As different types of sleep are associated to different levels of responsiveness [45], we hypothesized that birds are exposed to higher risks of predation when sleeping tucked and therefore only resort to this posture to conserve energy when in poorer condition. We predicted therefore that tucked birds would react to potential danger with a longer latency than those untucked would. To test this hypothesis, we measured the reaction time of sleeping birds when exposed to the sound of crunched leaves, a noise that might indicate the approach of a terrestrial predator. Garden warblers were housed singly in sound-proof boxes to minimize external disturbances, and their behavior was video recorded with infrared-sensitive cameras (Figure 4). An external trigger simultaneously activated the playback of the sound and an infrared flash, invisible to the birds but recorded by the camera (Figure 4A). We then analyzed the time elapsed between the activation of the trigger and the first reaction of the bird, i.e., the onset of the transition to an alert posture with visible reduction of the feather volume and opening of the eyes. The analysis clearly showed that birds sleeping with their head untucked reacted significantly faster than those sleeping with their head tucked did (Figure 4B), indicating that birds sleep more deeply when their head is tucked. Albeit not tested, response to visual threats could also be reduced in the tucked position in which feathers cover the eyes [46], further adding to the risks of sleeping in this position.

Taken together, our studies show that migratory garden warblers making a stopover after a long flight over sea adopt

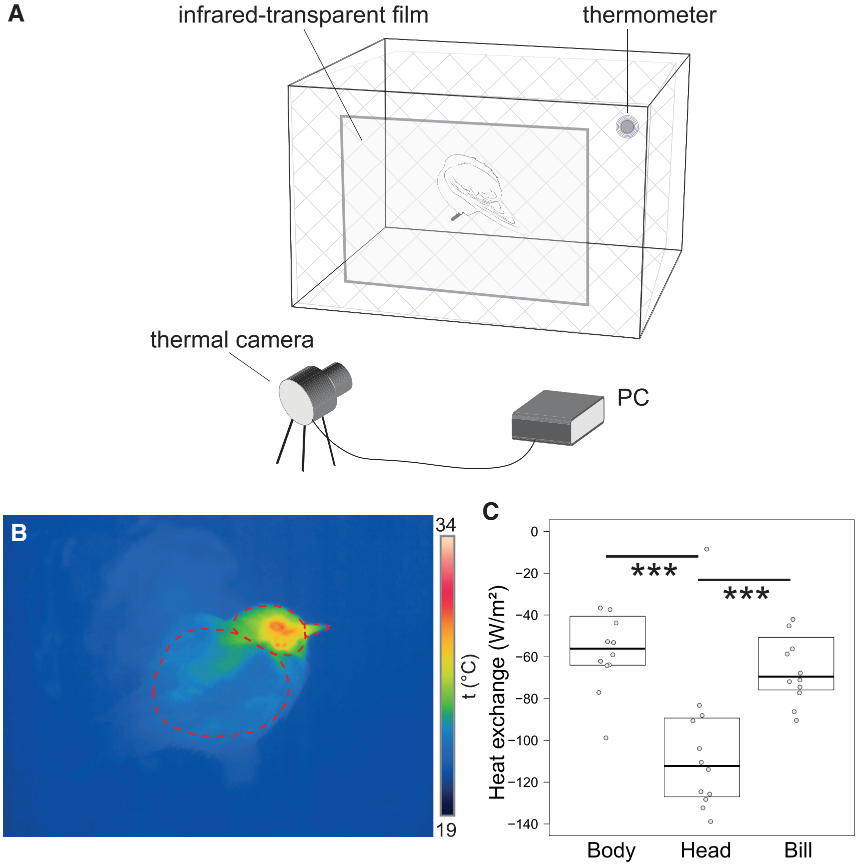

Figure 3. In Garden Warblers Sleeping Untucked, the Eye Region Is the Part of the Body with the Highest Heat Dissipation

(A) Birds (N = 12) were placed in a special cage with one side made of an infrared-transparent polymer that allowed the recording of the birds' surface temperature by means of a thermovision camera.

(B) Thermal imaging showed noticeable differences in the temperature of the birds' surface between different parts of the body: body (largest area), head with a patch of higher temperature around the eye (medium-sized area), and the bill (smallest area). Birds noticeably decrease the maximum surface temperature by tucking the eye region in the scapular feathers (Video S1).

(C) In birds sleeping untucked (Figure 2B), the estimated heat exchange was significantly higher for the head than for the beak and the body (pairwise post hoc test; head vs. bill: $p < 0.001$; head vs. body: $p < 0.001$; body vs. bill = 0.106).

different sleep postures according to their condition. Birds in good condition have a lower need to refuel and therefore sleep more during the day and show more nocturnal activity. Birds in poorer condition, on the contrary, sleep less during the day, as they need to forage. They compensate with a higher proportion of sleep at night, during which they preferentially display a tucked posture associated with reduced heat loss and metabolic rate, resulting in important energy savings. However, it is unclear whether tucking the head accounts for all of the energy savings or simply contributes to a strategic drop in metabolic rate associated with sleeping deeper in energetically compromised warblers. Regardless, the energy saved by sleeping with the head tucked in is paid for by reduced awareness and a likely resulting increase in the risk of predation. This trade-off between energy saving and predation risk seems to be confirmed by the finding that most birds sleep in the untucked posture during the daylight hours when higher ambient temperatures reduce the need to prevent heat loss and the risk of predation might be higher.

Our findings have implications for understanding the functions of sleep. The greater investment in deeper sleep with lower energy expenditure by warblers in poor condition supports an energy conservation function for sleep [47] that seemingly challenges the dominant view that "*Sleep is of the brain, by the brain, and for the brain*" [48]. Nonetheless, it is conceivable that by reducing heat loss in the tucked position, songbirds can also reallocate some of the energy used for thermoregulation in the untucked position toward sleep-dependent maintenance processes that benefit the brain [49] while still saving some energy overall. Indeed, birds in poor condition might have previously faced cognitively taxing circumstances (e.g., difficult foraging, longer flights) and therefore have a greater need for investment in brain restitution. The fact that warblers in poor condition did not lower their metabolic rate even further through becoming torpid [50] is consistent with energetic investment in such restorative processes as well as avoidance of the impairment in antipredator escape behavior associated with torpor [46, 50, 51]. Consequently, in addition to conserving some energy, sleeping tucked in may benefit the brain. In this regard, migratory songbirds may serve as a novel system for exploring the interplay between the systemic and brain-related aspects of sleep. Finally, our findings underscore the importance of considering sleep when attempting to understand the migratory strategy of millions of birds.

STAR★METHODS

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

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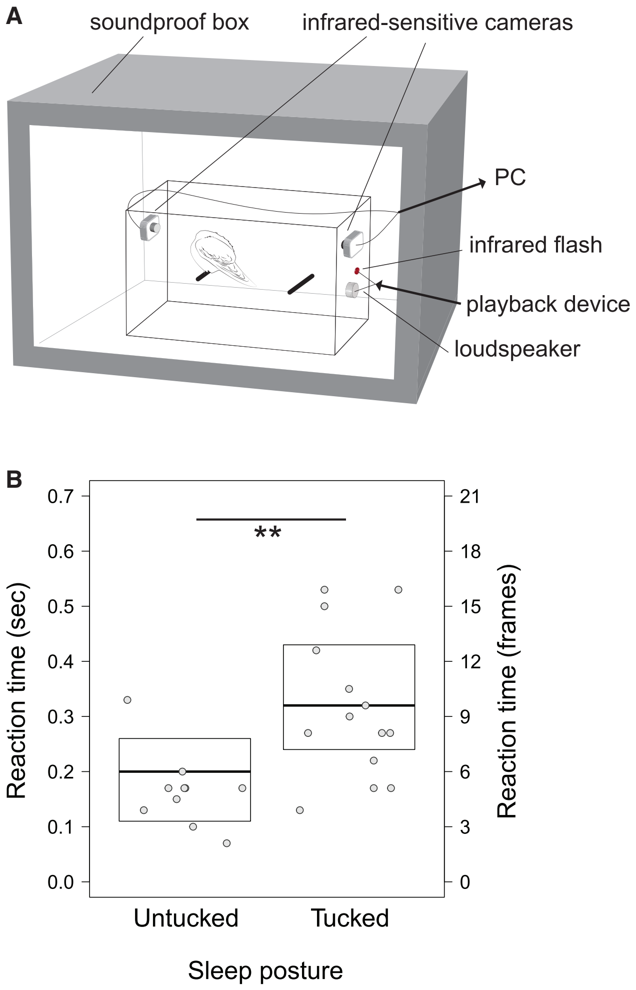

Figure 4. Birds Sleeping with the Head Tucked in the Feathers React More Slowly to External Stimuli than Do Those Sleeping Untucked

(A) Experimental setup used to measure reaction time. The birds were held in individual cages within sound-proof boxes. Inside the box, a loudspeaker played the acoustic stimulus (sound of crunched leaves) activated by the operator outside the box. An infrared flash, invisible to the bird, was triggered together with the sound and used to measure the latency of reaction from videos recorded by an infrared-sensitive camera.

(B) Response latency measured by counting the number of frames elapsed between the activation of the trigger and the onset of the transition to an alert posture. Birds (sleep untucked: 14 trials from 10 individuals; sleep tucked: 23 trials from 14 individuals; 3 individuals were tested in both postures) sleeping with their head tucked had a significantly longer response latency than did those with their heads untucked (LMMs; estimate = −0.175; SE = 0.060; t value = −2.933). Boxplots show the median and inter-quartiles calculated on the entire sample, and dots show the individual mean values for each sleep posture.

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.07.028.

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AUTHORS CONTRIBUTIONS

A.F. and L.F. conceived the study; A.F. and M.C. conducted the experimental work; A.F. and T.R. analyzed the data; and A.F., L.F., N.C.R., S.R.M., and T.R. wrote the paper.

DECLARATION OF INTERESTS

The authors declare that they have no conflict of interest.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
d-ROMs test	Diacron International	Code: MC002
OXY-Adsorbent test	Diacron International	Code: MC435
Software and Algorithms		
R version 3.5.1	The R Foundation	https://www.r-project.org/
Solomon Coder Version beta 16.06.26	Solomon Coder	https://solomoncoder.com/
VirtualDub Version 1.10.4	VirtualDub	http://www.virtualdub.org/
ThermaCAM Researcher 2.10	FLIR System	https://www.flir.com/
IBM SPSS Statistics 25	IBM, New York	https://www.spss.it/
Other		
FLIR SC325	FLIR System	https://www.flir.com/

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources, raw images and recordings should be directed to, and will be fulfilled by, the Lead Contact, Andrea Ferretti (andrea.ferretti1989@gmail.com). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Experimental model

In this experiment garden warbler (*Sylvia borin*) were used. This is a nocturnal, long-distance migrant that perform long migratory flights over the Sahara Desert and the Mediterranean Sea to reach its breeding grounds in Northern Europe.

Sex

The garden warbler is a monomorphic species and for this reason it was not possible to assess the sex of individuals.

Developmental stage

All studied individuals were adult birds. A more precise determination of age based on morphological traits is not possible in this species.

Health/immune status

All animals used in this study were caught during migration and did not present any obvious symptom of illness.

Drug or test naive

N/A

Previous procedure

All animals used in this study had not been used for other experiments.

Housing

Birds were placed in custom-made fabric cages (50 × 25 × 30 cm) containing 2 perches at different heights. The cages were fitted inside custom-made ventilated soundproof boxes, in order to isolate animals from external acoustic stimuli. The soundproof boxes were illuminated through a window and by a light system synchronized with natural light/dark cycle. For further information see Method details.

Ethical note

All experimental procedures including the permission to trap and temporarily hold birds in temporary captivity were authorized by the Regional Government (Determina Regione Lazio N. G02278 of 06 JUN 2015 and N. G03435 of 20 MAR 2018) in accordance with EU and Italian laws, and were communicated to, and performed according to the guidelines of, the Ethic and Animal Protection Committee (ETK) of the University of Veterinary Medicine, Vienna.

METHOD DETAILS

General method

This study was carried out on the island of Ponza, the largest of the Pontine islands, during spring. This island is located in the Tyrrhenian Sea (40°55' N, 12°58' E) and, due to its location, is an important stopover place for many European-African migratory birds.

Using mist nets, we caught garden warblers during spring migration, from March to May. After capture, an experienced ringer (MC) scored the subcutaneous fat score on a 0–8 scale, the size of the pectoral muscles on a 0–3 scale, and measured the length of the 3rd primary feather to 0.5 mm and the body mass to 0.1 g following standardized European methods [28]. After the ringing procedure, birds were caged overnight until the following morning.

Sleep pattern and postural preference

We used 63 garden warblers caught on Ponza in spring 2015 and 2016. Upon capture, the brachial vein was punctured and a small blood sample was collected for other studies described elsewhere. After measurements and sampling, the birds were rapidly transported to the recording room and placed in custom-made fabric cages (50x25x30 cm) containing 2 perches at different heights. The cages were fitted inside custom-made ventilated soundproof boxes, in order to isolate animals from external acoustic stimuli. The soundproof boxes were illuminated through a window and by a light system synchronized with natural light/dark cycle. All birds were placed in their cages by 12:00 CET and held inside the cage until the following sunrise. During the housing period, behavior was recorded by infrared-sensitive cameras connected to a recording system (700line ccd camera; HANDIKAM, Redruth, Cornwall, United Kingdom, 16 frm/s). Cage temperature was not measured in 2015-2016, and was therefore estimated from environmental temperature (recorded by C.O.Met., Centro Operativo per la Meteorologia, Aereonautica Militare) using a linear regression model built on environmental temperatures and cage temperatures recorded in 2018, with the same experimental setup. We used this estimate in the models as it reflects better the temperature experienced by the birds during the experiment.

Video analysis of sleep

The video analysis was conducted using Solomon Coder (version beta 16.06.26, developed by Andras Peter). The behavioral coding was slightly different between experiments. In the study of the relationships between condition and sleep, video-recordings were analyzed by focal, instantaneous sampling for 1 min each 5 min of recording. We divided the experimental period in 2 intervals: day (from 1 pm until sunset) and night (from sunset until sunrise). We categorized behaviors into two main states: 'awake' and 'asleep'. A bird was coded as asleep when it showed immobility for longer than 5 s and increased feather volume. The 5 s rule is based on the fact that EEG signs of slow-wave sleep occur within a few seconds after onset of immobility in a sleep posture in a variety of avian species, including songbirds (see figures in [22, 23, 52–54]). When possible, sleep was confirmed by observation of the closed eyes. Birds were coded as awake in all other cases. Asleep birds were further classified in two substates: in the untucked posture, the neck is retracted and the head is pulled toward the body facing forward; in the tucked posture, the neck is turned backward and the head tucked in the scapular feathers [32] (see Figure 1D of main text). The state 'Out of sight' was coded in the rare cases in which the bird was outside the surveilled area (i.e., the bird sat on the highest camera). To control for inter-observer variability, 3 entire days were analyzed independently by the three observers blind to the condition data and an Inter Observer Reliability was calculated by performing a Kruskal-Wallis test (Chi-sq = 0.136; p = 0.987).

In the Energy expenditure experiment, the smaller cage size allowed a more precise evaluation of the behavior of the animals. Therefore, the birds were coded in four mutually exclusive states. Active birds were moving around the cage. Birds scored as in 'quiet wakefulness' were awake as they displayed small movements of the body and limbs and had open eyes but did not change their position within the cage. The definition of tucked and untucked was the same as described above, although in this case the closure of the eyes in the untucked position was always verified and allowed to distinguish between untucked and quiet wakefulness. In this experiment, continuous video recordings during the whole night were coded by a single observer.

Metabolic rate

In order to investigate energy expenditure in different sleep postures, we measured oxygen consumption in 18 garden warblers caught on Ponza between the 4th and the 14th of May 2018. After capture and ringing, birds were brought to the recording room and placed in fabric cages (50x25x30 cm). At sunset, birds were moved to a custom-built metabolic chamber (15x15x15 cm, volume = 3.4 L), provided with a perch. During the whole experiment, birds were at temperatures between 21.2 and 24.5°C (mean = 22.6°C; SD = 0.695). Rates of oxygen consumption were measured continuously on an open circuit system, using an oxygen analyzer. Air was supplied to the chamber and air flow rate was measured with a calibrated electrochemical oxygen analyzer (OxBox 4.02.22, Research Institute of Wildlife Ecology, Vienna, see [55, 56]. The oxygen analyzer was calibrated with nitrogen/air mixtures produced by a high-precision proportioning pump (H. Wösthoff, Bochum, Germany, type 55A27/7a). During measurements a pump creates an air flow (ca, 30 L/h; measured by a thermal mass flow meter; AWM5101, Honeywell, Morris Plains, USA) aspirating the air from the chamber and conveying it into the analyzer to quantify the amount of oxygen in the air stream. The analyzer measured oxygen every minute, but switched air flow to fresh reference air regularly, alternating 5 min of reference air with 15 min of measurement air from the chamber. The environmental temperature was recorded by a sensor connected with the oxygen analyzer (HIH9131, Honeywell International Inc. Morristown, New Jersey, USA), as well as by a logger (developed and built at Research Institute of Wildlife Ecology, Vienna; Resolution 0.0625°C; Accuracy ± 0.1°C) fixed inside the metabolic chamber. The air humidity was recorded by the oxygen analyzer (HIH9131, Accuracy: ± 1.7% RH, Honeywell International Inc. Morristown, New Jersey, USA) and taken into account during the calculation of oxygen consumption. The rate of oxygen consumption was computed according to Lighton (2018), assuming and RQ of 0.85. While the bird was placed in the metabolic chamber, behavior was recorded by an infrared-sensitive video-camera (700line ccd camera; HANDIKAM, Redruth, Cornwall, United Kingdom, 16 fps) connected to a recording system.

Estimation of instantaneous oxygen consumption

We estimated the instantaneous oxygen consumption applying the instantaneous transformation to the raw respirometry data using the Z-transformed method [57]. The adequate Z-value was determined empirically by fitting instantaneous oxygen consumption to a rectangular oxygen pulse, generated by a proportioning pump. From the full night recording, we focused our analysis on 5 min-blocks during which animals displayed a given behavioral state (see above) without interruptions (active: 188 blocks from 14 individuals; quiet wakefulness: 33 blocks from 5 individuals; sleep untucked: 116 blocks from 12 individuals; sleep tucked: 315 blocks from 16 individuals). The measurement of oxygen consumption was calculated by averaging the instantaneous oxygen consumption of the 3 central minutes for each 5 min-block: we considered only the central minutes in order to reduce the influence of behaviors displayed immediately before, or possible changes of metabolic rate in preparation for activity after the 5 min-block. Data collected within one hour after caging (habituation period) and after 3:30am (anticipation of diurnal behavior) were excluded from the analysis.

Respiratory rate

Forty-nine 5-min video segments of a subset of 7 birds from the metabolic rate experiment (sleep untucked: 24 blocks displayed by 7 individuals; sleep tucked: 25 blocks displayed by 7 individuals) were used to calculate the respiratory rate. The subset was randomly selected by considering only videos with a side view of the birds. For each video segment, we counted the total number of breaths in the third minute of the recording.

Reaction time

In spring 2017 and 2018, we tested arousal threshold in 24 garden warblers displaying different sleep postures (sleep untucked: 14 trials from 10 individuals; sleep tucked: 23 trials from 14 individuals; 3 individuals were tested in both postures). We measured the reaction time to an acoustic stimulus as an indicator of anti-predator alertness. After capture and ringing, birds were brought to the recording room and kept inside fabric cages described above fitted inside soundproof boxes. The cage was equipped with 2 infrared-sensitive video-cameras (700line ccd camera; HANDIKAM, Redruth, Cornwall, United Kingdom, 30 fps) and a custom-made speaker. While the bird displayed a particular sleep posture, the operator played back an acoustic stimulus, an audio recording of crunched leaves (recorded in a soundproof room at the University of Vienna). To synchronize the acoustic stimulus and the video recording, we coupled the speaker with an infrared LED that was triggered simultaneously with the acoustic stimulus. Raw recordings were analyzed using VirtualDub (Version 1.10.4) by counting the number of frames from the onset of the stimulus until when the animal first showed a reaction. The start of the reaction was defined as the onset of the transition to alert posture, which was characterized by a visible reduction of the feather volume and open eyes. In order to measure the reaction time, the total number of frames elapsed between stimulus and response was then converted to seconds. Some birds were tested multiple times (maximum 3 times, minimum 30 min between trials) and we controlled for habituation to the noise adding the order of trials as covariate in the statistical model.

Thermovision-camera

During spring 2018, thermic images of garden warblers resting in different positions were recorded using a modified metabolic chamber in which one of the Plexiglas walls was substituted with a polyethylene film (CAP-T, manufactured by IRISS, Terrance Bradenton, Florida, USA) in order to measure heat loss using a thermovision-camera (FLIR SC325; FLIR System). At the same time, birds were filmed using an infrared-sensitive video-camera to monitor their behavior.

Estimation of heat exchange

We calculated heat exchange using only thermo-images of birds sleeping in the untucked posture (N = 12). We selected only images with a lateral view on the bird in order to standardize for the position of the body axes in relation to the camera. Based on the temperature recorded by the thermal-camera, we compared heat dissipation through different areas of the bird surface: the body, the head and the bill. For these areas we calculated the average temperature and estimated heat exchange (W/m^2). Heat exchange was calculated using the Termimage package in R [58, 59], incorporating independent measurements of ambient temperature (from an external temperature logger), relative humidity (from the respirometry system) and assuming an emissivity of 0.96 [58]. Heat exchange (loss = negative, gain = positive) was calculated as the sum of the convective and radiative heat exchange from the different body areas [60].

Replication

The number of biological replicates for each experiment is indicated in the corresponding method section and figure legends

Strategy for randomization and/or stratification
N/A

Blinding at any stage of the study
Video coding was conducted by researchers blind to the condition of the birds.

Sample-size estimation and statistical method of computation
N/A

Inclusion and exclusion criteria of any data or subjects
N/A

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis
Except for the relationships between condition and sleep, we analyzed our data using Linear Mixed Effect Models (LMMs) to take into account repeated-measurements from the same individuals.

Relationships between condition and sleep
In order to test the effects of condition on time spent sleeping and in each sleep posture during both day and night, we used Beta Regression Models. A condition index was defined as the first component of a Principal Component Analysis that included the variables fat score, muscle score and body mass (SPSS Statistics 25, IBM, New York). For the study of sleep patterns, the target variable was the ratio between total time spent sleeping and total time analyzed to control for differences in day/night length. With regard to sleep posture, the target variable was the ratio between the time spent in a given posture and the total time asleep to control for differences in total time spent sleeping. The estimated cage temperature was considered in the model as a covariate.

Reaction time
We tested for differences in reaction time between sleep postures using Linear Mixed Effect Models (LMMs; 'lmer' function in 'lme4' R package [61]. We controlled for repeated-measurements by computing random intercepts for each bird ID and we considered the order of sampling as a fixed effect covariate in the model.

Heat exchange
We tested for differences in heat exchange between sleep postures using LMMs. In this case, we adjusted for repeated-measurements by computing random intercepts for each bird ID and entering the posture as a fixed effect. We did not include the ambient temperature in the model because it was taken into account in the calculation of heat exchange.

Metabolic rate
We tested for differences in rates of oxygen consumption using LMMs. We considered body mass at sunset, average cage temperature and behavioral state as fixed effects. We adjusted for repeated-measurements by computing random intercepts for each bird ID nested in cage ID in the model.

Respiratory rate
We tested for differences in respiratory rate between sleep postures using LMMs. We considered the posture as a fixed effect and we adjusted for repeated-measurements by computing random intercepts for each bird ID in the model.

DATA AND CODE AVAILABILITY

For requests of the R scripts and data used in this study, please contact the corresponding authors.

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Behavioural Ecology: Sleeping Safely Carries Energetic Costs

Lauren Tworkowski and John A. Lesku*
School of Life Sciences, La Trobe University, Melbourne 3086, Australia
*Correspondence: J.Lesku@latrobe.edu.au
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Trade-offs shape animal behaviour. For decades, the study of trade-offs has provided insight into how animals make decisions, but they have rarely been explored in relation to sleep. A new study reveals a role for sleep in saving energy in garden warblers on a stopover during a northward migration, but with ecological costs.

Indulge yourself to answer these three familiar questions. Do you spend more time at work to increase your productivity at the expense of spending more time at home? When you leave work late, do you drive faster than you should to recover lost time, but risk getting a fine? When you arrive home, do you cook a more nutritious, but time-consuming meal, or a quick microwaveable meal packed with fattening sugars? You make behavioural decisions, every day, by weighing the benefits of an action against its likely costs, across each possible response. Your decision will (ideally) be the one that has the least cost for the most benefit. In a series of experiments, Ferretti *et al.* [1] demonstrate in this issue of *Current Biology* that a small songbird makes decisions in much the same way. In this case, whether to maximize energy savings by sleeping deeply and suffer increased vulnerability to terrestrial predators, or to sleep more safely, but by doing so, expend extra energy.

Animals, broadly, reach a decision by optimizing the ratio of costs-to-benefits [2]. Notably, small birds in winter must eat a substantial amount of food during the day to stave off starvation at night. However, eating by day increases the risk of being eaten themselves [3]. Leaving the safety of vegetation to find food makes little birds more visible to watchful predators, and keeping their head down to forage makes it harder to look out for those predators [2,4]. Moreover, eating too much can hamper an agile escape

Figure 1. Behavioural decision faced by garden warblers.
Choosing between two sleep postures, head tucked (left) and untucked (right), allows warblers to navigate a trade-off balancing energy homeostasis, sleep intensity, and anti-predator vigilance. The leaner birds choose the former: they sleep more deeply and save more energy, but are also more vulnerable to predators. Conversely, birds with greater energy reserves opt to sacrifice the extra energy in order to better escape approaching threats. Illustration ©Damond Kyllo.

from airborne attackers [3]. Some birds, including mourning doves (*Zenaida macroura*), can lower their night-time body temperature to conserve energy, but in doing so, compromise their ability to fly [5].

Similar trade-offs have long been thought to exist in sleeping animals, but with very little actual research. Firstly, what are the costs of being asleep? The disadvantages of sleep are chiefly its defining characteristics. A sleeping animal is largely unaware of the local environment. Therefore, the decreased responsiveness that best characterizes the behavioural shutdown associated with sleep leaves animals vulnerable to attack [6]. Furthermore, sleep has a 'missed opportunity' cost, as animals aren't performing other vital activities, such as finding food, avoiding predators, defending territory, or securing mates [7]. How do animals mitigate these costs? Rats (*Rattus norvegicus*) delay sleep onset, reduce sleep duration, and shift to safer (lighter) sleep states when threatened [8]. Similarly, barbary doves (*Streptopelia risoria*) sleep less by interrupting sleep more following the brief appearance of a potential predator [9]. Mallard ducks (*Anas platyrhynchos*) keep an eye out for threats while sleeping on the edge of a group, compared to those safely flanked by conspecifics [10]. Great frigatebirds (*Fregata minor*) soaring over the ocean [11], and male pectoral sandpipers (*Calidris melanotos*) on their Arctic breeding grounds [12] sleep very little in favour of long-distance foraging flights and securing mating opportunities, respectively. Thus, when faced with situations that require vigilance or sustained performance, animals commonly resolve the trade-off by simply waking up and reducing sleep.

In a series of experiments, Ferretti *et al.* [1] explore a more nuanced trade-off in the garden warbler (*Sylvia borin*). The garden warbler is a small, long-distance migrant. Normally day active, they switch to flying at night during their spring migration north. Mid-trip, the birds land on the island of Ponza in the Mediterranean Sea. When they arrive, there is substantial variation across birds in terms of body condition, with some birds having greater muscle mass and more body fat, whereas others are leaner. To save energy, lean birds sleep more and tuck their head into feathers on their back (Figure 1). In this posture, they reduce their metabolic rate and conserve heat. Furthermore, the leaner birds sleep more deeply, as revealed by a slower response to the sounds simulating the approach of a terrestrial predator. They respond a fraction of a second slower. The lapse might seem trivial, but when facing predatory attacks from cats and weasels, any advantage matters. Conversely, birds of better condition do not adopt this strategy. Instead, these heavier birds, with their more substantial energy reserves, sleep less deeply with head faced forward, but respond more quickly to the approaching acoustic threat. By doing so, they lose more heat through their exposed head. In this way, each bird solves the trade-off to prioritize either energy homeostasis [13] or anti-predator vigilance [6], depending on its energetic state.

The lean warblers use deep sleep to save energy, but what are other benefits of sleep? The strategy adopted by the heavier birds suggests added value, otherwise they would have remained awake, rather than sleeping lightly. Indeed, the ecological persistence of sleep under risk of predation attests to its undeniable role [6]. In addition to saving energy [13], sleep is also involved in the maturation [14] and upkeep of the brain [15–17]. Although some animals endure extended periods of sleep loss without decrements in performance [11,12], sleep restores the ability to function adaptively in humans (and most other animals) while awake. We are more alert and attentive, better motivated and coordinated, and our memory improves [17,18]. Therefore, beyond reducing energy expenditure, the migrating warblers may encounter neurologically demanding conditions *en route* to Ponza, for which other sleep functions are required.

It is interesting to speculate that the relative value of each sleep function may differ across individuals and species depending on their sex, age, metabolic rate, health, energetic and reproductive state, and broader life-history and ecology [7]. This 'changing priorities' idea with regards to sleep benefits shouldn't be surprising. There is great diversity in how animals use wakefulness. For example, antelope spend most of their waking effort grazing [19], male sandpipers spend exhaustive time pursing females and deterring rivals during the breeding season [12], and weaverbirds are preoccupied building their structurally complex nests [20]. Similarly, the chief purpose of sleep may differ across ontogeny and between individuals. Lean garden warblers use deep sleep to save energy, while other benefits appear to be ancillary [1], thermally stressed animals might primarily use sleep to reduce body (and brain) temperature [13], and food-caching animals might need to sleep to consolidate spatial memories regarding the locations of thousands of stored food items [7,17]. While time budgets show how awake animals allocate their time, owing to conspicuous behaviours with unambiguous aims, creating analogous visualizations for sleeping animals is much harder. To this end, Ferretti *et al.* [1] provide a window into how animals make decisions with respect to sleep, and by doing so, reveal their priorities with respect to sleep function.

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Plant Development: How Leaves Take Shape

James W. Satterlee and Michael J. Scanlon*
School of Integrative Plant Science, Cornell University, Ithaca, NY, USA
*Correspondence: mjs298@cornell.edu

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Live imaging, genetics, and computational modeling reveal how simple versus compound leaves are formed. Cross-species differences in leaf-wide growth determine the outcome of a locally-acting patterning process.

Deciphering the mechanisms by which genes control form is one of the central goals of developmental biology. Despite the tremendous technical advances made in this field, it remains challenging to relate genetic control of cellular growth to organ-level morphology [1]. New work from Kierzkowski and coworkers combines molecular genetic analysis, live imaging, and modeling to explain how differences in growth give rise to the simple, serrate leaves of *Arabidopsis thaliana* versus the compound leaflets of its close relative *Cardamine hirsuta* (Figure 1A) [2]. The