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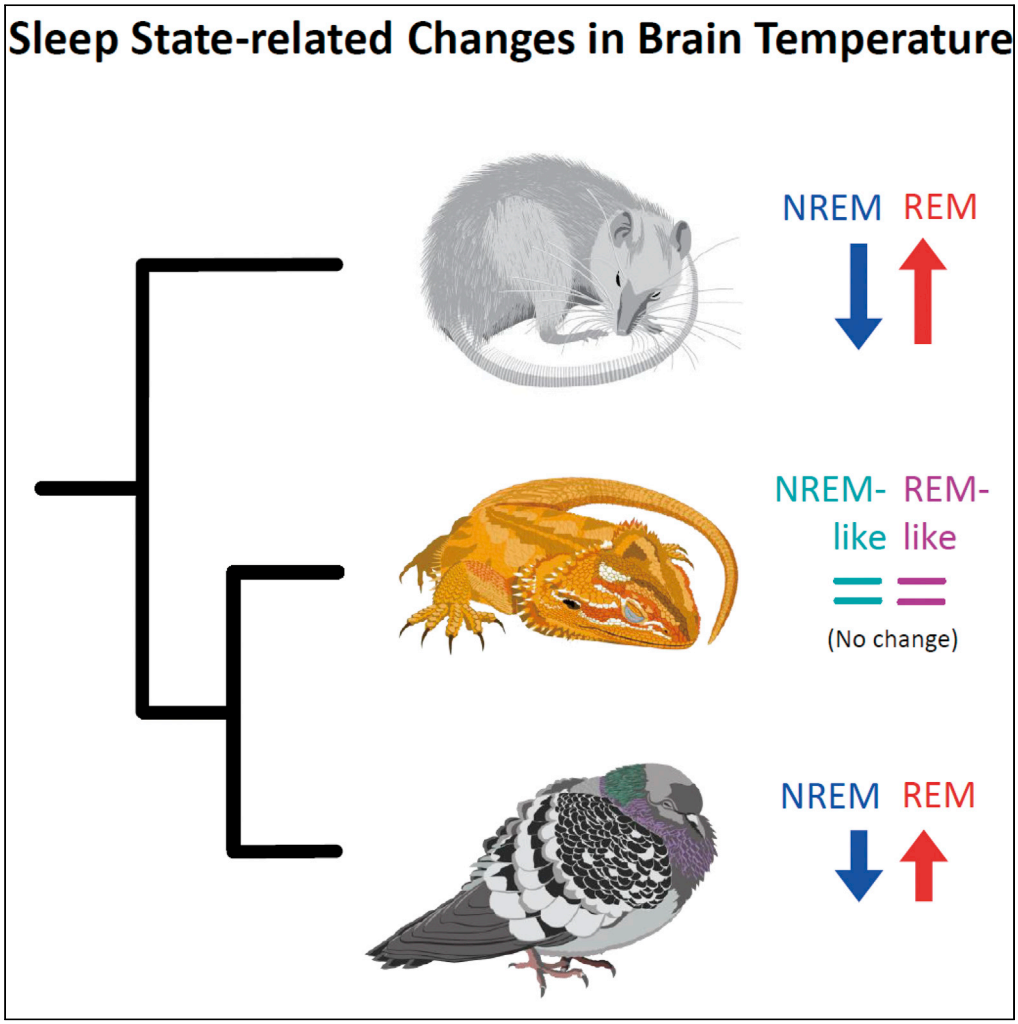

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HIGHLIGHTS

In many mammals, the brain cools during non-REM sleep and warms during REM sleep

Pigeons exhibit similar changes in cortical temperature during non-REM and REM sleep

Brain temperature does not increase during REM-like sleep in bearded dragon lizards

Brain warming is not a universal outcome of sleep states with wake-like brain activity

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Comparative Perspectives that Challenge Brain Warming as the Primary Function of REM Sleep

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SUMMARY

Rapid eye movement (REM) sleep is a paradoxical state of wake-like brain activity occurring after non-REM (NREM) sleep in mammals and birds. In mammals, brain cooling during NREM sleep is followed by warming during REM sleep, potentially preparing the brain to perform adaptively upon awakening. If brain warming is the primary function of REM sleep, then it should occur in other animals with similar states. We measured cortical temperature in pigeons and bearded dragons, lizards that exhibit NREM-like sleep and REM-like sleep with brain activity resembling wakefulness. In pigeons, cortical temperature decreased during NREM sleep and increased during REM sleep. However, brain temperature did not increase when dragons switched from NREM-like to REM-like sleep. Our findings indicate that brain warming is not a universal outcome of sleep states characterized by wake-like activity, challenging the hypothesis that their primary function is to warm the brain in preparation for wakefulness.

INTRODUCTION

Sleep is a mysterious state of reduced environmental awareness found in animals ranging from jellyfish to humans (Joiner, 2016; Libourel and Herrel, 2016; Blumberg and Rattenborg, 2017; Nath et al., 2017; Anafi et al., 2019; Iglesias et al., 2019; Kelly et al., 2019). Sleep in mammals is composed of two states, non-rapid eye movement (NREM) sleep and REM sleep, typically distinguished from one another and wakefulness by changes in brain and muscle activity. NREM sleep is characterized by the slow alternation between periods of cortical neuronal silence (down-states) and periods with high firing rates (up-states) that collectively give rise to high-voltage slow waves (0.5–4 Hz) in the electroencephalogram (EEG) (Timofeev et al., 2001; Vyazovskiy et al., 2009). REM sleep is typically characterized by continuous high firing rates comparable to wakefulness (Evarts, 1962; Mukhametov and Strokova, 1977; Timofeev et al., 2001; Vyazovskiy et al., 2009; *reviewed in* Jones, 2016), resulting in low-voltage, high-frequency EEG activity. In addition, muscle tone is reduced during NREM sleep, when compared with wakefulness, and absent during REM sleep (Peever and Fuller, 2017). Nonetheless, brief muscle twitches occur during REM sleep resulting in movements, such as rapid eye movements. The patterns of brain activity that characterize NREM and REM sleep have been implicated in various forms of synaptic plasticity (Chauvette et al., 2012; Tononi and Cirelli, 2014; Boyce et al., 2016; Li et al., 2017; Klinzing et al., 2019), and twitching during REM sleep is thought to play a role in mapping the sensorimotor cortex during development and possibly adulthood (Dooley et al., 2020; Blumberg et al., 2020).

In addition to brain and muscle activities, other physiological processes also differ between NREM and REM sleep in mammals. Notably, in most mammals examined, cortical and sub-cortical brain temperature (T_{br}) decreases during NREM sleep and increases during REM sleep (Kawamura and Sawyer, 1965; Hayward and Baker, 1969; Kovalzon, 1973; Obal et al., 1985; Wehr, 1992; Franken et al., 1992; Gao et al., 1995; Landolt et al., 1995; Csernai et al., 2019; Hoekstra et al., 2019; Komagata et al., 2019; see Hayward and Baker, 1968 and Hayward and Baker, 1969 for conflicting results in rhesus monkeys [*Macaca mulatta*]). Brain warming during REM sleep is thought to result from an increase in blood flow from the warmer body core to the brain to support this activity (Denoyer et al., 1991; Wehr, 1992; Parmeggiani, 2007; Pastukhov and Ekimova, 2012; Bergel et al., 2018). In this regard, brain warming might simply be a functionless by-product of functions that require increased neuronal activity, such as brain development and other types of synaptic plasticity. Alternatively, it has been proposed that increased neuronal activity is the mechanism the sleeping brain employs to periodically warm itself (Wehr, 1992; Lyamin et al., 2018). According to this hypothesis,

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brain warming is beneficial because it counteracts cooling occurring during preceding NREM sleep and thereby prepares the animal to awaken and rapidly interact adaptively with the environment (Wehr, 1992; Lyamin et al., 2018). The brain warming hypothesis remains largely untested, and it is unclear whether the brain warms during similar sleep states in other taxonomic groups.

Birds exhibit NREM and REM sleep characterized by changes in EEG activity similar to those observed in the mammalian cortex, despite differences in the organization of homologous neurons. In contrast to the laminar organization of neurons, with apical dendrites spanning the layers of the mammalian cortex, developmentally and functionally homologous ("cortical") regions of the avian brain, such as the visual hyperpallium (homolog of the mammalian primary visual cortex) (Medina and Reiner, 2000; Güntürkün et al., 2017; Briscoe and Ragsdale, 2018; Stacho et al., 2020), are composed of nuclear structures consisting of densely packed stellate neurons (Olkowicz et al., 2016). Similar nuclear structures comprise the dorsal ventricular ridge (DVR), a large brain region only found in sauropsids (birds and non-avian reptiles), involved in performing cortex-like functions (Güntürkün et al., 2017). Despite this fundamental difference in cytoarchitecture, as in mammals, an increase in EEG and local field potential (LFP) slow waves (approximately 1–5 Hz with a peak at 2 Hz) in the hyperpallium distinguishes NREM sleep from wakefulness and REM sleep in birds (van der Meij et al., 2019). As in mammals (Massimini et al., 2004; Huber et al., 2004), NREM slow waves travel through the hyperpallium (van der Meij et al., 2019) and are homeostatically regulated in a local use-dependent manner in pigeons (*Columba livia*) (Lesku et al., 2011b; Rattenborg et al., 2019). In addition to wake-like EEG activity, as in mammals, avian REM sleep is characterized by (at least partial) reductions in muscle tone (Dewasmes et al., 1985; Rattenborg et al., 2019); twitching, including rapid eye movements; suppressed thermoregulatory responses (Heller et al., 1983; Scriba et al., 2013); and its prevalence in young altricial birds (Scriba et al., 2013). One notable difference between birds and mammals is the short duration (typically <10 s) of bouts of REM sleep in birds (Tisdale et al., 2018b).

Interestingly, NREM-like and REM-like sleep were recently described in bearded dragon (*Pogona vitticeps*) lizards (Shein-Idelson et al., 2016; Libourel et al., 2018; Norimoto et al., 2020). NREM-like sleep was characterized by high-voltage slow LFP "sharp-waves" in the DVR, and REM-like sleep was characterized by LFP activity resembling wakefulness and eye movements occurring under closed eyelids. Although recent work has implicated the claustrum in the genesis of NREM sleep slow waves in mice and the slow "sharp waves" in bearded dragons, the claustrum appears to generate these field potentials via different mechanisms; in mice, the claustrum coordinates the synchronous inhibition of cortical neurons across several cortical areas, resulting in the down-state of slow waves (Narikiyo et al., 2020), whereas in bearded dragons, bursts of neuronal activity (giving rise to LFP sharp waves) propagate as traveling waves of excitation from the claustrum to the rest of the pallial DVR (Norimoto et al., 2020; see also Tisdale et al., 2018a). Given this apparent difference and the fact that not all of the components that typically characterize REM sleep in mammals and birds have been established in bearded dragons, we refer to these states as *NREM-like* and *REM-like* sleep (Libourel and Barrillot, 2020).

As in mammals, during REM sleep in pigeons and REM-like sleep in bearded dragons, hyperpallial and DVR neurons, respectively, fire at an elevated rate when compared with preceding sleep (Shein-Idelson et al., 2016; van der Meij et al., 2019), and therefore might cause T_{br} to increase via increased blood flow from the animal's warmer core. However, few studies have examined sleep state-related changes in T_{br} in birds, and none have examined bearded dragons. In birds, four studies examined hypothalamic temperature and one measured temperature in the nidopallium, a structure in the DVR. Graf et al. (1987) present a 4-h plot of hypothalamic temperature occurring during wakefulness, NREM sleep, and REM sleep in pigeons recorded at 10°C ambient temperature, but hypothalamic temperature did not vary systematically with state. In rooks (*Corvus frugilegus*) recorded at 8°C and 15°C, hypothalamic temperature declined during NREM sleep, but a significant increase was not detected during REM sleep (Szymczak et al., 1989). By contrast, Pastukhov et al. (2001) reported decreases and increases in hypothalamic temperature during NREM and REM sleep, respectively, in pigeons recorded at 21°C. It is unclear whether these differences are due to ambient temperature or the temporal resolution and sensitivity of the thermistors used in the respective studies. Hypothalamic temperature was also measured in sleeping ostriches (*Struthio camelus*), but the temporal resolution (once every 2 min) of the thermistor was too low to identify sleep state-related changes in temperature (Lesku et al., 2011a). In pigeons recorded at 22°C, temperature declined during NREM sleep, but a consistent change in temperature was not detected during REM sleep in the nidopallium (Van Twyver and Allison, 1972). Given that variations in T_{br} are smaller near the surface of the brain in

chickens (*Gallus gallus domesticus*) (Aschoff et al., 1973), the thermistor may not have been sensitive enough to detect changes in temperature during REM sleep in the nidopallium, a more superficial structure than the hypothalamus. Thus, apart from one study of hypothalamic temperature in pigeons, a consistent increase in temperature has not been detected during REM sleep in the birds examined. Moreover, the study reporting an increase in hypothalamic temperature during REM sleep did not characterize how temperature changed throughout and following bouts of REM sleep of different durations. It is also unknown whether temperature increases during REM sleep in "cortical" portions of the avian brain, an apparent requisite for brain warming to have a positive impact on cognitive performance upon awakening (Wehr, 1992). Finally, it is unknown whether T_{br} varies with the type of sleep in bearded dragons. To address these questions, and thereby expand our understanding of T_{br} regulation during sleep in non-mammals, we examined sleep state-related changes in temperature in the visual hyperpallium of pigeons and DVR of bearded dragons.

RESULTS

Across the night, pigeons experienced nearly 1,000 (979.6 ± 100.4) bouts of REM sleep lasting 6 ± 0.6 s (range, 1–30.7 ± 7.1 s, N = 7). Figures 1A and 1B illustrate the relationship between brain state and changes in T_{br} on 90- and 10-min time scales. Decreases and increases in T_{br} were related to the occurrence of NREM and REM sleep, respectively, with the peak T_{br} associated with a bout of REM sleep occurring after the bout ended (Figure 1B). To characterize the precise time course of T_{br} change during bouts of NREM and REM sleep of varying durations (Figures 1C and 1D), we examined isolated bouts of each state. Longer bouts of NREM sleep resulted in greater decreases in T_{br} (linear mixed effect model, Table S1; β_0 [intercept] ± SE = $-7.30 \times 10^{-3} \pm 8.70 \times 10^{-4}$, $p = 1.56 \times 10^{-4}$; β (slope) ± SE = $3.11 \times 10^{-3} \pm 3.31 \times 10^{-4}$, $p = 8.55 \times 10^{-5}$) (Figures 1C and 2A) and longer bouts of REM sleep resulted in greater increases in T_{br} (β_0 ± SE = $7.86 \times 10^{-3} \pm 5.70 \times 10^{-4}$, $p = 6.48 \times 10^{-6}$; β ± SE = $3.37 \times 10^{-4} \pm 5.59 \times 10^{-5}$, $p = 1.04 \times 10^{-3}$) (Figures 1D and 2B). T_{br} peaked on average 9 s after a bout of REM sleep, regardless of bout duration (β_0 ± SE = 9.22 ± 1.93, $p = 3.05 \times 10^{-3}$; β ± SE = $3.37 \times 10^{-4} \pm 5.59 \times 10^{-5}$, p = 0.1) (Figures 1D and 2C). This hysteresis accounts for the increase in T_{br} during short episodes of NREM sleep preceded by REM sleep (Figure 1B). Finally, we found that REM sleep was more likely to occur (density per 15 s) at lower T_{br} (β_0 ± SE = 2.18 ± 0.18, $p = 2.21 \times 10^{-5}$; β ± SE = −0.50 ± 0.08, $p = 1.02 \times 10^{-3}$) (Figure 2D).

In bearded dragons, brain (DVR) activity alternated between NREM-like and REM-like sleep throughout most of the night (Figure 3A). The bearded dragons experienced 436 ± 133.1 bouts of REM-like sleep lasting 49 ± 12.8 s (range, 8.8 ± 6.3–209.2 ± 81.9 s, N = 4). T_{br} either showed little change or decreased across the night (Figure S1). Despite exhibiting bouts of REM-like sleep that lasted on average 8.2 times longer than bouts of REM sleep in pigeons, average T_{br} did not increase when the bearded dragons switched from NREM-like to REM-like sleep (Figures 3B and S1).

DISCUSSION

It has been proposed that brain warming during REM sleep enhances performance upon awaking (Wehr, 1992). For this to be true, warming should occur in sub-cortical and cortical structures, as adaptive performance likely depends on the entire brain. Our results demonstrate that "cortical" warming occurs during REM sleep in pigeons, but not during a REM-like sleep in bearded dragons.

In pigeons, temperature in the visual hyperpallium decreased during NREM sleep and increased during REM sleep. Although this pattern is consistent with one study of hypothalamic temperature in pigeons, the average maximum increase was 10 times less in the hyperpallium (see Figure 1A in, Pastukhov and Ekimova, 2012). The smaller change in temperature in the hyperpallium, the most dorsal part of the avian brain, is consistent with the observation that although temperature covaries throughout the brain in awake and sleeping chickens, it varies less near the dorsal surface (Aschoff et al., 1973). Also, the small magnitude of state-related changes in T_{br} might explain why a consistent increase in nidopallial temperature was not detected during REM sleep in the earlier study of pigeons (Van Twyver and Allison, 1972), as the average maximum increase in T_{br} (0.01°C) in the hyperpallium is equivalent to the minimum sensitivity of the thermistor used to measure temperature in the nidopallium.

Although the sleep-state-dependent changes in T_{br} in pigeons are qualitatively similar to those observed in several mammals, the magnitude is much smaller. For example, the rate of cooling during NREM sleep and the rate of warming during REM sleep are approximately 10–20 and 5–12 times slower, respectively, than in

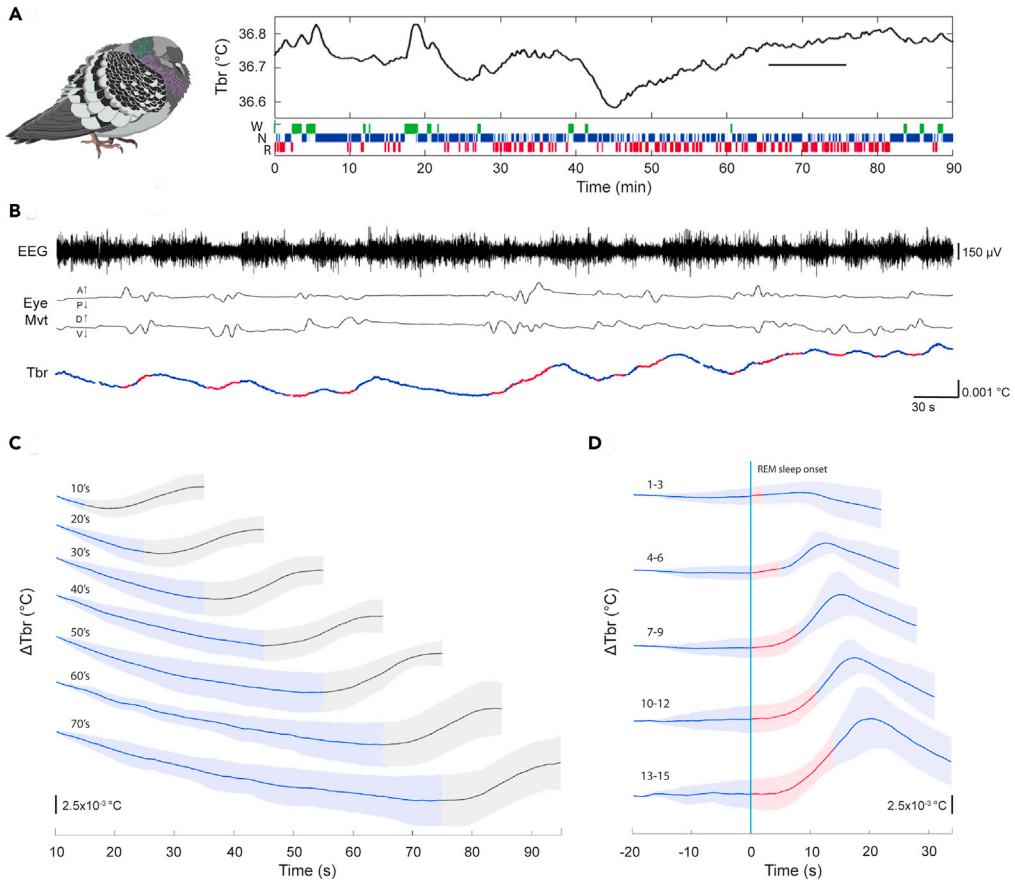

Figure 1. Brain Temperature (T_{br}) during NREM and REM Sleep in Pigeons

(A) T_{br} dynamics related to brain state across a 90-min period in a pigeon. Hypnogram (bottom plot) of brain state showing wakefulness (W, green), rapid eye movement (REM) sleep (R, red), and NREM sleep (N, blue). Note the frequent short bouts of REM sleep.

(B) Expanded 10-min period, demarcated by the bar in (A), showing electroencephalogram (EEG) activity and T_{br}, both recorded from the hyperpallium, and eye movements during NREM and REM sleep, color coded as in (A) on the bottom T_{br} recording. Lower-amplitude EEG activity and eye movements occur during REM sleep. Eye Mvt: left eye movements in the vertical (D, dorsal; V, ventral) and horizontal (A, anterior; P, posterior) planes determined from pupillometry.

(C) Longer bouts of NREM sleep are associated with greater brain cooling. Lines show the change in T_{br} (mean ± 1 standard deviation) relative to T_{br} 10 s after NREM sleep onset for NREM sleep bouts in 10-s duration categories (i.e., 10's = 10–19 s; 20's = 20–29 s; etc.). The 20 s after the middle of each category is plotted in gray, as it includes a mixture of the terminating state (wakefulness or REM sleep) and any subsequent states.

(D) Longer bouts of REM sleep are associated with greater brain warming. Lines show the change in T_{br} (mean ± 1 standard deviation) during 20 s of NREM sleep (blue) before REM sleep onset (vertical blue bar), during bouts of REM sleep (red) in 3-s duration categories (i.e., 1–3 s, 4–6 s, etc.), and during 20 s of NREM sleep following REM sleep offset, defined as the middle of each duration category. For each duration category, T_{br} is plotted relative to T_{br} 20 s before REM sleep onset. Pigeon illustration by Damond Kyllo.

rodents (Franken et al., 1992; Hoekstra et al., 2019). Moreover, the maximum average temperature increase associated with long (13–15 s) bouts of REM sleep, including the 9-s lag after REM sleep offset, is only 0.01°C in the hyperpallium. The reasons for this difference are unknown, but might include insulation (feathers verses hair), the level of neuronal activity and associated blood flow in the respective states, and the regulation of body temperature and the temperature of blood reaching the brain (Porter and Witmer, 2016).

In contrast to the sleep state-related changes in T_{br} in pigeons, DVR temperature did not increase when bearded dragons switched from NREM-like to REM-like sleep. Based on research in mammals, the absence

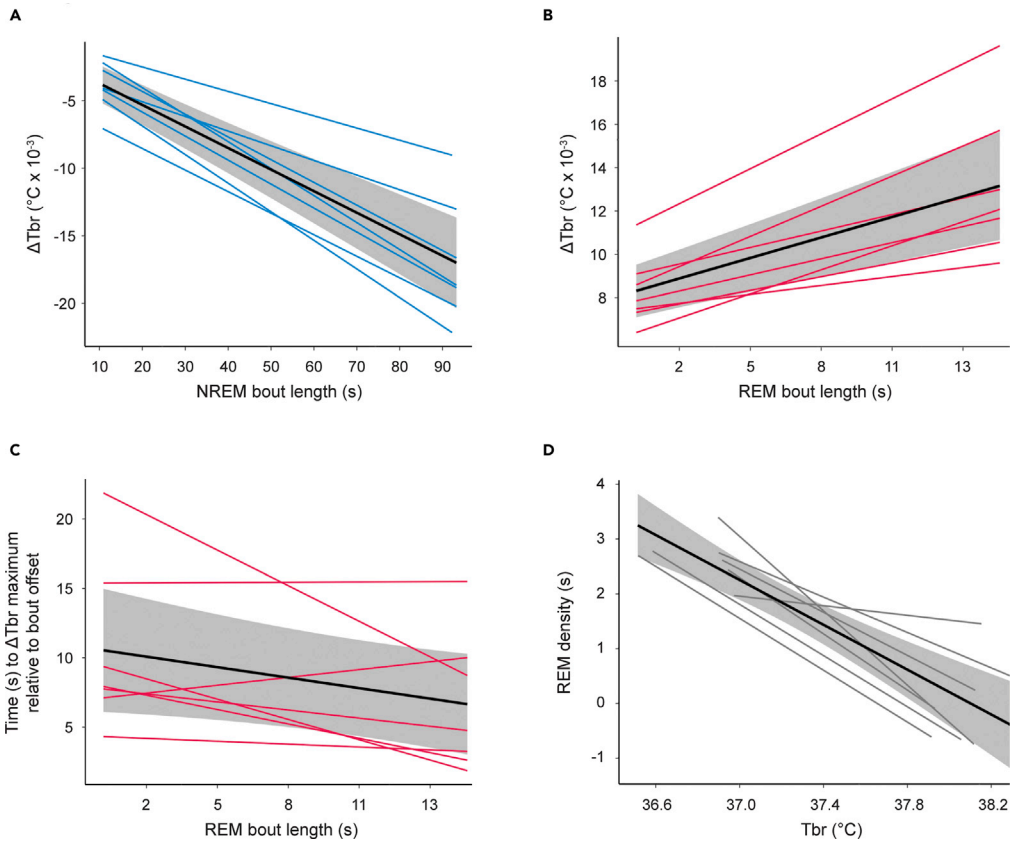

Figure 2. Relationships between Sleep States and Brain Temperature (T_{br})

(A and B) Relationship between (A) NREM and (B) REM sleep bout length and change in brain temperature in pigeons. y Axis values are the peak decreases and increases in T_{br} associated with bouts of NREM and REM sleep, respectively. (C) Relationship between REM sleep bout length and the time to the peak maximum T_{br} relative to the bout offset. (D) Relationship between REM sleep density (seconds per 15 s, see Methods) and T_{br}. Each plot shows the regressions for each pigeon (in blue and red for NREM and REM sleep, respectively) and the estimates (in black) with 95% confidence intervals (gray).

of brain warming might be due to blood flow to the brain. Either the increase in neuronal firing rate during REM-like sleep does not induce changes in blood flow or blood flow does increase, but a sufficient temperature gradient does not exist between the brain and body core for this flow to cause brain warming. In this regard, it would be important to know if dragons retain more heat acquired through basking and activity in the body than the head, due to the lower surface area to volume ratio of the body. It would also be informative to determine whether blood flow increases, as shown in rats (Bergel et al., 2018), during REM-like sleep in bearded dragons.

Our findings are consistent with the notion that sleep state-related changes in temperature are a trait unique to endotherms. However, more species of birds, as well as mammals, need to be examined to determine whether this is a general pattern. In addition, more reptiles need to be examined, especially given that the changes in brain activity that define sleep states in bearded dragons have not been found in all reptiles examined (Libourel and Herrel, 2016; Libourel et al., 2018). It would also be interesting to examine small and large reptiles, as T_{br} during sleep might depend on a reptile's size-dependent ability to retain core heat acquired through basking and physical activity (Tattersall, 2016). Investigating sleep and T_{br} in tegu lizards (*Salvator merianae*) that exhibit facultative endothermy during the reproductive season (Tattersall et al., 2016; Tattersall, 2016) or in Galapagos marine iguanas (*Amblyrhynchus cristatus*) that undergo periods of cooling while feeding at sea (Bartholomew, 1966; Butler et al., 2002), would also be informative. Finally, T_{br} should also be examined in other ectothermic animals, such as zebrafish and

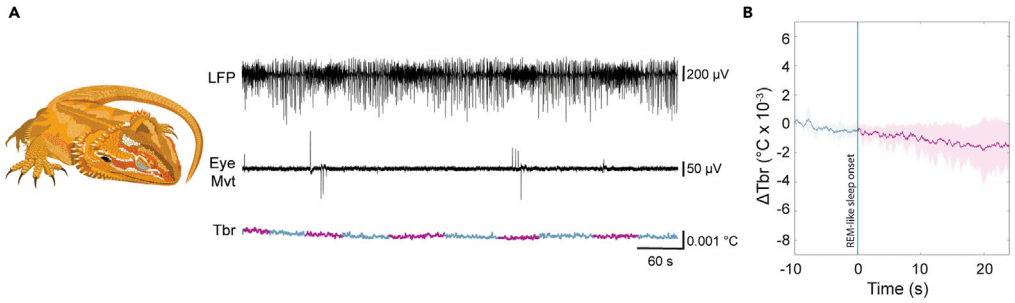

Figure 3. Brain Temperature (T_{br}) during NREM-like and REM-like Sleep in Bearded Dragons

(A) 10-min local field potential (LFP) recording from the dorsal ventricular ridge (DVR) of a sleeping bearded dragon showing state-related changes in brain activity. NREM-like sleep is characterized by higher LFP amplitude and δ [0.5–4 Hz]/β [11–30 Hz] ratio, when compared with REM-like sleep. The brain states are color coded (NREM-like sleep, cyan; REM-like sleep, magenta) in the bottom T_{br} recording. The electrooculogram (Eye Mvt) shows the association between eye movements and brain state.

(B) T_{br} recorded from the DVR of sleeping bearded dragons 10 s before and 30 s after the transition (vertical blue bar) between NREM-like and REM-like sleep. T_{br} values (mean ±1 standard deviation; see Figure S1 for individual data) are expressed relative to T_{br} 10 s before the transition. Note the absence of an increase in T_{br} following the transition between sleep states. The slight drop in T_{br} across the two states reflects the decline in T_{br} across the night (Figure S1). Bearded dragon illustration by Damond Kyllo.

cephalopods, wherein REM-like sleep states have been described (Leung et al., 2019; Iglesias et al., 2019).

Our findings contribute to, but do not resolve, the debate over proposed homology between REM-like sleep in bearded dragons and REM sleep in mammals and birds (Libourel and Barrillot, 2020). The interpretation of our findings depends on the emphasis given to specific components of REM sleep present in mammals and birds (Blumberg et al., 2020). If an increase in T_{br} is considered an essential feature of REM sleep, then our findings suggest that REM-like sleep in bearded dragons is not homologous to REM sleep in mammals and birds, and that REM sleep evolved independently in mammals and birds, perhaps in association with the independent evolution of endothermy. Alternatively, it is possible that the states are homologous and brain warming during REM sleep secondarily emerged independently in mammals and birds. Clearly, further studies are needed to define the essential components of REM sleep and their evolution (Blumberg et al., 2020; Libourel and Barrillot, 2020).

Brain warming might be an unimportant or even costly epiphenomenon of REM sleep in endotherms or an advantageous component of this state. According to the brain warming hypothesis, warming during REM sleep counteracts cooling during prior NREM sleep, and thereby prepares the animal to rapidly interact adaptively with its surroundings upon awakening (Wehr, 1992; Lyamin et al., 2018). The brain warming hypothesis is challenged by several lines of evidence (Gao et al., 1995; Lima et al., 2005; Ungurean and Rattenborg, 2018). Notably, mammals and birds sleeping in riskier situations suppress REM sleep (Lesku et al., 2008; Gravett et al., 2017; Lyamin et al., 2018; Tisdale et al., 2018a, 2018b), which, according to the brain warming hypothesis, would render them less likely to respond adaptively to a threat upon awakening. Nonetheless, it is worth considering the implications that our findings have for this hypothesis. In pigeons, brain cooling during NREM sleep and warming (albeit small) during REM sleep, as well as the increased density of REM sleep when T_{br} is low, are consistent with the brain warming hypothesis. However, even though ecologically important behaviors, such as courtship song in male zebra finches (*Taeniopygia guttata*), can occur faster at naturally occurring higher brain temperatures (Aronov and Fee, 2012), behavioral tests are needed to determine whether the small increase in T_{br} during REM sleep in pigeons has an ecologically meaningful impact on performance upon awakening. Finally, regardless of whether REM-like sleep in bearded dragons is homologous to REM sleep in mammals and birds, the absence of brain warming suggests that the primary function (if one exists) of periods of increased neuronal activity during sleep is not to warm the brain. Other processes linked to increased neuronal activity, such as learning, memory consolidation, and

brain development, are likely candidates for a shared function of increased neuronal activity during sleep.

Limitations of the Study

Our study has several limitations that should be taken into consideration. As we only studied pigeons and bearded dragons, more species need to be examined to determine whether our findings apply to other birds and reptiles. Additional studies are also needed to determine whether the relationship, or lack thereof, between brain state and T_{br} depends on ambient temperature and the animal's energetic status. The impact that REM sleep-related brain warming has on waking performance should also be examined in pigeons. Finally, additional research on bearded dragons is needed to establish the extent to which NREM-like and REM-like sleep are homologous to NREM and REM sleep in mammals and birds.

Resource Availability

Lead Contact

Further information and requests should be directed to and will be fulfilled by the Lead Contact, Niels C. Rattenborg (rattenborg@orn.mpg.de).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The brain temperature and pre-processed data used in this study are available at https://edmond.mpdl.mpg.de/imeji/collection/cWlHbILzSs1mhNKQ.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101696.

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AUTHOR CONTRIBUTIONS

G.U., P.-A.L., and N.C.R. designed the pigeon experiment. G.U. and D.M.-G. performed the pigeon surgeries. In consultation with P.-A.L. and N.C.R., G.U. conducted the experiment and analyzed the pigeon data. B.B. and P.-A.L. designed the bearded dragon experiment, performed the surgeries, conducted the experiment, and analyzed the data. G.U., B.B., P.-A.L., and N.C.R. contributed to writing the manuscript, and D.M.-G. provided comments.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Comparative Perspectives that Challenge Brain Warming as the Primary Function of REM Sleep

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Supplemental Information

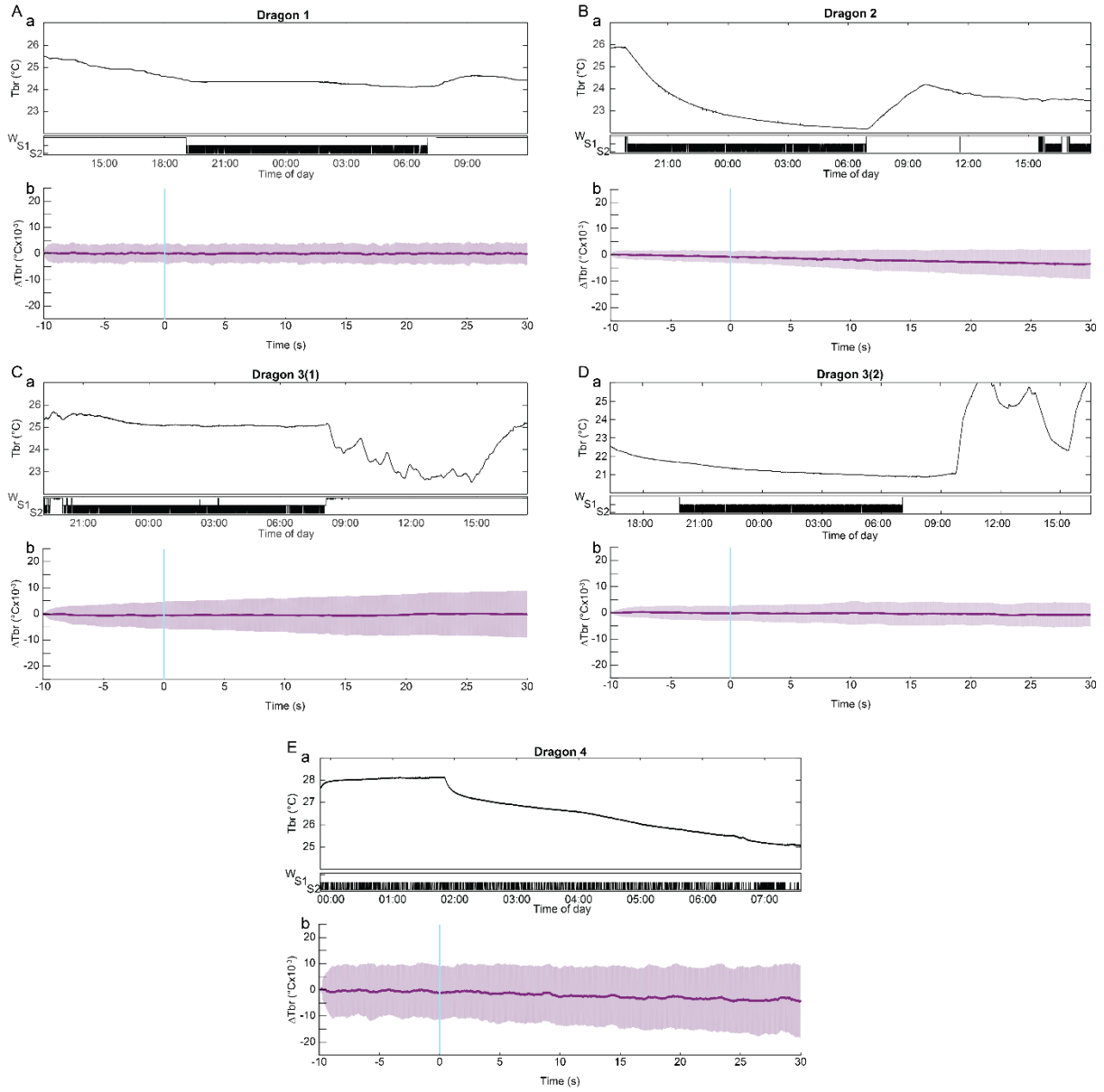

Figure S1. Brain temperature (T_{br}) across the main night-time sleep period for individual bearded dragons, Related to Figure 3B. For each individual (A-E; two nights (C and D) are shown for Dragon 3), (a) shows T_{br} with a hypnogram (bottom) showing brain state (wakefulness, W; NREM-like sleep, S1; REM-like sleep, S2) and (b) shows T_{br} recorded from the dorsal ventricular ridge 10 s before and 30 s after the transition (vertical blue bar) between NREM-like and REM-like sleep. T_{br} values (mean ± 1 standard deviation) are expressed relative to T_{br} 10 s before the transition.

Table S1. Results of the linear mixed effect models, Related to Figure 1C and D. For the last four models the predictors were scaled upon fitting the model.

Category	Response	Predictor	Fixed effects			Random effects	
						Proportion (%) of variance explained by:	
			Estimate ± SE	t-val	p-val	random intercept	random slope
Effect of bout length on ΔT_{br}	ΔT_{br}	*Intercept*	$-7.30*10^{-3} \pm 8.70*10^{-4}$	-8.39	$1.56*10^{-4}$	12.5	1.68
		NREM bout length	$3.11*10^{-3} \pm 3.31*10^{-4}$	-9.40	$8.55*10^{-5}$		
	ΔT_{br}	*Intercept*	$7.86*10^{-3} \pm 5.70*10^{-4}$	13.80	$6.48*10^{-6}$	2.80	0.02
		REM bout length	$3.37*10^{-4} \pm 5.59*10^{-5}$	6.04	$1.04*10^{-3}$		
Effect of bout length on the occurrence of min/max ΔT_{br} relative to bout offset	ΔT_{br} min occurrence	*Intercept*	-0.56 ± 0.55	-1.02	0.35	1.56	3.36
		NREM bout length	-5.93 ± 0.79	-7.54	$2.77*10^{-4}$		
	ΔT_{br} max occurrence	*Intercept*	9.22 ± 1.93	4.79	$3.05*10^{-3}$	6.33	0.30
		REM bout length	$3.37*10^{-4} \pm 5.59*10^{-5}$	-1.98	0.1		
Effect of T_{br} on the REM density	REM density	*Intercept*	2.18 ± 0.18	12.03	$2.21*10^{-5}$	1.85	0.34
		T_{br}	-0.50 ± 0.08	-5.99	$1.02*10^{-3}$		

Transparent Methods

Pigeons

Surgical procedures: Adult domestic pigeons (*Columba livia*; Budapest variety; 1 female, 6 males) were surgically instrumented for recording the electroencephalogram (EEG) from both hemispheres, neck electromyogram (EMG), and eye movements under isoflurane anesthesia (1-3% in 0.5 LPM oxygen). The birds were administered an injection of Diazepam (2 mg/kg) into the breast muscle 15 min prior to surgery, an injection of Metamizol (150 mg/kg) into the breast muscle shortly before isoflurane

anesthesia, 4 drops of Procaine (2%) for local analgesia prior to skin incision, and an intramuscular injection of Meloxicam (0.5 mg/kg) and Lidocaine gel to the wound at the end of the surgical procedure. The electrodes consisted of round-tipped gold pins (0.5 mm diameter) for the EEG and electrooculogram (EOG), and gold-plated electrode balls (1 mm diameter) for the EMG. The EEG electrodes were placed over the visual hyperpallium which is visible through a distinct window of thin bone on the surface of the cranium. Two EEG electrodes were placed on the dura overlying each (left and right) hyperpallium (2.5 and 3 mm lateral of the midline for the anterior and posterior electrode, respectively, and separated by 5 to 6 mm along the anterior-posterior axis). As part of another study, left and right pupil size and movements were detected through the closed eyelids via head-mounted micro camera modules (NanEye®, ams AG, 1 mm x 1 mm) connected to an image processing board (BAP Image Systems GmbH, AC62KUSB), and an infra-red LED (Kingbright®, 940 nm, 50 mA, 1.2 V) placed on the skull that illuminated the back of the eyes (Yüzgeç et al., 2016). For each hemisphere, a thermistor (Semitec®, 223Fμ3122-07U015, 0.28 mm diameter, thermal time constant of 20 ms, relative sensitivity 0.001°C) was inserted approximately 1 mm into the dorsal surface of the left and right hyperpallium, mid-way between the two EEG electrodes. Each thermistor was calibrated prior to implantation using a water bath and a digital thermometer.

Housing and recording: The pigeons were housed individually in an indoor aviary (1 m x 2 m x 2 m; 12 h:12 h light:dark photoperiod; 21°C) adjacent to, and in visual and auditory contact with, two other pigeons, each housed in similar aviaries. Food and water were provided *ad libitum*. After at least one week of post-surgical recovery and habituation to the recording equipment, the birds were recorded during the 12-h night while confined to their preferred sleeping perch. Electrophysiological data and head movements (accelerometry) were recorded at 256 Hz via a 2.6 g head-mounted transmitter. Images from the micro cameras were recorded via a thin, light-weight tether that allowed the pigeon to

freely move on its perch. The birds were disconnected from the tether and were free to move throughout their aviary once the lights came on in the morning.

Sleep scoring: The recordings were visually scored for wakefulness, NREM sleep, and REM sleep using standard criteria (Martinez-Gonzalez et al., 2008; Lesku et al., 2011; Tisdale et al., 2018). However, unlike previous studies which employed 4 s epochs, we used 1 s epochs to gain a more precise measure of the onset and offset of bouts of REM sleep. A bout of a given state was defined as one or more epochs of that state uninterrupted by an epoch of any other state; i.e. bouts could be as short as 1 s.

Eye movement tracking: The videos recordings of the birds' eyes were used to detect the pupil area through binary thresholding using a custom Matlab script. Variations in the position of the pupil area centroid were used as a proxy for eye movements. To correct for variation in camera position, the axes were rotated such that horizontal movements were along and vertical movements were perpendicular to the eyelid closure plane. Positive increases in signal values represent anterior and dorsal movements, respectively.

Brain temperature analysis: Although temperature was recorded from both hemispheres, in some birds, state-related changes in temperature were less evident in one hemisphere than the other, even though the changes in EEG activity were largely synchronous between the hemispheres. This variation likely reflects variation in the depth of the thermistor. Consequently, the thermistor that appeared the most responsive to state changes was selected for detailed analysis. Temperature signals were first filtered using a moving mean with a 1.0 s window. Due to frequent state changes and the fact that temperature usually peaked after a bout of REM sleep, we took the following measures to isolate the relationship between sleep state and T_{br}. For NREM sleep, we analyzed temperature starting 10 s after the onset of a bout. For REM sleep, we selected for analysis the bouts that were preceded and followed by at least 20 s of NREM sleep to avoid the overlapping effect of REM sleep bouts occurring in close succession. For

both states, changes in T_{br} were expressed relative to the temperature at the start of the analysis window. For Figure 1C, NREM sleep bouts were grouped into 10 s duration categories, and for Figure 1D, REM sleep bouts were grouped into 3 s categories. We only report data for categories that had at least 10 bouts that met the inclusion criteria for every bird. This resulted in seven categories for NREM sleep (10-19, 20-29, 30-39, 40-49, 50-59, 60-69, and 70-79 s) and five categories for REM sleep (1-3, 4-6, 7-9, 10-12, and 13-15 s). All reported values are the mean (± 1 standard deviation) of the individual bird means for each category.

Statistical analyses: On the non-grouped dataset shown in Figures 1C and D, we detected the timing and the values of the maximum decrease/increase in T_{br} for each T_{br} response curve associated with a bout of NREM/REM sleep. In the case of NREM sleep, the detection was limited to 10 s over the maximal length of the respective bout category to filter out potential effects of states occurring after the offset of a NREM sleep bout. We then tested whether longer NREM/REM sleep bouts were associated with higher decreases/increases in T_{br} using linear mixed effect models with the relative change in T_{br} (ΔT_{br}) as the response variable and the bout length as predictor. Individual identity was added as a random effect. A similar analysis was performed to test for a relationship between bout duration and the timing of the positive peaks relative to the offset of REM sleep bouts.

If one function of REM sleep is to warm the brain, then REM sleep should be expressed in higher proportions at lower T_{br}. We therefore tested for a relationship between T_{br} and the density of REM sleep during 15 s windows. For each bird, we divided the recording into 15 s windows. We then calculated the amount of REM sleep expressed in each 15 s window, and the average T_{br} for the first 7 s of the associated T_{br} data. The average for T_{br} was restricted to the first 7 s to account for the lag in response related to REM sleep bouts. The relationship was tested using a linear mixed effect model with REM sleep density as the response variable and T_{br} as predictor. Individual identity was added as a random effect. All statistical analyses were performed using RStudio. For mixed model analyses, we used

the lme4 package (Bates et al., 2015). The denominator degrees of freedom were computed with Satterthwaite's method.

Bearded dragons

Surgical procedures: 4 adult bearded dragons (*Pogona vitticeps*, 2 males, 2 females) were surgically instrumented for recording local field potentials (LFP) in the DVR, neck EMG, and eye movements under injectable ketamine (100 mg/kg) and medetomidine (200 µg/kg) anesthesia. The LFP were recorded using a bundle of six 35-µm diameter tungsten electrodes. The EMG was recorded using two stainless steel electrodes implanted in the neck muscles. The eye movements were recorded using one screw fixed in the skull on the supraorbital ridge of each eye. A calibrated thermistor (Semitec®, 223Fµ3122-07U015; or Micro-Betachip, GA100K6MCD1; the sensitivity of both sensors was around 0.001°C with a time constant of 20 ms) was inserted in the telencephalon 1 mm into the brain, targeting the DVR. The thermistor and LFP electrodes were positioned relative to the pineal eye (anterior-posterior (AP) 0 mm, medial-lateral (ML) 0 mm) and the surface of the skull (dorsal-ventral (DV) 0 mm). The thermistor positions were as follows: Dragon 1, AP -1 mm, ML 0 mm, DV -2.5 mm; Dragon 2, AP +1 mm, ML -1 mm, DV -2.5 mm; Dragon 3, AP -2 mm, ML -2 mm, DV -2.5 mm; and Dragon 4, AP +1 mm, ML +1mm, DV -2.5 mm. The LFP electrodes were placed bilaterally (AP -2 mm, ML +2 mm and -2 mm, and DV -5 mm).

Housing and recording: The lizards were housed individually in a glass terrarium (50 cm x 50 cm x 100 cm; 12 h:12 h light:dark photoperiod). Water was provided *ad libitum* and food (crickets, mealworms, and vegetables) was given every other day. After at least one week of post-surgical recovery and habituation to the recording equipment, the lizards were recorded for 3 to 7 d in their terrarium. Electrophysiological data and head movements (accelerometry) were recorded at 256 Hz via a 15g head-mounted transmitter (Massot et al., 2019). The animals were recorded at different room temperatures. Dragon 1 was recorded at a constant temperature (25°C), while the others were maintained with a more

natural temperature gradient between a ‘hot spot’ (set at 40°C) and a ‘cold spot’ (25°C). Lizards housed with a gradient exhibited a natural decrease of their core temperature across the sleep period (Figure S1).

Sleep scoring: The recordings were visually scored for wakefulness and sleep using body position, head elevation, and eye state (open or closed). The animals were considered asleep when in a “sleeping” position (lying on the ground with hind and front limbs relaxed) with the head on the ground and both eyes closed for more than a minute. The two sleep states were scored using a method previously developed for lizards (Libourel et al., 2018).

Brain temperature analysis: Temperature signals were first filtered using a moving mean with a 0.5 s window. To estimate the impact of sleep state on T_{br}, we examined T_{br} 10 s before the onset of REM-like sleep to 30 s after onset. All episodes of REM-like sleep occurring across the night were included in the analysis. The values plotted in Figure 3B are the mean of the nightly means (N = 5; one night for Dragons 1, 2, and 4, and two nights for Dragon 3); for individual bearded dragon data, see Figure S1.

Ethics statement

All experiments were conducted according to the 3R principles of animal experimentation and in accordance with the European Community Council Directive for the use of research animals. The protocols and procedures used were approved by the local ethics committee for animal experimentation (i.e., Government of Upper Bavaria for the pigeons and University Lyon 1 for the bearded dragons).

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