Survivin silencing improved the cytotoxicity of carboplatin and melphalan in Y79 and primary retinoblastoma cells

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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 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 the 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 to 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 activity, 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 biproduct 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, 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 (homologue 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 eye lids. 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 to 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 and colleagues (1987) present a 4-h plot of hypothalamic temperature occurring during wakefulness, NREM 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 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 and colleagues (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 one thousand (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 B 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 (Figure 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 = $-7.30*10^{-3} \pm 8.70*10^{-4}$, p = $1.56*10^{-4}$; β (slope) ± SE = $3.11*10^{-3} \pm 3.31*10^{-4}$, p = $8.55*10^{-5}$) (Figures 1C and 2A) and longer bouts of REM sleep resulted in greater increases in T_{br} (β_0 ± SE = $7.86*10^{-3} \pm 5.70*10^{-4}$, p = $6.48*10^{-6}$; β ± SE = $3.37*10^{-4} \pm 5.59*10^{-5}$, p = $1.04*10^{-3}$) (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 = $3.05*10^{-3}$; β ± SE = $3.37*10^{-4} \pm 5.59*10^{-5}$, 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 = $2.21*10^{-5}$; β ± SE = -0.50 ± 0.08, p = $1.02*10^{-3}$) (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 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 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 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., 2018) 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 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.

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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 Interest

The authors declare no competing interests.

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Figure Legends

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 (0 °C) 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 grey, 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) prior to 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 (0 °C) prior to REM sleep onset. Pigeon illustration by Damond Kyllo.

Figure 2. Relationship between (A) NREM and (B) REM sleep bout length and change in brain temperature (T_{br}) 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 (grey).

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 to 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.