8

THE EVOLUTION OF SLEEP: A PHYLOGENETIC APPROACH

JOHN A. LESKU
Indiana State University, Terre Haute, Indiana

NIELS C. RATTENBORG
Max Planck Institute for Ornithology—Seewiesen Starnberg, Germany

CHARLES J. AMLANER, JR.
Indiana State University, Terre Haute, Indiana

INTRODUCTION

Although scientists and physicians have been pursuing the meaning of sleep for decades, the functions of sleep remain elusive [1]. There is strong consensus that the comparative method is a powerful, yet underutilized, approach for illuminating sleep function [2–5]. Under the comparative paradigm, sleep is compared across the animal kingdom with the aim of revealing the evolutionary history of sleep. Studies utilizing this approach have demonstrated extensive variation in both the amount and phasing (e.g., monophasic—diurnal or nocturnal; or polyphasic—crepuscular or arrhythmic) of sleep across taxonomic groups [2]. Although most, if not all, vertebrates sleep, the study of sleep in these organisms is not likely to yield the fundamental function(s) for which sleep evolved. Additional adaptations likely became associated with sleep after its initial evolution. Sleep in mammals may therefore perform a tapestry of functions, making the dissection of sleep's original evolutionary function that much more difficult to understand or recognize.

The usefulness of the comparative method is clear, it is impeded by a lack of information on sleep in nonmammalian vertebrates and sleep-like behavior in invertebrates. Consequently, the electrophysiological hallmarks of mammalian sleep are often used as the ''gold standard'' for sleep in nonmammals, while sleep in other vertebrate classes has largely been neglected. Although the relative lack of information on sleep in reptiles, amphibians, and fishes has been the main obstacle to wide-ranging comparative sleep work, the relative wealth of knowledge on mammalian species has allowed for detailed comparisons among mammalian taxa [6].

Sleep is foremost a behavioral state. At the organismal level, sleep is readily identifiable by (1) behavioral quiescence, (2) increased arousal threshold, (3) rapid reversibility to wakefulness [7], (4) a species-specific sleep site and posture [8], (5) circadian organization, and (6) homeostatic regulation [9]. Both circadian rhythm and homeostatic regulation dictate propensity to sleep [5]. The circadian rhythm aligns sleep with a period(s) of the 24-hour day, whereas homeostatic regulation is a function of prior time awake. Sleep deprivation results in a ''debt that is repaid'' during successive sleep periods. In essence, sleep loss is compensated for by an increase in sleep time and intensity during recovery sleep. Intensity of sleep is measured via slow-wave activity (i.e., mean electroencephalogram power density between 0.75 and 4.0 Hz) [5] and is correlated with an increased arousal threshold following sleep deprivation.

Sleep: A Comprehensive Handbook, Edited by T. Lee-Chiong.

This review aims to provide insight into variation in sleep architecture among the vertebrate classes as well as an overview of the limited, yet increasing information on sleep-like behavior in invertebrates. We focus on the evolution of sleep as measured by electrophysiological attributes using extant organisms as models for ancestral forms.

MAMMALS

Marsupials and Terrestrial Placentals

All mammals studied to date show some form of sleep [6]. Although total sleep time and the relative proportions of the sleep stages vary greatly among taxa, all exhibit an alternating cycle of slow-wave sleep (SWS; also called quiet sleep or non-rapid eye movement (NREM) sleep), punctuated by episodes of rapid eye movement. (REM) sleep also called active sleep or paradoxical sleep). SWS is characterized by an electroencephalogram (EEG) of high-amplitude, low-frequency waves, which are the result of synchronous neuronal firing between adjacent neurons in the neocortex and thalamocortical interactions. In humans, SWS refers only to stages 3 and 4 of NREM; whereas in the animal literature, SWS usually refers to all stages of sleep other than REM sleep. During SWS, heart and respiratory rate remain steady, and thermoregulation remains functional. Eye movements (measured by the electro-oculogram (EOG)) are absent, while muscle tone (measured by the electromyogram (EMG)) persists.

REM sleep is distinguished from SWS by a low-amplitude, mixed-frequency or activated EEG. Heart and respiratory rate are irregular and thermoregulation is suspended; therefore an endotherm is essentially poikilothermic during REM sleep. Rapid eye movements are present and, unlike in wakefulness, there is EMG atonia. The mammalian sleep cycle consists of an alternation between SWS and REM sleep. Normally, the sleep cycle is entered through SWS and terminates after an episode of REM sleep with a brief awakening.

Insectivores, carnivores, and ungulates engage in drowsiness, a stage intermediate between wakefulness and sleep. Although it is probable that all terrestrial mammals exhibit drowsiness to varying degrees, it is most notable in the aforementioned groups. The EEG of a drowsy animal usually shows sleep spindles or slow waves superimposed on a background of waking EEG activity. Arousal thresholds remain low and eye states are intermediate between open and closed. The function of drowsiness is unknown, drowsiness may permit vigilance during sleep in perilous environments [6].

Monotremes

Monotremes are the earliest offshoot of the mammalian evolutionary line [10]. There are only three living representatives of monotremes: the short-beaked echidna (*Tachyglossus aculeatus*), the long-beaked echidna (*Zaglossus bruijni*), and the duck-billed platypus (*Ornithorhynchus anatinus*). EEG sleep studies have been conducted on the first and third. Understanding sleep in these egg-laying mammals may clarify the earliest sleep state from which all existing sleep states evolved.

The first sleep study on a monotreme (the short-beaked echidna) concluded that although the mammal engaged in SWS, it did not exhibit the EEG correlates and common features of REM sleep [11]. However, a reanalysis of sleep in *T. aculeatus* revealed concurrent cortical features of SWS and subcortical signs of REM sleep [12]. Specifically, the forebrain generated a high-amplitude, low- frequency EEG typical of SWS, while brainstem neurons fired with an irregular pattern similar to that observed in placental mammals during REM sleep. Thus the echidna exhibited a state composed of markers common to both SWS and REM sleep, suggesting that the two temporally distinct states arose in the placental and marsupial mammalian clade from a single, heterogeneous sleep state. Some controversy persists, however, over the characterization of sleep states in the echidna. In a subsequent study, Nicol et al. [13] reported SWS and REM sleep characterized by an activated EEG, reduced tonic EMG, intermittent EOG activity, and decreased heart rate, typical of REM sleep in placental and marsupial mammals.

The only study on sleep in the platypus identified both SWS and REM sleep [14]. As in the echidna, sleep was characterized by a high-amplitude, low-frequency EEG, typical of SWS. Although brainstem neuronal units were not recorded, the platypus showed bursts of rapid eye movements and twitching of the bill and head, similar to phasic skeletomuscle activity in other mammals during REM sleep. Arousal thresholds were higher in REM sleep than in SWS, again consistent with placental and marsupial mammals. Calculations of REM sleep time, based on the temporal distribution of eye movements and twitches, suggest that the platypus spends more time in REM sleep than any other animal studied.

Aquatic Mammals

There are three extant aquatic mammalian orders: Cetacea, Pinnipedia, and Sirenia. Aquatic mammals are conflicted by the need to simultaneously sleep, surface to breathe, and maintain vigilance. Several species of aquatic mammals appear to have overcome this conflict by engaging in unihemispheric slow-wave sleep (USWS), a unique state during which one cerebral hemisphere shows EEG activity indicative of SWS, while the other hemisphere shows activity indicative of wakefulness (Figure 8.1; reviewed in [15]). Interhemispheric asymmetries in the

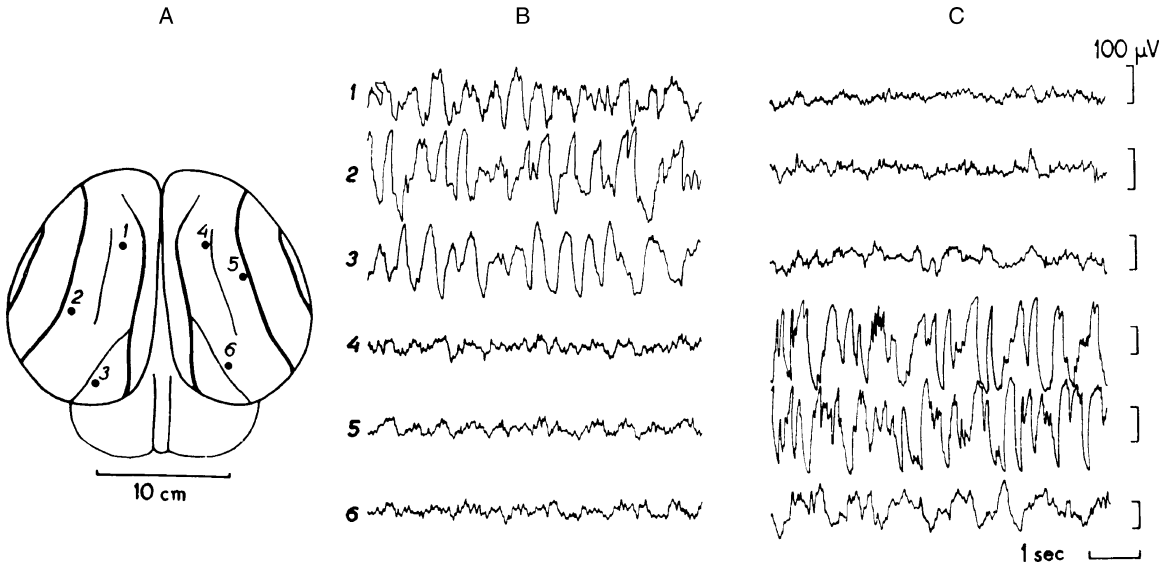

Figure 8.1 EEG recorded from the parieto-occipital cortex (A) of a bottlenose dolphin (*Tursiops truncatus*) during unihemispheric slow-wave sleep (USWS). (B) The left hemisphere (1–3) shows high-amplitude, low-frequency EEG activity denoting SWS, whereas the right hemisphere (4–6) is awake with low-amplitude, high-frequency EEG activity. (C) The interhemispheric asymmetry is reversed and the left hemisphere is awake while the right hemisphere sleeps. (Reprinted from Mukhametov et al., Interhemispheric asymmetry of the electroencephalographic sleep patterns in dolphins, *Brain Research* **134**:581–584. Copyright © 1977, with permission from Elsevier Science.)

EEG are associated with interhemispheric asymmetries in temperature, with the sleeping hemisphere having a lower parietal cortex temperature relative to the awake hemisphere [16]. During USWS, the eye contralateral to the sleeping hemisphere is usually closed, while the eye contralateral to the awake hemisphere is open.

USWS has been identified in five cetacean species: pilot whale (*Globicephala scammoni*), bottlenose dolphin (*Tursiops truncates*), common porpoise (*Phocoena phocoena*), Amazonian dolphin (*Inia geoffrensis*), and the beluga whale (*Delphinapterus leucas*). In all cetaceans examined, USWS is the predominant form of SWS; only rarely do cetaceans engage in unambiguous bihemispheric slow-wave sleep (BSWS). Interestingly, REM sleep either is absent, is greatly reduced, or occurs in a modified form in cetaceans; therefore the majority of sleep time is USWS. Bottlenose and Amazonian dolphins may swim slowly or hover at the surface of the water during USWS, while periodic fin movements maintain a stable posture. Furthermore, they breathe periodically without arousing to bilateral wakefulness. Interestingly, BSWS induced via pentobarbital administration, inhibit respiration [17], suggesting that USWS in cetaceans is, in part, an adaptation to maintain motor activity to allow surfacing to breathe. However, dolphins may also engage in USWS to monitor their environment. During USWS in a captive bottlenose dolphin, a visual stimulus presented to the open eye elicited a behavioral and electrophysiological response, such that the animal aroused to bilateral wakefulness. Indeed, Goley [18] found that Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) kept their open eye on adjacent dolphins, perhaps to maintain relative position within the pod. Thus USWS may be used during long-distance migrations as it allows both vigilance and motor control concurrent with sleep.

Selective sleep deprivation studies in dolphins have been instrumental in identifying the biological targets benefiting from sleep. USWS allows for one hemisphere to be deprived of sleep while permitting sleep in the other. As deprivation continues, only the deprived hemisphere increases its attempt to fall asleep and when allowed to recover from sleep deprivation, only the deprived hemisphere exhibits a rebound in SWS, indicating that sleep is homeostatically regulated independently within each hemisphere [19] and that sleep benefits primarily the brain and not the body, a finding consistent with the fact that cetaceans may continue to swim while sleeping.

Within the order Pinnipedia there are three families: Odobenidae (walruses), Otariidae (eared seals: fur seals and sea lions), and Phocidae (true seals). Electrophysiological sleep studies have been conducted in eared and true seals, yet no such study has been attempted in walruses. Pinnipeds, unlike cetaceans, partition their time between terrestrial and aquatic environments and may therefore sleep both in and out of the water. In contrast to cetaceans, much of SWS in eared seals is BSWS, rather than USWS. Eared seals engage in USWS both in and out of the water, with the proportion of SWS composed of USWS being greater during sleep in the water. Also unlike cetaceans, eared seals engage in unequivocal REM sleep.

Interestingly, REM sleep as a percent of total sleep time decreases in the water, when compared to sleep on land. Like cetaceans, however, eared seals engage in USWS to allow respiration concurrent with sleep. For example, fur seals assume a stereotypic sleep posture in the water with three flippers in the air while one flipper paddles in order to keep the nares above the water's surface. Conversely, true seals only display BSWS and REM sleep and must hold their breath while asleep at sea. Periodic, brief awakenings permit motor control and the seal surfaces to breathe. On the other hand, elephant seals floating near the surface simply raise their heads above the water and breathe without arousing to wakefulness.

Manatees (Order: Sirenia) also engage in USWS [20]. A captive Amazonian manatee (*Trichechus inunguis*) exhibited REM sleep (1% of recording time) and SWS (27% of recording time) with 25% of SWS being unihemispheric. However, during bouts of USWS the manatee remained motionless underwater [20]. USWS in the manatee was not used to allow respiration concurrent with sleep; rather the manatees aroused to bilateral wakefulness for each respiratory act. In this instance, USWS may serve another function, such as predator detection.

Correlates of Sleep Architecture in Mammals

Correlational studies have led to various hypotheses on sleep function and factors influencing sleep architecture. For example, there are two clear energy conservation hypotheses about mammalian sleep [6]. Energy conservationists suggest that sleep limits energy expenditure by reducing metabolic rate below that accomplished by rest alone. Alternatively, sleep may enforce rest, which, in turn, limits energy expenditure. If so, one would predict that mammals with higher mass-special metabolic rates would sleep more, and although this prediction was initially supported (Table 8.1) [21], the relationship was negative after controlling for body weight via partial correlation (as mammals with higher mass-specific metabolic rates actually sleep less) [22]. Elgar et al. [22] suggest that animals with higher mass- specific metabolic rates need to allocate more time to foraging than those with lower mass-specific metabolic rates. Foraging time may thus limit sleep time.

Potential predation may restrict REM sleep time as REM sleep is negatively correlated with an index of ''overall danger'' [23]. Under these conditions, prey species should not engage in lengthy bouts of REM sleep and it's associated high arousal thresholds due to increased predation threat. Elgar et al. [22] did not address the potential role of predation in influencing sleep architecture; however, they did demonstrate that geographic latitude correlated positively with cycle length after controlling for body weight. Presumably, the inhibition of thermoregulatory mechanisms (which occurs during REM sleep) limits the amount of time an animal in a temperate climate can endure long periods of uninterrupted REM sleep. Theoretically, extended bouts of REM sleep would be detrimental to an endothermic animal if the ambient temperature were significantly below that of thermoneutrality. Precocial species, those that are relatively self-sufficient at birth, exhibit less REM sleep as adults than altricial species, those that are relatively helpless upon birth or hatching [22, 23]. However, since precocial animals are generally larger than their altricial counterparts, Elgar et al. [24] reanalysized a revised version of their 1988 dataset and revealed that although altricial families have significantly more REM sleep than precocial families, this correlation was no longer significant after controlling for body weight.

Mammals with greater mass-specific metabolic rates may sleep longer [21], leading Siegel and co-workers to hypothesize that increased sleep requirements are necessary as a consequence of increased metabolic rate and the associated production of free radicals [25, 26], which are detrimental to protein structure. Finally, since sleep is likely of principal importance to the brain rather than to the body [1], one would expect larger brains to require more sleep; however, brain weight negatively correlated with total sleep time, suggesting that larger brained organisms may be able to handle sustained periods of wakefulness better than smaller brained organisms.

TABLE 8.1 Summary of Correlational Studies[a]

Variables	Cycle Length (min)	Metabolic Rate (cm^3 O_2/g/h)	Body Weight (kg)	Brain Weight (g)	Life Span	Overall Danger Index	Geographic Latitude
Total sleep time (h/day)	−[21]	+[21]/−[22]	−[22]	−[21, 22]	−[21]	?	?
SWS (h/day)	−[21]	+[21]/−[22]	−[22, 23]	−[21–23]	−[21, 23]	−[23]	?
REM sleep (h/day)	ns [21]	+[21]/−[22]	−[22, 23]	−[21–23]	−[21, 23]	−[23]	?
Cycle length (min)	n/a	−[21]/+[22]	+[22]	+[21, 22]	+[21]	?	−[22]

[a]A plus (+) denotes a significant ($p < 0.05$) positive correlation between the two variables, a minus (−) denotes a significant ($p < 0.05$) negative correlation, and ns means that the correlation was not significant. A question mark (?) indicates that the correlation was not calculated. For the correlations of metabolic rate with various sleep characteristics, Elgar et al. [22] calculated a partial correlation between total and SWS times with metabolic rate, after controlling for adult body weight, whereas Zepelin and Rechtschaffen [21] correlated sleep characteristics with mass-specific metabolic rate.

All of these correlational studies, however, are fraught by a common concern. Zepelin and Rechtschaffen [21] and Allison and Cicchetti [23] used each species as a statistically independent unit, whereas Elgar et al. [22] pooled data at the family level. However, the simple phylogenetic framework used by Elgar et al. [22] is no longer considered valid. Thus all studies experienced pseudoreplication since phylogenetic relationships between taxa were neglected. Data from mouse to elephant, bat to human, and horse to kangaroo were weighted equally against one another. Future work should readdress these datasets under an explicit, phylogenetic context using independent contrasts to control for pseudoreplication where possible.

BIRDS

The EEG of a sleeping bird is similar to that of a sleeping mammal; and yet, birds are more closely related to extant reptiles (i.e., crocodilians) than they are to mammals. A sleeping bird meets all of the behavioral criteria listed for mammals (Figure 8.2) and exhibits both SWS and REM sleep. Birds, like mammals, may be classified as nocturnal, diurnal, crepuscular, or arrhythmic with respect to their timing of sleep. A waking bird exhibits bilateral eye opening, complex body movements, an activated, low-amplitude, mixed-frequency EEG, a highly active EOG, highly tonic EMG, variable heart rate, and low arousal threshold. As in mammals, avian SWS is characterized by high-amplitude, low-frequency EEG activity (reviewed in [27]). Interestingly, sleep spindles and K-complexes during SWS are absent. Neck EMG is typically tonic without phasic events. Eye movements are infrequent with the exception of brief, high-frequency oscillations of the eye. Heart, respiratory, and metabolic rate are all stable and reduced relative to waking.

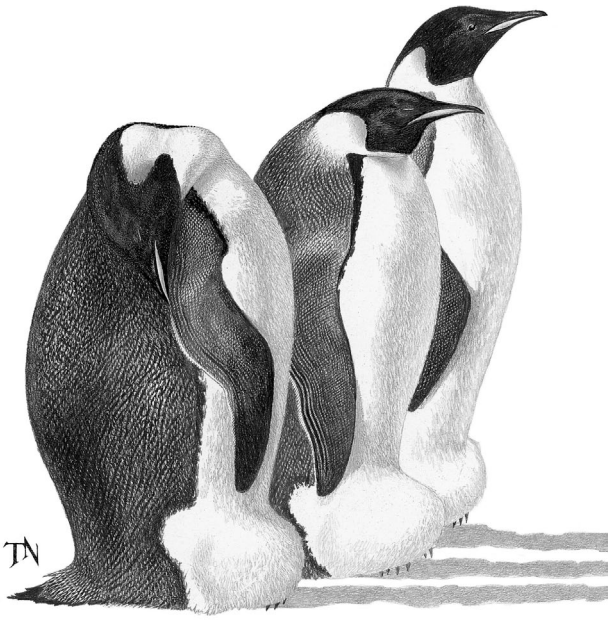

Figure 8.2 Emperor penguins (*Aptenodytes forsteri*) displaying the typical avian head postures associated with wakefulness (right) and sleep (left and middle). (Courtesy of Grass-Telefactor, An Astro-Med, Inc. Product Group.)

REM sleep is characterized by the highest arousal threshold and a relatively high-frequency, low-amplitude EEG. Birds typically show much less REM sleep than mammals and periods of REM sleep are usually shorter than 10 s. Eye movements are present in clusters. Hippocampal theta waves and PGO spikes have not been recorded during avian REM sleep. Heart rate is either variable or may increase or decrease. Finally, as in mammals, thermoregulation is inhibited during REM sleep in birds.

In mammals, sleep intensity is gauged by slow-wave activity (SWA). Unlike in mammals, sleep deprivation does not appear to result in an increase in SWA in subsequent bouts of sleep in birds. However, sleep duration and amount of REM sleep all increase during recovery after sleep deprivation. Interestingly, migratory songbirds, such as the white-crowned sparrow (*Zonotrichia leucophrys gambelii*), appear to reduce their amount of time sleeping by over 60% during migration [28].

The ontogenesis of avian sleep is similar to that of mammalian sleep patterns. Domestic fowl exhibit EEG components of an adult bird (i.e., SWS and REM sleep) while in the egg, but unlike most natal mammals, birds experience little REM sleep prehatch. SWS is identifiable by day 17 and is of typical form by day 18. REM sleep appears during day 18 or 19, just before hatching on day 20. REM sleep declines rapidly in chickens from 16.5% to 6.4% by the end of posthatch day 3.

Like aquatic mammals, birds engage in USWS [15, 27, 29]. Avian USWS is defined by unilateral eye closure (UEC) and associated interhemispheric asymmetries in SWS-related EEG activity. However, the degree of interhemispheric asymmetry in birds is small compared to aquatic mammals. USWS has been reported in 8 species across 6 avian orders and UEC has been reported in 29 species from 13 orders. Spooner [30] was the first to demonstrate an association between asynchronous eye closure and interhemispheric asymmetries associated with USWS. However, the function of avian USWS remained unclear until Rattenborg et al. [31, 32] showed that USWS allows birds to maintain vigilance for predators while sleeping. Using the ''group edge effect'' paradigm (birds on the periphery of a group perceive greater danger than those in the center), birds on the outside of the group increased their use of USWS by 150% relative to the birds in the middle and showed a strong preference for directing their open eye

away from the other birds and toward potential threats. Not only does this show the adaptive significance of USWS, it also reveals the plasticity of the trait under changing predation regimes. Unihemispheric REM sleep has never been reported in birds (or mammals).

Correlates of Sleep Architecture in Birds

Only two comparative studies exist that examine the correlates ofsleep architecture in birds [33, 34]. Amlaner and Ball [33] calculated correlations between total sleep time (TST) and environmental (latitude and hours of daylight) and ecological (sleep exposure, social sleep index, and an index of vulnerability or exposure to potential predation) factors thought to affect sleep architecture. However, they did not differentiate between SWS and REM sleep as their dataset was based totally on a behavioral definition of sleep. (At the time, there existed very little information on sleep architecture in wild birds—an ongoing limitation in comparative bird studies even today; see [34].) Like the correlational studies in mammals, there was great interspecies variability of TST. The average TST was 7 h. There was a negative correlation between TST and latitude and for species experiencing longer relative day length. Birds at arctic latitudes ($>68.00°$) during the summer, when day length is virtually 24 h, averaged a TST of 3.7 ± 1.3 h (mean $\pm$ s.e. $n = 12$). This may reflect (1) a bird's need to increase vigilance during the long daylight hours, (2) the need to accomplish more important behaviors at the expense of sleeping (a natural form of sleep deprivation), and/or (3) the direct alerting effects of light. A stepwise multiple regression based on these variables revealed that day length accounted for 49.1% ($r = -0.7$) of the variance contributing to TST; however, latitude was also a good predictor of TST ($r = -0.62$).

Schmidt [34] used an electrophysiological dataset that did differentiate between SWS and REM sleep. Like mammals, SWS time correlated negatively with resting mass-specific metabolic rate. However, the correlation between SWS time and body weight was not significant. SWS time was highly conserved between taxonomic orders, whereas REM sleep time varied significantly between orders. Since altricial mammals have more REM sleep than precocial mammals, one would expect birds to exhibit a large proportion of REM sleep, as birds are extremely altricial at birth. However, although birds have comparable amounts of SWS as mammals, they exhibit half the amount of REM sleep [34]. Interestingly, precocial birds (Order: Sphenisciformes, Anseriformes, and Galliformes) exhibited more REM sleep (but not SWS) than altricial orders (Order: Columbiformes, Psittaciformes, Strigiformes, Passeriformes). Arboreal birds require less REM sleep than either terrestrial or aquatic birds, or mammals. Most passerines appear to have extremely small amounts of REM sleep (3–10 min per 24 h), although 16% of sleep is REM sleep in white crowned sparrows [28]. Amlaner and Ball [27] speculated that passerines engage in less REM sleep because perching, which requires muscle tone, is inhibited during REM sleep. Alternatively, Schmidt [34] suggests that since passerines have larger optic lobes (relative to other avian orders) they require less REM sleep, although details and empirical support for this hypothesis are lacking. Diurnal birds did not differ in amounts of either SWS or REM sleep relative to nocturnal or polyphasic birds.

REPTILES

The class Reptilia is composed of four orders: Crocodilia (alligators, caimans, crocodiles, and gharials), Chelonia (tortoises and turtles), Squamata (lizards and snakes), and Rhynchocephalia (tuataras). Sleep has been investigated in all orders with the exception of Rhynchocephalia and all representatives studied exhibited sleep, according to behavioral criteria. However, the electrophysiological correlates of behavioral sleep in reptiles are often inconsistent and contradictory, sometimes within the same species [2, 3, 5, 35]—thus inferring the evolutionary pathway of sleep in birds and mammals from reptilian studies has been impeded and the need for future reptilian work is apparent.

Order: Crocodilia

Animals of this order are the closest extant relatives to modern-day birds. One might therefore expect crocodilian sleep architecture to be similar to that of birds (i.e., SWS and REM sleep). Studies in the caiman (*Caiman sclerops*) have been most telling. High-voltage sharp spikes in the EEG were prominent during periods of behavioral quiescence and were reduced upon arousal [36]. After sleep deprivation, there was an increase in spike activity similar to the rebound in slow-wave activity (SWA) in mammals. Interestingly, some studies have reported SWA (i.e., high-amplitude, low-frequency activity) reminiscent of mammalian SWS [37, 38]. Warner and Huggins [37] attribute the difference between their findings and that of Flanigan et al. [36] to the presence of other caimans in their study, which, presumably, relaxed their animals. REM sleep was not observed in either study. Unilateral eye closure (UEC) has been observed in caimans, but the possibility of unihemispheric sleep was not investigated [37].

Order: Testudines

Although turtles and tortoises sleep, their sleep apparently does not resemble mammalian and avian SWS or REM sleep. Box turtles (*Terrapene carolina*) [39] and the red-

footed tortoise (*Geochelone carbonaria*) [40] both meet the behavioral criteria for sleep: a species-specific sleep posture, behavioral quiescence, increased arousal threshold, rapid reversibility, and homeostatic regulation. Additionally, both had an EEG of high-amplitude spiking activity superimposed over a low-voltage background. EEG spikes disappeared with spontaneous or induced arousal. Spiking activity increased after sleep deprivation and was associated with increased arousal thresholds. Neither classical high-amplitude slow waves nor REM sleep was observed in either species. However, administration of atropine sulfate—a cholinergic blocking agent that increases mammalian slow waves—increased spiking activity in red-footed tortoises [41]. Furthermore, administration of parachlorophenylalanine—a serotonin synthesis inhibitor that suppresses mammalian slow waves—reduced spiking activity in the three tortoises studied, suggesting that these spikes are homologous to mammalian SWS [42]. UEC was reported for both box turtles and the red-footed tortoise [39, 40]. In the marginated tortoise (*Testudo marginata*), quiescence was associated with bilateral eye closure, body relaxation, increased arousal threshold, reduced EMG activity, and high-voltage slow waves. Likewise, the EEG of the yellow-footed tortoise (*Testudo denticulata*, now *Geochelone denticulata*) exhibited high-voltage spiking activity during sleep. In contrast, the loggerhead sea turtle (*Caretta caretta*) did not show spiking activity or slow waves during behavioral sleep. One study on the European pond turtle (*Emys orbicularis*) reported both SWS and REM sleep, a result that remains unreplicated.

Order: Squamata

Sleep in the desert iguana (*Dipsosaurus dorsalis*) [43] and spiny-tailed iguana (*Ctenosaura pectinata*) [44] consisted of a reduction of EEG amplitude and frequency relative to waking. Upon arousal, EEG amplitude increased in brainstem and forebrain electrodes [44]. Eye movements occurred at 4–25 min intervals, reminiscent of mammalian REM sleep [44]; however, this may be recording artifacts due to brief awakenings or nictitating membrane activity and not reflective of true REM sleep. Following sleep deprivation, the green iguana (*Iguana iguana*) and spiny-tailed iguana exhibited a rebound in sleep as indicated by total sleep time and by the increased frequency of high-voltage spikes. Chameleons (*Chamaeleo jacksoni* and *Chamaeleo melleri*) also exhibited bursts of high-voltage spikes during sleep.

REM sleep in reptiles appears to be ambiguous based on historic reports. Although REM sleep has been reported in the chameleon (*Chamaeleo* sp.) [45], desert iguana [43], and spiny-tailed iguana [44, 46], in the desert iguana, REM sleep was characterized by an increase in EEG amplitude to that of wakefulness with a concurrent atonic EMG [43]. REM sleep was temperature sensitive and appeared with greater incidence at higher temperatures. Conversely, other studies have not observed REM sleep in either the green or spiny-tailed iguana [47] and SWS has never been reported in squamates.

Despite the pervasiveness of UEC in squamates, only one behavioral study has explicitly investigated UEC in reptiles. Mathews et al. [48] demonstrated that the frequency of UEC increased in the western fence lizard (*Sceloporus occidentalis*) following exposure to a predator. Furthermore, lizards preferentially directed their open eye toward the last known position of the predator, suggesting at least a predator-detection function for UEC in reptiles similar to that of birds [31, 32].

Reptiles exhibit unambiguous behavioral sleep that is associated with concurrent changes in brain activity. However, the inconsistencies among and within species and between studies make interpretation difficult from many perspectives. High-voltage spikes are present in most studies, yet absent in others, which may reflect underlying methodological differences between studies, such as laboratory adaptation, electrode placement, and ambient temperature. Interestingly, it has been proposed that high-voltage spikes may occur in a state that is the precursor to mammalian and avian SWS. Sleep in reptiles appears to be homeostatically regulated as deprivation results in a reduction of sleep latency and an increase in sleep time when allowed to proceed. Furthermore, following sleep deprivation there is often an increase in spiking activity, leading some to suggest that spiking may reflect sleep intensity. Along these lines, it has been suggested that the spiking activity of reptiles resembles subcortical spiking during SWS in mammals. The fact that reptiles have a comparatively small cortex has also led some to suggest that reptiles lack the neuroanatomy necessary to generate SWS-related SWA. However, the minimal amount of cortex required to generate SWA remains unclear. Finally, the prevalence of UEC in crocodilians [37], chelonians [39, 40], and squamates (Family: Chamaeleonidae [45], Iguanidae [44, 47], Phrynosomatidae [48]) suggests that reptiles are able to engage in unihemispheric sleep; however, no study to date has examined the EEG correlates of UEC in reptiles.

AMPHIBIANS AND FISHES

Evidence for the existence of sleep in both amphibians and fishes is ambiguous [3, 5]. Although some species may show signs of behavioral sleep, others do not. Three species of tree frog (*Hyla cinerea*, *H. septentrionalis*, and *H. squirrella*) were monitored in the lab with EEG. Behavioral sleep was associated with decreased amplitude and increased frequency relative to waking (opposite of the typical mammalian/avian pattern). Arousal thresholds were not measured. The EEG of the common frog (*Rana temporaria*) showed

increased low-frequency activity during periods of behavioral quiescence and reduced muscle tone and bradycardia. Alternatively, captive bullfrogs (*Rana catesbeiana*) may not sleep; a tentative conclusion based on a lack of reduction in responsiveness to electrical stimuli and continuous bilateral eye opening. Furthermore, no EEG changes were noted during quiescence, although bullfrogs exhibited an EEG similar to that of sleeping tree frogs (i.e., decreased EEG activity). Sleep-like behavior in the western toad (*Bufo boreas*) was accompanied by a slowing of the EEG. However, high-voltage, fast spiking activity has also been reported during sleep-like behavior in the toad.

Behavioral sleep in amphibians may occur in the absence of changes in brain activity. Conversely, electrophysiological changes associated with sleep may be observed in the absence of accompanying behavioral measures of sleep. The few amphibian studies that report EEG correlates of behavioral state also contain significant interstudy differences that impede interpretation. It is also possible that recorded sleep patterns may not be representative of natural patterns. Experimental animals may have been stressed due to novel laboratory conditions and they may require a more prolonged acclimation period. Of course, these criticisms could be said of many laboratory studies that involve wild animals belonging to all taxa.

Electrophysiological data on fishes is scarce. Indeed only two studies have recorded neuronal activity during periods of behavioral quiescence. Sleep-like periods in the catfish (*Ictalurus nebulosus*) were defined by decreased behavioral motility and, unlike wakefulness, were associated with EEG slow-wave activity and spiking. Spiking disappeared upon arousal. Conversely, a study on the tench (*Tinca tinca*) did not find an association between behavioral state and variation in EEG activity, but observed decreased muscle tone and respiratory rate during sleep-like behavior. One laboratory reported decreased sleep latency in carp following 96 h of sleep deprivation. Similarly, sleep-deprived perch exhibited rebound in sleep during recovery. Thus, as in amphibians and reptiles, although the electrophysiological correlates of sleep in fishes are ambiguous, fish show behavioral signs of a homeostatically regulated sleep-like state.

INVERTEBRATES

It is now evident from behavioral, pharmacological, and electrophysiological data, mostly on terrestrial arthropods, that invertebrates engage in sleep or a state homologous to sleep [3, 5]. The fruit fly [49] (reviewed in [50]), cockroach, scorpion, honey bee, butterfly, locust, and mosquito all exhibit sleep-like behavior, characterized by increased arousal thresholds and associated postures. The deprivation of sleep-like behavior in cockroaches resulted in a subsequent rebound effect. Scorpions are the oldest extant arthropod group and exhibit three distinct vigilant states: (1) activity, (2) alert immobility, and (3) relaxed immobility. Arousal thresholds, measured by response to mechanical stimulation, were lowest for alert scorpions and highest for relaxed scorpions [51]. Like cockroaches, sleep-like behavior deprivation enhanced sleep-like behavior when allowed to proceed unimpeded. Honey bee (*Apis mellifera*) sleep-like behavior follows a circadian rhythm. Furthermore, it is monophasic and persists in constant darkness, indicating that it is not a direct response to photoperiod. Optomotor interneurons in the optic lobes of honey bees showed circadian rhythm in response to moving visual stimuli [52]. Sensitivity was higher during the day than during the night and corresponded with an increase and decrease in locomotor activity, respectively. Head position was the lowest and antennal immobility was the greatest during sleep-like behavior [53]. Like mammals and birds, sleep-like behavior decreased with increasing age. EEG recordings have been achieved from the mushroom bodies of sleeping honey bees [54]. Spiking activity increased with behavioral rest, characterized by antennal immobility. Following 12 h sleep deprivation via forced activity, bees showed a decreased latency and increased total time of antennal immobility, suggesting sleep in honey bees is homeostatically regulated [55].

Sleep in *Drosophila* has been characterized by behavioral quiescence, increased arousal threshold, and homeostatic regulation [49, 56]. Sleep deprivation studies using per^{01} mutant fruit flies (i.e., flies that lack a circadian rhythm) have revealed *Drosophila* sleep to be homeostatically regulated (Figure 8.3). Furthermore, stimulants and hypnotics affect *Drosophila* sleep in a manner similar to mammalian sleep. Young fruit flies exhibit significantly higher total sleep time than older fruit flies, a pattern typical of mammals and birds. Interestingly, chronic sleep deprivation results in fly death after approximately 70 h [57]. *Drosophila* also exhibit stereotypic correlates of neuronal activity depending on behavioral state. Nitz et al. [58] recorded local field potentials (LFPs) from the medial brain between the mushroom bodies of *Drosophila* in various behavioral states. Awake, interactive flies exhibited spike-like potentials in their LFPs, which disappeared with behavioral quiescence. Taken in concert, this suggests that behavioral quiescence in *Drosophila* is homologous to vertebrate sleep.

Many genes are differentially expressed in *Drosophila* due to changes in behavioral state independent of the circadian clock [59]. Sleep architecture and timing appear to have a strong genetic component [60]. Similar genes are up- and down regulated within sleep/wake states in *Drosophila* [61] and in the rat [62], further suggesting that sleep in the fruit fly is homologous to mammalian sleep. Specifically, certain ''waking'' genes in *Drosophila* are activated

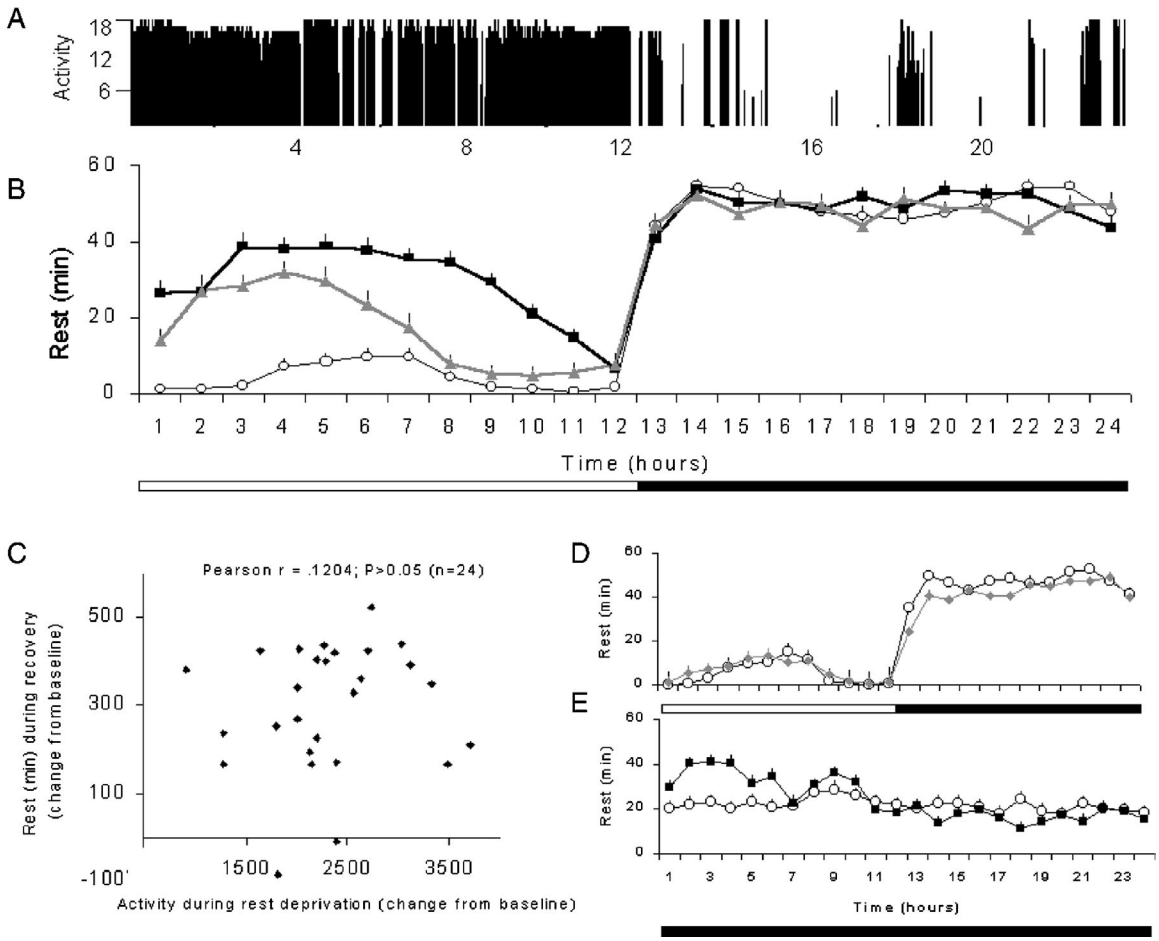

Figure 8.3 (A) The activity record for *Drosophila* maintained on a 12L:12D (open horizontal bar:dark horizontal bar) light cycle. (B) Sleep–activity cycle of undisturbed flies (circles) and flies sleep deprived via manual stimulation (squares) or by an automated system (triangles). Sleep-deprived flies showed an increase in sleep during the subsequent light period. (C) The amount of sleep during the 12 h recovery period was not correlated with the amount of activity during sleep deprivation. (D) Stimulation of flies during the light period did not result in a compensatory increase in sleep during recovery (diamonds) relative to baseline (circles). (E) Under constant darkness, per^{01} flies had the same amount of sleep as under a light–dark photoperiod but sleep was evenly distributed across the 24 h (circles). Twelve hours of automated sleep deprivation resulted in an increase in sleep during the first 6 h of recovery (squares) compared to baseline (circles). (Reprinted with permission from Shaw et al. Correlates of sleep and waking in *Drosophila melanogaster*. Science **287**:1834–1837. Copyright © 2000 AAAS.)

in the first few hours after waking. These genes are functionally homologous to ''waking'' genes in the rat. One such shared ''waking'' gene is *BiP* (*Hsc*70-3) that codes for an endoplasmic reticulum chaperone protein. Chaperone proteins are thought to promote proper folding and shaping of other proteins. Their expression at the onset of wakefulness and during sleep deprivation may very well hold an important clue to sleep function [57]. Some genes are upregulated after wakefulness and more so after sleep deprivation. Three hours of sleep deprivation results in an increase in the transcription of genes coding for transcription factors and/or genes involved in energy metabolism. After 8 h of deprivation there is an upregulation of growth factors, molecular chaperones, higher mRNA levels of heat shock proteins, neurotransmitters, transporters, and enzymes [62] and the synthesis of cholesterol, myelin structural proteins, and myelin-related receptors [61]. The increase in molecular chaperone protein expression suggests an increase in the mobilization of either newly synthesized proteins or those destined for catabolism. Furthermore, the upregulation of synaptic plasticity genes (e.g., brain-deprived neurotrophic factor (BDNF)) suggests the remodeling of neuronal configurations [62] or memory acquisition [61]. Wakefulness in *Drosophila* is also characterized by an increase in the levels of mRNA for arylalkylamine *N*-acetyltransferase (aaNAT1, also called dopamine acetyltransferase (DAT), an enzyme responsible for the catabolism of monoamines. Although rats lack this enzyme, arylsulfotransferase serves a similar function. Interestingly, an increase or decrease in gene expression can occur in

brain regions where EEG correlates of sleep are reduced or nonexistent [61].

A recent study reported behavioral sleep accompanied by slow-wave activity in crayfish [63]. Behavioral sleep was associated with specific postures (i.e., floating, lying on one-side) and this ''sleep-posture'' was associated with the highest arousal threshold to a vibratory stimulus. Sleep deprivation resulted in a compensatory increase in total sleep time during subsequent sleep bouts. Interestingly, the authors presented a waking EEG of high-voltage spikes and a sleeping EEG of continuous slow-wave activity. Slow-wave activity disappeared upon arousal. REM sleep was not observed.

The cephalopod, *Octopus vulgaris*, displays color patterns and associated postures that correlate with rest during the nocturnal phase of the photoperiod. While resting, arms are upturned, skin texture is smooth, and chromatophores are relaxed, resulting in a gray-green color to be expressed only on the dorsal body surface and purple on the ventral arms [64]. Furthermore, specific postures are associated with elevations in arousal threshold [2]. Another study revealed a rest/activity cycle in cuttlefish. Captive cuttlefish would lie still for 10–15 min periods interrupted by flashes of bold color from their chromatophores and twitches of the tentacles resembling mammalian and avian REM sleep.

CONCLUSION

A phylogenetic evaluation of sleep demonstrates that all mammals, birds, and reptiles engage in sleep, and evidence for sleep in amphibians, fishes, and invertebrates is strong if not certain. In critically important sleep studies of rats and fruit flies, it has been shown that chronic sleep deprivation is fatal [57, 65], attesting to the necessity for sleep and to its ancient evolutionary age.

Sleep in mammals and birds can be divided into two states: SWS and REM sleep. Although it is necessarily intuitive that sleep evolved from wakefulness, it is unclear which of the two sleep states (if either) evolved first. An understanding of this sequence can have significant consequences for our understanding of the functions of sleep. For example, the SWS–REM sleep cycle may be unique to mammals and birds, suggesting an association with a shared character of the two groups, such as endothermy or an enlarged forebrain. Below we summarize three theories currently circulating on the evolution of the sleep cycle exhibited in mammals and birds (Figure 8.4) [4, 35, 66].

The first hypothesis, henceforth called *SWS-first* (Figure 8.4a) is advanced in direct opposition to the *REM sleep-first* hypothesis [35]. The first sleep study in monotremes concluded that the short-beaked echidna did not exhibit REM sleep [11], supporting the notion that REM sleep evolved after SWS in the evolutionary lineage of mammals and birds. SWS was therefore assumed to be the ancestral sleep state and REM sleep subsequently evolved twice: once in birds (or their dinosaur ancestors) and once in the placental and marsupial mammalian clade, a finding consistent with reptilian studies at the time [36, 47] (see Reptiles section). However, slow waves, which are the hallmark of SWS, originate in the mammalian neocortex. Reptiles, perhaps void of necessary telencephalic structures, may not be able to generate slow-wave activity. Nevertheless, sleep deprivation experiments and pharmacological evidence suggest that the high-voltage spikes often reported in reptilian sleep studies may occur in a state that is the precursor to mammalian and avian SWS.

The second hypothesis, REM sleep-first, states that REM sleep is the ancestral sleep state [66] and that SWS is derived in the mammalian and avian lineages through convergent evolution (Figure 8.4b). This hypothesis is supported by a great deal of correlational data leading to the

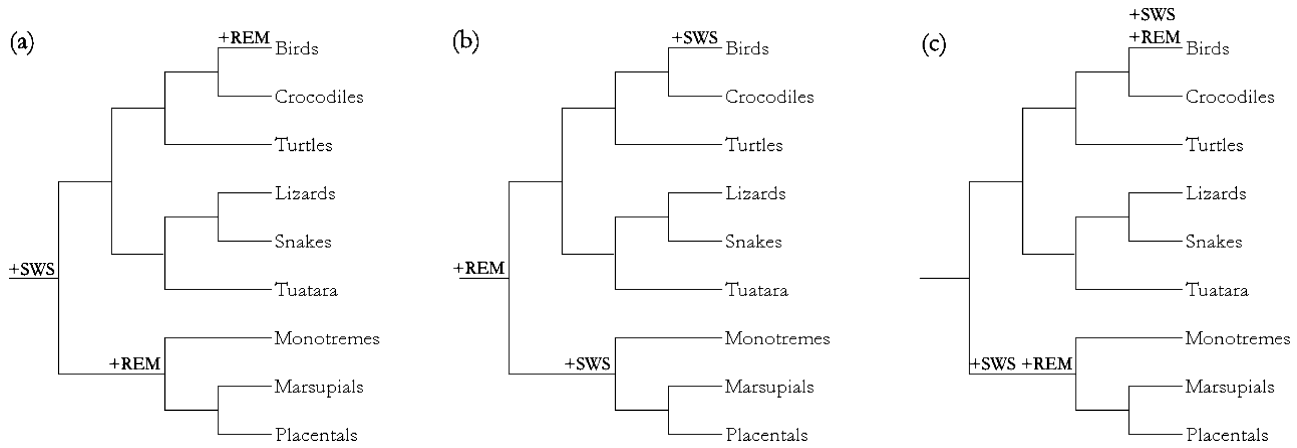

Figure 8.4 Three hypotheses for the evolutionary origins of SWS and REM sleep seen in endotherms (i.e., birds and mammals; see text for description). Phylogeny adapted from [10].

following four conclusions. (1) Monotremes, the oldest extant group of mammals, exhibit REM sleep (see Monotremes section) and, interestingly, the platypus, the oldest of the monotremes, may engage in more REM sleep than any other mammal. The identification of REM sleep in both the echidna and platypus suggests that REM sleep originated earlier in mammalian evolution than had previously been thought. Furthermore, it suggests that REM sleep, or a REM sleep-like state, was present in the reptilian ancestor to both the mammalian and avian lineage. (2) During REM sleep, an endotherm's thermoregulatory mechanisms are either suspended or impaired. Therefore an endothermic animal is essentially poikilothermic during REM sleep. Assuming that REM sleep is indeed the antecedent sleep state exhibited by our reptilian ancestors, such heterothermic animals would not be adversely affected by the inhibition of, albeit, nonexistent metabolic heat production mechanisms. However, with the evolution of endothermy, REM sleep became detrimental to survival. A second sleep state (i.e., SWS) evolved to permit thermoregulation concurrent with sleep. (3) The ontogenesis of the sleep cycle may also be used as evidence for the REM sleep-first hypothesis. REM sleep is the dominant sleep state of the mammalian fetus. After birth, REM sleep time decreases rapidly until it stabilizes at adult levels. The decrease in REM sleep is marked by a concurrent increase in SWS and wakefulness throughout early maturation. (4) Lastly, whereas the slow waves of mammalian SWS are propagated from the neocortex, REM sleep originates in the rhombencephalon (pons) of the brainstem—the most ancient caudal brain structure. Thus evolution of the SWS/REM sleep cycle may have begun with REM sleep and not SWS as once thought. The obvious drawback of this hypothesis is the absence of unequivocal REM sleep in extant reptiles. However, REM sleep or a REM sleep-like state may be highly temperature sensitive as it is in mammals, and future studies of sleep in reptiles must examine this factor further.

The existence of alternating SWS and REM sleep in the mammalian and avian lineages may be due to convergent evolution and not to inheritance from a common reptilian ancestor (Figure 8.4c), since evidence for unequivocal SWS and REM sleep in reptiles is controversial. Although REM sleep in placental and marsupial mammals is characterized by low-amplitude EEG activity, REM sleep in the platypus was associated with SWS-like cortical activity [14]. REM sleep with an activated EEG may have thus evolved in marsupial and placental mammals after divergence of the monotreme line. Moreover, due to the absence of unambiguous REM sleep and SWS in extant reptiles, SWS and REM sleep with cortical activation may have evolved twice: once in the mammalian and once in the avian clades.

Sleep differs from wakefulness on all levels of organization: behavioral, electrophysiological, cellular, molecular, and genetic. Indeed, sleep and wakefulness favor different cellular processes [61]. Present information suggests that a proximate sleep function is likely focused at the level of the neuron or synapse rather than the organ or tissue. Current criteria for sleep are based largely on changes in neural activity. In instances where such indicators are cryptic or absent (see Reptiles section and Amphibians and Fishes section), new criteria are needed, perhaps at the level of gene expression. This does not discount a functional significance for the EEG correlates of sleep in mammals and birds; it merely acknowledges that since invertebrates also sleep, the physiological criteria for sleep should not be limited to EEG correlates, which are clearly not shared by invertebrates, fishes, amphibians, and reptiles. Such investigations can shed much light on the nature and functions of sleep.

ACKNOWLEDGMENTS

We would like to thank Steven Lima and Peter Scott for their thoughtful and constructive comments on this chapter.

REFERENCES

1. Rechtschaffen A. Current perspectives on the function of sleep. *Perspect Biol Med* **41**:359–390(1998).
2. Campbell SS, Tobler I. Animal sleep: a review of sleep duration across phylogeny. *Neurosci Biobehav Rev* **8**:269–300(1984).
3. Hartse KM. Sleep in insects and nonmammalian vertebrates. In: Kryger MH, Roth T, Dement WC (Eds), *Principles and Practice of Sleep Medicine*, 2nd ed. Saunders, Philadelphia, 1994, pp 95–104.
4. Nicolau MC, Akaârir M, Gamundí A, González J, Rial RV. Why we sleep: the evolutionary pathway to the mammalian sleep. *Prog Neurobiol* **62**:379–406(2000).
5. Tobler I. Phylogeny of sleep regulation. In: Kryger MH, Roth T, Dement WC (Eds), *Principles and Practice of Sleep Medicine*, 3rd ed. Saunders, Philadelphia, 2000, pp 72–81.
6. Zepelin H. Mammalian sleep. In: Kryger MH, Roth T, Dement WC (Eds), *Principles and Practice of Sleep Medicine*, 3rd ed. Saunders, Philadelphia, 2000, pp 82–92.
7. Piéron H. *Le Problème Physiologique du Sommeil*. Masson, Paris, 1913 (in French).
8. Flanigan WF. Behavioral states and electroencephalograms of reptiles. In: Chase MH (Ed), *The Sleeping Brain. Perspectives in the Brain Sciences.* Brain Information Service/Brain Research Institute, UCLA, Los Angeles, pp 14–18.
9. Tobler I. Deprivation of sleep and rest in vertebrates and invertebrates. In: Inoué S, Borbély AA (Eds), *Endogenous Sleep*

Substances and Sleep Regulation. Japan Scientific Societies Press, Tokyo, 1985, pp 57–66.

10. Meyer A, Zardoya R. Recent advances in the (molecular) phylogeny of vertebrates. *Annu Rev Ecol Evol Syst* **34**:311–338(2003).
11. Allison T, van Twyver H, Goff WR. Electrophysiological studies of the echidna, *Tachyglossus aculeatus*. I. Waking and sleep. *Arch Ital Biol* **110**:145–184(1972).
12. Siegel JM, Manger PR, Nienhuis R, Fahringer HM, Pettigrew JD. The echidna *Tachyglossus aculeatus* combines REM and non-REM aspects in a single sleep state: implications for the evolution of sleep. *J Neurosci* **16**:3500–3506(1996).
13. Nicol SC, Andersen NA, Phillips NH, Berger RJ. The echidna manifests typical characteristics of rapid eye movement sleep. *Neurosci Lett* **283**:49–52(2000).
14. Siegel JM, Manger PR, Nienhuis R, Fahringer HM, Shalita T, Pettigrew JD. Sleep in the platypus. *Neuroscience* **91**:391–400(1999).
15. Rattenborg NC, Amlaner CJ, Lima SL. Behavioral, neurophysiological and evolutionary perspectives on unihemispheric sleep. *Neurosci Biobehav Rev* **24**:817–842(2000).
16. Kovalzon VM, Mukhametov LM. Temperature variations in the brain corresponding to unihemispheric slow wave sleep in dolphins. *J Evol Biochem Physiol* **18**:307–309(1982) (in Russian).
17. Mukhametov LM, Polyakova IG. EEG investigation of sleep in porpoises (*Phocoena phocoena*). *J High Nerve Activ* **31**:333–339(1981) (in Russian).
18. Goley PD. Behavioral aspects of sleep in Pacific white-sided dolphins (*Lagenorhynchus obliquidens*, Gill 1865). *Mar Mam Sci* **15**:1054–1064(1999).
19. Oleksenko AI, Mukhametov LM, Polyakova IG, Supin AY, Kovalzon VM. Unihemispheric sleep deprivation in bottlenose dolphins. *J Sleep Res* **1**:40–44(1992).
20. Mukhametov LM, Lyamin OI, Chetyrbok IS, Vassilyev AA, Diaz R. Sleep in an Amazonian manatee, *Trichechus inunguis. Experientia* **48**:417–419(1992).
21. Zepelin H, Rechtschaffen A. Mammalian sleep, longevity and energy metabolism. *Brain Behav Evol* **10**:425– 470 (1974).
22. Elgar MA, Pagel MD, Harvey PH. Sleep in mammals. *Anim Behav* **36**:1407–1419(1988).
23. Allison T, Cicchetti DV. Sleep in mammals: ecological and constitutional correlates. *Science* **194**:732–734(1976).
24. Elgar MA, Pagel MD, Harvey PH. Sources of variation in mammalian sleep. *Anim Behav* **40**:991–995(1990).
25. Ramanathan L, Gulyani S, Nienhuis R, Siegel JM. Sleep deprivation decreases superoxide dismutase activity in rat hippocampus and brainstem. *Neuroreport* **13**:1387–1390 (2002).
26. Siegel JM. Why we sleep. *Sci Am* **Nov**:72–77(2003).
27. Amlaner CJ, Ball NJ. Avian sleep. In: Kryger MH, Roth T, Dement WC (Eds), *Principles and Practice of Sleep Medicine*, 2nd ed. Saunders, Philadelphia, 1994, pp 81–94.
28. Rattenborg NC, Mandt BH, Obermeyer WH, Winsauer PJ, Huber R, Wikelski M, Benca RM. Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLoS Biol* **2**:924–936(2004).
29. Ball NJ, Amlaner CJ, Shaffery JP, Opp MR. Asynchronous eye-closure and unihemispheric quiet sleep of birds. In: Koella WP, Obál F, Schulz H, Visser P (Eds), *Sleep '86*. Gustav Fischer Verlag, New York, 1988, pp 151–153.
30. Spooner CE. *Observations on the Use of the Chick in the Pharmacological Investigation of the Central Nervous System*. Ph.D. dissertation, University of California, Los Angeles.
31. Rattenborg NC, Lima SL, Amlaner CJ. Half-awake to the risk of predation. *Nature* **397**:397–398(1999).
32. Rattenborg NC, Amlaner CJ, Lima SL. Unilateral eye closure and interhemispheric EEG asymmetry during sleep in the pigeon (*Columba livia*). *Brain Behav Evol* **58**:323–332(2001).
33. Amlaner CJ, Ball NJ. A synthesis of sleep in wild birds. *Behaviour* **87**:85–119(1983).
34. Schmidt DF. *A Comparative Analysis of Avian Sleep*. Ph.D. dissertation, University of Arkansas, Fayetteville, 1994.
35. Taylor L, Vana A, Givon L. The evolution of sleep: a reconsideration of the development of the quite sleep/active sleep cycle. *Med Hypotheses* **54**:761–766(2000).
36. Flanigan WF, Wilcox RH, Rechtschaffen A. The EEG and behavioral continuum of the crocodilian, *Caiman sclerops. Electroencephalogr Clin Neurophys* **34**:521–538(1973).
37. Warner BF, Huggins SE. An electroencephalographic study of sleep in young caimans in a colony. *Comp Biochem Physiol* **59**:139–144(1978).
38. Meglasson MD, Huggins SE. Sleep in a crocodilian, *Caiman sclerops. Comp Biochem Physiol* **63A**:561–567(1979).
39. Flanigan WF, Knight CP, Hartse KM, Rechtschaffen A. Sleep and wakefulness in Chelonian reptiles. I. The box turtle, *Terrapene carolina. Arch Ital Biol* **112**:227–252(1974).
40. Flanigan WF. Sleep and wakefulness in Chelonian reptiles. II. The red-footed tortoise, *Geochelone carbonaria. Arch Ital Biol* **112**:253–277(1974).
41. Hartse KM, Rechtschaffen A. Effect of atropine sulfate on the sleep-related EEG spike activity of the tortoise, *Geochelone carbonaria. Brain Behav Evol* **9**:81–94(1974).
42. Hartse KM, Rechtschaffen A. The effect of amphetamine, nembutal, alpha-methyl-tyrosine, and parachlorophenylalanine on the sleep-related spike activity of the tortoise, *Geochelone carbonaria*, and on the cat ventral hippocampus spike. *Brain Behav Evol* **21**:199–222(1982).
43. Huntley AC. Electrophysiological and behavioral correlates of sleep in the desert iguana, *Dipsosaurus dorsalis hallowell. Comp Biochem Physiol* **86A**:325–330(1987).
44. Tauber ES, Rojas-Ramírez J, Hernández-Peon R. Electrophysiological and behavioral correlates of wakefulness and sleep in the lizard, *Ctenosaura pectinata. Electroencephalogr Clin Neurophysiol* **24**:424–433(1968).
45. Tauber ES, Roffwarg HP, Weitzman ED. Eye movements and electroencephalogram activity during sleep in diurnal lizards. *Nature* **212**:1612–1613(1966).

46. Ayala-Guerrero F, Huitrón-Reséndiz S. Sleep patterns in the lizard *Ctenosaura pectinata. Physiol Behav* **49**:1305–1307(1991).
47. Flanigan WF. Sleep and wakefulness in iguanid lizards, *Ctenosaura pectinata* and *Iguana iguana. Brain Behav Evol* **8**:401–436(1973).
48. Mathews CG, Amlaner CJ. Eye states and postures of the western fence lizard (*Sceloporus occidentalis*), with special reference to asynchronous eye closure and behavioral sleep. *J Herp* **34**:472–475(2000).
49. Shaw PJ, Cirelli C, Greenspan RJ, Tononi G. Correlates of sleep and waking in *Drosophila melanogaster. Science* **287**:1834–1837(2000).
50. Cirelli C. Searching for sleep mutants of *Drosophila melanogaster. BioEssays* **25**:940–949(2003).
51. Tobler I, Stalder J. Rest in the scorpion—a sleep-like state? *J Comp Physiol A* **163**:227–235(1988).
52. Kaiser W, Steiner-Kaiser J. Neuronal correlates of sleep, wakefulness and arousal in a diurnal insect. *Nature* **301**:707–709 (1983).
53. Sauer S, Kinkelin M, Herrmann E, Kaiser W. The dynamics of sleep-like behavior in honey bees. *J Comp Physiol A* **189**:599–607(2003).
54. Schuppe H. Rhythmic brain activity in sleeping bees. *Wien Med Wochenschr* **145**:463–464(1995) (in German).
55. Sauer S, Herrmann E, Kaiser W. Sleep deprivation in honey bees. *J Sleep Res* **13**:145–152(2004).
56. Hendricks JC, Finn SM, Panckeri KA, Chavkin J, Williams JA, Sehgal A, Pack AI. Rest in drosophila is a sleep-like state. *Neuron* **25**:129–138(2000).
57. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF. Stress response genes protect against lethal effects of sleep deprivation in *Drosophila. Nature* **417**:287–291(2002).
58. Nitz DA, van Swinderen B, Tononi B, Greenspan RJ. Electrophysiological correlates of rest and activity in *Drosophila melanogaster. Curr Biol* **12**:1934–1940(2002).
59. Hendricks JC, Sehgal A. Why a fly? Using drosophila to understand the genetics of circadian rhythms and sleep. *Sleep* **27**:334–342(2004).
60. Tafti M, Franken P. Functional genomics of sleep and circadian rhythm: invited review: genetic dissection of sleep. *J Appl Physiol* **92**:1339–1347(2002).
61. Cirelli C, Gutierrez CM, Tononi G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron* **41**:35–43(2004).
62. Mackiewicz M, Pack AI. Functional genomics of sleep. *Respir Physiol Neurobiol* **135**:207–220(2003).
63. Ramón F, Hernández-Falcón J, Nguyen B, Bullock TH. Slow wave sleep in crayfish. *Proc Natl Acad Sci* **101**:11857–11861(2004).
64. Mather JA, Mather DL. Skin colours and patterns of juvenile *Octopus vulgaris* (Mollusca, Cephalopoda) in Bermuda. *Vie Milieu* **44**:267–272(1994).
65. Rechtschaffen A, Gilliland MA, Bergmann BM, Winter JB. Physiological correlates of prolonged sleep deprivation in rats. *Science* **221**:182–184(1983).
66. Siegel JM, Manger PR, Nienhuis R, Fahringer HM, and Pettigrew JD. Monotremes and the evolution of rapid eye movement sleep. *Philos Trans R Soc London B* **353**:1147–1157(1998).