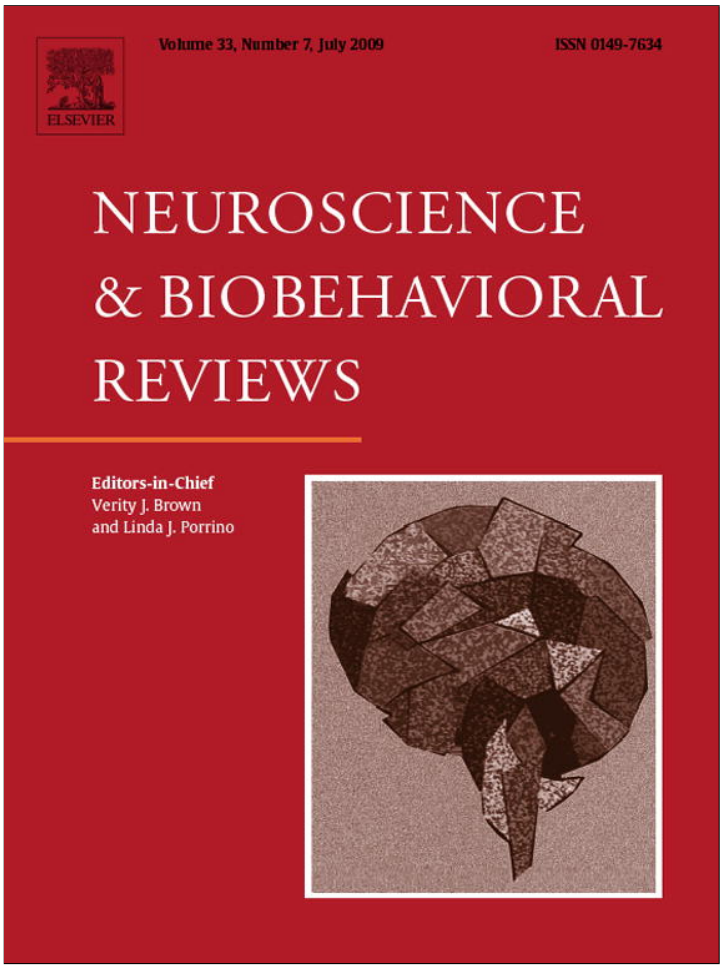

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Review

History and future of comparative analyses in sleep research

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ABSTRACT

The comparative methods of evolutionary biology are a useful tool for investigating the functions of sleep. These techniques can help determine whether experimental results, derived from a single or few species, apply broadly across a specified group of animals. In this way, comparative analysis is a powerful complement to experimentation. The variation in the time mammalian species spend asleep has been most amenable for use with this approach, given the large number of mammals for which sleep data exist. Here, it is assumed that interspecific variation in the time spent asleep reflects underlying differences in the need for sleep. If true, then significant predictors of sleep times should provide insight into the function of sleep. Many such analyses have sought the evolutionary determinants of mammalian sleep by relating the time spent in the two basic states of sleep, rapid eye movement (REM) and non-REM sleep, to constitutive variables thought to be functionally related to sleep. However, the early analyses had several methodological problems, and recent re-analyses have overturned some widely accepted relationships, such as the idea that species with higher metabolic rates engage in more sleep. These more recent studies also provide evolutionarily broad support for a neurophysiological role for REM sleep. Furthermore, results from comparative analyses suggest that animals are particularly vulnerable to predation during REM sleep, a finding that lends further support to the notion that REM sleep must serve an important function. Here, we review the methodology and results of quantitative comparative studies of sleep. We highlight important developments in our understanding of the evolutionary determinants of sleep and emphasize relationships that address prevailing hypotheses for the functions of sleep. Lastly, we outline a possible future for comparative analyses, focusing on work in non-mammalian groups, the use of more physiologically meaningful variables, and electrophysiological sleep studies conducted in the wild.

Contents

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1. Introduction

The toolkit of sleep researchers is ever-increasing. Investigations into sleep were once achieved only through behavioral observation (Piéron, 1913) or low resolution measures of brain activity (Loomis et al., 1937; Aserinsky and Kleitman, 1953). However, recent technological advances, such as the use of functional magnetic resonance imaging (fMRI, Kaufmann et al., 2006) and high-density electroencephalography (EEG, Tucker, 1993) allow us to see how activity varies across different parts of the brain during wakefulness and sleep (Huber et al., 2004; Massimini et al., 2004; Gais et al., 2007). Moreover, advances in molecular genetics (Tafti and Franken, 2002; Mackiewicz and Pack, 2003) indicate that, compared to wakefulness, sleep favors the expression of different classes of genes, some of which appear to be evolutionarily conserved (Cirelli, 2003; Cirelli et al., 2004). The recent development of miniature digital technology for measuring the EEG from free-ranging animals in the wild (Vyssotski et al., 2006) will allow the exploration of sleep under ecologically realistic circumstances (Rattenborg et al., 2008a). In addition to this suite of techniques, sleep researchers also have the comparative methods of evolutionary biology as a tool for investigating the functions of sleep.

Although sleep appears to serve a vital function, there is still no consensus on the specific functions of sleep (Siegel, 2005; Stickgold, 2005; Tononi and Cirelli, 2006; Krueger et al., 2008; Mignot, 2008). Ideally, the most straightforward way to determine sleep's function would be to identify animals that sleep and those that do not, and then identify traits that are unique to each group. Unfortunately, all species studied sleep, making such comparisons impossible (Cirelli and Tononi, 2008; Lesku et al., 2009). A second strategy for illuminating the function of sleep is to compare species that sleep differently in some way, and then identify the factors responsible for maintaining those differences. One popular and potentially insightful approach is to determine why some species sleep a great deal and others only very little. Such among-species (or interspecific) variation has been best documented in the time that mammals spend in rapid eye movement (REM) and non-REM (or slow wave) sleep (McNamara et al., 2008), the two basic types of sleep in mammals. For example, large hairy armadillos (*Chaetophractus villosus*) spend 16 h per day in non-REM sleep (Affanni et al., 2001), whereas horses (*Equus caballus*) spend just 2 h in non-REM sleep (Ruckebusch, 1972); Virginia opossums (*Didelphis virginiana*) engage in REM sleep for more than 7 h per day (Walker and Berger, 1980a), but sheep (*Ovis aries*) spend just half an hour in that state (Ruckebusch, 1972). If we assume that such interspecific variation reflects underlying differences in the need for sleep, then identifying the evolutionary factors responsible for maintaining such variation should provide clues to the functions of sleep. This is the essence of comparative sleep research. A unique strength of this comparative approach is that it can be used to assess whether the results from experiments obtained from only a single or few species might be applied to a broader group of animals. Exploratory comparative analysis can also be of value for the identification of new (potentially unexpected) relationships that might lead to new hypotheses for the function of sleep, much as genome-wide screening has been used to identify novel genes that are only expressed in the brain during sleep (Cirelli, 2005).

Here, we review the methodology and results from quantitative comparative studies of sleep, beginning with the influential work of Zepelin and Rechtschaffen (1974), the first large-scale statistical analysis of interspecific variation of mammalian sleep. Throughout, we highlight important developments in our understanding of the evolutionary determinants of sleep and emphasize relationships that address prevailing hypotheses for the functions of sleep. We also discuss recent results from comparative work on birds. Lastly, we outline a possible future for comparative analyses of sleep that includes using more physiologically meaningful variables and conducting EEG-based sleep studies in the wild.

2. History of comparative analyses in sleep research

The value of a comparative approach to understanding sleep has been recognized for at least four decades (e.g., Weiss and Roldán, 1964; van Twyver, 1969). The first comparative studies analyzed sleep times in only a handful of species, hence their results were necessarily descriptive in nature. Perhaps the most substantive contribution of these early studies was simply the identification of interspecific variation in some aspects of EEG-defined sleep (Weiss and Roldán, 1964; van Twyver, 1969), suggesting that at least some features of sleep are (in part) genetically determined (see also Tafti and Franken, 2002; Mackiewicz and Pack, 2003), a necessary prerequisite for traits used in comparative analyses. Subsequent work would expand greatly upon these first (descriptive) studies by quantifying relationships among sleep parameters and constitutive (Section 2.1) and ecological (Section 2.2) variables.

2.1. The first quantitative comparative sleep analysis: Zepelin and Rechtschaffen

Zepelin and Rechtschaffen (1974) provided the first formal comparative analysis of sleep. Their chief motivation was to determine whether hypotheses for the function of mammalian sleep applied broadly across mammals. Such hypotheses included the idea that sleep in some way promotes longevity, and that sleep plays a role in reducing energy expenditure through enforcing inactivity and lowering the metabolic rate of an animal. As such, species with longer life spans and species with relatively higher metabolic rates were expected to engage in more sleep.

Zepelin and Rechtschaffen compiled a dataset based on electrophysiologically and behaviorally derived sleep data for 53 species. Their analysis was part hypothesis-testing and part exploratory; consequently, they included numerous variables beyond those required to evaluate the longevity and energy conservation hypotheses. The sleep-related variables included estimates of the time spent in non-REM sleep and REM sleep per 24 h day, total sleep time, and the percentage of total sleep time allocated to REM sleep (or %REM sleep). %REM sleep could be particularly informative if there are constraints on the amount of time an animal can sleep. Presumably, under such a constraint, the allocation of time to one sleep state would increase at the expense of the other, reflecting a tradeoff between the specific costs and benefits involved in engaging in more non-REM or REM sleep. These sleep-related variables were then correlated with variables related to anatomy (brain mass), physiology (mass-specific basal metabolic rate, BMR), and life-history (maximum life span and gestation period, the latter a proxy for postnatal brain maturity), collectively referred to as constitutive variables. As noted by Zepelin and Rechtschaffen (1974), these variables are not

necessarily the most-informative that one could imagine, but were included because they were readily available from the literature for many of the species for which sleep data were available.

Overall, many significant correlations were identified between sleep and constitutive variables. Indeed, the majority of correlations were fairly strong with effect sizes often explaining over 25% of the variance in each bivariate comparison. Counter to expectations under the longevity hypothesis, long-lived species slept little whereas shorter-lived species slept more, suggesting that sleeping *per se* does not increase (maximum) life span. This negative relationship between sleep duration and life span disappeared when Zepelin and Rechtschaffen controlled statistically for brain mass or mass-specific BMR, suggesting that the correlation was only significant by virtue of strong correlations among constitutive variables. Conversely, in accordance with expectations under the energy conservation hypothesis (Berger and Phillips, 1995), species with a higher mass-specific BMR slept more than species with a lower mass-specific BMR (but see Section 3.1), perhaps to offset the high-energy expenditure during wakefulness (see also Zepelin et al., 2005).

The study by Zepelin and Rechtschaffen is important for many reasons. First, it illustrates the potential power of comparative analyses in sleep research. That is, the relationships identified here (and later by other researchers) demonstrate the taxonomic breadth at which insights about sleep based on individual species can be applied. Another important contribution is the recognition of the need for some type of statistical control for non-independence of species within their comparative dataset; this last point will be addressed in more detail below (see Section 3.2). Overall, Zepelin and Rechtschaffen (1974) is arguably the most influential comparative sleep study to date. Despite the strengths of this study, it also had several shortcomings, perhaps the most important of which concerns the idea that species with higher relative BMRs engage in more sleep (see Section 3.1).

2.2. A role for ecology in the evolution of sleep

Although Zepelin and Rechtschaffen (1974) included only constitutive variables in their analysis, there is good reason to believe that many aspects of sleep might also be determined by ecological factors, such as the risk of predation. While asleep, an animal is relatively unresponsive to its local environment. Thus, a sleeping animal is unlikely to detect an approaching predator or mount an effective response should that predator attack. Despite this fundamental reality of the dangers associated with sleeping, remarkably little work has been done on the way in which predators influence the structure of sleep in mammals or any other taxa (reviewed in Lima et al., 2005). This matter, however, was considered early in the comparative study of sleep. One brief report by Zepelin (1970) compared the sleep of jaguars (*Panthera onca*) to that of tapirs (*Tapirus* spp.) in a zoological garden. Zepelin found that tapirs slept about half that of the jaguars, and that sleep in tapirs was heavily fragmented as the animals were more responsive to the sounds made by other animals. This basic (descriptive) comparison between the sleep of a predatory mammal and that of its prey is certainly consistent with the idea that sleeping is dangerous. In a larger-scale study, Allison and van Twyver (1970) categorized species as "good" or "poor" sleepers based on how well they slept in the laboratory. They found that "good" sleepers were often predators or had a relatively secure sleep site relative to "poor" sleepers. These early observations suggest that trophic status (predator or prey) has played an important role in shaping the structure of mammalian sleep.

Allison and Cicchetti (1976) provided the first quantitative comparative study of sleep to incorporate ecological factors as predictors of mammalian sleep duration. Based upon their earlier observations (Allison and van Twyver, 1970), they focused on the predatory environment as a potential determinant of sleep times. Because absolute measures of predation risk were unavailable, Allison and Cicchetti created predation-related indices in an attempt to capture the vulnerability species may face during sleep. Briefly, their "predation index" ranked the likelihood of predation as observed in the wild, and a "sleep exposure index" categorized sleep sites into those that are risky (i.e., open) and those that are relatively secure (e.g., burrows); "overall danger" was a combination of the two. Ultimately, species subjected to a higher risk of predation in the wild spent less time in non-REM sleep and REM sleep in the laboratory relative to more safely sleeping species (see also Meddis, 1983 for a subsequent analysis with similar results). A stepwise regression that included constitutive variables in addition to the predation risk indices revealed that the best predictor of REM sleep time was the index of overall danger, which was also the second best predictor of non-REM sleep time (after body mass). These results suggest that predators act as a selection pressure favoring the evolution of short-sleeping prey. Alternatively, more vulnerable species might habituate poorly to the laboratory environment (perceiving it as potentially dangerous) and so engage in less non-REM sleep and REM sleep to maintain anti-predator vigilance. Regardless of the specific mechanism for this relationship, the risk of predation appears to strongly influence how long mammals sleep. We return to this matter of sleep and predators (see Section 4.2) after discussing some important methodological considerations in comparative analyses of sleep.

3. Some important methodological considerations

The analyses discussed so far each suffered from several methodological problems that influence the evolutionary patterns identified in comparative analyses. Here, we discuss the most problematic methodological aspects of these early analyses, such as the statistical control of body mass (Section 3.1), controlling for shared evolutionary history among species (Section 3.2), and the inclusion of debatable sleep data (Section 3.3).

3.1. Statistical controls of body mass: ratios vs. residuals

One shortcoming of Zepelin and Rechtschaffen (1974) was their statistical handling of body mass in the BMR-related relationships. Specifically, while evaluating interspecific support for an energy conservation role for sleep, they correlated the time spent asleep with "relative" BMR, calculated as BMR/body mass. Overall, Zepelin and Rechtschaffen (1974) identified the predicted positive relationship between the two variables (e.g., Fig. 1A) and concluded that a function of sleep is the reduction of energy expenditure to offset increased mass-specific BMR. This result has been replicated in a much larger dataset (Siegel, 2004, 2005), and is frequently cited as support for sleep's role in energy conservation or other metabolically based processes (e.g., Siegel, 2005; Harbison and Sehgal, 2008; Mignot, 2008). However, this positive relationship between the time spent asleep (and in non-REM sleep) and mass-specific BMR is a consequence of the inadequate statistical control of body mass inherent in ratio-based measures like BMR/body mass (Beaupre and Dunham, 1995). Such a ratio-based approach to the statistical control of a variable (e.g., body mass) is only appropriate when the two variables (e.g., body mass and BMR) vary as a constant proportion of one another (Packard and Boardman, 1988, 1999). When this is not the case, the control will be ineffective, as evidenced by a non-zero correlation between body mass and mass-specific BMR (Fig. 1B); one would expect no such correlation with an effective statistical control. In such situations, the use of residuals (as obtained from a log–log regression) is the more effective statistical control, since residual BMR will not correlate with body mass at all (Fig. 1D).

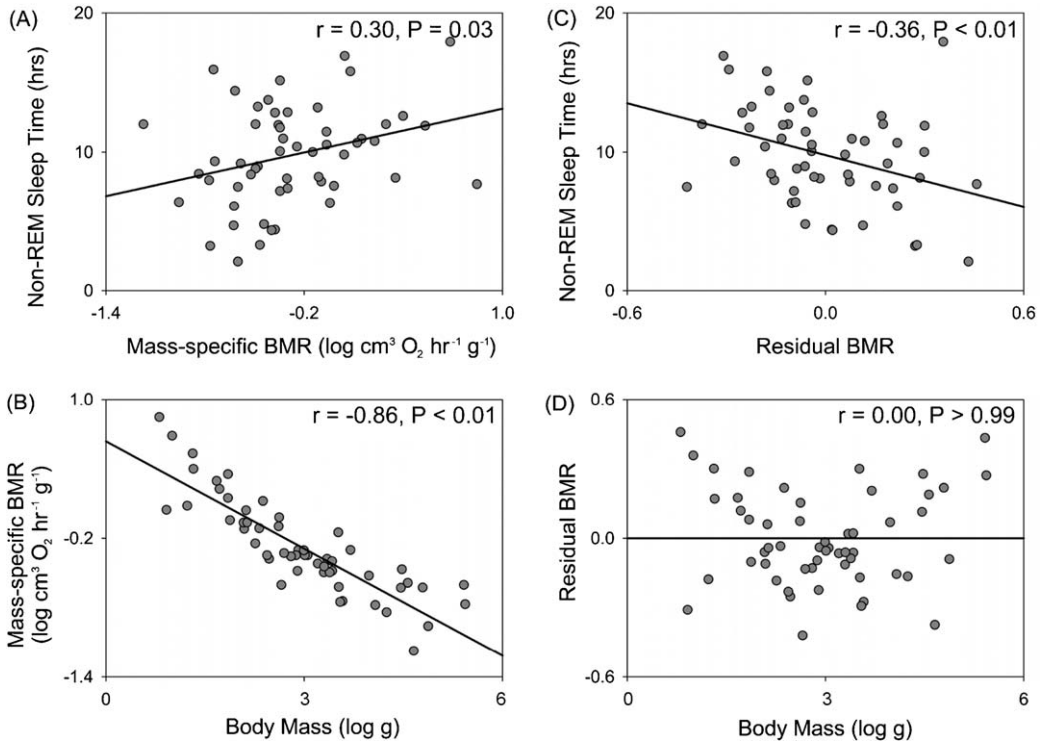

Fig. 1. Scatterplots comparing the effectiveness of two different statistical controls of body mass: a mass-specific ratio (A and B) and a residual-based approach (C and D). (A) The time spent in non-REM sleep increases with increasing mass-specific basal metabolic rate (BMR). However, (B) the control of body mass is incomplete as mass-specific BMR still correlates strongly with body mass. Conversely, (C) the time spent in non-REM sleep decreases with increasing residual BMR (i.e., residuals obtained from a log BMR-log body mass regression). (D) Residual BMR is an effective control of body mass as residual BMR does not correlate with body mass at all. Consequently, the finding that species with relatively higher BMRs engage in *less* non-REM sleep (C) is more accurate than the positive relationship in panel A.

Importantly, when one re-evaluates the relationship between the time spent in non-REM sleep and BMR while controlling for body mass using a more appropriate residual-based approach, the relationship flips signs and is significantly *negative* (Fig. 1C, see also Elgar et al., 1988; Lesku et al., 2006, 2008a; Capellini et al., 2008a). Consequently, species with a relatively higher BMR engage in less sleep, a result which does not provide phylogenetically broad support for an energy conservation role for sleep. Various authors have proposed that this negative relationship is attributable to the fact that animals with increased metabolic rates need to spend more time foraging, hence less time is available for sleep (Elgar et al., 1988; Lesku et al., 2006, 2008a; Capellini et al., 2008a). However, it is unclear how these animals would increase wakefulness without affecting sleep as well, which itself is dependent upon the duration and intensity of wakefulness (Huber et al., 2007; Vyazovskiy et al., 2008). Perhaps, as in short-sleeping humans, such animals have evolved the capacity to remain awake longer despite the homeostatic pressure to sleep (Aeschbach et al., 2001). Ultimately, how this potential demand for increased wakefulness interacts with the need for sleep is an interesting topic for future work.

3.2. Controlling for shared evolutionary history among species

Inherent in any comparative analysis is the issue of non-independence of data resulting from shared evolutionary history among species. That is, closely related species are genetically more similar to one another than to a third more distantly related species simply because the former share a more recent common ancestor. The comparative analyses mentioned so far (Zepelin and Rechtschaffen, 1974; Allison and Cicchetti, 1976; Meddis, 1983) treated each species as an independent statistical unit and thus implicitly assumed a phylogenetic tree such as that shown in Fig. 2A. Here, each of the seven species has an evolutionary history that is independent of that experienced by others since the time of the common ancestor, such that the patas monkey (*Erythrocebus patas*) is as closely related to the vervet monkey (*Chlorocebus aethiops*) as it is to the house mouse (*Mus musculus*). This sort of situation is obviously incorrect, as some species will inevitably be more closely related to some than to others (e.g., Fig. 2B). Although some researchers have argued that this basic tenet of evolutionary biology does not apply to sleep characteristics (Siegel, 2004, 2005; Allada and Siegel, 2008), mounting statistical evidence confirms that closely related species sleep more similarly than more distantly related species (Capellini et al., 2008a; Lesku et al., 2008a), a finding consistent with the observation that many sleep traits are heritable.

Elgar et al. (1988, 1990) were the first to explicitly recognize the problem of phylogenetic non-independence in a comparative analysis of sleep. They controlled for evolutionary relatedness by averaging species data to the taxonomic level at which the most variation in sleep variables existed (Harvey and Pagel, 1991), which in this case occurred at the family level. Zepelin and Rechtschaffen (1974) also analyzed their dataset at the family level, in addition to their primary species-level analysis, in order to adjust for sampling bias caused by the disproportionately large number of rodent and primate species in their dataset, but any reference to phylogenetic non-independence was made only tangentially. Importantly, unlike previous studies, Elgar et al. (1988) found that the relationships between REM sleep time and body mass, brain mass, and BMR were non-significant; however, their family-level analysis resulted in a great reduction of sample size (e.g., Fig. 2). Although the results of Elgar et al. (1988) hint at the importance of incorporating a phylogenetic control into comparative analyses of sleep, their procedure weighted all taxonomic families equally and therefore only partially resolved the problem of non-independence. The procedure has thus since been replaced by more powerful phylogenetically based compara-

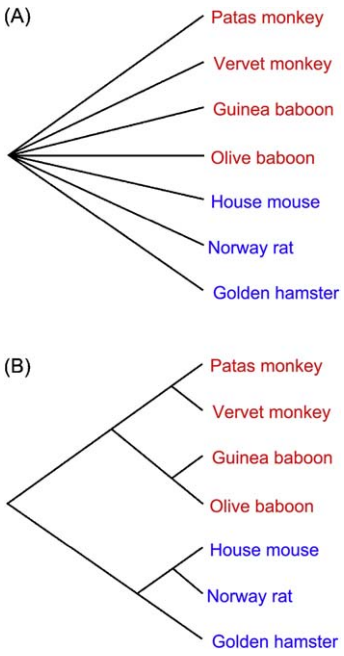

Fig. 2. Two phylogenetic trees depicting hypothetical evolutionary relatedness among several species of rodent (family Muridae, blue) and primate (family Cercopithecidae, red). (A) A highly unrealistic tree where each species has an independent history following their decent from the same common ancestor and (B) a more realistic phylogenetic tree derived from molecular analysis; tree structure was taken from Page et al. (1999) and Michaux et al. (2001). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

tive methods, such as independent contrasts (Martins, 2000; Garland et al., 2005).

Recently, we revisited the correlates of mammalian sleep and controlled for phylogenetic non-independence using independent contrasts (Lesku et al., 2008a, see also Lesku et al., 2006). Independent contrasts are calculated as a series of sister-taxa comparisons (Felsenstein, 1985, see also Nunn and Barton, 2001). In order to assess possible phylogenetic effects, we compared the sleep-related correlations based on non-phylogenetically controlled (raw) data to those obtained using phylogenetically controlled (independent contrast) data. After controlling for shared evolutionary history among species, many of the significant raw data correlations became non-significant, and over 60% of the correlations decreased in magnitude, suggesting that much variation in mammalian sleep is explained by phylogenetic relatedness alone. Indeed, Capellini et al. (2008a) quantified the degree to which closely related mammalian species resemble one another with respect to various sleep-related traits (or phylogenetic signal, see Blomberg et al., 2003) and found the signal to be high in all sleep variables examined. Consequently, the results stemming from "non-phylogenetic" sleep analyses should be viewed with caution as patterns identified in their comparative datasets are confounded with patterns of phylogenetic relatedness.

As in Elgar et al. (1988), we found that the relationships between REM sleep and body mass, brain mass, and BMR became less clear when using phylogenetically controlled data (Lesku et al., 2008a). How do these differences come about? Fig. 3A shows the relationship between REM sleep time and brain mass observed in several non-phylogenetically controlled analyses (Zepelin and Rechtschaffen, 1974; Allison and Cicchetti, 1976; Meddis, 1983; Siegel, 2004, 2005), which is strongly negative despite the fact that this relationship is non-significant (and non-negative) within the two well-represented taxonomic orders, Rodentia and Primates. This phenomenon simply reflects a grade shift between rodents and primates as evidenced by the clumping of data within groups (Nunn and Barton, 2001). The overall negative relationship identified in Fig. 3A (raw data) is no longer significant after controlling for phylogeny (Fig. 3B), a finding which is in agreement with the relationships within both rodents and primates. Also, note that as the degree of relatedness among species has now been controlled for statistically, data generated within these two orders are no longer clumped and separated as they were in Fig. 3A, indicating a good control.

The application of a phylogenetic control can also flip the sign of a relationship between sleep and constitutive variables, and this is the case when looking at the relationship between the percentage of total sleep time allocated to REM sleep (or %REM sleep) and relative brain mass (i.e., residuals obtained from a regression between log brain mass and log body mass). In analyses based on non-phylogenetically controlled data, the relationship between %REM sleep and relative brain mass is negative, even though the same relationship within rodents and primates is positive (Fig. 3C). Accordingly, after controlling for phylogeny, the overall relationship between %REM sleep and relative brain mass is now positive as well (Fig. 3D). The reversal of this particular relationship is noteworthy, because it provides comparative support for a neurophysiological role for REM sleep, possibly related to memory consolidation (see Section 4.1). Thus, the incorporation of a phylogenetic control into comparative analyses of sleep can be critically important for the accurate identification of evolutionary patterns related to mammalian sleep. We revisit this potentially important relationship between %REM sleep and relative brain mass in the context of a multivariate path model below (see Section 4.1).

3.3. What to do with behavioral sleep data, cetaceans, and monotremes?

The results from comparative analyses of sleep are only as reliable as the data which support them. Consequently, it is important to evaluate the criteria for the inclusion of data in comparative sleep datasets (Capellini et al., 2008a). Given the somewhat limited availability of data, the earliest analyses used both sleep data obtained from EEG recordings and behavioral observations of captive mammals (Zepelin and Rechtschaffen, 1974; Allison and Cicchetti, 1976). Some recent analyses have accepted these criteria, such that sleep data derived from behavioral observations constituted over 20% of the species in the dataset (Siegel, 2005; Savage and West, 2007). Although the use of such behavioral data has the advantage of allowing for the inclusion of the largest terrestrial mammalian species, such as elephants and giraffes, for whom EEG recordings are difficult to obtain, behavioral observations alone may give inaccurate estimates of sleep duration (Fig. 4A). Furthermore, as noted by Tobler (1992), the validity of scoring REM sleep based on posture, muscular twitches, and eye movements remains unconfirmed in most species. Thus, many comparative studies have opted to include estimates of sleep parameters based only on EEG recordings of sufficient duration (Fig. 4B, Elgar et al., 1988; Lesku et al., 2006, 2008a; Capellini et al., 2008a,b).

The existence of unihemispheric sleep in a few mammalian groups complicates the matter of which data to include in comparative analyses. Unihemispheric sleep occurs when one hemisphere shows non-REM sleep-related high-amplitude slow waves (or high slow wave activity) and the other shows a pattern similar to wakefulness and is associated with an open and responsive eye (Lyamin et al., 2008). In mammals, such sleep is most evident in cetaceans, but eared seals and manatees also show some degree of interhemispheric asymmetry in the level of slow wave activity (Lyamin et al., 2008). Although lower-amplitude

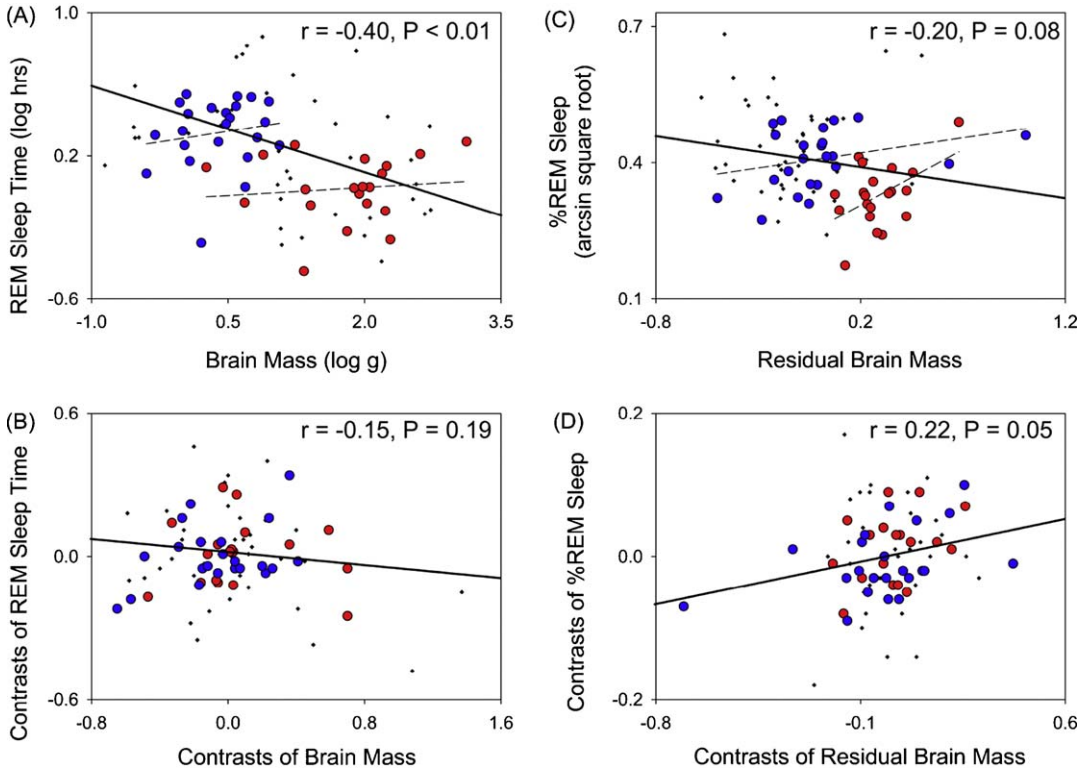

Fig. 3. Scatterplots illustrating two possible effects of controlling for phylogeny in comparative analyses, (A and B) weakening the magnitude of a relationship or (C and D) reversing the direction of a relationship. (A) The significant negative (raw data) relationship between REM sleep time and brain mass becomes (B) non-significant after controlling for phylogeny. (C) The negative (raw data) relationship between the percentage of total sleep time allocated to REM sleep (or %REM sleep) and residual brain mass (or encephalization) becomes (D) significantly positive after controlling for phylogeny. Data for rodents (blue) and primates (red) are emphasized; plus symbols (+) denote data for other taxa. The solid line in each plot reflects the regression line; the dashed lines in panels A and C reflect a regression line generated within rodents and primates. Reprinted from Lesku et al. (2008a) *Sleep Medicine Reviews*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

slow waves can occur bilaterally in cetaceans, deep non-REM sleep occurs only unihemispherically (Lyamin et al., 2008). Thus, it is unclear how to express unihemispheric sleep in terms of time spent in non-REM sleep, since the sleeping hemisphere is presumably obtaining the benefits of sleep while the other hemisphere is not (Oleksenko et al., 1992). Moreover, cetaceans often swim during periods of unihemispheric non-REM sleep, raising questions about whole animal metabolic rate during this state. Cetaceans are also problematic because they do not appear to exhibit REM sleep typical of terrestrial mammals (Lyamin et al., 2008). Given that it is unclear how to proceed with cetaceans in a comparative analysis of sleep, most recent analyses have excluded these mammals (Elgar et al., 1988; Siegel, 2005; Lesku et al., 2006, 2008a; Capellini et al., 2008a,b, but see Savage and West, 2007).

The egg-laying monotremes also appear to lack some of the typical features that characterize REM sleep in marsupial and placental mammals, most conspicuous of which is the apparent lack of REM sleep-related cortical activation. The first EEG-based sleep study on a monotreme found only non-REM sleep occurring in the cortex of sleeping echidnas (*Tachyglossus aculeatus*, Allison et al., 1972). A subsequent investigation that included brainstem neuronal recordings in addition to the epidurally seated cortical electrodes found that brainstem neurons fired with an irregular burst-pause pattern similar to that observed in placental mammals engaged in REM sleep, but such activity occurred concurrently with cortical non-REM sleep (Siegel et al., 1996, 1998). This finding led to the hypothesis that REM sleep with cortical activation evolved only after the appearance of the marsupial-placental lineage, a hypothesis that was strengthened by recordings of sleep in another monotreme, the duck-billed platypus (*Ornithorhynchus anatinus*). Although brainstem activity was not recorded, only non-REM sleep was observed in the cortex of sleeping platypuses. However, during non-REM sleep, the platypuses showed rapid movements of the eyes, neck, and bill, suggestive of a REM sleep-like state (Siegel et al., 1999). If one defines REM sleep as a quiescent period with at least one eye movement per minute concurrent with non-REM sleep EEG activity, then platypuses spend up to 8 h in REM sleep (Siegel et al., 1999), more than any other animal studied. However, the appropriateness of comparing the time spent in REM sleep based on EEG activation in the cortex seen in marsupial and placental mammals to the REM sleep data derived only from the temporal pattern of twitching from the platypus is unclear. A more recent study of sleep in the echidna revealed a temperature-dependent expression of REM sleep with cortical activation, such that temperatures outside of their thermoneutral range appeared to suppress REM sleep (Nicol et al., 2000). Unfortunately, it is not clear whether the purported episodes of REM sleep were indeed a sleep state or simply an animal sitting quietly awake as eye state and arousal thresholds were not determined. Because of these inconsistencies regarding the EEG correlates of sleep in monotremes, data for echidnas and platypuses have been excluded from most comparative analyses (Elgar et al., 1988; Lesku et al., 2006, 2008a; Capellini et al., 2008a,b, but see Siegel, 2005; Savage and West, 2007). Overall, more work is needed on sleep in monotremes to reconcile the evolutionary history of mammalian REM sleep.

4. A multivariate approach

Most of the analyses discussed to this point have been done with simple statistical procedures, mainly correlation. As correla-

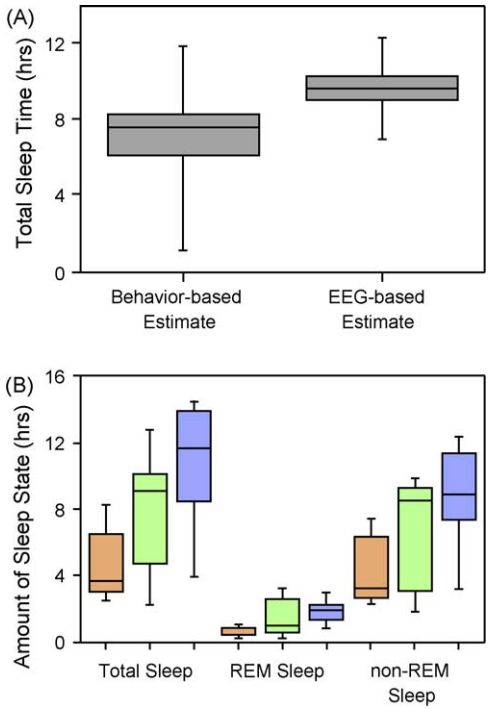

Fig. 4. A quantitative assessment of sleep data quality. (A) EEG-based estimates of total sleep time tended to be higher than estimates based only on behavioral observations ($P = 0.09$). (B) Estimates of total sleep time, REM sleep time, and non-REM sleep time per 24 h day from EEG recordings less than 12 h in duration underestimated these sleep parameters (orange); estimates of sleep parameters from recordings greater than 12 h, but shorter than 24 h (green), were not significantly different from those greater than 24 h in duration (blue). Boxes reflect lower and upper quartiles; the median is denoted by the horizontal line within each box. Reprinted from Capellini et al. (2008a) *Evolution*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

tion is the simplest statistical model, it can also be somewhat misleading, particularly when dealing with complex systems such as sleep and evolutionary processes. Sleep is arguably multifunctional, thus features of sleep will likely be determined by a combination of factors. The best way to model such a complex system is through multivariate statistical techniques, such as path analysis, a form of structural equation modeling (Mitchell, 1992). A comparative analysis within a single multivariate model (such as a path model) is advantageous because it quantifies the relationships among variables simultaneously, such that any redundant explanation of variation is taken into account. This is important because non-sleep traits are often correlated with one another (e.g., body mass and BMR). Moreover, unlike correlation or multiple regression, path analysis allows for the use of mediator variables through which the effect of an independent variable is channeled (Baron and Kenny, 1986). Such a model thus allows for indirect relationships among variables, which can better reflect reality. When a variable is treated as a mediator, relationships (paths) that flow from it reflect the influence of relative (or residual) values, provided some basic assumptions are first met (see Baron and Kenny, 1986), thus automatically eliminating the problem inherent in ratio-based statistical controls (see Section 3.1). Lastly, path analysis is an explicit hypothesis-testing procedure, such that model structure should be determined by *a priori* predictions. Conversely, this can also be a limitation of path analysis as it prohibits exploratory analysis. Below we outline the structure of recently published path models and briefly discuss important relationships between sleep and constitutive and ecological variables (see Lesku et al., 2006 for a more detailed discussion).

Two models were created and the structure of each was identical except for a difference in dependent (sleep) variables. The first model examined the relationships among constitutive and ecological variables on the time spent in non-REM sleep and REM sleep (Fig. 5), whereas the second structurally identical model (not shown) examined total sleep time and the percentage of total sleep time allocated to REM sleep (or %REM sleep). %REM sleep essentially reflects a time allocation problem, such that total sleep time would remain constant, but the allocation of time to non-REM or REM sleep would increase depending on a species-specific tradeoff. First, we will discuss relationships between sleep and constitutive variables, followed by those between sleep and ecological variables in Section 4.2.

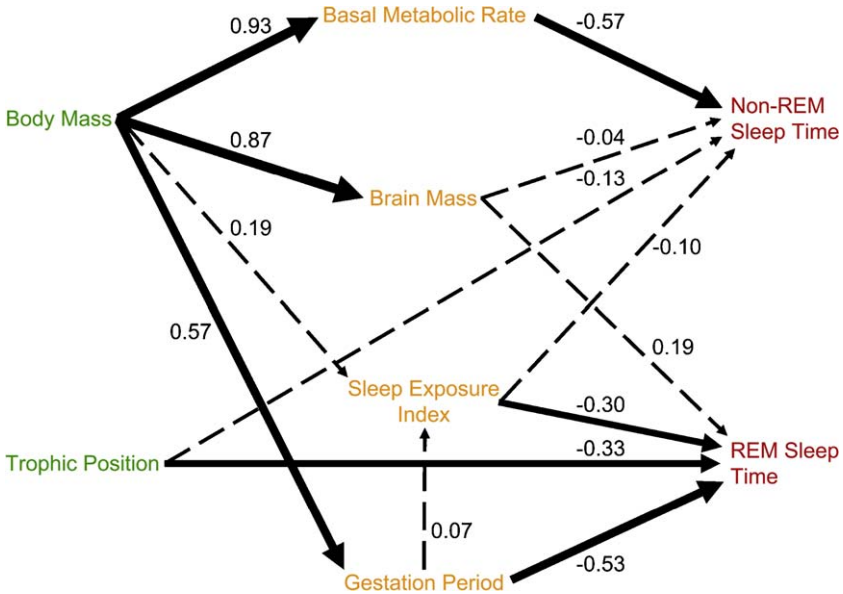

Fig. 5. A multivariate path model among independent (green), mediator (yellow), and dependent (red, sleep) variables reflecting hypotheses taken from the literature (see text for details). The number above each path represents a standardized regression coefficient, which quantifies the magnitude (bound by −1 and 1) and direction of a relationship. Non-significant paths are dashed, significant paths are solid and the thickness of each path is proportional to the strength of the relationship. Data were phylogenetically controlled using independent contrasts. Reprinted from Lesku et al. (2006) *American Naturalist*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

We posited that body mass has no direct relationship with sleep (Fig. 5), an idea which is consistent with the literature as no hypothesis for the function of sleep has a mechanistic relationship between sleep parameters and body size. Instead, we assumed that body mass might influence sleep via its influence on other constitutive traits, such as BMR and brain mass (Fig. 5), which conceptually have clearer functional relationships with sleep than body mass *per se*. For instance, under the energy conservation hypothesis (or other metabolically based hypotheses), one might expect species with a higher relative BMR to engage in more non-REM sleep (Zepelin et al., 2005). REM sleep on the other hand might be involved in the development of the central nervous system (Roffwarg et al., 1966). Specifically, the brain activation occurring during REM sleep might provide endogenous stimulation necessary for the normal development of the central nervous system, including the neocortex (Shaffery et al., 2002). This hypothesis stems (in part) from the observation that altricial species – those born relatively immature and dependent on their parents – engage in higher amounts of REM sleep at birth when compared to precocial species, a pattern that continues in adults (Jouvet-Mounier et al., 1970). Consequently, we predicted that comparative data would show that species more precocial at birth (higher relative gestation period) would engage in less REM sleep as adults than more altricial species. Conveniently, because BMR and gestation period (and brain mass, see Section 4.1) are mediator variables, paths from them reflect the influence of residual values similar to those obtained from an analysis of covariance (Garcia-Berthou, 2001).

Overall, our path models found support for some, but not all, of the above ideas (Fig. 5). Species with a higher relative BMR engage in less non-REM sleep, which does not provide phylogenetically broad support for non-REM sleep's role in energy conservation or other metabolically based hypotheses (Fig. 5, see also Section 3.1). Our models also revealed that species more precocial at birth (higher relative gestation period) have less REM sleep as adults, in both absolute and relative measures, than more precocial species (Fig. 5), which could be interpreted as comparative support for the hypothesis that REM sleep is important for the development of the central nervous system (Shaffery et al., 2002), although it remains unclear why this difference, most evident at birth, persists into adulthood (Siegel, 2005). We discuss other aspects of the path models below, first dealing with its implication for the function of REM sleep.

4.1. Phylogenetic data bearing on the sleep-learning connection

Experimental work indicates that non-REM sleep and REM sleep play a role in memory processing and plasticity (Stickgold, 2005); however, studies have been performed only on mammalian species of limited phylogenetic diversity (mainly rodents and primates). Thus, it is unclear whether sleep is important in facilitating enhancements in cognitive performance across mammals in general. If memory processing is a universal function of mammalian sleep, then species possessing greater cognitive abilities might be expected to engage in more sleep. Because of the mediator status of brain mass in our path models, paths from brain mass are conceptually similar to residual (relative) brain mass (or encephalization), which is a possible measure of interspecific cognitive ability (Jerison, 2001). Counter to the above prediction, variation in the time spent in non-REM or REM sleep was not determined strongly by variation in encephalization (Fig. 5). However, in the second path model (not shown) with total sleep time and %REM sleep as dependent variables, species with greater encephalization were found to allocate a higher percentage of time to REM sleep than those of lower encephalization (standardized regression coefficient = 0.51).

This REM sleep result is in contrast to those from other analyses. Specifically, the finding of an inverse (raw data-based) relationship between REM sleep and encephalization caused some to reject a memory consolidation function for sleep (Siegel, 2000, 2001, 2004). Our results, however, suggest that this inverse relationship stems from a lack of control for shared evolutionary history among species (see Fig. 3C and D). Furthermore, in a phylogenetically controlled (bivariate) analysis, Capellini et al. (2008a) did not detect a positive relationship between REM sleep and encephalization, a result they attributed to quality differences in dataset composition; however, we used similar criteria for the inclusion of data as Capellini et al. (2008a). Moreover, our positive relationship between %REM sleep and encephalization was identified using two different datasets of either 54 or 83 species, and in bivariate and multivariate analyses (see Lesku et al., 2006, 2008a). The reason for the divergent outcomes between Capellini et al. (2008a) and our own work is unclear.

Cetaceans were excluded from both the Lesku et al. (2006, 2008a) and Capellini et al. (2008a) analyses, because it is not clear how best to quantify the time spent asleep in these unihemispherically sleeping mammals (see Section 3.3). Importantly, in addition to sleeping with only one half of their brain at a time, cetaceans also lack cortical signs of REM sleep (Lyamin et al., 2008). The apparent secondary loss of REM sleep in cetaceans is surprising given that some cetaceans reach a level of encephalization shared by some anthropoid primates (Marino, 1998). Thus, if REM sleep is indeed important for information processing, then cetaceans have either found a different mechanism other than REM sleep to support their advanced cognition or cetaceans are not as intelligent as previously thought (Manger, 2006, but see Marino et al., 2008).

If %REM sleep increases with increasing encephalization, as suggested in our path model (Lesku et al., 2006), then %non-REM sleep necessarily decreases, yet non-REM sleep has also been implicated experimentally in memory processing and plasticity (Huber et al., 2004). Despite this fact, a positive relationship between non-REM sleep and encephalization has not been identified in comparative studies (Siegel, 2004; Lesku et al., 2006). Interestingly, mounting evidence suggests that the time spent in this state may not be the most neurophysiologically meaningful metric, such that a combination of time in, and intensity of, non-REM sleep may be the more relevant measure. Unfortunately, non-REM sleep intensity (i.e., low-frequency EEG power density or slow wave activity) has been reported for only a few species (Tobler and Jaggi, 1987). Nonetheless, in light of experimental data suggesting a connection between non-REM sleep and learning, the lack of a positive relationship between non-REM sleep and encephalization would seem to say more about the inadequacy of those two variables than to the connection itself (see Section 6.1).

4.2. Revisiting the risk of predation

In an early comparative analysis, Allison and Cicchetti (1976) showed that species subjected to higher risks of predation in the wild engaged in less non-REM sleep and REM sleep in the laboratory (see Section 2.2). We also predicted the same effect, as sleeping is dangerous irrespective of the state considered (Fig. 5). Our sleep exposure index estimated risk associated with where animals slept in the wild; trophic position estimated risk based on diet, with herbivores more susceptible to predators than carnivores. The sleep exposure index was set as a mediator variable to both body mass and gestation period since large mammals rarely sleep in burrows and small animals rarely sleep in the open, and precocial species often sleep in more open, riskier environments (Eisenberg, 1981). Overall, we found that species sleeping in more open locations and more herbivorous species engage in less REM

sleep relative to their secure-sleeping and more carnivorous counterparts, respectively (Fig. 5). Moreover, REM sleep in these species was disproportionately reduced (i.e., lower %REM sleep). Thus, this reduction of REM sleep in more vulnerable species might reflect an evolutionary strategy to minimize sleep-related risk, as arousal thresholds can be highest during REM sleep (Lima et al., 2005). Although cetaceans were excluded from this analysis (see Section 3.3), the absence of REM sleep in cetaceans, which sleep in the open water is consistent with this idea. Interestingly, as comparative and experimental data indicate that REM sleep is both dangerous for prey species *and* important for animals with relatively large brains, an interesting tradeoff may exist between minimizing REM sleep-related risk and maximizing REM sleep-related benefits of memory processing. How this (potential) tradeoff is resolved is an open area for future research.

The REM sleep results outlined above refute the sentinel hypothesis first proposed by Snyder (1966), which posits that REM sleep is the safer state (relative to non-REM sleep) as animals are better prepared for wakefulness when aroused from REM sleep. Moreover, experimental evidence indicates that REM sleep is selectively reduced following an increase in risk (Lesku et al., 2008b). The sentinel hypothesis also proposes that the adaptive significance of the brief awakenings that sometimes occur after a REM sleep bout allow the animal to periodically monitor the local environment for danger (Snyder, 1966). If true, then species subjected to higher risks of predation would be expected to have a faster sleep cycle so as to increase the number of brief awakenings. Capellini et al. (2008b) recently evaluated interspecific support for this aspect of the sentinel hypothesis by correlating sleep cycle length with indices of risk, but ultimately found no support for the idea, suggesting that the frequency of arousals is probably too low to be of much use for anti-predator vigilance (Lima et al., 2005).

Lastly, Capellini et al. (2008a) investigated the influence of the social environment on sleep. Socially sleeping animals may benefit from the early-warning and risk-dilution benefits of groups (Lendrem, 1983; Krause and Ruxton, 2002). Capellini et al. (2008a) created a 3-point scale to estimate risk related to group size while asleep. Species were categorized as (i) solitary sleepers, (ii) 'partially' social sleepers, or (iii) social sleepers. They hypothesized that species that sleep safely in a group would engage in more sleep than those that sleep alone (Capellini et al., 2008a). In a phylogenetically controlled (bivariate) analysis, group sleeping species were found to sleep less than species sleeping alone. Although this correlation ran counter to their expectation, it is consistent with the idea that sleeping is dangerous, if species that sleep in groups in the wild perceive the solitary-housing of the laboratory environment to be dangerous. If these animals never fully habituate to the laboratory (e.g., the "poor" sleepers of Allison and van Twyver, 1970), then poor habituation might manifest as reduced time spent asleep.

5. Beyond the mammalian paradigm

The study of sleep has been dominated by work on mammals (mainly rodents and primates). Not surprisingly, almost all comparative work on sleep has thus focused on mammals. The expansion of comparative analyses to non-mammalian taxa might reveal similar evolutionary patterns between distantly related groups (e.g., Manger et al., 2008), suggestive of similarities at a functional level as well. Currently, birds are the only other taxon with sufficient data for comparative work on sleep. Birds are a particularly interesting group with which to study sleep, because they exhibit non-REM and REM sleep comparable to that observed in mammals. Importantly, this similarity appears to be the result of convergent evolution, since the cortex of sleeping reptiles does not show similar sleep states (Rattenborg et al., 2009).

Given the broad similarities of sleep states between mammals and birds, it seems likely that these taxa share the same evolutionary determinants of sleep. As in mammals, there is reason to believe that non-REM sleep might be important in reducing energy expenditure in birds (Rashotte et al., 1998). Indeed, this was one of the early ideas for why mammals and birds, as homeotherms with high energetic demands, are the only animals known to exhibit non-REM sleep (Walker and Berger, 1980b). There is also reason to think that avian sleep is important in learning and facilitating enhancements in cognitive performance (Solodkin et al., 1985; Derégnaucourt et al., 2005; Margoliash, 2005; Crandall et al., 2007). In addition to sharing non-REM sleep and REM sleep, birds and mammals also share complex brains (Medina and Reiner, 2000) and in some species, primate-like cognitive abilities (Emery and Clayton, 2004), suggesting that the convergent evolution of sleep states, complex brains, and advanced cognition are functionally interrelated (Rattenborg et al., 2008b, 2009).

We recently conducted the first quantitative comparative analysis of sleep duration in birds (Roth et al., 2006). As with our previous comparative work on mammals, the avian analysis was based only on EEG-derived sleep data from adults. Despite the basic prediction that the correlates of sleep should be similar between birds and mammals, none of the correlates (whether phylogenetically controlled or not) previously identified in mammals were found in our avian analysis (Roth et al., 2006). Indeed, all of the avian relationships were markedly non-significant, with the exception that avian species sleeping in more open (potentially risky) locations have less non-REM sleep than those sleeping in more secure locations. This relationship was relatively strong ($r = -0.60$, $P = 0.003$) and robust to re-analysis using a newly published phylogenetic tree for birds (Hackett et al., 2008; Roth et al., unpublished data). These marked dissimilarities in the correlates of sleep between birds and mammals are difficult to interpret. They could be due to a lower range of variation in avian constitutive traits than in the mammalian dataset, or birds could be more variable in their responses to novel laboratory conditions, such that available sleep values do not reflect those in freely roaming birds.

Another issue is that avian sleep states are less clearly differentiated than in mammals, a condition that may render the quantification of avian sleep more open to interpretation, thereby adding more variation to the avian sleep dataset. For instance, the difference in EEG wave amplitude between wakefulness and non-REM sleep is smaller in birds than in mammals (Tobler and Borbély, 1988). Moreover, quiescent birds often exhibit EEG slow waves (the defining feature of non-REM sleep) in the light, a state that has been interpreted as "drowsiness" or outright non-REM sleep. For example, Tobler and Borbély (1988) and Martinez-Gonzalez et al. (2008) reported that pigeons spent 38% and 42% of the light phase of the photoperiod in non-REM sleep, respectively; however, Berger and Phillips (1994) reported that pigeons do not engage in non-REM sleep in the light at all. Instead, such periods of non-REM sleep were interpreted as drowsiness. When deprived of daytime non-REM sleep (or drowsiness, depending on the interpretation), pigeons show a compensatory increase in sleep intensity (or slow wave activity) during recovery sleep at night, indicating that irrespective of what we call them, the slow waves occurring during the light reflect homeostatically regulated non-REM sleep-related processes (Martinez-Gonzalez et al., 2008). Collectively, these studies reveal the great subjectivity in the scoring of avian non-REM sleep.

Avian REM sleep scoring is similarly open to some interpretation. As in mammals, transitions into and out of REM sleep are characterized by EEG features intermediate between REM sleep and the preceding or following state. Although such transitional episodes are open to interpretation in mammals, they constitute a relatively small proportion of the recording time when compared

to the overall amount of unambiguous REM sleep. Thus, transitional episodes have a minimal influence on the quantification of REM sleep. In birds, however, episodes of REM sleep are very short, typically lasting less than 10 s. Consequently, the ratio of time spent in transitional (ambiguous) states to time in unambiguous REM sleep is much greater, such that subjective interpretations of transitional episodes may impact estimates of avian REM sleep. Such a problem may be reflected in highly conflicting values of REM sleep reported in white-crowned sparrows (*Zonotrichia leucophrys gambelii*) in a non-migratory state, with one study reporting 16% REM sleep (Rattenborg et al., 2004), a value consistent with recent work in other songbirds (19% in the house sparrow, *Passer domesticus*, Costa et al., 2008; 15% in the blackbird, *Turdus merula*, Szymczak et al., 1993; see also Fuchs, 2006; Low et al., 2008), and another reporting less than 2% REM sleep (Jones et al., 2008). This anomalous finding probably reflects different perspectives on how to handle such transitional episodes. Consequently, perhaps a definitive comparative analysis of avian sleep must await standardized scoring criteria for EEG-defined sleep in birds.

6. Future of comparative analyses in sleep research

Comparative analyses of sleep have been conducted over the last 40 years, expanding the taxonomic applicability of some hypotheses for the functions of sleep. There are certainly more insights to be gained from additional work along these lines. This is also a good time to consider the ways in which comparative sleep analyses might be expanded to provide new insights into sleep. Here we suggest a few directions in which to proceed. Although our focus is largely on mammals, our suggestions could apply broadly to comparative analyses on any taxonomic group.

6.1. Hypothesis-testing and more physiologically meaningful variables

Future comparative studies of sleep should re-evaluate the many variables typically included in such analyses. The "traditional" constitutive variables, first used by Zepelin and Rechtschaffen (1974), have been used in virtually every analysis since. However, as acknowledged by Zepelin and Rechtschaffen (1974), these variables were selected simply because they were available, and not because they were the most precise or informative. Accordingly, other more meaningful variables would probably provide more insight into the functional basis for sleep. For instance, encephalization is relatively easy to obtain for a wide range of species, but may not be the most meaningful variable with which to assess interspecific support for sleep-dependent memory processing (Healy and Rowe, 2007; Capellini et al., 2009). More telling would be specific neurocytoarchitectural variables, such as measures of synaptic density or strength (see Tononi and Cirelli, 2006; Krueger et al., 2008). Gathering such data will not be easy, but the choice of 'new' variables must take specific hypotheses for the function of sleep into consideration, and cannot be based solely on ease of collection.

Preston et al. (2009) provide a good first step in this direction. Motivated by the idea that sleep in mammals maintains the immune system and protects against infection (see Imeri and Opp, 2009), they matched sleep data to species-specific white blood cell count, which is an index of investment in the immune system. In phylogenetically controlled analyses, they found that species that engage in more sleep have more white blood cells (Fig. 6A). In addition to total white blood cell count, this positive relationship extended to specific cell types functionally involved in an immune response, such as neutrophils, lymphocytes, eosinophils, and basophils. Importantly, other cell types not directly involved with the immune system, such as red blood cells and platelets, did not vary as a function of sleep duration. Perhaps as a result of enhanced immune defenses, species that slept longer were also found to be less parasitized (Fig. 6B). The increased immobility of these longer-sleeping species might also have lowered their encounter rate with parasites further reducing the incidence of parasitism. Collectively, these results are consistent with the idea that sleep allows (in part) for the re-allocation of energy to the immune system. Such an immune function for sleep probably reflects cellular processes that are best accomplished during the quiescent periods of sleep, and does not appear directly related to sleep-related changes in brain activity (Opp, 2009).

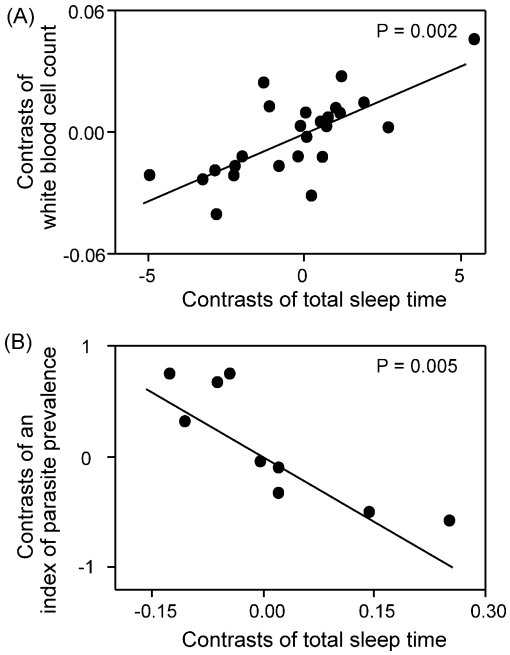

Fig. 6. An examination of the possible benefits sleep may serve for the immune system. (A) Species that sleep more have more white blood cells and (B) a lower occurrence of infection. Data were phylogenetically controlled using independent contrasts. Reprinted from Preston et al. (2009) *BMC Evolutionary Biology* (BioMed Central, publisher).

Not only are new constitutive variables needed, but so are new sleep variables. The mean duration of time over 24 h spent in non-REM sleep and REM sleep has been widely collected for the last 50 years, but few hypotheses posit a specific mechanism to relate a particular trait (neurophysiological or other) with the time spent in a given state *per se*. Indeed, many hypotheses for the function of non-REM sleep suggest that it is the time spent at a particular *intensity* of sleep that is sleep's most functionally important feature (e.g., Benington, 2000; Tononi and Cirelli, 2006; Krueger et al., 2008). Unfortunately, values for the intensity of non-REM sleep (or slow wave activity) are available for too few species to conduct a comparative analysis (Tobler and Jaggi, 1987), but could be collected provided regional differences in the level of slow wave activity (Vyazovskiy et al., 2002; Zavada et al., 2009) were taken into account. The incorporation of more physiologically meaningful variables represents the biggest challenge and opportunity for future comparative analyses of sleep.

6.2. Moving sleep research into the field

In addition to more physiologically meaningful variables, it is also important to record animals in the environments in which they evolved. All of the animals included in the mammalian and avian sleep datasets were recorded in captivity. An assumption of

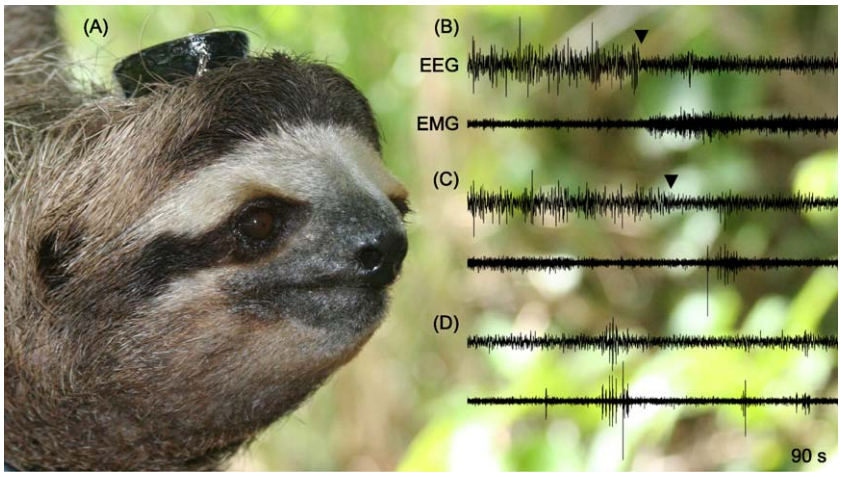

Fig. 7. The first electroencephalogram (EEG) and electromyogram (EMG) recordings obtained from a sleeping animal recorded in the wild. (A) An instrumented brown-throated three-toed sloth (*Bradypus variegatus*); the black cap on the head contains the miniaturized lightweight EEG/EMG logger. A recording showing representative non-REM sleep and a transition (arrow) to (B) wakefulness or (C) REM sleep. (D) A recording showing representative REM sleep. Reprinted from Rattenborg et al. (2008a) *Biology Letters*.

all comparative analyses is that interspecific differences in sleep in the laboratory reflect similar differences in sleep in the wild. This critical assumption remains untested.

There are, however, some data suggesting that at least some aspects of sleep in the wild are not well reflected in the laboratory. Rattenborg et al. (2008a) recently conducted the first EEG-based sleep study of an animal in the wild (Fig. 7). Wild brown-throated three-toed sloths (*Bradypus variegatus*) inhabiting a tropical rainforest were found to sleep 6 h *less* than the same species recorded in captivity, a 40% difference (Galvão de Moura Filho et al., 1983). This discrepancy could be due to differences in the age of implanted animals between the laboratory and field-based studies, but the definitive reason has yet to be demonstrated (Rattenborg et al., 2008a). If anything, captive sloths might be expected to engage in less sleep than those in the wild, as the need for sleep is determined (in part) by the duration and intensity of wakefulness (Huber et al., 2007; Vyazovskiy et al., 2008), such that the more sterile laboratory environment might be less stimulating than a more wild setting. Alternatively, the reduction of sleep seen in wild sloths may reflect a tradeoff between sleep and other behaviors, such as foraging and maintaining anti-predator defenses. In the laboratory, some demands are probably minimized (e.g., foraging), while others might be (perceived to be) heightened or reduced (e.g., predation risk) depending on the species. How a species perceives the laboratory environment will likely determine the degree to which its sleep reflects that observed in the wild. Nevertheless, if laboratory-housed sloths do indeed sleep more than their wild counterparts, then an examination of the specific costs associated with short- and long-term sleep restriction in response to other demands is an important avenue for future work (e.g., Horne, 1988, 2008; Patel et al., 2004; Patel and Hu, 2008).

7. Conclusions

The value of comparative analyses of sleep is clear. For instance, several comparative studies have overturned the commonly held view that species with relatively high metabolic rates engage in more non-REM sleep. Results from more recent analyses that controlled for shared evolutionary history among species are particularly important. These truly phylogenetic analyses suggest that REM sleep is important for the normal development of the central nervous system and also for memory processing and plasticity in adults. A recent analysis suggests that sleep allows for the re-allocation of energy to the immune system. Still other analyses highlight the importance of ecological processes, such as the risk of predation and energetic demands, on mammalian sleep. As these studies suggest, phylogenetic comparative methods should not be used to the exclusion of other lines of research, but rather should be viewed as a powerful complement to experimentation.

The productive history of comparative analyses of sleep suggests that it should also have a productive future. There are many possible avenues down which to proceed. More work is generally needed on sleep in non-mammalian animals, such as birds, which have independently evolved sleep states remarkably similar to those observed in mammals. We also encourage more hypothesis-testing coupled with the use of more physiologically meaningful variables, as well as studies of sleep recorded on free-living animals in the wild. Research on the functional significance of drowsiness would also be beneficial, as some animals, such as ruminants, appear to spend much more time in this mixed state than others (Ruckebusch, 1972). Collectively, such endeavors are important to our broader understanding of sleep, and will do much to maintain comparative analyses in the toolkit of sleep researchers.

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