2.06 The Evolution of Mammalian Sleep

S Herculano-Houzel, Vanderbilt University, Nashville, TN, United States

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Abstract

Mammalian brains and bodies tend to increase in size in evolution, requiring increasing hours of feeding per day—which is only feasible when daily sleep requirement is low. Mammalian species can sleep as little as 3 h day^{-1} and as much as 19 or 20 h day^{-1}, and typically, small species sleep longer hours than large species. Given that early mammals were very small, it can be expected that larger mammals appeared as sleep requirement evolved, decreasing with increasing body and brain mass. However, the correlation between brain (or body) mass and total daily sleep duration is not straightforward, with many examples of large mammals that sleep many hours per day, such as carnivorans. Besides, how could body mass possibly be related to brain mechanisms that control sleep duration? This chapter reviews the recent finding that daily sleep duration scales universally with the ratio between the density of neurons in the cerebral cortex and the total surface area of the cortex (D/A), which is presumed to determine the rate with which sleep-inducing metabolites accumulate in the cortical parenchyma during waking, both across adult species and in rat development. Because D/A decreases with increasing numbers of neurons, I propose that increasing numbers of cortical neurons in early mammalian evolution led to a decreased sleep requirement that allowed for more hours of feeding and increased body mass, which would then facilitate further increases in numbers of cortical neurons through a larger caloric input. Such coupling of increasing numbers of neurons to a decreased sleep requirement may thus have not only allowed but also driven the trend for increasing brain and body mass in mammalian evolution.

2.06.1 The Problem With Studying the Evolution of Sleep

Sleep is a seemingly universal behavior across animals. All mammalian species that so far have been examined alternate between sleep and waking states (Zepelin and Rechtschaffen, 1974; Campbell and Tobler, 1984), from which it can be safely inferred that sleep was a behavior already exhibited by the common ancestor to all mammals. Sleep also seems nearly universal among non-mammalian vertebrates. All reptiles, including birds, as well as amphibians and fish are also believed to sleep (Campbell and Tobler, 1984). The commonality of this behavior across extant vertebrates means that it reasonable to push it back to the common ancestor to all vertebrates. But sleep seems to go even further back in evolutionary history: insects sleep (Shaw et al., 2000), and even small-brained nematodes such as *C. elegans* are known to undergo a lethargy period that is similar to sleep in many ways (Trojanowski et al., 2015).

Given its ubiquity, sleep may well be a property so fundamental to nervous tissue that any brain will necessarily display it—and recent evidence is consistent with the possibility that sleep is an obligatory, alternate brain state triggered by metabolites that accumulate during waking (Xie et al., 2013). If this is the case (and determining whether it is will require examining the occurrence of sleep/waking alternation in a widely diverse range of animals, particularly those with simple or poorly organized brains, such as hydras, jellyfish, and flatworms), then it could be argued that sleep never "evolved" in the sense of being an "optional" feature that may or may not occur; rather, it emerges from the basic properties of brain tissue, and in that sense, it was always there, as a property that comes with a brain.

On the other hand, sleep is not universal in its features, and they may vary across species. Sleep is not a homogeneous period of rest and insensitivity to stimuli; it is subdivided into stages that have characteristic electrical signatures (Loomis et al., 1937). The structure of sleep into slow wave sleep (SWS) and rapid eye movement (REM) periods is strongly conserved across species, even beyond mammals: recently, birds (reviewed in Ref. Beckers and Rattenborg, 2015) and even a lizard, the Australian dragon, have been reported to alternate between SWS and REM sleep (Shein-Idelson et al., 2016). However, despite the similarity of sleep patterns, there is evidence suggesting that REM-like sleep evolved independently in birds and mammals (Lesku and Rattenborg, 2014).

Sleep diversity is most obvious when sleep duration is considered. Transitioning from waking to sleep is not simply a matter of passively shutting down the senses in the absence of light or other strong stimuli. It is by now well established that, at least in vertebrates, sleep is actively turned on by brain structures in the basal forebrain and hypothalamus (Saper et al., 2005), and reports across a wide range of species suggest that sleep duration is highly variable in both vertebrates and invertebrates, ectotherm or endotherm. Cockroaches sleep 14 h d^{-1}, through the entire daytime and extending into the night (Campbell and Tobler, 1984), and the laboratory fruit fly sleeps around 12 h d^{-1}, in bouts of 20–40 min, both during day and night (Van Alphen et al., 2013). Fish may sleep as little as 1 h or as much as 14 h day^{-1}, some concentrating sleep during the dark period (red-eye), some during daylight (tench; Campbell and Tobler, 1984). Likewise, among amphibians, the lake frog has been reported to sleep only 2.4 h day^{-1}, while the Western toad may sleep as much as 14.6 h day^{-1} (Campbell and Tobler, 1984). Among heterotherm reptiles, sleep has been reported to vary between 3 h day^{-1} in the caiman and as much as 20 h day^{-1} in the African python (Campbell and Tobler, 1984). Birds can also sleep as little as 4 h day^{-1} (hawk) and as much as 16 h day^{-1} (owls), and mammalian adults sleep between as little as 3 h day^{-1} (horse, elephant) and as much as 20 h day^{-1} (bats; Campbell and Tobler, 1984). Additionally, in different species, sleep may be concentrated during either daytime or nighttime or else happen in short cycles throughout the day. Assuming that technical issues that could interfere with the ability to detect sleep encephalographically as well as inconsistencies with behavioral observations were not the source of variation, it thus appears that variable sleep duration, or sleep requirement, is a widespread feature of all animals. Such wide variations in sleep duration across species point to the existence of variation in whatever yet-to-be-identified mechanisms control the number of daily hours of sleep in each brain. Because variation in biological features over the geological timescale that generates different species is the very definition of evolution, it appears, then, that sleep requirement has evolved.

But how to study the evolution of sleep, when it is a behavior that leaves no fossil evidence about its occurrence, much less its timing or duration? How can one determine when, and how, did sleep first appear, or who were the first animals to sleep? Absent a time machine, the only way to gain insight into the evolution of sleep is to examine its pattern of distribution across extant species to infer a phylogenetic history.

Similarly, examining how sleep requirement has changed over evolutionary time requires: (1) examining how much modern animals of different species sleep, (2) hoping to find a strong correlation with at least one parameter that can indeed be examined in the fossil record, then (3) looking for phylogenetic relationships in sleep duration patterns across clades that might allow inferences to be made about evolutionary patterns and mammalian ancestors. Steps (1) and (2) would contribute to establishing what determines sleep duration—that is, what is the variation in sleep requirement related to. Step (3), in turn, might determine whether there is an evolutionary pattern to variation in sleep duration: Has it changed systematically in mammalian evolution, or haphazardly, according to parameters that escaped major evolutionary trends?

2.06.2 The Elusive Scaling of Sleep

What do variations in total sleep duration correlate with is an issue that has been mostly addressed in mammals. Fig. 1 illustrates that sleep duration is variable not only across mammalian clades but also within them. While short sleep durations are found in the

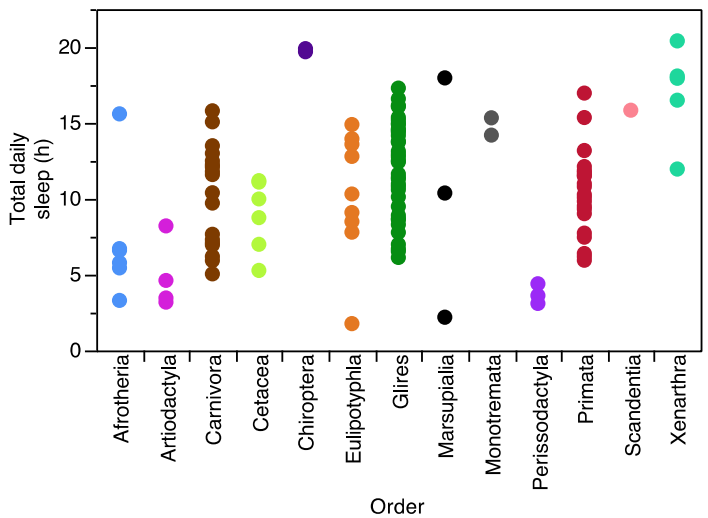

Figure 1 Average total number of hours of sleep per day across mammalian species. Each point represents one species, color coded across orders in the same manner throughout all figures in this chapter. Data from McNamara, P., Capellini, I., Harris, E., Nunn, C.L., Barton, R.A., Preston, B., 2008. The phylogeny of sleep database: a new resource for sleep scientists. Open Sleep J. 1, 11–14.

evolutionarily recent *Perissodactyla* and *Artiodactyla*, they also occur in the elephant, a member of the older clade *Afrotheria*—although elephants (Proboscidea) are, indeed, a fairly "young" group, of 5 million years (Meredith et al., 2011). Similarly, very long sleep durations occur in *Chiroptera* and in *Xenarthra*, two widely unrelated clades (Murphy et al., 2001).

One possible source of diversity in sleep duration in adult mammals of different species is metabolism, which in turn is related to body mass and longevity (Hofman, 1983). Considering that sleep enforces rest and conservation of energy, Zepelin and Rechtschaffen (1974) reasoned that daily sleep duration should be significantly correlated with longevity and metabolic rate—both of which are also related to body mass, the former positively, and the latter, negatively. Arguing that sleep restricts activity, those authors implied that it would be beneficial for smaller species, whose metabolic rates are elevated, to spend a greater portion of the day asleep than larger mammals, which have lower metabolic rates and live longer.

That argument was based on their finding that across a sample of 53 mammalian species, total sleep time correlated negatively with life span and brain weight, with coefficients of −0.52 and −0.71, respectively, and positively with metabolic rate estimated from body mass (and thus also with body mass itself), with a coefficient of 0.64. Those coefficients mean that, in general, smaller mammals, which have higher metabolic rates, sleep less per day than larger mammals. The negative correlations between total sleep time and body mass or brain mass are shown in **Fig. 2** for the updated data set provided by McNamara et al. (2008).

However, the correlations found by Zepelin and Rechtschaffen (1974) did not hold within the seven mammalian clades examined and in some cases had opposing signs across them. For example, total sleep time showed the expected positive correlation with metabolic rate (and thus body mass) in marsupials and perissodactyls, with coefficients of 0.67 and 0.65, respectively, but negatively in insectivores and artiodactyls, with coefficients of −0.87 and −0.65, respectively (Zepelin and Rechtschaffen, 1974).The authors note that these disparities "discourage a firm conclusion about the role of metabolic rate" in determining sleep duration across mammalian species.

Fig. 2 illustrates these discrepancies with the modern data set of McNamara et al. (2008). Across all mammalian species in the data set, total daily sleep duration decreases with increasing brain mass with a correlation coefficient of −0.4595 ($p < .0001$), but while a similar correlation is found in Afrotheria (−0.9000, $p = .0374$) and Carnivora (−0.5172, $p = .0335$), the correlation in positive in Glires (0.4528, $p = .0155$); across the latter species, total sleep time increases together with brain mass. Further, while total sleep time decreases with increasing body mass across all mammalian species in this updated data set (−0.4046, $p < .0001$), no significant correlation is found within any mammalian order at the $p < .05$ level. The finding of no consistent correlation between total daily sleep time and body mass or brain mass within mammalian clades should, however, not come as a surprise: to expect whatever mechanisms control sleep duration to be tightly linked to some aspect of bodily physiology that would depend on body mass, or even brain mass, seems far-fetched. It is understandable, however, that correlations would be sought for those variables, as for many decades, they were the only available for scrutiny across enough mammalian species to warrant studies of scaling.

A later study by Capellini et al., in 2008, also using the McNamara et al. (2008) data set, took into consideration the conditions under which sleep had been recorded and reached a different conclusion: that, across mammalian species, *residual* basal metabolic rate (that is, the variation in metabolic rate that remains once the variation explained by body mass is accounted for) actually correlates negatively, not positively, with daily sleep duration. In other words, those species with higher metabolic

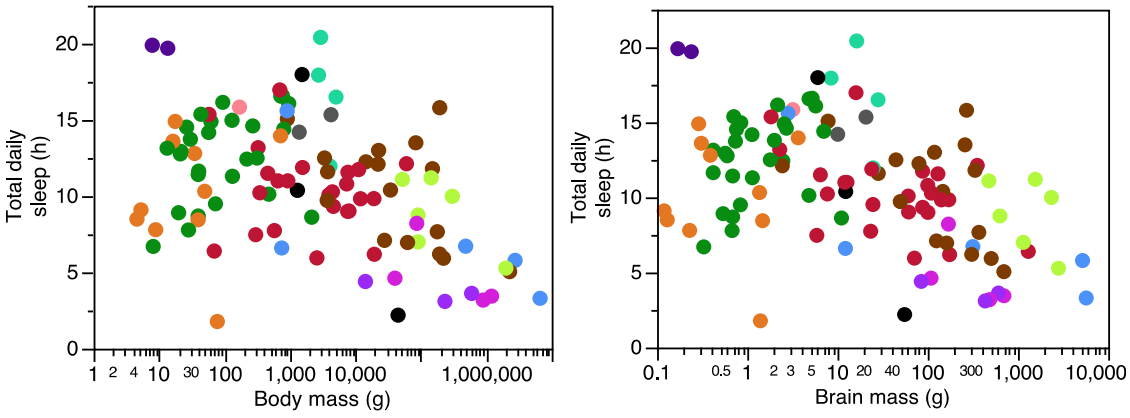

Figure 2 Average total number of hours of sleep per day tends to decrease with increasing body (left) or brain mass (right) across mammalian species. Each point represents one species, color coded across orders in the same manner throughout all figures in this chapter. Correlation coefficients across the entire data set: −0.4046 and −0.4595, respectively, $p < .0001$ (Spearman correlation). Breaking down the analysis per mammalian order, however, yields inconsistent results, with the following Spearman correlation coefficients, where $p < .05$, for the relationship brain mass x total daily sleep: *Afrotheria*, −0.9000, $p = .0374$ ($n = 5$); Artiodactyla, not significant ($p = .4000$, $n = 4$); *Carnivora*, −0.5172, $p = .0335$ ($n = X$); Cetacea, not significant($p = .4685$, $n = 6$); Eulipotyphla, not significant ($p = .8548$, n = X); *Glires*, 0.4528, $p = .0155$ ($n = X$); Primata, not significant($p = .0896$, $n = X$); Xenarthra, not significant($p = .4000$, $n = 4$). For the relationship body mass x sleep, no correlation is significant within an order: Afrotheria, $p = .1881$; Artiodactyla, $p = .4000$; Carnivora, $p = .0551$; Cetacea, $p = .4685$; Eulipotyphla, $p = .7514$; Glires, $p = .0710$, Primata, $p = .5989$.

rates than predicted for their body mass tended to sleep less—the opposite of the expected by Zepellin and Rechtschaffen (1974). Given the inconsistencies, Capellini et al. proposed that variation in sleep duration primarily reflects the ecological constraints characteristic of each species, with no physiological basis to determine sleep duration. Thus, maybe sleep does not scale; rather, it just varies according to a large number of variables, which is why its quantitative correlation with anything (that is, its scaling) for a long time remained elusive. And if sleep duration varies haphazardly, one should not expect to find systematic changes in sleep duration in mammalian evolution.

2.06.3 The Necessary Link Between Sleep Duration and the Evolution of Body Mass

The one consistent finding across the scaling studies of Zepellin and Rechtschaffen (1974) and Capellini et al. (2008) was that, across all mammalian species, there was some correlation between body mass and sleep. Whether focusing on the negative correlation between basal metabolic rate and sleep (Zepellin and Rechtschaffen, 1974) or the positive correlation between residual basal metabolic rate (after accounting for body mass) and sleep (Capellini et al., 2008), both studies agreed that larger mammals tend to sleep less. That correlation, however weak and possibly inconsistent across mammalian orders, does have a possible consequence for evolution, given an important trend found in all clades: that animals with larger body masses tend to appear over evolutionary time (Cope, 1887; Stanley, 1973; Alroy, 1998; Finarelli, 2007).

Although it is improbable that body mass exerts direct control over sleep duration, one link between body mass and sleep duration exists in the fact that, whatever the function of sleep (and there is now strong suspicion that it serves a very fundamental one for brain physiology; see below), the number of hours that an animal spends in this state per day has one very limiting consequence: by definition, how many hours that animal is awake and thus able to eat. The problem here is that larger animals cost more energy per day, at a steep rate of roughly $M_{BD}^{0.75}$ (Kleiber, 1947), but even though the capacity of ingesting calories per hour also increases with body mass (Fonseca-Azevedo and Herculano-Houzel, 2012), it does so more slowly than the cost of running the body. As a result, larger animals must spend longer hours feeding.

The corollary of the slower increase in energy intake than energy expenditure with increasing body mass is that the only way for larger animals to appear in evolution is through an increase in feeding time. Take the elephant, for example: maintaining a body of 4–6 tons requires feeding for 18 h day^{-1}, every single day (Estes, 1991). The only way for an elephant-sized animal to evolve was if its ancestral could sleep little enough to afford to eat ¾ of the day.

According to the fossil record, the earliest mammals were the size of the smallest extant shrews and bats (Rowe et al., 2011). While small mammals never disappeared, the largest mammals found at any point in time have increased in size over the last 65 million years (Cope, 1887; Stanley, 1973). Determining whether sleep duration was a constraint in mammalian evolution that limited the first mammals to small body sizes and allowed larger mammals to appear only slowly depends on establishing the relationship between sleep duration, body mass, and evolution. If ancestral mammals were frugal sleepers, sleep would not have been a limiting factor for increasing body mass, and larger bodies—even very large bodies—could possibly have appeared at any point in mammalian evolutionary history.

But the rough correlation that exists between sleep duration and body size in extant mammals suggests otherwise. As shown in **Fig. 2**, there is an overall correlation between body size and sleep duration, albeit with many large discrepancies: for instance, large carnivores sleep many more hours per day than similarly large ungulates. At around 100 kg of body mass, a lion sleeps an average of 14 h day^{-1}, whereas a pig sleeps only 8. At some 200 kg, a tiger sleeps around 16 h day^{-1}, whereas a donkey sleeps only 3 (McNamara et al., 2008).

It could simply be the case that animals sleep "when they can," that is, when they become idle—and while large carnivores, top predators by definition, can afford the luxury of many hours of sleep during the day, their prey, ungulates, must remain constantly on their toes. Such a behavioral difference could arguably place strong selection pressure against sleep in ungulates. However, sleep research has shown that it is not the case that sleep occurs whenever animals become idle or isolated from sensory stimuli; rather, sleep must be actively induced, a process that relies on an intricate system of interconnected structures distributed across the brainstem, hypothalamus, and basal prosencephalon (Saper et al., 2005). Moreover, there is a certain number of daily hours of sleep that are required for health, to the point that sleep seems subject to homeostatic control: in case of lack of sleep, sleep will last longer when it occurs (Porkka-Heiskanen et al., 1997).

Such discrepancies aside, it is the case that the largest mammals are those who sleep the least, and many of the smallest mammals sleep long hours (**Fig. 2**). If early mammals were long sleepers like equally small modern shrews and bats, then the evolution of larger bodies must have been tied to the evolution of shorter sleep duration. However, the loose correlation between increasing body mass and decreasing total sleep time in **Fig. 2** suggests that the evolutionary history of sleep has not been that simple: if the current relationship between sleep duration and body mass is not very strong, then it does not warrant the assumption that the two variables were strongly linked in the past.

But why should they, really? There is no physiological basis for expecting body mass to regulate sleep hours directly, other than through the correlation between body mass and metabolic rate. Because sleep is a behavior regulated by the brain like any other behavior, whatever controls sleep duration and its variation across species must be found in the brain. If sleep duration scales across species, then the brain feature that controls it must scale as well.

2.06.4 Sleep Serves a Function Related to Brain Scaling: Metabolite Clearance

Sleep brings a number of benefits to brain function and cognition, such as promoting learning and memory consolidation (Walker and Stickgold, 2004; Diekelmann and Born, 2010) and resetting levels of synaptic activity (Gilestro et al., 2009). Those, however, are functions that might not necessarily require scaling as a function of brain size or some other variable; a set number of hours might suffice—although comparative studies on memory-related sleep requirement across species are lacking.

Sleep, however, is a biological imperative, a brain state whose sustained and complete lack causes death in a few days through mechanisms yet to be identified (Rechtschaffen and Bergmann, 2002). In that regard, improving learning and memory might be considered "bonus" functions of sleep; it is hard to envision how a deterioration of these cognitive processes would lead to physiological breakdown and death in a matter of days.

A recent breakthrough indicated that sleep does serve a fundamental function that not only might lead to impaired brain physiology and possibly death but also scale with brain size: sleep allows the clearance of metabolites accumulated during the day (Xie et al., 2013). While investigating the flow of cerebrospinal fluid (CSF) through the glymphatic system in the awake mouse, the authors observed that CSF perfusion of the cortical parenchyma was enormously increased when the animal accidentally fell asleep underneath the two-photon microscope. This was due, the authors found, to a constriction of the interstitial space of the cerebral cortex during waking compared to sleep, possibly induced by the release of neuromodulators such as noradrenaline specifically during waking, which restricts CSF flow through the interstitial space during waking to only 5% of the flow found in sleep. As a consequence, metabolites accumulate during waking and get cleared away from the parenchyma during sleep (Xie et al., 2013). The restorative function of sleep, the authors proposed, is thus due to switching of the brain into a state that facilitates the clearance of those metabolites accumulated during waking.

Some of the metabolites that accumulate during waking might indeed be the very triggers of the switch into sleep. While some of these metabolites are toxic, one such sleep-inducing metabolite is adenosine (Porkka-Heiskanen et al., 1997; Halassa, 2011). The concentration of adenosine in the brain increases during waking, accumulates even more with sleep deprivation, and decreases rapidly during sleep (Porkka-Heiskanen and Kalinchuk, 2011). Thus, based on the findings of Xie et al. (2013), it can be postulated that sleep-inducing metabolites accumulate during waking up to the point when the switch to sleep is triggered and are cleared from the parenchyma during sleep.

2.06.5 A Hypothesis: Sleep Duration Scales With Metabolite Clearance

With such a function in the clearance of metabolites accumulated during sleep, particularly if some of them are sleep-inducing, it can be expected that sleep duration should scale with some aspects of brain size. CSF perfusion of the interstitial space is limited to the surface of the brain during waking (Xie et al., 2013), whereas brain volume increases faster than surface area across species, even with the folding of the cortical surface (Hofman, 1985, 1988). Thus, it is possible that the amount of time that elapses until a critical concentration of sleep-inducing metabolites accumulates (and thus the waking duration), and the amount of time required for clearance (and thus sleep duration), change with increasing brain size both in evolution and in development.

Brain mass, however, should not be the critical factor, and also not the number of neurons in the cortex, but rather the ratio between average neuronal density and the total surface area of the cortex. I have predicted (Herculano-Houzel, 2015) that sleep-inducing metabolites produced during waking hours should accumulate more slowly not in smaller cortices or in cortices with fewer neurons or a smaller density of neurons in the parenchyma, but rather in those cortices that have a smaller density of neurons underneath a unit surface area that gets preferentially washed by CSF during waking. Thus, those animals with a smaller ratio of cortical neuronal density to surface area should be able to stay awake longer and sleep less during the day, both in evolution and in the development of individuals. Although the prediction that sleep-inducing metabolites accumulate more slowly in cortices with a smaller density of neurons per surface area has not yet been tested, it leads to a second, more easily tested prediction: that daily sleep requirement should decrease together with the ratio between neuronal density and surface area both in development and in evolution.

The rationale for both predictions is illustrated in **Fig. 3**. According to the previous finding that mass-specific metabolic cost does not vary significantly with neuronal density across species (Herculano-Houzel, 2011), it can be assumed that sleep-inducing metabolites are produced at similar rates by neurons across species. The faster the rate of accumulation of sleep-inducing metabolites, the sooner a critical concentration (rather than total amount of metabolites) will be reached; the shorter the length of time that an animal should be able to spend in the waking state; and the longer the length of time spent in the sleeping state in which metabolites are cleared. The rate of accumulation of sleep-inducing metabolites, in turn, should depend not on the total number of neurons in the cortex (that would define the total amount of metabolites produced, but not their concentration), but on the density of neurons in the cortex. That is, how many neurons per milligram of cortex produce sleep-inducing metabolites at a constant rate per neuron should determine how the concentration of those metabolites changes over time spent in the awake state (Herculano-Houzel, 2015).

At the same time, because the surface of the parenchyma is constantly cleared of its metabolites, even during waking, the rate of metabolite accumulation should be inversely proportional to the pial surface area above the tissue volume: the larger the ratio

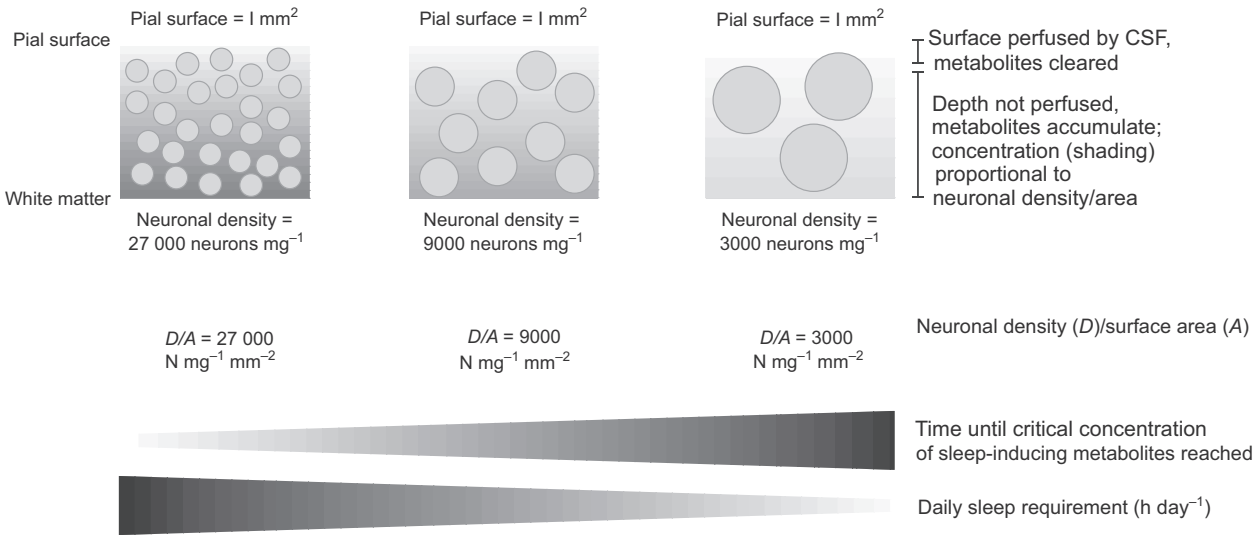

Figure 3 Daily sleep requirement is predicted to correlate with the ratio between neuronal density and surface area (D/A) in the cerebral cortex. The three images illustrate blocks of cortical tissue of similar pial surface area but different neuronal densities (either in different species or in different developmental stages of a same species). Because the production of metabolites per neuron per unit time is presumed to be similar across them (see text), the concentration of sleep-inducing metabolites produced per time and unit area (shading in each block) should be proportional to the density of neurons. Thus, when both the average neuronal density in the tissue and the total pial surface area vary across species (not shown), the rate of increase of the concentration of sleep-inducing metabolites should accompany D/A, the ratio between neuronal density and surface area in the cerebral cortex. The bottom part of the Figure shows how a smaller D/A would then allow for longer times spent in waking until a critical concentration of sleep-inducing metabolites is reached and thus lead to a smaller daily sleep requirement. *CSF*, Cerebrospinal fluid

between surface area and volume of the tissue, the larger the fraction of the parenchyma that should be kept clear of sleep-inducing metabolites during waking. Combining the two factors, the rate at which the concentration (that is, amount per volume) of sleep-inducing metabolites increases in the tissue should depend on the ratio between the density of neurons in the tissue (which determines the concentration of metabolites) and the total surface area of the tissue (which determines what fraction of those metabolites still gets cleared away during waking). The bottom part of Fig. 3 illustrates how a smaller ratio of neuronal density per surface area (D/A) should, in the scenario above, allow more time to elapse in the waking state until a critical concentration of sleep-inducing metabolites is reached, and thus lead to a smaller daily sleep requirement.

2.06.6 Sleep Duration Does Scale Across Mammalian Species

At this moment, the first prediction that the rate of concentration of sleep-inducing metabolites in the awake brain depends on D/A awaits direct testing in a variety of mammalian species. However, the prediction that total sleep duration decreases together with D/A across mammalian species and during the development of each individual has already been tested in a data set of 24 species belonging to six mammalian clades for which cortical numbers of neurons, neuronal density (collected in Herculano-Houzel et al., 2015), and surface area were available (Herculano-Houzel, 2015), as well as data for total number of sleep hours per day from the data set compiled by McNamara et al. (2008). All data used in the analyses below are available in Herculano-Houzel (2015). Only one update was made in the data set: I now consider the recent measurement by Yetish et al. (2015) that humans in preindustrial societies sleep an average of 6.4 h day^{-1}, rather than the average of 8.5 h in the original data set (Herculano-Houzel, 2015).

2.06.6.1 Sleep Duration Scales with D/A Across Species

As predicted by the hypothesis that awake duration should depend on the rate of accumulation of sleep-inducing metabolites, which in turn should depend on the ratio between neuronal density and surface area of the cerebral cortex (D/A), this is indeed the variable that best correlates with the total number of daily sleep hours across mammalian species (Herculano-Houzel, 2015), of all nine variables examined (brain mass or volume, cerebral cortical mass or volume, number of cortical neurons, neuronal density in the cerebral cortex, cortical surface area, number of neurons per mm^2 of cortical surface, neuronal density per mm^2 of cortical surface, cortical thickness and glia/neuron ratio; Fig. 4). Decreasing D/A ratios should correlate with slower accumulation of sleep-inducing metabolites, and as such, with shorter sleep duration. The predicted positive correlation is indeed observed, as shown in Fig. 4A.

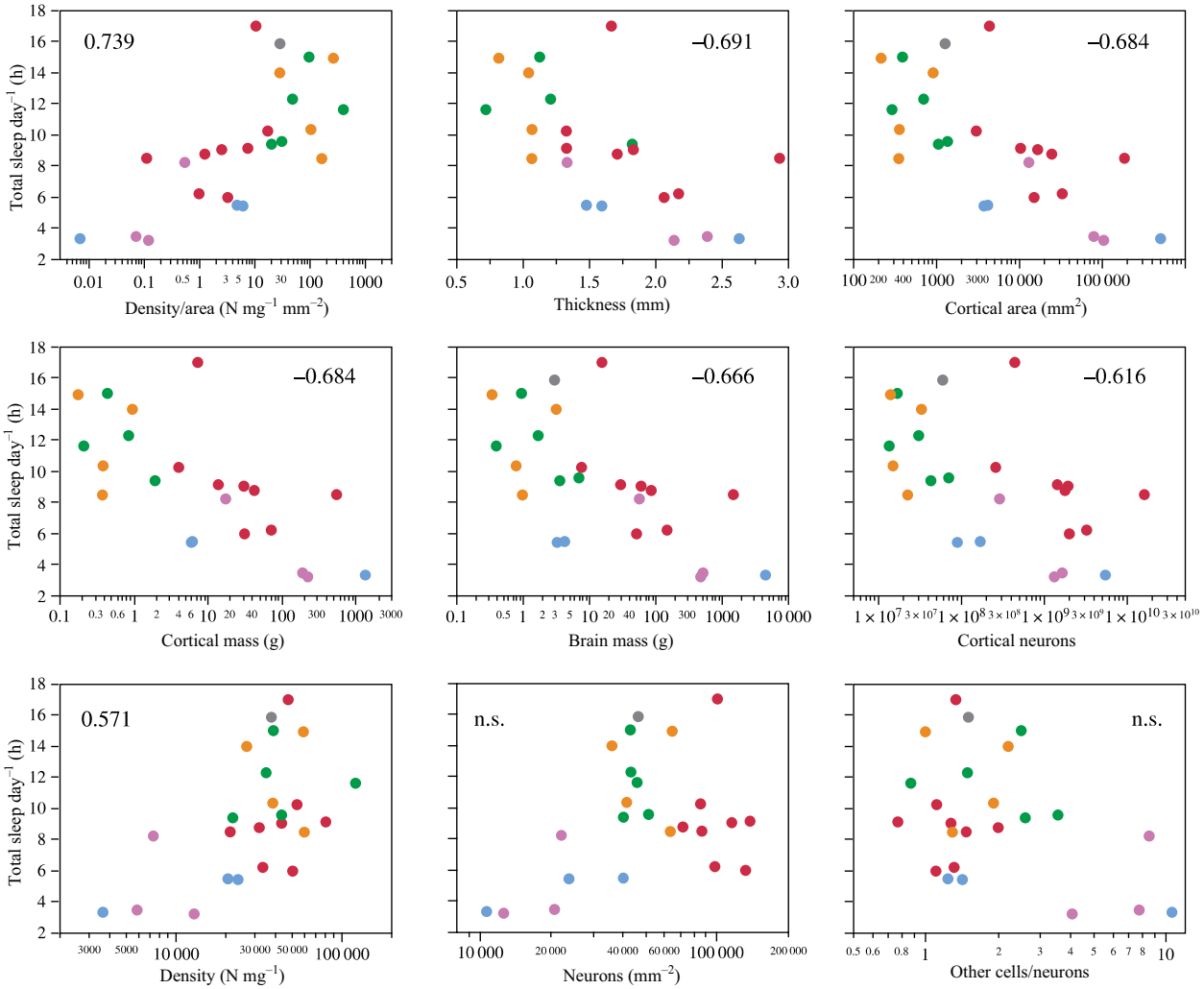

Figure 4 Ratio between cortical neuronal density and surface area (D/A) is the parameter that best correlates with total sleep hours per day across mammalian species. Each data point corresponds to one species. Values shown indicate the Spearman correlation coefficient for each graph. All values of $p < .005$ except for the two correlations in the bottom row, where $p > .1$. *Red*, primates; *orange*, eulipotyphlans; *green*, glires; *blue*, afrotherians; *pink*, artiodactyls; *gray*, scandentia.

A possible alternative mechanism for determining sleep duration is that awake time increases with the ratio of glial cells per neuron, as more glial cells would be available to offer metabolic support for neuronal activity. The finding that there is no significant correlation between daily sleep duration and the glia/neuron ratio (**Fig. 4I**) implies that this alternative can be excluded. Importantly, the number of neurons per cortical surface area (that is, the surface density of cortical neurons) also fails to show any significant correlation with variations in daily sleep duration (**Fig. 4H**), which indicates that the relevant parameter is indeed the *density* of neurons per surface area, not simply the number of neurons per surface area.

One constant problem when analyzing variables that scale with brain size is that most variables have some level of correlation among themselves, making it difficult to separate those that are directly responsible for variations in daily sleep duration. Indeed, although D/A is the parameter that best correlated with daily sleep duration (with a correlation coefficient of 0.739; **Fig. 4A**), several other parameters related to brain size or known to correlate with brain size (Herculano-Houzel et al., 2014) also showed correlation coefficients between 0.6 and 0.7 with daily sleep duration (**Fig. 4B–F**).

A principal components analysis, however, showed that D/A is the factor that most closely correlates with variations in daily sleep duration (Herculano-Houzel, 2015). When the five variables that best correlate with daily sleep time across all 24 species were considered together with total sleep duration, principal component analysis identified a first factor, which accounted for 53.2% of variation, composed of brain mass, cortical mass, and cortical surface area, which are known to be directly correlated with each other (Hofman, 1985). Daily sleep duration did not load significantly in this factor, but only in the second factor, together with D/A and cortical thickness (Herculano-Houzel, 2015). Together, the two factors explained 86.6% of the variation in all parameters.

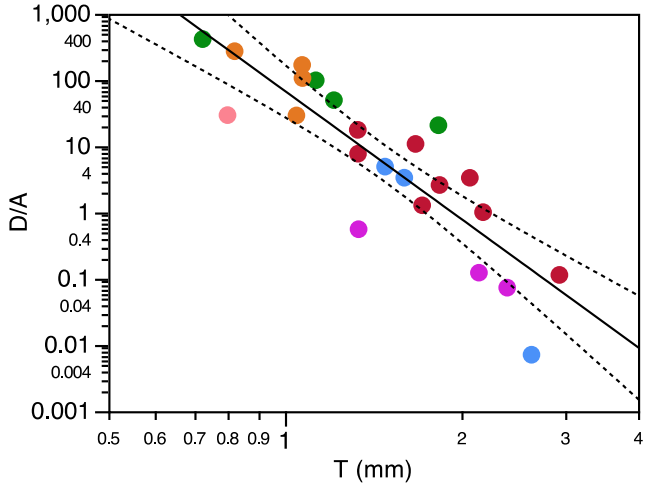

Figure 5 Ratio between cortical neuronal density and surface area (D/A) decreases with increasing cortical thickness across mammalian species. Each data point corresponds to one species. Exponent, -6.424 ± 0.810 ($r^2 = 0.946$, $p < .0001$). *Red*, primates; *orange*, eulipotyphlans; *green*, glires; *blue*, afrotherians; *pink*, artiodactyls; *gray*, scandentia.

There is a strong negative correlation between D/A and cortical thickness (Fig. 5; Spearman correlation, $r = -0.8953$, $p < .0001$), which explains the joint loading of the two variables in the second factor of the principal component analysis. Removing cortical thickness from the analysis actually increases the percent of variation explained from 86.6% to 88.3%, indicating that cortical thickness is not the key variable, while sleep duration continues to be the sole variable to load together with D/A.

Fig. 5 shows that thicker cortices are associated with lower D/A ratios, and as such, with *shorter* sleep duration, not longer sleep, as shown in Fig. 4B. Interestingly, the opposite would have been expected if thickness was the main variable determining the rate of metabolite clearance, as a thicker cortex should decrease metabolite clearance during waking even further and thus speed up the accumulation of sleep-inducing metabolites. Thus, the negative correlation observed between average cortical thickness and daily sleep duration is better explained through the inverse correlation between D/A and cortical thickness across species.

Although indirect, the correlation between cortical thickness and daily sleep duration through D/A is quite useful, as data on cortical thickness are available for more species than the 24 collected in Herculano-Houzel (2015) for which data on D/A were available. Thus, it is possible to use data on cortical thickness found in the literature to test for a correlation with daily sleep duration across a wider sample of 38 mammalian species. Fig. 6 shows that cortical thickness is indeed also inversely correlated with daily sleep duration across this wider data set.

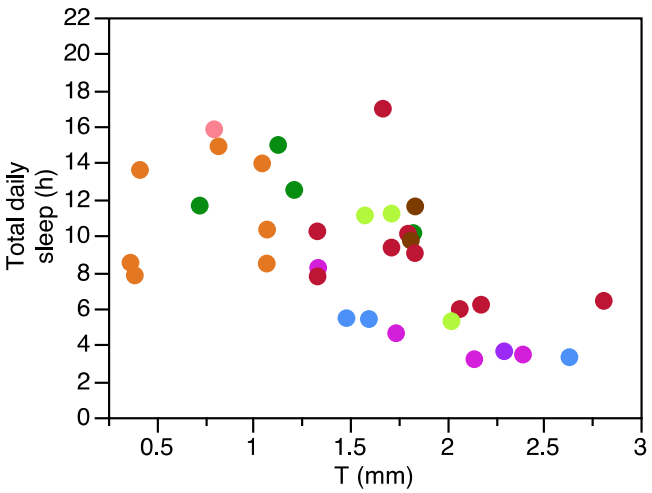

Figure 6 Although average cortical thickness is not the variable that PCA identifies as the main correlate of sleep duration, it shows a moderate negative correlation with sleep duration (Spearman correlation -0.5765, $p = .0004$) across a wider range of species ($n = 38$) than can be examined with D/A data at present ($n = 24$, Fig. 4).

2.06.6.2 Scaling of Sleep With Number of Cortical Neurons

Although the rationale for the hypothesis that sleep duration scales with the rate of metabolite accumulation still needs to be examined, the findings above indicate that sleep duration does scale across a wide range of mammalian species with a physiologically relevant parameter: D/A. Across the 24 mammalian species examined, total sleep duration was found to scale with D/A with a small but highly significant exponent of 0.139 (Fig. 7). Given what can be inferred at this point about how both cortical neuronal density and surface area have scaled with varying numbers of neurons in mammalian brain evolution (Herculano-Houzel et al., 2014), it becomes possible to examine how sleep duration may have scaled at the same time as mammalian brains varied in numbers of cortical neurons.

Early mammals were very small (Rowe et al., 2011) and thus presumably had a very small number of very small (that is, highly dense) cortical neurons (Herculano-Houzel et al., 2014). The strong trend toward the appearance of species with increasing numbers of neurons in mammalian evolution (Herculano-Houzel et al., 2014) raises the question of how daily sleep requirement would be impacted by increasing numbers of neurons, and possibly have evolved in parallel.

As shown in Fig. 4F, the number of daily sleep hours is only moderately correlated with numbers of neurons across all 24 species, and primates show no overlap with nonprimate species. However, it is now known that primates diverged in evolution from the nonprimate way of adding neurons to the cerebral cortex: while average neuronal density in the cerebral cortex decreases steeply with increasing numbers of neurons across nonprimate species, cortical neuronal density barely decreases significantly with increasing numbers of neurons in the primate cerebral cortex (Herculano-Houzel et al., 2014). A similar steeper decrease in the density of neurons in the cortical gray matter of nonprimates than in primates with increasing numbers of neurons is shown in Fig. 8A. The different consequences of changing numbers of cortical neurons on neuronal densities would thus be expected to impact D/A differently in primates and nonprimates. Indeed, when the relationship between D/A and number of cortical neurons is examined separately across primates and nonprimates, D/A is found to decrease steeply with increasing numbers of cortical neurons among nonprimates, and less steeply among primate species (Fig. 8B).

The lack of a universal correlation between D/A and numbers of cortical neurons thus explains why the correlation between daily sleep duration and numbers of cortical neurons is not universal: increasing numbers of neurons are associated with a steep enough decrease in D/A to be in turn associated with decreased sleep time only in nonprimates. Indeed, analyzing the relationship between D/A and number of cortical neurons separately among primate and nonprimate species reveals that it is only across the latter that daily sleep duration scales significantly with numbers of cortical neurons, decreasing with increasing numbers of cortical neurons across species as a power function of exponent -0.266 ± 0.034 ($r^2 = 0.809$, $p < .0001$, Fig. 9). The high r^2 of the fit for nonprimates indicates that 80.9% of the variation in total sleep duration across species is explained by variation in the number of cortical neurons.

Thus, as nonprimate species gain neurons in the cerebral cortex, their D/A decreases and they also sleep less per day; but as primates gain neurons, their smaller decrease in D/A is not enough to translate into significantly less sleep per day. Given the tendency in mammalian evolution toward increasing numbers of cortical neurons (Herculano-Houzel et al., 2014), it can be inferred that the first nonprimate mammals, with small cortices with very few neurons, had long daily sleep durations that decreased as new species with more cortical neurons (and thus larger cortices and brains) appeared. In contrast, as primates diverged away from the ancestral way of adding neurons to the cortex, larger number of neurons in their cortices came with no significant decrease in their daily sleep duration. The larger values of D/A in primate cortices compared to other mammals of similar brain size or number of cortical neurons thus explain the ca. 3 times larger sleep requirement of primates compared to artiodactyls and the elephant (Fig. 9).

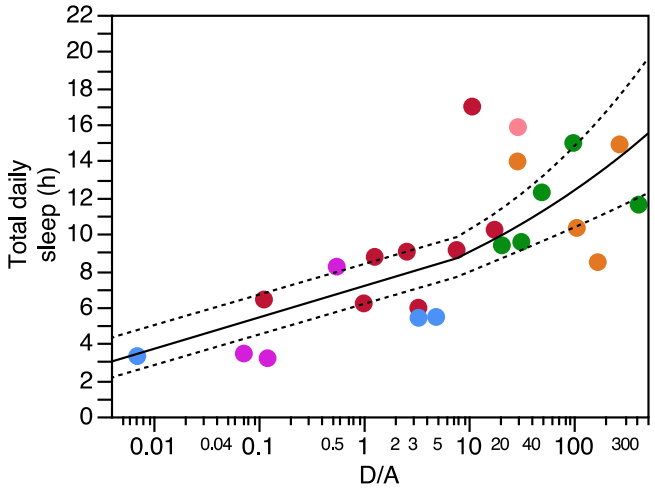

Figure 7 Daily sleep duration scales across the 24 mammalian species in the data set as a power function of D/A with an exponent of 0.139 ± 0.022 ($r^2 = 0.648$, $p < .0001$).

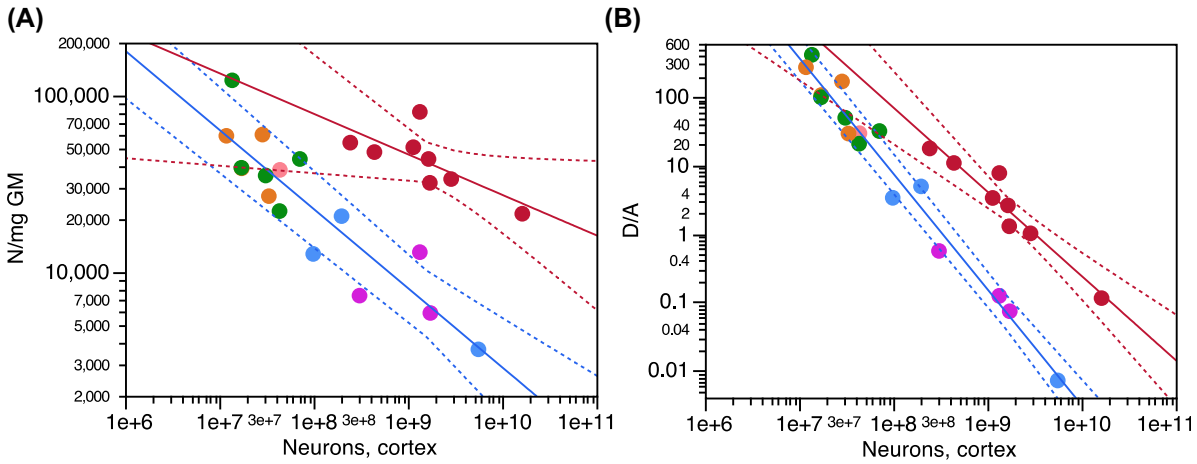

Figure 8 (A) Average neuronal density in the cerebral cortical gray matter decreases steeply with increasing numbers of neurons across nonprimate species in the data set (exponent, -0.448 ± 0.059, $p < .0001$), but less so across primates (exponent, -0.230 ± 0.090, $p = .0004$). (B) D/A also decreases more steeply with increasing numbers of cortical neurons across nonprimates (exponent, -1.694 ± 0.080, $p < .0001$) than across primates (exponent, -1.233 ± 0.144, $p = .0001$).

Daily sleep duration is therefore well predicted, and universally, by variations in D/A, which in turn are well predicted in evolution by changes in numbers of cortical neurons. It thus follows that daily sleep duration can be inferred to have changed in evolution as numbers of cortical neurons changed. Primates, like other mammals, sleep as much as can be predicted from their values of D/A (Fig. 7)—but predictably from their higher D/A, they sleep more than other mammals with similar numbers of cortical neurons.

2.06.6.3 Scaling of Sleep With Body Mass

Across the 24 species analyzed in Herculano-Houzel (2015), daily sleep duration is inversely correlated with body mass (Spearman correlation, -0.746, $p < .0001$; Fig. 10, left). This is a marginally stronger correlation than that of 0.739 found between daily sleep duration and D/A for the same data set (Fig. 4A). However, a principal component analysis once more shows that daily sleep duration loads only with D/A and cortical thickness in the second factor, whereas body mass loads with brain mass and cortical mass in the first factor.

As with the correlation between cortical thickness and daily sleep duration, which can be explained through the strong correlation between cortical thickness and D/A, the correlation between body mass and daily sleep duration can also be attributed to a strong correlation between body mass and D/A (Fig. 10, right).

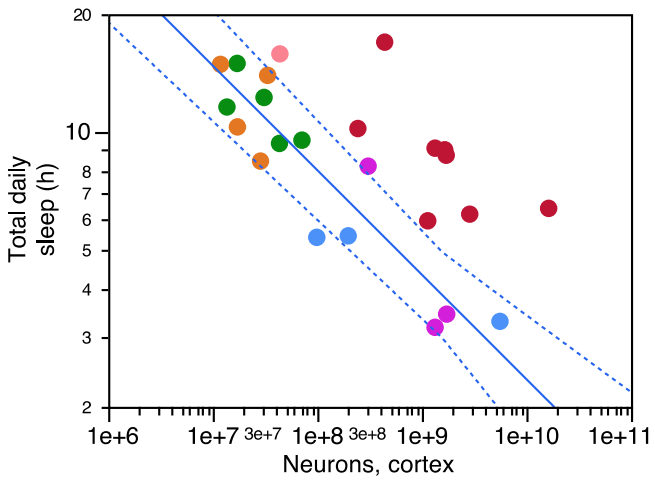

Figure 9 Daily sleep duration decreases steeply with increasing numbers of neurons across nonprimate species in the data set (exponent, -0.266 ± 0.034, $r^2 = 0.809$, $p < .0001$), but does not change significantly with increasing numbers of neurons across primates ($p = .0814$).

2.06.7 A Test of the Hypothesis: Sleep Duration Scales as Predicted in Development

Besides the enormous variation across species, sleep also varies over the lifetime of each individual: total sleep duration is maximal in newborn mammals and decreases rapidly over early postnatal development in both rats (Gramsbergen et al., 1970) and humans (Roffwarg et al., 1966). As shown in **Fig. 11**(top), rat pups spend more than 90% of the time asleep, but their total sleep duration decreases dramatically between postnatal days 4 and 10 (Gramsbergen et al., 1970), stabilizing around 50% of the day. If sleep duration is determined by a universal mechanism, then the same correlation with D/A found to apply across mammalian species should also apply to the dramatic change in sleep duration as the brain develops.

The most obvious change that the brain undergoes in development is an increase in brain size, with an ensuing expansion in surface area, accompanied by a decrease in neuronal density (Bandeira et al., 2009). However, and as predicted by the model detailed above, **Fig. 11** shows that the ontogenetic decrease in total sleep duration in rats mirrors closely developmental changes in D/A, but not in cortical mass, number of neurons, or the glia/neuron ratio (Herculano-Houzel, 2015). In ontogeny, as in phylogeny, a principal component analysis reveals that daily sleep duration loads with D/A and neuronal density, whereas brain mass, cortical mass, cortical area and neuronal density load in a separate factor that does not include sleep duration or D/A (Herculano-Houzel, 2015). Together, these factors account for 95.9% of all variation in the data, and the variation explained actually improves slightly to 96.5% when neuronal density is removed from the analysis, in which case daily sleep duration still loads together with D/A.

Fig. 12 shows that the fraction of the day that a rat pup spends asleep (and therefore total sleep duration) is directly correlated with D/A. Thus, as D/A decreases over postnatal development (**Fig. 11**), animals spend more and more time awake. A decreasing D/A ratio in ontogeny thus explains the initially large amount of time spent asleep and the rapidly decreasing sleep duration over development.

2.06.8 Implications for the Evolution of Sleep and Mammalian Body Size

Mammalian evolution is generally thought to occur through changes in the developmental program that leads to the formation of the adult body. Because total daily sleep duration decreases with increasing number of neurons in the cerebral cortex of nonprimate mammals (Herculano-Houzel, 2015), and early mammals had very small cortices with very few neurons (Herculano-Houzel et al., 2014), it seems likely that the first mammals slept most of the day and that requirement evolved as increasing numbers of cortical neurons presumably led to a decreasing D/A, and thus and ever decreasing number of hours spent asleep per day. Daily sleep requirement thus evolved together with increasing numbers of brain neurons.

Interestingly, to account for the evolution of sleep requirement in mammals, one does not need to invoke changes in a developmental program other than those that link increasing numbers of neurons to increasing average neuronal size (and thus decreasing neuronal density) and expanding cortical surface area. As seen in the developing rat, these changes, which cause D/A to decrease, explain the increase in awake time over the first weeks of life. Moreover, the range of total number of daily sleep hours in the developing rat, from 23 to 12 h day^{-1}, for corresponding values of D/A of between 2000 and 50 neurons/mm^5 (**Fig. 12**), overlaps with the distribution of daily sleep hours and D/A obtained across species (**Fig. 7**). This overlap supports the proposition that a single mechanism is at work to control daily sleep time both in developing and in adult brains, and this mechanism has not changed over time—that is, it has not evolved. Nevertheless, the behavioral result of this

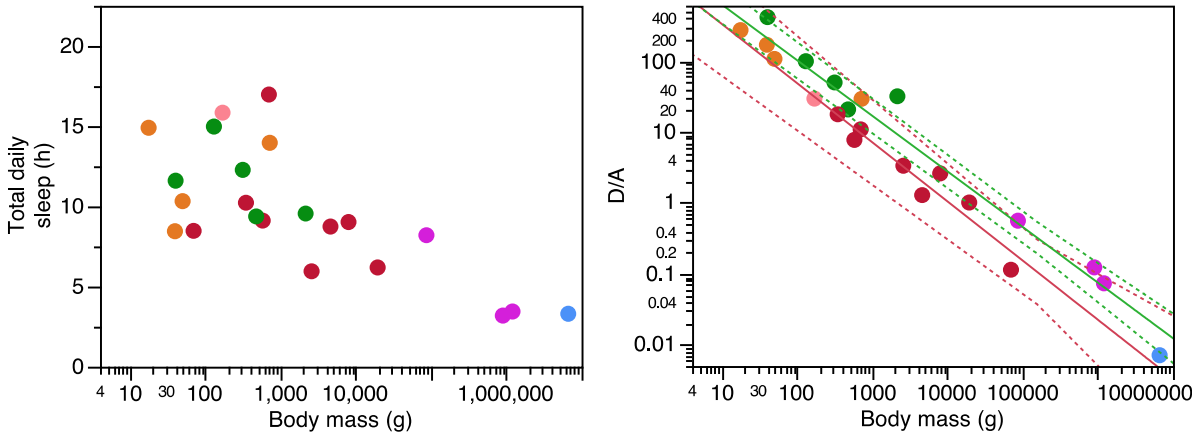

Figure 10 Daily sleep duration is negatively correlated with body mass in the data set of 24 species(Spearman correlation −0.746, $p < .0001$). This correlation can, however, be explained through a strong correlation between body mass and D/A (right), which in turn correlates strongly with sleep duration. Notice that primates show smaller values of D/A than predicted for nonprimates of a similar body mass.

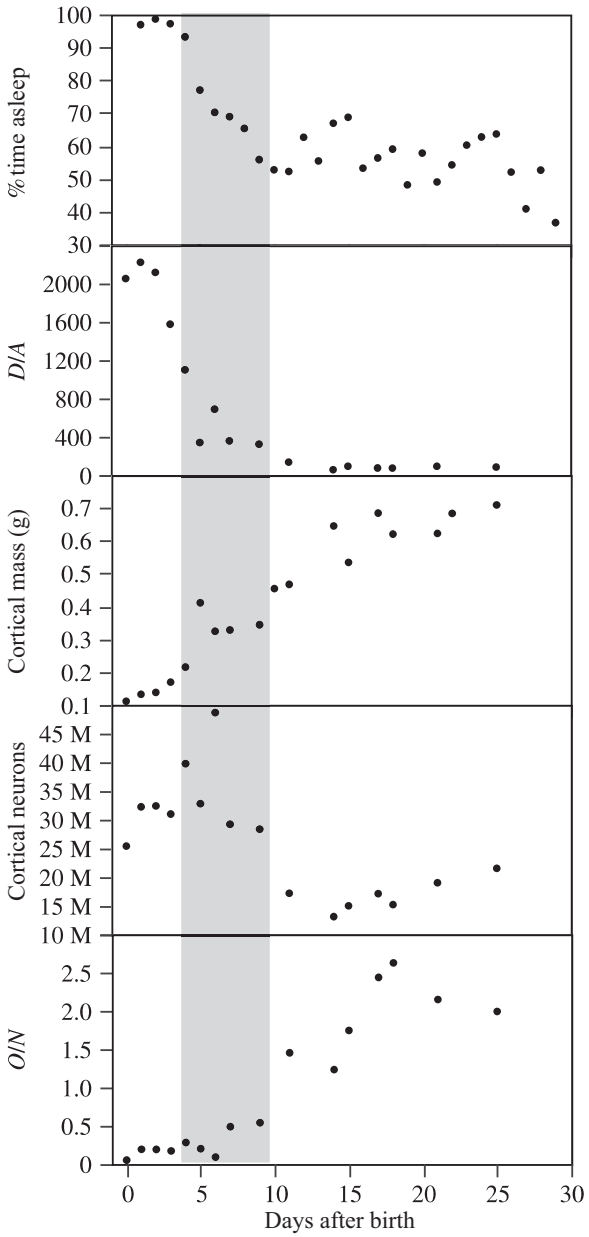

Figure 11 Decrease in total sleep time in early postnatal development in the rat is associated with a decrease in D/A. Graphs show the ontogenetic variation in different parameters pertaining to cortical morphology and cellular composition from birth (day 0) onwards. The shaded bar indicates the period of rapid decrease in % time spent asleep and in neuronal density/mm^2 (D/A). *O/N*, other cells/neurons ratio, which approximates the maximal glia/neuron ratio. Figure reproduced from Herculano-Houzel, S., 2015. Decreasing sleep requirement with increasing numbers of neurons as a driver for bigger brains and bodies in mammalian evolution. Proc. R. Soc. B 282, 20151853.

conserved mechanism—daily sleep time—does evolve as those mechanisms that regulate how numbers of neurons are related to neuronal density (or average neuronal cell size) and cortical surface area (Herculano-Houzel et al., 2014) change in evolution.

The decrease in daily sleep time that accompanies the decrease in D/A in early brain development has the important consequence of allowing the growing animal to feed for longer and longer periods of time, thus supporting continued growth. Thus, the very mechanisms that cause the brain to growth in development, with decreasing neuronal densities and increasing surface area (Bandeira et al., 2009), actually allow growth to be energetically possible through increasingly longer times spent awake, and thus feeding. Maternal care makes surviving on very little feeding time possible for the young, but a brain and body that grew without an accompanying decrease in the number of hours of sleep required per day would have a growing energy requirement that could, however, not be met.

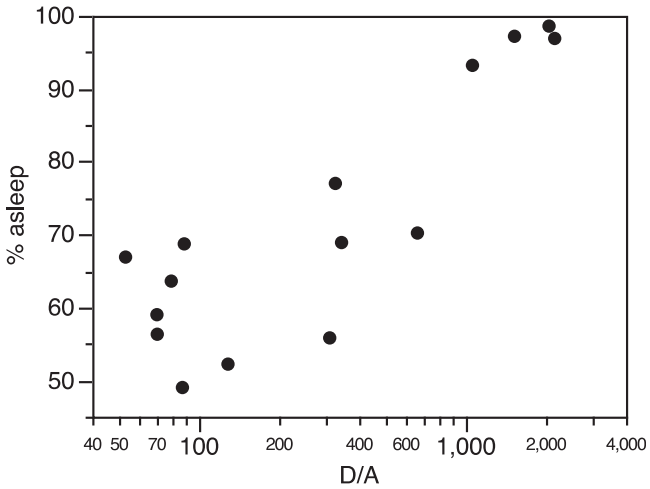

Figure 12 The percent time that a baby rat spends asleep varies together with D/A: animals are born with high D/A (to the right), and as D/A decreases accompanying the expansion of the growing cortical surface and a drop in neuronal densities, so does the daily duration of sleep.

Similar reasoning shows that the decrease in daily sleep time that accompanies the drop in D/A brought about by increasing numbers of neurons in mammalian evolution is not a side effect of increasing brain size without much consequence. For early mammals, nonprimates by definition, increasing numbers of neurons with the ensuing decrease in feeding time (**Fig. 13**) must have brought along the important advantage that more time became progressively available for the new species to feed, and thus afford both a larger body and a larger number of neurons. Spending more time awake and thus capable of feeding is particularly important when the earliest mammals, predicted to have the smallest numbers of cortical neurons in the data set, presumably approached 20 total hours of sleep per day. Because of its link to decreasing D/A and thus total daily sleep time, adding neurons to the cerebral cortex would thus bring not only increased information processing capabilities (Williams and Herrup, 1988) but also the means to afford both, the energy required to sustain more neurons and the time to use that increased number of neurons to explore the environment. Simultaneously, the increased body mass made affordable by the availability of longer hours to feed (because of shorter sleep hours) allows an increase in the amount of energy uptake per hour, which in turn allows supporting increasing numbers of neurons. I propose that this self-reinforcing spiral of increasing numbers of neurons leading to decreased sleep time which in turn allows more time to feed which makes larger bodies possible that obtain more energy per unit time and thus can afford even larger numbers of neurons has been a major driver of the tendency for brains and bodies to become larger in mammalian evolution. Had the early mammalian brain not added neurons to its cortex with a decreasing D/A, large mammals would probably not have evolved.

The limitation that results from adding neurons to the cortex without a rapid enough drop in D/A that would allow significant decreases in daily sleep time is illustrated by primates. These animals first appeared around 70 million years ago

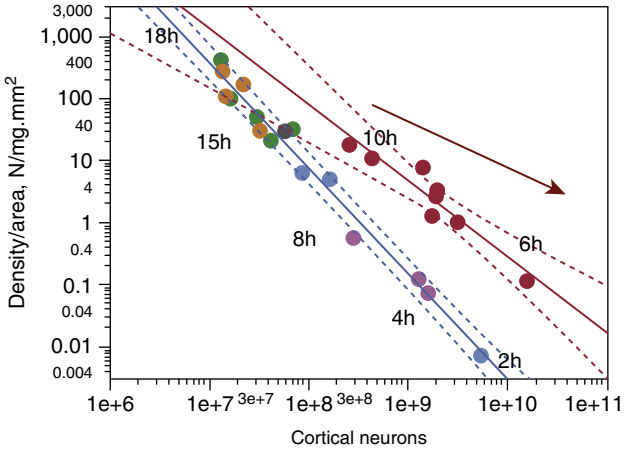

Figure 13 Approximate impact of increasing numbers of cortical neurons in mammalian brains on the number of total daily sleep hours, given the relationship between the number of cortical neurons and the D/A ratio in primates (*red*) and nonprimate species (*other colors*).

(Meredith et al., 2011), presumably with large neuronal densities and cortical surface areas still small enough that resulted in a large value of D/A and thus sleep times around 10 h/day^{-1}. Because the addition of neurons to the primate cortex does not cause D/A to drop fast enough, modern large primates still sleep 6–8 h day^{-1}, which limits the daily number of hours spent feeding to not much more than another 8 h (Fonseca-Azevedo and Herculano-Houzel, 2012). This is a constraint that we have shown to limit the maximal possible body size of primates to not more than 200 kg and also explains how come great apes do not have the large brains expected for their large body mass: because they cannot afford both. Incidentally, this is a constraint that human ancestors escaped by radically changing their diet, possibly with cooking (Fonseca-Azevedo and Herculano-Houzel, 2012), which allowed much larger numbers of cortical neurons—but with a small drop in D/A that still did not suffice to decrease their sleep requirement. Still, had primates appeared earlier in evolution, from a common ancestor with fewer neurons and an even higher D/A and therefore daily sleep requirement, their body mass and number of brain neurons would probably have been even more restricted than they already are.

Given the relationship between D/A and daily sleep requirement, the order-specific scaling of neuronal density with increasing numbers of neurons N and of cortical surface area with increasing numbers of cortical neurons explains how come animals of similar body size or even similar brain mass can have such different sleep requirements. It will be interesting to examine whether the present hypothesis also explains the long sleep hours of species of the order *Carnivora*, compared to artiodactyls and even primates of similar brain size (McNamara et al., 2008), once data on numbers of neurons and neuronal density become available for carnivores.

Several other issues also remain to be determined. The hypothesis that sleep-inducing metabolites accumulate in the parenchyma of waking mammals at a rate that is proportional to the ratio between neuronal density and cortical surface area still needs to be tested. It also remains to be determined whether daily sleep requirement also varies together with D/A in birds and other animals. Still, the finding that decreases in the D/A ratio explain remarkably well both ontogenetic and phylogenetic decreases in the daily sleep requirement of mammals opens a number of new possibilities for the comparative study of sleep requirement in developing and adult animals and its implications for evolution.

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