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Decreasing sleep requirement with increasing numbers of neurons as a driver for bigger brains and bodies in mammalian evolution

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Mammals sleep between 3 and 20 h d^{-1}, but what regulates daily sleep requirement is unknown. While mammalian evolution has been characterized by a tendency towards larger bodies and brains, sustaining larger bodies and brains requires increasing hours of feeding per day, which is incompatible with a large sleep requirement. Mammalian evolution, therefore, must involve mechanisms that tie increasing body and brain size to decreasing sleep requirements. Here I show that daily sleep requirement decreases across mammalian species and in rat postnatal development with a decreasing ratio between cortical neuronal density and surface area, which presumably causes sleep-inducing metabolites to accumulate more slowly in the parenchyma. Because addition of neurons to the non-primate cortex in mammalian evolution decreases this ratio, I propose that increasing numbers of cortical neurons led to decreased sleep requirement in evolution that allowed for more hours of feeding and increased body mass, which would then facilitate further increases in numbers of brain neurons through a larger caloric intake per hour. Coupling of increasing numbers of neurons to decreasing sleep requirement and increasing hours of feeding thus may have not only allowed but also driven the trend of increasing brain and body mass in mammalian evolution.

1. Background

Sleep is seemingly universal among vertebrates [1], and also occurs in invertebrates [2]. How much sleep is needed, however, varies across mammalian species from a total number of as much as 20 h d^{-1} in bats to as little as 3–4 h in giraffes and elephants [3,4]. Daily sleep requirement also changes during the development of each individual, with sleep occupying most of the hours of the day in newborns but a rapidly decreasing proportion of the day with development [5,6]. It remains unknown what determines daily sleep hours across species or what makes it decrease in postnatal life, as there is currently no reasonable physiological hypothesis to explain this variation [4]. There is a rough correlation of the daily sleep requirement of a species with brain mass, but large primates are clearly outliers, sleeping 8–9 h d^{-1}, compared with smaller sleep requirement of artiodactyls of similar brain mass, such as cattle and giraffes, which sleep around 3 h d^{-1} [4,7].

Although sleep brings a number of benefits to brain function and cognition, such as promoting learning and memory consolidation and resetting levels of synaptic activity [8–10], a recent breakthrough indicates that the fundamental function of sleep is to allow the clearance of metabolites accumulated during the day [11]. Because of a constriction of the interstitial space of the cerebral cortex during waking compared with sleep, cerebrospinal fluid (CSF) flow through the interstitial space during waking is 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.* [11] thus propose that the

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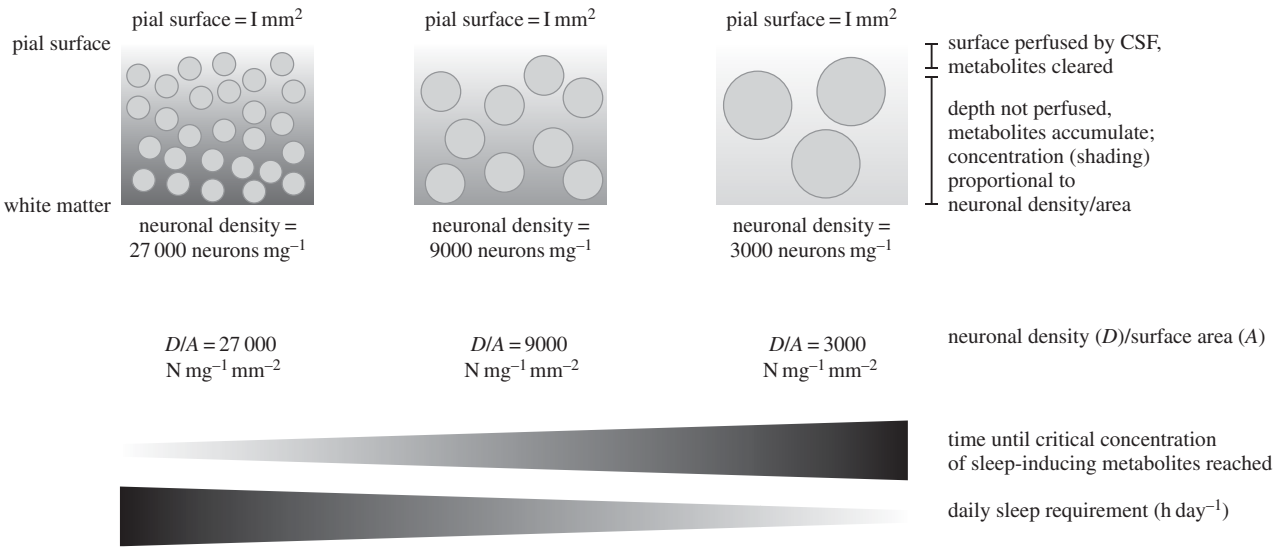

Figure 1. Daily sleep requirement is predicted to correlate with the ratio between neuronal density and surface area (*D*/*A*) in the cerebral cortex. The three images illustrate blocks of cortical tissue of similar pial surface area but different neuronal densities (either in different species or in 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.

restorative function of sleep is due to switching of the brain into a state that facilitates the clearance of accumulated metabolites.

While some of these metabolites are toxic, others are sleep-inducing, such as adenosine [12,13]. The concentration of adenosine in the brain increases during waking, accumulates even more with sleep deprivation and decreases rapidly during sleep [14]. This is well in line with the findings of Xie *et al.* [11], and indicates 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. Because CSF perfusion of the interstitial space is limited to the surface of the brain during waking and brain volume increases faster than surface area across species, even with the folding of the cortical surface [15,16], 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 mass both in evolution and in development.

More specifically, I predict 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. Similarly, the amount of time required in the sleep state to allow the clearance of the metabolites accumulated in waking should decrease as the density of metabolite-producing neurons per surface area also decreases, 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 is not tested in this study, 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 figure 1. The previous finding that average metabolic cost per neuron does not vary significantly with neuronal density across species [17] warrants the assumption 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. On the one hand, 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 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. On the other hand, the total amount of metabolites produced per time should depend on the number of neurons in the tissue, not simply on its volume. Thus, the rate at which the concentration (that is, amount per volume) of sleep-inducing metabolites increases in the tissue should depend neither on the simple number of neurons in the tissue, nor on the density of neurons in the tissue (which does determine the concentration of metabolites produced per time for a similar unit area of CSF-perfused pial surface), and also not on the number of neurons underneath a unit surface area (for this determines the total amount of metabolites per surface area, but not the concentration of those metabolites in the tissue). Rather, the concentration of sleep-inducing metabolites should increase at a rate that depends 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. The bottom part of figure 1 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.

Here I examine this second prediction that total sleep duration decreases together with neuronal density mm^{-2} across mammalian species and in their development. To this end, I present an analysis of a set of 24 species belonging to six mammalian clades for which cortical numbers of neurons, neuronal density and surface area were available [18–29], as well as data for total number of sleep hours per day [7]. Data are provided in table 1.

We were recently able to infer that, in mammalian evolution, the earliest animals had highly neuron-dense cortices and as new non-primate species gained neurons, these neurons also became larger [30]. By contrast, primates emerged in mammalian evolution with changes that resulted in much smaller increases in average neuronal size as neurons were added to the brain [30]. As a consequence, the scaling of the ratio of cortical neuronal density to surface area is expected to occur at different rates in primate and non-primate evolution, a hypothesis also examined here. Furthermore, because early mammals were very small [31], with very few neurons, and the number of cortical neurons tended to increase in mammalian evolution [30], I also determine how increasing numbers of neurons correlate with the total number of daily sleep hours, to gain insight into the evolution of sleep. Finally, I examine whether the ontogenetic decrease in sleep hours is also best explained by a decreasing ratio of neuronal density per surface area in the rat, by making use of published data on the ontogeny of sleep in the rat [5] and of our own data on the developmental changes in the cellular composition of the rat brain ([32]; data available in table 2).

2. Results

(a) Daily sleep requirement decreases together with the neuronal density/surface area ratio across adult mammals

Across all nine variables examined in 24 mammalian species (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 square millimetre of cortical surface, neuronal density per square millimetre of cortical surface, cortical thickness and glia/neuron ratio), the one that best correlates with the total number of daily sleep hours across species is neuronal density per square millimetre of cortical surface or density per area (D/A; figure 2). As predicted, total sleep duration decreases together with D/A. There is no significant correlation between daily sleep time and the glia/neuron ratio, which excludes the alternative hypothesis that sleep time decreases as more glial cells per neuron are available to support their activity. Importantly, the number of neurons per cortical surface area also fails to show any significant correlation with variations in daily sleep time (figure 2), which indicates that the relevant parameter is indeed the *density* of neurons per surface area, not simply the number of neurons per surface area. However, there is the concern 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 time.

A principal components analysis, however, can identify those variables that cluster with daily sleep time. When the five variables that best correlate with daily sleep time across all 24 species are considered together with total sleep duration, principal component analysis identifies two factors that together explain 86.6% of the variation in all parameters. The first factor, which accounts for 53.2% of variation, includes brain mass, cortical mass and cortical surface area, which are known to be directly correlated with each other [15] (factor loading: 0.9762, 0.9724 and 0.9685, respectively). Importantly, daily sleep time only loads significantly in the second factor, together with D/A and cortical thickness (factor loading: 0.7127, 0.8738 and -0.7741, respectively). The joint loading of D/A and cortical thickness in the second factor can be explained by a strong correlation between increasing D/A and decreasing cortical thickness across species (Spearman's correlation, $r = -0.8953$, $p < 0.0001$). Removing cortical thickness from the analysis actually increases the per cent of variation explained to 88.3%, and sleep duration continues to load only in the second factor together with D/A (loading, 0.7060 and 0.8998, respectively). Principal component analysis thus confirms that D/A is the parameter most closely associated with daily sleep time across species, as predicted. As shown in figure 3*a*, daily sleep requirement scales across all 24 mammalian species as a power function of the D/A ratio with a small but highly significant exponent of 0.133 ± 0.023 ($r^2 = 0.601$, $p < 0.0001$).

(b) Daily sleep requirement decreases with increasing numbers of neurons across non-primate species in evolution

Early mammals were very small [31], and thus presumably had a very small number of cortical neurons [30]. The strong trend towards the appearance of species with increasing numbers of neurons in mammalian evolution [30] 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 figure 1, the number of daily sleep hours is only moderately correlated with numbers of neurons across all 24 species. However, it is now known that while there is a single relationship between the number of cortical neurons and the cortical volume across non-primates, primates have their own scaling relationship, with neuronal densities that decrease only little as the cortex gains neurons [30]. It is thus possible that the reason why the overall correlation between daily sleep requirement and numbers of cortical neurons is not strong is that the relationship between D/A and numbers of cortical neurons is not universal across mammalian species.

Indeed, figure 3*b* shows that increasing numbers of cortical neurons are strongly associated with decreasing D/A across species, but as different power laws across primates and non-primate species. The D/A ratio falls steeply as numbers of neurons increase across non-primate species (with an exponent of -1.694 ± 0.080; $r^2 = 0.971$, $p < 0.0001$), but less so across primate species (exponent, -1.233 ± 0.144, $r^2 = 0.924$, $p = 0.0001$). Because daily number of sleep hours is best correlated with D/A, the different scaling of D/A across primates and other mammals indicates that the daily sleep requirement should scale as different functions of numbers of neurons across the two groups.

Table 1. Dataset used in this study. (Values refer to both hemispheres together. Daily sleep hours from [7]. When more than one estimate was available, the value presented here is the average for the species. For the other columns, numbers in parentheses refer to references in the main text. DN_{CX}, neuronal density in the cerebral cortical grey matter, except where indicated with an asterisk (*) (density includes the white matter), in neurons mg^{-1} (our own data) or neurons mm^{-3} (data taken from other authors). A_{CX}, total surface area of the cerebral cortex (in mm^2). *D/A*, ratio between neuronal density in the cerebral cortex (in neurons mg^{-1}) and total cortical surface area (in mm^2). N_{CX}, number of neurons in the cerebral cortex. *O/N*, ratio between numbers of other cells (non-neuronal cells, presumed to be mostly glial cells) and neurons in the cortical grey matter, except where indicated with an asterisk (*) (ratio includes the white matter). *T*, thickness of the cortical grey matter(in mm), from V_{CX}/A_{CX}. M_{CX}, mass (in g, our data) or volume (in cm^3) of the cortical grey matter, except where indicated with an asterisk (*) (mass includes white matter). n.a., not available.)

species	brain mass (g or cm^3)	daily sleep (h)	*D/A* (N mg^{-1} mm^{-2})	N_{CX}	DN_{CX} (N mg^{-1})	A_{CX} (mm^2)	*O/N*	*T*	M_{CX} (g or cm^3)
Primates									
Aotus trivirgatus	15.750 [19]	16.97	10.831	441.90×10^6 [20]	47 960 [20]	4428 [20]	1.340 [20]	1.670	7.396 [20]
Callithrix sp.	7.780 [19]	10.22	17.679	244.72×10^6 [20]	54 240 [20]	3068 [20]	1.114 [20]	1.331	4.084 [20]
Cebus apella	52.508 [19]	5.95	3.337	1.14×10^9 [20]	51 080 [20]	15 306 [20]	1.106 [20]	2.067	31.640 [20]
Saimiri sciureus	30.216 [19]	9.11	7.707	1.34×10^9 [19]	80 920 [20]	10 500 [20]	0.768 [20]	1.333	13.992 [20]
Macaca mulatta	87.346 [19]	8.74	1.284	1.71×10^9 [19]	32 110 [20]	25 000 [15]	2.011 [20]	1.714	42.860 [19]
Macaca radiata	61.470 [25]	9.02	2.595	1.66×10^9 [20]	43 810 [20]	16 882 [20]	1.278 [20]	1.835	30.986 [20]
Papio cynocephalus	151.190 [25]	6.19	1.011	2.88×10^9 [20]	33 730 [20]	33 378 [20]	1.317 [20]	2.177	72.668 [20]
Homo sapiens	1509 [24]	8.47	0.114	16.34×10^9 [24]	21 450 [24]	188 100 [29]	1.480 [29]	2.938	552.7 [29]
Eulipotyphla									
Blarina brevicauda	0.340 [23]	14.90	271.927	11.88×10^6 [23]	59 280* [23]	218[a]	1.000* [23]	0.821	0.179* [23]
Condylura cristata	0.802 [23]	10.32	106.832	17.25×10^6 [23]	38 780* [23]	363[a]	1.924* [23]	1.072	0.389* [23]
Erinaceus europaeus	3.230 [15]	13.96	29.221	33.53×10^{6b}	27 000 [48]	924 [15]	2.214 [48]	1.047	0.967 [15]
Scalopus aquaticus	0.999 [23]	8.45	168.291	28.68×10^6 [23]	60 252* [23]	357[a]	1.296* [23]	1.070	0.382* [23]
Glires									
Cavia porcellus	3.656 [26]	9.37	20.729	43.51×10^6 [26]	22 222 [26]	1072 [26]	2.606 [26]	1.827	1.959 [26]
Mesocricetus auratus	0.965 [18]	14.98	98.985	17.14×10^6 [18]	39 099* [18]	395[a]	2.507 [18]	1.129	0.446* [18]
Mus musculus	0.402 [26]	11.60	46.316	13.69×10^6 [18]	122 232 [26]	296 [4]	0.870 [26]	0.724	0.214 [26]
Oryctolagus cuniculus	7.100 [15]	9.55	31.739	71.45×10^6 [21]	43 800 [47]	1380 [15]	3.566	n.a.	n.a.
Rattus norvegicus	1.724 [26]	12.27	49.685	31.02×10^6 [18]	35 330 [26]	711 [4]	1.497 [26]	1.212	0.862 [26]
Afrotheria									
Dendrohyrax sp.	3.328 [27]	5.40	6.308	98.97×10^6 [27]	12 709 [27]	3791 [27]	1.426 [27]	1.598	6.058 [27]
Loxodonta Africana	4618 [22]	3.30	0.007	5.59×10^9 [22]	3661 [22]	514 134 [22]	10.628 [22]	2.633	1353.9 [22]
Procavia sp.	4.317 [27]	5.45	4.920	197.92×10^6 [27]	20 794 [27]	4226 [27]	1.241 [27]	1.482	6.263 [27]
Artiodactyla									
Bos taurus	486 [15]	3.19	0.123	1.34×10^{9c}	13 000 [47]	105 500 [15]	4.085 [47]	2.142	226.0 [15]

(*Continued.*)

Table 1. (*Continued.*)

species	brain mass (g or cm^3)	daily sleep (h)	D/A (N mg^{-1} mm^{-2})	N_{Cx}	DN_{Cx} (N mg^{-1})	A_{Cx} (mm^2)	O/N	T	M_{Cx} (g or cm^3)
Giraffa camelopardalis	537 [28]	3.44	0.073	1.73×10^9 [28]	5882 [28]	80 256 [28]	7.763 [28]	2.394	192.1 [28]
Sus scrofa	64.180 [28]	8.20	0.559	307.08×10^6 [28]	7375 [28]	13 188 [28]	8.543 [28]	1.336 [28]	17.614 [28]
Scandentia									
Tupaia sp.	3.040 [15]	15.84	29.457	60.39×10^6 [19]	38 000 [48]	1290 [15]	1.511 [20]	0.798	1.030 [20]

[a]Values estimated as $7.34118 \times V_{CX}^{0.654}$, according to [15].
[b]Value estimated from cortical mass according to [30].
[c]Value estimated from brain mass according to [28].

Figure 3*c* shows that this is the case. When non-primates are considered separately, total daily sleep duration decreases very significantly with increasing numbers of cortical neurons across species, as a power function of exponent -0.266 ± 0.034 ($r^2 = 0.809$, $p < 0.0001$). Across primates, on the other hand, the less steep scaling of D/A with numbers of neurons results in a non-significant scaling of daily sleep hours with numbers of neurons ($r^2 = 0.205$, $p = 0.2597$; figure 3*b*).

Importantly, given the tendency in mammalian evolution towards increasing numbers of cortical neurons [30], it can be inferred that such increases resulted in decreasing daily sleep hours across the first, non-primate species, presumably through the accompanying decrease in D/A. By contrast, primates diverged away from the ancestral way of adding neurons to the cortex in a manner that causes no significant decrease in their daily sleep requirement as their cortex gains neurons. The larger values of D/A in primate cortices compared with other mammals of similar brain size or number of cortical neurons thus explain the *ca* three times larger sleep requirement of primates compared with artiodactyls and the elephant (figure 3).

(c) Ontogenetic decrease in sleep requirement with a decreased neuronal density/area ratio

Newborn rats spend more than 90% of the time asleep, but their daily sleep requirement decreases dramatically between postnatal days 4 and 10 [5], stabilizing around 50% of the day (figure 4). The ontogenetic decrease in sleep requirement mirrors closely the ontogenetic decrease in D/A, but not in cortical mass, number of cortical neurons or the O/N ratio (figure 4). In ontogeny, as in phylogeny, a principal component analysis of the per cent of time spent asleep and four variables related to cortical development (cortical mass, cortical surface area, neuronal density and D/A) reveals that daily sleep requirement loads with D/A and neuronal density in the second factor (loading, 0.886, 0.760 and 0.827, respectively), which accounts for 43.9% of the variation. Brain mass, cortical mass, cortical area and neuronal density load in the first factor (loading, 0.904, 0.906, 0.872 and -0.610, respectively), accounting for 52.0% of variation. Together, these two factors account for 95.9% of all variation in the ensemble of the five parameters examined (per cent time asleep, cortical mass, cortical surface area, neuronal density and D/A) in rat postnatal development. Remarkably, removing neuronal density from the analysis does not decrease the per cent variation explained, and actually improves it slightly to 96.5%. In this case, daily sleep requirement still loads in the second factor together with D/A (loading, 0.904 and 0.803, respectively). A decreasing D/A ratio in ontogeny thus explains the initially large amount of time spent asleep and the rapidly decreasing sleep requirement between days 4 and 10 in the rat.

(d) Implications for mammalian evolution

Here I show that total sleep time decreases with increasing number of neurons in the cerebral cortex of non-primate mammals, which indicates that in early mammalian evolution, increasing numbers of cortical neurons must have led to a decreasing requirement for sleep time. This probably happened due to the decrease in D/A that accompanies increases of numbers of neurons in non-primate mammals,

Table 2. Dataset on the ontogeny of sleep and cellular composition of the rat brain used in this study. (Values refer to both hemispheres together. Per cent of hours asleep from [5], age in days after birth; all other data from [32]. M_{BR}, mass of the brain (excluding the olfactory bulbs). M_{CX}, mass of cerebral cortex (including white matter). N_{CX}, number of neurons in the cerebral cortex. *O/N*, ratio between numbers of other cells (non-neuronal cells, presumed to be mostly glial cells) and neurons in the entire cortex, including the white matter. D_{CX}, neuronal density in the cerebral cortex, including the white matter. A_{CX}, total surface area of the cerebral cortex (in mm^2). *D/A*, ratio between neuronal density in the cerebral cortex (in neurons mg^{-1}) and total cortical surface area (in mm^2). n.a., not available.)

age	% asleep	M_{BR} (g)	M_{CX} (g)	N_{CX}	*O/N*	D_{CX} (N mg^{-1})	A_{CX} (mm^2)	*D/A*, (N mg^{-1} mm^{-2})
1	96.9	0.309	0.132	32 247 500	0.193	271 127	125	2163
2	98.6	0.326	0.138	32 406 667	0.191	265 856	129	2059
3	97.2	0.387	0.169	30 995 000	0.171	225 940	148	1528
4	93.2	0.473	0.215	39 763 333	0.281	184 538	174	1063
5	77.0	0.826	0.411	32 790 000	0.200	87 112	267	326
6	70.2	0.683	0.324	48 660 000	0.091	150 880	228	661
7	68.9	0.675	0.328	29 213 333	0.487	79 039	230	344
9	55.8	0.785	0.344	28 370 000	0.540	73 702	237	310
10	52.7	0.934	0.454	n.a.	n.a.	n.a.	n.a.	n.a.
11	52.2	1.008	0.467	17 200 000	1.452	37 561	291	129
14	66.9	1.342	0.645	13 140 000	1.232	19 238	361	53
15	68.7	1.136	0.534	15 030 000	1.744	28 133	318	88
17	56.3	1.382	0.684	17 140 000	2.436	26 293	375	70
18	59.0	1.306	0.620	15 225 000	2.626	24 564	352	70
21	49.0	1.342	0.622	19 030 000	2.148	30 662	352	87
22	54.2	1.411	0.683	n.a.	n.a.	n.a.	375	n.a.
25	63.6	1.536	0.709	21 545 000	1.992	30 402	384	79

with the ensuing slower rate of accumulation of sleep-inducing metabolites during waking hours and thus the ability to remain awake for longer stretches of time.

Such a decreasing requirement for sleep with increasing numbers of cortical neurons in evolution brings the important advantage that more time becomes available for the new species to feed, and thus afford both a larger body and a larger number of neurons. The increased availability of time to feed is particularly important when the sleep requirement predicted for the smallest numbers of cortical neurons in the dataset, and thus presumably the earliest mammals approaches 20 h d^{-1}. Adding neurons to the cerebral cortex would thus bring not only increased information processing capabilities [33] but, perhaps even more importantly, also the means both to afford the energy required to sustain more neurons and to afford 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.

Because of the low caloric content of plant foods in the wild, short sleep times are fundamental for large mammals to become viable. Being elephant-sized, or even artiodactyl-sized, is incompatible with long sleep hours. An African elephant, for instance, eats 18 h d^{-1} [34], which is possible because a very small D/A ratio that, I propose, results from the accumulation of a large number of neurons over its evolutionary history.

Humans, by contrast, have three times more neurons in the cerebral cortex than the African elephant [22], but require a much larger 8–9 h of sleep daily [7]. I propose that the reason why humans and other primates as a whole require many more hours of sleep for a similar number of cortical neurons than non-primates is that because of the way the primate cortex evolved to be built, this number of neurons is accompanied by a larger D/A in primates than in non-primates. Increasing numbers of neurons in primate evolution does not lead to a steep enough drop in D/A to cause a significant decrease in total sleep requirement. Primates must thus sleep 8–9 h d^{-1}, which limits the daily number of hours spent feeding to not much more than another 8 h [35]. This is an important constraint, which we have shown to limit the maximal possible body size of primates, as well as the number of neurons that they can afford—a constraint that human ancestors escaped by radically changing their diet, possibly with cooking [35], but that still did not suffice to decrease their sleep requirement. It will be interesting to examine whether the present hypothesis also explains the long sleep hours of species of the order *Carnivora* compared with artiodactyls and even primates of similar brain size [7], once data on numbers of neurons and neuronal density become available for carnivores.

The different scaling of D/A with number of neurons in primates compared with non-primates can be explained by our finding that the initial evolutionary coupling between increasing numbers of neurons and average neuronal cell size found in non-primates no longer occurs in primates

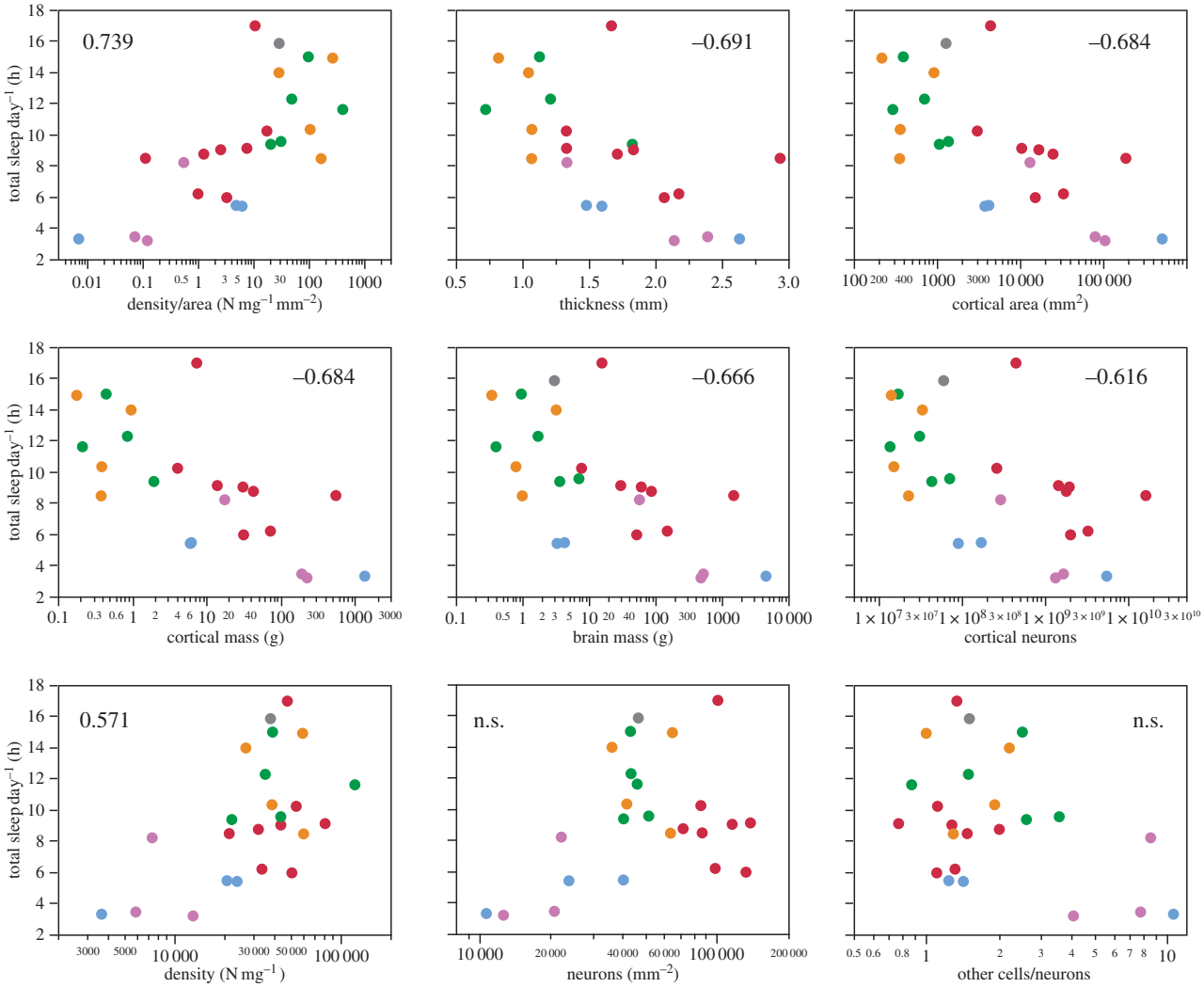

Figure 2. Neuronal density mm^{-2} (D/A) is the parameter that best correlates with total sleep hours per day across mammalian species. Each data point corresponds to one species, as in table 1. Values shown indicate the Spearman correlation coefficient for each graph. All values of $p < 0.005$ except for the two correlations in the bottom row, where $p > 0.1$. Red, primates; orange, eulipotyphlans; green, glires; blue, afrotherians; pink, artiodactyls; grey, scandentia. (Online version in colour.)

[30]. Why would increasing numbers of neurons be coupled to increasing average neuronal cell size in non-primate evolution in the first place? The fact that primate brains are not built with increasingly large neurons indicates that the coupling between more neurons and larger neurons is not mandatory (i.e. it does not reflect basic neuroanatomical constraints) and raises the possibility that having neuronal densities decrease as neurons are added to the earliest mammalian brains is not a just-so scenario but rather one that has an advantage. I suggest that this advantage is the decrease in daily sleep requirement that ensues when the addition of neurons occurs in such a way that causes a decrease in D/A and thus a smaller rate of accumulation of metabolites underneath the surface of the parenchyma, in the self-reinforcing spiral described above. Primates, in turn, must have branched out at a point in mammalian evolution when the D/A ratio of their exclusive common ancestor was already small enough to allow only 8–9 h of sleep per day, and thus a number of daily hours of feeding that, while it later would show itself to be limiting at the upper end of body mass, still allowed a significant range of body mass and number of brain neurons [35].

Similarly, non-primate mammals appear to be born with very high neuronal densities concentrated underneath a very small cortical surface [32,36]. Given the correlation between D/A and sleep requirement, newborns are expected to not only require many daily hours of sleep, but also to be able to remain awake only for short periods at a time—which is viable for mammals because of maternal care. Several studies show that small-brained newborn mammals indeed sleep most of the time [37]. For the same reason, a similar need for many daily hours of sleep interspersed with only short bouts of waking is expected for very small mammals, which are predicted to have large D/A ratios. Again, this is indeed the case for small bats and shrews, which are known to sleep as much as 20 h d^{-1}.

The discovery that sleep has the fundamental function of allowing the clearance of accumulated metabolites from the cortical parenchyma [11] inspired the present hypothesis (which still requires direct testing) that the daily sleep requirement of a species or a developing animal is determined by the rate at which sleep-inducing metabolites accumulate during waking. This quantity is expected to depend on the D/A ratio between neuronal density in the cortex and the area of the surface above it through which metabolites are cleared during waking. It is possible that other factors, such as the rate of production of CSF, also play a role in determining the rate of accumulation of sleep-inducing metabolites. However, while it is safe to conclude that larger brains produce

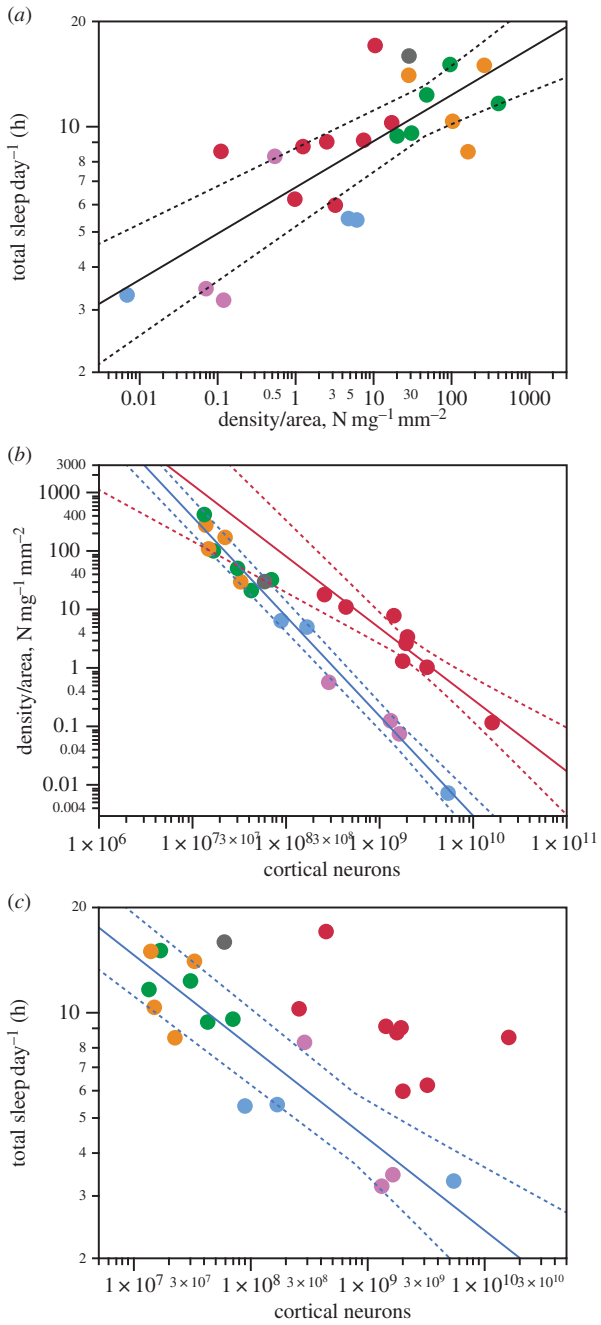

Figure 3. Total daily sleep requirement decreases with increasing number of neurons in the cerebral cortex across non-primate species through a decrease in *D*/*A* (neuronal density mm^{-2}). (*a*) Total daily sleep requirement scales as a power function of neuronal density mm^{-2} with an exponent of 0.133 $\pm$ 0.023 across all 24 mammalian species in the dataset ($r^2 = 0.601$, $p <$ 0.0001). (*b*) Neuronal density mm^{-2} decreases steeply with increasing number of cortical neurons as a power function of exponent $-1.694 \pm$ 0.080 across non-primates ($r^2 = 0.971$, $p < 0.0001$) and less steeply across primates (exponent -1.233 ± 0.144, $r^2 = 0.924$, $p < 0.0001$). (*c*) Total daily sleep requirement decreases as a power function of the number of cortical neurons across non-primates (exponent, $-0.266 \pm$ 0.034, $r^2 = 0.809$, $p < 0.0001$), but not across primates ($p = 0.2597$). Red, primates; orange, eulipotyphlans; green, glires; blue, afrotherians; pink, artiodactyls; grey, scandentia. (Online version in colour.)

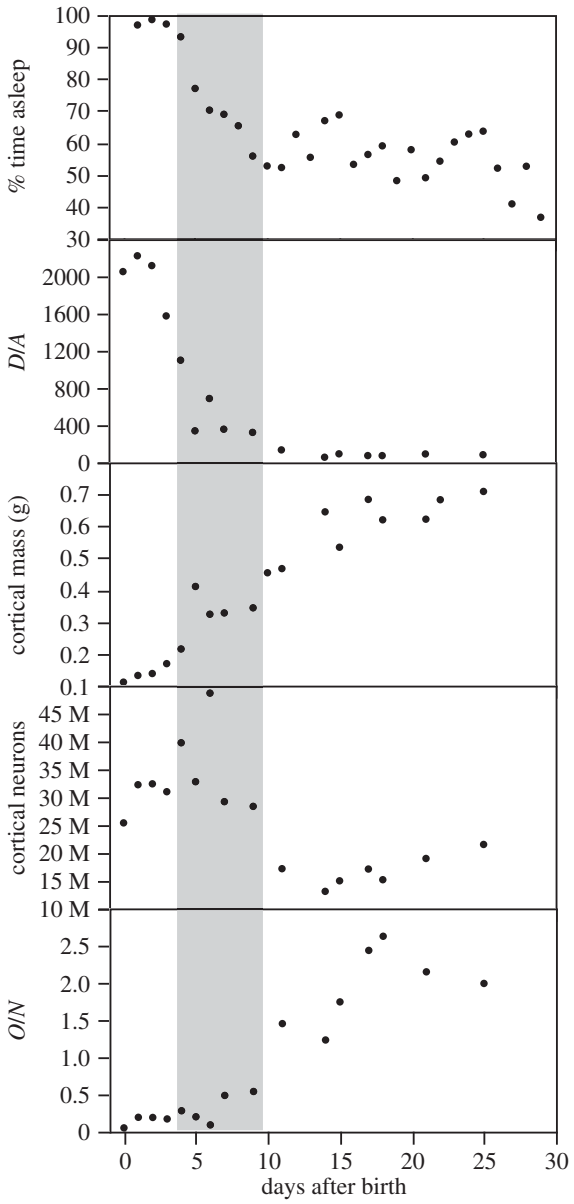

Figure 4. Decrease in total sleep time in early postnatal development in the rat is associated with a decrease in neuronal density mm^{-2}. Graphs show the ontogenetic variation in different parameters pertaining to cortical morphology and cellular composition from birth (day 0) onwards. The shaded bar 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. Data available in table 2.

much larger volumes of CSF per minute, from 1–4 µl min^{-1} in the rat [38,39], 6–19 µl min^{-1} in the rabbit [38,40], 11–17 µl min^{-1} in the cat [41], 42 µl/min in the dog [42] to 305–694 µl min^{-1} in humans [43,44], data are not yet available on enough species to examine the relationship between CSF production rate and sleep requirement. In any case, the present 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 study of sleep requirement in evolution, development and both normal and abnormal brain function, as well as for the development of drugs that alter this requirement.

3. Material and methods

I compiled data for 24 species on average total daily number of sleep hours from McNamara *et al.* [7], and brain mass, numbers of neurons, neuronal density, cortical surface area, thickness of the cortical grey matter and glia/neuron ratio in the grey matter for one single cortical hemisphere from the sources indicated in table 1, but mostly from our own previous studies

[18–29]. Only in a handful of cases, identified in the table, were numbers of cortical neurons or cortical surface area estimated from brain mass or cortical volume, according to the scaling rules known to apply. The main criterion for including a species in the table was the availability of direct estimates of neuronal density. Numbers of neurons in our dataset were obtained using the isotropic fractionator [45], which has been shown to yield estimates that are similar to those obtained with stereology [46].

All mathematical analyses (correlations, function fitting, principal components analysis) were performed in JMP v. 9.0 (SAS, USA). Non-parametric Spearman's rank correlations were used as they do not assume a normal distribution of the data (Spearman's ρ and p-value reported). All power functions were calculated using log-transformed data (r^2 and p-value for the exponent are reported).

Authors' contributions. S.H.H. conceived and designed the study, collected and analysed data, and wrote the manuscript.

Competing interests. We declare we have no competing interests.

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