Connectivity-driven white matter scaling and folding in primate cerebral cortex

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Contributed by Jon H. Kaas, August 24, 2010 (sent for review February 6, 2010)

Larger brains have an increasingly folded cerebral cortex whose white matter scales up faster than the gray matter. Here we analyze the cellular composition of the subcortical white matter in 11 primate species, including humans, and one Scandentia, and show that the mass of the white matter scales linearly across species with its number of nonneuronal cells, which is expected to be proportional to the total length of myelinated axons in the white matter. This result implies that the average axonal cross-section area in the white matter, *a*, does not scale significantly with the number of neurons in the gray matter, *N*. The surface area of the white matter increases with $N^{0.87}$, not $N^{1.0}$. Because this surface can be defined as the product of *N*, *a*, and the fraction *n* of cortical neurons connected through the white matter, we deduce that connectivity decreases in larger cerebral cortices as a slowly diminishing fraction of neurons, which varies with $N^{-0.16}$, sends myelinated axons into the white matter. Decreased connectivity is compatible with previous suggestions that neurons in the cerebral cortex are connected as a small-world network and should slow down the increase in global conduction delay in cortices with larger numbers of neurons. Further, a simple model shows that connectivity and cortical folding are directly related across species. We offer a white matter-based mechanism to account for increased cortical folding across species, which we propose to be driven by connectivity-related tension in the white matter, pulling down on the gray matter.

brain size | number of neurons | small-world networks | evolution

Larger mammalian brains have relatively larger cerebral cortices that become increasingly folded, such that the overall cortical surface increases more quickly than the exposed cortical surface, presumably as a result of fast expansion of the gray matter (1). The fastest expanding structure, however, is not the cortical gray matter (GM), but the subcortical white matter (WM), which contains the axons that interconnect nearby as well as distant areas in the GM and their subcortical targets. Across mammalian species, the WM comprises as little as 5% of the cerebral cortex in the smallest insectivores, but >40% of the cerebral cortex of dolphins, whales, elephants, and humans (2). The faster increase in WM volume V_W than in GM volume V_G is to be expected from their spatial characteristics, one as the core, and the other as the shell, of the cortex; if the WM were a perfect sphere surrounded by a spherical shell of GM of constant thickness, V_W should increase with $V_G^{3/2}$ or $V_G^{1.5}$. However, WM has been found to increase more slowly than expected across mammalian species, with $V_G^{1.22}$ (3), $V_G^{1.24}$ (4), $V_G^{1.33}$ (2), or $V_G^{1.23}$ (5), which raises the possibility that connectivity through the WM does not increase proportionally with increases in GM.

It has been argued that an exponent of 1.23 would follow naturally once the exponent of 1.33, or 4/3, consequence of the local uniformity of the cortex (in number of neurons beneath a unit surface area), and of the requirement for compact arrangement of long axonal fibers, is corrected to account for variations in cortical thickness (5). This model considered explicitly that a constant fraction of cortical neurons sends axons into the WM, that is, that connectivity does not scale with brain size. Alternatively, another model, which also considered the number of neurons beneath a cortical surface unit to be constant, and additionally assumed that synaptic density is invariant, estimated that the connectivity between neurons and cortical areas should decrease with increasing brain size (6). Such models with opposing views on the scaling of connectivity considered the distribution of neurons in the GM to be uniform out of necessity, as a means to estimate scaling of the total number of neurons in the GM and hence scaling of the number of fibers in the WM.

Assuming neuronal uniformity in the cortex, however, is no longer necessary or appropriate to model cortical connectivity. By means of a method that we developed, the isotropic fractionator (7), we have recently been able to determine the number of neurons in the cortical gray matter of a number of primate species (8), including humans (9). A comparative analysis of the ratio between the total number of neurons in the GM and the total GM surface showed that the number of neurons beneath a unit surface area varies by as much as 3×, depending on neuronal density in a manner that does not correlate with brain size (because, in primates, neuronal density does not vary significantly with cortical size), and therefore cannot be considered uniform (10).

Here we examine how cortical WM size and connectivity scale with the number of neurons in the cortex without making assumptions about its cellular composition or fraction of neurons with axons in the WM, by determining the cellular composition of the subcortical white matter of various primate brains and investigating how it scales with the number of neurons in the gray matter (10). Because the number of myelinating oligodendrocytes is considered to increase linearly with axon length (11), and the density of nonneuronal cells has been shown not to vary with brain size (8), we use the number of nonneuronal cells that compose the subcortical white matter to determine how total axon length in the white matter scales with the number of cortical neurons, to infer whether and how average axonal diameter varies with cortical size, to deduce how the fraction of cortical neurons with axons in the WM scales with GM size and number of neurons, and to examine how changes in connectivity relate to changes in cortical folding.

Results

All analyses reported refer to the 11 species in the dataset and the closely related *Tupaia*, because its exclusion from the dataset had only negligible effects on the results (Table S1). Because scaling exponents were little affected by accounting for phylogenetic relatedness in the dataset (Table S1), the exponents reported below

Author contributions: S.H.-H. designed research; S.H.-H. performed research; S.H.-H., P.W., and J.H.K. contributed new reagents/analytic tools; S.H.-H., B.M., and J.H.K. analyzed data; and S.H.-H., B.M., P.W., and J.H.K. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1012590107/-/DCSupplemental.

refer to the uncorrected relationships. Across species, we find that WM mass scales with GM mass raised to an exponent of 1.148 ± 0.037 including humans ($P < 0.0001$; from here on $\bar{a} \pm \sigma_a$ signifies expected exponent $\bar{a}$ with SD σ_a; Fig. 1*A*) or 1.147 ± 0.055 (without humans; $P < 0.0001$), confirming that WM size scales faster than GM size in the larger primate brains in our sample. Accordingly, the relative size of the WM increases with cortical size from 27.0% of cortical mass in the tree shrew to 47.8% in humans (Table S2). WM volume V_W also scales faster than GM volume V_G, with $V_G^{1.184\pm0.063}$ ($P < 0.0001$; does not include humans and macaque monkeys, for which V_G was not available). Because mass and volume for each structure were found to be linearly related (GM, $r^2 = 0.982$, $P < 0.001$; WM, $r^2 = 0.896$, $P < 0.0001$), and measurements of mass, but not volume, were available for all species, heretofore we use solely GM and WM mass in the analysis and interchangeably with V_W and V_G where called for in the models.

As reported previously (10), cortical GM increases linearly in mass as a function of its number of neurons, *N*. This relationship is linear even when humans are included in the comparison (the exponent is 1.043 ± 0.073, $P < 0.0001$ and $r^2 = 0.991$ including humans, and 0.956 ± 0.084, $P < 0.0001$ and $r^2 = 0.896$ excluding humans). Whole cortical mass (GM + WM) increases as a power of the number of neurons in the GM, with exponents 1.097 ± 0.081 if we include humans ($P < 0.0001$; Fig. 1*B*), or 1.000 ± 0.091 if humans are excluded ($P < 0.0001$).

White Matter Scaling. White matter mass, M_W, increases across primate brains as a linear function of its number of nonneuronal cells ("other cells," *O*) whether humans are included (exponent, 1.032 ± 0.040; $P < 0.0001$; Fig. 2*A*) or excluded from the comparison (exponent, 1.019 ± 0.057, $P < 0.0001$; linear regression, $r^2 = 1.000$ and 0.970, respectively, $P < 0.0001$). M_W increases as a power function of the number of neurons in the gray matter *N* raised to an exponent of 1.197 ± 0.091 if humans are included ($P < 0.0001$; Fig. 2*B*) or 1.096 ± 0.111 if humans are excluded from the comparison ($P < 0.0001$).

The number of nonneuronal cells in the subcortical WM increases as a power law of *N* with exponent 1.165 ± 0.070 (including humans, $P < 0.0001$; Fig. 2*C*) or 1.081 ± 0.074 if humans are excluded from the comparison ($P < 0.0001$). Assuming the number of nonneuronal cells in the WM, *O* (presumably mainly oligodendrocytes), varies linearly with total myelinated axonal length (*L*) in the WM (11), we can therefore infer that, for primates including humans, $L \propto O \propto N^{1.165\pm0.040}$.

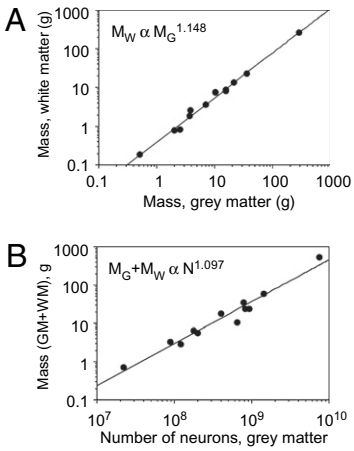

Fig. 1. White matter scales faster than gray matter. (*A*) White matter mass (M_W) increases faster than gray matter mass (M_G), with $M_G^{1.148}$ ($P < 0.0001$). (*B*) Total cortical mass ($M_W + M_G$) increases with the number of GM neurons $N^{1.097}$ ($P < 0.0001$). Each point indicates the average for one species. Power functions include the human datapoint.

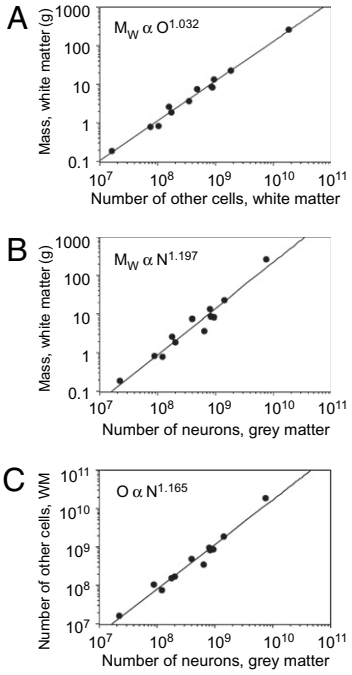

Fig. 2. White matter scaling. (*A*) White matter mass (M_W) increases linearly with the number of nonneuronal (other) cells in the WM (*O*), with $O^{1.032}$ ($P < 0.0001$). (*B*) White matter mass (M_W) increases faster than the number of neurons *N* in the GM, with $N^{1.197}$ ($P < 0.0001$). (*C*) The number of nonneuronal cells (*O*) in the WM increases faster than the number of neurons *N* in the GM, with $N^{1.165}$ ($P < 0.0001$). Each point indicates the average for one species. Power functions include the human datapoint.

Scaling of Average Axon Cross-Sectional Area. The average cross-sectional area *a* of myelinated axons in the WM can be estimated by using the same assumption about the proportionality between *L* and *O*. Considering that $V_W \propto n.N.a.l$ (where *n* is the fraction of gray matter neurons connected through the GM and *l* is the average axonal length in the WM) and $L = n.N.l$, it follows that $a \propto V_W/L \propto V_W/O$. Using the observed relations for V_W and *O* as (very similar) power laws of *N* shown above, we obtain $a \propto N^{\alpha}$, where $\alpha = 0.032 \pm 0.049$, i.e., close to, and statistically indistinguishable from, zero. In other words, because V_W is linearly related to *O*, it follows that V_W/O is constant, that is, that *a* is invariant for primate brains. The same relationship holds if we consider *O* to be proportional to total axonal surface, rather than length (*SI Methods*). We can infer, therefore, that the average myelinated cross-sectional area in the white matter does not vary significantly with increasing number of neurons in the gray matter.

Scaling of Gray Matter Connectivity Through the White Matter. The surface area of the GM/WM interface, A_W, can be defined as $A_W \propto n.N.a$. Given that $a \propto N^{\alpha}$, then $A_W \propto N^{1+c+\alpha}$. We find that $A_W \propto N^{0.873\pm0.102}$ ($P < 0.0001$; Fig. 3*A*), which implies that *n* is not invariant with *N*, but rather changes with $N^{-0.159\pm0.113}$. This result suggests that gray matter connectivity through the white matter, *n*, decreases slightly with increasing number of neurons in the GM, as $n \propto N^c$, such that $c = -0.159 \pm 0.113$.

Scaling of WM Volume, Surface, and Radius. Visual inspection of brain sections of different species shows that the surface of the WM is convoluted and does not scale isometrically with cortical size. Were this the case, the volume of the WM, V_W, would scale with $A_W^{3/2}$. In contrast, we find that $V_W \propto A_W^{1.243\pm0.036}$ ($P <$

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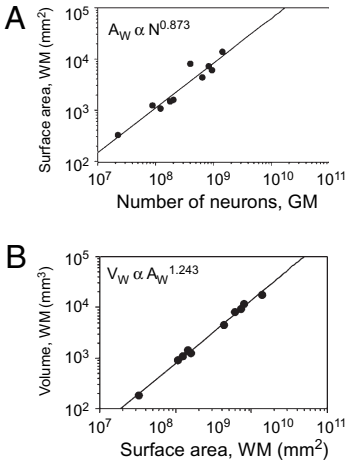

Fig. 3. Scaling of the white matter surface area. (*A*) White matter surface area (A_W) increases with the number of neurons in the GM with $N^{0.873}$ ($P < 0.0001$). Each point indicates the average for one species. (*B*) White matter volume (V_W) increases with WM area A_W more slowly than expected for an isometric surface, with $A_W^{1.243}$ ($P < 0.0001$) instead of $A_W^{1.5}$, as if growing under tension. Power functions do not include human or rhesus datapoints, for which measurements of A_W are lacking.

0.0001; Fig. 3*B*), with an exponent significantly <1.5, which means that the volume of the WM increases significantly more slowly than expected, as if growing under tension. This scaling relationship implies that the WM surface A_W grows faster than its volume—that is, it becomes more convoluted as the cortex grows.

Under very general conditions (*SI Methods*), the average length of myelinated axons in the WM is given by $l = 2V_W/(pA_W)$, where p is approximately the average of the cosine of the incidence angle of the axons into the GM–WM interface. An economically built brain (i.e., one folded only as much as it needs to be able to accommodate all its WM axons as tightly packed as possible) would have $p \approx 1$, so we assume that p is close to unity and constant across species. In any case, because $p \leq 1$, $2.V_W/A_W$ can always be taken as a lower bound for the value of l. Models of cortical scaling often assume that l varies linearly as a function of the cortex radius R [a length scale defined as $(3V/4\pi)^{1/3}$]. Instead, we find that l varies with R^{λ}, with $\lambda = 0.662 \pm 0.186$ ($P < 0.0001$; Fig. 4), with an exponent well below linearity, and therefore with $N^{0.242\pm0.085}$. This result strongly suggests that, contrary to expectations, the average axonal length in the WM grows more slowly than the radius of the cerebral cortex, again as expected if its axons were under tension.

Cortical Folding. The degree to which the WM becomes convoluted as the GM gains neurons can be expressed by its folding index F_W, which we define as the ratio A_W/A_E (where A_E is the exposed surface of the cerebral cortex). As expected from the approximately isometric external shape of the brain, A_E is found to vary in our sample with $V^{0.676\pm0.010}$ ($P < 0.0001$; Fig. 5*A*) and $N^{0.652\pm0.079}$ ($P < 0.0001$; Fig. 5*B*). Given these similar exponents and considering that V varies approximately linearly with N ($V \propto N^{1.097\pm0.081}$), A_E can be considered to scale as $N^{2/3}$ and R to scale as $N^{1/3}$. F_W, defined as A_W/A_E, scales approximately as $n.a.N/N^{2/3} \propto n.a.R$. Because a does not vary systematically with N, F_W thus increases linearly with the product of cortical radius and connectivity through the WM. The larger the number of cortical neurons interconnected through the WM is, the more the WM surface is folded.

Applying the relationship $F_W \propto N^{1/3+c+\alpha}$, we find that in hypothetical cortices in which F_W did not change with N (that is, cortices that gained neurons in the GM without becoming more convoluted), cortical connectivity n would decrease steeply with $c = -1/3$. In contrast, in hypothetical primate brains in which

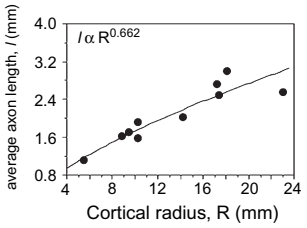

Fig. 4. Scaling of average axonal length, l. The lower bound for average axonal length l, calculated as $2.V_W/A_W$, increases with approximate cortical radius $R^{0.662}$ [where R is defined as $(3V/4\pi)^{1/3}$, for one hemisphere only; $P < 0.0001$]. Each point indicates the average for one species. Power function does not include human or rhesus datapoints, for which measurements of A_W are lacking.

cortical connectivity did not change with N (that is, with $c = 0$), F_W should vary with $N^{1/3}$. In other words, increasing the WM without increasing the folding of its surface can occur only in the face of a steep decline in cortical connectivity through the WM; on the other hand, increasing the WM without decreasing cortical connectivity through it, or with an actual increase in cortical connectivity, would be accompanied by in a sharp increase in WM folding. Therefore, the higher degree of folding of the WM in larger cortices can be seen as a feature that accompanies either maintenance of connectivity or only a slight decrease in it.

In our primate sample, we find that $F_W \propto N^{0.220\pm0.026}$ ($P < 0.0001$; Fig. 6), with an exponent that is both significantly >0 and <1/3. Given the relationship $F_W \propto N^{1/3+c+\alpha}$, this exponent suggests that cortical connectivity decreases in larger primate brains with $c = -0.145$, consistent with our previous estimates of $c = -0.159 \pm 0.113$.

Considering that the WM is a structure under tension because of the axons that compose it (12), and that this tension might force the folding of the WM surface A_W in a way that increases together with the number of cortical neurons interconnected through the WM, the folding of A_W might consequently force the folding of the external surface of the GM. In this manner, the folding index of the external surface of the GM, F_G, would be expected to follow as a consequence of the folding of A_W. Using our model, it can be shown that F_W and F_G should be related by the formula $F_W = F_G + (T/R)F_G[(T/R)F_G - 2]$ (*SI Methods* and Fig. S1). We

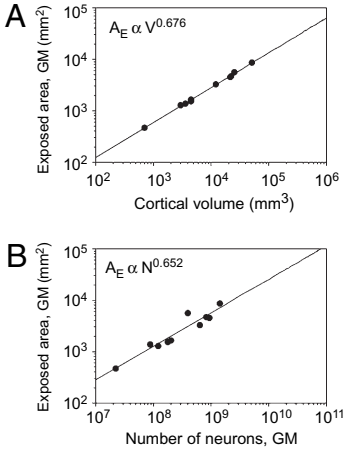

Fig. 5. Scaling of the exposed cortical surface area. (*A*) Exposed GM surface area (A_E) increases with total cerebral cortical volume V as expected for an isometric surface, with $V^{0.676}$ ($P < 0.0001$). (*B*) Exposed GM surface area (A_E) increases with the number of neurons in the GM with $N^{0.652}$ ($P < 0.0001$). Each point indicates the average for one species. Power functions do not include human or rhesus datapoints, for which measurements of A_W are lacking.

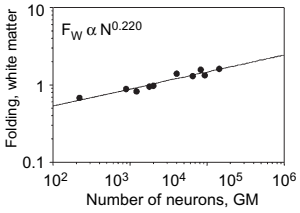

Fig. 6. Folding of the white matter as a function of cortical neurons. Folding of the WM surface (F_W) increases with the number of cortical neurons $N^{0.220}$ ($P < 0.0001$). Each point indicates the average for one species. Power functions do not include human or rhesus datapoints, for which measurements of A_W are lacking.

find that the values of F_W obtained by applying this formula are very close to the measured values of F_W: $F_W = 1.000 \times$ expected $F_W^{1.007}$ ($P < 0.0001$), indicating that the formula that relates F_W to F_G is accurate despite being based on a very simple model. This coordinated increase in F_G and F_W is consistent with our proposition that the folding of the external surface of the cerebral cortex is a consequence of the folding of the WM surface, which is in turn proportional to GM connectivity through the WM.

Discussion

Making no assumptions about cortical uniformity, neuronal composition of the cerebral cortex, the fraction of GM neurons connected through the WM, or how average axonal length in the WM scales with brain size, and assuming only that the number of nonneuronal cells in the WM is proportional to its total length of myelinated axons, here we show that, for primate brains, average axon length in WM increases more slowly than cortical radius, and total axonal length in WM increases more slowly than expected if a constant fraction of neurons had axons in the WM. This result implies that GM connectivity (n, or the fraction of GM neurons that sends an axon through the WM) decreases as the GM gains neurons, in a manner that we estimate as $n \propto N^{-0.159}$. Supposing, for the sake of argument, that 50% of all cortical neurons were connected through the white matter in the marmoset, a decrease in n as $N^{-0.159}$ would imply that in a monkey-sized cortex with 10 times more neurons than the marmoset, WM connectivity would fall to $10^{-0.159} = 0.59 \times 50\% = 30\%$ of all neurons; and a human-sized cortex with about 100 times more neurons than a marmoset would have only 18% of its neurons interconnected through the white matter.

Note that decreased connectivity occurs in the face of an increased total number of axons in the WM, which is proportional to $n.N$, or N^{1+c}. In the exercise scenario above, the total number of axons in the WM would increase from ~122 million in the marmoset, to 510 million in the macaque, to 2.9 billion in the human cortex. Larger primate cortices, therefore, increase in size proportionally to N^1 neurons in the GM, of which a number proportional to $N^{0.841}$ send axons into the WM. Given that the average axonal length in the WM increases with $N^{0.242}$, and given our inference that the average axonal diameter does not change appreciably with N, WM volume (being proportional to $N.n.a.l$) is expected to increase with $N^1.N^{-0.159}.N^{0.032}.N^{0.242} = N^{1.114}$, which is close to the exponent obtained empirically.

Decreasing Connectivity in Small-World Networks. The decrease in connectivity in larger cortices is compatible with the decrease predicted by previous models of cortical scaling (6, 13–15) and calls into question popular scaling models that assume constant cortical connectivity (5, 16, 17). Such a decrease in connectivity in larger cerebral cortices is also compatible with the view that the cerebral cortex displays among its neurons the connectivity properties of a small-world network (18, 19), even though the cerebral cortex may be densely connected at the level of functional areas (20). A small-world network is a network in which distance between nodes (neurons) is small and grows with the addition of mostly local connectivity (through horizontal connections in the GM) and only a relatively small number of long-range connections (21) (through long fibers in the WM, which still guarantees fast global communication) (6, 22–25). A decrease in neuronal connectivity is, indeed, an expected feature of growing small-world networks (26).

Constant Axon Diameter. From the relationship between n, N, and A, we deduce that average axon cross-sectional area a in the WM does not scale with N or brain size and is therefore approximately constant across the species in our sample. This result is compatible with the finding that the distribution of fiber diameters in the splenium of the corpus callosum is conserved across species of different mammalian orders (27). In that study, Olivares et al. found that, across species, the average diameter of the widest callosal axons (of up to 0.8 μm in diameter, which are a minority of callosal fibers) increases with brain size, albeit slowly, with brain size raised to an exponent of 0.2. In a more recent study (17), Wang et al. described much larger axons (up to >10 μm in diameter) in two other regions of the callosum, but the distribution of the widest axons across a variety of species with brains of the size of the macaque and larger did not seem to accompany brain size (their figure 2d), despite the authors' claim that the diameters of the widest callosal axons increase (across all species, including least shrew, mouse, and rat) with brain diameter.

An increase in the axon diameter of a small subpopulation of WM axons, such as callosal axons, has been considered as a requirement to allow synchronous activity at the level of the whole neocortex (28). The alternative of maintaining constant conduction delays in larger brains by widening all axons would result in an unsustainable increase in the volume of the WM (29) and is clearly not the case in primates. Our finding of an invariant average axonal diameter in the WM as a whole implies that, if an increase in axonal diameter does occur, it must apply to only very few WM axons, such that the average axonal diameter is not altered even in large brains such as ours. Alternatively, if one considers the evidence that the corpus callosum increases in volume more slowly than intrahemispheric WM (30), our finding of no scaling of average axonal diameter combined with the decrease in connectivity points to the possibility that it is mostly the longest-range (callosal) connectivity that decreases in larger cortices (which would balance out the increase in widest axon diameter of a few fibers).

White Matter Scaling. Here we use the described linear relationship between axonal length and numbers of myelinating oligodendrocytes (11, 31) to infer, from the finding of a linear scaling between WM volume and number of nonneuronal cells in the WM, that total axonal length in the WM also increases linearly with V_W. We presume, like scaling models usually do (5, 32), that, at least in our sample of primate brains, the majority of axons in the WM are myelinated (~70% of all axons) (27), that the relative number of myelinated fibers does not vary significantly across species of different brain sizes (27), and that most of the WM volume amounts to myelinated axons (17). In this scenario, our findings regarding the scaling of the cellular composition and total myelinated axonal length of the WM can be considered representative of the whole WM, even though they do not take into consideration the volume of WM that is occupied by unmyelinated fibers.

Implications for Scaling of Conduction Velocity. Average conduction delay T in the brain scales with average length of global connections l and average axon diameter D such that $T \propto l/D$. A previous model of scaling of conduction times, which assumed a fixed number of connections per neuron and therefore presumed that volume of the whole brain (or WM) is proportional to

$N.D^2.l$, estimated that T increases with $N^{0.5}$ (32). Considering our finding that global connectivity through the WM decreases as a function of N, our updated estimate is that global T for the whole cerebral cortex actually increases more slowly, with $N^{0.242\pm0.085}$. Decreased connectivity in larger cortices, therefore, effectively decreases average conduction delays along global connections.

Cortical Folding and Connectivity. The increasing folding of the cerebral cortex in larger brains has been attributed to various factors such as space limitations inside the skull (1), the growth of the cortical sheet relative to its subcortical core, and the sheer expansion of the cortical sheet (33). However, partial removal of the skull during development does not have a dramatic effect on the fissure pattern, and lesion experiments suggest that cortical folding is not primarily dependent on a disproportionate growth between cortical and subcortical structures (33). Thus, the primary source of fissure formation must be sought in factors within the cortex itself.

One probable such factor to promote cortical folding is the mechanical tension along the axons that course in the WM, such that more densely interconnected cortical areas would tend to buckle together, forming a gyrus between them (12). The qualitative, tension-based theory of morphogenesis that takes into consideration the patterns of connectivity between cortical areas, proposed by David Van Essen, accounts for the consistent formation of convolutions in a species-specific pattern (12), but does not explain the increased cortical folding that accompanies increasing cortical size across species.

We propose an extension of Van Essen's tension-based theory of cortical folding that takes into consideration how GM connectivity through the WM changes across primate species to explain how increased folding accompanies increasing cortical size across primate species. In our view, rather than *driving* the folding of the WM surface, the folding of the external surface of the GM *results* from folding of the WM surface, which, in turn, results from increased tension within the WM due to increased numbers of axons composing the WM (although other contributing factors such as cortical growth and molecular factors cannot yet be ruled out). According to our model, cortical folding begins in the WM as a consequence of the elastic tension of its axons and proceeds as a function of both the number of axons that it contains and their length: the larger the number of axons (which varies as a function of N and c) is, the larger the tension in the WM, the more folded the WM surface, and, therefore, the more folded the GM surface. Given the different scaling relationships observed between the number of cortical neurons and cortical size across mammalian orders (8, 34, 35), such a connectivity-based model of cortical folding might account for the differences in the relationship between cortical folding and brain size across orders (36).

Methods

Animals. We analyzed the cellular composition of the WM, the WM surface area, and its folding in the same cortical hemispheres for which we had determined the cellular composition of the GM before (10), namely tree shrews (*Tupaia belangeri*, $n = 2$), galagos (*Otolemur garnetti*, $n = 2$), marmosets (*Callithrix jacchus*, $n = 3$), owl monkeys (*Aotus trivirgatus*, $n = 3$), squirrel monkeys (*Saimiri sciureus*, $n = 3$), capuchin monkeys (*Cebus apella*, $n = 2$), baboons (*Papio* sp., $n = 2$), one Goeldi's marmoset (*Callimico goeldii*), one long-tailed macaque monkey (*Macaca fascicularis*), and one bonnet macaque monkey (*Macaca radiata*). Additionally, one rhesus monkey (*Macaca mulatta*) cerebral cortical hemisphere was also analyzed (8), although we could not subject it to topological or volumetric analyses because we received it already cut into pieces. All animals were young adults at the time of the experiments (10). Data for the human brain were obtained from ref. 9 (no surface or volume measurements available). All GM values are from ref. 10.

Dissection. All animals were killed by a lethal injection of sodium pentobarbital, weighed, and perfused transcardially with 0.9% PBS followed by 4% phosphate-buffered paraformaldehyde. Brains were removed from the skull, weighed, and postfixed for 2 wk to 18 mo by immersion in 4% phosphate-buffered paraformaldehyde. Only one cerebral hemisphere of each animal (13 right hemispheres, 7 left hemispheres) was available for analysis.

Cerebral Cortical Reconstruction. Reconstruction of the WM volume and surface areas was performed in the same 2-mm-thick serial coronal brain sections for which GM volume and surface areas were determined before (10). All sections had their anterior surface digitalized in a 1,200-dpi desktop scanner. Subcortical white matter was defined as the entire WM contained between the inferior surface of the GM and the external surface of the striatum. Surface area A_W of the WM was estimated as $2 \times$ the sum of all perimeters of the WM in the sections, and WM volume (V_W) was estimated as the sum of the coronal WM area of all sections multiplied by 2 mm.

Isotropic Fractionator. Once all sections were scanned, the white matter in the coronal sections was dissected away from the gray matter under a stereoscope and weighed to determine WM mass (M_W). Total numbers of neurons in the GM were estimated separately in the GM and WM and considered jointly as the total number of cortical neurons (N) to avoid loss of gray matter neurons due to imprecision in the dissection (10). Total numbers of nonneuronal cells in the white matter of each cortical hemisphere were estimated as described previously using the isotropic fractionator method (7).

Data Analysis. Statistical analyses and regressions were performed in Statview (SAS), using the average values obtained from the individuals of each species. Least-squares regressions of the data to linear and power functions were calculated and reported. The uncertainties in the model parameters were propagated in the usual way from the variance associated with the power law best fit for the experimental variables, assuming uncorrelated residues and the existence of power law relations between the various quantities.

Phylogenetic Analysis. Phylogenetic independent contrasts were calculated to examine the scaling of the primate brain structures as a function of their cellular composition in the expanded dataset of 12 primate species, including humans, while controlling for effects of phylogenetic relatedness in the dataset (37). Standardized independent contrasts were calculated using the PDAP:PDTREE module of Mesquite software version 2.7 (38). Contrasts were calculated from both log-transformed and raw data, to evaluate how well they are described by power and linear functions, respectively. Phylogenetic relationships, shown in Fig. S2, are based on ref. 39. Branch lengths were transformed according to the method of Pagel (40), which assigns all branch lengths to 1 with the constraint that tips are contemporaneous. The reported values for the linear regressions of independent contrasts on log-transformed or raw data are reduced major axis (RMA) slope, r^2, and P value.

ACKNOWLEDGMENTS. We thank Christine Collins for collecting most of the brains used and for continued collaboration; Alexandre Valotta, Esper Cavalheiro (Universidade Federal de São Paulo, São Paulo, Brazil), and Laila Torres (Instituto Evandro Chagas, Belém, Brazil) for donating the hemispheres of one *Cebus* and one *Callimico*; and Drs. Terrence Sejnowski, Henry Kennedy, Jan Karbowski, Dmitri Chklovskii, and Georg Striedter for their comments on the manuscript. This work was supported by Fundação de Amparo á Pesquisa do Estado do Rio de Janeiro (FAPERJ)-Jovem Cientista and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)–Edital Universal (S.H.-H.), FAPERJ (B.M.), and National Eye Institute Grant 002686 (to J.H.K.).

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