White matter volume and white/gray matter ratio in mammalian species as a consequence of the universal scaling of cortical folding

Bruno Mota[a], Sandra E. Dos Santos[b], Lissa Ventura-Antunes[b], Débora Jardim-Messeder[c], Kleber Neves[c], Rodrigo S. Kazu[c,d], Stephen Noctor[e], Kelly Lambert[f], Mads F. Bertelsen[g], Paul R. Manger[h], Chet C. Sherwood[i,j], Jon H. Kaas[b,1], and Suzana Herculano-Houzel[b,k,l,1]

[a]Instituto de Física, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 21941-909, Brazil; [b]Department of Psychology, Vanderbilt University, Nashville, TN 37203; [c]Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 21941-901, Brazil; [d]Department of Psychology, University of Sheffield, Reading RG6 6AH, United Kingdom; [e]Department of Psychiatry and Behavioral Sciences, University of California, Davis, CA 95616; [f]Department of Psychology, University of Richmond, Richmond, VA 23173; [g]Center for Zoo and Wild Animal Health, Copenhagen Zoo, 2000 Copenhagen, Denmark; [h]School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Braamfontein 2000, Johannesburg, South Africa; [i]Department of Anthropology, The George Washington University, Washington, DC 20052; [j]Center for the Advanced Study of Human Paleobiology, The George Washington University, Washington, DC 20052; [k]Department of Biological Sciences, Vanderbilt University, Nashville, TN 37212; and [l]Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN 37232

Contributed by Jon H. Kaas, June 3, 2019 (sent for review October 6, 2017; reviewed by Terrence J. Sejnowski, Wolf Singer, and David C. Van Essen)

Because the white matter of the cerebral cortex contains axons that connect distant neurons in the cortical gray matter, the relationship between the volumes of the 2 cortical compartments is key for information transmission in the brain. It has been suggested that the volume of the white matter scales universally as a function of the volume of the gray matter across mammalian species, as would be expected if a global principle of wiring minimization applied. Using a systematic analysis across several mammalian clades, here we show that the volume of the white matter does not scale universally with the volume of the gray matter across mammals and is not optimized for wiring minimization. Instead, the ratio between volumes of gray and white matter is universally predicted by the same equation that predicts the degree of folding of the cerebral cortex, given the clade-specific scaling of cortical thickness, such that the volume of the gray matter (or the ratio of gray to total cortical volumes) divided by the square root of cortical thickness is a universal function of total cortical volume, regardless of the number of cortical neurons. Thus, the very mechanism that we propose to generate cortical folding also results in compactness of the white matter to a predictable degree across a wide variety of mammalian species.

scaling | white matter | cortical folding | brain size | evolution

The folding of the cortical surface and the segregation of the tissue of the cortical hemispheres into gray and white matter are the 2 most evident characteristics of the cerebral cortex. The proportion of the cerebral cortical volume that consists of white matter tends to increase with total cortical volume (V_T), varying from 3% in the least shrew to 50% in the African elephant (1). The segregation of the cerebral cortical volume into gray and white matter is often assumed to lead to optimized axonal propagation times (2). However, the telencephalon of avian brains shows no clear separation into gray and white matter, even though its network consists of even more neurons than found in mammalian cortices of similar mass (3). Whether or not segregated into a compartment of its own, and all else remaining equal, the importance of minimizing the volume of the white matter is clear: the shorter the axons, the smaller the propagation times, and therefore the faster that activity will be integrated across distant sites.

In line with the obvious importance of minimizing the volume of cortical white matter, V_W, previous models have considered that white matter volume is subject to universal principles that lead to its minimization (4–6). However, more recent data have shown that the assumptions made by those models turned out to be incorrect. By analyzing a sample of 59 species of various mammalian clades pooled together, Zhang and Sejnowski (4) found that V_W scales as a power function of the volume of cortical gray matter, V_G, with an exponent of 1.23 ± 0.01. Using a simple geometrical model that assumed 1) that each unit area of cortical surface receives a constant total cross-sectional area of long-distance fibers from the white matter; 2) that the geometry of the cortex minimizes the total length of fibers; and 3) that the average thickness of the cortical gray matter T varies universally with V_G, Zhang and Sejnowski calculated that V_W should scale with V_G raised to a theoretical exponent of 1.33. Deeming this exponent close to the empirical exponent of 1.23, the authors concluded that the universal scaling of V_W with V_G "might arise naturally as a consequence of the local uniformity of the cortex and the requirement for compact arrangement of long axonal fibers" (4).

The study by Zhang and Sejnowski (4), however, did not examine the scaling of V_W with V_G separately within each mammalian clade in the dataset. We have recently shown that, contrary to their assumption that T scales universally with V_G (which amounts to a universal correlation between T and total cortical surface area A_T), T scales as a power function of total

Significance

The white matter of the cerebral cortex contains all axons that support long-range cortical connectivity and increases faster in volume than the gray matter, which contains the connected cortical neuronal cell bodies. We show that the ratio between volumes of white and gray matter scales universally according to the same factors that account for the degree to which the cortex folds, that is, the combination of cortical surface area and thickness. We postulate that the relative white matter volume is determined as the developing cortex settles in the most energetically favorable folded conformation, regardless of its number of neurons.

Author contributions: B.M. and S.H.-H. designed research; B.M. and S.H.-H. performed research; B.M., S.E.D.S., L.V.-A., D.J.-M., K.N., R.S.K., S.N., K.L., M.F.B., P.R.M., C.C.S., J.H.K., and S.H.-H. contributed new reagents/analytic tools; B.M. and S.H.-H. analyzed data; and B.M., J.H.K., and S.H.-H. wrote the paper.

Reviewers: T.J.S., Salk Institute for Biological Studies; W.S., Ernst Strüngmann Institute for Neuroscience; and D.C.V.E., Washington University School of Medicine.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. Email: jon.h.kaas@vanderbilt.edu or suzana.herculano@vanderbilt.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1716956116/-/DCSupplemental.

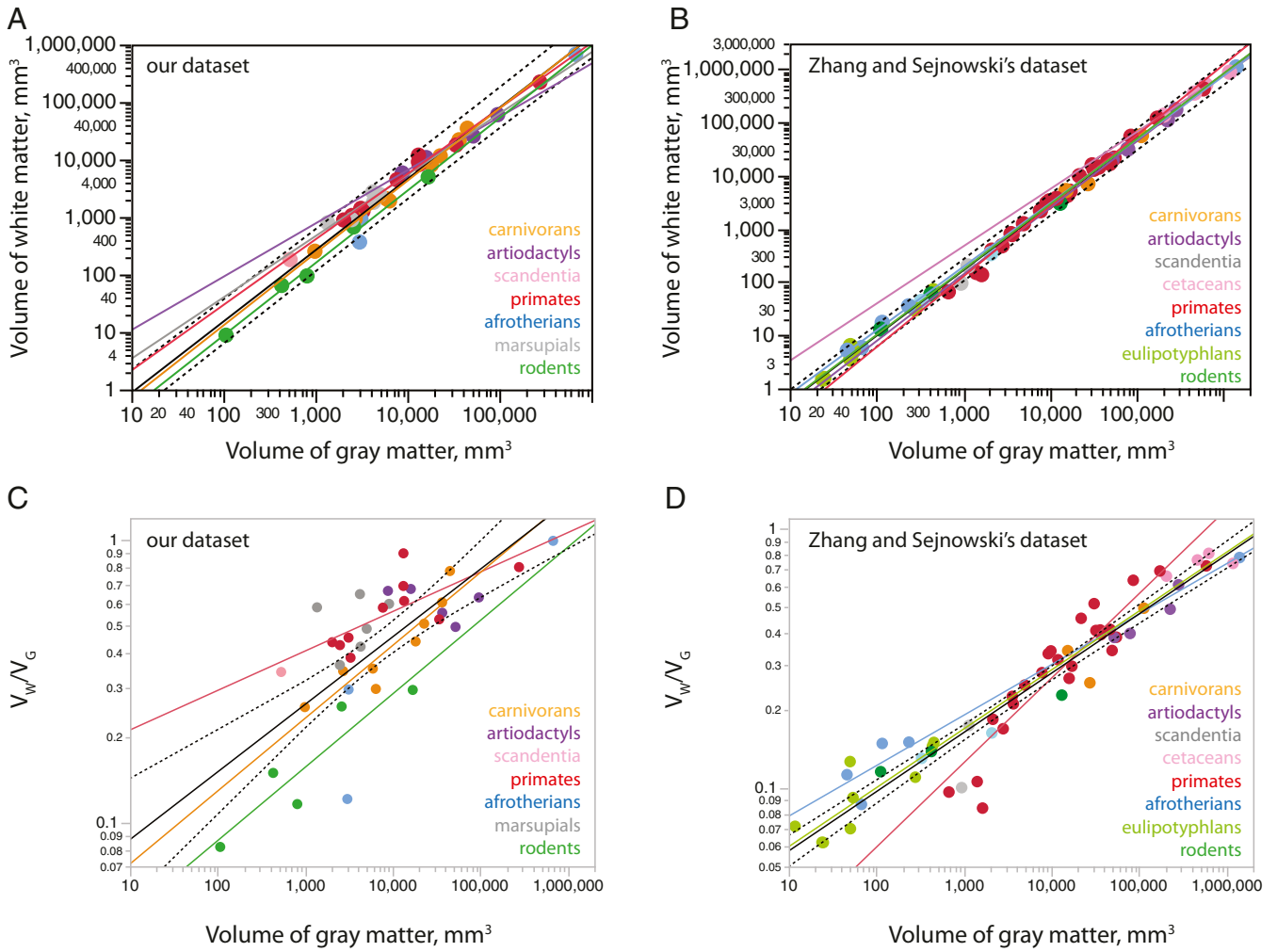

Fig. 1. V_W scales as different functions of V_G across mammalian clades. Each point represents 1 species according to the key in the graph (values in *SI Appendix*, Dataset S1). (*A*) Variation in V_W across species in our dataset can be fit with a single power function of V_G with exponent 1.240 ± 0.036 ($r^2 = 0.970$, $P < 0.0001$, $n =$ 38 species; plotted in black). However, V_W varies as a different function of V_G in each mammalian clade, with exponents as follows: artiodactyls, $V_W \sim V_G^{0.923 \pm 0.068}$, $r^2 = 0.984$, $P = 0.0009$ (lavender); marsupials, $V_W \sim V_G^{1.066 \pm 0.171}$, $r^2 = 0.907$, $P = 0.0034$ (gray); primates, $V_W \sim V_G^{1.140 \pm 0.047}$, $r^2 = 0.987$, $P < 0.0001$ (red); carnivorans, $V_W \sim V_G^{1.258 \pm 0.045}$, $r^2 = 0.992$, $P < 0.0001$ (orange); rodents, $V_W \sim V_G^{1.260 \pm 0.062}$, $r^2 = 0.993$, $P = 0.0003$ (green). Notice that all r^2 values per clade are higher than the r^2 value for the entire dataset, despite the smaller number of species per clade. (*B*) Variation in V_W across species in Zhang and Sejnowski's dataset (57 of the reported 59 species) can be fit with a single power function of V_G with exponent 1.228 ± 0.010 ($r^2 = 0.996$, $P < 0.0001$, $n = 57$ species; plotted in black). In their dataset, as in ours, V_W varies as a different function of V_G in each mammalian clade, with exponents as follows: artiodactyls, $V_W \sim V_G^{1.250 \pm 0.062}$, $r^2 = 0.995$, $P =$ 0.0025 ($n = 4$; lavender); cetaceans, $V_W \sim V_G^{1.075 \pm 0.064}$, $r^2 = 0.993$, $P = 0.0035$ ($n = 4$; pink); primates, $V_W \sim V_G^{1.326 \pm 0.028}$, $r^2 = 0.990$, $P < 0.0001$ ($n = 25$; red); afrotherians, $V_W \sim V_G^{1.195 \pm 0.022}$, $r^2 = 0.999$, $P < 0.0001$ ($n = 5$; blue); eulipotyphlans, $V_W \sim V_G^{1.227 \pm 0.054}$, $r^2 = 0.986$, $P < 0.0001$ ($n = 9$; green). Notice that all r^2 values per clade are higher than the r^2 value for the entire dataset, despite the smaller number of species per clade. (*C*) In our dataset, the increase in the ratio between V_W and V_G together with V_G can be described as a power function of exponent 0.240 ± 0.036 across all species ($r^2 = 0.549$, $P < 0.0001$, $n = 38$; plotted in black, with the 95% confidence interval for the fit shown as dashed lines). However, as expected from the different exponents in *A*, V_W/V_G scales as functions of V_G with different exponents across rodents (0.260 ± 0.062, $r^2 = 0.855$, $P = 0.0244$; plotted in green), primates (0.140 ± 0.047, $r^2 = 0.527$, $P = 0.0175$; plotted in red), and carnivorans (0.258 ± 0.045, $r^2 = 0.847$, $P = 0.0012$; plotted in orange), and does not scale across marsupials ($P = 0.7198$) or artiodactyls ($P = 0.3606$). (*D*) In Zhang and Sejnowski's dataset, the increase in the ratio between V_W and V_G together with V_G can be described as a power function of exponent 0.228 ± 0.010 across all species ($r^2 = 0.906$, $P < 0.0001$, $n = 57$; plotted in black, with the 95% confidence interval for the fit shown as dashed lines). However, as expected from the different exponents in *A*, V_W/V_G scales as functions of V_G with different exponents as follows: artiodactyls, not significant, $P = 0.0565$ (lavender); cetaceans, not significant, $P =$ 0.3612 (pink); primates, $V_W/V_G \sim V_G^{0.326 \pm 0.028}$, $r^2 = 0.857$, $P < 0.0001$ (red); afrotherians, $V_W/V_G \sim V_G^{0.195 \pm 0.022}$, $r^2 = 0.964$, $P = 0.0029$ (blue); eulipotyphlans, $V_W/V_G \sim V_G^{0.227 \pm 0.054}$, $r^2 = 0.715$, $P = 0.0041$ (green). Notice that, in *C* and *D*, data points for most species fall outside the 95% confidence interval.

cortical surface area A_T with different exponents across mammalian clades (7). By mathematical necessity, therefore, T does not scale universally with V_G, invalidating their third assumption (4). Moreover, we have found that, while the proportion of cortical neurons that are connected through the white matter is fairly constant across rodent species (8), it decreases with increasing numbers of cortical neurons (and thus cortical volume) across primate species (9, 10). These findings make the first assumption of Zhang and Sejnowski's model unlikely. Considering additionally that the discrepancy between their calculated and observed exponents is a substantial 10 times the SD of the observed exponent, it thus seems improbable that V_W and V_G scale universally as proposed. In that case, the source of the relationship(s) between V_W and V_G remains to be determined.

Optimization, by itself, is not a well-posed fundamental principle for biology, for 2 reasons. First, the choice of utility function that is maximized is not necessarily obvious and may be far too intricate to be estimated empirically. Second, and more

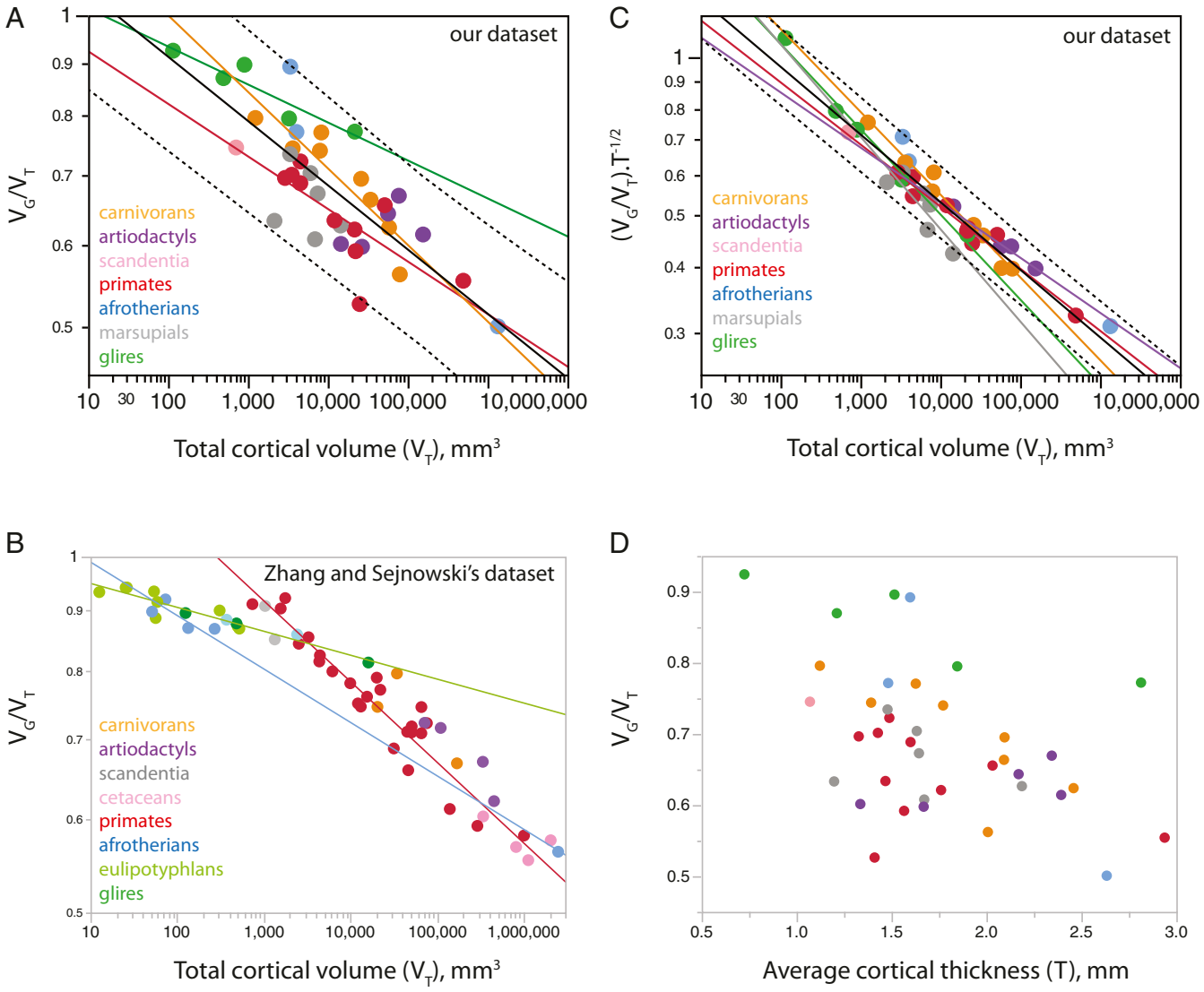

Fig. 2. The ratio V_G/V_T scales differently with V_T in each mammalian clade, but the ratio $(V_G/V_T)\cdot T^{-1/2}$ scales universally with V_T. Each point represents 1 species according to the key in the graph (values in *SI Appendix*, Dataset S1). (*A*) In our dataset, the ratio V_G/V_T can be fitted across all species with a single power function of exponent -0.062 ± 0.008 ($r^2 = 0.631$, $P < 0.0001$, $n = 38$; black). However, V_G/V_T varies as a different function of V_T in each mammalian clade, with exponents as follows: artiodactyls, not significant ($P = 0.3908$; lavender); marsupials, not significant ($P = 0.5809$; gray); primates, $V_G/V_T \sim V_T^{-0.051 \pm 0.016}$ ($r^2 = 0.555$, $P = 0.0134$; red); carnivorans, $V_G/V_T \sim V_T^{-0.074 \pm 0.014}$ ($r^2 = 0.823$, $P = 0.0019$; orange); rodents, $V_G/V_T \sim V_T^{-0.037 \pm 0.008}$ ($r^2 = 0.880$, $P = 0.0182$; green). Notice that the latter 2 r^2 values per clade are higher than the r^2 value for the entire dataset, despite the smaller number of species per clade. (*B*) In Zhang and Sejnowski's dataset, the ratio V_G/V_T can be fitted across the entire dataset with a single power function of exponent -0.045 ± 0.002 ($r^2 = 0.869$, $P < 0.0001$, $n = 57$; not plotted). However, here V_G/V_T varies even more clearly as a different function of V_T in each mammalian clade, as a shallow function in eulipotyphlans (exponent, -0.020 ± 0.004, $r^2 = 0.740$, $P = 0.0029$, $n = 9$; green), as a less shallow function in afrotherians (exponent, -0.045 ± 0.002, $r^2 = 0.992$, $P = 0.0003$, $n = 5$; blue), and as a steeper function in primates (exponent, -0.068 ± 0.005, $r^2 = 0.903$, $P < 0.0001$, $n = 25$; red). Exponents for cetaceans and artiodactyls are not significant ($P = 0.3427$ and 0.0597, respectively). (*C*) The folding-dependent ratio $(V_G/V_T)\cdot T^{-1/2}$, in comparison, is very well fitted across all clades in our dataset by a single power function of V_T of exponent -0.128 ± 0.006 ($r^2 = 0.919$, $P < 0.0001$, $n = 38$; black), with more overlap across species. Additionally, the ratio $(V_G/V_T).T^{-1/2}$ varies as clade-specific power functions of V_T that are significant for each clade with the following exponents: artiodactyls, -0.104 ± 0.014 ($r^2 = 0.945$, $P = 0.0055$; lavender); marsupials, -0.175 ± 0.050 ($r^2 = 0.758$, $P < 0.0001$); primates, -0.118 ± 0.008 ($r^2 = 0.960$, $P < 0.0001$; red); carnivorans, -0.157 ± 0.010 ($r^2 = 0.978$, $P < 0.0001$; orange); rodents,-0.162 ± 0.011 ($r^2 = 0.985$, $P = 0.0008$; green). Notice the higher values of r^2 compared with the fits in *A*. The 95% confidence intervals for individual values are plotted in both graphs. (*D*) The V_G/V_T ratio in our dataset decreases with increasing average thickness of the cortical gray matter (Spearman correlation, -0.407; $P = 0.0112$). That is, the thinner the gray matter, the smaller the white matter fraction of the cortex tends to be.

important, biological form results from evolution through spontaneous variation and natural selection rather than by ab initio design, so any optimization is constrained by what can be achieved by changing a relatively few degrees of freedom in the context of largely conserved developmental processes and pre-existing conditions inherited from ancestors of a given lineage.

One possible such set of processes through which constrained optimization of white matter connectivity might occur in evolution (thereby possibly creating a link between the volumes of gray and white matter in the cortex) are the push–pull forces that are both subject and cause of cortical growth and development, and have been previously proposed to lead to folding (11). Cortical morphology, including cortical folding, which results in smaller cortical volumes than expected for surface area, is ultimately a consequence of tension (push–pull) forces at play during development, and therefore is presumably highly conserved, subjected to natural selection, and constrained by physics. It is thus possible that whatever mechanism determines the degree of folding of the

cortical surface also determines the relationship between the volumes of gray and white matter. We have recently developed a model that predicts the existence of a universal relationship between total surface area (A_T), exposed surface area (A_E), and average cortical thickness (T, calculated as V_G/A_T) for lissencephalic and gyrencephalic species alike (7). According to this model, this relation $A_T \cdot T^{1/2} = k \cdot {A_E}^{1.25}$ is derived from the minimization of the effective free energy associated with cortical shape based on known mechanisms of axonal elongation and the self-avoiding nature of the cortical surface. This is a very simple model that, like the original one (4), seeks only to relate global cortical morphological variables (i.e., areas, volumes, and average thickness that pertain to the entire cortex), favoring generality over detail, with a widely applicable (cross-species) but coarse-grained (whole-cortex) description of cortical folding.

Empirically, by measuring the cortices of dozens of mammalian species belonging to different orders and varying over 4 orders of magnitude in total cortical surface area, we verified that lissencephalic and gyrencephalic species alike follow closely the theoretical prediction, with $A_T \cdot T^{1/2} = k \cdot {A_E}^{1.305 \pm 0.010}$ and an r^2 of 0.998 (7), although the empirical exponent of 1.305 differs from the theoretical 1.25 by a small but statistically significant value. The goodness of the fit of our model to empirical data strongly suggests that A_T and T are 2 independent evolutionary degrees of freedom, from which V_G, A_E, and the folding index $FI = A_T/A_E$ result directly. Additionally, the relationship between the exposed surface area of a cortical hemisphere and total cortical volume V_T can be very precisely estimated across species (see below) using the relation between area and volume of a solid hemisphere, $V_T = (2/9\sqrt{3\,\pi}){A_E}^{3/2} = 0.0724{A_E}^{1.5}$, even though cerebral cortices are obviously not perfect hemispheres. Thus, from T, A_T, and A_E we can obtain both V_T and $V_W = V_T - V_G$, which implies that V_G and V_W cannot be taken as independent variables.

One of the terms in our equation for free energy that defines the degree of cortical folding (that is, A_T and A_E) is related to self-avoidance, while the other is proportional to V_W (*SI Appendix*). Thus, since we do not start by assuming V_W minimization, a constrained minimization of white matter volume is a consequence, rather than a postulate, of our model (7).

Here, we examine the propositions that 1) the scaling of V_W with V_G is clade-specific, contrary to the report by Zhang and Sejnowski (4), but nevertheless 2) there is a universal relationship between V_G and V_T (or V_W) as a consequence of the mechanism that leads to the folding of the cerebral cortex through the minimization of the effective free energy of the cortex depending on its surface area and thickness (7). We examine prediction 1 by revisiting the dataset of Zhang and Sejnowski, for which we could retrieve the original data cited for 57 of the 59 species. We also examine prediction 1, and additionally prediction 2, using our own dataset (*SI Appendix*, Dataset S1), which includes V_G, V_W, A_T, T, folding index ($FI = A_T/A_E$), and numbers of cortical neurons, N, and consists of the data examined in ref. 7 plus new data for 6 marsupial and 8 carnivoran species for which numbers of neurons were reported in refs. 12 and 13.

Results

We find that the relationship $V_W \times V_G$ can be well-fit across the 38 mammalian species in our dataset (*SI Appendix*, Dataset S1) with a single power function of exponent 1.240 ± 0.036 (r^2 = 0.970, $P < 0.0001$). However, our dataset includes enough species of primates (10), marsupials (6), carnivorans (8), artiodactyls (5), and rodents (5) to allow the calculation of power functions for each order separately. We then find that V_W scales as different functions of V_G in each clade, with exponents that vary from 0.923 ± 0.048 in artiodactyls to 1.260 ± 0.062 in rodents (Fig. 1*A*). Exponents for artiodactyls (0.923 ± 0.048) and primates (1.140 ± 0.047) have 95% confidence intervals that exclude the exponent of 1.240 that applies to all species in the dataset. The exponent that applies to artiodactyls is not significantly different from unity, which implies that, contrary to other mammalian clades, larger artiodactyl cortices do not have proportionately more white matter than gray matter. Importantly, the fits that apply to each clade have higher r^2 values than the fit for all species, despite the smaller number of species per clade (Fig. 1*A*). Likewise, while we obtain a similar exponent of 1.228 ± 0.010 across the 57 species in the dataset from Zhang and Sejnowski, we find that breaking down by clade the analysis of the $V_W \times V_G$ relationship in their dataset also reveals clade-specific relationships (Fig. 1*B*). Strikingly, the exponents of each of the clade-specific relationships differ between the 2 datasets (compare Fig. 1 *A* and *B*), which indicates that, unlike a true universal relationship, the precise set of species included affects the exponents obtained for each clade. These findings indicate that the relationship between V_W and V_G is not universal across mammalian clades as previously thought.

The large variation in V_G and V_W, spanning 5 and 6 orders of magnitude, respectively, may have masked the real extent of divergence from different clades to the universal scaling rule proposed by Zhang and Sejnowski (4). If such a universal scaling rule applied as those authors proposed, and with an exponent significantly above linearity, then the ratio V_W/V_G should increase homogeneously with increasing V_G across all species, conforming in each clade to a power function of V_G raised to an exponent of 0.240 in our dataset, or 0.228 in theirs. Instead, we find in our dataset that V_W/V_G does not increase significantly together with V_G across marsupial or artiodactyl species, and whereas similar exponents of 0.258 ± 0.045 and 0.260 ± 0.062 apply to carnivorans and rodents, a significantly smaller exponent of 0.140 ± 0.047 applies to primates (Fig. 1 *C*, red). In Zhang and Sejnowkski's dataset, exponents are also clade-specific, and as in our dataset, V_W/V_G does not scale significantly with V_G in all clades (Fig. 1*D*). Importantly, in both datasets, data points for most species fall outside the confidence interval that applies to all species; and clade-specific exponents differ between the 2 datasets, which include different species subsets (compare Fig. 1 *C* and *D*). Thus, as the volume of the gray matter increases across species, the volume of the white matter does tend to increase, but differently across clades; not in all clades; and differently depending on the precise dataset.

The relative scaling of the white and gray matter portions of the cortex can more readily be captured as the ratio V_G/V_T. Although power functions are not additive, the relationship $V_G \times V_T$ across all species in the range of our dataset is indistinguishable from a power function of exponent 0.938 ± 0.008 ($r^2 = 0.997$; $P < 0.0001$; $n = 38$). If a universal scaling rule applied, then the gray matter fraction V_G/V_T should decrease homogeneously with increasing V_T across all species as a single power function of V_T with a negative exponent of −0.062, and species with similar V_T should display similar gray matter volume fractions. We find that the relationship between V_G/V_T and V_T can indeed be described across all 38 species in our dataset by a single power function of exponent −0.062 ± 0.008, although with a fairly low r^2 = 0.631 ($P < 0.0001$; Fig. 2*A*). Still, once more we find that the gray matter fraction V_G/V_T is better described as clade-specific power functions of V_T, with exponents ranging from −0.037 ± 0.008 in rodents to −0.074 ± 0.014 in primates (Fig. 2*A*). For similar values of V_T, we find that the gray matter fraction of the cerebral cortical volume is much smaller in most primates than in carnivorans, rodents, and afrotherians. For example, V_G represents 77% of V_T in the capybara, the largest rodent in the dataset, but only 59% of V_T in the bonnet monkey, whereas both species have values of V_T around 22,000 mm^3 (*SI Appendix*, Dataset S1). Likewise, we find that in Zhang and Sejnowski's dataset, there are clade-specific relationships between V_G/V_T and V_T, with a clear distinction between eulipotyphlans, afrotherians, and primates (Fig. 2*B*). As above, the clade-specific exponents differ across the

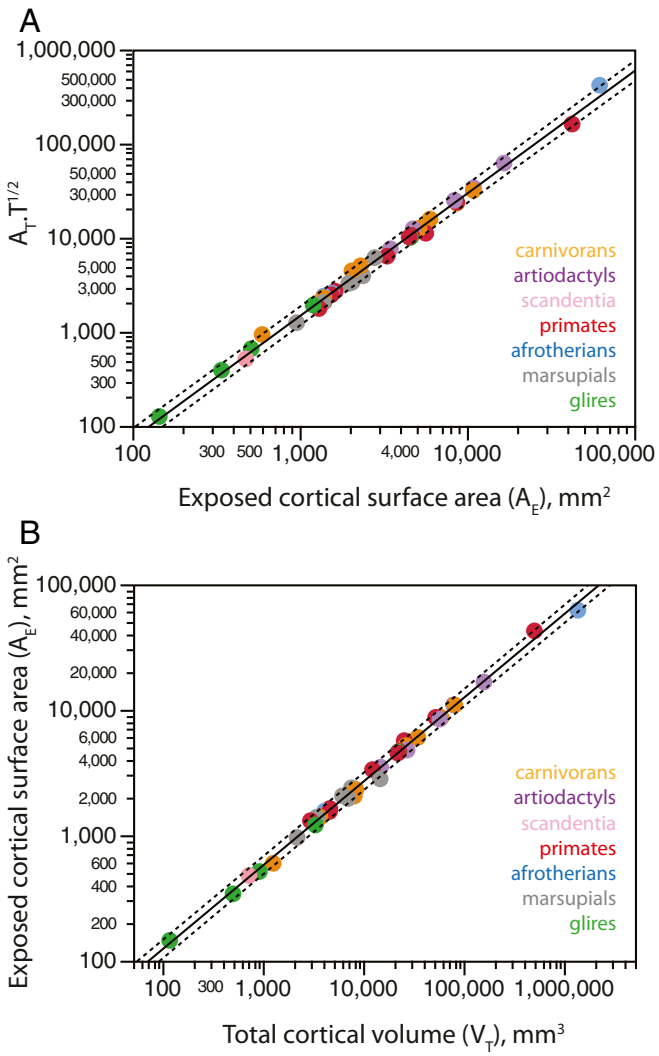

Fig. 3. The equation that describes the degree of cortical folding applies universally across mammalian species. Each point represents 1 species according to the key in the graph (values in *SI Appendix*, Dataset S1). (*A*) The product $A_T{\cdot}T^{1/2}$ varies across all 38 species in our dataset as a power function of A_E with exponent 1.302 ± 0.014 (r^2 = 0.996; P < 0.0001). (*B*) The exposed cortical surface area, A_E, varies in turn as a universal power function of V_T with exponent 0.668 ± 0.007 (r^2 = 0.996; P < 0.0001; n = 38). The 95% confidence intervals for individual values are plotted in both graphs.

2 datasets, which include different species, in contrast to what would be expected of a truly universal relationship between V_G/V_T and V_T.

We next examine our prediction that the distribution of V_T into V_G and V_W should scale universally, but depending on the relationship between A_T, T, and A_E that determines the degree of cortical folding. The rationale behind this expectation is that the product $A_T{\cdot}T$ describes V_G, whereas A_E is tied to V_T (7). Thus, the same physical process of cortical folding that links A_T and T to A_E should tie V_G to V_T, determining V_W. Confirming our previous report (7), we find that the product $A_T \times T^{1/2}$ scales as a universal function of ${A_E}^{1.302\ \pm\ 0.014}$ (r^2 = 0.996; P < 0.0001) across all 38 species (Fig. 3*A*), 14 more species than in our original study. Data on surface areas and thickness were not available for the dataset used in Zhang and Sejnowski (4).

Our model of cortical folding (7) assumes that A_E scales with ${V_T}^{2/3}$, according to a simple geometric approximation. Fig. 3*B* shows that this assumption is warranted: We find that A_E scales with ${V_T}^{0.668\ \pm\ 0.007}$ across all 38 species in the dataset (r^2 = 0.996; P < 0.0001), with an exponent that is virtually identical to the expected 2/3 and with very little deviation from the expected values across clades. This relationship can be rewritten as $V_T = 0.0756{\cdot}{A_E}^{1.493}$, which is very close to the relation between area and volume of a solid hemisphere, $V_T = (2/9\sqrt{3\ \pi})A_E{}^{/2} = 0.0724{A_E}^{1.5}$.

Because $A_T = V_G/T$ and $A_E \sim {V_T}^{0.668}$, the initial equation $A_T{\cdot}T^{1/2} \sim {A_E}^{1.302}$ can be rewritten as $V_G/T{\cdot}\ T^{1/2} \sim {V_T}^{0.668\ \times\ 1.302}$ or $V_G{\cdot}T^{-1/2} \sim {V_T}^{0.870}$. As shown in Fig. 4*A*, we find that $V_G{\cdot}T^{-1/2} \sim {V_T}^{0.872\ \pm\ 0.006}$ (r^2 = 0.998; P < 0.0001), a function that applies universally across all clades. The exponent for this function has a coefficient of variation of 0.69%, compared with 0.85% for the exponent of the function $V_G \sim V_T$. The normalized residuals obtained for $V_G{\cdot}T^{-1/2}$ are not significantly correlated with V_T (Spearman, ρ = 0.096, P = 0.5675; Fig. 4*B*) and are not significantly different between clades at the P < 0.01 level (Wilcoxon pairwise comparisons), which indicates that variation in $V_G{\cdot}T^{-1/2}$ is indeed universally and well described as a function of V_T across species. Thus, the product $V_G{\cdot}T^{-1/2}$ (that is, gray matter volume

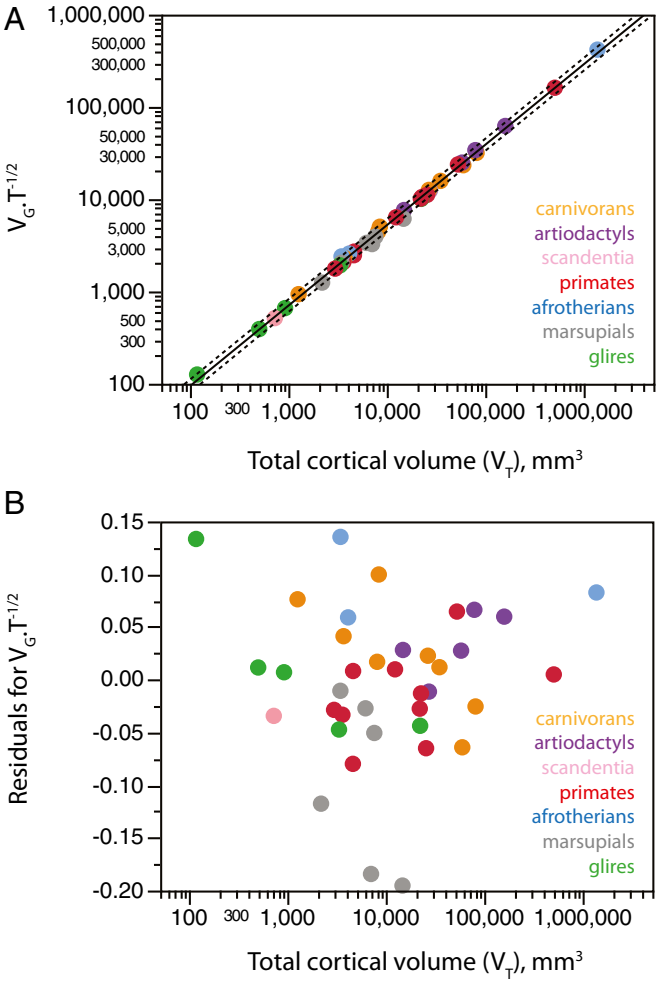

Fig. 4. The product $V_G{\cdot}T^{-1/2}$ varies universally with V_T as predicted by the equation that describes the degree of cortical folding. Each point represents 1 species according to the key in the graph (values in *SI Appendix*, Dataset S1). (*A*) The product $V_G{\cdot}T^{-1/2}$ varies across all 38 species as a power function of V_T with exponent 0.872 ± 0.006 (r^2 = 0.998; P < 0.0001). The dotted line indicates the 95% confidence interval for individual values. (*B*) The residual values obtained from the fit in *A*, after normalization for the product $V_G{\cdot}T^{-1/2}$ for each species, are not significantly correlated with variation in V_T (Spearman correlation coefficient, 0.096; P = 0.5675).

adjusted for cortical thickness) is well predicted by our model of cortical folding to scale as a universal function of V_T.

As shown above, given the scaling over several orders of magnitude, a better test of the universality of the relationship $V_G \sim V_T$ is to examine how the ratio V_G/V_T varies with V_T. We find that while the gray matter fraction V_G/V_T scales as a poor universal function of V_T, with $r^2 = 0.631$, and the ratio V_G/V_T did not scale significantly with V_T in all clades (Fig. 2*A*), the gray matter fraction adjusted for cortical thickness according to our equation that describes folding, $(V_G/V_T)\cdot T^{-1/2}$, does scale universally both across all clades, with $V_T^{-0.128 \pm 0.006}$ ($r^2 = 0.919$; $P < 0.0001$), and within each clade (Fig. 2*C*). The gray matter fraction adjusted for cortical thickness shows much more overlap across species and clades, and mostly overlapping clade-specific exponents (Fig. 2*C*). This indicates that average cortical thickness impacts the V_G/V_T ratio, which is illustrated by the significantly decreasing gray matter fraction (i.e., a smaller V_G/V_T ratio) as cortical thickness increases (Fig. 2*D*).

The relationship $(V_G/V_T)\cdot T^{-1/2} = k\cdot A_E^{\,a}$, which is directly predicted from our model of cortical folding, is also a much better (and universal) fit of the data (Fig. 5*B*) than the relationship uncorrected for T, $V_g/V_T = k\cdot A_E^{\,a}$ (Fig. 5*A*). Accordingly, the values of the constant k calculated for each species for the relationship between (V_G/V_T) and $V_T = A_E^{\,a}$ are much less variable both within and across clades when T is taken into consideration ($k = T^{-1/2}\cdot V_G/V_T\cdot A_E^{\,-a}$; Fig. 5*D*) than when it is not ($k = V_G/V_T\cdot A_E^{\,-a}$; Fig. 5*C*).

We previously found that the degree of cortical folding is not a universal function of the number of neurons in the cerebral cortex (7). Here, we find that neither the gray matter ratio V_G/V_T nor the gray matter ratio adjusted for cortical thickness, $(V_G/V_T)\cdot T^{-1/2}$, is a universal function of the number of neurons in the cortex: as shown in Fig. 6, distinct functions apply to primates, rodents, and carnivorans in our dataset. For similar numbers of cortical neurons, rodents have larger ratios of V_G/V_T than carnivorans and primates (Fig. 6*A*), while primates have larger values of $(V_G/V_T)\cdot T^{-1/2}$ than most other species (Fig. 6*B*). In contrast, for a same V_T, the different mammalian species analyzed have comparable gray matter fractions once adjusted for cortical thickness (Fig. 2*C*).

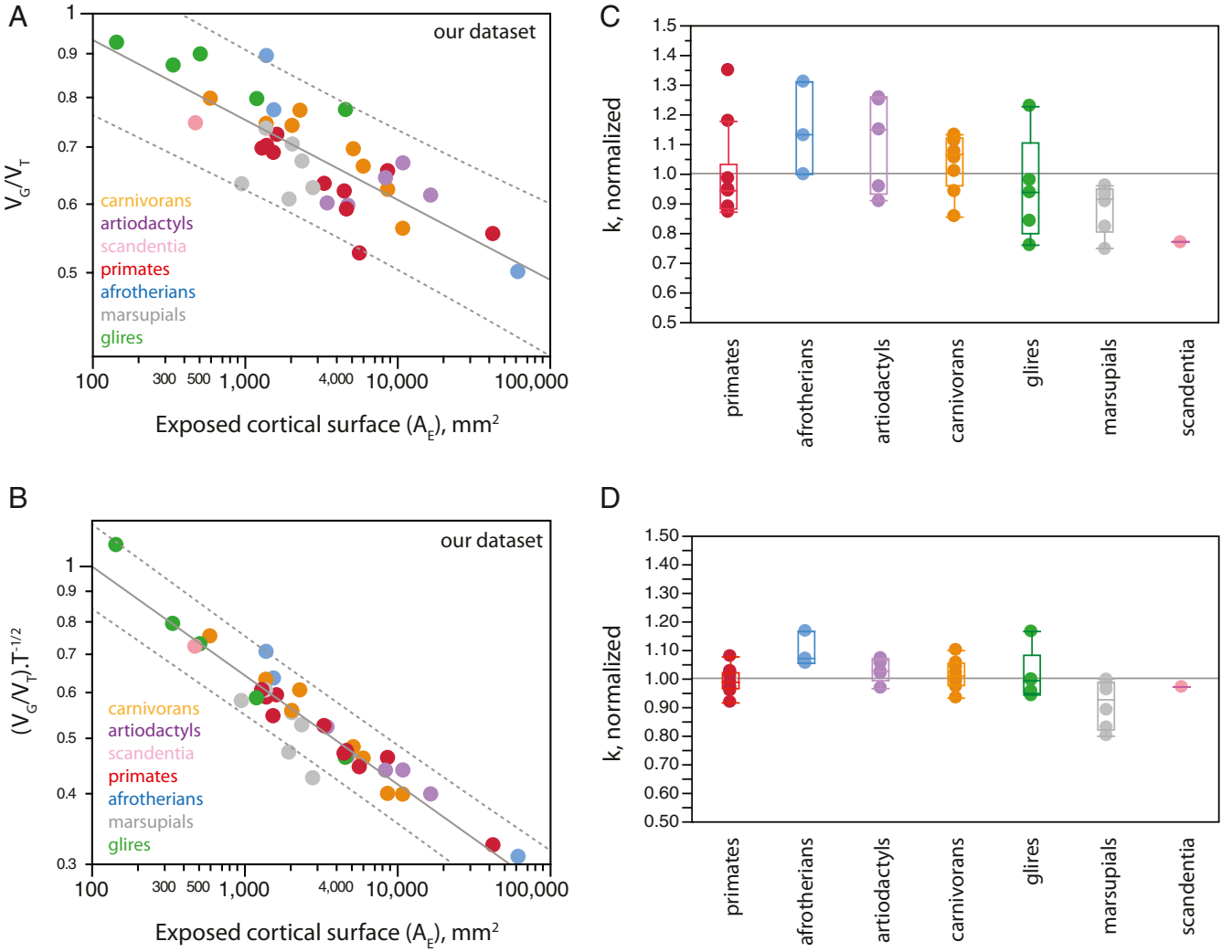

Fig. 5. The ratio $(V_G/V_T)\cdot T^{-1/2}$ varies universally with the exposed surface area of the cerebral cortex. Each point represents 1 species according to the key in the graphs. (*A*) The ratio V_G/V_T decreases as a power function of the exposed surface area of the cerebral cortex, A_E, with exponent -0.093 ± 0.012, but with a moderate $r^2 = 0.631$ ($P < 0.0001$; $n = 38$). (*B*) In contrast, the V_G/V_T ratio normalized for T, $(V_G/V_T)\cdot T^{-1.2}$, decreases as a power function of A_E with a much higher $r^2 = 0.910$ and a steeper exponent of -0.191 ± 0.010 ($P < 0.0001$; $n = 38$). *C* and *D* show the constants that apply to each species according to the power functions depicted in *A* and *B* after normalization, for the sake of comparison, by the average value obtained for all 38 species, with a larger spread in *C* (nonadjusted for T according to our folding model) than in *D* (adjusted for T).

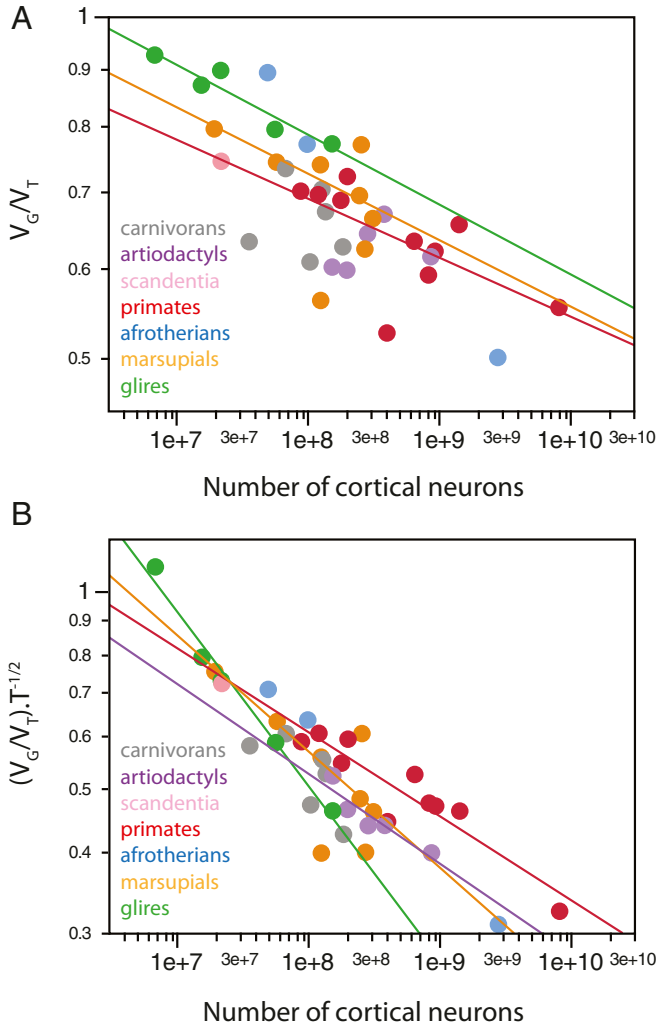

Fig. 6. The ratio $(V_G/V_T)\cdot T^{-1/2}$ does not vary universally with the number of neurons in the cerebral cortex. Each point represents 1 species according to the key in the graph (values in *SI Appendix*, Dataset S1). (*A*) The ratio V_G/V_T does not vary significantly as a function of the number of neurons in the cerebral cortex across all clades. Scaling is significant in rodents (exponent –0.061 ± 0.011; $r^2 = 0.908$; $P = 0.0121$) and in primates (exponent –0.052 ± 0.021; $r^2 = 0.438$; $P = 0.0370$), but not across carnivorans ($P = 0.2580$), marsupials ($P = 0.9205$), or artiodactyls ($P = 0.6048$). (*B*) The ratio $(V_G/V_T)\cdot T^{-1.2}$ varies as clade-specific functions of the number of neurons in the cerebral cortex, with the following exponents: rodents, –0.266 ± 0.020 ($r^2 = 0.983$; $P = 0.0009$); primates, –0.128 ± 0.018 ($r^2 = 0.860$; $P = 0.0001$); carnivorans, –0.171 ± 0.067 ($r^2 = 0.518$; $P = 0.0441$); artiodactyls, –0.137 ± 0.032 ($r^2 = 0.743$; $P < 0.0001$). The scaling is not significant in marsupials ($P = 0.1136$).

Finally, we examined whether, under the constraints of a self-avoiding cortical surface and of a specified value of A_T (which is related to the cross-sectional area of white matter axons; ref. 7), the observed value of T minimizes V_W. By using the universal relation depicted in Fig. 2*C*, we can express V_W solely as a function of A_T and T (which is not a power function; *SI Appendix*) and find the value of T that minimizes V_W while keeping A_T constant. If the value of V_W is minimized, then the expected value of T should be given by the following:

$$T_{opt} = 2^{-\frac{2ab}{-1+2ab}} a^{-\frac{2ab}{-1+2ab}} A_T^{-\frac{2a\left(1-\frac{1}{ab}\right)^b}{-1+2ab}} b^{-\frac{2ab}{-1+2ab}} c^{-\frac{2ab}{-1+2ab}} k^{-\frac{2}{-1+2ab}},$$

where a, k, b, and c are taken from the relations $A_T\cdot T^{1/2} = k\cdot A_E{}^a$ and $V_T = cA_E{}^b$. For the empirical values we have obtained, this reduces to $T_{opt} \cong 17/A_T{}^{0.65}$. We find for our data that the optimal values T_{opt} and the actual values of T can differ by a factor of 5 or more. The best-fit power law for T as a function of A_T has an exponent of 0.19 ($r^2 = 0.64$), far from the –0.65 expected if T were close to T_{opt}. As a result, the ratio T_{opt}/T ranges from 4.2 for the pygmy shrew to 0.002 for the African elephant. This discrepancy indicates that, despite the intuition that wiring length and volume should be minimized in evolution, cortical diversity does not even approximately reflect minimization of V_W, and thus presumably wiring length, under a naive constraint of A_T. It is natural to expect wiring length optimization to be present in some form, from evolutionary reasoning alone, but if that is true, then we have not identified the set of constraints under which minimization happens.

Discussion

Here, we show that once enough species of different mammalian clades are analyzed separately, there is not a single universal relationship that relates V_G and V_W directly, in contrast to the previous suggestion by Zhang and Sejnowski (4) that this relationship is universal. The existence of clade-specific scaling of the relationship between V_W and V_G that is also dependent on the particular species analyzed is confirmed by the lack of a universal scaling of the gray matter fraction V_G/V_T as V_T increases, or of the V_W/V_G fraction as V_G increases across mammalian species.

On the other hand, we show that there is an empirical universal relationship between $V_G\cdot T^{-1/2}$ and V_T, as well as between $V_G\cdot T^{-1.2}/V_T$ and V_T, both in the form of power laws. More importantly, these relationships are predicted by the same mechanism of minimization of the effective free energy of the cortical volume that we have shown to explain the degree of cortical folding universally across mammalian species and even within an individual cerebral cortex (7). We note that taking into account clade-specific variation in T, which Zhang and Sejnowski (4) dismissed by assuming universal variation of T with V_T, is the very factor that makes the empirical relationship between $V_G\cdot T^{-1/2}$ and V_T universal, when the relationship between V_G and V_T is not. Notice that while V_W is predicted universally by our model, the equation that describes the relationship between V_W and V_G (*SI Appendix*) is not a simple power function.

Fig. 7 illustrates how the 2 universal relationships that we have uncovered are sufficient to determine all coarse-grained morphological variables that describe the cerebral cortex: A_T, T (and therefore V_G), A_E, V_T, and therefore V_W. The universal relationship between A_T, T, and A_E is described by our model of cortical folding (7); the universal relationship between A_E and V_T is described in the present study and indicates that hemisphericity is maintained as cortices vary in size. V_G results from the product of the number of cortical neurons N and the average neuronal volume v_N (14), assuming that the volume contributed by glial cells scales proportionately to this product (15). The volume V_G is spread laterally in the growing cortex into clade-dependent combinations of A_T and T, which we consider to be independent biological variables during cortical development. According to our cortical folding model, depending on the specific combination of A_T and T into which the growing volume V_G is spread, A_E is determined as the cortex settles, at each point during development, into the folded conformation that minimizes its effective free energy according to the relationship $A_E{}^{1.305} \sim A_T\cdot T^{1/2}$ (7). Because V_T accompanies A_E as $A_E{}^{3/2}$, as hemisphericity is maintained, V_T is also determined by cortical folding, depending on the combination of A_T and T. It thus follows that V_W, the other component of the expanding volume that by definition amounts to V_T-V_G, is also determined as a consequence of the folding of the cortical volume and surface depending on the combination of A_T and T. Thus, while the ratio V_G/V_T does not vary as a single, universal function of the variable V_T (Fig. 2*A*), it is well described as a combined function of

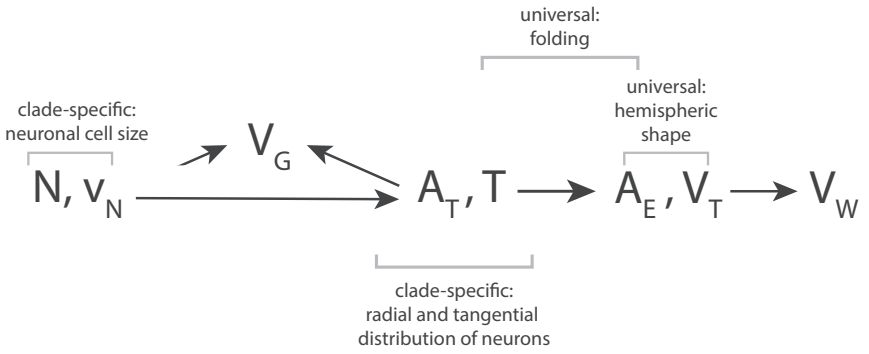

Fig. 7. The combination of A_T and T determines both the degree of cortical folding and V_G, V_T, and V_W. Schematics showing how A_T and T universally define A_E (according to our model, as the cortex settles into the folded conformation that minimizes its effective free energy) and therefore also V_T (given that V_T and A_E are tightly in a universal relationship that indicates that hemispheric shape is maintained across all clades). Because the product $A_T{\cdot}T$ (which consists of N neurons of average cell volume v_N) is by definition V_G, the volume V_W is defined simultaneously as the cortex settles into a folded conformation. Notice that, while A_T and T (and therefore V_G) depend on clade-specific relationships between numbers of neurons, average neuronal size, and how these neurons are distributed tangentially and radially forming the cortex, the morphological variables A_E, V_T, and by consequence V_W are independent of the numbers of neurons that form the cortex.

2 variables, A_T and T. Similarly, while V_W is not a universal power function of V_T or of $V_T{\cdot}T^{1/2}$, it is a universal mathematical function of $V_T{\cdot}T^{1/2}$ nevertheless—just not a simple power function. We conclude that while V_G/V_T (and therefore V_W/V_T) does not vary as a simple universal function of V_T across all mammals, the reciprocal fractions of the cortical volume that are constituted by gray and white matter are indeed mathematically and universally predictable across mammals as a consequence of the physical mechanism of minimization of the effective free energy of the cortical volume that leads to cortical folding, depending simply on the combination of A_T and T that define V_G. Although at this point our model addresses only folding at the level of the whole cortex, it does predict and explain global cortical morphology across species.

There are obviously advantages to keeping V_W small, but an implication of our findings is that this quantity is not directly optimized in mammalian cortices, or else is minimized under constraints that remain to be identified. Rather, A_T and T are independent variables, from which all other global morphological variables that characterize the geometrically hemispherical cortical hemispheres, including V_W, can be derived through a universal folding mechanism. This mechanism effectively minimizes V_W, but only given A_T and T, and far from optimizes it. To the extent that a small V_W is selected for, it can happen only through minimizing A_T and/or keeping T small relative to A_T (both of which contribute to more folding) through varying the neuronal proliferative processes that determine A_T and T, the 2 main degrees of freedom of cortical morphology. Although changes in the plastic and elastic mechanical properties of cerebral cortical matter might also lead to decreased V_W, that is unlikely due to the existence of a universal relationship between A_T, T, and A_E across adult mammalian cortices (7), which suggests that properties are largely conserved across species.

de Lussanet (16) has rearranged the terms in our theoretical equation, $k{\cdot}A_E{}^{1.25} = A_T{\cdot}T^{1/2}$, to show that the ratio between V_T and V_G should be inversely proportional to the folding index A_T/A_E. However, this elegant relation comes with no theoretical motivation or alternative folding mechanism attached, and is only strictly true for the theoretical exponents predicted by our model. As mentioned, the empirical exponent of 1.305 across different species is statistically different from 1.25. The exponent obtained across

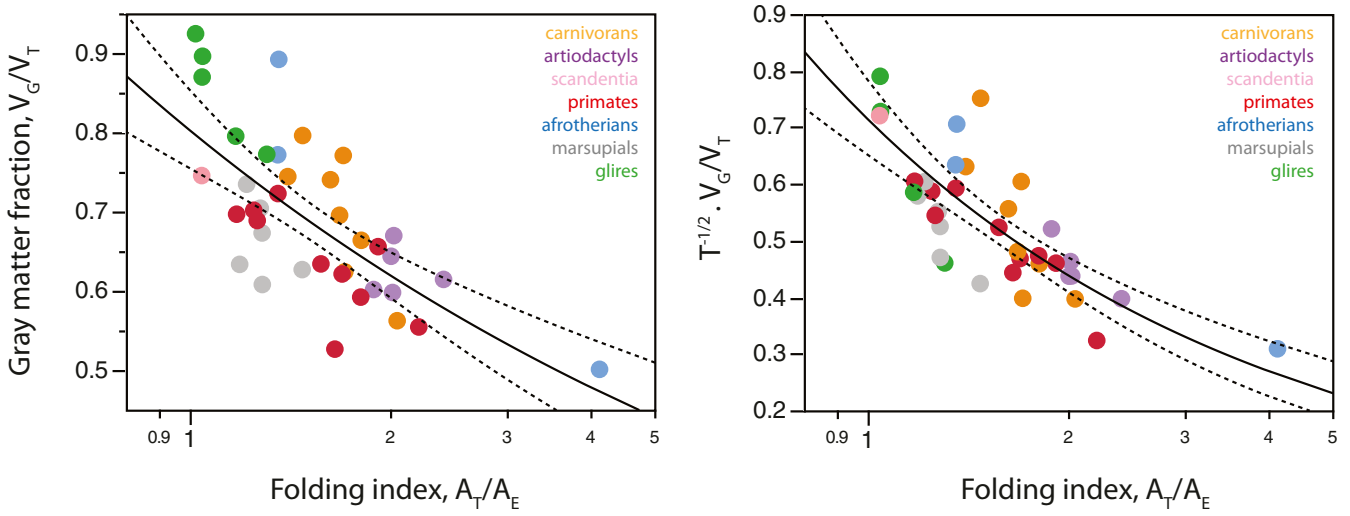

Fig. 8. The ratio V_G/V_T varies with A_T/A_E, but not universally. (*A*) The relationship across all species can be fit with a power function of exponent −0.372 ± 0.059 ($P < 0.0001$), but with a low r^2 value of 0.524. Notice that carnivoran species (in orange) have higher gray matter fractions (V_G/V_T) than primate species of similar folding index (A_T/A_E), just like carnivoran species have higher gray matter fractions than primate species for the same cortical thickness (Fig. 2*D*). (*B*) Adjusting V_G/V_T according to T improves the fit ($r^2 = 0.634$), but still leaves many outliers (compare with Fig. 5*B*, the truly universal relationship).

individuals for healthy human cortices is statistically indistinguishable from 1.25 once subjects are separated by age (17); across different age groups, there is a small but systematic decrease in the value of k with aging, possibly due to age-related changes in axonal mechanical plasticity. This suggests that the discrepancy between theoretical and empirical exponents in our comparative neuroanatomy data may be due to a "smearing" of the data along the direction of age-related changes in the morphological variables, as our dataset is not controlled by (species-equivalent) age. This discrepancy is, however, enough to make de Lussanet's equation (16) not universal: As shown in Fig. 8, most carnivorans have larger gray matter fractions than primates and marsupials for the same folding index, just like carnivorans have a larger V_G/V_T than these other species for the same cortical thickness (Fig. 2*D*). In contrast, we find empirically that the relationship $(V_G/V_T)\cdot T^{-1/2} = k\cdot A_E{}^a$, which we predict from our cortical folding model, is indeed universal (Fig. 5*B*).

Another main implication of our finding for brain evolution is that the enormous diversity of cortical morphology (in terms of variation in neuronal density, surface area, volume, thickness, and degree of cortical folding) can be quantitatively determined by a small handful of variables: N, the average volume of individual neuronal cells v_N (which together define V_G), and whatever defines how V_G is spread laterally into a given combination of A_T and T. From these 4 variables, A_E is defined (Fig. 7), and with that come folding index and V_T, and therefore V_W. In this context, it is noteworthy that V_G/V_T is not related to the number of cortical neurons by any single function across all species in our dataset, with primates as obvious outliers, which makes it unlikely that an alternative folding mechanism can be obtained directly from the mechanics of neuronal proliferation, that is, directly from numbers of neurons alone without taking into consideration the factors that contribute to cortical geometry. There is therefore no generalizable interplay between the numbers of neurons that build a cortical network and functionally relevant parameters of the network, such as average fiber length and thus signal propagation time, that do not depend on the particular combination of A_T and T that apply to each clade. In other words, there are as many possible ways to assemble a cortex as there are recognizable clades, which makes it unlikely that any particular one is more or less optimal or adapted than the others. It is thus plausible that most of the quantitative variation in cortical morphology encountered in evolution has resulted from natural variation in a restricted number of genes that regulate just 4 variables: the number of neurons in the cerebral cortex; their average size (that is, their cellular volume in the gray matter, including dendritic and axonal arbors, which together with the number of neurons defines the volume of the gray matter); and the combination of surface area and thickness into which the growing cortical volume is spread. Other variables of course must act to define internal, spatial aspects of the cortical morphology, such as local cortical thickness, the distribution of neurons into thicker or thinner layers, the precise placement of functional areas and of the spatial pattern of sulci and gyri, and the precise connectivity of white matter tracts that are characteristic of primates, artiodactyls, or carnivores, for instance. We are currently working on the formalization of this quantitative model of cortical morphology.

Materials and Methods

We compiled our own published data on numbers of neurons, total cortical surface area, exposed cortical surface area, volume of the cortical gray matter, volume of the subcortical white matter (external to the striatum), average thickness of the cortical gray matter, and folding index for 1 single cerebral cortical hemisphere for 24 species: 10 primates (18–20), 1 scandentia (18), 5 artiodactyls (21), 3 afrotherians (22, 23), and 5 rodents (8). We also collected morphometrical data from cerebral cortices of 6 marsupials and 8 carnivoran species, for which cellular composition was reported in refs. 12 and 13. In our own dataset, all data (including numbers of cortical neurons) were thus available for 38 species. Additionally, we obtained the original data of 57 of the 59 species analyzed by Zhang and Sejnowski (4). Numbers of neurons are not available for that dataset. Numbers of neurons in our dataset were obtained using the isotropic fractionator (24), which has been shown to yield estimates that are similar to those obtained with stereology (25–27). All morphological measurements were obtained as described in detail in ref. 21.

ACKNOWLEDGMENTS. We thank all our collaborators who participated in previous studies to collect the data shown here. This work was supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Conselho Nacional de Desenvolvimento Científico e Tecnológico grants and a James McDonnell Award to S.H.-H.; a FAPERJ stipend and crowdfunding support to S.E.D.S.; and a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior stipend to D.J.-M.

1. M. A. Hofman, Size and shape of the cerebral cortex in mammals. I. The cortical surface. *Brain Behav. Evol.* **27**, 28–40 (1985).
2. Q. Wen, D. B. Chklovskii, Segregation of the brain into gray and white matter: A design minimizing conduction delays. *PLoS Comput. Biol.* **1**, e78 (2005).
3. S. Olkowicz *et al*., Birds have primate-like numbers of neurons in the forebrain. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 7255–7260 (2016).
4. K. Zhang, T. J. Sejnowski, A universal scaling law between gray matter and white matter of cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 5621–5626 (2000).
5. D. B. Chklovskii, T. Schikorski, C. F. Stevens, Wiring optimization in cortical circuits. *Neuron* **34**, 341–347 (2002).
6. J. Protherom, Cortical scaling in mammals: A repeating units model. *J. Hirnforsch.* **38**, 195–207 (1997).
7. B. Mota, S. Herculano-Houzel, BRAIN STRUCTURE. Cortical folding scales universally with surface area and thickness, not number of neurons. *Science* **349**, 74–77 (2015).
8. L. Ventura-Antunes, B. Mota, S. Herculano-Houzel, Different scaling of white matter volume, cortical connectivity, and gyrification across rodent and primate brains. *Front. Neuroanat.* **7**, 3 (2013).
9. S. Herculano-Houzel, B. Mota, P. Wong, J. H. Kaas, Connectivity-driven white matter scaling and folding in primate cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19008–19013 (2010).
10. B. Mota, S. Herculano-Houzel, How the cortex gets its folds: An inside-out, connectivity-driven model for the scaling of mammalian cortical folding. *Front. Neuroanat.* **6**, 3 (2012).
11. D. Van Essen, A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature* **385**, 313–318 (1997).
12. S. E. Dos Santos *et al*., Cellular scaling rules for the brains of marsupials: Not as "primitive" as expected. *Brain Behav. Evol.* **89**, 48–63 (2017).
13. D. Jardim-Messeder *et al*., Dogs have the most neurons though not the largest brain: Trade-off between body mass and number of neurons in the cerebral cortex of large carnivoran species. *Front. Neuroanat.* **11**, 118 (2017).
14. S. Herculano-Houzel, P. R. Manger, J. H. Kaas, Brain scaling in mammalian evolution as a consequence of concerted and mosaic changes in numbers of neurons and average neuronal cell size. *Front. Neuroanat.* **8**, 77 (2014). Erratum in: *Front. Neuroanat.* **9**, 38 (2015).
15. B. Mota, S. Herculano-Houzel, All brains are made of this: A fundamental building block of brain matter with matching neuronal and glial masses. *Front. Neuroanat.* **8**, 127 (2014).
16. M. H. de Lussanet, Comment on "Cortical folding scales universally with surface area and thickness, not number of neurons." *Science* **351**, 825 (2016).
17. Y. Wang, J. Necus, M. Kaiser, B. Mota, Universality in human cortical folding in health and disease. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 12820–12825 (2016).
18. S. Herculano-Houzel, C. E. Collins, P. Wong, J. H. Kaas, Cellular scaling rules for primate brains. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 3562–3567 (2007).
19. M. Gabi *et al*., Cellular scaling rules for the brains of an extended number of primate species. *Brain Behav. Evol.* **76**, 32–44 (2010).
20. F. A. Azevedo *et al*., Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J. Comp. Neurol.* **513**, 532–541 (2009).
21. R. S. Kazu, J. Maldonado, B. Mota, P. R. Manger, S. Herculano-Houzel, Cellular scaling rules for the brain of Artiodactyla include a highly folded cortex with few neurons. *Front. Neuroanat.* **8**, 128 (2014). Erratum in: *Front. Neuroanat.* **9**, 39 (2015).
22. K. Neves *et al*., Cellular scaling rules for the brain of afrotherians. *Front. Neuroanat.* **8**, 5 (2014).
23. S. Herculano-Houzel *et al*., The elephant brain in numbers. *Front. Neuroanat.* **8**, 46 (2014).
24. S. Herculano-Houzel, R. Lent, Isotropic fractionator: A simple, rapid method for the quantification of total cell and neuron numbers in the brain. *J. Neurosci.* **25**, 2518–2521 (2005).
25. J. Bahney, C. S. von Bartheld, Validation of the isotropic fractionator: Comparison with unbiased stereology and DNA extraction for quantification of glial cells. *J. Neurosci. Methods* **222**, 165–174 (2014).
26. D. J. Miller, P. Balaram, N. A. Young, J. H. Kaas, Three counting methods agree on cell and neuron number in chimpanzee primary visual cortex. *Front. Neuroanat.* **8**, 36 (2014).
27. S. Herculano-Houzel, C. S. von Bartheld, D. J. Miller, J. H. Kaas, How to count cells: The advantages and disadvantages of the isotropic fractionator compared with stereology. *Cell Tissue Res.* **360**, 29–42 (2015).