

TECHNICAL RESPONSE

BRAIN STRUCTURE

Response to Comments on “Cortical folding scales universally with surface area and thickness, not number of neurons”

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De Lussanet claims that our model that accounts for the degree of folding of the cerebral cortex based on the product of cortical surface area and the square root of cortical thickness is better reduced to the product of gray-matter proportion and folding index. Lewitus *et al.*, in turn, claim that the assumptions of our model are in conflict with experimental data; that the model does not accurately fit the data; and that the ancestral mammalian brain was gyrencephalic. Here, we show that both claims are inappropriate.

In our original Report (1), we showed for the first time that the relationship between total surface area (A_T) and exposed surface area (A_E) of the mammalian cerebral cortex can be universally described, across lissencephalic and gyrencephalic species alike, by the power function $A_T T^{1/2} = k A_E^{1.305 \pm 0.010}$, where T is the average thickness of the cortical gray matter (Fig. 1A). Our model predicts the degree of folding of the mammalian cerebral cortex (that is, the relationship between A_T and A_E) with an r^2 of 0.998; that is, 99.8% of the variance in $A_T T^{1/2}$ across species is explained by the variation in $k A_E^{1.305}$. The empirical function is very close to our theoretical function $A_T T^{1/2} = k A_E^{1.25}$ that expresses the relationship between A_T and A_E , given T , that minimizes the effective free energy of a growing cortical volume—that is, the function that describes the most stable conformation of a growing, elastic, deformable cerebral cortex given its surface area and thickness. We thus propose that folding occurs as the expanding cortex, during development, settles at each moment in time into the most energetically favorable conformation, one that depends simply on its current combination of A_T and T , not number of neurons.

Based on the calculation from our data of values of k for the theoretical exponent of 1.25, de Lussanet notes that the residuals of the function $A_T T^{1/2} = k A_E^{1.25}$, that is, k , vary systematically with increasing A_E across species (2). This indicates a (presumably hidden) significant discrepancy between the exponent for A_E predicted by our effective free energy-minimization theory and the empirical exponent 1.305 (excluding cetaceans) that is found in the data. Our original Report

makes it clear that, with a value of 1.305 ± 0.010 , the exponent for A_E that best fits the data is close to, but statistically distinguishable (given the standard error) from, the theoretical exponent of 1.250, which is not surprising for a mean-field model based on a number of simplifying assumptions.

The origin of the discrepancy of 0.055 in the scaling exponent is an important question. In purely theoretical terms, the use of A_E as an order parameter was a necessary simplification, based on the expected relationship $V_T \sim A_E^{3/2}$ [made explicitly in the supplementary information in (1), and a good empirical approximation, given that we obtain $V_T \sim A_E^{1.52}$]. Whether modifications to correct for Gaussian curvature of the surface and the presence of deep nuclei would suffice to close the gap of 0.055 between theoretical and empirical exponents is an open question.

That our theory-based empirical relationship of exponent 1.305 nevertheless successfully predicts a single relationship that applies universally to both lissencephalic and gyrencephalic cortices can be shown using de Lussanet's own recourse of calculating k and examining for systematic variation. As shown in Fig. 1B, we find that $k = (A_T T^{1/2})/A_E^{1.305}$, calculated for each data point, does not vary significantly with A_E across non-cetacean species [cetaceans are examined in the supplementary information in (1)].

Next, de Lussanet assumes, as we did, that the total cortical volume V_T and exposed area A_E are related as $V_T \sim A_E^{3/2}$ and rearranges the terms in our scaling law to give $\kappa = (A_T/A_E)(V_G/V_T)$. This equation, however, is tautological: It carries exactly the same information as the original relationship in our model, because it is simply a rewriting based on no distinct biological foundation. Moreover, the rewriting is explicitly dependent on an exponent of precisely 1.25. Given that de Lussanet himself shows (and we agree) that the empirical exponent is significantly different from

1.25, it follows that his rearrangement is not mathematically valid. That his proposed “model” with no theoretical basis other than our own fails to fully account for the data by his own standards is shown in Fig. 1C: The constant κ predicted by his formula is not invariant but rather varies systematically with A_E , in contrast to the constant k calculated using our empirical relationship (Fig. 1B). We thus believe that our empirical scaling relationship with an exponent of 1.305 ± 0.010 , which is a very good approximation of the theoretical equation that describes the combination of parameters that minimize the effective free energy of the cortical volume, offers for the first time a useful and robust predictive account of the degree of cortical folding across mammalian species. This is in contrast to the work of Hofman (3), who identified not one universal function between A_T and A_E , but three, which applied separately to lissencephalic species, terrestrial gyrencephalic species, and marine gyrencephalic species.

We used exclusively data on surface area and cortical thickness that had already been published. Contrary to the claim of Lewitus *et al.* (4), all original reports were cited in the text, and those reports explained extensively how A_T , A_E , and T were obtained according to the standards in the neuroanatomy literature: respectively, as the total extent of the pial surface area of the gray matter; the total exposed gyral surface area not included in sulci; and the ratio between total gray-matter volume and total pial surface area. Most important is the fact that, whether traced from images of brain slices or from magnetic resonance imaging data, from tissue fixed by immersion or by perfusion, and regardless of the exact method of measurement, all values converge on the same, single relationship for which 99.8% of the variance in $A_T T^{1/2}$ is explained by the variation in $k A_E^{1.305}$. This goes to show (i) that data collected in these various ways are robust and method-insensitive enough to be comparable across laboratories, and thus really useful, and (ii) that our model indeed applies universally across species and data sets.

Importantly, these are precisely the same data that yield an r^2 of only 0.751 for the relationship between folding index of gyrencephalic species and brain mass, given in our original figure 1A. An r^2 of 0.751 is much smaller than an r^2 of 0.998, and we thus consider the former to be “fairly low,” indeed. Only gyrencephalic species are considered in the analysis of scaling of folding index with brain mass because the folding index, expressed as the ratio A_T/A_E , is not a smooth function of brain mass but a two-tiered trait, because it has a hard lower bound of 1.0 and, by definition, no variation across all lissencephalic species. Including lissencephalic species would only worsen the already poor fit of a power function to the data. Remarkably, our analysis of the relationship between $A_T T^{1/2}$ and A_E carried out for all species, lissencephalic and gyrencephalic alike, yields an r^2 of 0.998 that is even higher than the r^2 of 0.996 obtained for gyrencephalic species alone (1). We thus concluded that the relationship between $A_T T^{1/2}$ and A_E is much stronger than the relationship

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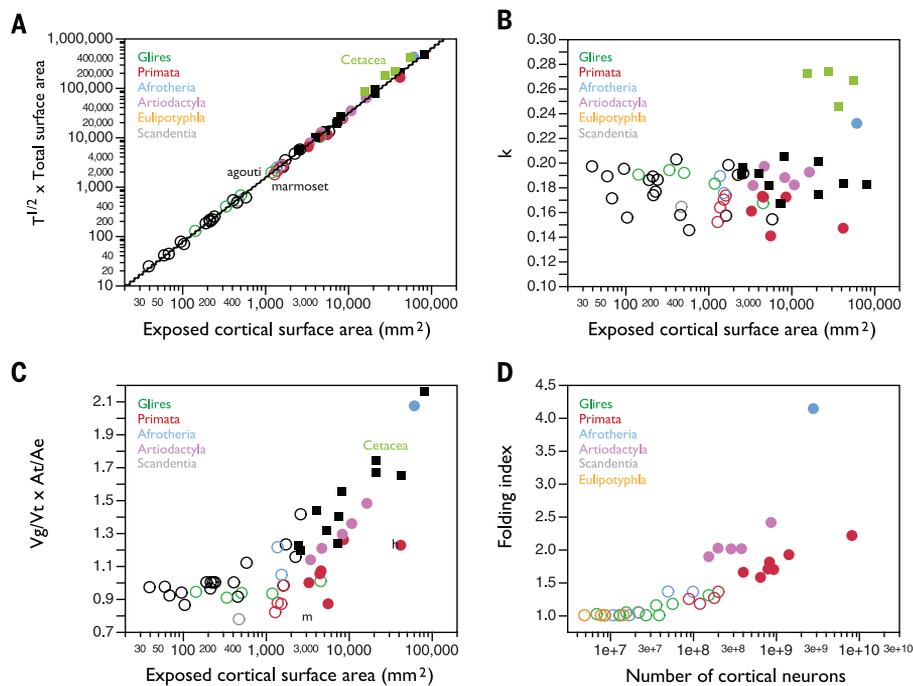


Fig. 1. Universal scaling relationship of exponent 1.305 describes cortical folding across mammalian species. All figures were plotted from data in (1), showing species with folding index <1.5 as open circles and species with folding index >1.5 as solid symbols. (A) $A_T T^{1/2}$ varies with $A_E^{1.305 \pm 0.010}$ ($r^2 = 0.998$, $P < 0.0001$) across lissencephalic and gyrencephalic species (cetaceans were excluded here; inclusion changes the exponent slightly to 1.325 ± 0.009). Marmoset and agouti, which are gyrencephalic species, are indicated. (B) $k = (A_T T^{1/2})/A_E^{1.305}$ calculated for each data point does not vary significantly with A_E across species (Spearman correlation, $P = 0.8883$) nor across species with folding below or above 1.5 (Wilcoxon, $P = 0.5477$). (C) $\kappa = (V_g/V_t) \times (A_T/A_E)$, which de Lussanet predicts to be constant, varies significantly with increasing A_E (Spearman correlation, $\rho = 0.771$, $P < 0.0001$). (D) Folding index is not a single function of the number of cortical neurons (shown for a single hemisphere) and also not simply as two clusters for species with folding index above (solid circles) or below (open circles) 1.5. Rather, species with folding indices above 1.5 show different scaling of folding, with the number of cortical neurons depending on whether they are artiodactyls (pink) or primates (red).

between folding index (A_T/A_E) and any other variable examined.

That “the degree of gyrification is much larger in artiodactyls than in primates for similar numbers of cortical neurons” is shown very obviously in our original figure 1B, reproduced here as Fig. 1D. There is no overlap between primates and artiodactyls. For instance, the cerebral cortex in the pig (an artiodactyl) and baboon (a primate) have the same folding index of 1.9, whereas one cortical hemisphere has only 146 million neurons in the former but 1.6 billion neurons in the latter, a difference of more than one order of magnitude. Statistical analyses are hardly necessary to prove that the two groups do not overlap.

Our assertions that “a better fit is found for [folding index against] total surface area” than against total brain mass and that “the precise relationship between T and A_G across gyrencephalic species differs across orders” [“with a much smaller exponent for primates than for artiodactyls (Fig. 3B, red and pink lines),” our original text continues] are founded on the r^2 values, exponents, standard errors, and 95% confidence intervals provided in the respective figure legends.

The criticisms by Lewitus *et al.* thus require no further comment.

Regarding the so-called “clusters of gyrencephaly,” there are indeed different groups in the relationship between folding index and number of cortical neurons, as we show in our original figure 1B, reproduced here (Fig. 1D), in line with the different, clade-specific relationships between A_T and T , as shown in our original figure 3B (although we don’t call these “clusters”). The two candidate groups to form different “clusters of gyrencephaly,” primates and artiodactyls, are however not the “two mammalian groups” to which Lewitus *et al.* refer in (5). Figure 1D shows that, for similar numbers of cortical neurons, artiodactyls have much larger folding indices than primates. In contrast, Lewitus *et al.* (5), who did not analyze our data on artiodactyls, arbitrarily defined two groups as those with folding index above 1.5 (the larger primates in their sample, including humans) and below 1.5 (rodents and the smallest primates, which includes lissencephalic species). This is a finding that essentially replicated our previous demonstration that the relationship between folding index and number of neurons differs across

primates and rodents (6), without giving credit to it. Lewitus *et al.* (5) went on to propose that this arbitrary threshold of 1.5 corresponds to $\sim 10^9$ neurons. Figure 1D redraws our data identifying species with folding indices above and below 1.5 and illustrates how the “adaptive threshold” of gyrencephaly and the clustering around a threshold of folding index 1.5 proposed by Lewitus *et al.* (5) fail to hold in the face of data. Four of the five artiodactyls in our data set have fewer than 10^9 cortical neurons in both cortical hemispheres but folding indices of 1.9 and higher; cortices with similar numbers of neurons, all well below 10^9 , have much higher folding indices in artiodactyls than in primates; and primates exhibit a continuous relationship between folding index and numbers of cortical neurons well below and well above 10^9 , as we had already shown in (6) and reported again in our original figure 1B. Most importantly, there is no difference in our empirical k between species with folding indices above or below 1.5 (Fig. 1B). Thus, there are no “two clusters of gyrencephaly”: All species, regardless of numbers of cortical neurons, of being lissencephalic or gyrencephalic, and of how gyrencephalic they are, share the same relationship $A_T T^{1/2} = k A_E^{1.305}$ (Fig. 1A).

The statement by Lewitus *et al.* that “there is formidable corroboration for a positive role of the developmental neurogenic program in determining the folding pattern of the adult cortex” is also incorrect, in the face of the data that we presented, here shown in Fig. 1D. More neurons do not necessarily translate into a more folded cortex, nor are they a mandatory requirement. These are fundamental realizations for the study of cortical development and evolutionary scaling (1). Developmental models of cortical expansion and evolution, including that of Lewitus *et al.* (7), can no longer consider that cortical surface area (and folding) grows proportionately across species as a single function of increasing numbers of neurons.

Still, in regard to development, it is in light of the clade-specific relationships between A_T and T that we state that “there is no a priori reason for lissencephaly.” There is no known developmental mechanism that necessarily ties A_T to kT^2 , the necessary condition for lissencephaly, because A_T and T are most likely controlled by different developmental mechanisms. That the relationship between A_T and T that maintains lissencephaly is not obligatory is shown by the very fact that not all species are lissencephalic.

The claim by Lewitus *et al.* that “the most recent common mammalian ancestor was gyrencephalic” (5) is based on unfortunate phylogenetic inferences instead of actual fossils and indeed flies in the face of the ample fossil evidence of lissencephalic ancestral mammals (8–11). We did, however, cite the wrong paper in regard to there being “no secondary lissencephaly”; we should have cited (5) instead of (12). Incidentally, we note that the marmoset, a species cited by Kelava *et al.* (12) as an example of secondary lissencephaly, is actually gyrencephalic, with a folding index of 1.175, similar to the agouti, and both fit perfectly on the universal scaling relationship that we identified (Fig. 1A).

The reason that the inference by Lewitus *et al.* (5) of a common gyrencephalic ancestor to all mammals is unfounded is that it was based on the finding that clades of extant mammalian species have average folding indices larger than 1.36. This is akin to calculating the average body mass of extant mammalian clades, finding that in all clades this average is larger than 1 kg, and thus concluding that the ancestral mammal also had a body mass larger than 1 kg. Actually, it is worse; given that folding indices are by definition never smaller than 1.0, their mathematical analysis could never obtain an average folding index of 1.0 for any clade, given that all of them contain gyrencephalic species. O'Leary *et al.* (13), cited by Lewitus *et al.* (5), used a similarly flawed inference to conclude that the ancestral placental mammal was gyrencephalic.

In the context of the ample fossil evidence of lissencephalic ancestral mammals (8–11) and of our finding that extant lissencephalic and gyrencephalic species alike obey the same relationship (1), $A_T T^{1/2} = k A_E^{1.305}$, and given the small size of those ancestral brains, it would be extraordinary indeed if any of the small ancestral cortices were found to be folded as Lewitus *et al.* suggest. They would stand out as the sole, major outliers to the universal scaling relationship that we identified.

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