

3.12 How to Build a Bigger Brain: Cellular Scaling Rules in Rodent Brains

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Glossary

<u>g0005</u> <i>allometry</i>	Modification in the proportion of the various parts of an organism or structure, like the brain, as it increases in overall size.	<i>neuronal cells</i>	When determined by the isotropic fractionator, 'neuronal' refers to cells whose nuclei express neuronal nuclear antigen (NeuN), detected immunocytochemically. <u>g0030</u>
<u>g0010</u> <i>anisotropic</i>	Any structure whose components – neuronal cells, in the case of the brain – are distributed unevenly in different directions. The brain, with its white matter tracts and gray matter regions organized in laminae and nuclei, is a highly anisotropic structure.	<i>neuronal density</i>	Number of neuronal cells per mg or ml of tissue, measured as the number of neuronal somata or nuclei found per unit volume or mass. <u>g0035</u>
<u>g0015</u> <i>glial density</i>	Number of glial cells per mg or ml of tissue, measured as the number of glial somata or nuclei found per unit volume or mass.	<i>neuronal size</i>	Has often been used in the literature as shorthand for 'size of neuronal somata'. We define it here as the total size of a neuronal cell, including the whole of its dendritic and axonal arborizations and the immediately surrounding extracellular space. <u>g0040</u>
<u>g0020</u> <i>hypermetric growth</i>	Growth of a structure that surpasses the rate of growth of another. cerebral cortex is a typical example of hypermetric growth at the expense of other brain regions.	<i>neuropil</i>	The ensemble of axonal and dendritic arborizations in a brain region. <u>g0045</u>
<u>g0025</u> <i>isometric growth</i>	Growth of a structure at the same rate of growth of another structure.	<i>non-neuronal cells</i>	All the cells in the brain that do not express a neuronal phenotype. This includes glial and endothelial cells. When determined by the isotropic fractionator, 'non-neuronal' refers, by exclusion, to cells whose nuclei do not express NeuN, detected immunocytochemically. <u>g0050</u>

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g0055 *power function*

Any mathematical equation of the form $Y = aX^b$, meaning that Y varies as a power function of X , that is, according to the exponent b of X . When b is larger than 1, Y grows faster than X . If b is a positive value below 1, Y grows slower than X . Negative values of b mean that Y actually decreases as X grows. If b is unity, the function becomes linear, as Y varies directly as X multiplied by a .

g0060 *stereology*

The study of the three-dimensional properties of brain structures, or of any other tissue, through the measurement of microscopic sections of the whole. Usually, stereology involves the measurement of parameters such as cell or synaptic density within a tissue section of known volume, and extrapolation to the volume of the entire tissue.

s0005 3.12.1 Introduction

p0005 Brain size varies by a factor of 100,000 across mammalian species (Count, 1947). Several variables probably contribute to determine adult brain size within and across species: number of neurons, number of glial cells, cell body size, dendritic and axonal arborization volume, vasculature, and extracellular space. Although the cellular composition of the brain is one of the major determinants of its computational capacities (Williams and Herrup, 1988), little is known about how it varies with brain size. What are the cellular scaling rules that determine brain allometry? How do number of neuronal and non-neuronal cells contribute to structure size? What are their relative contributions across species of different brain sizes? Do different brain structures gain neurons and overall mass at the same rate?

s0010 3.12.2 Where Volumetry and Stereology Have Gotten Us

s0015 3.12.2.1 Bigger Animals Have Bigger Brains, and Bigger Brains Have More and More Cortex

p0010 Comparative studies of mammalian brain anatomy have been largely limited to analysis of volumetric data on large brain divisions of different species published by a small number of labs (Stephan *et al.*, 1981; Frahm *et al.*, 1982), often based on measurements of only one brain of each species. These have established that brain size is related to body size by a power law of exponent inferior to 1.0 (Martin, 1981; Fox and Wilczynski, 1986), such that brain size increases with body size, but at a slower pace (Figure 1a). The cerebral cortex increases hypermetrically in volume in relation to

the remaining brain structures (Figure 1b), such that, within each order, the relative size of the cerebral cortex increases with brain size (Figure 1c): larger brains are more and more dominated by cortex (Frahm *et al.*, 1982).

In comparison, larger brains have isometrically larger cerebella, which accompany almost linearly the size of the cerebral cortex (Figure 1d), and retain a stable relative size with increasing brain size: larger brains have cerebella of the same relative volume (Figure 1e).

3.12.2.2 Bigger Brains Have Decreasing Neuronal Density

From visual inspection only, Nissl (1898) observed that neurons are distributed more sparsely in larger brains. Stereological measurements soon confirmed his observation, showing that neuronal density declines in the cerebral cortex as a power function of increasing brain volume with a small, negative exponent of -0.32 (Tower and Elliot, 1952). Further stereological studies showed that the numerical relationship between brain size and neuronal density is valid from the smallest mammalian species, such as insectivores (Stolzenburg *et al.*, 1989) to species with brains much larger than human, such as dolphin, elephant, and whale (Tower, 1954; Garey and Leuba, 1986; Haug, 1987; Figure 2a). Nonstereological measurements have found the same relationship in rodents (Herculano-Houzel *et al.*, 2006a). The direct, negative relation between cortical neuronal density and brain size is an interesting indication that intelligence does not bear a simple relationship to neuron density and thus to the degree of axodendritic complexity in the cerebral cortex (Tower and Elliot, 1952).

In principle, two factors might account for the decreased neuronal density in larger cerebral cortices: increased neuronal size (including the neuropil), and increased relative number of the interspersed glial cells. Both seem to apply: Ghouse Shariff showed in 1953 that smaller neuronal densities are associated with larger neuronal somata in the primate cerebral cortex, a finding that was later replicated in insectivores (Stolzenburg *et al.*, 1989); and it was soon confirmed that larger cortices do have larger and larger relative number of glial cells to each neuron (see 00010).

3.12.2.3 Bigger Brains Have Much More Glia

Friede (1954) defined the glial index of the cerebral cortex: the ratio between number of glial and neuronal cells (g/n ratio), which he believed to

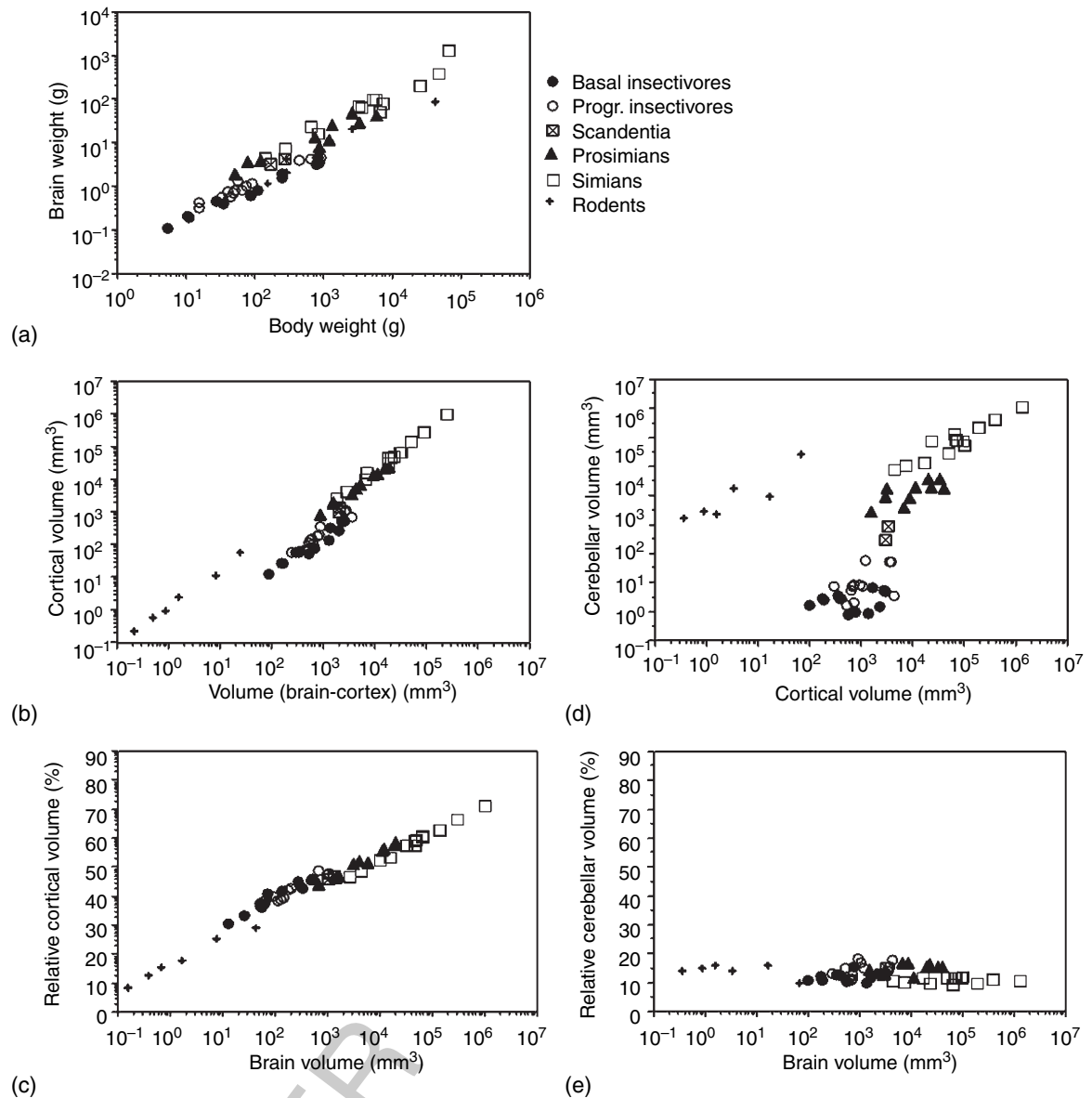


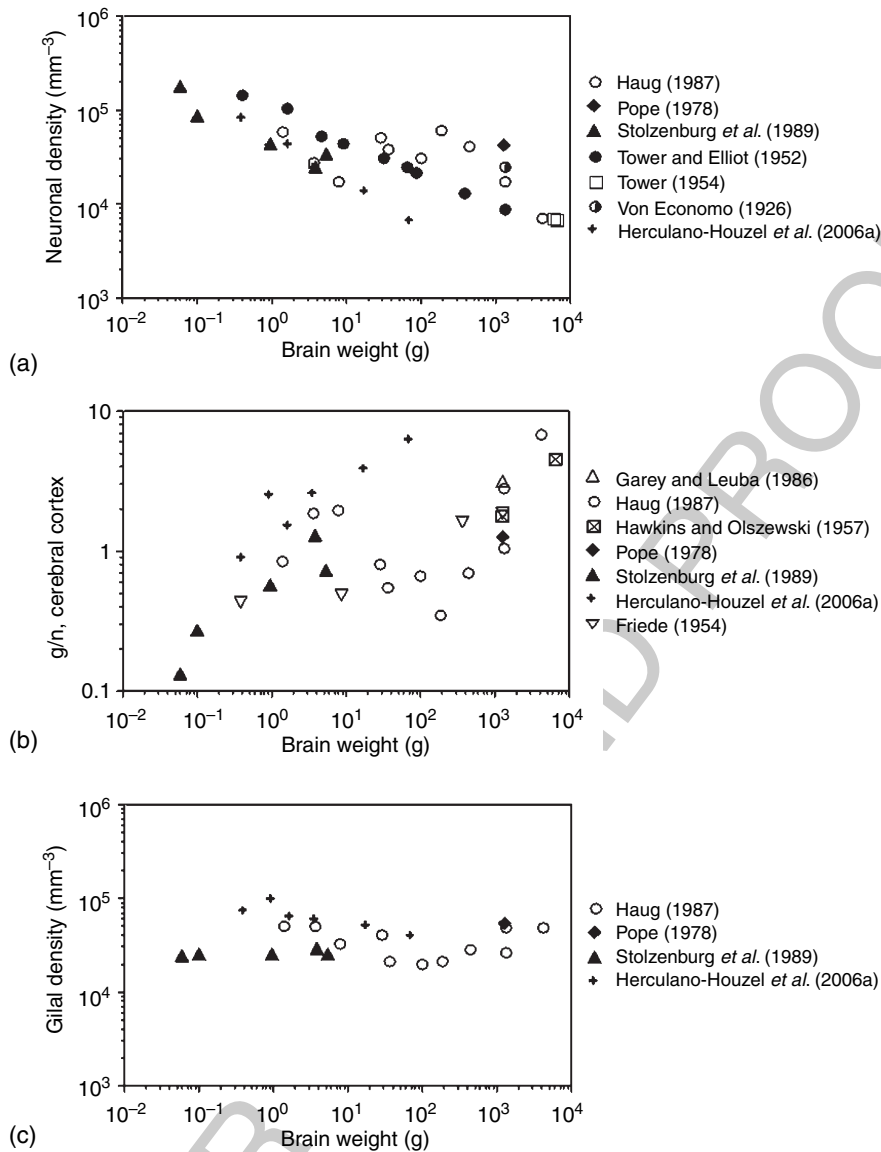
Figure 1 Brain scaling across mammalian species. a, Brain size scales hypometrically with body weight. Power law exponents relating the two parameters vary between 0.60 and 0.80. b, Absolute cortical volume (or mass, for rodents) scales hypermetrically compared with the remaining, noncortical brain structures. Power law exponents relating the two parameters vary between 1.10 and 1.20. c, Relative cortical volume (or mass, for rodents) increases with increasing brain size within each group. d, Absolute cerebellar volume (or mass, for rodents) scales hypometrically compared with cerebral cortex, with power law exponents of 0.8–1.0. e, Relative cerebellar volume (or mass, for rodents) remains invariant among all groups, regardless of increasing brain size. Data from Stephan, H., Frahm, H., and Baron, G. 1981. New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol.* 35, 1–29; Frahm, H. D., Stephan, H., and Stephan, M. 1982. Comparison of brain structure volumes in insectivora and primates. I: Neocortex. *J. Hirnforsch.* 23, 375–389; and Herculano-Houzel, S., Mota, B., and Lent, R. 2006a. Cellular scaling rules for rodent brains. *Proc. Natl. Acad. Sci. USA* (submitted).

vary according to the phylogenetic position of the species, such that an increasing proportion of glia would indicate a more highly developed cortex. It was soon demonstrated, however, that the g/n ratio in the cerebral cortex increases with brain size rather than with an ill-defined phylogenetic scale that placed the human species on top (Figure 2b; see 00022). Fin whales have a cortical

g/n ratio of 4.54, compared to 1.78 in humans (Hawkins and Olszewski, 1957). Stolzenburg *et al.* (1989) later proposed that the g/n ratio actually increases with increasing thickness of the cortical wall rather than with total brain or cortical weight.

In any case, it has been widely believed that the relative glial expansion has trophic and metabolic

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f0010 **Figure 2** a, Scaling of neuronal density; b, g/n ratio; and c, glial density in the cerebral cortex of different species. All graphs were drawn from data reported in the references listed.

meaning, in accordance with the traditional view that these cells have a supportive function for neurons (Friede, 1954; Reichenbach, 1989). However, the numeric expansion of glial cells relative to neurons seems to contradict the observation that the neuronal need for metabolic support remains similar across species (Nedergaard *et al.*, 2003). Hypothetically, this discrepancy might be settled if, as the brain increases in size, a larger number of glial cells were a compensation for their small size relative to increasingly larger neurons. However, data on the relative scaling of neuronal and glial cells in the brain are lacking in the literature.

Interestingly, the increased g/n ratio is not accompanied by any major variation in glial density, which has been reported either to vary widely but independently of brain size (Haug, 1987), to remain stable (Stolzenburg *et al.*, 1989), or to show a marginally significant decrease (Herculano-Houzel *et al.*, 2006a) across mammalian species of increasing brain size. Inspection of data pooled from these sources shows that, compared to neuronal density and given the relatively high variation in experimental data obtained by different authors, glial density can be considered to remain relatively invariant across species of different brain size (Figure 2c).

s0030 **3.12.3 The Need for Cell Counts, and the Trouble with Estimating Total Number of Cells with Stereology**

p0045 Glia are widely said to be the most numerous cell type in the brain (Doetsch, 2003; Nishiyama *et al.*, 2005), and to be $10\text{--}50\times$ more numerous than neurons in humans (Kandel *et al.*, 2000). Evidence for this assertion, however, is scant. Stereology, the only available tool for addressing cell numbers until recently, relies on the determination of cell densities in small sectors of tissue. Total cell numbers can in principle be estimated by multiplying cell density and volume of given brain regions, which is how mouse neocortex was estimated to have about 10 million neurons (Schüz and Palm, 1989), for instance.

p0050 There are, however, several problems with this approach to determining total number of cells in large brain regions or even the whole brain. First, brain tissue is highly anisotropic: neuronal density varies widely across cortical areas and subcortical nuclei, which would have to be sampled separately. This problem is solved in part by techniques with ingenious sampling strategies such as the optical fractionator, which allowed a recent estimate of the total number of neurons in the human cerebral cortex at about 20 billion (Pakkenberg and Gundersen, 1997). However, precise determination of neuronal density in the samples is still a key issue. Neuronal densities in human cerebral cortex have been estimated to be as low as 8750 mm^{-3} (Tower and Elliot, 1952), and as high as $48\,100\text{ mm}^{-3}$ (Shariff, 1953), with intermediate estimates of $24\,500\text{ mm}^{-3}$ (Von Economo, 1926), $25\,000\text{ mm}^{-3}$ (Haug, 1987) and $41\,300\text{ mm}^{-3}$ (Pope, 1978). Even if cortical volume were assumed to remain constant across samples, estimates of total number of neurons in cortex based on these densities would vary about $5\times$.

p0055 Precision of measurements of cortical volume, however, is also an issue: even with the highest estimate of neuronal density found in the literature, Shariff's value of 115.2 cm^3 for one cortical hemisphere would yield a total of 11 billion neurons in both hemispheres, a value that is still too low compared to Pakkenberg and Gundersen's (1997) more recent estimate of about twice that.

p0060 Even if measures of neuronal density and volume could be considered precise, stereological determination of total number of cells in the whole brain would require its parcellation into a prohibitive number of structures of defined volume and homogeneous density. This incompatibility explains why there are data in the literature on densities and total cell numbers in well-defined brain nuclei, but no

attempts to use stereology to determine total number of neurons or non-neuronal cells in the brain of any mammalian species.

3.12.3.1 Why Stereological Estimates of Total Cell Number Yield Invalid Relationships

A further limitation of stereological determinations of total cell numbers is that these will, by definition, depend on the volume of the structure of interest, and therefore variations in number of cells estimated in this way cannot be compared to variations in structure volume across species. Stereological methods, thus, should not be applied to the examination of cellular brain scaling rules across species. Haug (1987) employed neuronal densities to calculate the total number of neurons in the cerebral cortex of various mammals, and reported it to vary as a power function of cortical volume with an exponent of 0.95. However, this is an invalid power law relationship that seems only to reflect variations in volume of the cerebral cortex, and does not reveal any real relationship with the number of cells. This is so because, since neuronal density varies as a very small power of cortical volume, the latter always varies much more than its density. Total number of neurons estimated as the product of density and volume will thus necessarily reflect mostly the variation of structure volume and be very little affected by variations in neuronal density, yielding invalid power law relationships of exponent close to 1, as Haug found. This is in strong contrast to the much larger exponent of 1.760 obtained when total number of cortical neurons are determined independently of cortical volume (Herculano-Houzel *et al.*, 2006; see below).

Similarly, Stevens (2001) has used the product of structure volume and neuronal density to examine how variations in total number of neurons correlate between the lateral geniculate nucleus (LGN) and primary visual area (V1) of several primate species (Stevens, 2001). The estimated exponent of $3/2$, however, mirrors the $3/2$ exponent relating LGN and V1 volumes across the species, since thalamic and V1 neuronal densities vary little compared to their volumes. It is most probable, therefore, that this exponent does not reveal a true relationship between number of neurons in the two structures. The questionable nature of the relationships reported by Haug (1987) and Stevens (2001) can be confirmed by shuffling neuronal density values across the species being compared, which, as expected, does not affect the exponents obtained, as they reflect mostly the variations in structure

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volume. Volume-independent estimates of total number of neurons are required in order to determine how they really relate to brain volume.

s0040 **3.12.3.2 The Problem with the Idea That Volume Indicates Computational Power and the Need of Total Cell Numbers**

p0075 Many authors are interested in allometric rules of brain scaling from a functional point of view, as brain size, or encephalization, has long been accepted as an indicator of computational and cognitive capabilities and even intelligence (Jerison, 1985; Reader and Laland, 2002; Sol *et al.*, 2005). In the absence of data on total number of neurons, different authors have focused their studies on the analysis of published volumetric data, derived mostly from Heinz Stephan's group (Stephan *et al.*, 1981; Frahm *et al.*, 1982). Thus, a number of studies have compared the absolute size of brain regions (Finlay and Darlington, 1995), their proportional size relative to one another (Barton and Harvey, 2000; de Winter and Oxnard, 2001), and relative to the whole brain (Clark *et al.*, 2001). Based on the same data, these authors have proposed, respectively, that different brain regions evolve concertedly, in mosaic, or even as scalable versions of a same set of proportions, called cerebrotypes, within a given taxon but not among them.

p0080 Strikingly, conclusions drawn from the same data can be conflicting. For instance, while the neocortical fraction of the brain increases from 14% in basal insectivores to 80% in humans (Frahm *et al.*, 1982), the cerebellar fraction of brain volume varies little across species of various mammalian orders, a discrepancy taken to argue against the hypothesis that the cerebellum works in service of the neocortex (Clark *et al.*, 2001). However, cerebellar and cerebral cortices increase concertedly both in surface area (Sultan, 2002) and in volume (Barton, 2002). Given that these parameters are adopted by most authors to indicate computational capacity, this evidence has been taken to suggest a functional dependence of one structure upon the other. A conciliatory view holds that cerebellum and neocortex evolved together, but with the cerebellum evolving more slowly than neocortex (Barton, 2002).

p0085 These conflicting interpretations demonstrate that cortical volume and surface, although informative measurements and widely used in the literature, particularly in relation to intelligence, cognitive abilities, and versatility, are only indirect indicators of computational capacity. As both cerebral (Douglas and Martin, 2004) and cerebellar (Leiner *et al.*, 1991) cortices have modular structures, their computational capacity can be related more directly

to the number of modules in each structure, and thus to the number of neurons in each structure, independently of total cortical surface or volume, characteristics that are affected by other variables such as non-neuronal volume. Thus, one way of clarifying the issue of how cerebral and cerebellar cortices are structurally, functionally, and evolutionarily related might be through the comparative analysis of the number of neurons in these structures.

s0045 **3.12.3.3 A Nonstereological Way Out: The Isotropic Fractionator**

The isotropic fractionator is a novel method developed recently in our lab which allows the nonstereological determination of the absolute number of neuronal and non-neuronal cells in different brain regions (Herculano-Houzel and Lent, 2005). It consists in transforming highly anisotropic brain structures into homogeneous, isotropic suspensions of fixed cell nuclei which can then be counted and identified immunocytochemically as neuronal or non-neuronal. The method can be applied either to the brain as a whole or to its dissected parts, such as cerebral cortex or cerebellum, and their respective number of cells can next be added up in order to obtain a whole-brain estimate. Estimates of total cell, neuronal and non-neuronal numbers in any brain structure can be obtained in 24 h, and vary by less than 10% among animals. Since the estimates obtained are independent of brain volume, they can be used in comparative studies of brain volume variation among species and in studies of phylogenesis, development, adult neurogenesis, and pathology. We have used the isotropic fractionator to compare the cellular composition of cerebral cortex, cerebellum, and remaining areas of the adult brain of six species of the order *Rodentia*, from mouse to the giant Amazonian capybara (Herculano-Houzel *et al.*, 2006a), and are currently expanding this analysis to primate species (Herculano-Houzel *et al.*, 2006b).

s0050 **3.12.4 How to Build a Bigger Brain**

Across the six rodent species examined, body mass varies over 1000-fold, from about 40 g in mouse to over 40 kg in capybara, while brain mass varies by less than 200 \times , accompanied by a smaller increase of 45 \times in total number of cells, an even smaller 23 \times increase in total number of neurons but a relatively large 86 \times increase in total number of non-neuronal cells (Figure 3). All data mentioned henceforth







	Species	Body mass	Brain mass	#Cells	#Neurons	#Non-neuronal
	Mouse	40 g	0.416 g	109 M	71 M	38 M
	Hamster	168 g	1.020 g	166 M	90 M	76 M
	Rat	315 g	1.802 g	331 M	200 M	131 M
	Guinea pig	311 g	3.759 g	478 M	240 M	238 M
	Agouti	2843 g	18.365 g	1941 M	857 M	1084 M
	Capybara	47500 g	76.036 g	4870 M	1600 M	3270 M
	Variation	1188×	183×	45×	23×	86×

Figure 3 Total number of neuronal and non-neuronal cells in the brain of six rodent species. Average body mass, brain mass (in grams), total number of cells, neurons and non-neuronal cells (in millions) in the brain of six rodent species, shown in the same scale. Relative variations in each parameter from mouse to capybara are listed in the bottom.

regarding the cellular composition of rodent brains were reported in [Herculano-Houzel *et al.* \(2006a\)](#).

3.12.4.1 Bigger Brains Have a Constant Relative Number of Neurons in Cortex

As reported previously for other mammalian orders, relative size of the cerebral cortex increases significantly with brain size among rodent species ([Frahm *et al.*, 1982](#); Figure 4a). Interestingly, this expansion in size is not reflected in the distribution of total brain neurons. Regardless of total brain size, cerebral cortex in all six species contains a relatively stable 18% of all brain neurons. This is in contrast to the distribution of total brain non-neuronal cells, which become relatively more numerous in the cortex as it expands in larger-brained species (Figure 4a). The long-acknowledged cortical expansion in bigger brains, thus, at least in the order Rodentia, does 'not' reflect any increasing allocation of brain neurons to the cortex, but only of non-neuronal cells, presumably glia.

3.12.4.2 Bigger Brains Have Relatively More Neurons in Cerebellum

The cerebellum represents a steady 14% of brain mass across rodent species, as reported for other mammalian orders ([Clark *et al.*, 2001](#)). Remarkably, its fraction of total brain neurons is 'not' stable: larger cerebella concentrate more and more of all brain neurons, from 59% in mouse to

72% in capybara (Figure 4b) but, in contrast to the cerebral cortex, they concentrate a steady fraction of all brain non-neuronal cells.

The increase in the relative number of cerebellar neurons can be explained through the addition of neurons to the cerebellum as a power function of the number of cortical neurons with an exponent >1.0 , estimated at 1.113. The total number of neurons actually increases faster in the cerebellum than in the remaining of the brain, with an exponent of 1.181. Contrary to all expectations from volumetric data, the fact that the cerebellum gains neurons at a faster rate than all other brain structures, cerebral cortex included, and concentrates increasing fractions of all brain neurons shows that the total number of functional integrative units – neurons – increases faster in cerebellum than in cortex. This finding calls into question the validity of using surface and volume measurements as indicators of computational capacity. Given the modular structure of both cerebral and cerebellar cortices, the addition of neurons – and therefore supposedly of more modules – increases the computational capabilities of both networks, and therefore their total number of neurons should be a far more direct indicator of functional capacity than structure volume and surface, which are inflated by non-neuronal cells and connecting fibers of larger caliber. Thus, it has to be concluded that despite the volumetric expansion of the cerebral cortex as brain size increases within the order Rodentia, its

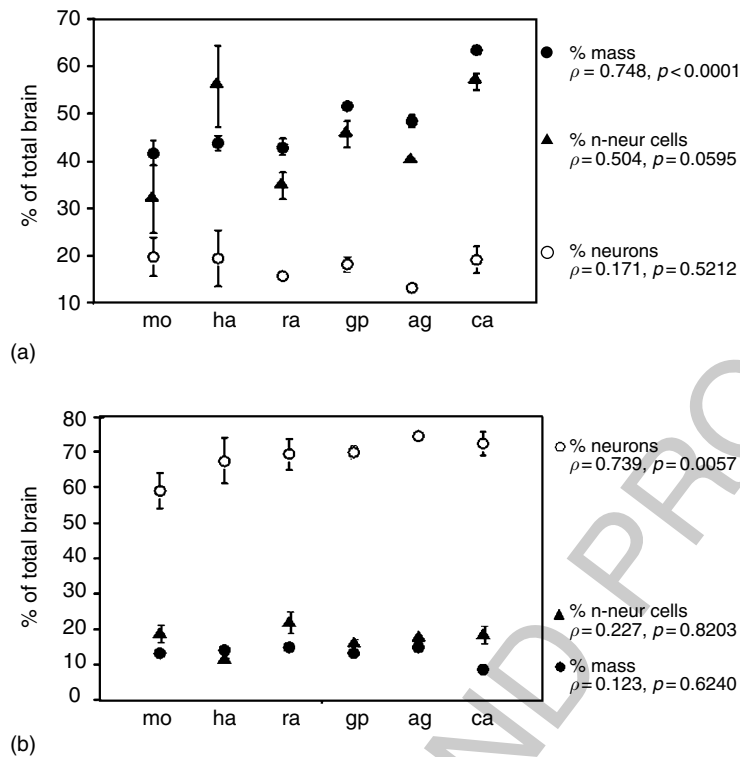


Figure 4 Cerebral cortex expands in relative volume, but retains stable relative number of neurons, while larger cerebella hold increasing relative number of neurons in an unchanging relative volume. Points represent relative distribution of mass, number of neurons, and number of non-neuronal cells in the cerebral cortex (a), and cerebellum (b) of six rodent species (mouse (mo), hamster (ha), rat (ra), guinea pig (gp), agouti (ag), and capybara (ca)). Each point corresponds to one individual animal. Correlation coefficients and p -values for Spearman rank correlations are given.

computational capacities may remain stable, while those of the cerebellum may actually increase. Interestingly, this does not seem to correlate with the motor abilities of the species, as, for instance, agoutis, which manipulate food with their front paws, have a richer motor repertoire than the much larger capybara, endowed with a larger relative number of cerebellar neurons. However, this may just come to show that, as noted recently (Leiner *et al.*, 1991), the cerebellum is much more than a merely motor structure.

3.12.5 Cellular Scaling Rules for Rodent Brains

As shown in Figure 3, larger rodent brains contain increased number of neurons, as expected, and this is true for cerebral cortex, cerebellum, and the remaining areas separately. In striking contrast to Haug's (1987) estimate based on neuronal densities and volume (see above), the results obtained non-stereologically with the isotropic fractionator show that the power law relating cortical mass to its number of neurons has a large exponent of 1.760.

This means that a $10\times$ larger rodent cortex would have only $3.6\times$ more neurons, or that a $10\times$ increase in the number of cortical neurons would result in a $58\times$ larger cortex. Other factors, such as increased neuronal size and increased non-neuronal mass, must therefore also contribute significantly to cortical expansion.

3.12.5.1 More Neurons, Many More Non-Neuronal Cells

Direct estimation of number of cells with the isotropic fractionator shows that larger brains are built with more neurons, but with even larger number of non-neuronal cells. The latter are related to the total number of neurons by a power law with exponent > 1 (1.554 in cerebral cortex, 1.254 in cerebellum). This proportional expansion of the non-neuronal cell population results in the increasing g/n ratio observed both in cerebral cortex (Figure 2c) and cerebellum as these structures become larger.

Interestingly, the mass of all the structures examined increase as power laws of their respective number of non-neuronal cells with similar exponents of close to 1.0, and non-neuronal cell

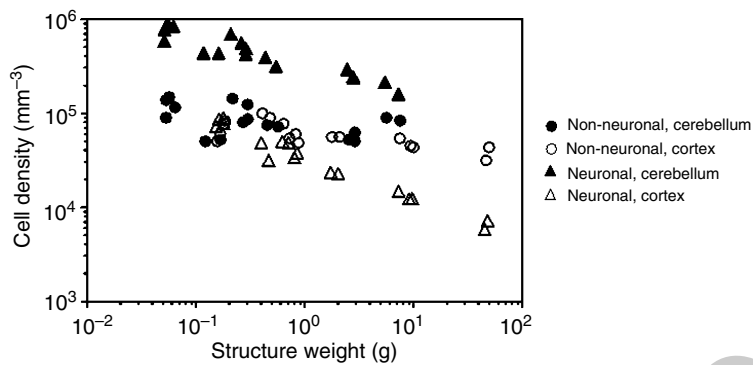


Figure 5 Cerebral cortex and cerebellum have different neuronal densities across rodent species, but similar non-neuronal cell densities. Neuronal (triangles) and non-neuronal (circles) densities are plotted on the y-axis against weight of the respective structure, cerebellum (filled symbols), and cerebral cortex (open symbols).

densities are strikingly similar both across brain structures and species. In fact, although across all species the cerebellar g/n ratio is much smaller than the cortical ratio, these structures share similar non-neuronal densities (Figure 5).

3.12.5.2 Neurons Become Larger As They Are Added; Glia Do Not

The different scaling of neuronal and non-neuronal cell densities with brain size suggests that these two cell types vary differently in average size: as they become more numerous, neurons must increase in size faster than non-neuronal cells.

Indeed, mathematical analysis of all the power laws obtained experimentally suggests that average neuronal size increases proportionally to the total number of neurons raised to the power of 0.760 in cerebral cortex and 0.370 in cerebellum. In comparison, the estimated variation in average non-neuronal cell size among rodents is much smaller: it follows a power law function of total number of non-neuronal cells with very small exponents of 0.114 in cerebral cortex, and 0.063 in cerebellum. This means that if the number of cortical neurons is multiplied by 100, their average size increases 33.1 \times , while a similar increase in the number of cerebellar neurons is accompanied by an increase in average neuronal size of only 5.5 \times . In contrast, 100 \times more numerous non-neuronal cells in the cerebral cortex will be on average only 1.7 \times larger, and if they become 100 \times more numerous in the cerebellum, their average size will increase only 1.3 \times .

We find that capybaras have 22 \times more cortical neurons than mice, and the average neuronal size is estimated to be 10 \times larger. The larger rodent has even more cerebellar neurons than the mouse (a 27 \times increase), but the average neuronal size is estimated to be only 3.4 \times larger. At the same time, capybaras

have 153 \times and 82 \times more non-neuronal cells than mice in the cerebral cortex and cerebellum, respectively, but these cells are estimated to be only 1.8 \times and 1.3 \times larger.

Since increasing number of neurons result in larger brain size, it is expected that these neurons increase in size, with longer, highly branched processes to maintain long-distance connectivity as brain size increases, as well as larger somata to support them. However, increases in average neuronal size are observed to contribute ‘less’ to final structure size than changes in total number of neurons, and the ‘larger’ the increase in this number between two species, the ‘smaller’ the relative contribution of average neuronal size to final structure size. This is consistent with a strong selective pressure against increased neuronal size, lest the brain becomes too large too fast as it gains neurons (Harrison *et al.*, 2002). The faster increase in cortical neuronal size compared to cerebellum is in good agreement with the known architecture of cerebral and cerebellar cortices, the former composed of relatively large number of neurons with large cell bodies and extensively arborized processes that span long distances (Douglas and Martin, 2004), the latter composed mostly of much smaller neurons with a single, long, and comparatively local arborization (Leiner *et al.*, 1991).

In contrast, increasing number of non-neuronal cells are added to the brain in the absence of large changes in average non-neuronal cell size. Compared to neurons, glial cells act locally, so it is reasonable to expect that, as the brain grows and glial cells are added in large numbers, they retain a volume that is small enough to perform local functions of regulating the microcirculation and synaptic transmission (Nedergaard *et al.*, 2003).

It will be interesting to see whether data on neuronal and non-neuronal cell size will match our estimates as they become available by direct

measurement. According to our estimates, a rodent brain with a human-sized cerebellum would be expected to have $c. 900\times$ more cerebellar non-neuronal cells that are on average only $1.5\times$ larger than in mouse cerebellum. Recent measurements of human astrocytes have shown that they are only $3\times$ larger than mouse astrocytes (Oberheim *et al.*, 2005), which seems to be good evidence that non-neuronal cell size indeed changes very little with cell number. Similarly, Purkinje cells are $50\times$ more numerous in human (Andersen *et al.*, 1992) than in rat cerebellum (Korbo *et al.*, 1993), and would therefore be expected to be $4.2\times$ larger in the former, according to our estimates; in the literature, these cells have been found to have a $2.5\times$ bigger perykaryon (Korbo and Andersen, 1995), which falls close enough to the expected value, given that the dendritic and axonal arborizations were not considered in that study.

s0080 **3.12.5.3 Glial Mass to Match Neuronal Mass: Something Remains Constant**

p0160 In the midst of all the power laws that relate changing number of cells, their average volumes, and the resulting size of brain structures, it is interesting to find that one parameter remains constant: the ratio between ‘total’ neuronal and ‘total’ non-neuronal ‘masses’ in a given brain structure, that is, the ratio between the product of total number of neurons and their average mass, and the product of total number of non-neuronal cells and their average mass in a structure. This follows mathematically from the relationships between number of neuronal and non-neuronal cells and their relationships with structure size, and occurs simultaneously with the increased ratio between number of non-neuronal and neuronal cells (the g/n ratio) with larger brain size.

p0165 The constant total g/n mass ratio is achieved as the increased neuronal mass, resulting from larger number of neurons that increase significantly in size, is matched by the addition of much larger number of non-neuronal cells of only slightly larger size. In this way, a $2\times$ increase in total neuronal mass is accompanied by an equal $2\times$ increase in total non-neuronal mass, and yields a $2\times$ increase in brain size. The overall mass constraint suggested by our data is compatible with the recent notion that glial cells serve as dynamic regulators of neuronal production, function and phenotype, and organize brain tissue into functional compartments (Nedergaard *et al.*, 2003). On the other hand, an increase in number of glial units would favor a growing participation of these cells in neural computation, as has been proposed recently (Allen and

Barres, 2005; Volterra and Meldolesi, 2005), without compromising their role in regulatory and support functions. The constant neuronal/non-neuronal mass ratio also settles the apparent discrepancy between the numeric expansion of glial cells compared to neurons, while the neuronal need for metabolic support remains similar across species (Nedergaard *et al.*, 2003).

We have proposed this constant balance between total neuronal and non-neuronal mass in the brain to be a major mechanism driving changes in brain size. The constant total g/n mass ratio could be achieved economically if gliogenesis were regulated according to the number of neurons generated in each structure. This would take place during the development of each individual, as the increased neuronal proliferation that has been proposed to drive cortical growth across species (Rakic, 1995) is followed by gliogenesis, which is largely postnatal (Sauvageot and Stiles, 2002). Glial precursor proliferation is density-dependent and ceases once a steady-state glial density has been achieved, most likely by cell-cell contact inhibition (Zhang and Miller, 1996). Given the relatively invariant non-neuronal densities observed both across brain structures and species, we have suggested that continued gliogenesis until confluency is reached in a formerly purely neuronal tissue, such as newborn cerebral cortex, is a likely candidate mechanism by which the number of neuronal and non-neuronal cells are related and by which the ratio between total neuronal and non-neuronal mass could be kept constant across species.

3.12.6 Conclusions

Comparative analysis of the cellular composition of the mammalian brain is starting to confirm some trends expected from volumetric and stereological studies, and to reveal novel principles of brain scaling. Our studies with the isotropic fractionator have confirmed that neuronal density decreases with increasing size of cerebral cortex and cerebellum while non-neuronal cell density remains relatively stable; further, the g/n ratio in these structures increases with structure size. Additionally, these studies have revealed that:

1. variations in total number of neurons contribute more than variations in neuronal size towards final structure size;
2. average non-neuronal cell size changes very little across brains of different sizes;
3. the number of non-neuronal cells seems to be regulated according to the number of neurons in

the structure such that, as a result, total g/n mass ratio remains constant within a structure as its size varies; and

4. unexpectedly, the cerebellum gains neurons at a faster rate than the cerebral cortex, and concentrates increasing fractions of all brain neurons as brain size increases, despite the volumetric expansion of the cerebral cortex.

p0180 We have suggested that this latter finding is a consequence of a greater increase in average neuronal size in the cerebral cortex than in the cerebellum, matched by a corresponding increase in total non-neuronal mass. Although the cerebellum gains neurons faster than cerebral cortex, the average size of its neurons increases much more slowly, and once the non-neuronal population expands to match the total neuronal mass in these structures, the result is a much inflated cerebral cortex that still holds the same number of neurons relative to the whole brain. In this manner, volumetric expansion is dissociated from expansion of the neuronal population. This latter finding has important implications for the functional relationship and computational capacity of these structures, as discussed above.

p0185 It is important to realize that the current view of encephalization and neocorticalization as adaptive and selected traits in evolution (Jerison, 1985) are based on volumetric relationships that do not hold at the cellular level of brain composition, and therefore may not be reliable indicators of function. The very concept of encephalization carries the built-in assumption that brain size is indeed a measure of computational capacity as it puts forward the notion that a larger than expected brain size endows species with better cognitive capabilities. However, our data indicate that, at least in rodents, neocorticalization is only apparent; when it comes to number of neurons, it is the cerebellum that becomes expanded in larger brains.

p0190 It is interesting to wonder how the view of neocorticalization would be different today if studies of cellular scaling rules had been available earlier: given the increasing concentration of neurons in the cerebellum, perhaps we would deal today with concepts of cerebellarization of the brain, instead of neocorticalization as traditionally considered. Indeed, in light of the recent discoveries on the functional contribution of glial cells to intercellular signaling, in concert with neurons (Allen and Barres, 2005; Volterra and Meldolesi, 2005), there is actually the novel possibility that it is the increasing numerical predominance of glial cells in the cerebral cortex that accounts for the expected increase in computational power of larger brains.

Further Reading

Kaas, J. H. 2000. Why is brain size so important: Design problems and solutions as neocortex gets better or smaller. *Brain Mind* 1, 7–23. [b9025](#)

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