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REVIEW
How many neurons do you have? Some dogmas of quantitative neuroscience under revision

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Abstract

Owing to methodological shortcomings and a certain conservatism that consolidates wrong assumptions in the literature, some dogmas have become established and reproduced in papers and textbooks, derived from quantitative features of the brain. The first dogma states that the cerebral cortex is the pinnacle of brain evolution – based on the observations that its volume is greater in more 'intelligent' species, and that cortical surface area grows more than any other brain region, to reach the largest proportion in higher primates and humans. The second dogma claims that the human brain contains 100 billion neurons, plus 10-fold more glial cells. These round numbers have become widely adopted, although data provided by different authors have led to a broad range of 75–125 billion neurons in the whole brain. The third dogma derives from the second, and states that our brain is structurally special, an outlier as compared with other primates. Being so large and convoluted, it is a special construct of nature, unrelated to evolutionary scaling. Finally, the fourth dogma appeared as a tentative explanation for the considerable growth of the brain throughout development and evolution – being modular in structure, the brain (and particularly the cerebral cortex) grows by tangential addition of modules that are uniform in neuronal composition. In this review, we sought to examine and challenge these four dogmas, and propose other interpretations or simply their replacement with alternative views.

Introduction: Four dogmas of quantitative neuroscience

Quantitative neuroscience should not, perhaps, be properly defined as a discipline, within the broad field of the neural sciences, as the whole field employs quantitative methods to investigate the morphology, physiology, cell biology and molecular interactions in the nervous system. It should rather be considered as an important, normative collection of quantitative methods and data that can be used by neuroscientists to approach evolutionary and developmental issues, and to ask new questions about these and other aspects of brain structure and function.

However, over time, because of methodological limitations (Box 1) and strong adherence to tradition, some beliefs derived from quantitative features of the nervous system have been extensively reproduced in papers and textbooks, becoming undisputed dogmas of neuroscience. For instance, if asked how many neurons the human brain has, the reader will probably answer – 100 billion. However, this figure is not soundly supported by empirical evidence, and its origin in the literature is unknown.

In this review, we analyze and question four dogmas of quantitative neuroscience, and propose new interpretations or alternative views. These dogmas were chosen and brought together because they have a common relationship with the evolution and development of the nervous system. They are probably not the only ones, but are perhaps those most commonly quoted in the literature.

The first dogma states that the cerebral cortex is the highest achievement of brain evolution, on the basis of the observations that brain volume is greater in more 'intelligent' species, and that cortical surface area seems to grow more than any other brain region, to reach the largest proportion in higher primates and humans. According to this view, the traditional concept of evolutionary encephalization could actually be translated as 'corticalization', to express the volumetric predominance of the cerebral cortex as the brain has evolved in vertebrates. The second dogma targets the human brain and its computational 'units' – our brain would contain 100 billion neurons, plus about 10 times more glial cells. These round, 'magic' numbers have become largely adopted in the literature, despite the fact that estimates by different authors vary as much as 10-fold in the cortex and 1.5-fold in the cerebellum, leading to a broad discrepancy range of 75–125 billion neurons in the whole brain (a 50% range around the adopted number). As a sort of corollary, the third dogma states that the human brain is structurally and functionally special, and exquisitely sophisticated as compared with those of other primates. Its large and highly convoluted structure would give it the status of a unique construct of evolution, somehow unrelated to comparative scaling. Such a concept places the human brain apart from mammalian evolutionary rules, in contradiction to most, if not all, empirical evidence. Finally, the fourth dogma connects evolution with

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development to explain the considerable growth of the brain along both ontogenetic and phylogenetic timelines – the existence of structural modules in the brain (and particularly in the cerebral cortex) allows size increases simply by the addition of modules that would be uniform in neuronal composition.

Box 1. Simple methods, complex science

Most of the mentioned dogmas derive from gross volume and/or weight measurements of the brain or its parts, and from regional cell counts in brain sections. Gross measurements, however, albeit simple and straightforward, tell us very little about the functional abilities of brains. This led to measurements designed to quantify the computational 'units' of the nervous tissue – cells, especially neurons – and relate them to size, function, and other parameters.

To count cells in the brain, however, is not a simple task, owing to the pronounced anisotropy of the nervous tissue. In a typical brain sector (Fig. 1A), cell density can vary from highly populated, cell-packed layers, to neuropil and white matter sectors, which are fiber-rich and cell-scarce. The main methods used to estimate cell numbers are stereological techniques (reviewed by Schmitz & Hof, 2005) – cell densities are quantified in sample sectors, and the average is multiplied by the measured volume or mass of the region or of the whole brain. When the variance is large, however, the resulting absolute number becomes highly uncertain (von Bartheld, 2001; Benes & Lange, 2001; Farel, 2002), which explains the broad range of results in the literature (see main text for references). Stereological techniques have the advantage of allowing quantification of more restricted cytoarchitectonic regions, such as cortical areas and subcortical nuclei, as well as their subdivisions, e.g. layers. They remain powerful tools for quantifying brain sectors of functional relevance.

In order to increase the precision of high-scale measurements, a new method was proposed – the isotropic fractionator (Herculano-Houzel & Lent, 2005) – by which the anisotropic tissue is rendered isotropic [compare (A) with (B) in Fig. 1], so that cell densities quantified from tissue samples yield very representative averages. The technique starts with homogenization of the tissue sufficient to rupture cell membranes but not nuclear membranes. A nuclear suspension is obtained (Fig. 1C), DNA-stained for better visualization (Fig. 1D), and immunostained to reveal a neuron-specific antigen (Fig. 1E), enabling discernment of neuronal from non-neuronal nuclei. From the nuclear suspension, aliquots are taken, placed into a hemocytometer, and counted under a fluorescence microscope. Similar results have been obtained by running the suspension through a flow cytometer (Collins *et al.*, 2010).

The isotropic fractionator has the advantage over stereology of yielding less variable (and therefore more precise) absolute cell numbers, especially for large brains or brain regions. However, it depends on the possibility of standardizing the dissection of target brain structures for different specimens, restricting its use mostly to anatomical regions, and usually not for their histological, cytoarchitectonic sectors. For instance, the cerebral cortex as a whole can be easily dissected apart from subcortical regions in almost all brains of different species. For this reason, estimates of its global cell composition, as obtained by use of the isotropic fractionator, are statistically precise and reliable. In contrast, this is not so for each of the cortical layers, because they cannot be perfectly separated by dissection from neighboring layers. In this case, stereological methods are recommended.

Isotropic fractionation and stereology, in fact, are complementary techniques. The first is best applied to large, dissectable anisotropic regions (e.g. the cerebellum and the cerebral cortex), and the latter to small anisotropic brain sectors with imprecise borders (e.g. cytoarchitectonic cortical areas), or to relatively isotropic regions of any dimension (e.g. individual subcortical nuclei).

First dogma – the cerebral cortex is the highest achievement of brain evolution

> 'The cerebral cortex holds two-thirds of the brain's neurons and thus appears to be a promising candidate for determining the primary neuroanatomical correlates of intelligence' (Luders *et al.*, 2009)

Most theories on brain evolution and development assume that the size of the brain or the sizes of particular regions are sufficient correlates of cognitive abilities (Hofman, 1985; Luders *et al.*, 2009), although neurons, and more recently glial cells, have been recognized, in essence, to be the minimal computational units of nervous systems (Williams & Herrup, 1988; Roth & Dicke, 2005).

Indeed, there is a great wealth of evidence that neurons are involved in different aspects of cognition, either because of their properties as single units, or because of emergent functions deriving from their association in circuits and networks that include glial cells (see Dehaene & Changeux, 2011; Sporns, 2011; and Wig *et al.*, 2011 for recent reviews). Perhaps two good examples are the 'gnostic cells', discovered in the monkey inferotemporal cortex (Gross *et al.*, 1972), and the mirror neurons of the premotor cortex (Rizzolatti *et al.*, 1996). Both have been reported in humans as well (Quiroga *et al.*, 2005; Mukamel *et al.*, 2010). The functional role of these cells in complex perception and other cognitive operations (reviewed by Gross, 2009; and Rizzolatti & Sinigaglia, 2008) was interpreted initially in a rather reductionist way as self-sufficient (that is, they would be able individually to code complex stimuli), and has more recently been interpreted as ensemble-dependent (that is, they would work cooperatively in interconnected circuits), forming face-recognition and mirror systems, respectively (Freiwald & Tsao, 2010; Heyes, 2010). Whatever one's view on the way in which neurons operate, it is undisputed that they play the most crucial role in determining the functions of the nervous system, increasingly so for the larger brains of mammals that display highly complex behavior, and that have recently appeared in evolution. Possibly, in the future, new techniques that quantify absolute numbers of synapses, circuits and even multi-circuit networks will be developed, thus allowing us to approach more closely the computational operations of the nervous system.

Nevertheless, despite the evidence for neuronal prevalence over brain size, evolutionists have often correlated cognition (which equals 'intelligence' in many authors' words) with the absolute volume or mass of the brain as a whole or the relative proportions of its main sectors (Hofman, 1985; Luders *et al.*, 2009), implying that larger brain regions will necessarily contain more neurons. Furthermore, they have used these structural variables to derive scaling relationships that would predict how complex a brain or brain region will become in evolution (Clark *et al.*, 2001). Along these lines, the relative size of the cerebral cortex reveals a clear increase (Fig. 2A, green dots) – in rodents, the cerebral cortex relative representation in the whole brain goes from about 40% in mice to 60% in capybaras (Herculano-Houzel *et al.*, 2006); in primates, it represents about 67% of the galago (*Otolemur*) brain (Herculano-Houzel *et al.*, 2007), and approaches 82% in humans (Azevedo *et al.*, 2009). On the other hand, the relative size of the cerebellum remains constant across phylogenetic groups, occupying about 10–15% of the total brain mass in different orders (Hofman, 1988) (Fig. 2B, green dots).

On the basis of these relationships, the evolutionary predominance of the cerebral cortex became a dogma, and because its relative volumetric proportion in humans reaches the peak among mammals, this brain region came to be considered as the pinnacle of evolution (Luders *et al.*, 2009). However, if neurons, glial cells and their

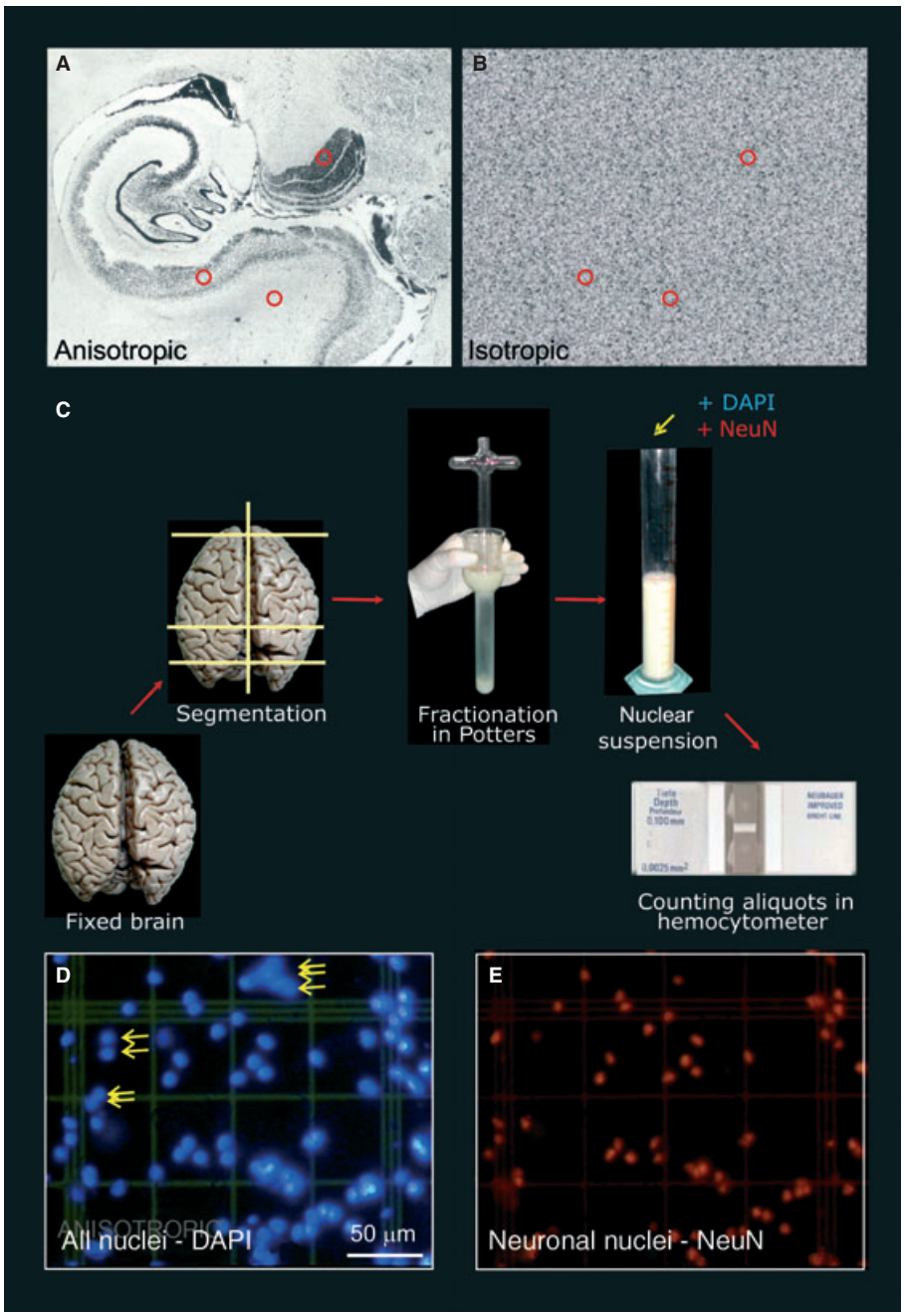

FIG. 1. It is possible to count absolute cell numbers in the brain, by use of the isotropic fractionator (Herculano-Houzel & Lent, 2005). (A) A sector of the human brain, with its high anisotropy. Cell counts within the three red circles give largely different values. (B) If this same sector is rendered isotropic, cell counts within the same circles give very similar values. (C) The brain can be rendered isotropic by fractionation in potters, to arrive at a nuclear suspension, aliquots of which can be counted under the microscope in hemocytometers. (D and E) The same field, where some of the 4′,6-diamidino-2-phenylindole (DAPI)-labeled nuclei do not appear to be labeled by NeuN (yellow arrows) – these are non-neuronal nuclei.

networks are responsible for cognitive abilities, relying on brain cell numbers to calculate relative proportions among brain regions and define 'cerebrotypes' among taxa (Clark *et al.*, 2001) would be an advance relative to relying simply on size. The picture that emerges from this exercise tells us that it is the cerebellum that underwent greater growth in phylogeny (Fig. 2B, blue dots). In rodents, cerebellar neurons represent about 60% of all brain neurons in the small-brained mouse, increasing to about 70% in the capybara (Herculano-Houzel *et al.*, 2006). In primates, cerebellar neurons represent about 83% in the macaque (Herculano-Houzel *et al.*, 2007) and about 80% in humans (Azevedo *et al.*, 2009).

Analysis of 19 species of four mammalian orders has recently revealed that the absolute neuronal composition in the cortex covaries significantly with that of the cerebellum (Herculano-Houzel, 2010), showing that these two brain structures display coordinated growth during phylogenesis in mammals (Fig. 2C). According to these data, each neuron in the cerebral cortex corresponds to about four in the cerebellum, irrespective of the species considered.

Such a coordinated evolution of the cerebral cortex and cerebellum fits well with the recent clinical and experimental evidence suggesting an important role of the cerebellum in cognitive and affective functions, in close connection with cortical associative areas (reviewed by Schmah-

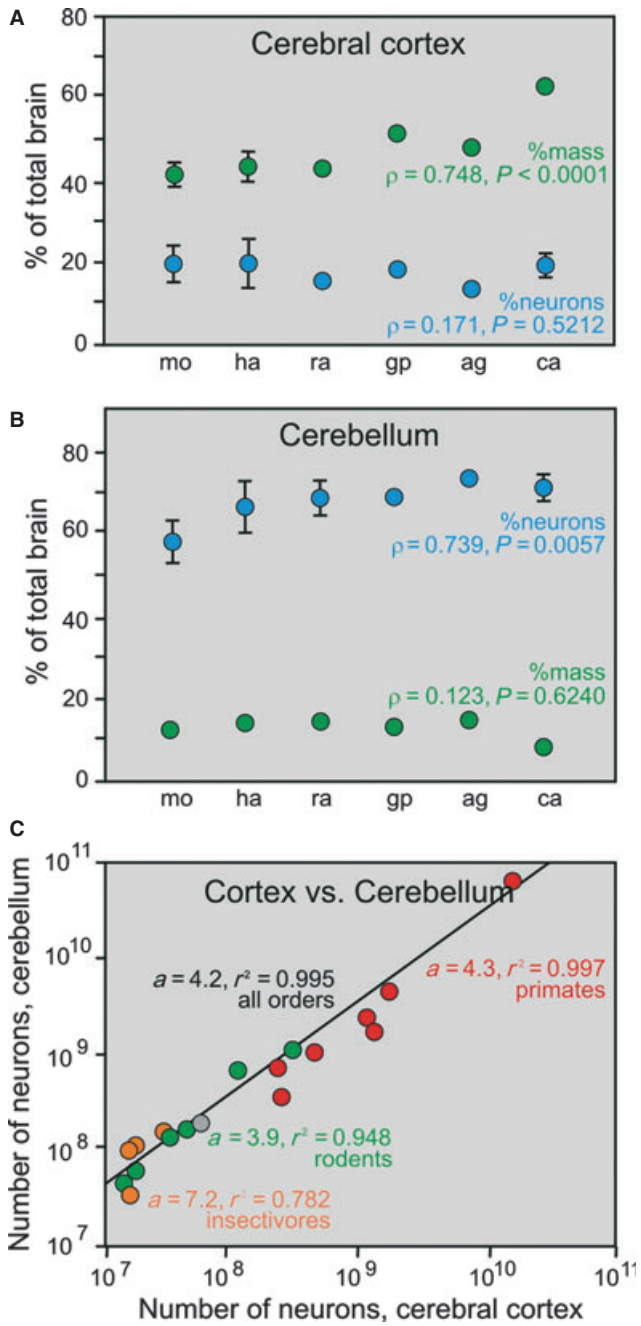

FIG. 2. Concerted increase in the proportion of neurons between the cerebral cortex and the cerebellum. (A and B) Whereas the proportion of cerebral cortex mass increases more than that of the cerebellum in rodents (green dots in top and middle graphs), the proportion of neurons shows a greater increase in the latter (blue dots). Data from Herculano-Houzel *et al.* (2006). (C) The absolute numbers of neurons in both structures, however, show a significant correlation among many mammalian orders (bottom graph; data from Herculano-Houzel, 2010). ag, agouti; gp, guinea pig; ca, capybara; ha, hamster; mo, mouse; ra, rat. Statistical parameters: a = slope; r^2 = regression; ρ = Spearman correlation coefficient.

mann, 2010). Much as motor dysmetria is caused when the anterior lobe of the cerebellum is damaged, dysmetria of thought appears when the posterior lobe becomes lesioned (Schmahmann, 1998). Besides clinical, physiological and neuroimaging data, the proposal is substantiated by findings of extensive connections between the associative cortex and cerebellum (Middleton & Strick, 1994; Schmahmann & Pandya, 1997).

The internal circuitry of the cerebral cortex and that of the cerebellum have been thoroughly studied for a long time (Eccles *et al.*, 1967; Ito, 1972), and display differences that certainly explain their different functional strategies. Despite some controversy (e.g. Horton & Adams, 2005; Da Costa & Martin, 2010), both are considered to be organized in modules – neocortical modules (columns, as they are often called) have been regarded either as varying considerably (Da Costa & Martin, 2010) or being homogeneous and consistent in their cell composition and internal circuitry (Douglas *et al.*, 1989; Lund *et al.*, 2003; Innocenti & Vercelli, 2010). On the other hand, cerebellar modules (or microzones, as they are also called) are considered to display a homogeneous circuit performing similar types of computation that differ only in input and output information (Andersson & Oscarsson, 1978; Apps & Hawkes, 2009; Dean *et al.*, 2010). Synaptic plasticity in both the cerebral cortex (Kirkwood & Bear, 1994; reviewed by Feldman, 2009) and the cerebellum (Ito *et al.*, 1982; Carey, 2011) provides the biological substrate for their involvement in complex cognitive operations.

However, the developmental control of neuronal populations is known to depend on functional interactions between connected regions that influence neurogenesis and programmed cell death (Piñón & Linden, 1996; Sherrard & Bower, 1998; Davies, 2003; Madalosso *et al.*, 2005). It is therefore conceivable that the coordinated regulation of cell numbers between the cortex and cerebellum took place in evolution as a consequence of the abundant connections that formed between both structures, rather than resulting from their intrinsic differences, which would tend to drive them into independent evolutionary trends.

It seems more appropriate, therefore, to question the view that the mammalian cerebral cortex was selected in evolution for greater growth, as a result of growing environmental pressure for more sophisticated abilities to orient behavior. Instead, coordinated evolution of both the neocortex and the cerebellum should be viewed as a more realistic evolutionary 'investment' that resulted in the cognitive computations of higher primates, including humans.

Second dogma – the human brain has one hundred billion neurons and 10 times more glial cells

> 'The mature brain is composed of 100 billion to 200 billion neurons and perhaps 10 times as many glial cells' (Hubel, 1979)

The 'magic number' of 100 billion neurons in the human brain has been widely sustained in papers (Hubel, 1979; Fischbach, 1992; Noctor *et al.*, 2007) and textbooks (Kandel *et al.*, 2000; Bear *et al.*, 2007; Purves *et al.*, 2008), although a broad range is arbitrarily adopted, from 10 billion to 1 trillion (reviewed by Soper & Rosenthal, 1988). However, little direct evidence for it has been produced. In fact, stereological estimates have yielded numbers of 3 billion, 7 billion, 14 billion, 19–23 billion, 21–26 billion and 28–39 billion neurons for the cerebral cortex (Pakkenberg, 1966; Pakkenberg & Gundersen, 1997; more extensively reviewed by Azevedo *et al.*, 2009). The same has been the case for the cerebellum, for which counts have produced numbers from 70 billion to 109 billion neurons (Lange, 1975; Andersen *et al.*, 1992, 2003).

Using the isotropic fractionator, we contributed to reducing the uncertainty of these numbers (Azevedo *et al.*, 2009) – absolute counts yielded an average of 86 billion neurons in male human brains 50–70 years old (Fig. 3), about 15% less than the 'magic number'. Interestingly, it is noticeable that the cerebral cortex contains only 19% of this total neuronal number, despite occupying 81% of the brain mass. On the other hand, the cerebellum contains the impressive proportion of 80% of all cerebral neurons, packed within only 10% of the total brain mass.

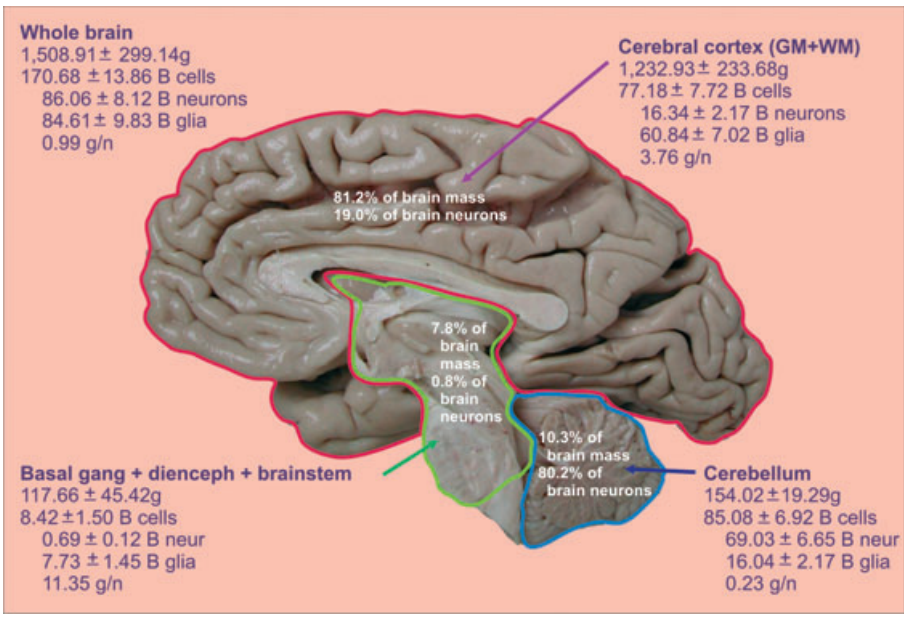

FIG. 3. Absolute cell composition of the human brain. Values represent mean ± standard deviation. Glia stands for non-neuronal cells, for simplicity. g/n, glia/neuron ratio; neur, neurons; GM, gray matter; WM, white matter. Modified from Azevedo *et al.* (2009).

A precise determination of the actual number of neuronal cells in the adult human brain is important for many reasons. It stands as a reference figure for comparisons with diseased brains (e.g. Alzheimer's), for a determination of the developmental oscillations until the mature number is reached, for a better understanding of the quantitative impact of aging in different brain regions, and for estimates of the number of neurons in different hominin brains (Box 2). The 'magic number' of 100 billion neurons has to be considered as an arbitrary midpoint between the broad range of estimates inferred from regional stereological counts that should now be replaced with the experimental numbers as determined by use of the isotropic fractionator.

For glial cells, the prevalent dogma poses that the glia/neuron ratio is approximately 10 : 1 in the brain (Hubel, 1979; Nauta & Feirtag, 1986; Soper & Rosenthal, 1988; Nishiyama *et al.*, 2005; reviewed by Hilgetag & Barbas, 2009). However, with the isotropic fractionator, the actual glia/neuron ratio for the whole human brain was shown to be close to 1 (Fig. 3) (Azevedo *et al.*, 2009). The cerebellum, which has a huge neuronal population, contains a much lower proportion of glial cells, with a glia/neuron ratio of 0.23, whereas the cerebral cortex has a higher number of glial cells, with a ratio of 1.48 for the gray matter alone, and a ratio of 3.76 for the associated gray and white matter. The remaining regions, altogether, have the highest glia/neuron ratio – 11.35. Concerning the cerebral cortex, in particular, stereological methods provided similar (Dombrowski *et al.*, 2001; Lidow & Song, 2001) or even lower proportions in monkeys (O'Kusky & Colonnier, 1982; Christensen *et al.*, 2007) and humans (Pakkenberg & Gundersen, 1997; Pakkenberg *et al.*, 2003; Pelvig *et al.*, 2008).

Unfortunately, so far there is no universal marker for the nuclei of specific glial types. For this reason, the absolute numbers and proportions of astrocytes, oligodendrocytes and microglial cells remain unknown.

Glial cells cooperate with neurons in the proper function of the nervous system. Their importance has increased recently, well beyond the early conception of their role as a structural 'glue' for the tissue (reviewed by Kettenmann & Ransom, 2005). During development, glial cells act as stem cells (Kriegstein & Alvarez-Buylla, 2009) and as guidance scaffolds for migrating neurons (Rakic, 2003) and growing axons (Chotard & Salecker, 2004). In adults, they play important roles in synapse physiology (Eroglu & Barres, 2010), neurovascular interactions (Nedergaard *et al.*, 2003), immune mechanisms and circuit stabilization (Graeber, 2010), signal conduction (Yamazaki *et al.*, 2010), fiber maintenance and regeneration (Nave, 2010), and higher information processing (Pereira & Furlan, 2010). As a whole, the evidence indicates that glial cells do participate actively in the functional computations performed by neurons, circuits, and networks.

Given the increasing importance of these cells, it is crucial to tackle the issue of what determines their quantitative relation with neurons. Why is it that the cerebellum needs fewer glial cells than the cortex, even though it contains the majority of brain neurons? Is this an indication that the glial cells therein are more supportive than computational? On the other hand, could it be the case that glial cells in the cerebral cortex acquired more sophisticated computational functions, thereby increasing in numbers to the extent of being more numerous than neurons? There are no clear answers to these questions – the next few years will probably clarify this issue.

Third dogma – the human brain is exceptionally complex, as compared with those of other primates

> 'There is a long tradition that ascribes properties to humans that are supposedly not found in other animals … It is assumed that animals with larger brains are more intelligent than those with smaller ones' (Roth & Dicke, 2005)

The belief that the human brain displays exceptional properties as compared with the brains of most other animals possibly derives, besides from our own anthropocentric tendencies, from brain–body relationships as represented by encephalization quotients (EQs) (Jerison, 1955, 1973; Marino, 1998) and other measures (reviewed by Deaner *et al.*, 2007). EQs are mathematical indices relating brain size with body size, and became widely used to compare animals of different orders and to draw evolutionary trends from this comparison. Under this logic, humans are positioned as outliers among all animals, displaying an unusually great EQ (Marino, 1998; Klein, 2009), closely followed by some cetaceans and non-human primates.

This line of thought explains the view that the human brain is seven times larger than expected for any mammal, and about 3.5 times larger than expected for an anthropoid primate of its body size (Marino, 1998).

However, not only scaling rules may be different among orders (indeed, they are – see below), but also body size may not represent an appropriate parameter with which to explain the cognitive/affective achievements of the different species. Absolute brain measures, on the other hand, have been reported as being significantly correlated with cognitive measures (Deaner *et al.*, 2007). Additionally, when the absolute number of neurons is employed to study the relationships between brain evolution and development, the conclusion is that orders, in fact, differ in scaling rules. Whereas neuronal number correlates with brain size on the basis of a power equation in rodents (Herculano-Houzel *et al.*, 2006), the same correlation is linear for primates (Herculano-Houzel *et al.*, 2007; Azevedo *et al.*, 2009).

The power equation of rodents means that, to reach the number of neurons that humans have, a rodent would need a brain weighing about 40 kg, in a body of about 100 tons! Humans of 75 kg, if built under rodent scaling rules, would have brains no bigger than 150 g, with only about 3 billion neurons. On the other hand, when the linear equation of primates is applied to a human with a brain of about 1500 g, one arrives at a figure of 90 billion neurons, very close to what has been found experimentally (Azevedo *et al.*, 2009). It can be concluded, therefore, that a change in scaling from rodents to primates (from allometric to isometric) has made it possible to achieve a great growth in the number of neurons, maintaining both brain and body size within dimensions more adapted to on-land locomotion.

We conclude that the human brain is not exceptional in the absolute composition of neurons and glial cells, the main operators of its computational functions. It is, rather, a result of the linear scaling rule characteristic of primates. We are not special in nature, but only big-brained primates. Having big brains and being primates (Box 2), we have acquired a gigantic number of computational units that have made us capable of superior cognitive performance (Deaner *et al.*, 2007).

Box 2. The long road to the current number of neurons in humans

The linear scaling law determined for primates allows one to estimate the number of neurons of human ancestors, using brain volumes as inferred from fossil cranial endocasts (Klein, 2009).

This exercise shows that ancestral primates living between 35 and 20 million years ago – arboricole and quadruped – did not have more than 20 billion neurons in their brains (Fig. 4). By the end of the Miocene period, between 7 million and 2 million years ago, neuronal numbers may have increased to about 40 billion in *Ardipithecus* and *Australopithecus*, just above the estimated 30 billion neurons of chimpanzees. These hominins became bipedal, and produced the first flaked stone tools. Another increase took place at the end of the Pliocene, about 2 million years ago, with the appearance of the genus

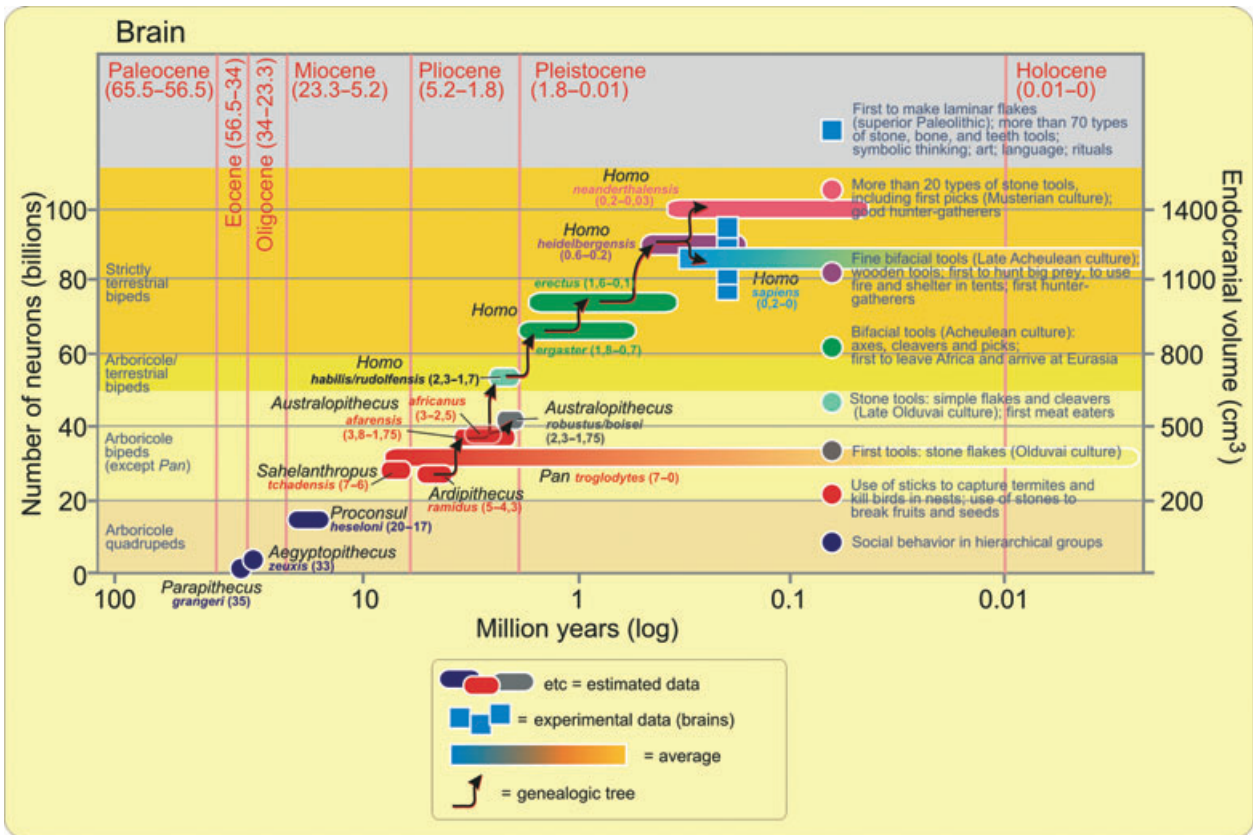

FIG. 4. The number of neurons of human ancestors (left ordinates) can be derived from endocranial volumes (right ordinates; taken from Klein, 2009). Time (in millions of years, at bottom) is segmented into paleontological periods (top). The different hominin species are represented as colored circles or bars; their main cognitive achievements are shown on the right, and their main motor behaviors on the left.

Box 2. Continued

Homo. The number of neurons in the brain grew to about 50 billion in *Homo habilis*, reaching about 70 billion in *Homo erectus*, and finally our 86 billion. It is still a puzzle that, on the basis of the estimated brain volumes, *Homo neanderthalensis* would contain about 100 billion neurons.

With such a great number of neurons, bipedal locomotion consolidated, and hand/finger movements acquired sophisticated abilities, which allowed *Homo* to produce more and more elaborate tools, dominate fire, and improve social interactions.

Fourth dogma – brains grow in evolution and development by the addition of uniform modules

> 'In fact, one of the puzzling dogmas in comparative studies on the mammalian cerebral cortex is the constant number of neurons in an arbitrary unit column' (Abdel-Mannan *et al.*, 2008)

A rising trend in neuroscience is to explain evolution by means of changes in developmental mechanisms (often called the evo-devo approach). Under this rationale, brain growth during phylogenesis is explained mainly by increases in the numbers of cell cycles of neuronal and glial precursors. The cerebral cortex, in particular, is a favorable structure with which to test this idea, not only because it grows considerably in evolution (about five orders of magnitude across mammals), but also because this growth takes place mainly in surface area rather than in thickness.

As the cortex is reportedly organized in modules (Douglas *et al.*, 1989; Lund *et al.*, 2003; Da Costa & Martin, 2010; Innocenti & Vercelli, 2010), as mentioned above, the prevalent view has been that precise developmental mechanisms control the number of modules that will later populate the cortical surface. A strong hypothesis has been put forward in this respect (Rakic, 1988), suggesting that the ventricular zone of the embryonic cortex was composed of radial units that would then, by centrifugal migration along a radial glial scaffold, give rise to the cortical columns. According to this hypothesis, a protomap of radial units would reside in the germinative layers, giving rise to the adult cortical map of different cytoarchitectonic areas.

A cortical column, therefore, should be composed of a given number of neurons, predetermined by the number of cell cycles undergone by ventricular precursors. Of course, neurons therein may associate according to their functional and output properties to form dendritic bundles (Vercelli *et al.*, 2004), and afferents may cluster in specific layers in consonance with the modular architecture (White & Peters, 1993; Sato *et al.*, 2007).

Investigation of the numerical composition of cortical columns (Rockel *et al.*, 1980) has led to the dogma that a constant number of neurons per column compose any cortical area in any mammalian species (except for area 17 of primates). According to this view, a constant number of about 147 000 neurons populate each cortical cylinder of 1 mm^2 at the surface. Despite the fact that different authors provided evidence in favor (Schüz & Palm, 1989) or against (Stolzenburg *et al.*, 1989; Poth *et al.*, 2005) this idea, it became rooted in the literature (Zhang & Sejnowski, 2000; Cheung *et al.*, 2007; but see Rakic, 2009 for a critical appraisal).

This dogma has been approached by use of the isotropic fractionator, to avoid the pitfalls of an arbitrary, histological definition of the counting module, as was the case in the work by Rockel *et al.* (1980). A variation as great as 300% was found in primates (Herculano-Houzel *et al.*, 2008) for the number of neurons within a unit volume under 1 mm^2 at the surface. In addition, the prediction that the total surface area of the cortex would increase linearly with the number of neurons (provided that uniform columns were added across species) did not prove true – on the contrary, surface area was found to increase more slowly than neuronal number (Herculano-Houzel *et al.*, 2008). Uniform modules would also require an inverse relationship between density and thickness, but, in fact, these two variables did not show any correlation (Herculano-Houzel *et al.*, 2008).

Questioning the presumed uniformity of cortical columns does not imply questioning either the radial unit hypothesis or the concept that the brain is organized in modules. In the course of development, the number of neurons that will form each module in the mature cortex will be necessarily attained by regulation of the number of cell cycles within each radial unit in the germinative layers. Such regulation, however, would be controlled and influenced by local variables of unknown nature, rather than being homogeneous across different cortical areas and species.

Concluding remarks

Dogmas are fundamental to science (Kuhn, 1970). They serve the purpose of being challenged, and eventually replaced by other transient truths. Neuroscience is no different. In addition to the four dogmas examined in this review, others can be mentioned, for future analysis. Is it true that the number of neurons in the brain declines with aging? To what extent would this be influenced by dementia? Do males have higher number of neurons than females? The near future will perhaps reveal the answers to these and other questions. Other, newer, dogmas will be generated for later challenge.

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