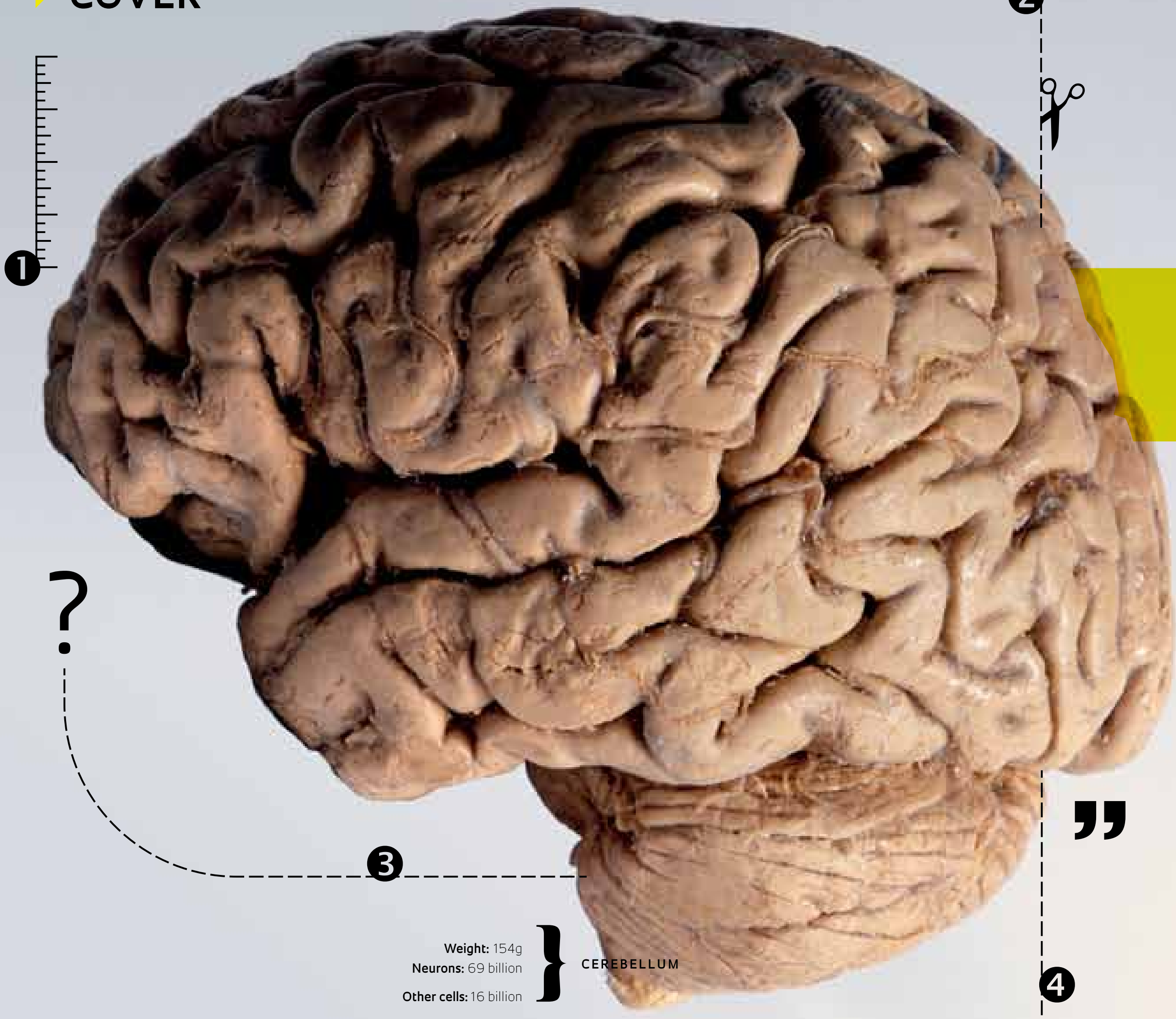
COVER



Numbers under review

Recounting neurons puts neuroscience ideas in check

TEXTO **Ricardo Zorzetto**

PUBLISHED IN FEBRUARY 2012

On the afternoon of Wednesday, January 11th, 2012, researchers Frederico Casarsa de Azevedo and Carlos Humberto Moraes conducted an uncommon task for neuroscientists. They covered a masonry bookcase with white cardboard to hide the window in the back, cleaned a granite table and moved glass recipients, pipettes and reagents to a nearby bench that also held glassware, pipettes and reagents. They prepared the laboratory, which was headed by the physician Roberto Lent from the Federal University of Rio de Janeiro (UFRJ), for a photo and filming session. They wanted to record in detail the functionality of a machine that they had begun creating seven years prior that was now ready: the automatic cell fractionator. They intended to patent the fractionator, and the scenery could not interfere.

The equipment, with its complicated name and a height of nearly one meter, was a type of family-sized waste disposal unit. It had electric motors that made six plastic pistons, which were fixed to a mobile base, rotate at 400 rpm. Each piston was immersed in a glass recipient containing samples of brain tissue bathed in a solution with detergent. Once the fractionator was turned on, its pistons stirred the colorless liquid, creating vortexes that broke up the samples. Two pieces of brain tissue were dissolved in a milky-colored mixture. This was what the researchers affectionately called "brain juice."

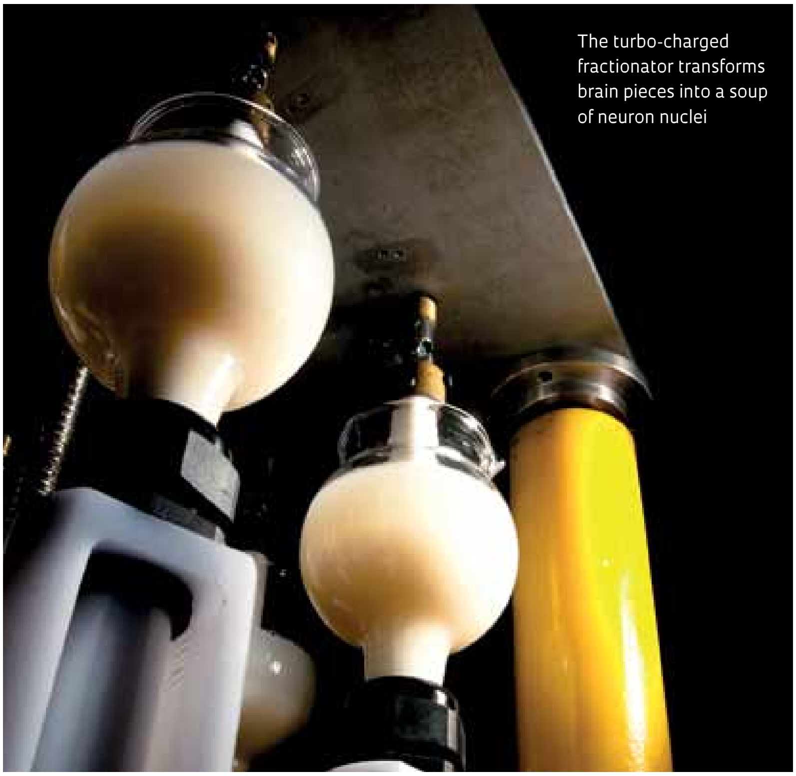
The machine that was undergoing tests in UFRJ's Neuroplasticity Laboratory at the Institute of Biomedical Sciences (ICB) was a turbo-charged version of a much simpler fractionator. It contained a tube and piston, which were both made of glass and manually activated, that Lent and neuroscientist Suzana Herculano-Houzel had used since 2004 for breaking up pieces of brain and counting the cells. This technique, which was created by Lent and Suzana, allowed them to more precisely know data that were supposedly already known, which was how many neurons there were in the brain and other encephalon organs housed in the skull.

Thanks in part to the work of Lent's and Suzana's groups, it is known today that there are 86 billion neurons in the human brain, not the 100 billion thought years ago. It can also be stated with more assurance that these neurons are accompanied by 85 billion glial cells, which are the other cell type comprised in the brain. This number is far lower than the previously touted one trillion.

These are not just details. More accurately knowing the number of brain cells and where they are located is important in understanding how the brain functions and what strategies were adopted by nature in the construction of a complex organ that, in the case of humans, allows for the existence of the self-conscious mind. These data also help identify characteristics that distinguish a normal brain from one that is sick.

However, just looking at the number of cells in the brain is not sufficient to understand one of the most intriguing and fascinating organs. Today, neuroscience considers the brain to be much more than just a collection of neurons, which are cells that communicate using electrical current. According to the Italian neuro-anatomist Alessandro Vercelli from the University of Tu-

PHOTOS LEO RAMOS


The turbo-charged fractionator transforms brain pieces into a soup of neuron nuclei

rin, perhaps even more important than the total number of neurons is the effective connections established among them, resulting in the creation of networks that process information in a distributed manner. "The number, pattern and quality of these connections vary in space and time," says Martín Cammarota, a neuroscientist from the Pontifical Catholic University of Rio Grande do Sul who studies the formation and recall of memories. He explains that "having more or fewer neurons does not necessarily make an individual or a species more or less intelligent than the other."

Despite these considerations, the results that Suzana and Lent have been collecting since 2005 have led them to question some ideas about the composition and structure of the brain that were once held as absolute truths. Last year, Lent decided that the data generated by his group and that of Suzana were sufficiently consistent and that the results should be consolidated into a more direct critique. Lent and three researchers from his laboratory wrote a review published in January in the *European Journal of Neuroscience* stating that at least four basic concepts of neuroscience needed to be rethought.

The first dogma discussed in the article is that the human brain and the rest of the encephalon together have 100 billion neurons. Known even by those who are not neuroscience specialists, this number has been circulating in scientific articles and textbooks for almost 30 years. Lent himself published a book in 2001 that is used in graduate courses entitled *'Cem bilhões de neurônios'* [One hundred billion neurons].

1º DOGMA

There are 100 billion neurons in the human brain

THE ORIGIN

This book, to a certain extent, created the doubts that have driven the researchers from UFRJ to investigate how many cells there are in the brain. Prior to its launch, Suzana began a study evaluating the knowledge of neuroscience in high school and university students. One of the 95 statements that they had to determine was right or wrong was as follows: we only use 10% of our brain.

Almost 60% of the 2,200 people interviewed replied that this statement was correct. This statement, which is incorrect because all of the brain is used all of the time, results from a statement made in 1979 by the Canadian neurobiologist David Hubel. Hubel received the Nobel Prize in Medicine or Physiology in 1981. He stated that there were 100 billion neurons in the brain and 1 trillion glial cells. This fact was repeated in other publications, and the information spread. Because neurons are the units that process information and represent only one tenth of brain cells, it was concluded that the other 90% of the brain was not used when walking, planning a trip or sleeping.

The results of the survey bothered Suzana. She looked for the original source of these figures in the scientific literature, but it could not be found. She had collaborated with Lent on his book and raised her doubts with him: "How do you know that there are 100 billion neurons?" Lent replied, "Look, everybody knows, every book says so." Many articles and books carried the information but did not state the source. Lent commented that these numbers "were apparently intuitive data that had become consolidated, and people cited them without thinking."

One of the reasons why these numbers are not easily found is that counting brain cells is not an easy task. In addition to being a large organ (the human brain weighs approximately 1,200 grams, and the encephalon weighs 1,500 grams), its architecture is complex. Different areas have varied cell concentrations, and the technique available for counting cells previously, stereology, only works well for small regions with a homogenous cell distribution. Its application in counting brain cells generated unreliable estimates, which varied by as much as 10-fold for some regions and left the human brain with somewhere between 75 and 125 billion neurons.

Having been recently hired by UFRJ, Suzana told Lent that she had a "bold and half crazy" idea for how to count neurons, but she had no laboratory. He invited her to work with him. Suzana's proposal was simple: make the brain regions homogenous before counting the cells. How? By breaking up the cells.

The main reason for the heterogeneity of the encephalon is that the cells and spaces between them vary in size. By dissolving the cells, the question of space would be resolved, provided that it was possible to preserve the cells' nuclei, which are the central organelle that houses the DNA. As each brain cell has just one nucleus, the count is simple. The sum of the nuclei would give the total number of cells. Dyes specific to neurons then allowed them to be distinguished from other brain cells.

Using chemical compounds that preserve cell structures, Suzana managed to destroy the external membrane without damaging the nucleus. She described this technique with Lent in 2005 in the *Journal of Neuroscience*. "It's an intelligent, simple and easy method to use and replicate," commented Vercelli. "I wonder why no one ever thought of this before." In the opinion of Zoltan Molnar, a neuroscientist from the University of Oxford in England, it was an important advance. He stated that "genomics, trasnscriptomics and proteomics are quantitative and accurate areas that have made a lot of progress, while we anatomists have remained in the dark ages. We haven't developed methods that can measure the number, density and variations in cell architecture."

The first test was with rat brains. The total number of cells in the encephalon was 300 million. Two hundred million of these cells were neurons. Unlike what was expected, only 15% of the neurons were in the brain, the most voluminous part of the encephalon. Most (70%) were found in a smaller organ in the back of the skull, the cerebellum.

2º DOGMA

The number of glial cells is 10-fold greater than the number of neurons

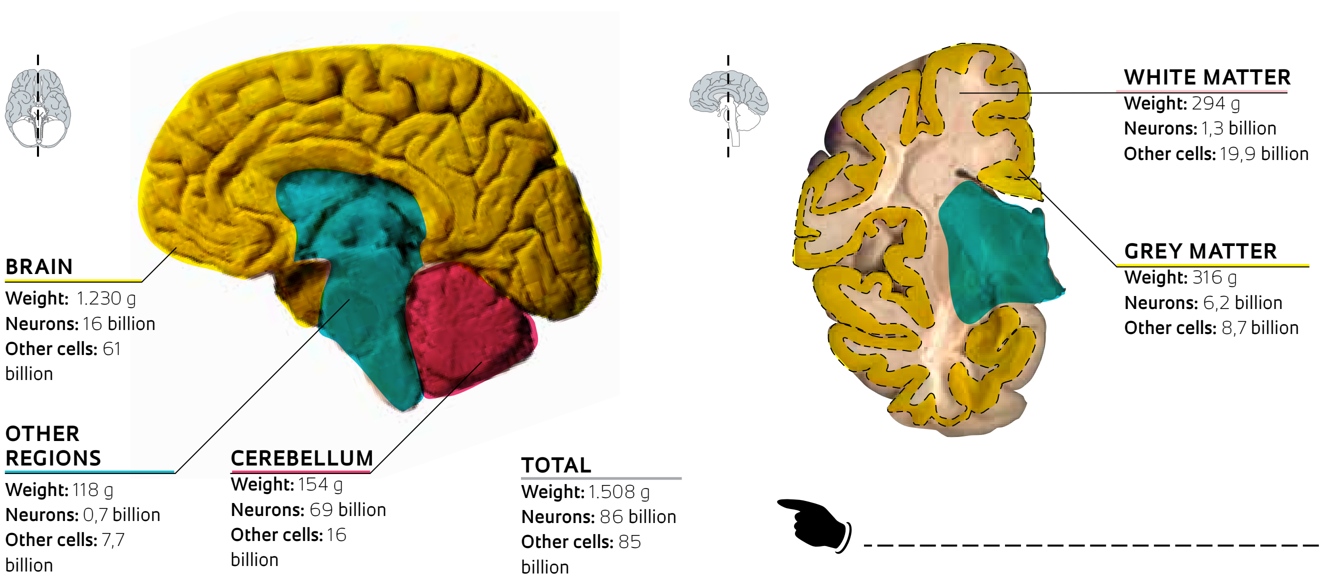
This was the composition in rats, but what about other species? Suzana then analyzed the brains of five other rodents (mice, hamsters, guinea pigs, pacas and capybaras). It was already known that bigger animals contained bigger brains and larger numbers of neurons. The mouse, weighing just 40 grams, is the smallest and has 71 million neurons stored in a brain weighing 0.4 grams. The capybara, which is almost 1,200-fold heavier than the mouse, has an encephalon that is 183-fold bigger (76 grams) but only contains 22-fold more neurons (1.6 million).

THE HUMAN BRAIN

Supervised by Suzana and Lent, biologist Frederico Azevedo counted the cells in human brains. First, however, he had to adapt the technique to humans. "What functioned with rodents didn't work for humans," he said. It took months before he discovered that the problem was in the method the tissue was fixed before fractionation. When the brain was immersed for too long in compounds that prevented its degradation, the researcher was unable to stain and count the neurons under a microscope. Frederico fractioned the brain samples of four individuals (ranging in age from 50 to 71) by hand. The samples were supplied by the brain bank at the University of São Paulo (USP). "This was when I began thinking of a way to make this work automatic," said the biologist, who was doing a Ph.D. at the Max Planck Institute in Germany.

The cell count revealed that the human brain has 86 billion neurons on average. This is 14% less than the previous estimate and close to the number suggested in 1988 by Karl Herrup from Rutgers University. "There are those who say that the difference is small, but I disagree," says Suzana. "It corresponds to the brain in a baboon

Where the neurons are



SOURCE ROBERTO LENT / UFRJ

or half the brain of a gorilla, which is one of the primates that, in evolutionary terms, is closest to human beings," explains the neuroscientist, who is the head of the Comparative Neuroanatomy Laboratory of ICB-UFRJ.

Lent cautiously commented that "we can't state that these numbers are representative of the human species. It's probable that they're representative of mature adults." This assumption may not even be the case because only four brain samples were analyzed. In younger people, the number of neurons could also be different. "Who knows whether individuals in the 20-year-old age group have 100 billion neurons that they lose over time?" the researcher wondered. His group is now studying the brain in younger individuals and comparing brains between men and women. Until he answers these questions, Lent has altered the title of the second edition of his book, published in 2010 to *Cem bilhões de neurônios? [One hundred billion neurons?]* by including a question mark at the end.

THE CEREBELLUM

Similar to rodents, most of the neurons in humans are not in the brain but in the cerebellum. The brain (more specifically, the cerebral cortex, which until recently, was considered primarily responsible for cognitive functions, such as attention, memory and language) is the part of the encephalon that grew the largest throughout evolution. In humans, the brain weighs 1,200 grams and occupies more than half of the skull but houses just 16 billion neurons. The cerebellum, however, weighs 150 grams and contains 69 billion cells (*see infographics on page 29*).

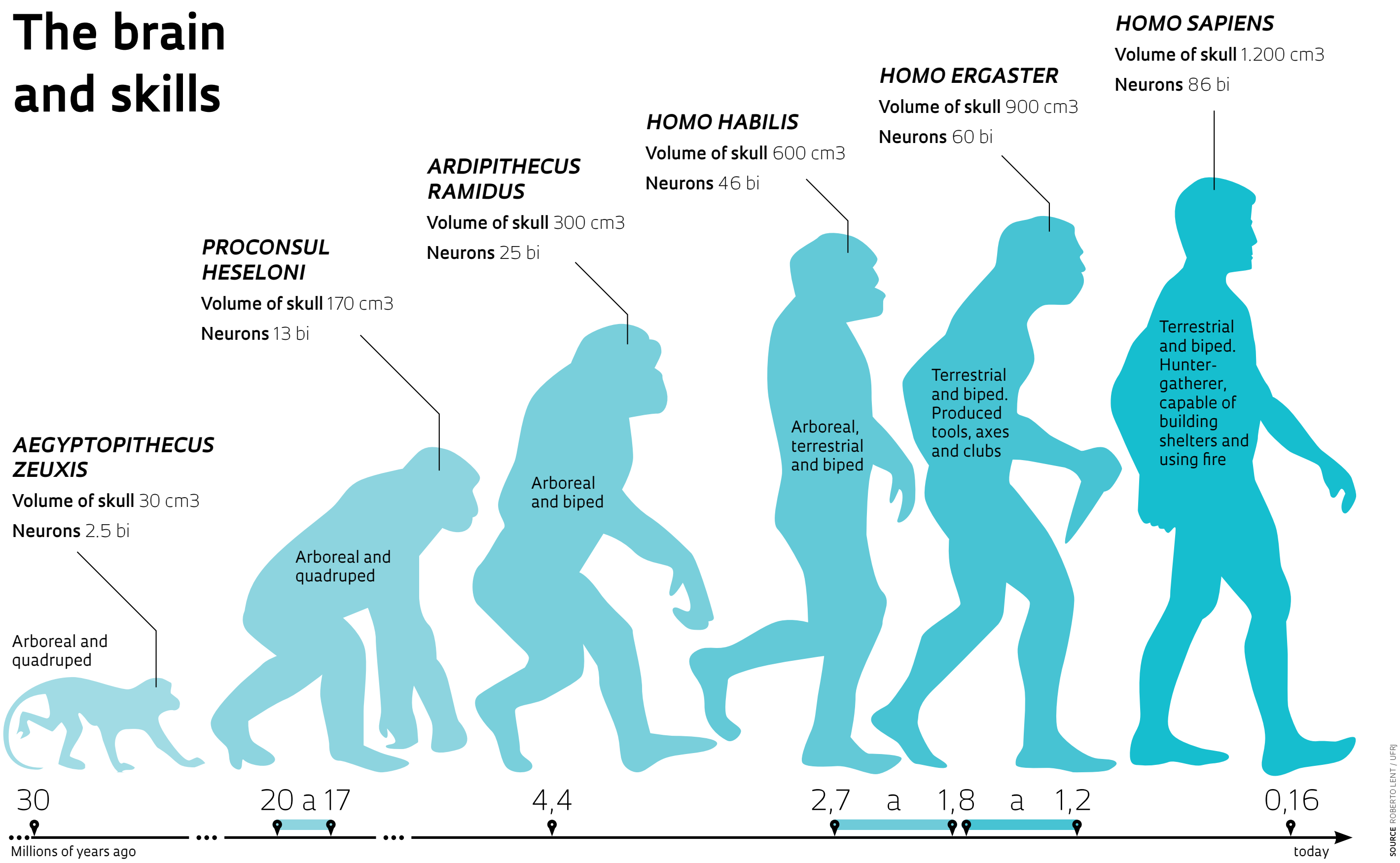
How can we explain the different sizes of these organs? The answer is twofold. First, although the brain has fewer neurons than the cerebellum, it contains almost four times more of the other cell types, such as glial cells. These cells, previously seen merely as the physical support for neurons, perform other essential functions, including helping in the transmission of impulses, feeding the neurons and defending the central nervous system from invading microorganisms. They also occupy space. Second, the brain and cerebellum are composed of different types of neurons that are connected differently.

In conducting this work, the group from Rio also found that evolution did not favor the development of just the brain. Among mammals, which is the class of animals that human beings belong to, the brain and cerebellum acquired neurons at the same pace. This result, according to Vercelli, corroborates research indicating that the role of the cerebellum is not merely to control movement. It is fundamental for learning, memory,

3º DOGMA

The human brain is more complex than that of other primates.

The brain and skills



the acquisition of language and the control of behavior and emotions. "It's being increasingly clear that the cerebellum participates in processes that we used to only associate with the cerebral cortex," comments Herrup from Rutgers.

THE STRATEGIES

Since the development of this technique, Suzana has already applied it in the study of the encephalon from 38 species of mammals. She found that over the last 90 million years, nature has adopted at least two strategies to build brains, one for rodents and another for primates.

In rodents, the increase in the number of neurons in the encephalon occurs logarithmically. Generally speaking, as the size of the species increases, the encephalon becomes larger in size, and the absolute number of neurons increases. However, as the rodent increases in size, it gains proportionally fewer neurons. Alternatively, among primates, which include monkeys and human beings, the number of neurons increases linearly with the cerebral volume. "There was an abrupt transition between lower mammals, like rodents, and the higher mammals, like primates," comments Vercelli. According to Lent, this change allowed the brain in primates to group more neurons in a smaller volume and accumulate more cells than rodent brains.

This pattern of encephalic development in primates led Suzana and Lent to question another idea in force for nearly 40 years: that the human brain is exceptionally large. In 1973, American paleoneurologist Harry Jerison said that our brain was seven times larger than what one might expect from a 70-kilogram mammal. Neuroscientist Lori Marino later said that the brain was large, even for a primate. At almost 1,500 grams, the human encephalon is the biggest among all primates. The gorilla, which is the biggest primate, weighs 200 kilos and has a 500-gram encephalon. However, the impetus for this notion was the principle that body size is a good indicator of brain dimensions. It now appears that this is not the case.

Aside from body mass and the number of cells, it appears that the human brain does not differ from that of primates. "Our brain has the number of cells to be expected for a primate of this size," says Suzana.

Based on the volume of the skull and on the assumption that the human brain does not differ from that of primates, in 2011, Suzana and neuroscientist Jon Kaas from Vanderbilt University in the United States published an estimate of the number of brain cells in nine other hominids in *Brain, Behavior and Evolution*. As was to be expected, the species that is closest to humans (*Homo sapiens*) in terms of neuron numbers is Neanderthal man (*Homo neanderthalensis*), who lived between 30,000 and 300,000 years ago in the region where Europe is today. Neanderthal man had 85 billion neurons according to Suzana and Kaas' estimate. With the help of bioanthropologist Walter Neves from USP, Lent expanded the projection to include other species of primates that belonged to the super-family of hominids and calculated that the Neanderthals may have had 100 billion neurons (*see infographics on page 30*).

The size of glial cells remained constant throughout evolution, while that of the neurons varied by up to 250-fold

Another dogma being questioned is that the total number of glial cells exceeds the number of neurons by ten-fold. This number led to the idea that only 10% of the brain is used. "This high rate of glial cells was taught in textbooks, although experiments were already indicating that the proportion was 1 to 1," says Helen Barbas from the University of Boston.

Rather than the number of glial cells (there are 85 billion in human beings, and they are concentrated more in the brain than in the cerebellum), what most surprised Suzana was that they underwent practically no morphological changes during evolution. Their size is almost constant among primates, while the size of neurons has varied by a factor of 250. "The functionality of glial cells must be adjusted in such a fundamental way that nature eliminated any change that arose," she comments.

It is expected that more exciting results will appear as the Brazilian technique spreads. "If it's widely used, it can simplify the tedious process of counting brain cells," says Herrup. Perhaps more hours can be saved if the turbo-charged version of the fractionator is as efficient as expected. ■

4º DOGMA

The modules (cell groupings) of the brain contain the same number of neurons

Scientific articles

1. LENT, R. *et al.* How many neurons do you have? Some dogmas of quantitative neuroscience under revision. **European Journal of Neuroscience.** v. 35 (1). Jan. 2012.

2. HERCULANO-HOUZEL, S.; LENT, R. Isotropic fractionator: a simple, rapid method for the quantification of total cell and neurons in the brain. **Journal of Neuroscience.** v. 25(10), p. 2.518-21. 9 Mar. 2005.