Mammalian Brains Are Made of These: A Dataset of the Numbers and Densities of Neuronal and Nonneuronal Cells in the Brain of Glires, Primates, Scandentia, Eulipotyphlans, Afrotherians and Artiodactyls, and Their Relationship with Body Mass

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Abstract

Comparative studies amongst extant species are one of the pillars of evolutionary neurobiology. In the 20th century, most comparative studies remained restricted to analyses of brain structure volume and surface areas, besides estimates of neuronal density largely limited to the cerebral cortex. Over the last 10 years, we have amassed data on the numbers of neurons and other cells that compose the entirety of the brain (subdivided into cerebral cortex, cerebellum, and rest of brain) of 39 mammalian species spread over 6 clades, as well as their densities. Here we provide that entire dataset in a format that is readily useful to researchers of any area of interest in the hope that it will foster the advancement of evolutionary and comparative studies well beyond the scope of neuroscience itself. We also reexamine the relationship between numbers of neurons, neuronal densities and body mass, and find that in the rest of brain, but not in the cerebral cortex or cerebellum, there is a single scaling rule that applies to average neuronal cell size, which increases with the linear dimension of the body, even though there is no single scaling rule that relates the number of neurons in the rest of brain to body mass. Thus, larger bodies do not uniformly come with more neurons – but they do fairly uniformly come with larger neurons in the rest of brain, which contains a number of structures directly connected to sources or targets in the body.

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Introduction

The availability of datasets on mammalian brains that make comparative studies possible has been instrumental for the advancement of evolutionary neuroscience. Most notable have been the datasets on the volumes of brain structures in 51 species of bats, 48 primates and 28 'insectivores' (currently recognized as a combination of afrotherians and eulipotyphlans) published by Heinz Stephan's group [Stephan et al., 1981a, b], on cortical sur-

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faces and volumes for 44 mammalian species compiled by Hofman [1985, 1988], and on neuronal and glial cell densities for 11 species studied initially by Tower and Elliott [1952] and Tower [1954], and later extended to another 42 species by Haug [1987].

Although restricted in their scope to mostly structure volumes and to cell densities in the cerebral cortex, those datasets were, for a few decades, the major references for studies on brain evolution that established the basic notions that there is both concerted [Finlay and Darlington, 1995] and mosaic [Barton and Harvey, 2000] scaling across brain structure volumes in evolution, that larger brains were composed of more and larger neurons, resulting in smaller neuronal densities and increasing glia/neuron ratios in a uniform manner across species [Tower and Elliot, 1952; Haug, 1987; Stolzenburg et al., 1989; Marino, 2006], and that larger brains have relatively larger cerebral cortices but a cerebellum of constant relative size [Stephan et al., 1981a, b; Clark et al., 2001], with presumably larger relative numbers of neurons in the cerebral cortex over the rest of the brain.

Since 2005, with the development of the isotropic fractionator, a new, nonstereological method to determine the numbers of neuronal and nonneuronal cells that compose brain structures [Herculano-Houzel and Lent, 2005] that gives results comparable to those obtained with careful stereological analysis [Herculano-Houzel et al., 2015], we have been able to expand our understanding of brain evolution by examining the scaling relationships between the mass of brain structures and the number of cells that compose them. Through the analysis of 42 species of primates (including the human) [Herculano-Houzel et al., 2007; Azevedo et al., 2009; Gabi et al., 2010; Ribeiro et al., 2014], glires [Herculano-Houzel et al., 2006, 2011; Ribeiro et al., 2014], eulipotyphlans [Sarko et al., 2009], scandentians [Herculano-Houzel et al., 2007], afrotherians [Herculano-Houzel et al., 2014a; Neves et al., 2014] and artiodactyls [Kazu et al., 2014], we have been able to challenge a number of the initial notions regarding mammalian brain evolution. Specifically, we could show that while there is indeed a shared, single relationship between numbers of nonneuronal cells and the mass of brain structures across species, with relatively unchanging nonneuronal densities, neuronal densities do not vary uniformly across all species and brain structures [reviewed in Herculano-Houzel, 2011a, 2014; Herculano-Houzel et al., 2014b], that glia/neuron ratios vary with average neuronal cell size, not brain structure mass, across different brain structures and mammalian species [Mota and Herculano-Houzel, 2014], that the relationship between the

number of brain neurons and body mass differs across mammalian orders [Herculano-Houzel, 2011b; Herculano-Houzel et al., 2014b], and that relatively larger cerebral cortices do not hold relatively more of all brain neurons [Herculano-Houzel, 2010; Herculano-Houzel et al., 2014b]. We could also show that the apparent uniform scaling of the energetic requirement of the brain with brain mass across species [Karbowski, 2007] is actually a spurious mathematical consequence of the apparent scaling of neuronal density across the brains included in that analysis, which conflated primates and nonprimates, then already known to have different relationships between brain mass and neuronal density [Herculano-Houzel et al., 2006, 2007]. Rather, the energetic requirement of the brain scales linearly with the number of neurons in the brain, and uniformly across rodents and primates, despite the different neuronal scaling rules that apply to these orders [Herculano-Houzel, 2011c].

The analysis of our new dataset on numbers of neurons and nonneuronal cells that compose mammalian brains allowed us to propose a new synthesis of the mechanisms of brain evolution [Herculano-Houzel et al., 2014b]. Briefly, we propose that the evolution of mammalian brains of a wide range of masses has been the result of both concerted and mosaic changes in the distribution of neurons across brain structures and in the relationship between number of neurons and average neuronal cell size (including the cell body and all arbors). In most mammalian groups, the addition of neurons to individual brain structures has been accompanied by predictable increases in the average size of neurons in each structure (as inferred from changes in neuronal cell densities), which allowed us to infer the ancestral neuronal scaling rules for each structure. From those ancestral scaling rules, we inferred that the primate cerebral cortex and cerebellum, the eulipotyphlan cerebellum, and the artiodactyl rest of brain (RoB) diverged with changes in the predicted mechanism that ties the number of neurons to the average size of the neurons generated. The distribution of neurons to the cerebral cortex and cerebellum, two structures generated by different progenitor cell populations, has varied little from what we infer to have been the ancestral mammalian rule of about 4 neurons in the cerebellum to every neuron in the cerebral cortex. At the same time, the allocation of neurons to the ensemble of these two structures has departed greatly from the inferred ancestral ratio of 2 neurons in the cerebral cortex (and 8 in the cerebellum) for every neuron in the RoB to much larger and variable ratios in primates and artiodactyls (while still maintaining the ratio between numbers of

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neurons in the cerebellum and cerebral cortex) [Herculano-Houzel et al., 2014b].

In the spirit of making this new body of data available for researchers with complementary interests and expertise to ours who will be able to advance the understanding of brain evolution in a much wider sense, here we provide the full dataset that we have generated on the mass and numbers of neuronal and nonneuronal cells that compose the brain as a whole and subdivided in its four major structures (cerebral cortex, cerebellum, olfactory bulb and RoB). All data have been thoroughly checked for consistency regarding the brain structures included, because of inconsistencies in a few of the original studies [Herculano-Houzel et al., 2006; Sarko et al., 2009], guaranteeing that comparisons across species are valid (for example, that numbers for 'cerebral cortex' always include the hippocampus, and that numbers for 'RoB' and 'whole brain' always exclude the olfactory bulb). We also report new observations on the scaling of neuronal density with body mass that shed light on the different factors that may control cell size across brain structures.

The Dataset

Our full dataset consists of 42 mammalian species across 5 orders (Glires, Primata, Scandentia, Eulipotyphla and Artiodactyla) and the superorder Afrotheria. For two of these species (the orangutan and gorilla), data were available only for the cerebellum, and although these allow the inference of numbers of neurons in the whole brain, and in the cerebral cortex in particular [Herculano-Houzel and Kaas, 2011], we have limited the data presented here to the cerebellum alone. The phylogenetic relationships amongst the species, compiled according to Price et al. [2005], Purvis [1995], Blanga-Kanfi et al. [2009], Douady et al. [2002], Shinohara et al. [2003] and Murphy et al. [2001], are illustrated in figure 1. A total of 86 brains (or hemispheres) were analyzed, and all data are provided in tables 1–6. All data provided are averages ± standard deviation across individuals where more than one individual of each species was available, or data obtained for single individuals. All data are reported for the two sides of the brain together, even when the original data were collected from a single hemisphere, in which case results were multiplied by 2.

Values are reported here for the cerebral cortex (defined as all structures lateral to the olfactory tract), which includes the hippocampus and subcortical white matter, the cerebellum, which includes the cerebellar cortex, sub-

cortical white matter and deep cerebellar nuclei, olfactory bulbs, where available, and RoB. The RoB amounts to the ensemble of brainstem, diencephalon and striatum. Because the olfactory bulbs are not always available for analysis, we chose to report values for 'whole brain' as the sum of cerebral cortex, cerebellum and RoB, excluding the olfactory bulbs.

All analyses were made across average values so as not to confound intraspecific and interspecific allometric relationships [Armstrong, 1990]. All analyses were performed with JMP 9.0 (SAS). Although we report the best currently known phylogenetic relationships across the species in the dataset (fig. 1), we do not correct the reported allometric relationships for phylogenetic relatedness across the species included. As shown before, accounting for phylogenetic relatedness hardly changes the exponent of these strong allometric relationships [Gabi et al., 2010]. Most importantly, however, we wish to address directly the mathematical relationships across some of the most basic variables related to how mammalian brains are built, and we do not wish these to be affected by assumptions of phylogenetic relationships that have been known to change upon reexamination, such as those for 'insectivores' (now assigned to the distant clades Afrotheria and Eulipotyphla).

Brain Structures

The mass of all brain structures reported refers to paraformaldehyde (PFA)-fixed brains postfixed for at least 2 weeks. The brains of glires, primates, scandentians and eulipotyphlans were stored in 4% PFA until processed; the brains of all afrotherians and artiodactyls were stored in an antifreeze solution after fixation and cryoprotection in 30% sucrose [Herculano-Houzel, 2012]. While the mass may vary slightly from the fresh mass depending on the time of postfixation, shrinkage and other alterations in tissue mass due to the substitution of water with the glycerol-based antifreeze are minor concerns in studies of allometric relationships, where data typically span 3 or more orders of magnitude, although future users of this dataset must keep in mind that they are likely sources of extraneous, nonbiological variation in tissue mass. Most importantly, however, any alterations in tissue mass or volume due to fixation or storage in antifreeze have no effect on the estimates of numbers of cells reported here, since they were obtained with the isotropic fractionator [Herculano-Houzel and Lent, 2005], a nonstereological method.
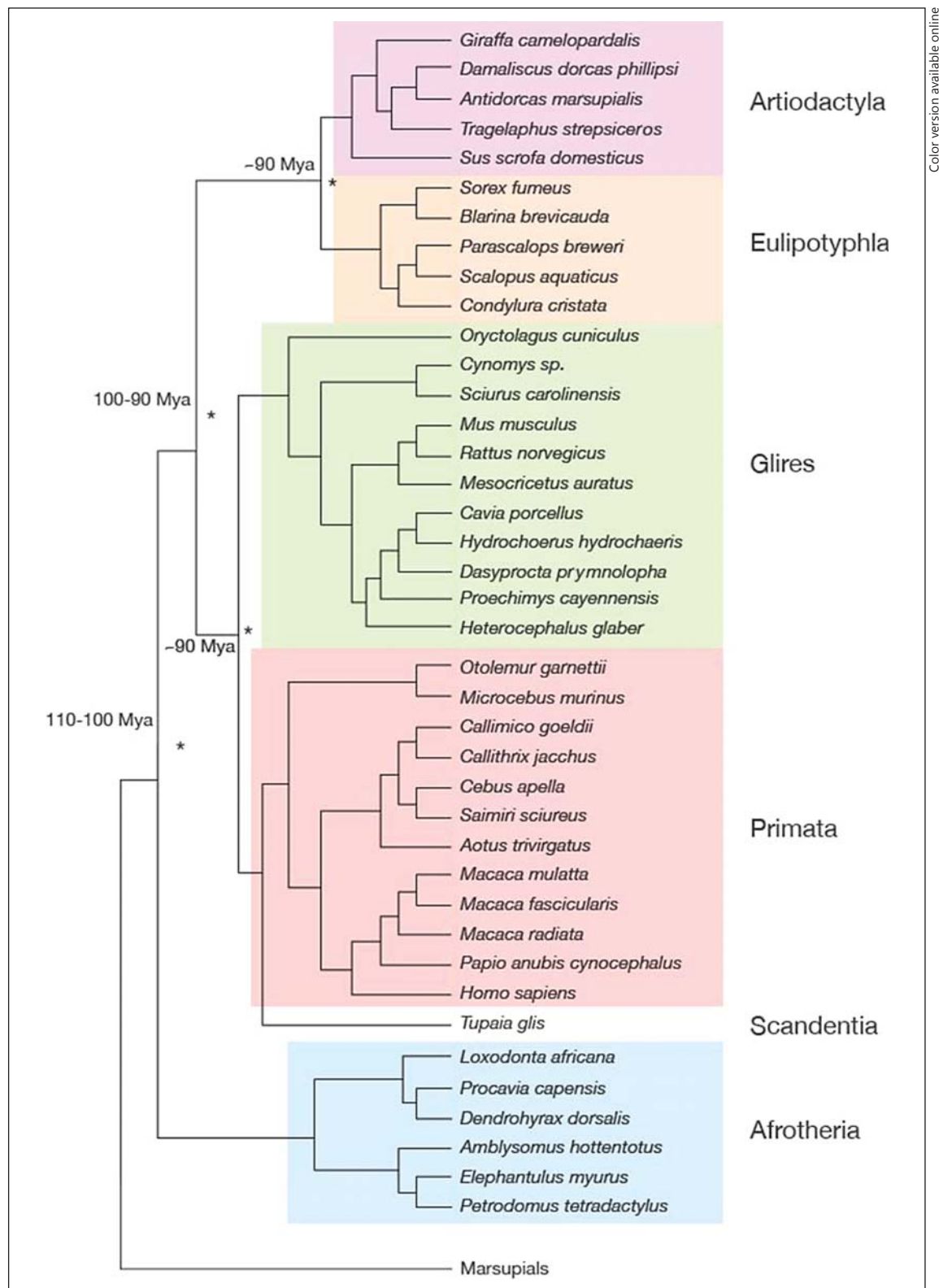
Fig. 1. Phylogenetic relationships between the 40 non-great ape species examined. Compiled according to Price et al. [2005], Purvis [1995], Blanga-Kanfi et al. [2009], Douady et al. [2002], Shinohara et al. [2003] and Murphy et al. [2001]. * = Divergence points to which the dates refer.

Table 1. Cerebral cortex

Species	Order	Mass, g	N, n	O, n	N/mg	O/mg	O/N	Source
Sorex fumeus	Eulipotyphla	0.084±0.009	9,730,000±352,000	9,290,000±1,112,000	116,727±9,387	111,754±18,566	0.958±0.135	Sarko et al., 2009
Mus musculus	Glires	0.173±0.015	13,688,162±2,242,257	12,061,838±3,668,594	78,672±7,683	68,643±15,807	0.870±0.177	Herculano-Houzel et al., 2006
Blarina brevicauda	Eulipotyphla	0.197±0.012	11,876,000±1,569,000	15,820,000±1,158,000	60,214±4,935	80,729±8,731	1.357±0.250	Sarko et al., 2009
Heterocephalus glaber	Glires	0.184±0.026	6,151,875±1,065,587	8,398,125±1,197,056	33,374±2,063	45,894±11,497	1.365±0.125	Herculano-Houzel et al., 2011
Condylura cristata	Eulipotyphla	0.420±0.024	17,250,000±3,105,000	32,010,000± 8,822,000	40,777±5,145	76,995±25,019	1.966±0.924	Sarko et al., 2009
Parascalops breweri	Eulipotyphla	0.429±0.019	15,690,000±2,611,000	39,870,000± 4,884,000	36,727±7,359	93,185±14,583	2.581±0.109	Sarko et al., 2009
Amblysomus hottentotus	Afrotheria	0.439±0.035	21,516,000±2,154,000	21,370,000±4,614,000	48,932±1,004	48,146±6,674	0.982±0.116	Neves et al., 2014
Scalopus aquaticus	Eulipotyphla	0.476±0.032	26,680,000±5,113,000	38,540,000±5,567,000	60,461±12,249	80,745±6,407	1.383±0.368	Sarko et al., 2009
Elephantulus myurus	Afrotheria	0.471±0.021	25,865,000±4,020,000	26,229,000±1,104,000	54,644±6,098	55,693±138	1.032±0.119	Neves et al., 2014
Mesocricetus auratus	Glires	0.446±0.048	17,140,000±3,619,934	41,870,000±1,350,121	39,099±12,332	94,271±7,136	2.507±0.608	Herculano-Houzel et al., 2006
Rattus norvegicus	Glires	0.769±0.113	31,017,192 + 3,034,654	45,687,808±5,678,958	41,092±7,999	60,426±12,477	1.497±0.328	Herculano-Houzel et al., 2006
Microcebus murinus	Primata	0.908	22,310,400	70,649,600	24,571	77,808	3.167	Gabi et al., 2010
Proechimys cayennensis	Glires	0.924±0.050	26,086,024±2,155,723	71,833,039±6,712,722	28,321±3,870	78,011±11,497	2.752±0.030	Herculano-Houzel et al., 2011
Petrodromus tetradactylus	Afrotheria	1.239±0.059	33,947,000±5,840,000	40,486,000±1,104,000	27,236±3,416	32,550±2,661	1.202±0.053	Neves et al., 2014
Tupaia glis	Scandentia	1.455±0.174	60,390,000±26,510,000	85,580,000±8,400,000	42,900±23,350	58,900±1,270	1.417	Herculano-Houzel et al., 2007
Cavia porcellus	Glires	1.938±0.231	43,510,525±3,169,924	108,614,475±12,775,334	22,508±1,050	56,036±93	2.492±0.112	Herculano-Houzel et al., 2006
Cynomys sp.	Glires	2.586±0.109	53,768,353±6,044,322	183,451,647±17,959,104	20,866±3,113	71,202±9,677	3.432±0.433	Herculano-Houzel et al., 2011
Sciurus carolinensis	Glires	2.730±0.178	77,334,617±2,634,444	209,654,717±13,003,170	28,384±1,487	76,877±3,859	2.709±0.076	Herculano-Houzel et al., 2011
Oryctolagus cuniculus	Glires	4.448	71,448,750	254,801,250	16,063	57,284	3.566	Herculano-Houzel et al., 2011
Callithrix jacchus	Primata	5.561±0.443	244,720,000±81,180,000	395,340,000±81,180,000	44,280±15,900	71,800±14,880	1.615	Herculano-Houzel et al., 2007
Otolemur garnettii	Primata	6.290±0.863	226,090,000±87,570,000	402,070,000±87,570,000	37,820±20,500	63,610±3,400	1.778	Herculano-Houzel et al., 2007
Dendrohyrax dorsalis	Afrotheria	7.56	98,960,000	183,540,000	13,098	24,291	1.855	Neves et al., 2014
Dasyprocta prymnolopha	Glires	8.913±1.214	110,641,950±2,576,768	416,208,050±950,422	13,250±1,633	49,939±7,422	3.763±0.096	Herculano-Houzel et al., 2006
Procavia capensis	Afrotheria	10.478±0.646	197,933,000±29,082,000	366,620,000±13,520,000	19,134±3,955	35,203±3,461	1.883±0.208	Neves et al., 2014
Aotus trivirgatus	Primata	10.617±0.610	441,900,000±111,310,000	695,420,000±130,000,000	41,990±12,900	65,330±5,950	1.574	Herculano-Houzel et al., 2007
Callimico goeldii	Primata	12.984	357,129,180	715,330,820	27,505	55,093	2.003	Gabi et al., 2010
Saimiri sciureus	Primata	20.652±0.368	1,340,000,000±20,000,000	1,610,000,000±40,000,000	64,930±7,420	77,840±790	1.201	Herculano-Houzel et al., 2007
Macaca fascicularis	Primata	36.226	800,955,000	2,758,845,000	22,110	76,156	3.444	Gabi et al., 2010
Cebus apella	Primata	39.178	1,140,000,000	2,550,000,000	29,180	64,980	2.237	Herculano-Houzel et al., 2007
Macaca radiata	Primata	48.274	1,655,707,140	3,808,672,860	34,298	78,897	2.300	Gabi et al., 2010
Sus scrofa domesticus	Artiodactyla	42.404	307,082,404	3,250,251,354	7,276	77,016	10.585	Kazu et al., 2014
Hydrochoerus hydrochaeris	Glires	48.175±2.714	306,501,565±62,726,120	1,847,818,435±512,392,109	6,336±945	38,117±8,489	5.983±0.447	Herculano-Houzel et al., 2006
Antidorcas marsupialis	Artiodactyla	68.806	396,896,159	4,126,259,275	5,768	59,969	10.396	Kazu et al., 2014
Macaca mulatta	Primata	69.832	1,710,000,000	5,270,000,000	24,470	75,400	3.082	Herculano-Houzel et al., 2007
Damaliscus dorcas phillipsi	Artiodactyla	111.310	570,673,431	6,762,256,227	5,127	60,760	11.851	Kazu et al., 2014
Papio anubis cynocephalus	Primata	120.214	2,875,028,372	7,569,751,628	23,916	62,969	2.633	Gabi et al., 2010
Tragelaphus strepsiceros	Artiodactyla	213.370	762,567,178	12,302,304,448	3,574	57,657	16.133	Kazu et al., 2014
Giraffa camelopardalis	Artiodactyla	398.808	1,730,513,460	27,513,706,540	4,339	68,990	15.900	Kazu et al., 2014
Homo sapiens	Primata	1,232.93±233.68	16,340,000,000±2,170,000,000	60,840,000,000±7,020,000,000	13,520±3,636	49,230±3,755	3.723±0.675	Azevedo et al., 2009
Loxodonta africana	Afrotheria	2,847.594	5,593,241,033	55,698,998,687	1,964	52,721	26.844	Herculano-Houzel et al., 2014

All values refer to the sum of gray matter, subcortical white matter, and hippocampus in the two hemispheres. N = Neurons; O = other cells.

As mentioned above, most of the data were obtained from single hemispheres and multiplied by 2 to refer to the entire structures or brain. This allowed one brain hemisphere to be kept for histological analysis, while the other was used for the quantitative analysis discussed here. In all cases, dissections started with a mid-sagittal section through the whole brain. From the available hemisphere, the olfactory bulb was dissected by a transverse cut at the olfactory tract immediately proximal to the bulb, which left the olfactory tract included in the RoB. The cerebellum was dissected next by cutting the cerebellar peduncles at the surface of the brainstem. The cerebral cortex in all animals was defined as all cortical regions lateral to the olfactory tract, including the hippocampus, amygdala and piriform cortex, and dissected from each hemisphere in small brains by peeling it away from the subcortical structures, as described earlier [Herculano-Houzel et al., 2006], or from a complete series of coronal sections after removing the brainstem by a transverse cut along the plane anterior to the superior colliculus and posterior to the hypothalamus. In this manner, the cerebral cortex includes the underlying white matter. All other brain structures (the ensemble of brainstem, diencephalon and striatum) were pooled and processed together as RoB.

The Method

Some authors have expressed concerns about the isotropic fractionator, the method whereby the numbers of cells reported here were obtained [e.g. Carlo and Stevens, 2013; Charvet et al., 2015]. Concerns about the validity of estimates obtained with the isotropic fractionator in comparison to stereology were dispelled when two groups established independently that the isotropic fractionator yields estimates of cell numbers that are comparable in value and variation to those obtained with stereology for matching [Miller et al., 2014] or neighboring [Bahney and von Bartheld, 2014] tissue. The data presented here can therefore be considered to be at least as reliable as data obtained with stereological methods. Most importantly, given the time and histological effort required for stereology, the determination of total numbers of neurons for structures that include widely different subregions such as those in the entire cerebral cortex, entire cerebellum or entire brainstem, would not have been possible without the isotropic fractionator [Herculano-Houzel et al., 2015].

It should be kept in mind that the numbers of neurons in the dataset correspond to the numbers of nuclei that express the universal neuronal nuclear marker NeuN [Mullen et al., 1992]. NeuN is known not to be expressed in some particular neuronal cell types such as Purkinje cells, mitral cells of the olfactory bulb, inferior olivary and dentate nucleus neurons [Mullen et al., 1992], neurons in the substantia nigra pars reticulata of the gerbil [Kumar and Buckmaster, 2007], and possibly others as yet unidentified. While this of course impacts the total number of cells identified as neurons, and unduly inflates the population identified as other cells (nonneurons), we expect this impact to be negligible, given that these specific neuronal subpopulations are very small compared to the structures that they integrate and which were analyzed here – the entire cerebral cortex, cerebellum or RoB.

It should also be kept in mind that, for most species, only one individual was available for study, and typically only one of the two brain halves was used for quantification with the isotropic fractionator. This means that this dataset does not address individual differences or scaling rules across individuals, which are known not to be an extension of allometric rules across species either in terms of brain × body mass [Armstrong, 1990] or in the relationship between brain structure mass and number of neurons [Herculano-Houzel et al., 2015]. Importantly, since only averages or single individual values for a species are reported in the dataset, their use in comparative studies will not confound intraspecific and interspecific variation. Moreover, although intraspecific variation can be as large as 50% in brain structure mass or number of neurons in the mouse [Herculano-Houzel et al., 2015], in the scope of comparative studies, which typically span several orders of magnitude, such variation is usually insignificant.

Numbers of Cells

Although our dataset still excludes the very extremes of brain size in mammals, it ranges from very small shrews *(Sorex fumeus, Blarina brevicauda)* to the African elephant *(Loxodonta africana)*, spanning body masses from 8 to 5,000,000 g and brain masses from 0.2 to over 4,000 g. Total numbers of neurons span from 36 million to 257 billion (that is, 36×10^6 to 257×10^9), and total numbers of other (nonneuronal) cells range from 23 million to 216 billion (table 5). Importantly, in all species, the majority of neurons (53–98%) are located in the cerebellum, leaving the cerebral cortex with typically 15–25% of all brain neurons, and the RoB with not more than 21% and often less than 10% of all brain neurons (ta-

Table 2. Cerebellum

Species	Order	Mass, g	N, n	O, n	N/mg	O/mg	O/N	Source
Sorex fumeus	Eulipotyphla	0.020±0.002	20,870,000±4,660,000	5,290,000±2,120,000	1,038,666±214,440	258,073±85,510	0.253	Sarko et al., 2009
Blarina brevicauda	Eulipotyphla	0.037±0.005	33,430,000±5,821,000	4,410,000±1,280,000	919,942±19,721	118,736±25,620	0.132	Sarko et al., 2009
Heterocephalus glaber	Glires	0.048±0.004	15,742,270±2,849,254	5,482,730±1,274,352	327,280±48,331	115,748±32,952	0.356±0.106	Herculano-Houzel et al., 2011
Mus musculus	Glires	0.056±0.005	42,219,708±9,277,647	6,947,791±1,502,773	746,691±128,541	123,493±25,715	0.165±0.017	Herculano-Houzel et al., 2006
Amblysomus hottentotus	Afrotheria	0.084	34,488,379±3,207,000	8,155,621±813,000	409,687±18,667	96,849±5,069	0.236±0.002	Neves et al., 2014
Parascalops breweri	Eulipotyphla	0.102±0.005	100,780,000±13,850,000	7,010,000±1,100,000	997,370±173,030	68,795±7,910	0.070	Sarko et al., 2009
Condylura cristata	Eulipotyphla	0.138±0.012	105,920,000±22,100,000	19,480,000±6,420,000	776,460±181,530	139,912±39,720	0.184	Sarko et al., 2009
Mesocricetus auratus	Glires	0.145±0.030	61,210,000±12,351,246	7,430,000±1,713,108	424,002±3,743	51,332±1,054	0.121±0.004	Herculano-Houzel et al., 2006
Scalopus aquaticus	Eulipotyphla	0.153±0.008	158,550,000±13,630,000	17,510,000±3,160,000	1,037,390±63,570	114,660±20,790	0.110	Sarko et al., 2009
Elephantulus myurus	Afrotheria	0.168	89,312,372±2,852,000	23,368,628±1,279,000	531,494±10,651	139,028±5,956	0.261±0.005	Neves et al., 2014
Rattus norvegicus	Glires	0.272±0.038	139,171,882±11,185,675	29,005,617±6,282,204	522,688±108,847	108,555±29,355	0.209±0.047	Herculano-Houzel et al., 2006
Petrodromus tetradactylus	Afrotheria	0.304	110,653,150±14,948,000	34,657,851±15,801,000	362,537±15,780	110,153±41,831	0.299±0.102	Neves et al., 2014
Tupaia glis	Scandentia	0.326±0.018	185,280,000±16,980,000	19,980,000±1,510,000	571,460±83,200	61,600±7,990	0.108	Herculano-Houzel et al., 2007
Proechimys cayennensis	Glires	0.330±0.026	162,512,050±3,553,848	36,372,950±5,094,068	494,338±28,466	110,122±6,716	0.224±0.026	Herculano-Houzel et al., 2011
Microcebus murinus	Primata	0.391	221,386,140	17,433,860	566,205	44,588	0.079	Gabi et al., 2010
Cavia porcellus	Glires	0.500±0.077	167,854,925±2,175,973	36,290,075±4,506,186	339,755±48,069	72,824±2,216	0.216±0.024	Herculano-Houzel et al., 2006
Callithrix jacchus	Primata	0.730±0.039	361,370,000±28,530,000	49,490,000±6,770,000	494,970±25,740	68,170±12,210	0.137	Herculano-Houzel et al., 2007
Cynomys sp.	Glires	0.789±0.093	350,084,813±72,177,851	66,155,187±39,268,821	440,658±39,554	84,802±54,286	0.192±0.123	Herculano-Houzel et al., 2011
Sciurus carolinensis	Glires	0.874±0.069	342,832,180±71,181,798	110,797,820±9,070,639	392,363±74,294	127,682±19,212	0.336±0.090	Herculano-Houzel et al., 2011
Otolemur garnettii	Primata	1.196±0.105	743,500,000±52,450,000	65,960,000±20,290,000	623,080±45,720	54,460±11,890	0.089	Herculano-Houzel et al., 2007
Oryctolagus cuniculus	Glires	1.412	396,671,250	124,578,750	280,929	88,229	1.222	Herculano-Houzel et al., 2011
Aotus trivirgatus	Primata	1.732±0.218	1,040,000,000±20,000,000	145,270,000±45,030,000	605,080±90,570	82,890±15,580	0.140	Herculano-Houzel et al., 2007
Dendrohyrax dorsalis	Afrotheria	1.918	360,929,350	77,570,650	188,180	40,444	0.215	Neves et al., 2014
Procavia capensis	Afrotheria	2.058	488,373,000±42,322,000	91,005,000±30,180,000	242,415±46,950	46,365±19,711	0.182±0.046	Neves et al., 2014
Dasyprocta prymnolopha	Glires	2.742	673,488,085±48,145,960	155,986,915±32,978,520	253,208±39,447	57,945±7,405	0.234±0.066	Herculano-Houzel et al., 2006
Saimiri sciureus	Primata	4.300	1,820,000,000	133,020,000	424,000	30,940	0.073	Herculano-Houzel et al., 2007
Cebus apella	Primata	4.6	2,490,000,000	245,810,000	540,310	53,440	0.099	Herculano-Houzel et al., 2007
Macaca fascicularis	Primata	5.642	2,572,600,000	135,400,000	455,973	23,999	0.053	Gabi et al., 2010
Macaca radiata	Primata	5.748	2,038,554,160	453,565,840	354,655	78,908	0.222	Gabi et al., 2010
Hydrochoerus hydrochaeris	Glires	6.632±1.312	1,157,810,000±5,515,433	570,940,000±81,105,148	177,982±34,889	86,574±4,902	0.493±0.068	Herculano-Houzel et al., 2006
Macaca mulatta	Primata	7.694	4,550,000,000	931,030,000	590,800	121,010	0.205	Herculano-Houzel et al., 2007
Sus scrofa domesticus	Artiodactyla	8.128	1,858,320,313	348,710,938	228,632	42,902	0.188	Kazu et al., 2014
Antidorcas marsupialis	Artiodactyla	11.458	2,257,214,074	467,244,676	196,999	40,779	0.207	Kazu et al., 2014
Damaliscus dorcas phillipsi	Artiodactyla	13.402	2,401,712,670	443,918,456	179,206	33,123	0.184	Kazu et al., 2014
Papio anubis cynocephalus	Primata	13.745	7,794,907,300	525,977,700	567,109	38,267	0.067	Gabi et al., 2010
Tragelaphus strepsiceros	Artiodactyla	31.776	4,042,494,141	1,266,099,609	127,218	39,845	0.313	Kazu et al., 2014
Pongo pygmaeus	Primata	35.06±4.34	26,300,000,000±2,470,000,000	2,200,000,000	750,143	62,750	0.084	Herculano-Houzel and Kaas, 2011
Gorilla gorilla	Primata	37.56	26,400,000,000	2,900,000,000	702,875	77,210	0.110	Herculano-Houzel and Kaas, 2011
Giraffa camelopardalis	Artiodactyla	67.73	8,878,076,563	5,520,360,938	131,080	81,505	0.622	Kazu et al., 2014
Homo sapiens	Primata	154.02±19.29	69,030,000,000±6,650,000,000	16,040,000,000±2,170,000	471,660±90,393	101,020±19,800	0.232±0.019	Azevedo et al., 2009
Loxodonta africana	Afrotheria	1,171.628	250,708,527,536	38,507,229,964	213,983	32,866	0.154	Herculano-Houzel et al., 2014

All numbers refer to the whole cerebellum (both brain halves), including the deep nuclei. The cerebellum of *Callimico goeldii* was not available for analysis. N = Neurons; O = other cells.

Table 3. RoB

Species	Order	Mass, g	N, n	O, n	N/mg	O/mg	O/N	Source
Sorex fumeus	Eulipotyphla	0.072±0.010	5,560,000±1,490,000	8,280,000±1,440,000	75,941±11,870	114,880±17,860	1.489±0.294	Sarko et al., 2009
Blarina brevicauda	Eulipotyphla	0.113±0.009	6,340,000±636,200	13,320,000±1,120,000	55,924±2,130	117,736±5,840	2.102±0.096	Sarko et al., 2009
Heterocephalus glaber	Glires	0.160±0.019	4,981,317±853,026	10,310,350±1,843,572	31,674±8,484	64,145±3,790	2.125	Herculano-Houzel et al., 2011
Mus musculus	Glires	0.172±0.019	11,960,000±1,577,022	14,850,000±3,590,591	64,301±6,623	79,839±11,249	1.242	Herculano-Houzel et al., 2006
Parascalops breweri	Eulipotyphla	0.228±0.009	7,130,000±920,000	31,380,000±1,500,000	31,276±3,180	137,960±7,180	4.401±0.375	Sarko et al., 2009
Condylura cristata	Eulipotyphla	0.244±0.026	8,160,000±2,180,000	31,550,000±5,950,000	33,475±8,560	128,990±17,820	3.866±1.692	Sarko et al., 2009
Amblysomus hottentotus	Afrotheria	0.289±0.013	9,070,358±1,069,000	16,834,642±3,276,000	31,616±5,124	58,880±13,983	1.839±0.144	Neves et al., 2014
Scalopus aquaticus	Eulipotyphla	0.370±0.042	16,560,000±2,990,000	45,690,000±4,820,000	44,620±4,900	123,600±2,550	2.759±0.379	Sarko et al., 2009
Mesocricetus auratus	Glires	0.375±0.057	5,870,000±1,162,059	21,330,000±8,878,857	15,611±715	55,804±15,174	3.556	Herculano-Houzel et al., 2006
Elephantulus myurus	Afrotheria	0.401±0.063	14,012,533±3,258,000	28,997,467±7,906,000	34,520±2,702	70,967±8,566	2.048±0.088	Neves et al., 2014
Microcebus murinus	Primata	0.500	11,014,640	50,865,360	22,029	101,731	4.618	Gabi et al., 2010
Rattus norvegicus	Glires	0.683±0.153	18,678,758±4,243,909	47,221,243±5,979,982	27,813±6,171	71,760±18,475	2.587	Herculano-Houzel et al., 2006
Proechimys cayennensis	Glires	0.824±0.006	13,492,039±1,533,120	73,068,898±2,691,449	16,381±1,973	88,667±2,658	5.462	Herculano-Houzel et al., 2011
Petrodromus tetradactylus	Afrotheria	0.894±0.022	12,231,811±188,000	28,204,189±5,408,000	13,696±547	31,716±6,830	2.300±0.407	Neves et al., 2014
Tupaia glis	Scandentia	0.919±0.072	22,480,000	87,080,000	25,900	100,320	3.874	Herculano-Houzel et al., 2007
Cavia porcellus	Glires	1.218±0.178	22,191,825±9,431,638	83,183,175±8,139,966	18,990±10,522	69,528±16,855	4.035	Herculano-Houzel et al., 2006
Callithrix jacchus	Primata	1.489±0.317	29,720,000±9,350,000	145,910,000±27,830,000	19,650±2,840	98,370	4.909	Herculano-Houzel et al., 2007
Sciurus carolinensis	Glires	1.943±0.156	33,493,401±17,128,790	210,023,933±3,415,060	17,616±9,989	108,482±7,808	7.246	Herculano-Houzel et al., 2011
Cynomys sp.	Glires	1.945±0.146	34,090,600±589,361	167,742,733±5,025,290	17,590±1,335	86,542±6,793	4.923	Neves et al., 2014
Callimico goeldii	Primata	2.086	53,911,480	194,528,520	25,844	93,254	3.608	Gabi et al., 2010
Otolemur garnettii	Primata	2.131±0.021	20,800,000±12,280,000	147,440,000±64,500,000	9,730±5,920	69,040	7.088	Herculano-Houzel et al., 2007
Aotus trivirgatus	Primata	3.104	49,340,000	313,460,000	15,900	100,990	6.353	Herculano-Houzel et al., 2007
Oryctolagus cuniculus	Glires	3.272	26,085,000	251,415,000	7,972	76,838		Herculano-Houzel et al., 2011
Dendrohyrax dorsalis	Afrotheria	3.328	44,671,471	152,466,529	13,423	45,813	3.413	Neves et al., 2014
Macaca fascicularis	Primata	4.294	65,449,620	260,170,380	15,242	60,589	3.975	Gabi et al., 2010
Procavia capensis	Afrotheria	4.317±0.625	69,358,240±762,000	162,984,760±6,105,000	16,436±2,556	38,353±4,138	2.351±0.114	Neves et al., 2014
Saimiri sciureus	Primata	5.004	65,530,000	302,590,000	13,090	60,470	4.618	Herculano-Houzel et al., 2007
Dasyprocta prymnolopha	Glires	5.972±0.514	43,204,320	356,835,680	6,830	56,408		Herculano-Houzel et al., 2006
Macaca radiata	Primata	7.448	61,359,000	616,641,000	8,238	82,793	10.050	Gabi et al., 2010
Cebus apella	Primata	8.430	61,850,000	506,110,000	7,340	60,040	8.183	Herculano-Houzel et al., 2007
Macaca mulatta	Primata	9.204±0.871	121,900,000	966,520,000	12,410	98,420	7.929	Herculano-Houzel et al., 2007
Sus scrofa domesticus	Artiodactyla	13.850	58,709,836	1,096,821,414	4,238	79,192	18.682	Kazu et al., 2014
Papio anubis cynocephalus	Primata	17.235	278,150,760	1,079,809,236	16,136	62,656	3.882	Gabi et al., 2010
Hydrochoerus hydrochaeris	Glires	19.927±0.270	108,250,000±4,400,000	779,170,000±381,900,000	5,434	39,234		Herculano-Houzel et al., 2006
Antidorcas marsupialis	Artiodactyla	25.810	70,485,000	1,319,280,624	2,731	51,115	18.710	Kazu et al., 2014
Damaliscus dorcas phillipsi	Artiodactyla	30.006	86,428,126	2,136,071,876	2,880	71,188	24.718	Kazu et al., 2014
Tragelaphus strepsiceros	Artiodactyla	61.716	106,590,230	3,408,779,523	1,727	55,233	31.980	Kazu et al., 2014
Giraffa camelopardalis	Artiodactyla	70.680	142,697,625	4,878,864,876	2,019	69,028	34.190	Kazu et al., 2014
Homo sapiens	Primata	117.660±45.42	690,000,000±120,000,000	7,730,000,000±1,450,000,000	6,560±2,115	69,850±20,026	11.203±2.352	Azevedo et al., 2009
Loxodonta africana	Afrotheria	564.674	741,704,844	27,404,306,156	1,314	48,531	36.948	Herculano-Houzel et al., 2014

All values refer to the ensemble of brainstem, diencephalon and basal ganglia, for both sides of the brain. N = Neurons; O = other cells.

Table 4. Olfactory bulb

Species	Order	n	Mass, g	N, n	O, n	N/mg	O/mg	O/N	Source
Callithrix jacchus	Primata	5	0.008±0.014	2,108,078±983,420	2,547,922±960,104	232,309±137,605	269,383±105,563	1.209	Ribeiro et al., 2014
Sorex fumeus	Eulipotyphla	3	0.012±0.002	3,330,000±1,050,000	2,760,000±130,000	289,806±124,350	235,249±44,620	0.829±214	Sarko et al., 2009
Mus musculus	Glires	4	0.014±0.004	3,893,300±1,246,396	5,456,700±1,154,502	257,475±34,036	371,204±75,573	1.454±0.260	Herculano-Houzel et al., 2006
Heterocephalus glaber	Glires	3	0.021±0.001	2,303,030±636,099	3,571,970±1,548,861	108,895±22,957	167,991±62,442	1.516±0.254	Herculano-Houzel et al., 2011
Blarina brevicauda	Eulipotyphla	5	0.026±0.003	8,090,000±935,900	4,910,000±730,000	318,164±34,950	193,631±31,450	0.607±0.125	Sarko et al., 2009
Microcebus murinus	Primata	2	0.030±0.008	7,636,912±119,088	9,723,088±842,576	270,894±61,946	341,900±54,311	1.273	Ribeiro et al., 2014
Condylura cristata	Eulipotyphla	4	0.040±0.005	10,550,000±4,290,000	7,470,000±970,000	254,720±74,620	185,124±19,370	0.708±0.328	Sarko et al., 2009
Parascalops breweri	Eulipotyphla	3	0.049±0.008	16,750,000±6,370,000	10,910,000±3,400,000	333,590±81,590	217,440±41,370	0.651±0.168	Sarko et al., 2009
Elephantulus myurus	Afrotheria	2	0.050±0.010	9,693,534±1,745,000	4,919,466	194,678±5,708	97,872	0.507	Neves et al., 2014
Aotus trivirgatus	Primata	6	0.050±0.012	7,925,468±3,114,924	8,360,532±3,213,106	155,879±62,241	162,922±44,209	1.055	Ribeiro et al., 2014
Mesocricetus auratus	Glires	2	0.055±0.011	5,747,930±347,203	5,507,170±2,277,605	105,418±27,498	96,197±21,237	0.972±0.455	Herculano-Houzel et al., 2006
Rattus norvegicus	Glires	5	0.074±0.022	11,103,272±3,202,766	9,238,728±2,250,728	152,373±26,913	126,210±8,033	0.848±0.145	Herculano-Houzel et al., 2006
Scalopus aquaticus	Eulipotyphla	3	0.082±0.005	34,610,000±5,960,000	17,780,000±2,060,000	423,520±94,950	215,230±14,600	0.514±0.154	Sarko et al., 2009
Macaca mulatta	Primata	1	0.088	8,473,800	11,006,200	96,293	125,070	1.299	Ribeiro et al., 2014
Tupaia glis	Scandentia	16	0.100±0.032	12,700,000±3,584,952	20,068,000±6,541,838	130,173±17,451	205,876±51,596	1.580	Herculano-Houzel et al., 2007
Cavia porcellus	Glires	2	0.103±0.013	6,065,700±1,295,335	10,154,300±4,220,098	58,560±5,340	96,793±29,011	1.637±0.346	Herculano-Houzel et al., 2006
Proechimys cayennensis	Glires	1	0.132	9,141,540	21,128,460	69,254	160,064	2.311	Herculano-Houzel et al., 2011
Oryctolagus cuniculus	Glires	1	0.156	18,765,000	22,935,000	120,288	147,019	1.222	Herculano-Houzel et al., 2011
Petrodromus tetradactylus	Afrotheria	2	0.159±0.009	12,828,365±380,000	14,775,635	80,805±3,084	91,369	1.141	Neves et al., 2014
Otolemur garnettii	Primata	11	0.200±0.016	30,237,060±9,645,480	34,244,000±7,155,556	149,219±47,590	170,505±39,463	1.133	Ribeiro et al., 2014
Sciurus carolinensis	Glires	9	0.212±0.022	28,845,724±7,903,412	39,015,942±12,812,316	137,532±38,236	185,359±58,050	1.478±0.776	Herculano-Houzel et al., 2011
Procavia capensis	Afrotheria	1	0.286	20,909,490	14,790,510	73,110	51,715	0.707	Neves et al., 2014
Dasyprocta prymnolopha	Glires	3	0.737±0.162	58,124,085±4,952,795	72,595,915±19,682,805	88,008±14,973	107,301±1,958	1.239±0.233	Herculano-Houzel et al., 2006
Sus scrofa domesticus	Artiodactyla	1	0.822	9,195,500	77,554,500	11,187	94,348	8.434	Kazu et al., 2014
Antidorcas marsupialis	Artiodactyla	1	1.200	15,998,400	105,201,600	13,332	87,668	6.576	Kazu et al., 2014
Hydrochoerus hydrochaeris	Glires	2	1.302±0.031	28,560,310±8,515,588	67,389,690±21,015,416	21,864±6,018	51,544±17,981	2.333±0.180	Herculano-Houzel et al., 2006
Giraffa camelopardalis	Artiodactyla	1	2.052	24,678,000	232,384,500	12,026	113,248	9.417	Kazu et al., 2014
Tragelaphus strepsiceros	Artiodactyla	1	5.546	38,331,562	362,731,438	6,912	58,913	8.523	Kazu et al., 2014
Loxodonta africana	Afrotheria	1	41.886	908,371,986	2,857,878,014	21,687	68,230	3.146	Herculano-Houzel et al., 2014

All values refer to both olfactory bulbs. N = Neurons; O = other cells.

Table 5. Whole brain

Species	Order	n	Body mass, g	Brain mass, g	Neurons	Other cells	% Neurons	Source
Sorex fumeus	Eulipotyphla	3	7.8±0.1	0.176±0.007	36,460,000±4,567,000	22,860,000±3,956,000	61.3±1.4	Sarko et al., 2009
Blarina brevicauda	Eulipotyphla	5	16.2±1.6	0.347±0.018	55,190,000±6,126,000	33,550,000±1,222,000	64.8±2.3	Sarko et al., 2009
Heterocephalus glaber	Glires	3	23.3±5.9	0.392±0.045	26,875,462±3,340,087	24,191,205±1,739,102	52.5±3.3	Herculano-Houzel et al., 2011
Mus musculus	Glires	4	40.4±11.6	0.402±0.028	67,873,741±10,406,194	33,858,759±6,657,119	65.3±2.3	Herculano-Houzel et al., 2006
Parascalops breweri	Eulipotyphla	3	42.7±9.1	0.759±0.024	123,600,000±12,470,000	78,260,000±6,095,000	61.2±4.1	Sarko et al., 2009
Condylura cristata	Eulipotyphla	4	41.1±4.7	0.802±0.046	131,330,000±21,229,000	83,040,000±19,046,000	61.3±6.3	Sarko et al., 2009
Amblysomus hottentotus	Afrotheria	2	79.0	0.812±0.044	65,074,000±2,124,000	46,631,000±527,000		Neves et al., 2014
Mesocricetus auratus	Glires	2	168.1±13.6	0.965±0.136	84,220,000±9,893,371	70,640,000±11,942,086	54.3±1.3	Herculano-Houzel et al., 2006
Scalopus aquaticus	Eulipotyphla	3	95.3±9.8	0.999±0.080	203,520,000±14,587,000	101,740,000±11,823,000	66.7±1.4	Sarko et al., 2009
Elephantulus myurus	Afrotheria	2	45.1	1.040±0.082	129,190,603±4,424,000	78,594,397±7,733,000		Neves et al., 2014
Rattus norvegicus	Glires	4	315.1±102.9	1.724±0.292	188,867,832±12,622,383	121,914,668±7,106,729	60.7±2.4	Herculano-Houzel et al., 2006
Microcebus murinus	Primata	1	60.0	1.799	254,711,180	138,948,820	64.7	Gabi et al., 2010
Proechimys cayennensis	Glires	2	223.485±16.6	2.078±0.071	202,090,113±2,931,245	181,274,887±4,310,103	52.7±1.0	Herculano-Houzel et al., 2011
Petrodromus tetradactylus	Afrotheria	2	132.5	2.440±0.109	156,830,795±20,600,000	103,349,000±15,610,000		Neves et al., 2014
Tupaia glis	Scandentia	2	172.5±3.5	2.752±0.011	261,400,000	199,650,000	56.7	Herculano-Houzel et al., 2007
Cavia porcellus	Glires	2	311.0±49.1	3.656±0.486	233,557,275±4,085,741	228,087,725±9,141,554	50.6±1.4	Herculano-Houzel et al., 2006
Cynomys sp.	Glires	3	1,515±230.6	5.321±0.197	437,943,767±78,742,230	417,349,567±27,350,023	51.0±4.2	Herculano-Houzel et al., 2011
Sciurus carolinensis	Glires	3	500	5.548±0.306	453,660,197±59,752,698	530,476,469±5,605,837	46.0±3.0	Herculano-Houzel et al., 2011
Callithrix jacchus	Primata	3	361.0±1.4	7.780±0.654	635,800,000±115,730,000	590,740,000±70,810,000	51.7	Herculano-Houzel et al., 2007
Oryctolagus cuniculus	Glires	1	4,600	9.132	494,205,000	630,795,000	43.9	Gabi et al., 2010
Otolemur garnettii	Primata	3	946.7±102.6	10.150±0.060	936,000,000±115,360,000	666,590,000±63,500,000	58.4	Herculano-Houzel et al., 2007
Dendrohyrax dorsalis	Afrotheria	1	1,150	12.800	504,572,834	413,574,000		Neves et al., 2014
Aotus trivirgatus	Primata	2	925±35	15.730	1,468,410,000	1,195,130,000	55.1	Herculano-Houzel et al., 2007
Procavia capensis	Afrotheria	2	2,517	16.853±1.495	755,653,000±72,145,000	620,622,000±37,616,000		Neves et al., 2014
Dasyprocta prymnolopha	Glires	3	2,843±196	17.628±1.900	795,112,070	951,677,930	45.5	Herculano-Houzel et al., 2006
Saimiri sciureus	Primata	2	859	30.216	3,246,430,000	2,073,030,000	61	Herculano-Houzel et al., 2007
Macaca fascicularis	Primata	1	5,700	46.162	3,439,004,620	3,154,415,380	52.2	Gabi et al., 2010
Cebus apella	Primata	1	3,340	52.208	3,690,520,000	3,297,740,000	52.8	Herculano-Houzel et al., 2007
Macaca radiata	Primata	1	8,012	61.470	3,755,620,300	4,878,879,700	43.5	Gabi et al., 2010
Sus scrofa domesticus	Artiodactyla	1	100,000	64.180	2,224,112,553	4,695,783,705	32.1	Kazu et al., 2014
Hydrochoerus hydrochaeris	Glires	2	47,500±3,536	74.734±3.756	1,572,560,385±72,641,426	3,197,929,615±974,583,717	33.6±5.8	Herculano-Houzel et al., 2006
Macaca mulatta	Primata	1	3,900	87.346	6,376,160,000	7,162,900,000	47.1	Herculano-Houzel et al., 2007
Antidorcas marsupialis	Artiodactyla	1	25,000	106.074	2,724,595,233	5,912,784,575	31.5	Kazu et al., 2014
Papio anubis cynocephalus	Primata	2	8,000	151.194	10,948,086,440	9,175,538,564	54.4	Gabi et al., 2010
Damaliscus dorcas phillipsi	Artiodactyla	1	60,000	154.718	3,058,814,227	9,343,246,559	24.7	Kazu et al., 2014
Tragelaphus strepsiceros	Artiodactyla	1	218,000	306.860	4,911,651,549	16,977,183,580	22.4	Kazu et al., 2014
Giraffa camelopardalis	Artiodactyla	1	470,000	537.218	10,751,287,650	37,912,932,350	23.5	Kazu et al., 2014
Homo sapiens	Primata	4	70,000	1,508.910±299.140	86,060,000,000±8,120,000,000	84,610,000,000±9,830,000,000	50.5±3.6	Azevedo et al., 2009
Loxodonta africana	Afrotheria	1	5,000,000	4,618.620	257,043,473,412	216,057,982,337		Herculano-Houzel et al., 2014

All values refer to the whole brain (both sides), not including the olfactory bulbs. Not listed are *Callimico goeldii*, *Gorilla gorilla* and *Pongo pygmaeus*, for which not all brain structures were available.

Table 6. Relative distributions of mass and numbers of neurons across brain structures

Species	Order	M_{BRAIN}, g	% M_{Cx}	% M_{Cb}	% M_{RoB}	% N_{Cx}	% N_{Cb}	% N_{RoB}	Source
Sorex fumeus	Eulipotyphla	0.176	47.6	11.4	41.0	26.9	57.7	15.4	Sarko et al., 2009
Blarina brevicauda	Eulipotyphla	0.347	56.7	10.6	32.6	27.9	60.6	11.5	Sarko et al., 2009
Heterocephalus glaber	Glires	0.392±0.045	46.9±2.3	12.3±1.5	40.8±1.0	23.2±5.1	58.3±3.9	18.5±1.5	Herculano-Houzel et al., 2011
Mus musculus	Glires	0.402±0.028	41.7±2.8	13.5±0.8	44.8±2.8	19.6±4.1	59.0±5.0	21.3±2.4	Herculano-Houzel et al., 2006
Parascalops breweri	Eulipotyphla	0.759	56.5	13.4	30.0	12.7	81.5	5.8	Sarko et al., 2009
Condylura cristata	Eulipotyphla	0.802	52.4	17.2	30.4	13.1	80.7	6.2	Sarko et al., 2009
Amblysomus hottentotus	Afrotheria	0.812±0.044	54.1	10.3	35.6	33.1	53.0	13.9	Neves et al., 2014
Mesocricetus auratus	Glires	0.965±0.136	46.3±1.5	14.9±1.1	38.8±0.5	20.7±6.7	72.3±6.2	6.9±0.6	Herculano-Houzel et al., 2006
Scalopus aquaticus	Eulipotyphla	0.999	47.6	15.3	37.0	14.1	77.8	8.1	Sarko et al., 2009
Elephantulus myurus	Afrotheria	1.040±0.082	45.3	16.2	38.6	20.0	69.1	10.8	Neves et al., 2014
Rattus norvegicus	Glires	1.724±0.292	44.8±1.8	15.8±1.1	39.4±2.6	16.4±1.2	73.7±3.5	9.9±2.2	Herculano-Houzel et al., 2006
Microcebus murinus	Primata	1.799	50.5	21.7	27.8	8.8	86.9	4.3	Gabi et al., 2010
Proechimys cayennensis	Glires	2.078±0.071	44.5±0.9	15.8±0.7	39.7±1.6	12.9±1.3	80.4±0.6	6.7±0.7	Herculano-Houzel et al., 2011
Petrodromus tetradactylus	Afrotheria	2.440±0.109	50.8	12.5	36.6	21.6	70.6	7.8	Neves et al., 2014
Tupaia glis	Scandentia	2.752±0.011	52.9±6.1	11.8±0.7	35.3±5.4	15.9	75.5	8.6	Herculano-Houzel et al., 2007
Cavia porcellus	Glires	3.656±0.486	53.1±0.7	13.6±0.3	33.3±0.4	18.6±1.7	71.9±2.2	9.5±3.9	Herculano-Houzel et al., 2006
Cynomys sp.	Glires	5.321±0.197	48.6±2.4	14.8±1.6	36.5±1.4	12.4±0.8	79.7±2.0	7.9±1.2	Herculano-Houzel et al., 2011
Sciurus carolinensis	Glires	5.548±0.306	49.2±2.1	15.8±1.3	35±1.1	17.2±1.6	75.1±6.2	7.7±4.8	Herculano-Houzel et al., 2007
Callithrix jacchus	Primata	7.780±0.654	71.6±3.0	9.4±0.4	19.0±3.2	37.8±5.6	57.6±6.0	4.6±1.0	Herculano-Houzel et al., 2007
Oryctolagus cuniculus	Glires	9.132	48.7	15.5	35.8	14.5	80.3	5.3	Herculano-Houzel et al., 2011
Otolemur garnettii	Primata	10.150±0.060	66.8±1.5	12.2±1.2	21.0±0.3	18.9±0.8	79±1.9	2.2±1.1	Herculano-Houzel et al., 2007
Dendrohyrax dorsalis	Afrotheria	12.800	59.0	15.0	26.0	19.6	71.5	8.9	Neves et al., 2014
Aotus trivirgatus	Primata	15.730	70.2	10.0	19.7	24.7	71.9	3.4	Herculano-Houzel et al., 2007
Procavia capensis	Afrotheria	16.853±1.495	62.2	12.2	25.6	26.1	64.7	9.1	Neves et al., 2014
Dasyprocta prymnolopha	Glires	17.628±1.900	50.5±1.6	15.6±0.6	33.9±1.1	14.1	80.4	5.4	Herculano-Houzel et al., 2006
Saimiri sciureus	Primata	30.216	69.2	14.2	16.6	41.8	56.2	2.0	Herculano-Houzel et al., 2007
Macaca fascicularis	Primata	46.162	78.5	12.2	9.3	23.3	74.8	1.9	Gabi et al., 2010
Cebus apella	Primata	52.208	75	8.8	16.1	31.0	67.4	1.7	Herculano-Houzel et al., 2007
Macaca radiata	Primata	61.47	78.5	9.4	12.1	44.1	54.3	1.6	Gabi et al., 2010
Sus scrofa domesticus	Artiodactyla	64.180	65.8	12.7	21.6	13.8	83.6	2.6	Kazu et al., 2014
Hydrochoerus hydrochaeris	Glires	74.734±3.756	64.5±0.4	8.8±1.3	26.7±1.7	19.4±3.1	73.7±3.1	6.9±0.0	Herculano-Houzel et al., 2006
Macaca mulatta	Primata	87.346	79.9	8.8	10.5	26.8	71.3	1.9	Herculano-Houzel et al., 2007
Antidorcas marsupialis	Artiodactyla	106.074	64.9	10.8	24.3	14.6	82.8	2.6	Kazu et al., 2014
Papio anubis cynocephalus	Primata	151.194	79.5	9.1	11.4	26.3	71.2	2.5	Gabi et al., 2010
Damaliscus dorcas phillipsi	Artiodactyla	154.718	71.9	8.7	19.4	18.7	78.5	2.8	Kazu et al., 2014
Tragelaphus strepsiceros	Artiodactyla	306.860	70.6	10.0	19.4	15.5	82.3	2.2	Kazu et al., 2014
Giraffa camelopardalis	Artiodactyla	537.218	74.2	12.6	13.2	16.1	82.6	1.3	Kazu et al., 2014
Homo sapiens	Primata	1,508.910±299.140	81.7±3.1	10.2±1.2	7.8±3.0	19.0±1.8	80.2±1.8	0.8±0.3	Azevedo et al., 2009
Loxodonta africana	Afrotheria	4,618.620	62.1	25.6	12.3	2.2	97.5	0.3	Herculano-Houzel et al., 2014

All values refer to the percentage of mass or number of neurons contained in the structure in comparison to the whole brain, not including the olfactory bulbs. Not listed are *Callimico goeldii, Gorilla gorilla* and *Pongo pyg-maeus*, for which not all brain structures were available. Cx = Cerebral cortex; Cb = cerebellum.

ble 6). This translates into a smaller range of 6–742 million neurons in the RoB (table 3), in contrast to 6 million to 16 billion neurons in the cerebral cortex (table 1), and 16 million to as many as 251 billion neurons in the cerebellum (table 2). In comparison to the cerebral cortex and cerebellum, the number of neurons in the RoB is thus remarkably small: no species has over 1 billion neurons in the RoB, even in the primate and artiodactyl brains with several billion neurons in the cerebral cortex and cerebellum.

Outliers

As described previously [Azevedo et al., 2009; Herculano-Houzel, 2009, 2012], the availability of data on the cellular composition of the cerebral cortex of humans and various other primates allowed us to establish that the human cerebral cortex is not an outlier in its cellular composition, when compared to other primate brains. The human cerebral cortex, in particular, is not an outlier in the number of neurons for its mass. As shown in figure 2,
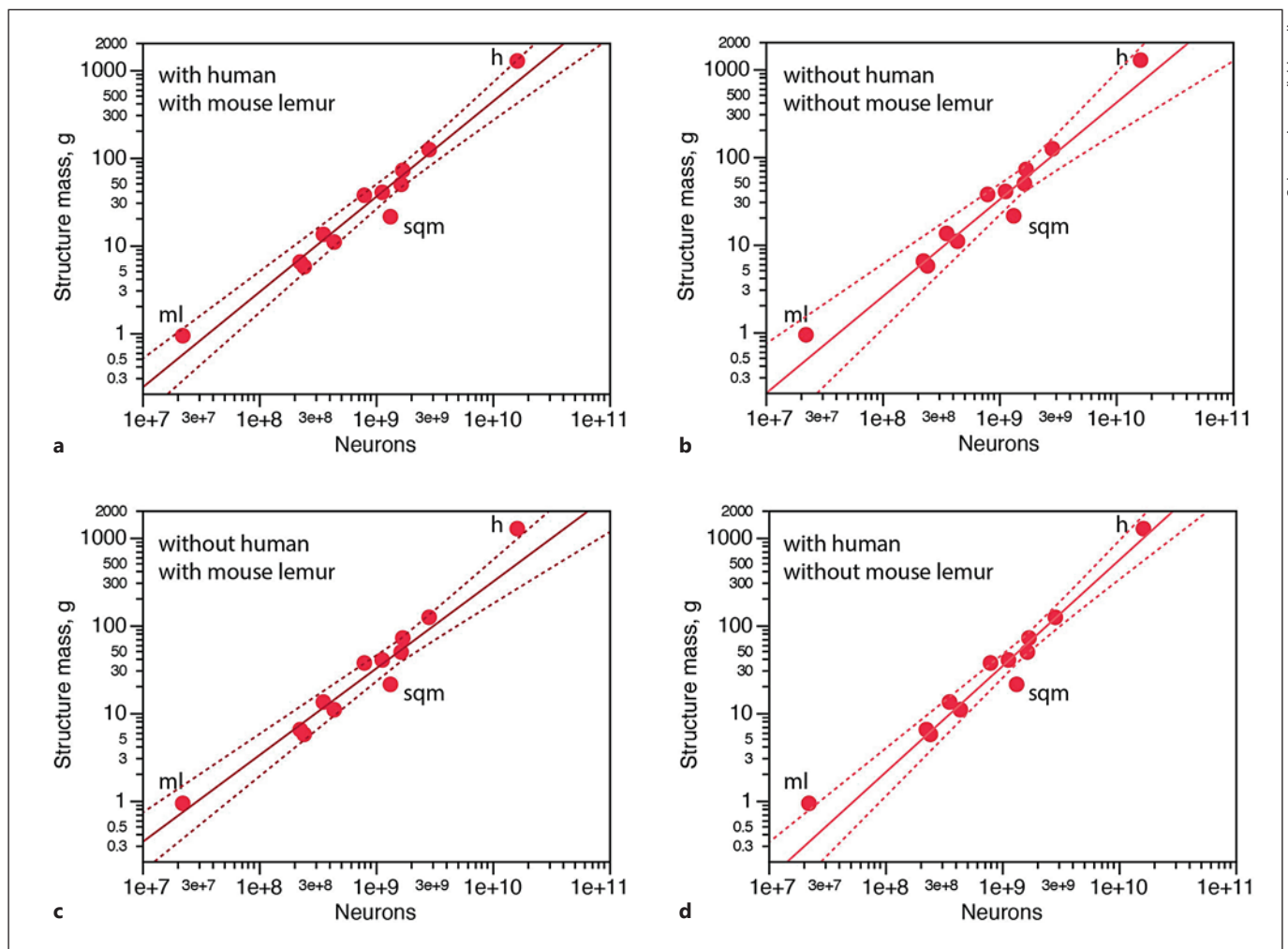
Fig. 2. The human cerebral cortex is not an outlier in its neuronal scaling rule. All graphs show how the mass of the cerebral cortex varies with the number of neurons in the structure for the same data points for the non-great-ape primate species in the dataset. Power functions plotted differ across graphs, as indicated: including the mouse lemur (ml) and human (h) data points (the best fit, with exponent 1.087 ± 0.073, $r^2 = 0.956$, $p < 0.0001$; **a**), excluding the mouse lemur and human data points (the worst fit, with exponent 1.105 ± 0.127, $r^2 = 0.904$, $p < 0.0001$; **b**), including the mouse lemur but excluding human (exponent 0.989 ± 0.080, $r^2 = 0.944$, $p < 0.0001$; **c**), and including human but excluding mouse lemur (exponent 1.210 ± 0.088, $r^2 = 0.944$, $p < 0.0001$; **d**). sqm = Squirrel monkey.

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when either all species (including the human and mouse lemur; fig. 2a) or only the center species in the distribution (excluding the two extremes, human and mouse lemur; fig. 2b) are used to calculate the relationship between cortical mass (including white matter) and number of cortical neurons, the human data point is well within the 95% confidence interval. The human cerebral cortex is only outside the confidence interval when the mouse lemur is included in the comparison (fig. 2c), but in turn the mouse lemur is the outlier in the relationship that excludes it but includes the human cerebral cortex (fig. 2d). The discordance reflects the influence of extreme data points in the calculation of fitted functions, but importantly neither mouse lemur nor human are outliers in comparison to the relationships that either include or exclude both. Instead, it is another species – of the genus *Saimiri* – that systematically sits outside the confidence intervals because of its atypically high neuronal density and absolute number of neurons in the cerebral cortex. Still, because of its relatively central position in the distribution of primate species, the inclusion or exclusion of *Saimiri* does not markedly affect the scaling rules that apply to primates. It is those species that have either very small or very large brains that possibly have a much larger impact on scaling relationships.

One such clear outlier in the allometric scaling rules that we have described previously is the naked mole-rat, which has only about half the number of neurons expected in a rodent cerebral cortex and cerebellum of its size, possibly due to regressive events such as reduced eyes, lateral geniculate nucleus and visual cortex [Catania and Remple, 2002, Xiao et al., 2006] caused by its strictly fossorial lifestyle [Jarvis and Sherman, 2002]. As shown in figure 3, calculating the neuronal scaling rules that apply to the rodent cortex with the exclusion of the two smallest species, mouse and naked mole-rat, places the latter, but not the former, outside the 95% confidence interval

(fig. 3a), and adding the mouse to the scaling relationship changes it little, while still excluding the naked mole-rat (fig. 3b). The naked mole-rat should therefore be included with caution in comparative studies of rodents.

Fig. 3. Naked mole-rat (nmr) and elephant are outlier species. **a** The power law that relates the mass of the cerebral cortex to its number of neurons calculated across glires species without the naked mole-rat and the mouse (exponent, 1.519 ± 0.112, $r^2 = 0.953$, $p < 0.0001$) still includes the mouse (m) data point in its 95% confidence interval, but excludes the naked mole-rat. **b** A better fit to the same data points is found when the mouse is included in the analysis (exponent, 1.699 ± 0.096, $r^2 = 0.975$, $p < 0.0001$), and still excludes the naked mole-rat. **c** The elephant is a clear outlier to the relationship that describes the variation of the number of cerebellar neurons as a power law of the number of neurons in the cerebral cortex across all species, with exponent 1.007 ± 0.054 ($r^2 = 0.905$, $p < 0.0001$), which is a linear relationship.

Another outlier in our dataset is the giraffe, probably because the individual in our dataset was still a juvenile, and therefore while its numbers of neurons had probably already reached adult levels, its brain mass was still below the average reported for the species, thus presumably skewing scaling relationships for numbers of cells and densities calculated with the inclusion of the giraffe [Kazu et al., 2014]. In agreement with the possibility that adult numbers of neurons had already been reached while brain structure mass was still growing, the giraffe matches the scaling rules across numbers of neurons in the cerebral cortex and cerebellum (fig. 3c).

Finally, we have reported that while the elephant cerebral cortex fits the neuronal scaling rules that apply to afrotherians and other nonprimates, its cerebellum is an obvious outlier, with over twice the number of neurons expected for an afrotherian cerebellum of its mass and 10 times the number of neurons that would be expected for the number of neurons in the elephant cerebral cortex, holding an extraordinary 98% of all brain neurons [Herculano-Houzel et al., 2014] (fig. 3c). Thus, we recommend not including the naked mole-rat, the giraffe and the elephant in comparative analyses, except for the purpose of examining these species directly.

Allometric Rules

Our dataset on the cellular composition of mammalian brain structures has made possible a number of discoveries on the scaling rules that apply to the construction and evolution of mammalian brains, many of which have been the subject of previous reviews [Herculano-Houzel, 2011, 2012; Herculano-Houzel et al., 2014b]. Amongst the most notable is the finding that distinct neuronal scaling rules apply to the primate cerebral cortex in comparison to all other mammalian species in the dataset. Nonprimate cortices scale with decreasing neuronal densities as the number of neurons increases, which suggests that the increases in neurogenesis across species that necessarily underlie increased numbers of neurons in evolution are coupled to an increasing average size of neurons
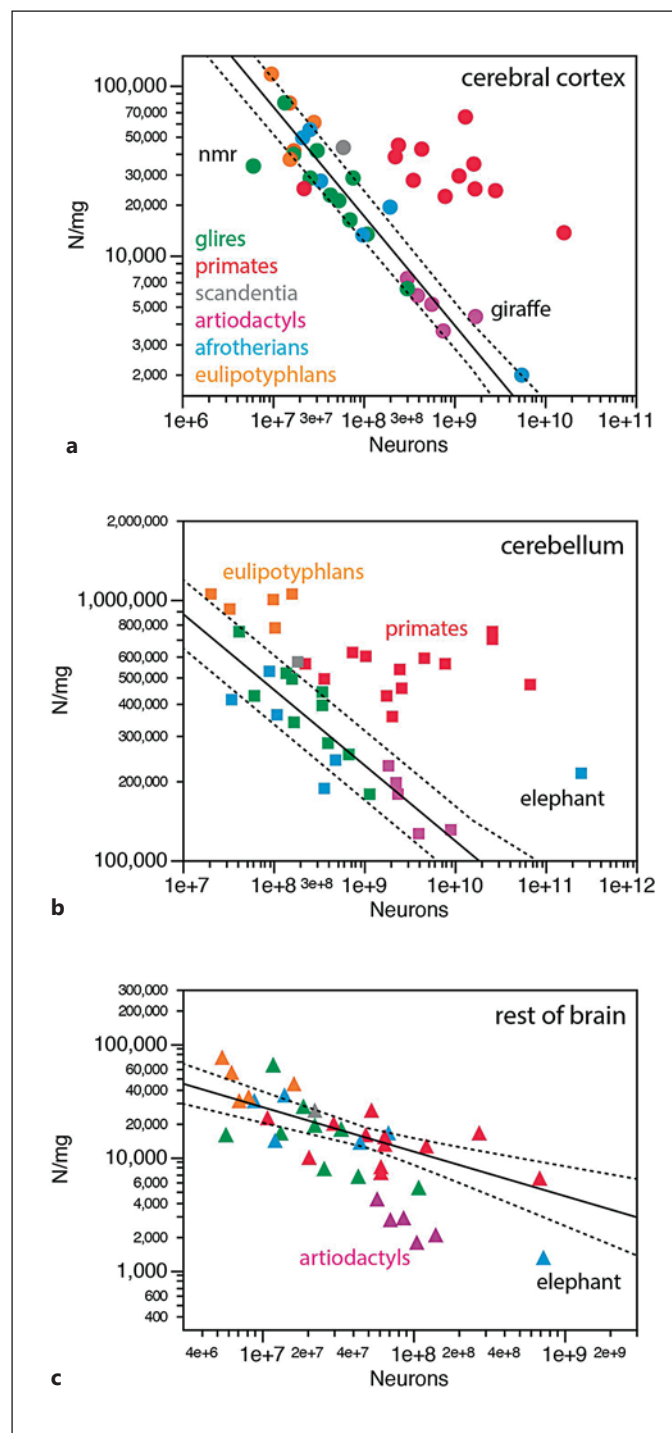

Fig. 4. Neuronal density does not scale uniformly with number of neurons across structures and clades. **a** Average neuronal density in the cerebral cortex (neurons per mg, N/mg) scales across nonprimate species as a power function of the number of cortical neurons with exponent -0.632 ± 0.042 ($r^2 = 0.904$, $p < 0.0001$, calculated without the naked mole-rat and the giraffe). **b** Average neuronal density in the cerebellum scales across nonprimate, non-eulipotyphlan species (also excluding the elephant) as a power function of the number of cerebellar neurons with exponent -0.290 ± 0.037 ($r^2 = 0.766$, $p < 0.0001$). **c** Average neuronal density in the RoB scales across nonartiodactyl species (also excluding the elephant) as a power function of the number of neurons in the structure with exponent -0.393 ± 0.080 ($r^2 = 0.439$, $p < 0.0001$).

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(which we define as including all of their arbors, besides the cell body). Primates have diverged away from the common ancestor with other lineages with an uncoupling between increased numbers of neurons and changed average neuronal cell size (fig. 4a) [Herculano-Houzel et al., 2014b]. As a result, primate cortices contain many more neurons than nonprimate cortices of a similar mass. The magnitude of the discrepancy can be observed in table 1, where the different species of all six orders and superorders have been listed in ascending order of cortical mass. Perusing table 1 makes clear the numerical advantage that primates have in comparison to other groups in terms of numbers of neurons in the cerebral cortex, even when the human cerebral cortex is compared to the much larger African elephant cortex.

We found that different neuronal scaling rules apply to the cerebellum of primates and eulipotyphlans in comparison to the ensemble of afrotherians, glires and artiodactyls, with neuronal densities that decrease with increasing numbers of neurons in the latter but not in the former (fig. 4b) [Herculano-Houzel et al., 2014b]. Again, perusing table 2 shows the larger number of neurons found in eulipotyphlan cerebella compared to even larger cerebella of glires and afrotherians. The much larger number of neurons in primate cerebella than in even larger artiodactyl cerebella is also documented in table 2.

In contrast, we reported recently that the neuronal scaling rules for the RoB are shared by primates, glires, afrotherians and eulipotyphlans, but not by artiodactyls [Herculano-Houzel et al., 2014b]. These latter animals have far fewer neurons in their RoB than nonartiodactyls in the dataset with an even smaller RoB (table 3). The difference translates into far smaller neuronal densities in the artiodactyl RoB than expected for its number of neurons or RoB mass, compared to the scaling rules that apply to the RoB of other species (fig. 4c). However, it will

be argued here that artiodactyls are not outliers in their neuronal scaling rules for the RoB; rather, once other relationships are taken into consideration, as shown below, once again it is primates who have deviated away from the scaling rule that applies to other mammalian clades.
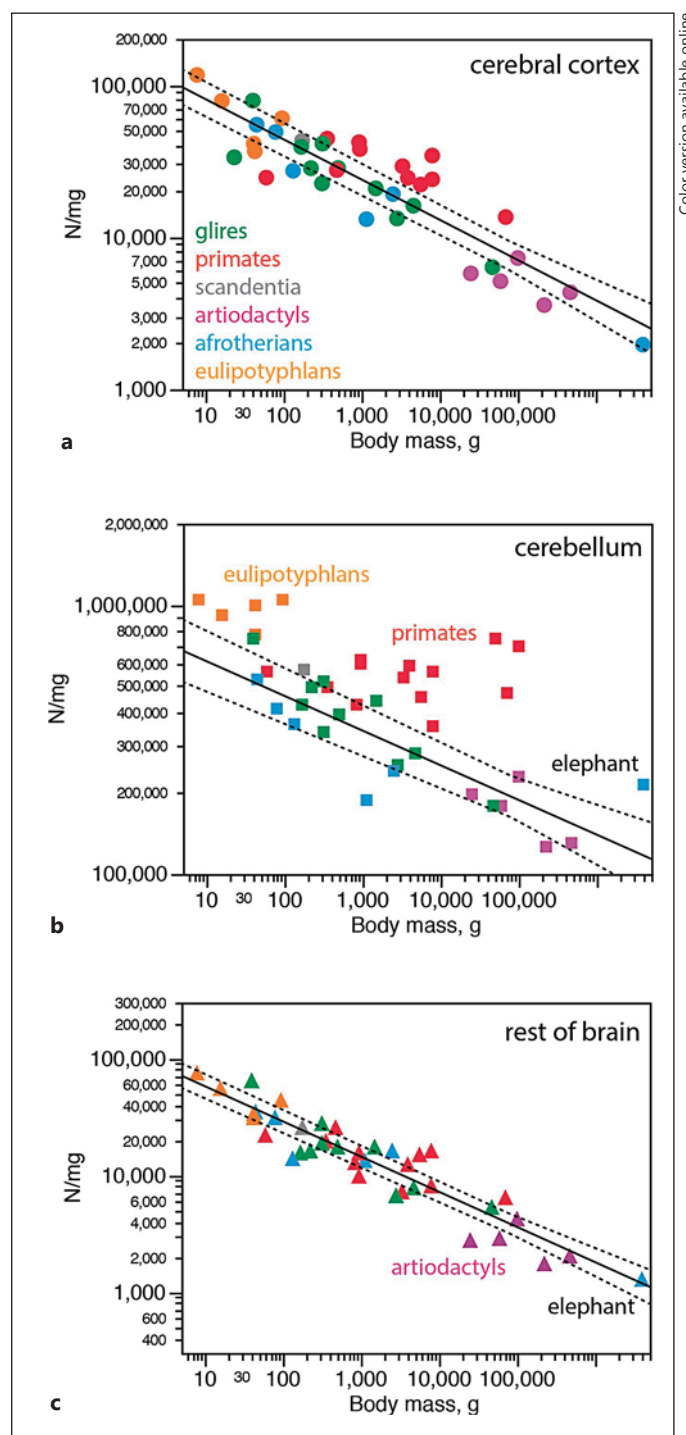

Fig. 5. Neuronal density in the RoB, but not in the cerebral cortex or cerebellum, scales uniformly with body mass. **a** The power law that fits the variation in average neuronal density in the cerebral cortex (neurons per mg, N/mg) as a function of body mass across the entire dataset excludes most primate species (exponent, -0.267 ± 0.021, $r^2 = 0.822$, $p < 0.0001$). **b** The power law that describes the variation in average neuronal density in the cerebellum as a function of body mass, calculated across nonprimate, noneulipotyphlan species, excludes both these orders as well as the elephant (exponent, -0.156 ± 0.017, $r^2 = 0.715$, $p < 0.0001$). **c** In contrast, the power law that describes the variation in average neuronal density in the RoB with increasing body mass, calculated across all species, includes many representatives of all clades, including artiodactyls and the elephant (exponent, -0.300 ± 0.019, $r^2 = 0.872$, $p < 0.0001$).

Larger Neurons in Larger Bodies
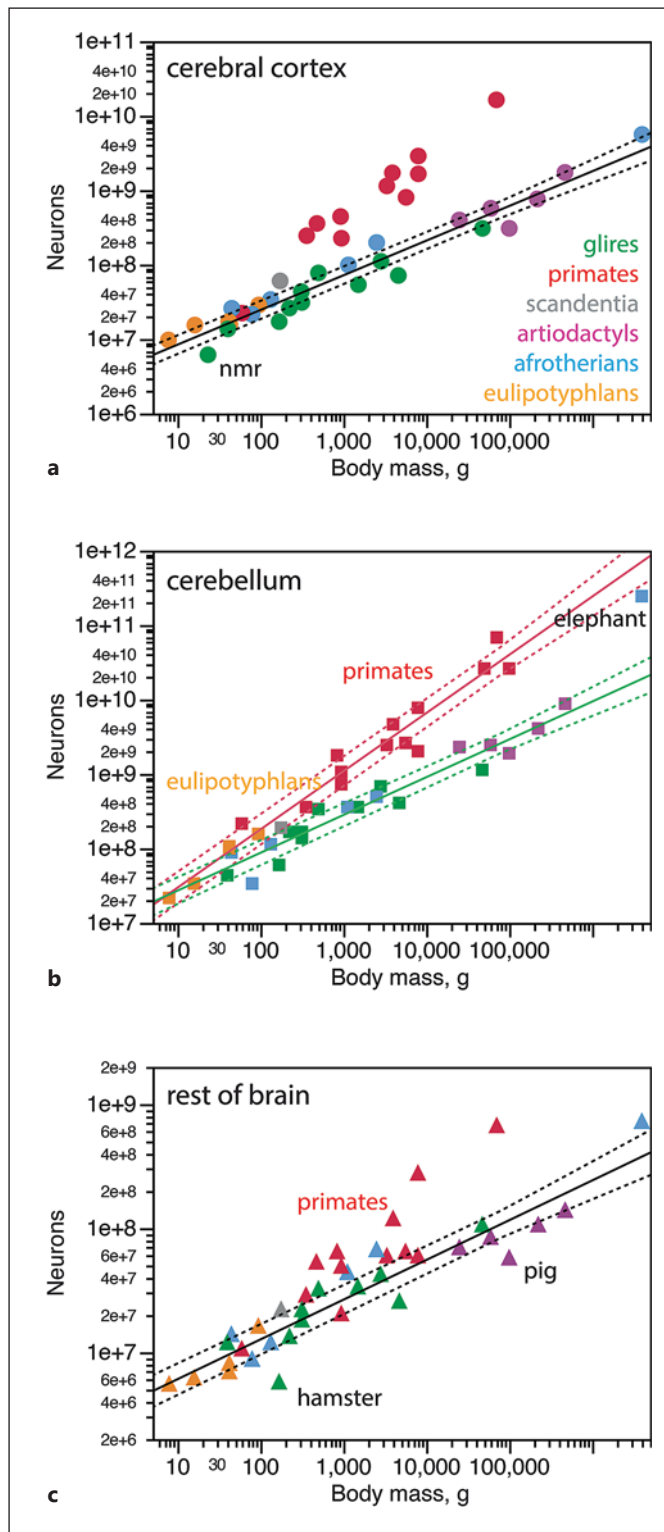
Although artiodactyls share a similar range of brain masses with primates, the former are typically much larger animals than primates of similar brain mass or number of neurons. Since the RoB includes a number of structures that are directly connected to targets or sensory sources in the body, we examined the possibility that the very low neuronal densities found in the artiodactyl RoB, which indicate very large average neuronal sizes [Mota and Herculano-Houzel, 2014], are related to the large body mass of these animals, in comparison to all other mammals in the dataset.

We found that neuronal densities in the artiodactyl RoB are indeed much better aligned across all species in the dataset as a function of body mass (fig. 5c), to the point that they can be well described by a single power function, with lower neuronal densities (and thus larger average neuronal mass) in animals with larger body mass. In contrast, although there is also an overall trend for lower neuronal densities in the cerebral cortex and cerebellum of larger animals, fitting a single power law to the entire dataset here excludes the primate cerebral cortex (fig. 5a). Similarly, the power law that fits the cerebellum of glires, afrotherians and artiodactyls excludes not only the cerebellum of primates and eulipotyphlans, but also the elephant (fig. 5b). Thus, while neurons in the RoB seem to increase uniformly in average mass with increasing body mass across all mammalian orders analyzed, neurons in the cerebral cortex and cerebellum vary significantly across mammalian orders in how average neuronal cell mass scales with increasing body mass. This is consistent with the existence of different neuronal scaling rules that govern how average neuronal cell size in the cerebral cortex in primates and in the cerebellum of pri-

Fig. 6. The number of neurons in each brain structure does not scale uniformly with body mass across all clades. **a** The number of neurons in the cerebral cortex scales across nonprimate species as a power function of body mass with exponent 0.474 ± 0.021 ($r^2 = 0.940$, $p < 0.0001$), which clearly excludes all primates in the dataset larger than the mouse lemur. **b** The number of neurons in the cerebellum scales across nonprimate, noneulipotyphlan species (also excluding the elephant) as a power function of body mass with exponent 0.535 ± 0.027 ($r^2 = 0.933$, $p < 0.0001$). In contrast, the number of cerebellar neurons scales across eulipotyphlans and primates jointly as a power function of exponent 0.782 ± 0.039 ($r^2 = 0.962$, $p < 0.0001$). **c** The number of neurons in the RoB scales across nonprimate species (including the elephant) as a power function of body mass with exponent 0.317 ± 0.021 ($r^2 = 0.875$, $p < 0.0001$) that excludes most primates.

mates and eulipotyphlans scale with numbers of neurons compared to other species, as we have suggested [Herculano-Houzel et al., 2014b].

If it remains the case that the scaling rules that link average neuronal cell size to numbers of neurons in the RoB have diverged in artiodactyls, as shown in figure 4c, then one possibility is that the driving force behind this divergence was a shift in the body × brain relationship in the species of this clade. However, as seen in figure 6, artiodactyls are a much closer fit to the scaling relationship between body mass and number of RoB neurons (as also found for the cerebral cortex and cerebellum) that applies to nonprimate species, while primates clearly have their own body × brain relationship. If artiodactyls shared with all mammals the relationship between neuronal density in the RoB and body mass (fig. 5c) but showed a faster decrease in neuronal density for the number of RoB neurons compared to other species (fig. 4c), as we had initially presumed [Herculano-Houzel et al., 2014b], then the number of neurons in the artiodactyl RoB should scale faster with body mass than in other species – but it does not (fig. 6c). In contrast, if artiodactyls shared with other nonprimate mammals both the scaling of neuronal density in the RoB and body mass (fig. 5c) and the scaling of neuronal density with the number of RoB neurons, and primates were instead the outliers as shown in figure 7, then artiodactyls would be expected to share with nonprimates the scaling of number of RoB neurons with body mass, as is indeed the case (fig. 6c). It thus appears more likely that the scaling rules that apply to the RoB have diverged not in artiodactyls, but rather in primates, as they did in the cerebral cortex and cerebellum, as indicated in figure 7.

While the neuronal scaling rules that apply to the RoB might thus have diverged not in artiodactyls, but in primates, it remains that for all species in the dataset, including primates, neuronal densities in the RoB decrease with increasing body mass, indicating that average neuronal mass in the RoB increases together with increasing body mass. Of all brain neurons, it is those situated in the RoB that are most directly related to the body, as many neurons in these structures, from the medulla to the diencephalon, are directly connected to structures in the body through sensory or motor nerves. Those neurons that are directly connected to bodily structures must have their fibers increase, at least in length, within the RoB (as in the body) as the body grows and those targets become more distant. Indeed, the exponent of the single power law that relates neuronal density in the RoB to body mass, -0.301 ± 0.019 ($r^2 = 0.873$, $p < 0.0001$), is not significantly different from 1/3 – the exponent that relates body length to
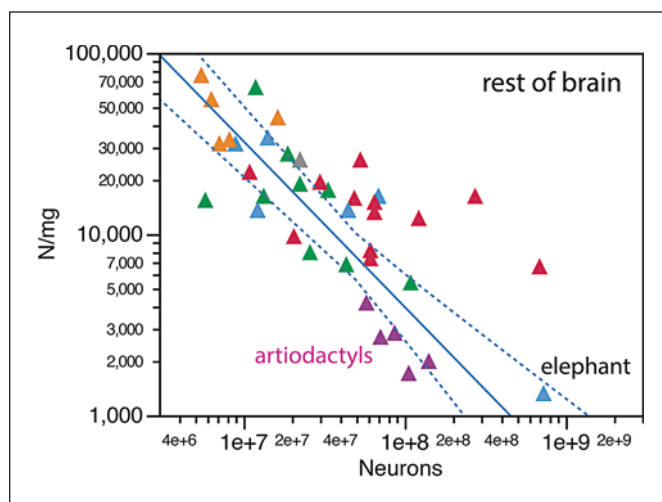

Fig. 7. Neuronal density in the RoB is better described to scale uniformly with number of neurons across nonprimates than across nonartiodactyls. Average neuronal density in the RoB (neurons per mg, N/mg) scales across nonprimate, nonelephant species as a power function of the number of neurons in the RoB with exponent -0.914 ± 0.118 ($r^2 = 0.712$, $p < 0.0001$). Notice that while the 95% confidence interval still excludes most artiodactyls, it explains much better the variation in neuronal density in the structure than the fit shown in figure 4c, which included primates but excluded artiodactyls.

body volume. It thus appears that all mammalian species in the dataset have neurons that become larger (longer) within the brain as body mass increases, with no distinction across orders. We suggest that it is this physical constraint that makes neurons in the RoB become larger (longer) with increasing body mass across all clades.

Importantly, and in contrast to the hypothesis that larger bodies require *more* neurons to operate them [Jerison, 1973], it is only the neuronal density in the RoB (and thus average neuronal cell mass) that varies uniformly with increasing body mass: as shown in figure 6c, primates are clear outliers, such that there is no single scaling rule that relates numbers of neurons in the RoB to body mass across all mammalian species in the dataset. Interestingly, although clear relationships exist between brain mass and the number of neurons in the cerebral cortex (fig. 6a), cerebellum (fig. 6b) or RoB (fig. 6c), primates are in all three cases subject to a different scaling rule, with more neurons for a given body mass compared to other mammalian clades. The clade specificity indicates that, while larger bodies have neurons in the RoB that are on average larger in proportion to the linear dimension of the body, the number of brain neurons is not dictated simply by body mass, either in the RoB or elsewhere.

Conclusion

As mentioned above, the main focus of our work has been the investigation of the scaling relationships that apply to mammalian brains and what they teach about the evolutionary origins of brain diversity in mammals. We expect the dataset that we have generated to be useful to researchers interested in many other aspects of diversity: how it is related to lifestyle, habitat, diet; how it evolved within particular clades; how it is constrained by physical aspects of brain morphology and function. As our research on brain diversity continues to grow, we will continue to expand our dataset on the cellular composition of different brain structures across mammalian species and clades and make it available to the scientific community. In the near future, we will be able to add chiropterans, carnivores, marsupials and cetaceans to the dataset, as well as a subdivision of nonneuronal 'other' cells into the underlying cell types (endothelium, astrocytes, oligodendrocytes and microglial cells).

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