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Faster Scaling of Auditory Neurons in Cortical Areas Relative to Subcortical Structures in Primate Brains

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Key Words
Allometry · Auditory cortex · Brain size · Evolution · Inferior colliculus · Medial geniculate nucleus

Abstract
Allometric studies in primates have shown that the cerebral cortex, cerebellum, and remaining brain structures increase in size as a linear function of their numbers of neurons and non-neuronal cells across primates. Whether such scaling rules also apply to functionally related structures such as those of the auditory system is unknown. Here, we investigate the scaling of brain structures in the auditory pathway of six primate species and the closely related tree shrew. Using the isotropic fractionator method to estimate the numbers of neurons and non-neuronal cells in the inferior colliculus, medial geniculate nucleus, and auditory cortex (Ac), we assessed how they scaled across species and examined the relative scaling relationships among them. As expected, each auditory structure scales in mass as a linear function of its number of neurons, with no significant changes in neuronal density across species. The Ac scales proportionately with the cerebral cortex as a whole, maintaining a relative mass of approximately 1% and a relative number of neurons of 0.7%. However, the Ac gains neurons faster than both subcortical structures examined. As a result, larger primate brains have increased ratios of cortical to subcortical neurons involved in processing auditory information.

Introduction

Recent studies have shown that primate brain evolution has occurred in the absence of systematic changes to cortical neuronal densities [Herculano-Houzel et al., 2007; Gabi et al., 2010]. The cerebral cortex has also maintained a fairly constant relative number of brain neurons despite an increasing absolute number of neurons in larger brains [Herculano-Houzel, 2011a]. Such initial studies necessarily addressed the scaling of the cerebral cortex as a whole, encompassing different functional areas, ignoring probable heterogeneities in the distribution of neurons across the cortical surface [later demonstrated by Collins et al., 2010] and the possibility that different scaling rules apply to the different functional cortical areas. Given the known systematic differences in morphological properties of neurons across sensory and associative areas in the primate cerebral cortex, including dendrite extension and number of spines [reviewed in Elston, 2003], it is plausible that functional cortical areas differ in their cellular scaling rules. For example, some areas may increase in volume as average neuronal size increases,

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whereas others may retain or even show a reduction in their average neuronal size. As a consequence, the relative number of neurons in a given cortical area cannot be gauged directly from its size relative to the entire cortex [Collins et al., 2010], as the area may have a different neuronal density than the cortex overall. Thus, while the importance of a given sensory system for a species has traditionally been inferred from the size of its cortical or subcortical structures in the brain [reviewed in Striedter, 2005], our evidence that structure size is not a reliable proxy for number of neurons – across either structures or species belonging to different mammalian orders – argues instead for a direct assessment and comparison of total numbers of neurons in these structures.

Another open issue is the scaling of numbers of neurons across brain structures within the same functional pathway, such as the visual or auditory system. By comparing indirect estimates of numbers of neurons in the primary visual cortex (V1) and the dorsal lateral geniculate nucleus (LGN) of the visual thalamus, Stevens [2001] found a relative increase in cortical over subcortical numbers of neurons with an exponent of 3/2. In a more recent study of structures in the visual system, Collins et al. [2012] also found that V1 adds neurons at a relatively faster rate than the subcortical structures, both the superior colliculus (SC) and LGN. Stevens [2001] attributed the functional significance of this increase to the shift from a 2-dimensional representation of horizontal and vertical positions within the visual field to a 3-dimensional representation that includes orientation. However, a similar scaling rule applies to the increase in the number of cortical and cerebellar neurons compared to those found in remaining brain structures [Herculano-Houzel, 2011a], suggesting that cortical areas may generally enjoy a faster addition of neurons over functionally related subcortical structures. Indeed, cortical structures generally increase in mass more quickly than subcortical ones [Finlay and Darlington, 1995]. To test whether the scaling rules for the visual pathway generalize, we considered the scaling of cortical relative to subcortical numbers of neurons in a different sensory modality, the auditory system.

In this study, we examined three brain structures in the auditory pathway, the inferior colliculus (IC), the medial geniculate nucleus (MGN), and the auditory cortex (Ac). These structures bear direct anatomical and functional relationships to one another. Brainstem auditory pathways terminate in the IC, where auditory information is relayed to the medial geniculate complex and then to the Ac [Hackett and Kaas, 2009]. The IC includes a large central nucleus with projections to the large principal or ventral nucleus of the MGN and a surrounding shell that has projections to other divisions of the MGN. The shell also receives auditory information from brainstem structures, as well as from the Ac. The MGN includes the ventral and medial nuclei, which project to the primary or core auditory cortical area, and the dorsal nucleus with projections to nonprimary auditory areas. Larger primates, with larger brains, do have slightly larger cochleas, with basilar membranes whose length increases with body mass raised to an exponent of 0.41 [Kirk and Gosselin-Ildari, 2009]. The longer basilar membrane of larger primates correlates with both an increase in sensitivity to low frequency sounds and a decrease in the upper limit of frequency hearing, but it is uncertain how these changes relate to the computational requirements of auditory structures. One might expect that an increased number of neurons in the longer basilar membrane of larger animals will lead to increases in the auditory structures, but auditory space does not scale with increasing body or brain size. Hence, Stevens' [2001] spatial dimension hypothesis provides no a priori functional reason to expect that the Ac in larger animals, or larger brains, should have a faster increase in mass or numbers of neurons relative to the IC and MGN.

Here we tested whether the auditory pathway followed the scaling rule of the visual pathway, albeit perhaps for different functional reasons, in six species of primates and the closely related tree shrew. The three structures of the auditory pathway (IC, MGN, and Ac) were dissected from the rest of the brain, and the numbers of neurons and nonneuronal cells for each structure were then estimated using the isotropic fractionator [Herculano-Houzel and Lent, 2005]. This method has proven to be a rapid and reliable way to estimate numbers of neurons in dissectable brain structures [e.g. Herculano-Houzel et al., 2006, 2007; Sarko et al., 2009], yielding numbers that are comparable to those obtained with stereological methods [Azevedo et al., 2009].

Materials and Methods

The brains used in the present study were obtained from 23 individuals of seven different species, originating from colonies at the Vanderbilt University or acquired from other facilities (see below). As some of the brains were not intact when received, not all of the auditory structures were available for some of the individuals. Available auditory structures were separated from each brain and processed by the isotropic fractionator method [Herculano-Houzel and Lent, 2005] to determine the numbers of neurons and nonneuronal cells. Eighty-five auditory structures were processed and analyzed (28 primary Ac, 32 MGN, and 25 IC) from six primate species and one tree shrew species.

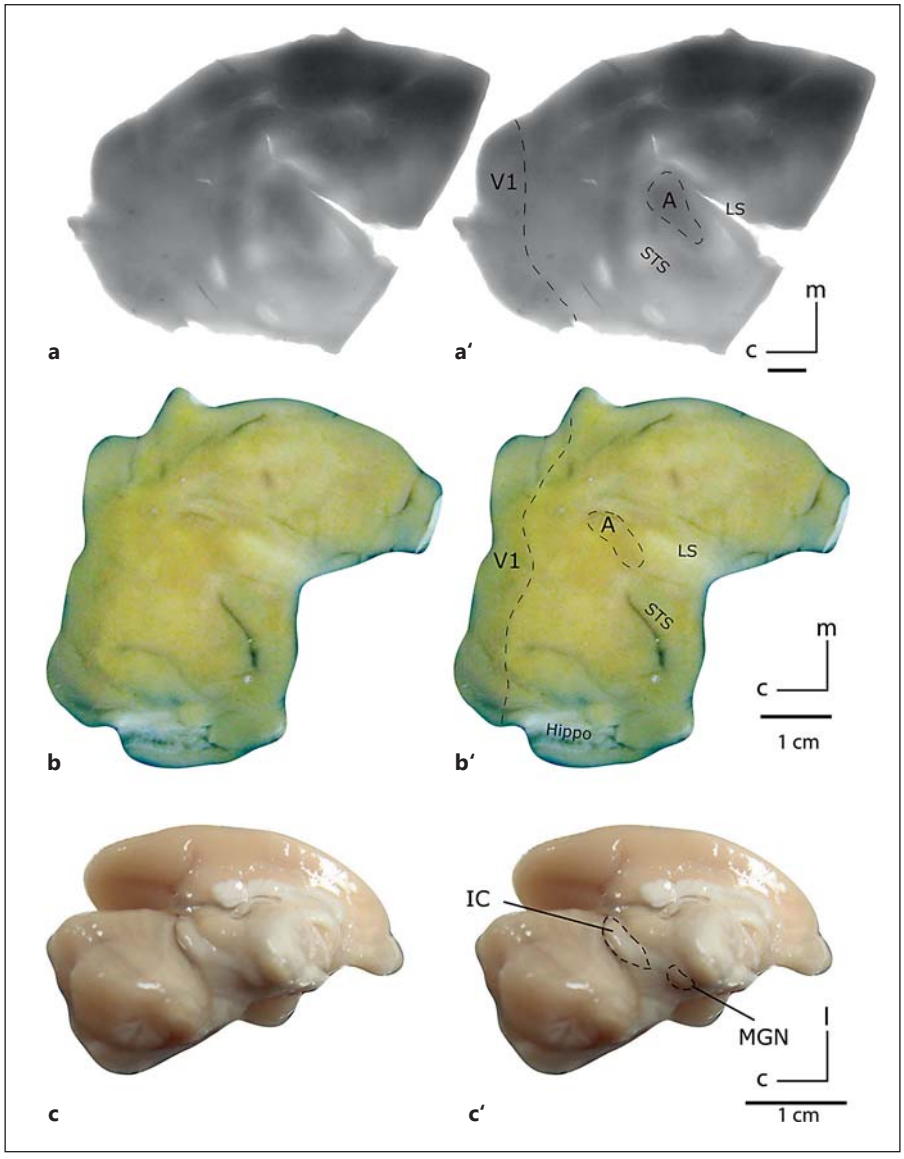

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Fig. 1. Dissection of auditory structures. **a** Flattened galago cortex, backlit. **a'** Same as in **a** showing the densely myelinated auditory cortex that likely includes auditory core cortex, R, and parts of the auditory belt. **b** Flattened galago cortex, not backlit. **b'** Same as in **b** showing still visible boundaries for auditory cortex even with no backlighting. **c** Medial view of a galago hemisphere after removal of the right cortical hemisphere. **c'** Same as in **c** showing the location of the dissected MGN and IC.

Animals

The brains of four prosimian galagos *(Otolemur garnetti)*, two marmosets *(Callithrix jacchus)*, six owl monkeys *(Aotus trivirgatus)*, two rhesus macaques *(Macaca mulatta)* and six tree shrews *(Tupaia belangeri)* were obtained from animals euthanized at the Vanderbilt University after terminal experimental procedures under protocols approved by the Vanderbilt Institutional Animal Care and Use Committee. These animals were sacrificed with a lethal dose of sodium pentobarbital, perfused with 2% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) followed by 2% paraformaldehyde plus 10% sucrose in PBS. Two baboon brains *(Papio cynocephalus anubis)* were purchased after perfusion from the University of Washington National Primate Research Center Tissue Program and the brain of one mongoose lemur *(Eulemur mongoz)* was purchased from the Duke University Lemur Center after natural death. These brains were removed and postfixed by immersion in 4% paraformaldehyde in PBS.

Tissue Preparation and Dissection

Our dissections were intended to include all of the IC, all of the MGN, and two primary areas of the Ac: the large primary cortical area Ac and the rostral primary-like area R. Ac is defined here as the densely myelinated area that likely includes Ac and R (both of which receive inputs from the ventral nucleus of the MGN) as well as parts of the auditory belt, but does not include the narrow, smaller, less densely myelinated rostrotemporal area [Morel and Kaas, 1992]. To remove the Ac from the rest of the brain, the region including the Ac was separated from subcortical structures and then manually unfolded and flattened so that the cortex could be viewed on a light box. The more densely myelinated primary audi-

tory area thus appeared as a dark region [Luethke et al., 1989]. Dissections of the MGN and IC in all animals were performed on the intact remainder of the brain, after removal of the cerebral cortex, under a magnifying lamp. The dissected MGN consisted primarily of the caudal pole that protrudes from the thalamus, where the ventral subdivision of the MGN is located, and its rostral extension, containing the dorsal and medial subdivisions. The IC was carefully peeled away from the rest of the brainstem, above the brachium of the IC and the tract of the mesencephalic nucleus of the trigeminal nerve (fig. 1). Unless one hemisphere was not available, the auditory structures for both sides of each brain were examined. All values are reported as averages for a single hemisphere structure. Average whole-brain mass and numbers of neurons for the seven species analyzed here were obtained from Herculano-Houzel et al. [2007] and Gabi et al. [2010]. Average cerebral cortex gray matter mass and numbers of neurons were obtained from Herculano-Houzel et al. [2008].

Isotropic Fractionator

The isotropic fractionator method [Herculano-Houzel and Lent, 2005] was used to estimate the total number of cells, neurons, and nonneurons in each dissected structure. After weighing, each auditory pathway structure was mechanically homogenized in a detergent solution (40 mM sodium citrate and 1% Triton X-100), yielding a nuclear suspension of known volume that was then made isotropic by agitation. To estimate total cell number, 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen), a DNA-specific fluorescent dye, was added to the suspensions after being diluted 20–100 times from a stock solution of 10 mg/l. To determine the average number of cell nuclei per milliliter of suspension, 4 aliquots of the suspension were placed on a hemocytometer and cell nuclei were counted using a fluorescence microscope at ×400 total magnification. By multiplying the count by the total suspension volume, the total number of cells in the original tissue was obtained. To determine the total number of neurons, a 1-ml sample of the nuclear suspension was centrifuged, resuspended in PBS, centrifuged, then resuspended in a 0.2 M solution of boric acid, pH 9.0, and heated for 45 min at 75°C for epitope retrieval. The suspension was centrifuged once more to collect the nuclei, washed with PBS, and immunoreacted overnight with anti-NeuN mouse IgG (1:300 in PBS; Millipore, Billerica, Mass., USA), a neuron-specific antibody that stains all neurons in the central nervous system except mitral cells, inferior olive cells, and Purkinje cells [Mullen et al., 1992]. The nuclei were then washed and resuspended in a solution consisting of 10% goat serum, 40% DAPI, 50% PBS, and a secondary antibody, Alexa Fluor 594 goat anti-mouse IgG (1:400; Invitrogen, Eugene, Oreg., USA). After washing in PBS, an aliquot of the stained nuclear suspension was again placed on the hemocytometer for the evaluation of at least 500 DAPI-positive nuclei to determine how many of those nuclei were NeuN positive. By multiplying the fraction of NeuN-positive neurons by the total number of cells, we obtained an estimate of the total number of neurons in the original tissue. The total number of nonneuronal cells in each structure was determined by subtracting its total number of neurons from its total number of cells.

Data Analysis

Average values (mass and number of neurons) for each structure were calculated from the individual cases of each species. Statistical analyses and regressions were performed using MATLAB 7.8.0 (MathWorks, Natick, Mass., USA). Best-fitting power function parameters (scaling exponent and a multiplier) were determined by least-square linear regression on the log-transformed data.

Results

Our sample consisted of seven species, including six primates: two Old World monkeys (macaque and baboon), two New World monkeys (marmoset and owl monkey), and two prosimians (galago and mongoose lemur). Together, these species represent the two major primate branches, prosimians and anthropoids. For the purposes of this study, the tree shrew *(T. belangeri)*, a closely related nonprimate species (order Scandentia), was included in all data analyses. In a previous study, its removal resulted in nonsignificant changes to the data [Herculano-Houzel et al., 2007]. All results presented below refer to a joint analysis of the seven species.

Variation in and Relationships between Body, Brain, and Auditory Structure Mass

Average body size in our sample varied 91-fold from the tree shrew (173 g) to the baboon (15,750 g; table 1). Brain mass had a significant scaling relationship with body mass across species (power function fit, $p = 0.0001$). Consistent with previous reports [Herculano-Houzel et al., 2007], this relationship was approximately linear, as the scaling exponent was close to 1 ($Mass_{Brain} \sim Mass_{Body}^{0.838}$) and the 95% confidence intervals included 1 (CI: 0.633–1.043). The masses of the brain and its subcomponents varied widely across species, with 55-fold variation in brain mass, 32-fold variation in Ac mass, 8.9-fold variation in MGN mass, and 7.9-fold variation in IC mass (table 1; fig. 2). The different mass ranges for the auditory structures suggest that they may scale differently with brain mass. Indeed, the mass of Ac scaled approximately linearly with total brain mass ($Mass_{Ac} \sim Mass_{Brain}^{0.801\ [CI:\ 0.481–1.134]}$, $p = 0.0024$; fig. 3a) and with cortical gray matter mass ($Mass_{Ac} \sim Mass_{Cortex}^{0.854\ [CI:\ 0.420–1.288]}$, $p = 0.0082$). In contrast, IC and MGN were found to grow more slowly than the brain overall ($Mass_{IC} \sim Mass_{Brain}^{0.510\ [CI:\ 0.363–0.658]}$, $p = 0.0003$; $Mass_{MGN} \sim Mass_{Brain}^{0.479\ [CI:\ 0.232–0.725]}$, $p = 0.0041$; fig. 3a), with confidence intervals for the scaling exponents that did not include 1. (The scaling relationships among the three auditory structures are compared directly below.)

Put another way, IC and MGN had relatively smaller masses in larger brains. The relative mass of the IC (ex-

pressed as a percentage of half brain mass) declined from 1.11% in the tree shrew to 0.16% in the baboon, varying significantly with brain mass across species ($Mass_{IC}/Mass_{Brain} \sim Mass_{Brain}^{-0.490\ [CI: -0.637\ to\ -0.343]}$, p = 0.0004; fig. 3b). Similarly, the relative mass of the MGN declined from 0.70 to 0.08% ($Mass_{MGN}/Mass_{Brain} \sim Mass_{Brain}^{-0.522\ [CI: -0.768\ to\ -0.275]}$, p = 0.0028; fig. 3b). Relative Ac mass, however, did not vary consistently as a function of either brain mass (range of 0.29–1.12%; $Mass_{Ac}/Mass_{Brain} \sim Mass_{Brain}^{-0.192\ [CI: -0.519\ to\ 0.134]}$, p = 0.18; fig. 3b) or cortical gray matter mass (range of 0.94–3.00%; $Mass_{Ac}/Mass_{Cortex} \sim Mass_{Cortex}^{-0.146\ [CI: -0.580\ to\ 0.288]}$, p = 0.36).

Variation in and Relationships between Brain and Auditory Structure Neuronal Counts

The neuronal count results were similar to those found for mass. The number of neurons across species varied 42-fold in the brain, 13-fold in the Ac, 9.2-fold in the MGN, and 4.4-fold in the IC (table 1). These different ranges corresponded to different scaling exponents, with Ac again scaling approximately linearly relative to neuronal counts in the brain ($Neurons_{Ac} \sim Neurons_{Brain}^{0.762\ [CI: 0.451–1.074]}$, p = 0.0025; fig. 4a) and cortical gray matter ($Neurons_{Ac} \sim Neurons_{Cortex}^{0.673\ [CI: 0.259–1.087]}$, p = 0.0140), whereas IC and MGN grew more slowly than the brain as a whole ($Neurons_{IC} \sim Neurons_{Brain}^{0.426\ [CI: 0.274–0.579]}$, p = 0.0015; $Neurons_{MGN} \sim Neurons_{Brain}^{0.579\ [CI: 0.293–0.865]}$, p = 0.0049; fig. 4a). The number of neurons in these latter structures became a smaller fraction of all brain neurons in larger brains, declining from 0.30 to 0.03% for IC ($Neurons_{IC}/Neurons_{Brain} \sim Neurons_{Brain}^{-0.574\ [CI: -0.726\ to\ -0.422]}$, p = 0.0005; fig. 4b) and from 0.12 to 0.02% for MGN ($Neurons_{MGN}/Neurons_{Brain} \sim Neurons_{Brain}^{-0.421\ [CI: -0.707\ to\ -0.136]}$, p = 0.0149; fig. 4b). As with mass, relative neuronal counts in the Ac did not consistently vary with the number of neurons either within the brain (range of 0.12–0.40%; $Neurons_{Ac}/Neurons_{Brain} \sim Neurons_{Brain}^{-0.238\ [CI: -0.549\ to\ 0.074]}$, p = 0.10; fig. 4b) or within the cortical gray matter (range of 0.50–2.39%; $Neurons_{Ac}/Neurons_{Cortex} \sim Neurons_{Cortex}^{-0.327\ [CI: -0.741\ to\ 0.087]}$, p = 0.09).

Together with the mass scaling findings above, the neuronal scaling results suggest nonlinear scaling relationships amongst the auditory structures and no consistent change in density within these structures (neurons per milligram of tissue). We next directly compared the relative scaling of the auditory structures and then determined the scaling rules within each structure.

Table 1. Variation in absolute mass and neuronal composition of auditory structures

Species	Individuals n	Hemispheres n	1/2 brain mass g	1/2 brain neurons millions	IC mass g	IC neurons millions	MGN mass g	MGN neurons millions	Area A mass g	Area A neurons millions	Cortex GM mass, g	Cortex GM neurons millions
Tupaia belangeri	6	8	1.38	131	0.015±0.002	0.39±0.18	0.010±0.001	0.16±0.06	0.015±0.002	0.53±0.09	0.515	22.0
Callithrix jacchus	2	2	3.89	318	0.017	0.36	0.007	0.12±0.04	0.019	0.87±0.35	2.04	120
Otolemur garnettii	4	6	5.08	468	0.028±0.010	0.47±0.41	0.018±0.014	0.26±0.26	0.042±0.048	0.58±0.18	2.56	88.5
Eulemur mongoz	1	2	7.70	NA	0.036±0.002	0.94±0.13	0.024±0.001	0.24±0.14	NA	NA	NA	NA
Aotus trivirgatus	6	7	7.87	734	0.036±0.014	0.60±0.24	0.018±0.005	0.40±0.20	0.050±0.020	1.84±1.06	3.70	200
Macaca mulatta	2	3	43.7	3,190	0.067	1.22	0.035±0.019	0.75±0.34	0.127±0.014	5.42±0.04	NA	NA
Papio cynocephalus	2	4	75.6	5,480	0.119±0.021	1.63±0.19	0.062±0.011	1.10±0.14	0.482±0.060	7.08±0.48	36.3	1,420
Variation			55	42	7.9	4.4	8.9	9.2	32	13	70	65

Mass and counts are given as average ± SD and refer to a single brain hemisphere. Values for brain mass, number of total brain neurons, and cortical gray matter (GM) mass and number of neurons are species averages from our prior studies [Herculano-Houzel et al., 2007, 2008; Gabi et al., 2010], except for brain mass in *E. mongoz*, which was obtained from Stephan et al. [1981]. Variation is calculated as the largest value in a column divided by the smallest. NA = Structure not available.

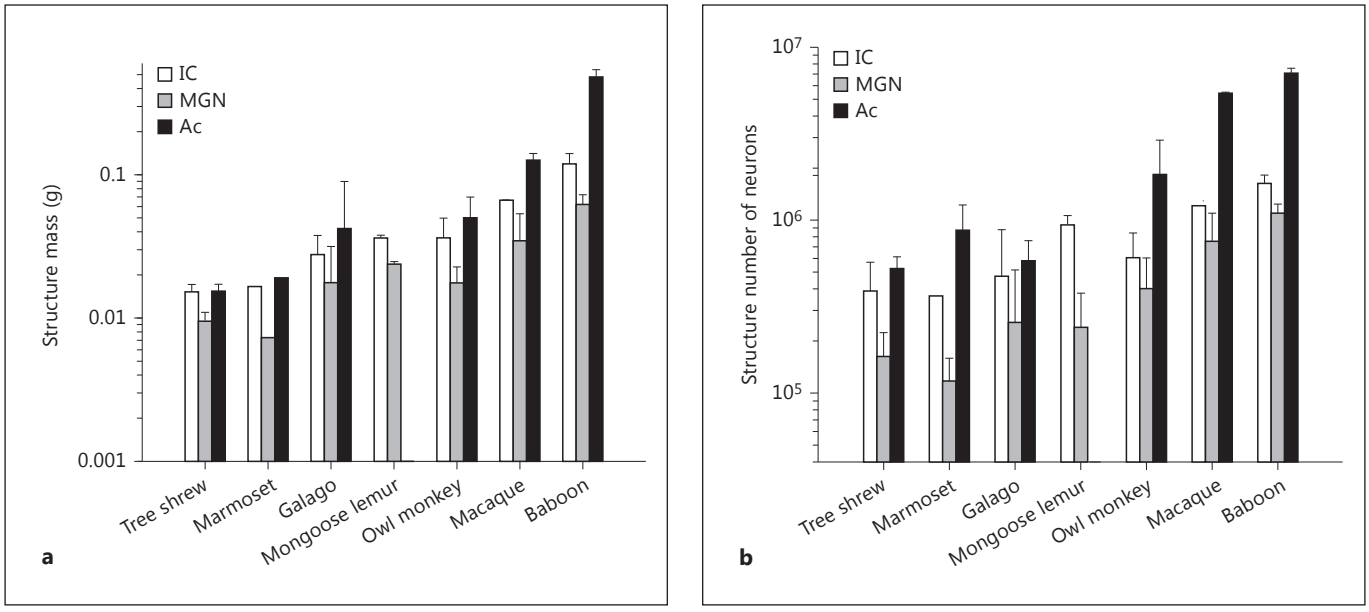

Fig. 2. Variation in the mass (**a**) and number of neurons (**b**) of auditory structures. Average mass (**a**) and numbers of neurons (**b**) and standard deviation of the Ac, MGN, and IC in each species. Note that in both cases, the y-axis is a log scale.

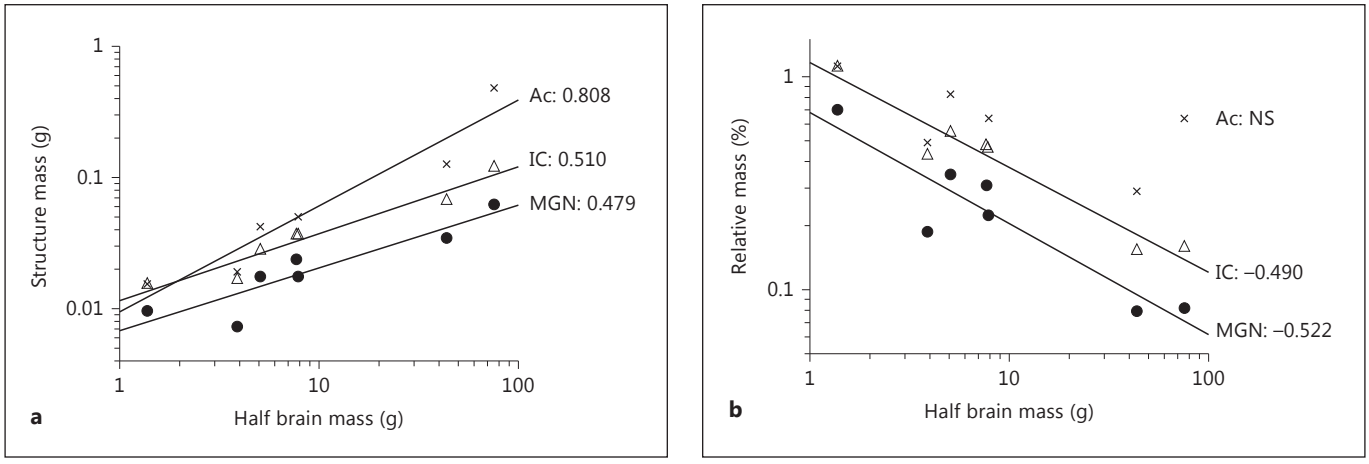

Fig. 3. Scaling relationships between the masses of auditory structures and the brain. Relationship between the mass (**a**) and the relative masses (**b**) of the Ac, MGN, and IC (in a single hemisphere) and half brain mass. The structures scale with brain mass raised to the indicated exponents. For this figure and the ones that follow, each point represents the average value for one species in a single hemisphere, and crosses represent the Ac, triangles represent the MGN, and circles represent the IC.

Relative Scaling of the Auditory Structures Ac, MGN, and IC

As suggested by the different scaling relationships between the brain and the three auditory structures, the auditory regions grew at significantly different relative rates. Across species, Ac increased in mass more rapidly than either IC or MGN ($Mass_{Ac} \sim Mass_{IC}^{1.584\ [CI:\ 1.303–1.865]}$, $p = 0.0001$; $Mass_{Ac} \sim Mass_{MGN}^{1.578\ [CI:\ 1.114–2.043]}$, $p = 0.0007$), whereas IC and MGN grew at approximately the same rate ($Mass_{MGN} \sim Mass_{IC}^{0.974\ [CI:\ 0.733–1.216]}$, $p = 0.0001$). These relationships can be illustrated by the observation that while the three structures have fairly similar sizes in

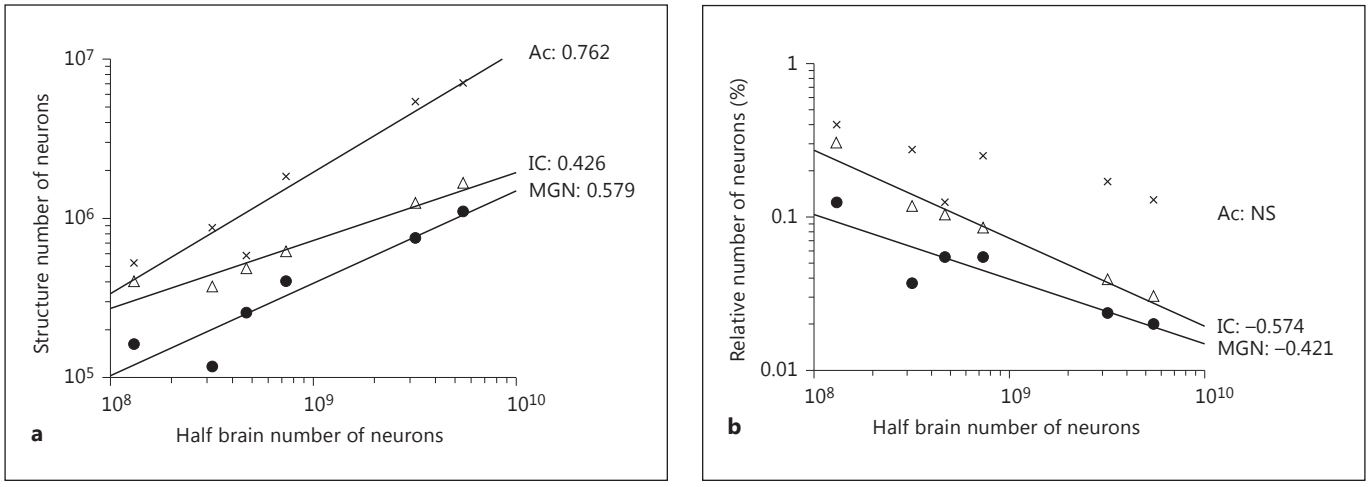

Fig. 4. Scaling of number of neurons in auditory structures with increasing brain mass. **a** Relationship between the number of neurons in the Ac, MGN, and IC (in a single hemisphere) and the number of neurons in a single brain hemisphere. **b** Relative number of neurons found in the Ac, MGN, and IC, expressed as a percentage of all neurons in a single brain hemisphere. The Ac does not vary significantly with increasing brain mass. In contrast, the relative numbers of neurons in the MGN and in the SC decrease with increasing brain mass raised to the exponents indicated.

the tree shrew and the marmoset, Ac is 4 times larger than the IC and 7 times larger than the MGN in the baboon (fig. 2a).

The scaling relationships for numbers of neurons were similar, albeit less consistent. Across species, the Ac grew significantly faster than the IC ($Neurons_{Ac} \sim Neurons_{IC}^{1.731\ [CI:\ 1.016–2.445]}$, $p = 0.0025$), but the relationship between Ac and MGN was not significantly nonlinear ($Neurons_{Ac} \sim Neurons_{MGN}^{1.184\ [CI:\ 0.461–1.907]}$, $p = 0.0104$). As before, MGN and IC scaled approximately linearly ($Neurons_{MGN} \sim Neurons_{IC}^{1.238\ [CI:\ 0.543–1.933]}$, $p = 0.0059$). These relationships can be illustrated by the observation that while the Ac has 2 times more neurons than the IC and 3 times more neurons than the MGN in the tree shrew, it contains 4 times more neurons than the IC and 6 times more neurons than the MGN in the baboon (fig. 2b).

Scaling within Each Auditory Structure IC, MGN, and Ac

Given the general agreement in scaling results for mass and number of neurons, we expected linear scaling between these measures across species and did not expect consistent changes in neuronal density. Indeed, mass and number of neurons scaled approximately linearly within our three auditory structures ($Mass_{IC} \sim Neurons_{IC}^{1.201\ [CI:\ 0.796–1.607]}$, $p = 0.0006$; $Mass_{MGN} \sim Neurons_{MGN}^{0.853\ [CI:\ 0.502–1.203]}$, $p = 0.0015$; $Mass_{Ac} \sim Neurons_{Ac}^{1.033\ [CI:\ 0.372–1.694]}$, $p = 0.0123$; fig. 5a). Correspondingly, neural density did not change consistently across species ($Neurons_{IC}/Mass_{IC} \sim Mass_{IC}^{-0.234\ [CI:\ -0.492\ to\ 0.025]}$, $p = 0.07$; $Neurons_{MGN}/Mass_{MGN} \sim Mass_{MGN}^{0.040\ [CI:\ -0.388\ to\ 0.468]}$, $p = 0.82$; $Neurons_{Ac}/Mass_{Ac} \sim Mass_{Ac}^{-0.202\ [CI:\ -0.712\ to\ 0.309]}$, $p = 0.34$; fig. 5b). The number of other cells (nonneurons) also scaled approximately linearly with mass ($Mass_{IC} \sim Others_{IC}^{0.982\ [CI:\ 0.662–1.303]}$, $p = 0.0005$; $Mass_{MGN} \sim Others_{MGN}^{0.857\ [CI:\ 0.554–1.159]}$, $p = 0.0008$; $Mass_{Ac} \sim Others_{Ac}^{1.157\ [CI:\ 0.738–1.575]}$, $p = 0.0016$; fig. 5c), with a corresponding lack of density changes across species ($Others_{IC}/Mass_{IC} \sim Mass_{IC}^{-0.058\ [CI:\ -0.365\ to\ 0.250]}$, $p = 0.65$; $Others_{MGN}/Mass_{MGN} \sim Mass_{MGN}^{0.067\ [CI:\ -0.310\ to\ 0.444]}$, $p = 0.67$; $Others_{Ac}/Mass_{Ac} \sim Mass_{Ac}^{-0.190\ [CI:\ -0.484\ to\ 0.103]}$, $p = 0.15$; fig. 5d).

Discussion

The present study addressed the question of how subcortical and cortical structures in the auditory system vary in mass and neuron number across primate species. Adhering to linear scaling rules for cellular density within primate brains, the structures examined in this study, Ac, MGN, and IC, were found to scale linearly in number of neurons relative to mass, such that the density of neu-

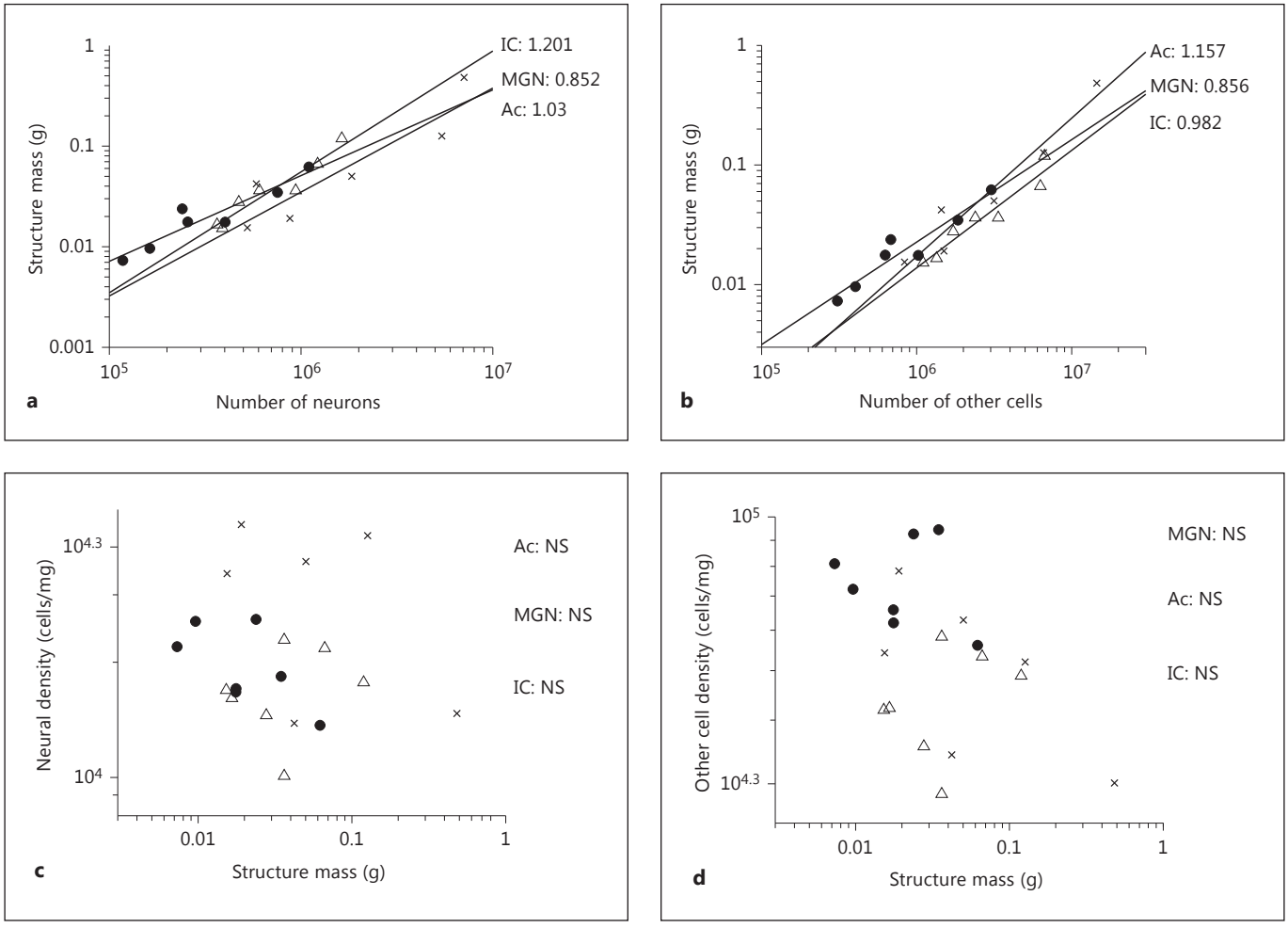

Fig. 5. Cellular scaling rules for the auditory structures Ac, MGN, and IC. The mass of each of the auditory structures increases approximately linearly as the structures gain neurons (**a**) and other cells also scale approximately linearly with mass (**b**). Neuronal densities (**c**) and other cell densities (**d**) in the Ac, MGN, and IC do not vary systematically as these structures increase in mass.

rons within each structure did not change across species. We also found that the mass and number of neurons in Ac scaled linearly with the brain and cortex, whereas the subcortical structures grew more slowly than the brain and Ac. These findings are consistent with the general scaling rule that cortical mass increases faster than either overall brain mass or subcortical structure mass [Finlay and Darlington, 1995; Herculano-Houzel et al., 2007]. In contrast, our results have key differences from what has been observed in the visual pathway [Collins et al., 2012].

The Ac, MGN, and IC are closely related functionally, as the large central nucleus of the IC provides driving activation to the large principal (ventral) subnucleus of the MGN, which in turn activates the auditory core cortex [Kaas and Hackett, 2000, 2008]. Such close functional correspondence may result in similar scaling relationships between these two subcortical structures and the Ac, a notion that our results support. The Ac increases in mass with an exponent of about 1.6 relative to both the MGN and the IC, and these latter two structures scale linearly relative to one another. In comparison, V1 mass increases linearly with the LGN (exponent of 0.988) and nonlinearly with the SC (exponent of 1.9), and LGN mass increases with an exponent of 1.356 relative to the SC [Collins et al., 2012]. These results suggest that relative scaling of masses amongst the structures is rather different in the two sensory pathways. The neuronal scaling rules further support a difference for SC scaling. V1 neu-

rons increase with an exponent of 1.2 relative to the LGN, but 3.0 relative to the SC. Indeed, the number of neurons in the LGN increases with an exponent of 1.7 relative to the SC. In the auditory system, however, A1 neurons increase with an exponent of 1.2 compared to the MGN and 1.7 compared to the IC, with MGN-IC scaling not significantly different from linear.

Taken together, the above findings are consistent with other evidence that the MGN, like its visual counterpart, is closely tied to its target cortical area [Kaas and Hackett, 2000, 2008]. However, the different scaling exponents for the IC and SC to their respective cortical areas may reflect closer ties of the IC to the MGN and Ac, than ties of the SC to the LGN and visual cortex. For example, the SC has a more limited functional relationship to V1, as the SC provides a small direct projection to the LGN, which then is relayed to V1. Instead, the SC projects more densely to parts of the visual pulvinar, especially subregions that project to temporal visual cortex, and to brainstem motor centers concerned with eye and head movements [Kaas, 2013]. In contrast, the IC projects densely to the MGN and receives strong feedback connections from the Ac [Kaas and Hackett, 2000, 2008]. Although the SC similarly receives a large proportion of its cortical inputs from the visual cortex, SC functions are otherwise less closely tied to V1 functions. As such, the difference in IC scaling relative to the auditory pathway and SC scaling relative to the visual pathway may be due to differences in information processing in the IC and SC.

Stevens [2001, 2002] proposed that a greater proportion of V1 neurons would be needed for increasing numbers of LGN neurons because V1 maps the additional dimension of orientation. V1 would therefore grow with a scaling exponent of 3/2 relative to the LGN, as the former represents three dimensions to the two of the latter. Alternatively, it was proposed that similar scaling rules may apply for neuron ratios between all thalamic nuclei and their cortical targets, as cortical areas would be expected to engage in more complex information processing more generally, perhaps elaborating upon dimensions or features that were more simply represented in the thalamus. This processing expansion might lead to scaling exponents around 3/2 as well, though for different functional reasons. The present results include scaling exponents rather close to 3/2, which may be due to an elaboration of information fed in from subcortical structures in the Ac, because major auditory deficits follow lesions of the Ac in primates [Heffner, 2005]. Even though the ability to discriminate between left and right sounds is retained, it has been suggested that the Ac is necessary for the integration of binaural time difference and intensity cures that are necessary for the perception of sound in space [Wesolek et al., 2010]. Another possible explanation for the 3/2 scaling exponent is that there are increased projections from the Ac compared to the subcortical structures, with increasing numbers of feedforward projections to other cortical areas and feedback projections to subcortical structures in larger brains [Kaas and Hackett, 2000, 2008].

We recently demonstrated that visual pathway scaling rules are different from those in the brain as a whole [Collins et al., 2012]. As discussed above, they also differ from the scaling in the auditory system. Additional differences between the two sensory systems are worthy of further consideration and analysis. First, V1 is consistently much larger and contained many more neurons (around 50× regardless of species) than the auditory core, even though the Ac contains two tonotopic representations, auditory core cortex and R [Kaas and Hackett, 2000, 2008]. While both visual and auditory systems have a number of cortical areas at several levels of processing, overall much more cortex and many more neurons are devoted to vision in primates. There are also neuronal density differences between the two sensory systems. The visual structures decrease in densities as they increase in mass, whereas auditory structures maintain constant neuronal density as they increase in mass.

Neuron numbers and structure sizes obtained in the present study were generated in the same way as those of the study by Collins et al. [2012] on V1, LGN, and SC; that is, Ac, MGN, and IC were dissected from the rest of the brain, weighed, and estimates of total cell and neuron numbers were obtained by the isotropic fractionator method [Herculano-Houzel and Lent, 2005]. This method is now in wide use, and results have been consistent with repeated measures [Herculano-Houzel, 2011b; Young et al., 2012]. We acknowledge that variability in the dissection is an inevitable source of error in this study, despite the great care that was taken to keep dissections consistent across cases. The dissection method has also been reasonably consistent with estimates based on traditional stereological methods [Tsai et al., 2009].

In summary, our results show that Ac scales linearly with the brain and cortex, and that subcortical auditory structures scale at a slower rate than the brain. The auditory pathway scaling relationships may reflect the increased complexity required of the Ac in larger animals, necessitating more ascending connections to higher order processing centers and more descending connections to subcortical structures. The scaling relationships among and within the three auditory structures are in line with

what has been shown for the brain as a whole, but they differ from what has been shown in the visual pathway [Collins et al., 2012]. For example, the auditory structures maintain constant cellular densities while densities decrease in the visual structures of larger brains. Given the different scaling rules in the auditory and visual pathways, we would like to be able to extend such comparisons to the somatosensory system. The dissection methods used here, however, would be difficult to apply in that system. Potentially, the primary somatosensory cortex, area 3b, could be separated from the flattened cortex on a light box in a range of primate species, but subcortical relays are largely inaccessible, and would require a standard stereological approach. Despite such challenges, we hope that such studies will be attempted in the near future.

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References

Azevedo FAC, Carvalho LRB, Grinberg LT, Farfel JM, Ferretti REL, Leite REP, Jacob Filho W, Lent R, Herculano-Houzel S (2009): Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol 513: 532–541.

Collins CE, Airey DC, Young NA, Leitch DB, Kaas JH (2010): Neuron densities vary across and within cortical areas in primates. Proc Natl Acad Sci USA 107:15927–15932.

Collins CE, Leitch DB, Wong P, Kaas JH, Herculano-Houzel S (2012): Faster scaling of visual neurons in cortical areas relative to subcortical structures in non-human primate brains. Brain Struct Funct, Epub ahead of print.

Elston GN (2003): Cortex, cognition and the cell: new insights into the pyramidal neuron and prefrontal function. Cereb Cortex 13:1124–1138.

Finlay BL, Darlington RB (1995): Linked regularities in the development and evolution of mammalian brains. Science 266:1578–1584.

Gabi M, Collins CE, Wong P, Torres LB, Kaas JH, Herculano-Houzel S (2010): Cellular scaling rules for the brains of an extended number of primate species. Brain Behav Evol 76:32–44.

Hackett TA, Kaas JH (2009): Audition; in Bernston GG, Cacioppo JT (eds): Handbook of Neuroscience for the Behavioral Sciences. New York, Wiley, pp 251–266.

Heffner (2005): The neurobehavioral study of auditory cortex; in König R, Heil P, Budinger E, Scheich H (eds): The Auditory Cortex: A Synthesis of Human and Animal Research. London, Erlbaum.

Herculano-Houzel S, Collins CE, Wong P, Kaas JH, Lent R (2008): The basic nonuniformity of the cerebral cortex. Proc Natl Acad Sci USA 105:12593–12598.

Herculano-Houzel S (2011a): Brains matter, bodies maybe not: the case for examining neuron numbers irrespective of body size. Ann NY Acad Sci 1225:191–199.

Herculano-Houzel S (2011b): Not all brains are made the same: new views on brain scaling in evolution. Brain Behav Evol 78:22–36.

Herculano-Houzel S, Collins CE, Wong P, Kaas JH (2007): Cellular scaling rules for primate brains. Proc Natl Acad Sci USA 104:3562–3567.

Herculano-Houzel S, Lent R (2005): Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. J Neurosci 25:2518–2521.

Herculano-Houzel S, Mota B, Lent R (2006): Cellular scaling rules for rodent brains. Proc Natl Acad Sci USA 103:12138–12143.

Kaas JH (2013): The evolution of brains from early mammals to humans. Wiley Interdiscip Rev Cogn Sci 4:33–45.

Kaas JH, Hackett TA (2000): Subdivisions of auditory cortex and processing streams in primates. Proc Natl Acad Sci USA 97:11793–11799.

Kaas JH, Hackett TA (2008): The functional neuroanatomy of the auditory cortex; in Dallos P, Oertel D (eds): The Senses: A Comprehensive Reference, vol 3: Audition. London, Elsevier, pp 765–780.

Kirk EC, Gosselin-Ildari AD (2009): Cochlear labyrinth volume and hearing abilities in primates. Anat Rec 292:765–776.

Luethke LE, Krubitzer LA, Kaas JH (1989): Connections of primary auditory cortex in the New World monkey, Saguinus. J Comp Neurol 285:487–513.

Morel A, Kaas JH (1992): Subdivisions and connections of auditory cortex in owl monkeys. J Comp Neurol 318:27–63.

Mullen RJ, Buck CR, Smith AM (1992): NeuN, a neuronal specific nuclear protein in vertebrates. Development 116:201–211.

Sarko DK, Catania KC, Leitch DB, Kaas JH, Herculano-Houzel S (2009): Cellular scaling rules of insectivore brains. Front Neuroanat 3:e8.

Stevens CF (2001): An evolutionary scaling law for the primate visual system and its basis in cortical function. Nature 411:193–195.

Stevens CF (2002): Predicting functional properties of visual cortex from an evolutionary scaling law. Neuron 36:139–142.

Striedter GF (2005): Principles of Brain Evolution. New York. Sinauer.

Tsai PS, Kaufhold JP, Blinder P, Friedman B, Drew PJ, Karten HJ, Lyden PD, Kleinfeld D (2009): Correlations of neuronal and microvascular densities in murine cortex revealed by direct counting and colocalization of nuclei and vessels. J Neurosci 29:14553–14570.

Wesolek CM, Koay G, Heffner RS, Heffner HE (2010): Laboratory rats *(Rattus norvegicus)* do not use binaural phase differences to localize sound. Hear Res 265:64–62.

Young NA, Flaherty DK, Airey DC, Varlan P, Aworunse F, Kaas JH, Collins CE (2012): Use of flow cytometry for high-throughput cell population estimates in brain tissue. Front Neuroanat 6:27.