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Neocortical Anatomy in the South American Plains Vizcacha, *Lagostomus maximus*, Reveals Different Strategies in Encephalic Development among Hystricomorpha and Myomorpha Rodents

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Plains vizcacha · Encephalization quotient · Gyrencephaly index · Rodentia · Hystricomorpha

Abstract
Depending on the presence or absence of sulci and convolutions, the brains of mammals are classified as gyrencephalic or lissencephalic. We analyzed the encephalic anatomy of the hystricomorph rodent *Lagostomus maximus* in comparison with other evolutionarily related species. The encephalization quotient (EQ), gyrencephaly index (GI), and minimum cortical thickness (MCT) were calculated for the plains vizcacha as well as for other myomorph and hystricomorph rodents. The vizcacha showed a gyrencephalic brain with a sagittal longitudinal fissure that divides both hemispheres, and 3 pairs of sulci with bilateral symmetry; that is, lateral-rostral, intraparietal, and transverse sulci. The EQ had one of the lowest values among Hystricomorpha, while GI was one of the highest. Besides, the MCT was close to the mean value for the suborder. The comparison of EQ, GI, and MCT values between hystricomorph and myomorph species allowed the detection of significant variations. Both EQ and GI showed a significant increase in Hystricomorpha compared to Myomorpha, whereas a Pearson's analysis between EQ and GI depicted an inverse correlation pattern for Hystricomorpha. Furthermore, the ratio between MCT and GI also showed a negative correlation for Hystricomorpha and Myomorpha. Our phylogenetic analyses showed that Hystricomorpha and Myomorpha do not differ in their allometric patterning between the brain and body mass, GI and brain mass, and MCT and GI. In conclusion, gyrencephalic neuroanatomy in the vizcacha could have developed from the balance between the brain size, the presence of invaginations, and the cortical thickness, which resulted in a mixed encephalization strategy for the species. Gyrencephaly in the vizcacha, as well as in other Hystricomorpha, advocates in favor of the proposal that in the more recently evolved Myomorpha lissencephaly would have arisen from a phenotype reversal process.

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Introduction

The anatomical macrostructure of the mammalian brain shows 2 different morphologies: smooth cortex or lissencephaly, and folded cortex or gyrencephaly. Sulci and gyri in gyrencephalic brains are highly conserved among species of the same order and just the smallest convolutions show species-specific or individual variations [Welker, 1990; Pillay and Manger, 2007].

The classic view of the evolution of neopallium has generated the concept of an ancestral mammal with a small and smooth-walled lissencephalic brain that gave rise to a mammal with a more developed, larger, and highly folded brain following a linear path throughout evolution [Striedter, 2005; Rakic et al., 2009]. However, more recent studies from living and fossil placental mammals have suggested that the common ancestor would have had a small folded gyrencephalic brain [O'Leary et al., 2013], supporting the idea that lissencephaly repeatedly derived from gyrencephaly through a phenotype reversal process [Borrell and Reillo, 2012; Kelava et al., 2013]. The presence of transient amplifying progenitor cells in the neocortex, together with changes in the proportion of basal radial glial cells, could have resulted in the great morphological variety of neocortexes in extant mammals [Kelava et al., 2013].

A lissencephalic brain is normally found in small mammals, particularly in small rodents such as mice and rats. Conversely, convolutedness in gyrencephalic brains is highly variable and increases in large mammals with an increasing brain mass [Pillay and Manger, 2007; Kelava et al., 2013, Zilles et al., 2013; Triarhou, 2017]. Despite being confined by the skull, cortical folding has enabled brain growth during evolution, increasing cortical surface in direct relation to the degree of gyrification.

The degree of cortical folding depends not only on the brain mass or volume, but also on the cortex thickness [Hoffman, 1985]. To understand the variation of the anatomical structure of the brain in phyletic lineages, the use of quantitative parameters such as the encephalization quotient (EQ) [Jerison, 1973, 1977], the gyrification index (GI) [Zilles et al., 2013], and the cortical thickness are invaluable tools. In particular, the use of exotic species with highly gyrated brains for comparative quantitative studies may help to test hypotheses on brain anatomical variation [Triarhou, 2017]. Most of our understanding of brain morphological variability has been founded on studies from a few mammalian species, especially in the so-called laboratory models and scattered species of primates, ungulates, carnivores, and cetaceans [Triarhou, 2017]. In the order Rodentia, which encompasses more than 2,270 living species [Wilson and Reeder, 2006], brain morphology has been extensively studied in mice and rats, and just a few anatomical descriptions are found for other species.

The South American plains vizcacha, *Lagostomus maximus*, is a caviomorph rodent, suborder Hystricomorpha, distributed mainly in the Pampean region of Argentina. *L. maximus* belongs to the family Chinchillidae which also comprises 2 other living species, the chinchilla (*Chinchilla lanigera*), and the mountain viscacha (*Lagidium viscacia*). Chinchillidae is one of the more recently evolved families from Hystricomorpha, a clade that radiated early in rodent phylogeny (Fig. 1) [Voloch et al., 2013; Steppan and Schenk, 2017]. We evaluated the gross brain morphology and morphometry in *L. maximus* to establish the degree of encephalic development and the encephalization strategy in comparison with evolutionarily related species of rodents of both the Hystricomorpha and Myomorpha suborders.

Materials and Methods

Ethics

All experimental protocols concerning animals were conducted in accordance with the guidelines published in the National Institutes of Health (NIH) guide for the care and use of laboratory animals [National Research Council USA, 2011], and were reviewed and approved by the Institutional Committee on the Use and Care of Experimental Animals from Universidad Maimónides, Argentina (resolution No. 16/2014).

Animals

South American plains vizcacha specimens were captured from a resident natural population at the Estación de Cría de Animales Silvestres (ECAS), Villa Elisa, Buenos Aires, Argentina, using live-traps placed at the entrance of their burrows. The capture and transport of animals were approved by the Ministerio de Agroindustria, Dirección de Flora y Fauna, Buenos Aires Province, Argentina. For the present work, brains of 22 vizcachas (11 males and 11 non-pregnant females), which had been previously captured for other studies, were used [González et al., 2012; Dorfman et al., 2013; Inserra et al., 2017; Gariboldi et al., 2019; Schmidt et al., 2019]. All animals ranged from 2.5 to 3.5 years old as determined by the dry lens weight according to Jackson [1986].

Tissue Collection and Processing

The animals were weighed, anesthetized by intramuscular injection of 13.5 mg/kg body weight ketamine chlorhydrate (Holliday Scott S.A., Buenos Aires, Argentina) and 0.6 mg/kg body weight xylazine chlorhydrate (Richmond Laboratories, Veterinary Division, Buenos Aires, Argentina), and sacrificed by intracardiac injection of 0.5 mL/kg body weight of Euthanyl™ (Sodic Pentobarbital, Sodic Diphenilhidanthoine, Brouwer S.A., Buenos Aires, Argentina). The brains were immediately removed, weighed, and

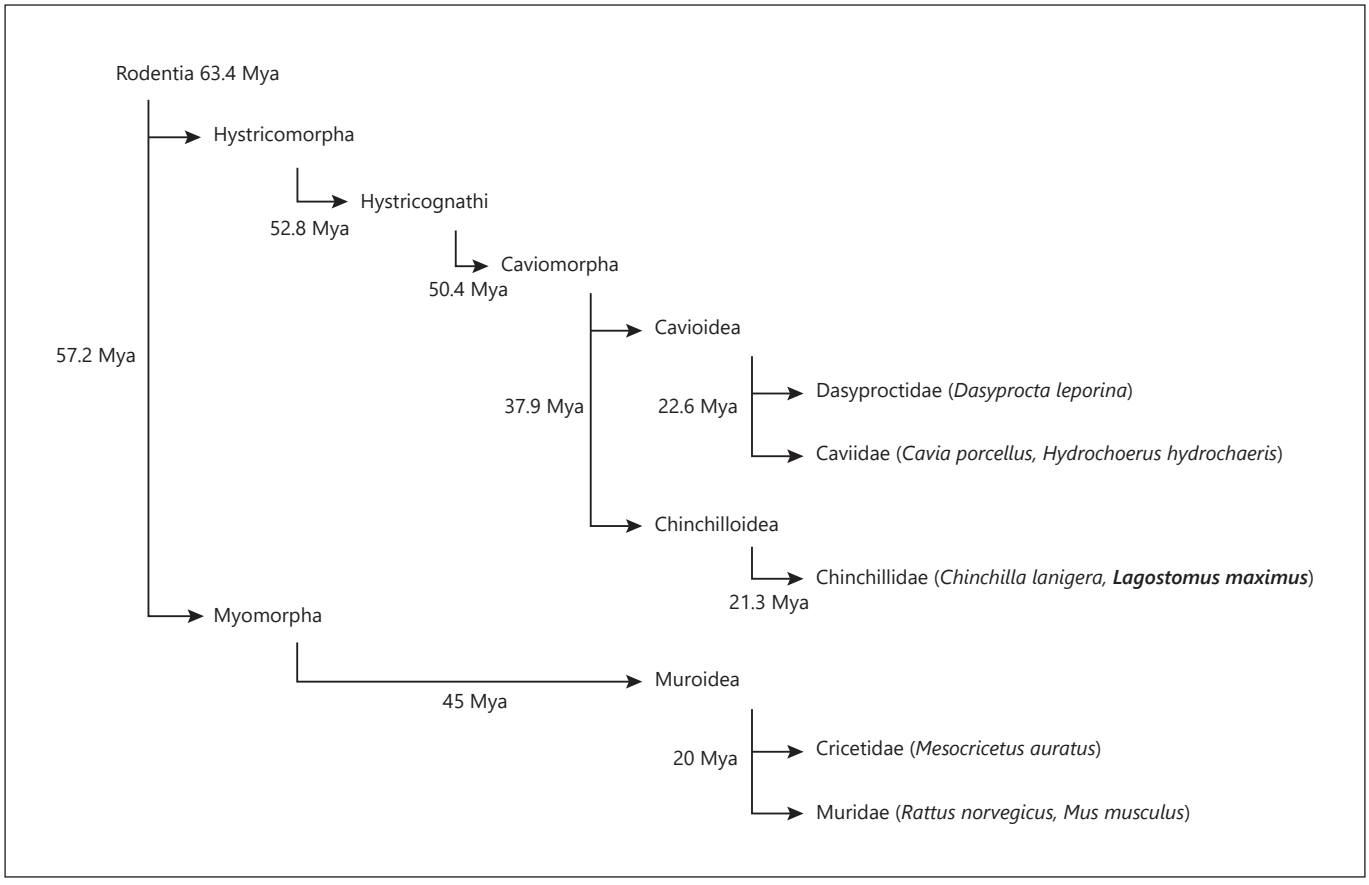

Fig. 1. Partial phylogenetic tree of Rodentia. Simplified cladogram of the order Rodentia (after Voloch et al. [2013] and Steppan and Schenk [2017]) showing phylogenetic relationships between *L. maximus* and the species used in this study for comparisons. Mya, million years ago.

photographed with a Coolpix L820 digital camera (Nikon Corp., Tokyo, Japan). After that, the brains were serially sectioned in a coronal plate in 5-mm-thick blocks and immediately fixed in cold 4% paraformaldehyde (Sigma Aldrich Inc., St. Louis, MO, USA) in 0.1 M phosphate buffer saline (pH 7.4) for 72 h. Then, the blocks were dehydrated through a graded series of ethanol and embedded in paraffin. The blocks containing the rostral temporo-parietal cortex and the caudal temporo-parietal cortex were cut in serial coronal sections (5 μm thick) with a microtome (Leica, Wetzlar, Germany) and mounted individually onto coated slides.

Histological Staining and Imaging

One from every 10 slices were separated to visualize the neurons by classical Nissl staining, according to the following procedure. Sections were dewaxed in xylene, rehydrated through a decreasing series of ethanol (100, 95, and 70%), and stained with Nissl solution using Cresyl violet (0.1 g/L) in 10% glacial acetic acid (pH 7.4) for 6 min. The images of histological staining were captured using an Olympus microscope (BX40, Olympus Optical Corporation, Tokyo, Japan) and a Nikon SMZ800 stereotactic magnifying glass equipped with 0.5× and 1× magnification (Nikon Corp.), both fitted with a digital camera (390CU 3.2 Megapixel CCD Camera, Micrometrics, Spain) and the Micrometrics SE P4 software (Standard Edition Premium 4, Micrometrics). Care was taken in selecting comparable areas among brains.

Neuroanatomical Comparison

The neuroanatomy of the brain of the vizcacha was compared with that of other rodents, including golden hamster (*Mesocricetus auratus*), rat (*Rattus norvegicus*), mouse (*Mus musculus*), chinchilla (*C. lanigera*), guinea pig (*Cavia porcellus*), agouti (*Dasyprocta leporina*), and capybara (*Hydrochoerus hydrochaeris*) available at the open-access digital brain collection of the Comparative Mammalian Brain Collections of the University of Wisconsin (http://www.brainmuseum.org).

Encephalization Quotient

For the calculation of EQ, we used a log-transformation of the allometric formula $E = KP^{\alpha}$, where E is the brain mass, K is the *y*-intercept (proportionality constant), P is the body mass, and α is the allometric exponent [Snell, 1891; Huxley, 1950; Jerison, 1985; Kruska, 2005]. To perform a phylogenetic generalized least squares (PGLS) regression analysis, we used our own data for vizcacha, the body and brain weights published by Spotorno et al. [2004] for

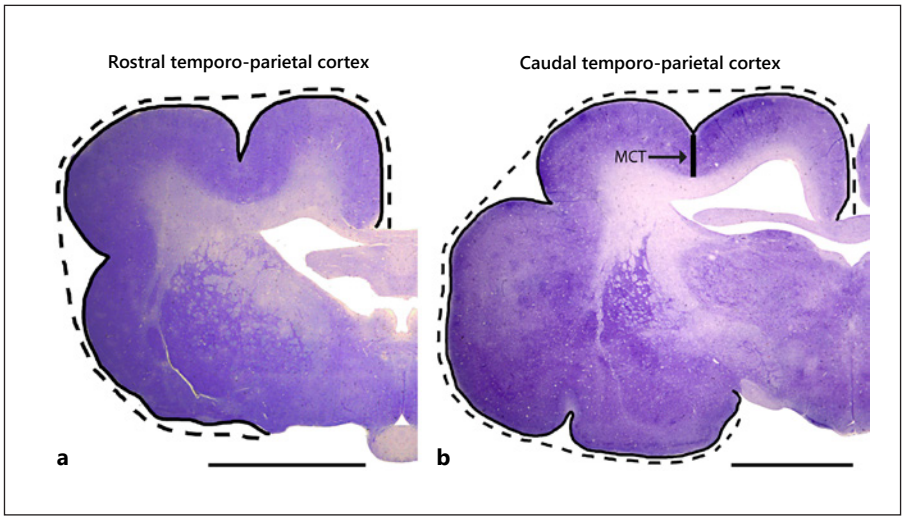

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Fig. 2. Temporo-parietal cortex of the plains vizcacha, *L. maximus*. Nissl-stained representative images of hemi-coronal slices at the rostral (**a**) and caudal (**b**) temporo-parietal cortex level. The CPC (solid line), the OCC (broken line), and the MCT are indicated. Scale bar, 5 mm.

chinchilla, and the information provided by Herculano-Houzel et al. [2006] for hamster, rat, mouse, guinea pig, agouti, and capybara. To determine whether the suborders Hystricomorpha and Myomorpha differ in their allometric patterning of brain mass and body mass, we used a phylogenetic analysis of covariance (pANCOVA). The analyses were carried out on the log-base-10-transformed body and brain mass data using Nmle and Evomap R Package [Smaers and Rohlf, 2016; Smaers and Mongle, 2018]. Based on our results (online suppl. Tables S1, S2; for all online suppl. material, see www.karger.com/doi/10.1159/000515638) the EQ used in this study was: EQ = brain mass/0.06 × body mass$^{0.65}$.

Gyrencephaly Index

To calculate the degree of cortical folding, the gyrencephaly Index (GI) was determined using Nissl-stained coronal brain slices (20 sections per animal) distributed throughout the temporo-parietal cortex, from the rostral to caudal temporo-parietal cortex. Both the complete pial contour (CPC) and the outer cortical contour (OCC) encompassing cortical structures were measured using the perimeter tool of the Image-Pro Plus 6.0 software. The GI value was determined as the quotient between CPC and OCC when the Zilles' formula was applied [Zilles et al., 1988]. To determine the GI for the species, the index for each brain slice was calculated and then the mean value was determined for each individual ($n = 22$). For vizcacha, the GI for the species was determined as the average of individual mean GI values. For the comparison of GI among species, images of coronal brain slices of hamster, rat, mouse, agouti, capybara, chinchilla, and guinea pig were obtained from the open-access digital brain collection of the Comparative Mammalian Brain Collections of the University of Wisconsin (http://www.brainmuseum.org) and BrainMaps.org (http://brainmaps.org). Besides, the obtained data for hamster, rat, mouse, agouti, and capybara were corroborated using the published literature [Pillay and Manger, 2007; Lewitus et al., 2014].

Minimum Cortical Thickness

Considering that the thickness of neopallium is inversely associated with the folding degree [Hogstrom et al., 2013], the minimum cortical thickness (MCT) was determined. MCT was calculated as the length between the pial surface and the external limit of the corpus callosum measured at the rostral lateral sulcus of the rostral temporo-parietal cortex where the neopallium shows its least thickness (Fig. 2). For vizcacha, the MCT was calculated as the average of all specimens examined ± SD ($n = 22$). For hamster, rat, mouse, chinchilla, guinea pig, agouti, and capybara, MCT was calculated using images of the rostral temporo-parietal cortex obtained from the open-access digital brain collection Comparative Mammalian Brain Collections (http://www.brainmuseum.org) and BrainMaps.org (http://brainmaps.org). Significant differences were not detected for MCT between both right and left hemispheres for all species analyzed.

Statistical Analysis

Significant differences for EQ, GI, and MCT values between the Myomorpha and Hystricomorpha suborders were evaluated using the Student *t* test or Mann-Whitney U test according to the distribution of the data. Differences were considered significant with $p < 0.05$. Pearson's correlation analysis for each suborder was performed between EQ and GI, and between MCT and GI. For statistical analysis, the Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA) was used.

Phylogenetic Analysis

To evaluate whether Hystricomorpha and Myomorpha differ in their allometric patterning of GI and brain mass, and MCT and GI, we used a PGLS regression analysis and a phylogenetic comparative method pANCOVA. Analyses were performed using Nmle and Evomap R Packages [Smaers and Rohlf, 2016; Smaers and Mongle, 2018] with logarithmically transformed (base 10) data.

Results

Gross Brain Morphology in the Vizcacha

The vizcacha has a gyrencephalic brain with a sagittal longitudinal fissure that divides both hemispheres.

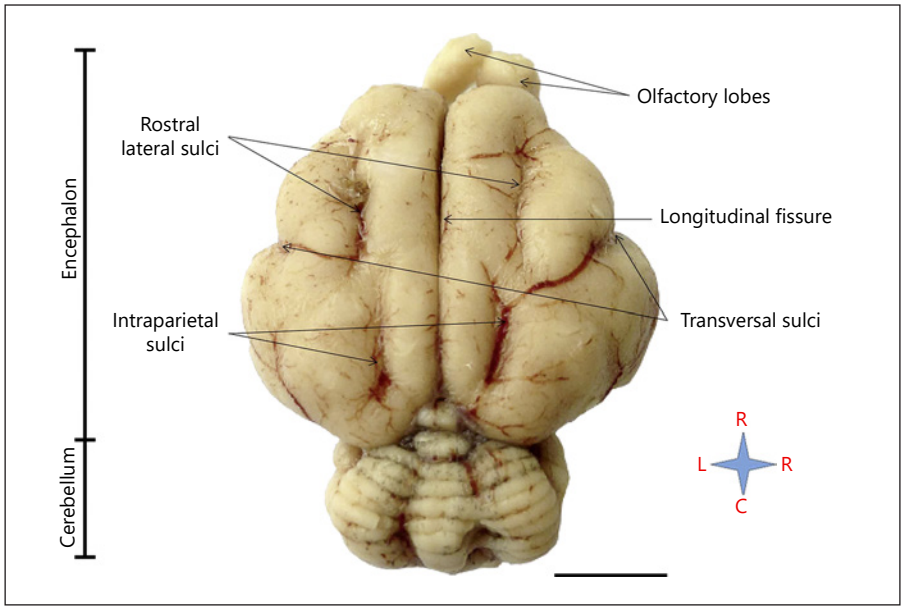

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Fig. 3. Gross brain morphology of the South American plains vizcacha, *L. maximus*. Dorsal view of the gyrencephalic encephalon and the cerebellum, showing the longitudinal fissure separating both hemispheres and symmetrical sulci at both sides of the brain. Scale bar, 1 cm.

The neocortical surface shows 3 pairs of sulci in the dorsal surface of the brain including one pair of lateral rostral sulci, one pair of intraparietal sulci, and one pair of transverse sulci, all of them with bilateral symmetry (Fig. 3). The morphometric parameter values were: EQ = 1.26 ± 0.06, GI = 1.11 ± 0.02, MCT = 0.63 ± 0.04 mm.

Comparative Neuroanatomy within Rodentia

Comparison of neuroanatomical parameters included 3 species of Myomorpha (golden hamster, rat, and mouse) and 4 species of Hystricomorpha (guinea pig, agouti, capybara, and chinchilla) in addition to vizcacha. All 3 Myomorpha species have a lissencephalic cortex and little variation in brain size. On the contrary, Hystricomorpha species show gyrencephalic brains with wide variability in size and a convoluted cortex (Fig. 4). Agouti and guinea pig show few and shallow sulci, whereas the capybara has the deeper and greatest number of sulci (Fig. 4). In chinchilla and vizcacha, both belonging to the same family, there are a similar number of sulci but they appeared deeper in vizcacha (Fig. 4). The lateral sulci in the vizcacha are divided into 2 sections, with one portion in the rostral cortex and the other one in the parietal cortex, as also found in the chinchilla and the guinea pig, whereas the lateral sulci in the capybara and the agouti are aligned in a single continuous sulcus (Fig. 4).

Relationships between EQ, GI, and MCT values revealed similarities and differences between Myomorpha and Hystricomorpha suborders as well as within each suborder (Table 1). The average EQ for Hystricomorpha was significantly higher than that for Myomorpha (1.51 ± 0.09 and 0.65 ± 0.08, respectively; Fig. 5a). EQ did not show a significant variability among the Myomorpha species; however, a great variability was observed among the Hystricomorpha, while the EQ of the vizcacha was below the average value of the suborder (Fig. 5b). Regarding GI, it was significantly higher for Hystricomorpha compared to Myomorpha (1.14 ± 0.04 and 1.02 ± 0.01, respectively; Fig. 5c). Whereas similar values of GI were observed among Myomorpha, Hystricomorpha displayed a great GI variability; the GI value of vizcacha was close to the average of the suborder (Fig. 5d). The MCT average was similar between Hystricomorpha and Myomorpha (0.80 ± 0.20 and 0.74 ± 0.02 mm, respectively; Fig. 5e). However, Hystricomorpha showed greater variability of MCT values, with the MCT value of the vizcacha close to the average value of the suborder (Fig. 5f).

Encephalization Strategy

To understand the encephalization strategy among rodents, and particularly the encephalization strategy of the vizcacha, the correlation between the neuroanatomical parameters was studied. For each species, Pearson's correlation was calculated between EQ and GI, and between MCT and GI, and independently analyzed for each suborder. The ratio between EQ and GI showed positive and negative correlations for Myomorpha ($r = 0.50$) and Hystricomorpha ($r = -0.65$), respectively (Fig. 6a, b). On the other hand, the ratio between GI and MCT showed a neg-

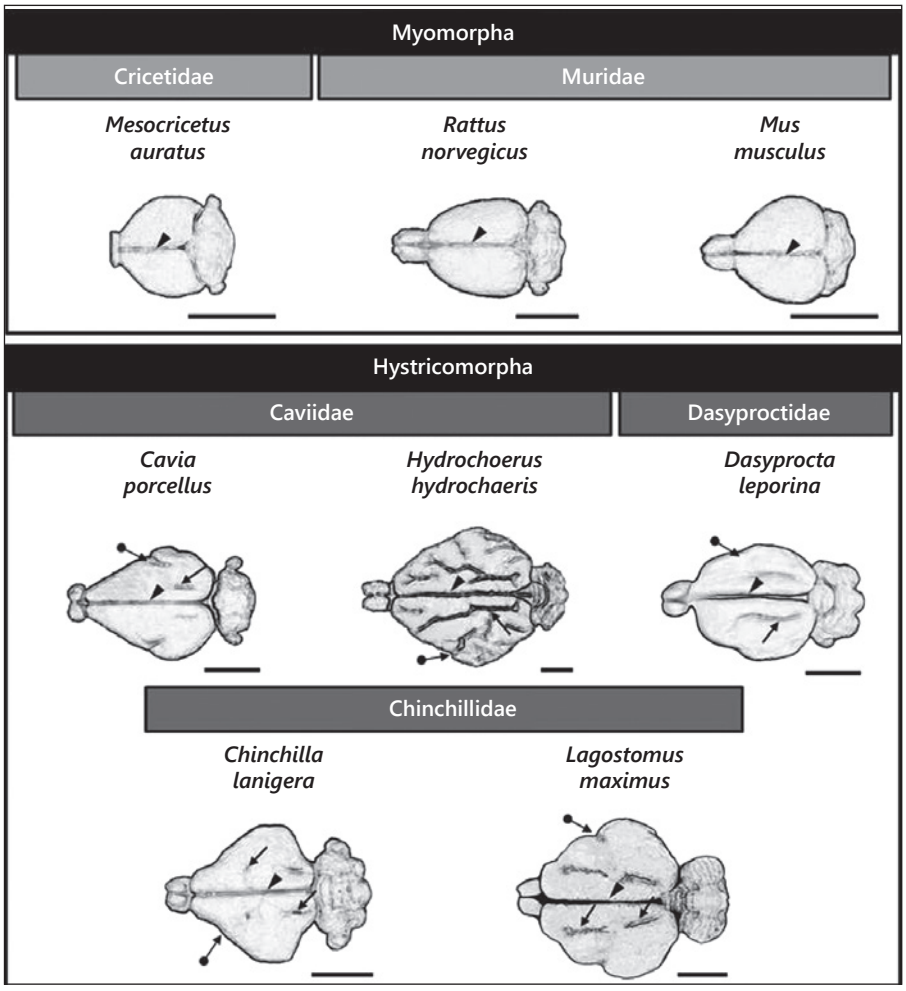

Fig. 4. Comparative brain drawing of rodents used in this study. Longitudinal fissure (arrowhead), transverse sulci (circle with arrow), and lateral sulci (arrow) are indicated. Drawings after the Comparative Mammalian Brain Collections (http://brainmuseum.org). Scale bar, 1 cm.

Table 1. Neuroanatomical indices in the rodent species analyzed in the current study

Species	Suborder	Brain morphology	EQ	GI	MCT, mm
Mesocricetus auratus	Myomorpha	Lissencephalic	0.61±0.07	1.01	0.74±0.03
Mus musculus	Myomorpha	Lissencephalic	0.63±0.20	1.03	0.71±0.04
Rattus norvegicus	Myomorpha	Lissencephalic	0.71±0.07	1.02	0.76±0.04
Cavia porcellus	Hystricomorpha	Gyrencephalic	1.48±0.02	1.05	0.92±0.03
Chinchilla lanigera	Hystricomorpha	Gyrencephalic	1.88±0.05	1.03	1.54±0.03
Dasyprocta leporine	Hystricomorpha	Gyrencephalic	1.74±0.21	1.23	0.50±0.04
Hydrochoerus hydrochaeris	Hystricomorpha	Gyrencephalic	1.16±0.07	1.30	0.45±0.05
Lagostomus maximus	Hystricomorpha	Gyrencephalic	1.26±0.06	1.11	0.63±0.04

EQ and MCT are average values ± SD. EQ, encephalization quotient; GI, gyrencephaly index; MCT, minimum cortical thickness.

ative correlation for Myomorpha ($r = -0.60$) and Hystricomorpha ($r = -0.81$; Fig. 6c, d). In addition, the vizcacha showed an intermediate position for both correlations (Fig. 6b, d).

Phylogenetic Analysis

PGLS regressions showed that while GI increases with increasing brain mass, MCT decreases with it (online suppl. Table S1). The difference between the suborders

Hystricomorpha and Myomorpha is not larger than expected by chance for either analysis (online suppl. Table S2).

Discussion

The current study reveals that the plains vizcacha has a gyrencephalic brain comparable to those found in other Hystricomorpha species distributed in South America (Caviomorpha). However, gyrification shows different patterns among the caviomorph species so far reported and compared herein. A few and shallow sulci characterize the brain morphology in agouti, guinea pig, and chinchilla, whereas the vizcacha shows 3 well-defined and deeper sulci, and the capybara has the most numerous and deepest sulci (Fig. 4). As found in Hystricomorpha, a wide variety of brain sizes and complexity of sulci and gyri has been previously described in other mammals at intra-order level [Jones and Peters, 1990]. Besides the variability of the gyrification pattern, the sulci in the vizcacha are divided into 2 sections while those of agouti and capybara are aligned in a single continuous space as reported previously for other species [Ono et al., 1990].

According to the number of sulci and its depth, the pattern of gyrification places the vizcacha at a middle position among Hystricomorpha analyzed herein. Quantitative analysis also indicates a middle position within the encephalization range. The EQ of the vizcacha showed a 26% increment above the expected brain volume according to its body weight. This level of encephalization is in agreement with the EQ >1.05 previously calculated for living caviomorphs [Bertrand and Silcox, 2016]. Despite belonging to the same family, the EQ of vizcacha is strikingly far lower than that of chinchilla. Similar differences were previously revealed by Herculano-Houzel [2007] for the guinea pig and the agouti. Although both species belong to different families of Caviidae, guinea pigs showed a higher EQ but lower cognitive abilities than agouti. In fact, all Hystricomorph rodents showed an EQ increment in comparison to Myomorpha. However, the pANCOVA revealed that this pattern of scaling was not significantly different from the allometric predictions.

The degree of cortical folding revealed by the GI reflected the lissencephalic condition of Myomorpha and the contribution to the increase of cortical surface in Hystricomorpha. As previously shown in primates [Zilles et al., 1988], GI increases allometrically with the increment of brain mass. GI showed great variability among the evaluated Hystricomorpha in agreement with previous works that showed variations in the number of sulci and gyri within the same taxonomic order [Manger, 2005; Pillay and Manger, 2007]. The GI determined for vizcacha revealed an 11% increment in the cortical invaginations related to lissencephaly, with the consequent increase in the cortical surface. This may indicate that cortical folds and fissures would allow better packing of the encephalon of the vizcacha inside the skull, as previously described for other species [Welker, 1990; Rakic, 1995; Albert and Huttner, 2015]. Moreover, at the macrostructural level, the increment in the GI of the vizcacha comes from a well-developed temporo-parietal cortex where the main sulci are located [Grewal et al., 2020]. In this lobular cortex, the brain structures corresponding to touch and hearing senses reside. This is consistent with the descriptions of Contreras [1984] who postulated that, as in the majority of animals that live underground, the vizcacha would present a great development of touch and hearing senses that play important roles in recognizing the sounds of the individuals of the colony and potential predators. Likewise, other structures associated with the neopallium, such as the olfactory bulb and striatum, show particular ecomorphological features [Schmidt et al., 2019], and this agrees with the wide ecological and morphological diversity of the caviomorph parvorder throughout evolution [Ferreira et al., 2020].

On the other hand, GI and thickness showed an inverse relation in Hystricomorpha ($r = –0.81$), as well as in Myomorpha, with both suborders not differing in their allometric patterning. This supports the idea that the thinner the brain cortex the greater the folding, allowing smaller and a greater number of gyri [Zilles et al., 1988; Pillay and Manger, 2007; Zilles et al., 2013].

Fig. 5. Relationships between neuroanatomical indices in Hystricomorpha and Myomorpha. **a** Box chart comparing EQ in Myomorpha and Hystricomorpha. EQ: Myomorpha = 0.65 ± 0.08, Hystricomorpha = 1.51 ± 0.09 (* $p = 0.0037$, Student t test). **b** Mean EQ in species of Myomorpha (hamster, rat, and mouse) and Hystricomorpha (chinchilla, guinea pig, vizcacha, agouti, and capybara). **c** Box chart comparing GI for Myomorpha and Hystricomorpha. GI: Myomorpha = 1.03 ± 0.01, Hystricomorpha = 1.15 ± 0.04 (* $p = 0.017$, Student t test). **d** Mean GI in species of Myomorpha and Hystricomorpha calculated according to the formula of Zilles et al. [1988]. **e** Box chart showing MCT in Myomorpha and Hystricomorpha. MCT for Myomorpha: 0.74 ± 0.02 mm; MCT for Hystricomorpha: 0.80 ± 0.20 mm ($p = 0.793$, Mann-Whitney U test). **f** Mean MCT for species of Myomorpha and Hystricomorpha. Light gray boxes and bars represent the Myomorpha suborder; dark gray boxes and bars represent the Hystricomorpha suborder.

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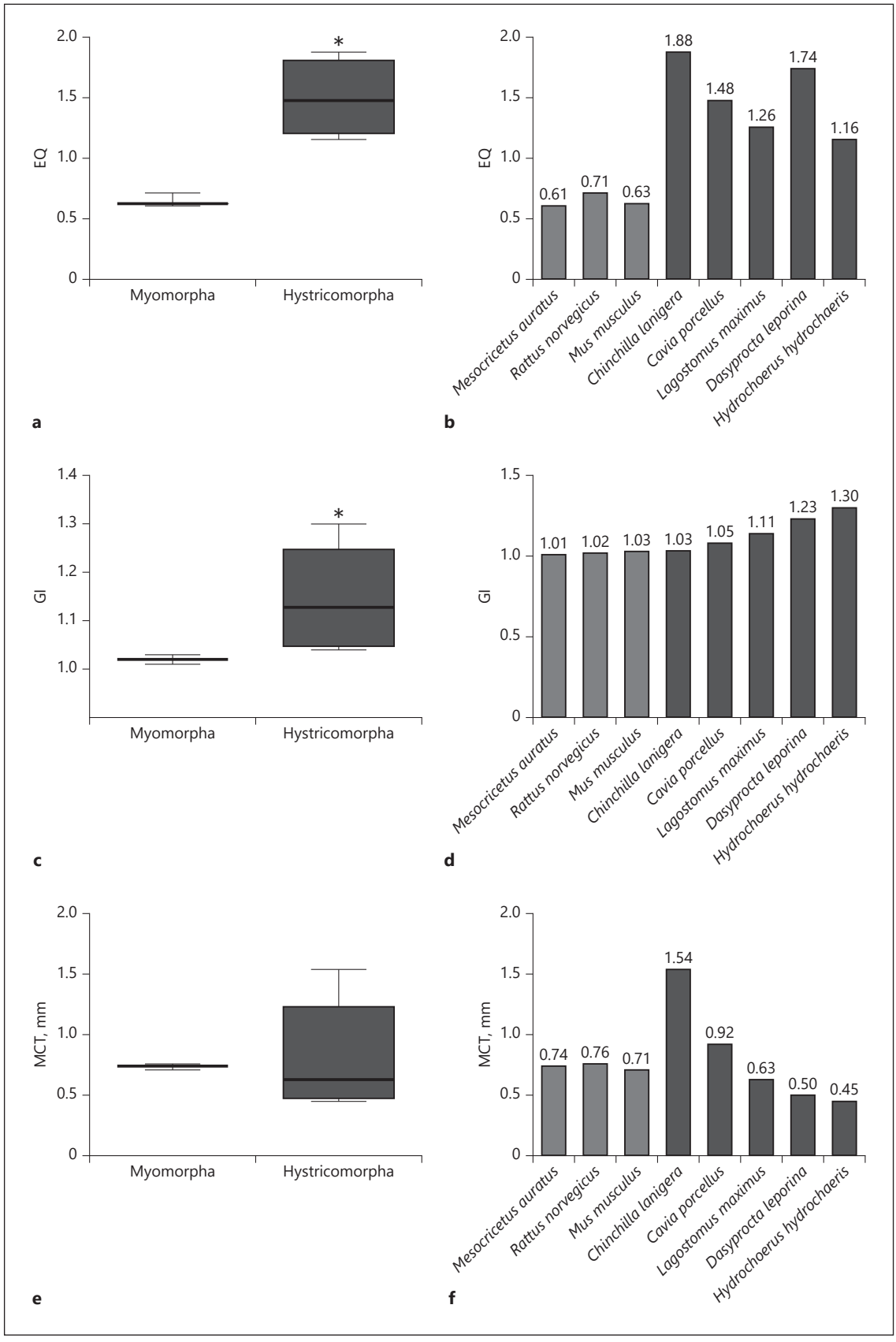
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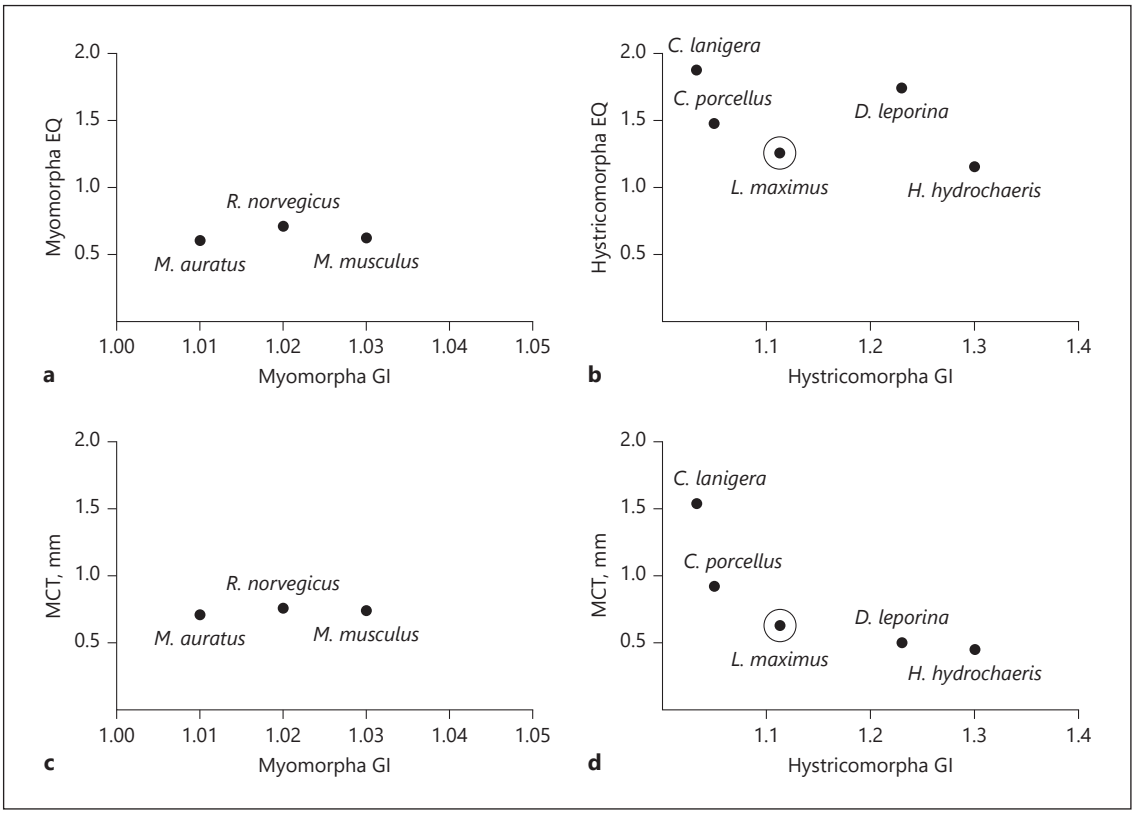

Fig. 6. Correlation analysis of neuroanatomical indices in Myomorpha and Hystricomorpha. Pearson's correlation analysis for EQ versus GI in Myomorpha ($r = 0.50$; **a**) and for Hystricomorpha ($r = -0.65$; **b**). Pearson's correlation analysis for MCT versus GI for Myomorpha ($r = -0.60$; **c**) and for Hystricomorpha ($r = -0.81$; **d**). EQ, encephalization quotient; GI, gyrencephaly index; MCT, mean minimum cortical thickness.

In fact, it has been previously described that cortical folding is inversely correlated with cortical thickness [Hogstrom et al., 2013]. Thin cerebral cortices are extremely folded, whereas thick cortices are completely smooth [Welker, 1990; Toro and Burnod, 2005; Pillay and Manger, 2007]. As a result of factors like cortical thickness, cell proliferation rate, and axons that tether the cortical plate at specific locations, cortical embryonic development is biased to buckle at specific locations [Striedter et al., 2015]. According to this, we hypothesized that the cortical thickness measured at the anterior lateral sulcus, which is mostly conserved among species, would be a reliable marker of the degree of invagination. Chinchilla and guinea pig, which show the smoothest brain of the analyzed species, displayed the greatest MCT values, whereas the highly folded brain of capybara showed the lowest value, close to those of vizcacha and agouti. The MCT value determined for the vizcacha was close to the average value of the group. As noticed above for gyrification, although the vizcacha and the chinchilla belong to the same family, their MCT values were disparate.

To establish the possible encephalization strategy, the specific correlation between indices indicated that Myomorpha, which does not show sulci except for the central fissure, showed a simple encephalization strategy with the brain size as the main variable. In contrast, Hystricomorpha showed different and more complex encephalization strategies, leading to a variety of brain morphologies in species with brain size as the main source of encephalization, such as chinchilla, or with a highly folded cortex and a lower brain size than expected for its body weight, as in the capybara. Correlation analysis of neuroanatomical parameters placed the vizcacha in a mid-position among Hystricomorpha, indicating a possible mixed strategy that includes a balance between the encephalic volume and folding as a combination of invaginations and cortical thickness.

Evolutionary Considerations

Studies on extant and extinct mammals have proposed a common ancestor with a small folded, gyrencephalic brain [O'Leary et al., 2013] in opposition to the classic view of a small and smooth-walled lissencephalic brain from which gyrencephaly derived repeatedly during evolution. In support of gyrencephaly as an ancestral condition, instead of being a primitive trait lissencephaly has been proposed as a derived trait emerging through a phenotypic reversal process following phyletic dwarfing [Kelava et al., 2013]. A good example is found in the small-bodied marmoset, a member of New World Platyrrhini, which evolved by dwarfism from big-bodied ancestors with a gyrencephalic cortex [Kelava et al., 2012, 2013, and references therein]. Moreover, weasels provide another example of progression towards lissencephaly since the smaller the species, the simpler the gyration pattern [Kelava et al., 2013]. In favor of this point of view, our results show that big-bodied South American Hystricomorpha rodents, such as capybara and vizcacha, have gyrencephalic brains, and it becomes simpler in species with smaller bodies, like chinchilla and guinea pig (Fig. 4).

Species of the late-evolved clade Myomorpha used for comparison in this study are very small-bodied Old World murids with a lissencephalic brain, a similar condition that can also be found in small-bodied South American cricetids, such as *Calomys* spp. and *Akodon* spp., among others [Vitullo, unpubl. observation]. This can be interpreted as an evolutionary trend toward secondary lissencephaly within the phyletic tree of Rodentia. However, there are examples of big-bodied nonmurid Myomorpha with a lissencephalic brain, such as the African giant pouched rat, *Cricetomys gambianus* [Ibe et al., 2014], which has a bodyweight intermediate to that of chinchilla and vizcacha. Moreover, the big-bodied Hystricomorpha African grasscutter, *Thryonomys swinderianus*, also has a lissencephalic brain [Ibe, pers. commun.]. Strikingly, South American Caviomorpha with a gyrencephalic brain, as shown in this study, are thought to have radiated from an African ancestor belonging to the infraorder Phiomorpha [Huchon and Douzery, 2001], which includes the family Thryonomyidae.

Conclusion

Even though rodents are the most numerous group of mammals, with more than 2,277 species distributed in around 480 genera in 3 suborders [Wilson and Reeder, 2006], our knowledge on neurocortical morphology and organization comes from a very small number of species. Paradoxically, laboratory mice and rats have been largely used in building most of our understanding of brain organization and function [Manger et al. 2008], neglecting the great variability that may hide in this group of mammals with so different lifestyles, occupying a wide variety of ecological niches, and displaying a plethora of adaptive behaviors surely associated with a highly variable size and number of cortical fields [Krubitzer et al., 2011]. Elucidating how many different strategies may have driven neocortical evolution in rodents, in perspective with extensive adaptation to different environments and lifestyles, still requires a comparative analysis of a large number of species.

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Statement of Ethics

All experimental protocols concerning animal handling were conducted in accordance with the guidelines published in the National Institutes of Health (NIH) guide for the care and use of laboratory animals and were reviewed and approved by the Institutional Committee on the Use and Care of Experimental Animals from Universidad Maimónides, Argentina (CICUAE-Res. 16/2014).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.R.S., M.C.G., S.A.C., S.P., M.C.C., P.I.F.I., and V.S.J. performed the experiments, data collection, and data analysis. J.H., A.D.V., and V.B.D. were responsible for the conception and design of the research, data analysis, and funding acquisition. V.B.D. performed project administration. A.R.S. wrote the original draft. A.D.V. and V.B.D. reviewed and edited the final version. All authors agreed to be accountable for the content of the work.

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