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Original Article

Stereology of spix's yellow-toothed cavy brain (*Galea spixii*, WAGLER, 1831)

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Spix's Yellow-toothed Cavy is a histrichomorphic rodent of the Caviidae family found in South American countries such as Brazil and Bolivia. It is a widely-used species as an experimental model in research in reproductive biology due to morphological and reproductive characteristics, such as the similarity in the placental development of *Galea spixii* and human species. However, there are no studies on the behavior of this species or on its brain morphology. Considering the lack of information in the literature about the brain and internal structures of *Galea spixii*, this study aimed to stereologic evaluate the brain as well as the volumetric proportions of the hippocampus and corpus callosum. Therefore, ten healthy animals were used from the Wild Animal Multiplication Center of the *Universidade Federal Rural do Semiárido*. The brains were measured in terms of external length, height and width, followed by fixation in paraformaldehyde solution 4% and coronary cuts with a thickness of 4mm and the rostral face of each cut was photographed, with the images being analyzed to determine the volumetric proportions of the required areas. There was no statistically significant difference between the means of volume, length, width and height when the right and left hemispheres were compared, and there was also no statistically significant difference between the volume of the corpus callosum and hippocampus in both hemispheres. The brain of *Galea spixii* is larger when compared to the rodent *Rattus norvegicus*. Volumetric differences may be responsible for distinct behavioral aspects between these species.

INTRODUCTION

The Spix's Yellow-toothed Cavy (*Galea spixii*, Wagler, 1831) is a wild synantrope rodent of the suborder Hystricognathi, family Caviidae and subfamily Caviinae, distributed throughout Brazil, Bolivia, the Andes mountain range (MOOJEN, 1952; OLIVEIRA et al., 2008; OLIVEIRA et al., 2012; WILSON; REEDER, 2007), and more precisely in the whole Caatinga biome (OLIVEIRA et al. 2012). It is an animal with an elongated body, being able to reach thirty centimeters in length and one kilogram of weight. It has uniform coloration with a dorsal gray-dark surface dorsal and white ventral surface. The head and eyes are large and the ears are short and rounded (OLIVEIRA et al., 2012).

This animal is much used in research in reproductive biology (SANTOS et al., 2015; SANTOS et al., 2016), in the development of spermatogenesis (SANTOS et al., 2012), as well as hormonal characteristics through the immunolocalization of steroidogenic enzymes (SANTOS, 2017). *Galea spixii* is an animal model suitable for studying the comparative development of trophoblastic processes in humans (BEZERRA, 2014).

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They belong to the genus *Galea* which has a complex taxonomic history with many controversies related to the taxon description, mainly because these comparisons are based on measurements and coloring patterns of the animals (BEZERRA; MARINHO-FILHO, 2010). Despite this, the species belonging this genus have been little studied.

Regardless wide geographic distribution of the species in South America, there are still few studies related to its behavior, as well as variations related to the environment. It is possible to find behavioral studies of some species of cavies in the literature such as the *Cavia intermedia*, and these studies suggest different learning strategies between male and female animals of this species (FURNARI, 2011). Bezerra; Marinho-Filho (2010) observed differences in cranial characters between males and females of the *Cavia intermedia* species, such as greater height in the mandibular branch and interorbital constriction in males, as well as greater incisive canal in females. These variations in the skull are certainly related to changes in the anatomy of the brain of this animal. However, studies on morphometry of this organ are necessary to confirm this hypothesis and will be extremely relevant to support behavioral studies on this species.

Morphometric knowledge of the encephalon pattern in *Galea spixii* becomes important for detecting diseases that affect this structure, in addition to allowing comparative anatomical analyses with other catalogued species, and may contribute to discussions about behavior and taxonomy among species of the same genus. Thus, the present study aimed to morphometrically evaluate the brain in Spix's Yellow-toothed Cavy, as well as the volumetric proportions of the hippocampus and corpus callosum.

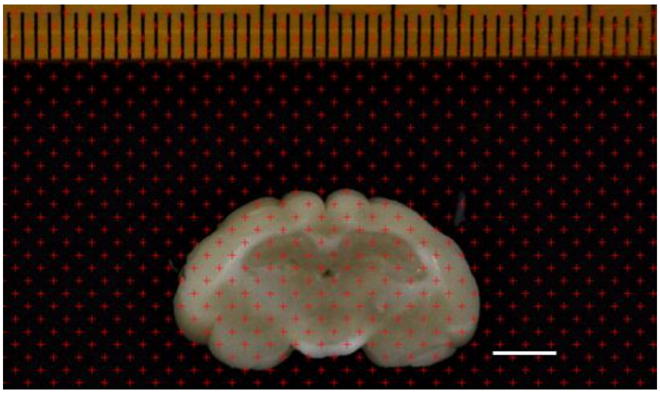
MATERIAL AND METHODS

The present study used ten female brains of healthy *Galea spixii* from the Wild Animal Multiplication Center (CEMAS) of the Universidade Federal Rural do Semi-árido (UFERSA), headquartered in Mossoró, and registered at *IBAMA* as scientific breeding under number 1478912. In addition, the research was submitted to the Animal Ethics Committee (*CEUA/UFERSA)* nº 48585-1/2015 after approval by the Chico Mendes Institute for Biodiversity Conservation (*ICMBio/SISBIO*) under the protocol number 23091.005451/2015-51.

To collect the samples, the animals were weighed and pre-medicated with a combination of 4 $mg.kg^{-1}$ xylazine hydrochloride and 60$mg.kg^{-1}$ ketamine hydrochloride, then after 10 minutes they were anesthetized and euthanized with a thiopental overdose (60 $mg.kg^{-1}$) and 2.56 $mEq.kg^{-1}$ of potassium chloride, both by intracardiac administration. Upon finding the death of the animal, they were perfused for 15 min through the ascending aorta with 4 % paraformaldehyde in 0.1 M phosphate buffer pH 7.4 at room temperature. The calvaria was subsequently opened for collecting the brains. An incision was made in the skin in a median line in order to reach the calvaria, extending from the supraorbital to the occipital region. After removing the skin and muscles on the calvaria, the skull was opened with a oscillating saw and pliers and the brain removed. To do this, an incision was made in the upper caudal part of the skull, in the interparietal bone region.

The brains were measured for external length, height and width. After the fixation period, coronary cuts were performed in the brains with a thickness of 4 mm, establishing an average of five sections per sample. Photographs of the rostral face of the slices were later subjected to analysis in Image ProPlus® software, which has the feature of a point grid projection on the captured image (Figure 1). In this way, data were extracted as the number of points that touched the analyzed structure and the area between them, enabling the volumetric determination of the required regions. The calculations were based on Cavalieri's principle. After it, the brains were fixed with 4 % paraformaldehyde in 0.1 M phosphate buffer pH 7.4 in order to conserve the samples.

Figure 1 – Point system of the Image ProPlus software over the brain section. Bar= 5mm



The volume of the structures was obtained by the formula: $[V]= t \times a(p) \times \sum p$. Where: [V] = volume, t = section thickness, a (p) = area associated with a test point, Σp = total number of points touching the cerebral section (GUNDERSEN et al., 1988).

Error coefficient (CE), at least 5 sections are sufficient to obtain a suitable CE, as shown in table 1.

Table 1 – Example of CE estimated from 5 coronal sections.

Section	PD	PE	TD	Mean	Pi.Pi	Pi.Pi+1	Pi.Pi+2
1	16	18	17	17	289	413,67	776,33
2	17	36	20	24,33	592,11	1111,22	1354,56
3	42	45	50	45,67	2085,44	2542,11	5251,67
4	48	64	55	55,67	3098,78	6401,67	0
5	125	110	110	115	13225	0	0
Total				257,67	19290,33	10468,67	7382,56
					A	B	C

$Nug = 0.0724 \cdot (b / \sqrt{a}) \cdot \sqrt{n \cdot \Sigma P} = 20.79$

$Var_{SPS} (\Sigma^N A) = [\,3\,(A - Nug) - 4B + C\,)\,] / 12 = 1943.04$

$CE (\sum P) = \frac{\sqrt{Total\ Var}}{\sum P} = 0.171$

The coefficient of variation (Cv) was obtained from 5 sections of three animals used along with the formula: $CV = \frac{Standard\ deviation\ (CE)}{\text{Mean}}$. CE and CV were estimated based on Gundersen and Jensen, 1987 and Sahin, 2001.

The following descriptive statistics were performed: mean, standard error of the volumetric proportions of the brain and brain structures, and inferential statistics were performed *a posteriori* to compare the volumes of the structures between the hemispheres by means of paired T-test (in case of normal distribution) or Wilcoxon test (in case of non-normal distribution). Normality was assessed using the Kolmogorov-Smirnov test, and the results were considered significant when the p-value was less than 0.05.

RESULTS AND DISCUSSION

The mean volume of the *Galea spixii* brain was 4631.96mm³, the error coefficient (CE) was 0.171 and the coefficient of variation (Cv) was 9.1%. The average length, width and height were 20.97mm, 23.74mm and 12.89mm, respectively. There was no statistical difference between the mean volume, length, width and height when the right and left hemispheres were compared. The mean length, width and height of the entire brain can also be seen in Table 2.

Table 2 – Length, width, height and volumetric proportions of the right and left hemispheres and the brain of *Galea spixii.*

	Length	Width	Height	Volume[1]
Right	20.54 ± 0.31	11.50 ± 0.31	12.96 ± 0.36	2360.39 ± 162.84
Left	20.41 ± 0.37	12.13 ± 0.40	12.81 ± 0.39	2292.09 ± 145.52
Brain	20.97 ± 0.41	23.74 ± 0.55	12.89 ± 0.31	4631.96 ± 295.21

Data expressed as mean ± SD and values expressed in mm [1]expressed in mm³.

There was no statistically significant difference in the volume of the corpus callosum and hippocampus of both hemispheres of *Galea spixii* (Table 3).

Table 3 – Mean volume of the corpus callosum and hippocampus of both hemispheres of *Galea spixii*.

Structure	Right	Left
Corpus callosum	144.97 ± 11.63	140.18 ± 12.12
Hippocampus	190.50 ± 26.09	184.51 ± 23.09

Data expressed as mean ± SD and values expressed in mm³.

This research is a pioneer study on the brain morphometry of *Galea spixii*. The data showed that the volume of the brain, corpus callosum and hippocampus, as well as their length, height and width do not have statistical differences when compared to the hemispheres. It was only possible to include females of this species in this research because it is a wild animal that is difficult to access and has a limited number of specimens. Limitation in the use of only one gender does not seem to interfere with the obtained results, since there are no reports of sexual differences regarding the volume of structures analyzed in studies with other rodents. Sahin et al. (2001) demonstrated that there is no significant difference between the volume of the hemispheres of the brains of males and females of *Rattus norvegicus* species. Likewise, Keeley et al. (2014) demonstrated that there were no statistical volumetric differences between the hippocampus of *Rattus norvegicus* males and females. Based on these findings, the inclusion of males in the present study is unlikely to significantly alter the volumetric results found.

It is interesting to note that the comparative volumetric study of the hemispheres of other species developed by Sahin et al. (2001) in using similar methodology verified that the volume of the brain of *Rattus norvegicus* (598.95 ± 10.59 mm³), comparing to Spix's Yellow-toothed Cavy, described in the present study, it was verified that the volume of the brain in Cavies were larger. The fact that Spix's Yellow-toothed Cavy has a larger body structure in relation to rats may partially explain the greater brain volume. In addition, Kruska (2014) evaluated that the mean volume of the brain of *Cavia aperea* and *Cavia porcellus* were 4764.31 mm³ and 4468.893 mm³, respectively. An one-sample t-test was performed, with a 95% confidence interval, stating that there was no significant statistical difference between the mean brain volumes of *Galea spixii* and *C. aperea*, as well as between *G. spixii* and *C. porcellus* (data not shown).

The hippocampus and corpus callosum were the most prominent encephalic structures during the analysis of the encephalic sections, which motivated measurement of their volumes and because they were structures of fundamental importance. The hippocampus as a component of the limbic system has the functional ability of memory and spatial orientation (MANDAL, 2014). The primary function of the corpus callosum is to provide a connection between homologous cortical areas (BLOOM; HYND, 2005), as well as to work together with these cortical visual maps, forming a coherent vision map and having an important role in coordination between the two sides of the body (GOOIJERS; SWINNEN, 2014). In addition and according to Hutchinson et al. (2008) and Luders et al. (2007), considering that this structure is related to cognitive functions, a larger corpus callosum area promotes better performance in difficult activities and better integration between cortical areas.

According to Zhao et al. (2012), the mean corpus callosum volume of *Rattus norvegicus* obtained by the Cavalieri principle was 68.9 mm^3, being lower than that found for *Galea spixii*. The cavies used in this research were raised/bred in enclosures with an area of 12.5 m^2 which mimic their natural environment containing rocks, twigs and dry leaves. The higher volume of corpus callosum in the Spix's Yellow-toothed Cavy may be associated to the enriched environment, which is according Zhao et al. (2012) who studied the effects of an enriched environment on the volume of this structure and its amyloinized nerve fibers, and concluded that the animals submitted to an improved environment presented a larger corpus callosum, and consequently greater spatial learning.

Using a methodology similar to the one used in the present study, Lu et al. (2014) demonstrated that the hippocampal volume of *Rattus norvegicus* is 99.8 ± 3.3 mm^3. While using magnetic resonance imaging (MRI), Luo et al. (2014) obtained values of 38.92 ± 1.95 mm^3 and 39.67 ± 1.84 mm^3 for the hippocampus volumes of the right and left hemispheres of the same species, respectively. These results are lower to those found in our study. This volumetric difference probably derives from the need for spatial orientation and memorization in a more complex habitat, which would give the cavies a larger hippocampus, in addition to the larger encephalon volume in relation to the *Rattus norvegicus*. The distinction between the volumes of this structure would explain that although the ability to establish and memorize trails is found in both *Galea spixii* and *Rattus norvegicus*, it is more present in *Galea Spixii*.

CONCLUSION

In conclusion, in the morphometric analysis of encephalon structures of females of the *Galea spixii* species, it was verified that there are no significant differences between the right hemisphere and the left hemisphere of the encephalon in this species. Moreover, in relation to the volume of structures such as the corpus callosum and hippocampus, no statistically significant differences were observed between the hemispheres. However, in view of this stereological study demonstrating the absence of asymmetries between the structures, it is not possible to establish a relation between the functional and structural differences between the hemispheres. This limitation should be the objective of future studies.

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