NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., March 2018 **Vol 39 (1): 66 - 74.**

https://dx.doi.org/10.4314/nvj.v39i1.8 **ORIGINAL ARTICLE**

Lobulation Pattern of the of the Cerebellum of African Grasscutter (*Thryonomys swinderianus*)

Obadiah, B. [1]***; Dzenda, T.** [2] **and Wanmi, N.**[1]

[1.] Department of Veterinary Anatomy, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria.
[2.] Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.
*Corresponding author: Email: byaneto@yahoo.com; Tel No:+2348062804899.

SUMMARY

Grasscutter is one of the largest species of rodents in Africa whose basic cerebellar folding pattern has not been documented. The aim of the research was to provide reference data on the lobulation morphometric of its cerebellar cortex. A quantitative analysis was conducted on the cerebella of seven African grasscutters. The results showed that the head weight had strong positive correlation with the cerebellar weight ($r = 0.8954$, $P < 0.001$). In the rostral lobe, the mean size of declive was significntly larger than *Lingula cerebelli* ($P < 0.01$) and culmen (*Pars caudalis*) ($P < 0.001$). Also, the culmen *Pars rostralis* differed signigifcantly with *Pas caudalis* ($P < 0.01$). For the *Lobus caudalis*, the mean folium vermis was significantly higher than those of the tuber vermis and nodulus ($Ps < 0.001$). The ovula vermis was the broadest lobule in this lobe, was significantly larger than the tuber vermis the pyramis, and nodulus ($Ps < 0.001$). The mean internal perimeter, including all primary and secondary sublobules (4643.3 ± 341.69 µm) was higher than the external perimeter (1621 ± 42.43 µm) significantly ($P < 0.0001$). The mean cerebellar foliation index was 2.86 ± 0.18; suggesting a relatively increased–in length of cerebellum in form of a folded structure. In conclusion, some of the structural development and behaviours in the grasscutter may likely be correlated with the relative sizes of individual cerebellar lobules.

Key words: Cerebellum, lobes, lobules, morphometric, *T. swinderianus*

INTRODUCTION

The African grasscuttes (AGC) is one of the Africa's largest rodents. The animals are nocturnal and usually forage in groups, with a male leading the group, as they travel through trails in reeds and grasses they created (NRC, 1991). The animals do not create burrows, but may scoop out shallow burrows created by other rodents, using their strong limbs, which also enable them to stand upright position when feeding or sensing predators.

Cerebellum is that complex structure of the brain that coordinate posture and voluntary movement in animals and man (Fletcher, 2006). It accounts for 13.5 % and 10% of the total brain weight in the grasscutter and humans, respectively (Knierim, 1997; Byanet *et al*., 2012). The morphological complexity of the cerebellum is shown to be associated with the degree of folding, or foliation, which is related to its regional function (Demaerel, 2002; Iwaniuk *et al*., 2005). The folding of laminated structures like cerebellum has been shown to increase its surface area for processing activities (Finger, 2000).

Sultan and Braitenberg (1993) reported that differences in shapes and sizes of cerebellar regions are related to peculiarities of body build, motor habits and skills. Welker (1990) had documented that species differences in cerebellar size and number of folia/lobules reflect behavioural and/or cognitive differences in mammals. For example, expansion of the paraflocculus and medial lobes regions in bats and whales are adaptation for echolocation (Paulin, 1993).

We have reported on the gross, histomorphology, morphometric (Byanet *et al*., 2008, 2012) and deep cerebellar nuclei (Byanet, 2015) of the cerebellum in grasscutter, without considering its degree of folding / foliation pattern. Therefore, the present work was aimed at determining the gross cerebellar lobulation pattern and its functional relationship in the grasscutter.

MATERIALS AND METHODS

Animal Source and study Location

A total of seven matured AGCs were used in this study. Brownish perineal staining in males was considered as an index of sexual maturity (Adu and Yeboah, 2003). The animals were purchased from a breeder farm in Otukpo town ($07^o13^/$N, $08^o05^/$E), Benue State, situated in the Southern Guinea Savannah zone of Nigeria. The animals were then transported by road in wooden constructed laboratory cages having two separate compartments; for males and females to avoid fighting. The dimension of the cages measured 50 cm (height) by 40 cm (width) and 40 cm (length). They were kept in the Research Laboratory, Department of Veterinary Anatomy, Ahmadu Bello University, Zaria, Nigeria, under room temperature for three days for acclimatization before the experiment. The rodents were fed with elephant grass (*Pennisetum purpureum*), supplemented with chick growth mash and given access to water and feed *ad libitum.*

The protocol for the research project was approved by the Ethics Committee of Ahmadu Bello University, Zaria, Nigeria. The environment, housing and management of the animals were similar to that stipulated in the Guide for the Care and Use of Laboratory Animals, 8th Edition, National Research Council, USA, downloaded from the National Academic Press, Washington D.C. (www.nap.edu.).

Brain Extraction and Fixation

The body weight of each animal was taken with a digital electronic balance (Mettler balance P 1210, instruments AG. Switzerland), after being anaesthetized with 10-25 mg/kg IM ketamine Hcl. The animals were then killed through decapitation at the atlanto-axial joint using a small sharp knife and forceps. Each head was weighed and its length measured before it was skinned and stripped of all muscles. The brain was then extracted from the skull according to the method described by Fletcher (2006), but with modifications due to peculiarity of the rodents. Briefly, each head was completely flayed and the surface of the skull cleared of all muscles using forceps and scissors. Little cuts were made on the frontal and occipital bones using small knife, but with care to avoid damaging the brain. Bones were gently removed starting from the cut areas of the frontal and advanced to the nasal bone rostrally and temporal bones laterally using small bone cutter. This gradually continued to the base of the skull, where the cranial nerves were cut through and finally the brain was removed and meninges were gently removed from the brain. The cerebellum of each rodent was separated from the brain stem by cutting through the cerebellar peduncles using forceps and surgical blade.

They were fixed in 10% formalin and later transferred to Bouin's solution.

The brains on gross examination were apparently normal without any pathological lesions. The brains were weighed with a mettler balance, (Model P 1210, AG, Switzerland, with sensitivity of 0.01g) and their lengths obtained using a digital vernier caliper (MG6001DC, General Tools and Instruments Company, New York). The cerebellar peduncles were cut using a surgical blade to separate the cerebellum from brainstem. The cerebellar weight and dimensions (such as the width and circumference) were obtained using a mettler balance and digital vernier caliper, respectively.

Measurement of lobule size

Before measuring the sizes of lobules, a mid-sagittal section of the vermis of each cerebellum was made to identify their lobes and lobules (Figure I). A Steindorff Hand Held Digital Microscope (New York Microscope Company) was then attached to a computer and macro-photographic pictures of the mid-sagittal view of each cerebellum were captured (640 x 480 jpg pixels). Each lobule was numbered in ascending order, from rostral to caudal, with the primary lobules identified by Roman numerals (I, II, III…) as described by Larsell (1952). Their sizes were then measured and expressed in micro millimeter (µm) using software, Motic Images Plus, 2.0 ML, Multi Language Version (Motic China Group Co., Ltd., 2001-2007 (http://www.motic.com). The cerebellar foliation index (CFI) was determined by measuring the total length of the Purkinje cell layer or internal perimeter divided by the length of the envelope or external perimeter, which follows the dorsal surface of the Purkinje cell layer without counting the depth of the lobules as described by Turner (1967) in geometry of the human cerebellar vermis and Iwaniuk *et al*. (2006). For terminology, Nomina Anatomica Veterinaria (2012), Edition was used.

Statistical analysis

The data (weights and dimensions) were tabulated and expressed as mean and standard error of the mean (SEM). Differences in mean weights and dimensions were analyzed using the student t - test. A correlation matrix was constructed, relating the weight and dimensions of the cerebellum to other variables (body, head and brain), and also relating the sizes of the lobules to each other in both the anterior and posterior lobes. Pearson's Correlation Coefficient (r) was used to compare the relationships in weights and dimensions of cerebellum and other variables and the lobule sizes."One-way ANOVA with Dunnett's post-test was performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA (www.graphpad.com). Value of $P \leq 0.05$ was considered as significant.

RESULTS

Table 1 shows the weight and dimensions of the body and brain of grasscutter. The mean weight of the body (1145.71 ± 338.84 g), the head (132.86 ± 43.55 g), brain (8.25 ± 0.64 g) and cerebellum (1.12 ±0.06 g) were observed. Also, the length of the body (469.14 ± 38.67 mm), brain (43.31 ± 1.35 mm) and cerebellum (14.79 ± 1.35 mm) were noted. The cerebellum width (22.43 ± 0.72 mm) and circumference (31.57 ± 0.69 mm) were wider than its length (14.79 ± 0.15mm). The correlation matrix computed for the weight of the body, head, brain and cerebellum are presented in Table 2. The body weight showed strong positive correlation with the brain ($r = 0.9828$, $P < 0.0001$) and with the head ($r = 0.9248$, $P < 0.01$) weights. The head weight also was positively correlated with the brain ($r = 0.9251$, $P < 0.01$) and cerebellar ($r = 0.8954$, $P < 0.001$) weights.

The results of the lobule sizes in the *Lobus rostralis* and *Lobus caudalis* are presented in Table 3. The size of rostralis (355.64 ± 29.48 µm) was smaller than the caudalis (405.35 ± 29.87µm), though not statistically

Table 1: Weights and dimensions of the body and brain components in the grasscutter

Parameter		**Grasscutter (n = 7)**	
		Min – Max	**Mean ± SEM**
Weight (g)	Body weight	460 -2500	1145.71± 338.84
	Head weight	50 -300	132.86±43.55
	Brain weight	6.89 -10.77	8.25±0.64
	Cerebellum weight	1.0 -1.47	1.12±0.06
Dimension (mm)	Body length	400-690	469.14±38.67
	Head length	82-124	106.57±5.87
	Brain length	35.5-46.2	43.31±1.35
	Cerebellum length	14-15	14.79±0.15
	Cerebellum width	20-25	22.43±0.72
	Cerebellum circumference	30-34	31.57±0.69

n = sample size, Min = minimum, Max = maximum, SEM = standard error of mean

TABLE 2: Comparison of correlation matrix of the body and brain weights in the grasscutter

Weight (g)	BDW	HDW	BRW	CBW
Body weight	1.0000	0.9248**	0.9828***	0.7022
Head weight	0.9248**	1.0000	0.9251**	0.8954
Brain weight	0.9828	0.9251	1.0000	0.7464*
Cerebellum weight	0.7022	0.8954	0.7464	1.0000

n = sample size, BDW = body weight, HDW = head weight, BRW = brain weight, CBW = cerebellar weight, * = P < 0.01, ** P < 0.001, *** = P < 0.0001

TABLE 3: Size of the lobules in the cerebellum of the grasscutter

Lobule	Grasscutter (n = 7)	
	Min-max	Mean ±SEM (µm)
Lingula cerebelli	332.10-568.80*	427.40±30.07 ^{a}
Lobulus centralis	393.70-637.20	486.41±36.37
Culmen (Pars rostralis)	480.10-787.40	596.86±37.60 ^{a1}
Culmen (Pars caudalis)	35.00-497.10	332.26±54.27 $^{a1, b}$
Declive	40.40-832.90	646.54±48.08 $^{a, b}$
Lobus rostralis	-	355.64±29.48
Folium vermis	566.20-858.3	754.23±35.34 ^{b}
Tuber vermis	271.20-521.20	394.41±39.90 $^{b, b1}$
Pyramis (vermis)	288.10-692.40	380.54±54.00 $^{c, b2}$
Ovula (vermis)	677.20-1046.9	887.73±55.18 $^{b1, b2, b3}$
Nodulus	345.00-507.70	420.57±24.69 $^{b, b3}$
Lobus caudalis	-	405.35±29.87
Overall mean	-	760.99±59.35
External perimeter (EP)	1420.5-1752.60	1621.0±42.43^{c}
Internal perimeter (IP)	3447.00-5703.60	4643.3±341.69^{c}
CFI=EP/IP	2.37- 3.54	2.86±0.18

n = sample size, Min = minimum, Max = maximum, SEM = standard error of mean, I –X = lobule number, CFI = cerebellar foliation index. a = P < 0.01, b, b^{1}, b^{2} and b^{3} = p< 0.001, c = p< 0.0001

TABLE 4: Comparison of correlation matrix of the lobule sizes in the anterior and posterior lobes of the grasscutter

Lobe		Lobule				
		I	II	III	IV	V
Lobus rostralis	Lingula cerebelli	1.0000	-0.1058	-0.1490	0.3106	0.3628
	Lobulus centralis	-0.1058	1.0000	0.6206	0.4410	0.0159
	Culmen (Pars rostralis)	-0.1490	0.6206	1.0000	0.5652	0.3639
	Culmen (Pars caudalis)	0.3106	0.4410	0.5652	1.0000	0.5460
	Declive	0.3628	0.0159	0.3639	0.5460	1.0000
		VI	VII	VIII	IX	X
Lobus caudalis	Folium vermis	1.0000	0.3342	0.2273	0.4901	-0.1835
	Tuber vermis	0.3342	1.0000	0.6509	0.3445	-0.0340
	Pyramis (vermis)	0.2273	0.6509	1.0000	0.0230	-0.1628
	Uvula (vermis)	0.4901	0.3445	0.0230	1.0000	0.7867*
	Nodulus	-0.1835	-0.0340	-0.1628	0.7867*	1.0000

n = sample size, I-V = lobule numbers of the anterior lobe, * = P <0.01, *** = P < 0.0001

significant (P > 0.25). In the *Lobus rostralis*, the individual lobules showed that the size of declive (646.54±48.08 µm) was significntly larger than *Lingula cerebelli* (427.40 ± 30.07 µm) (P < 0.01) and culmen (*Pars caudalis*) (332.26 ± 56.27 µm) (P < 0.001). Also, the mean culmen (*Pars rostralis* (596.86 ± 37.60 µm) differed signigifcantly with *Pars caudalis* (P < 0.01). For the *Lobus caudalis*, the mean folium vermis (754.23±35.34 µm) was significantly higher than those of the tuber vermis (394.41±39.90 µm) and nodulus (420.57±24.69 µm) (P< 0.001). The ovula vermis was the broadest lobule in this lobe, with mean size (887.73±55.18 µm), significantly larger than the tuber vermis (394.41±39.90 µm), the pyramis (380.54±54.00 µm) and nodulus (420.57±24.69 µm) (Ps < 0.001). The mean internal perimeter, including all primary and secondary sublobules (4643.3 ± 341.69 µm) was higher than the external perimeter (1621 ± 42.43 µm) significantly (P < 0.0001).

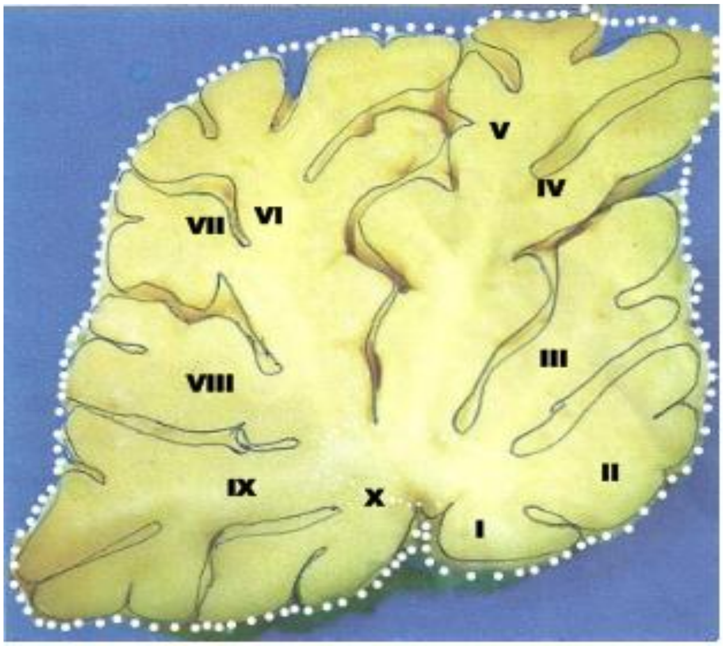

Figure 1: A mid-sagittal section of the grasscutter showing lobules I (*Ligula cerebelli*) to lobule X (Nodulus) in a rostro-caudal direction using the terminology of Larsell (1967). The *Lobus rostralis* consists of lobules I (*Lingula cerebelli*) to V (Declive), while Lobus caudalis has lobules VI (Folium vermis) to X (Nodulus). The total circumference or external perimeter of the section of the vermis was measured along the white dotted solid lines, while the internal perimeter was measured on individual lobules shown by dark faint lines

The mean cerebellar foliation index (CFI) of 2.86 ± 0.18 µm was recorded.

Table 4 presents the results of correlation matrix test for the relative size of the *Lobus rostralis* and *Lobus caudalis*. The correlation coefficient (r) was computed for every pair of variables independently without considering the other variables. Relationships between lobule sizes within the *Lobus rostralis* were non-significant ($P > 0.05$). In the *Lobus caudalis,* the uvula (vermis) was significantly correlated with nodulus ($r = 0.7867$; $P < 0.01$).

DISCUSSION

The macroscopic complexity of the cerebellar cortex in the grasscutter is an important parameter, which Sultan & Braitenberg (1993) related its form and size to the peculiarities of body build, motor habits and skills of an animal. Analysis from this study may provide clues to the special role of the cerebellum in motor and non-motor controls in this species. Similarly, Sultan & Braitenberg (1993) showed that cerebellar width increased with body size in smaller species of mammals, but almost remained constant in larger ones.

Elftman (1932) had earlier reported that *Lingula cerebelli* controls the caudopelvic muscles, such as pubo-caudalis, iliocaudalis and coccygeus that strengthen the floor of the pelvic region in primates. These muscles have their insertions in the tail and play important role in flexing and abducting the tail. The relative short, though big tail of the grasscutter may be related to the size of *Lingula cerebelli* and *Lobulus centralis* as reported in the present study. Chang and Ruch (1949) reported that *Lingula cerebelli* size is related to tail length in the spider monkeys. To support this argument, Bispo (2010) showed *Lingula cerebelli* in humans to be small and the only lobule that did not extend to the cerebellar hemispheres.

Even though grasscutter has a relatively short tail the base of the tail is usually large, leading to relatively large muscles within the perineum to compensate for the tail length, hence, relatively large size of *Lingula cerebelli* in grasscutter. *Lobulus centralis* in the present findings corresponded to the central lobule of Niadich *et al.* (2009). *Lobulus centralis* is also considered to play a vital role in the caudopelvic muscles like *Lingula cerebelli*; especially in supporting the viscera and in control of internal body pressure as well as the activity of the tail (Larsell, 1953).

The Culmen (*Pars rostralis*) in the present work was well developed in grasscutter, being the fourth largest lobule in this study. It is said to be correlated with hindlimb musculature or leg strength, walking and swimming abilities in animals and birds (Larsell, 1967; Iwaniuk et al., 2006). The wild grasscutters are good rock climbers and excellent swimmers (NRC, 1991; Mensay and Okeyo, 2005). These activities are done by the help of their limbs; suggesting a correlation between these lobules with the strength of their limbs. It has also been noted that when the grasscutter is alarmed, it stamps its hind feet, then gives a strange booming grunt sound before its runs (NRC, 1991). This behaviour still supports the strength of the limbs in escape from predators, which is related to the sizes of Culmen (*Pars rostralis*).

Grodd *et al.* (2001) showed that a relationship exists between the movement of the lower extremities of the forelimb, with the vermis and hemisphere of declive. In the similar vein, Stoodley & Schmahmannn (2009) reviewed the functional topography of the human cerebellum and stated that tasks which employed finger movements are associated with the activity of declive. Base on these reports, the ability of the grasscutter to stand upright or erect while feeding using their forelimb paws (homologues of fingers in humans) to grasp grasses or other food material, may be related to the size of declive.

Voogd and Barmack (2006) described *Folium vermis* as oculomotor vermis, because it receives proprioceptive, vestibular and auditory inputs and also plays a vital role in the guidance and modification of eye movements. The cerebellar hearing area has been argued to be located in lobules *Folium vermis* and *Tuber vermis* (Leiner *et al.*, 1986). The size of *Tuber vermis* has also been considered to be correlated animal visual cues, which is very important in guiding the whole body and the limbs (Pakan *et al.*, 2005). These lobules were relatively well formed in the present study. Mensah and Okeyo (2005) noted that grasscutter in the wild has good sight, delicate ear (capable of perceiving even small voice) and good sense of orientation. The uvula vermis was the broadest in this study. In humans, Bispo (2010) showed the uvula vermis to be the second largest lobule of the cerebellum. In another report, Sultan (2005) showed that the enlargement of this lobules reflect cognitive ability in birds.

Generally, in this study, more positive correlations were observed than negative correlations in the anterior lobe. Taken together, these results in the anterior lobe indicate that expansion of one lobule is generally correlated with increase in other lobules. Our results therefore agreed with Iwaniuk *et al*. (2006) who reported that folia in the anterior lobe tended to be positively correlated with one another in bird cerebellum. One of the most striking features of the cerebellar vermis is the great amount of in-folding of the cerebellar cortex. Finger (2000) showed that folding of laminated structures such as cerebellum increases its processing capacity and is correlated with behaviour or cognitive abilities. Striedter (2005) reported that cerebellum folding results in the larger surface area without necessarily affecting its weight.

CONCLUSION

In conclusion, some structural development and behaviours in grasscutter may likely be correlated with the relative size of individual cerebellar lobules. For more information of foliation pattern in this species further studies involving larger sample size, genetic analysis of the earliest stages of foliation may reveal how each lobule develops in such that some lobules expand while others contract in size.

ACKNOWLEDGMENT

This research was supported by grants from the Education Trust Fund (ETF), Federal Ministry of Education, Nigeria, through the Federal University of Agriculture, Makurdi, Nigeria.

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