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Dimensional sex differences in the cerebellum of the African giant pouched rat (*Cricetomys gambianus* – waterhouse, 1840)

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Abstract

Numerous scientific studies have revealed a number of striking sex differences in the mammalian cerebella. The present study was carried out to determine the effect of sex on 12 cerebella (6 males and 6 females) and also its relationship to other body variables in adult African giant pouched rats using a quantitative morphometric method. The results showed that cerebellum mean weight was slightly higher in females (0.82 ± 0.03 g) than males (0.76 ± 0.02 g). In females, the cerebellum weight was strongly positively correlated with the brain (r = 0.95) and the head (r = -0.49) weights. In males, the cerebellum weight was positively correlated with the body (r = 0.81), the head (r = 0.37) and brain (r = 0.30) weights. Generally, cerebellum weight and dimensions in both sexes tended to be positively correlated with the body, the head and the brain. In conclusion, the cerebellum in females may be estimated accurately from the brain mass, while in males, it may be used to estimate the body mass.

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Introduction

The African Giant pouched rat (*Cricetomys gambianus*- Waterhouse, 1840) (AGR), is one of the largest African wild rodent which form part of the bush meat trade in Nigeria and the West African sub-region (Asibey and Addo, 2000). These rodents are being exploited as a source of protein in the sub-region (Yeboah and Adamu, 1995), but their domestication is still at infant stage, owing partly to their wild nature and an incomplete knowledge of their biology (National Research Council, USA, 1991), particularly the nervous system that determines their behaviour in the wild or in captivity. The functions of the cerebellum, as part of brain that control movement behaviour in animals have been documented *(Ito et al., 1982; Ito, 2000; Han et al.*, 2006).

Many scientific studies have revealed a number of striking sex differences in both humans and animal brains (Cooke *et al.*, 1998; Murshed *et al.*, 2003; Majdic, 2009). For example, sex differences in neuroanatomy have been reported in cerebral and cerebella volumes (Andreasen *et al.*, 1993; Filipek *et al.*, 1994), gray matter proportion (Allen *et al.*, 2003), and in corpus callosum (Allen *et al.*, 1991; Luders *et al.*, 2003). In addition, Escalona *et al.* (1991) showed that women have a significantly smaller cerebellar volume than men. Similarly, Murshed (2003) showed a significant sex difference in the brain stem area which favoured males, but no significant difference was recorded in the cerebellar vermis area. In rodents like rat, Nguon *et al.* (2005) showed that female cerebellum had more extensive dendritic branching than that of the male. It has been suggested that sexual dimorphism in cerebellar size (if it exists) can be attributed to the effect of sex hormones (Luft *et al.*, 199920; Rhyu *et al.*, 1999).

The aspects of biology which have received attention by researchers in AGR in recent times among others include the skeletal (Olude *et al.*, 2010; Salami *et al.*, 2011), digestive (Ali *et al.*, 2008; Byanet *et al.*, 2010) and reproductive (Ali *et al.*, 2011) systems. Furthermore, sexual dimorphism has been reported in the body conformation and encephalization quotient (EQ) of AGRs; where females were observed to be larger (Byanet *et al.*, 2009) and having higher EQ (Byanet and Dzenda, 2014) than males. Information on sex differences in the cerebellum of the AGR is still lacking. Thus, the present study was aimed at determining the possible effect of sex on the size of the cerebellar cortex in apparently health adult AGRs. Provision of such information may form the neural bases for sex-specific behaviour observed in AGR in the wild and /or in captivity. The aim of the present research was to conduct a quantitative study on the sexual dimorphism of the cerebellum in the AGR.

Materials and methods

Animal Source and Research Location

A total of 12 adult AGRs, made up of six males and six females, were used in the study. The rodents were live-trapped in the wild, in Zaria (11^{o}10$^{/}$N, 07^{o}38$^{/}$E), Kaduna State in the Northern Guinea Savannah zone of Nigeria, and reafred under laboratory conditions in the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. They were obtained in this department and transferred in standard laboratory cages to a nearby Veterinary Anatomy Research Laboratory, of the same University, where the research was conducted.

The protocol for the research was approved by the Ethical Committee of Ahmadu Bello University, Zaria, Nigeria. The environment, housing and management of the animals were in accordance with 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. site www.nap.edu

Cerebellar Extraction

The body weight of each rodent was obtained with a digital electronic balance (Mettler balance P 1210, Mettler instruments AG. Switzerland) and the length using twine and meter rule. The rats were then euthanized by an overdose of chloroform anaesthesia (within 3-6 minutes) in a closed container. The head of each rodent was decapitated at the atlanto-axial joint using a small sharp knife and forceps. The 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), with some modifications due to the peculiarity of the rodents. The brains were grossly examined for pathological lesions and were found to be apparently normal. 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).

In order to separate the cerebellum from the brain stem, the cerebellar peduncles were cut through using a surgical blade. The cerebellar cortex gross features such as the vermis, paravermis and cerebellar hemispheres were examined. The cerebellar weight and dimensions (such as the width, circumference and vermal length) were obtained using a mettler balance and a digital venier caliper, respectively.

The terminology adopted in the present gross morphology is according to the Comparative Anatomy of the mammalian cerebellum, especially the white rat cerebellum (Larsell, 1952) and the Nomina Anatomica Veterinaria (2005) 5th Edition.

Statistical Analysis

All Statistical analyses were performed using the Statistical Analysis Package (Graph Pad Prism, Version 3.10, 2009). The weights and dimensions were expressed as mean and standard error of the mean (Mean ± SEM). The differences in weights and dimensions between the male and female cerebella, the brain, the body and head were analyzed using the student - t - test. Pearson's Correlation Coefficient (r) was used to compare the relationships in weights and dimensions between cerebellum and other body variables (the body, head and brain). Values of P ≤ 0.05 were considered significant.

Results

Gross Cerebellar Surface

The general gross feature of cerebellar cortex in both sexes showed no marked differences in terms of shape and folding pattern. Both cerebella were observed to have three divisions (the vermis, paravermis and bilateral hemispheres) and three lobes (the anterior, posterior and flocculonodular lobes). Also, connecting them to the brain stem were three cerebellar peduncles; the rostral or inferior (*Brachium conjunctiva)* peduncle, the middle cerebellar peduncle *(Brachium pontis*) and the caudal or superior (*Restiform body*) peduncle.

Table 1. Sex differences in the fixed brain, cerebellum, head and body of African giant pouched rat.

Weight (g)	Sex	Min-Max	Mean ±SEM	% Tbdw	% Tbrw	P value
Body	M	1120-1500	1356.67±51.74	-	-	0.71
	F	1120-1440	1313.33±43.40	-	-	
Head	M	120-140	126.67±4.22	9.3	-	0.03
	F	95-140	105.0±7.07	4.2	-	
Brain	M	4.24-5.12	4.64±0.14	0.3	-	0.32
	F	4.20-5.60	4.96±0.28	0.4	-	
Cerebellum	M	0.70-0.80	0.76±0.02	0.1	16	0.10
	F	0.74-0.89	0.82±0.03	0.1	17	

M =male, F= female, Min= minimum, Max= maximum, SEM= standard error of the mean, Tbdw = total body weight, Tbrw= total brain weight, P ≤ 0.05.

Morphometric Findings

Table 1 shows the sexual dimorphism in the weights of the cerebellum and other variables, such as the body, head and brain. The mean body weight of 1356.67 ± 51.74 g in males was slightly higher than 1313.33 ± 43.40 g in females, but was not statistically significant (P > 0.05). The mean head weight of the males (126.67 ± 4.22 g) was significantly (P < 0.05) larger than that of the females (105.0 ± 7.07 g) and these values accounted for 9.3% and 4.2% of the total body weight in the males and females, respectively. The cerebellum mean weight was slightly larger in the female (0.82 ± 0.03 g) than males (0.76 ± 0.02 g), (P > 0.05) and accounted for 16% and 17% of the total brain weight in males and females, respectively, though the differences was not statistically significant (P > 0.05).

Table 2. Sex differences in the dimensions of brain, cerebellum, body and head of the African giant pouched rat.

Length (mm)		Sex	Min-Max	Mean ±SEM	% Tbdl	% Tbrl	P value
Body		M	790-852	819.17±10.5	-	-	0.03
		F	650-810	754.83±23.17	-	-	
Head		M	100-120	111.5±3.06	13.6		0.11
		F	80-115	99.5±6.12	22.4		
Brain		M	34-37	35.0±0.63	4.3	-	0.56
		F	32-36	34.5±0.56	4.6	-	
Cerebellum							
a.	Length	M	11-13	12.0±0.26	1.5	34.3	0.69
		F	11-13	11.83±0.31	1.6	34.3	
b.	Width	M	20-24	21.83±0.75	2.7	62.4	0.85
		F	21-24	22.0±0.45	2.9	63.8	
c.	Circumferenc	M	26.89-30	26.63±1.19	3.3	76	0.37
e		F	24.94-30	28.06±0.95	3.7	81.2	
d.	Vermal length	M	13.73-17.73	15.64±0.64	1.9	44.7	0.62
		F	14.83-16	15.27±0.33	2.0	44.3	

M = male, F= female, Min= minimum, Max= maximum, SEM= standard error of the mean, Tbdl =total body length, Tbrl =total brain length, P ≤ 0.05.

The sex differences in dimensions of the cerebellum and other variables (body, head and brain) are presented in Table 2. The mean lengths of the body, head, brain and cerebellum in males were higher than those in the females, but the mean cerebellar circumference (28.06 ± 0.95mm) in the females was slightly larger than in the males (26.63 ± 1.19mm), though not statistically significant (P > 0.05).

Table 3. Correlation matrix of the weights of cerebellum in males and females.

Weight (g)	Sex	Body	Head	Brain	Cerebellum
Body	M	1.00	0.53	-0.74	0.81
	F	1.00	0.63	-0.03	-0.10
Head	M	0.53	1.00	-0.74	0.37
	F	0.63	1.00	-0.54	0.49
Brain	M	-0.02	-0.74	1.00	0.30
	F	-0.03	-0.54	1.00	0.95
Cerebellum	M	0.81	0.37	0.30	1.00
	F	-0.10	-0.49	0.95	1.00

M = male, F = female.

Data on the Pearson correlation coefficient (r) for the cerebellum and other components are shown in Table 3. The head weight in both sexes was positively correlated with the body, but females had a stronger correlation (r = 0.63) than males (r = 0.53). The brains in both sexes were negatively correlated to the body and the head. In general, there was a trend for cerebellum in both sexes to be positively correlated with the body, the head and the brain. For males, the cerebellum was strongly correlated with the body (r = 0.81), but weakly correlated with the head (r = 0.37) and brain (r = 0.30). In females, the cerebellum was strongly correlated with the brain (r = 0.95), but negatively correlated with the body (r = -0.10) and head (r = -0.49). Overall, for weight, there were thirteen positive correlations and eleven negative correlations.

The correlation matrix of dimensions of cerebellum and other variables in both sexes are presented in Table 4. Here, the male head length was positively correlated with the body length (r = 0.10), but the females were negatively correlated (r = -0.33). The male and female brains were negatively correlated with the body and the head, except the females that were positively correlated with the head length (r = 0.76). The cerebellum lengths in both sexes were all positively correlated with the head and brain lengths. Even though the cerebellum length in females were weakly positively correlated with the body length (r = 0.07), the males cerebella lacked correlation with the body length (r = 0.00). Overall, for dimensions, there were fourteen positive correlations and eight negative correlations and two that lacked correlations. Taken together, in both sexes shows that increase in the weights and lengths of these variables (body, head and brain) generally correlated with increase in the cerebellum weights and lengths.

Table 4. Correlation matrix of the dimensions of cerebellum and other body variables in males and females.

Length (mm)	Sex	Body	Head	Brain	Cerebellum
Body	M	1.00	0.10	-0.24	0.00
	F	1.00	-0.33	-0.37	0.07
Head	M	0.10	1.00	-0.50	0.34
	F	-0.33	1.00	0.76	0.88
Brain	M	-0.24	-0.50	1.00	0.41
	F	-0.37	0.76	1.00	0.68
Cerebellum	M	0.00	0.34	0.41	1.00
	F	0.07	0.88	0.68	1.00

M = male, F = female.

Discussion

The morphometric study in the present work was conducted on the brain and cerebellum weights and dimensions fixed in the 10% formalin of short duration to avoid significant effect on shrinkage. In the literature, the results on formalin fixed tissues have varied and accuracies are still being discussed (Peters *et al.*, 1998; 2000). Fixing brains of dogs using 7% formalin for 1-2 months showed a volumetric shrinkage factor of 1.25 and an area shrinkage factor of 1.16 (Mayhew *et al.* (1990). In another work Cutts (1988) reported a mean shortening of 1.06% (shrinkage factor: 0.0106) in the length of isolated skeletal muscle of laboratory rats fixed in 10% formalin for 3 days. Formalin therefore has the ability to cause minimal tissue shrinkage and the degree varies with duration of fixation period. Although imaging studies such as the Magnetic Resonance Imaging (MRI) technique pronounces to yield more accurate and acceptable results, but it is expensive and still not universally available.

There are many studies in the literature where anatomical structures in brain are measured quantitatively in terms of volume, area, width and length. In the present study, the cerebella of both sexes presented a common anatomical disposition, that is, no sex differences in the folial pattern. These findings are similar to those reported for rodents in the literature (Larsell, 1952; Chenn and Wash, 2002). To further support this observation, Sultan and Braitenberg (1993) stated that all mammalian cerebella conformed to the same general plan. These findings also agreed with Inouge and Oda (1980) who did not find sex related differences in the cerebellar folial pattern of inbred and a closed colony of mice. The authors were of the opinion that the morphological variations of the mouse cerebellum are strain-specific in the aspect of folial pattern which have intimate connection with the genetic control.

The shape of the cerebellar cortexes and the pattern of foliation in both sexes did not differ from each other. The cerebellar cortexes presented three main divisions; the vermis, paravermis and bilateral cerebellar hemispheres, with chains of folia extending continuously from the most anterior to the most posterior part of the cerebellum, similar to that described for rodents like the white rat (Larsell, 1952), chinchilla, squirrel (Sultan and Glickstein, 2007) and grasscutter (Byanet *et al.*, 2008, 2009). Therefore, sex differences with respect to the shape, foliations and morphological divisions of the cerebellum were not observed.

The mean body, head and brain weights in the present study were slightly higher in the males than females, the head being statistically significant ($P < 0.05$). The sex differences in the body and brain observed in this study are similar to the findings documented on the same African giant rats species by Ibe *et al.* (2010) and other rodents like African white-tailed rats (*Mystromys albicaudatus*) by Becker and Middleton (1979), who reported that the males were significantly heavier than the females. Contrariwise, Byanet *et al.* (2009) observed higher body and brain weights in the female than male grasscutters (*Thryonomys swinderianus).* Similar to these reports in ruminant, the females of Red Sokoto goats, West African Dwarf goats and West African Dwarf sheep were all observed to have higher mean brain weights than their male counterpart (Olopade and Onwuka, 2002; Onwuka *et al.*, 2003).

In similar studies in another large tropical Savannah rodent, the grasscutter, it was observed that even though the mean brain and head weights were higher in the males, the cerebellar mean weight and dimensions (cerebellar circumference, width) were higher in the females accounting for 81.2 % and 76% the total brain length in females and males, respectively (Byanet *et al.*, 2009). The results of the present study agreed with the previous results in grasscutter (Byanet *et al.* 2009) with respect to larger cerebellar circumference in the female than male.
The brain weight in the present study was negatively correlated with the body and head, but positively correlated with the cerebellar weight in both sexes, with females exerting a stronger positive effect ($r = 0.95$) than males ($r = 0.30$). Sultan and Braitenberg (1993) conducted a quantitative comparative neuroanatomical study on the shapes and sizes of cerebella in different groups of animals, such as rodents (mice, squirrel, chinchilla and guinea pig), birds (pigeon), primates (Macaque), ruminants (sheep and bovine), pets (cat and dog) and humans. Their results showed that the cerebellar weight and volume was greater in larger animals with higher body weight than in the smaller animals.

The cerebellar relationship with the body showed that the mean body weight was significantly larger in the males than females ($P < 0.05$) and cerebellar mean weight in the males was strongly positive correlated with the body weight ($r = 0.81$), but lacks correlation with body length ($r = 0.00$) and negatively correlated with the female mean body weight ($r = -0.10$). This means that increase in the body mass will lead to corresponding increase in the size of the cerebellum in the males, but lack correlation with respect to length and weakly positively correlated in the females.

In general, there was a trend for cerebellum weights and dimensions in both sexes to be positively correlated with the body, the head and brain, with effects tended to be stronger in the female than male African giant rat. Overall, there was 83% of the positive correlation between cerebellum and the other variables (the body, head and brain) in weights and dimensions in both sexes. Taken together, these results indicate that increase in the weight and length of these variables (the body, head and brain), correlate with increase in the weight and dimensions of the cerebellum, that is, coordinated increase in size and dimension of cerebellum are relatively common. Similarly, Sultan and Braitenberg (1993) reported that cerebellar width increases with increase in body size in the smaller species and tend to remain constant in larger ones.

The relatively larger mean weight and dimension of cerebellum with stronger correlation effect with the body, head and brain in females than males, may/or not necessary reflect the female's higher motor activities than their male counterparts. Paulin (1993) showed that the standard theory which considered the function of the cerebellum to be the control and coordination of movements, only partially characterizes cerebellar function. For apart from the coordination of voluntary movement, it also plays an important role in the coordination of eyes, hands, and in cognition.

Welker (1990) showed that species differences in the relative size and number of folia/lobules is thought to reflect behavioral and/or cognitive differences. Similar findings in birds showed that the enlargement and/ or reduction of individual folia is thought to relate to specific behavioral differences among the groups (Iwaniuk *et al*., 2006). Therefore, further study that involves comparing the cerebellar foliation patterns between sexes to relate the various cerebellar regions to function is needed in the AGR to ascertain the structural- behavioural differences in this species. In conclusion, the cerebellum in females was slightly heavier than in males and may be estimated accurately from the brain mass, while in females, it may be used to estimate the body mass.

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