Morphometric and Stereological Studies of the Pons and Medulla Oblongata of the African Striped Ground Squirrel (*Xerus erythropus*)

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Abstract

The study was undertaken to investigate the structure and functional relationships of the pons and Medulla Oblongata of African Striped Ground Squirrel (Xerus erythropus). Twenty (20) adult African striped ground squirrels were used for this study (10 males and 10 females). The ground squirrels were obtained from the surrounding villages of Zaria Local Government, Kaduna state Nigeria. Each Squirrel was euthanized using ketamine hydrochloride at 80mg/kg bw followed by gentle perfusion with neutral formal saline. A pair of scissors, chisel and scalpel blade were used to gently extract the brain (craniotomy). The extracted brain was fixed in Bouin's fluid for 24 hours and processed histologically. Morphometrically, the absolute brain weight was higher in male than female

while the brain length was higher in female. The absolute weight of medulla oblongata was significantly higher in male than female (p<0.05). Stereologically, the neuronal volume of medulla oblongata was higher in female squirrel than in male while male squirrel has higher neuronal number of pons and medulla oblongata. In conclusion, the higher brain morphometry in male squirrels may be suggestive of a higher locomotive activity and a good climbing ability than the female counterpart.

Keywords: African stripped ground squirrel, Medulla Oblongata, Pons.

Introduction

Squirrels are mammals which belong to order *rodentia*. They are member of the family *sciuridae* and subfamily *Xerinae*, genus Xerus (African ground squirrel), species *Xerus erythropus* consisting of small or medium size rodents (Thorington and Hoffmann, 2005). They are indigenous to America, Africa and Eurasia (Whatton, 2012).

Ground squirrel has an overall uniform appearance and their fur color varies with ages and season. Striped ground squirrels are diurnal herbivores, active during the day and spend almost their entire lives on the ground, although they are capable of climbing into bushes to reach their food. The juvenile has soft gravish tan fur while adults have buffy grey to sandy brown fur and light tan or buff around the head and underside. Their ears are small and closely appressed to the head; eyes are black, large and place high on the head enabling the animal to detect approaching danger from almost any direction. Their tail has hairs much longer than those on the body, which fan out to the sides, and are multi-coloured along their length, presenting a grizzled appearance (Wilson and Reeder, 2005). They have versatile claws for grasping, climbing and digging. Their weight varies during annual cycle but typical weight of adult is 0.5 to 1kg (Thorington and Hoffmann, 2005). The squirrels live in open woodlands, grassy area like cemeteries, pastures, parks, or rocky country.

In Nigeria (especially south Nigeria), squirrel is one of the most commonly consumed small mammal species (bush meat) by rural dwellers as supplementary protein diet (Adeola and Decker, 1987; Ajayi, 1979). They are also used by Nigerian farmers in cultural ceremonies, for medicinal purposes (i.e. components of anti-poison drug and as ingredients in prevention of convulsion in children and also used to enhanced fertility in men) (Ajayi, 1979; Adeola, 1992).

The pons is a broad, horseshoe shaped portion of the brain consisting of large bundles of nerve fibers. The pons connects the two halves of the cerebellum and can be broadly divided into two parts; the basilar part, located ventrally and the pontine tegmentum, located dorsally (Henry et al., 2002). A number of cranial nerve nuclei are present in the pons which include pontine and motor nucleus of the trigeminal nerve (CN V), located in the mid-pons while abducens nucleus (CN VI), facial nerve nucleus (VII) and vestibulocochlear nuclei (vestibular nuclei and cochlear nuclei) (VIII) are located at lower end in the pons (Alexander, 1983). The pons also contains nuclei that relay signals from the forebrain to the cerebellum, along with nuclei that deal primarily with sleep, respiration, bladder control and posture (Saladin, 2007). The medulla oblongata is the conical part of the brain that extends from the pons to the medulla spinalis (Al-Shehri, 2007). It is broad above where it joins the level of the foramen magnum (Inderbir, 2003). Medulla oblongata is divided into two parts: an opened part (closed to the pons) and a closed part (closed to the spinal cord), the most rostral part of the medulla oblongata is the corpus trapezoideum (**AI-Shehri, 2007**).

Although, several studies have been conducted on the Pons and Medulla oblongata of other rodents but little or no research has been carried out on the pons and medulla oblongata of squirrels, thus this study will provide baseline data for detailed neuroanatomical descriptions of the pons and medulla oblongata of ground squirrels. This will in turn provide further laboratory research opportunities usina ground squirrels as models and also provide additional information required for successful breeding as ground squirrels are good source of protein for domestic farmers in Nigeria.

Material and Methods

Experimental animals and management

Twenty (20) African striped ground squirrel (10 male and 10 female) were used for this study. The animals were captured live from the wild in Zaria and its environs. They were acclimatized for one month in standard laboratory cages in the animal pen of the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria, Nigeria. The animals were given access to food and water *ad libitum* throughout the experimental period. They were physically examined during the pre-experimental period and only apparently healthy ones were utilized.

Morphometric parameters

The body weights of each squirrel were obtained using a weighing balance model JJ1000, USA with a capacity of 1000g and sensitivity of 0.01g. The mean length, width and depth of the pons and medulla oblongata were obtained with a vernier caliper (MG6001DC, General Tools and Instruments Company, New York; sensitivity: 0.01mm). Gross pictures were taken using canon digital camera power shot (SX170 IS) with 64-megapixel sensor (focal length: 28-448mm, 7.5cm (3.0") TFT. Histological pictures were taken using light microscope (Amscope, T120B) and a digital microscope camera (DCM 510-megapixel, Scope Photo[®] China) at X40, X100, X250, X400,

Brain extraction

Each squirrel was euthanized using ketamine anaesthetic at (80mg/kg bw) David G. P. and Kangmei C. (1996). Each brain was perfused (intra-cardiac route) with 10% phosphate buffered formalin. Each skull was exposed after skinning and stripping off all the facial muscles within 30 minutes of euthanasia. Craniotomy was carried out through the calvaria to expose the dura matter, which was later cut with a curved pointed scissors. The falx cerebri and tentorium cerebelli were pulled from the longitudinal and transverse fissures by gentle traction. The cerebral vein was transected and at this stage, the brain was still in the cranium fixed in 10% phosphate buffered formalin for two days to enhance easy extraction as described by Ramaswamy (1978).

Harvest of Pons and Medulla oblongata

The pons and medulla oblongata were isolated from the rest of the brain by gently pulling apart the two cerebral hemispheres at the occipital lobe to expose the corpus callosum. The entire corpus callosum together with septum pellucidum and the body and rostral commissure of the fornix were severed in the midline and this separates the cerebrum from the brainstem and cerebellum. Then the flocculli of the cerebellum were raised manually to expose the cerebellar peduncle which was severed starting with the laterally located brachium restiformis, followed by the middle brachium pontis and then the brachium conjuctivum. The brainstem was free from the cranial nerves by simple trimming using scalpel blade, an incision was made at the transverse fissure between the pons and caudal colliculi to isolate the pons and medulla oblongata from the midbrain while incision made at the pontomedullary junction separates the medulla oblongata from the pons.

Histology

The extracted pons and medulla oblongata were fixed in Bouin's fluid for 24hour, dehydrated through series of ascending concentrations of ethanol (70%, 90%, 100%, 100%, 100%) for 2 hours at each concentration, cleared in xylene, embedded in paraffin wax and sectioned at 5µm. Hematoxylin & Eosin was used for general histological evaluation (Kiernan, 2007) while, Cresyl fast violet stain was used for Nissl substance and nerve nuclei evaluation (Drury, 1967).

Stereology

Isotropic uniform random (IUR) samples were obtained by the orientator method (Ali, *et al.*, 2012), Fig (1).

At first the pons and medulla oblongata each was placed at the center of the circle with equal divisions, and a random number (2) was calculated and selected from the random number table and the sample was cut here. Secondly, each part of the cut sample was again placed on a second circle with unequal divisions and another random number (6) was selected and the samples were cut here and then a trochar was used for getting the isotropic sections; then these sections were measured before and after processing with a digital vernier caliper (MG6001DC, General Tools and Instruments Company, New York; sensitivity: 0.01mm). They were fixed in Bouin's fluid for 24 hours: and thereafter dehydrated in a series of ascending concentration of alcohol, cleared in xylene, infiltrated with molten paraffin wax as described by Kiernan (2007). Degree of shrinkage were estimated by subtracting the final volume after processing from the initial volume before processing and then divided by the initial volume before processing (S_1-S_2/S_1) Braendgaard, et al. (1990).

Serial Sectioning

Serial Sections were cut with a rotatory microtome (LEICA) at 8µm. A random number 3 was selected from the random number table and sections were randomly picked and floated out in a hot water bath, mounted on glass slides, left to air dry and stained with hematoxylin and eosin.

Volume estimation

A test point counting grid (Cavalieri estimator) was superimposed on the pons and medulla oblongata tissue sections and single test points hitting the pons and medulla oblongata were counted and summed (fig 2).

The volume changes of the pons and medulla oblongata were calculated as described by Gundersen et al. (1988) using the following computations:

V (mm³) = \overline{T} x a/p x $\sum P_i$ (\overline{T} = distance from the 1st section to the 13th section; a/p = area per point; $\sum P_i$ = sum of test points).

Total neuronal number estimation

These was done on pons and medulla oblongata of the African striped ground squirrel (*xerus erythropus*) using an unbiased counting frame called the **Physical dissector.**

Two unbiased counting frames were superimposed on systematically random

tissue sections (7 sections from each half of the medulla oblongata and pons). According to the dissector rules; the counting frame defines neuronal cells to be completely outside the frame if the cells touch the exclusion line as being outside the frame and not counted (black arrow); whereas neuronal cells that are completely within the frame or that touch the inclusion line were counted as being within the frame (white arrow) Fig 3. In addition, only distinct neuronal cells which are seen in the sampling section frame but are not seen in the reference section frame were counted and summed as Q⁻. Each half, left or right is sample and reference section to each other (i.e. when counting the left as sample section, the right is reference section and vice versa). The total neuronal number was therefore estimated from the computations below as proposed by Schurmann et al. (1991).

Data analysis

All data obtained were expressed as mean ± standard deviation (n=20). Statistical comparison between pons and medulla Oblongata was made by subjecting the data to independent student t-test using GraphPad Prism version 5.0 for windows. Values of P < 0.05 was considered significant.

Results

Morphometric results

The mean body weight and body length of male ground squirrel was 504.41 ± 38.52 gm and 44.30 ± 0.68 cm while that of female squirrels was 458.78 ± 18.60 gm and 43.38 ± 0.85 cm respectively. The mean brain weight and length of male squirrel was 6.52 ± 0.54 gm and 32.76 ± 1.85 mm while, 6.39 ± 0.32 gm and 37.54 ± 2.49 mm was obtained for female squirrels respectively. The mean brain volume of male squirrels was 6.18 ± 0.56 ml while that of female squirrel was 6.10 ± 0.33 ml (Table 3).

The mean weight, length and volume of the medulla oblongata of male squirrel was 0.52 ± 0.02 gm, 13.41 ± 1.10 mm and 0.32 ± 0.02 cm³ respectively, while, that of female counter part was 0.41 ± 0.03 gm, 2.57 ± 1.23 ml and 0.34 ± 0.02 cm³ respectively. The mean weight, length and volume of the pons of male squirrel was 0.16 ± 0.02 gm, 6.37 ± 0.83 ml and 0.24 ± 0.02 cm³ respectively, while that of female squirrels was 0.18 ± 0.01 gm, 4.92 ± 0.56 ml and 0.20 ± 0.00 cm³ respectively (Table 4).

The brain of male squirrels constituted about 1.29% of their body weight while that of female squirrels constituted about 1.39% of their body weight. The brain length constituted about 73.95% of the body length in male and 86.54% of body length in females. The pons and medulla oblongata weight constituted about 2.45% and 7.98% of the brain weight in male squirrels while 2.82% and 6.42% in female squirrels respectively. The pons and medulla oblongata length constituted about 19.44% and 40.93% of the brain length in male and 13.11% and 33.48% in female squirrels respectively (Tables 3 and 4).

Stereological results

The result showed that the volume estimated for the left and right pons and medulla oblongata were 14610 mm³ and 9000 mm³, 24285 mm³ and 21435 mm³ for male and 13350mm³ and 12795 mm³, 27315 mm³ and 27270 mm³ for female respectively (Tables 5 and 6). The total neuronal estimates for the male and female pons and medulla oblongata respectively were14.00338 x10⁴ and 21.57704 x10⁴, for male 10.00446 x10⁴ and 14.27286 x10⁴ for female (Tables 7 and 8). However, data obtained for the volume of medulla oblongata showed that the female squirrel had higher volume than the male squirrel (Table 6) but the neuronal number in the pons and medulla oblongata of the male squirrel was higher than the female counterpart (Tables 7 and 8).

Discussion

The pons and medulla oblongata are the caudal extend of the brainstem. The pons is convex and smooth ventrally and triangular dorsally while the medulla oblongata opens rostrally and closed caudally. The mean body weight of squirrels (male: 0.18 ± 0.01gm; female: 458.78 ±18.60 gm) and percentage brain weight (male: 1.29%; female: 1.39%) was relatively higher than that reported in African giant rat by Nzalak et al., (2005) and in grasscutter by Ajayi et al., (2010). The ratio of brain weight to body weight (1:75) was higher than that reported in grasscutter (1:214) by Ajayi et al., (2010), in rabbit (1:300) by Russel, (1979) and in African giant rat (1:193) by Nzalak, et al., (2005). The body weight of male (504.41 ±38.52g) and female (458.78 ±18.60g) squirrels obtained in this study are statistically insignificance (p>0.05), this is similar to the findings of Nowak and Walker, (1999) who reported that the mean body weight of male and female African giant rats is statistically insignificance, this is equally in line with the findings of Becker and Middleton (1979) who reported that male and female African white-tailed rats show insignificant variation in body weight. Male squirrels were observed to have higher body weight than females, this agrees with the findings of Oto and Haziroglu, (2009), who reported that male rodents have higher body weight than females, but this is in contrary to the findings of Byanet, (2009) who reported that female grasscutters have higher body weight than male.

Male squirrels have larger brain size than female counterparts in this study,

which suggests that male squirrels navigate better in their habitats than females, this is in line with the findings of Byanet and Dzenda, (2014) who reported that large brain size is required to navigate structurally complicated habitats within species but not across species of rodents. Similarly, Hart, *et al.* (2001) and Mace, *et al.* (1981), reported that larger animal usually has larger brain than smaller animal and that rodents brain size is related to factors like complex habitat, specialized diet, nocturnal behavior, climbing and burrowing ability.

The weight of medulla oblongata (male: 0.52 ±0.02 gm; female: 0.41 ±0.03 gm) and pons (male: 0.16 ±0.02 gm; female: 0.18 ±0.01 gm) obtained in this study is lower than that reported in African giant rat by lbe, et al. (2010) and in grasscutter by Byanet and Dzenda (2014). The mean weight of the medulla oblongata of the male squirrel was statistically higher than that of the female squirrel (p<0.05), this is in agreement with the findings of Ibe, et al. (2010) who reported a higher medulla oblongata weight in male African giant rat than females. These sexual dimorphism in brain weight may not mean that sex with heavier brain is more intelligent, however, Pilleri, et al. (1982/5) reported that the internal structural complexity of the brain and interconnection of specific brain center are the most important factors in the evolution of intelligence and not brain size.

The pons and medulla oblongata of Male squirrels have higher neuronal number than their female counterparts which is suggestive of a better consciousness and alertness in male squirrels than females, this is in line with the neuronal estimate of the neocortex of brown rats by Korbo, et al. (1990), neocortex of domestic pigs by Jelsing et al. (2006), pons and medulla oblongata of African elephant by Suzana, *et al.* (2014).

Conclusion

The higher brain morphometry in male squirrels may be suggestive of a higher locomotive activity and a good climbing ability than the female counterpart. While, the higher neuronal number of pons and medulla oblongata in male squirrels confers them greater alertness and consciousness than female squirrels.

Conflict of interest: No conflict of interest

Ethical Statement

An ethical approval was given by Animal research committee of Ahmadu Bello University Zaria with an approval number of ABU/CAUC/2016/038.

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Authors contributions

All authors contributed substantially to the design, acquisition, and analysis of

the study. Writing and revising for intellectual consumption was also collectively done.

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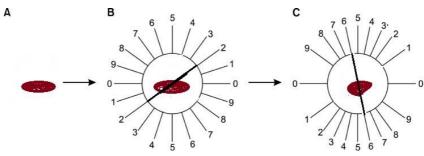


Fig (1): The Orientator 44 grid.

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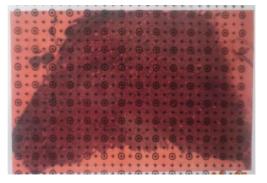


Fig (2): The Cavalieri estimator grid.

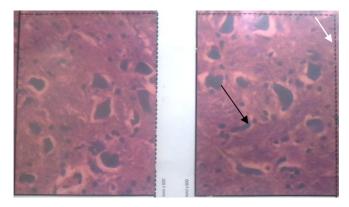


Fig (3): The physical dissector counting frames

Table (1): Volume	estimation	(Gundersen,	et al.,	1988),	cavalieri	estimator	point
counting method.							

Sections	P ₁	P ₁ x P ₁	P ₁ x P ₁₊₁	P ₁ x P ₁₊₂
1	283	80,089	100,748	52,921
2	356	126,736	66,572	112,921
3	187	34,969	59,279	53,482
4	317	100,489	90,662	_
5	286	81,796	_	_

 $\overline{\sum P_1 = 1,429 \sum P_1 x P_1 = 424,076(A) \sum P_1 x P_{1+1} = 317,261(B) \sum P_1 x P_{1+2} = 219,255(C)}$

Noise due to errors in the sampling:

Noise = 0.0724 x B/ \sqrt{A} x \sqrt{n} x $\sum P_1$ ($\sum P_1$ = sum of test points).

Variations due to the systematic random sampling of the serial sections were calculated: **VAR**_{SURS} = 3 (A-Noise) – 4(B + C) + C **Total variance (TVAR)** = Noise + VAR_{SURS} Coefficient of error due to the entire sampling process (CE) was calculated: **CE**= $\sqrt{TVAR}/\sum P_1$.

Dissector	Q ⁻ left	Q ⁻ right
1	2	1
2	4	3
3	4	2
4	8	8
5	3	7
6	10	9
7	ΣQ- = 39	3 = 33

N = N _v x V _{ref}	Vref = ∑Q⁻ / n x vdis	Vdis = t x a(frame)/magnification ² = 21
x 110 x 175 /600 x 60	0 n = 7 + 7	∑Q ⁻ = 39 + 33

Where Nv = numerical density, V(ref) = volume of the structure estimated with the cavalieri estimator, V(dis) = volume of all dissector probes placed in the structures, a(fra) = area of counting frame which is standardize from the grid as 110 x 175.

Noise due to errors in the sampling:

Noise = 0.0724 x B/ \sqrt{A} x \sqrt{n} x $\sum Q_1$.

Variations due to the systematic random sampling of the serial sections was calculated:

 $VAR_{SURS} = 3 (A-Noise) - 4(B + C) + C$

Total variance (TVAR) = Noise + VARSURS

Coefficient of error due to the entire sampling process (CE) was calculated: **CE**= $\sqrt{TVAR}/\Sigma Q_1$.

Parameters	Mean± SD	p-value	
	Male	Female	
BW (gm)	504.41±38.52	458.78±18.60	0.317
BL (cm)	44.30 ± 0.68	43.38 ± 0.85	0.424
BRW (gm)	6.52 ± 0.54	6.39 ± 0.32	0.845
BRL (mm)	32.76 ± 1.85	37.54 ± 2.49	0.162
BRV (ml)	6.18 ± 0.56	6.10 ± 0.33	0.906
% BRW	1.29%	1.39%	-
% BRL	73.95%	86.54%	-

Table (3): Mean ± SD values of body weight, length and brain parameters of African stripped ground squirrels (*Xerus erythropus*).

Key: BW= Body weight, BL= Body length, BRW= Brain weight, BRL= Brain length, BRV= Brain volume, % BRW= Percentage brain weight and % BRL= Percentage brain length. P<0.05 are statistically significant

Parameters	Mean± SD	p-value	
	Male	Female	
MW (gm)	0.52 ±0.02	0.41 ±0.03	0.026*
ML (mm)	13.41±1.10	12.57±1.23	0.513
MV (cm ³)	0.32 ±0.02	0.34 ±0.02	0.545
PW (gm)	0.16 ±0.02	0.18 ±0.01	0.484
PL (mm)	6.37 ±0.83	4.92 ±0.56	0.189
	0.24 ±0.02		
PV (cm3)		0.20 ±0.00	0.178
% MW	7.98%	6.42%	-
% ML	40.93%	33.48%	-
% PW	2.45%	2.82%	-
% PL	19.44%	13.11%	-

Table (4): Mean ± SD values of the dimensions of Pons and medulla oblongata of African stripped ground squirrels (*Xerus erythropus*).

Key: MW= Medulla weight, ML= Medula length, MV= Medulla volume, PW= Pons weight, PL= Pons length, PV= Pons volume, % MW= Percentage medulla weight, % ML= Percentage medulla length, % PW= Percentage pons weight, and % PL= Percentage pons length. P<0.05 are statistically significant

 Table (5): volume estimation of Pons of African striped ground squirrel

Side	Sex	Volume(mm ³)	Noise	VAR _{SURS} TVAR	CE
Left	Male	14610	1644.94	-539700.82	0.75
53805	0.00				0.56
24428	Female 4.14	13350	1184.07	-245468.21	0.66
Right -15775	Male 53.26	9000	751.63	-158504.89	
-37185	Female 51.18	12795	1390.09	-373241.27	0.71

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Sides	Sex	VOLUME (mm	3) NOISE	VAR _{SURS}	TVAR	CE
Left	male	24285	3469.85	_1472396.55	_1468926.70	0.74
	Female	27315	4259.10	-1782924.30	-1778665.20	0.73
Right	Male	21435	2981.17	-1102034.51	-1099053.34	0.73
	Female	27270	3993.99	-1924995.97	-1921001.98	0.76

Table (6): volume estimation of medulla oblongata of African striped ground squirrel

Table (7): Estimated total neuronal number of pons of African striped ground squirrel

Sex	Tot.neuronal estimate	NOISE	VAR _{SURS}	TVAR	CE
Male	14.00338x10 ⁴	20.87	-1442.61	-1421.74	0.80
Female	10.00446x10 ⁴	10.86	-577.58	-566.72	0.74

Table (8): Estimated total neuronal number of medulla oblongata of African striped ground squirrel

Sex	Tot.neuronal estimate	NOISE	VAR _{SURS}	TVAR	CE	
Male	21.57704x10 ⁴	13.75	-762.25	-748.5	0.70	
Female	14.27286x10 ⁴	3.59	-140.77	-137.18	0.55	

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Animal species in this Issue

African Striped Ground Squirrel (*Xerus erythropus*)



Kingdom: Animalia & Phylum: Chordata & Class: Mammalia & Order: Rodentia & Family: Sciuridae & Genus: Xerus & Species: *X. erythropus*

Striped ground squirrels are diurnal herbivores, and spend almost their entire lives on the ground, although are capable of climbing into bushes to reach food. They eat a range of seeds, nuts, and roots, and can be an agricultural pest, eating crops such as cassava, yams, cotton bolls, peanuts, and sweet potatoes. They may occasionally supplement their diet with eggs, insects, and other small animals. Their predators include servals, jackals, birds of prey, and common puff adders.

They forage throughout home ranges of about 12 hectares (30 acres) in semi-arid terrain, but their ranges overlap and they make frequent forays into surrounding areas in search of food. They mark their territories using scent glands on their cheeks, which they rub onto stones and tree trunks, although they do not appear to defend them from intruders.

The squirrels spend the night in burrows, which they dig with their large claws. Their burrows are usually simple in structure, with a central nest less than a meter below the surface, a single entrance tunnel, and a few blind-ending tunnels that almost reach the surface. The latter are used as escape routes, allowing the squirrel to rapidly break through to the surface; the main entrance tunnel is often also blocked with a temporary pile of dirt at night. Burrows may also contain caches of food, although these are more commonly located some distance away and concealed beneath stones or dead leaves. They also bury their urine, but not their dung.

Source: Wikipedia, the free encyclopaedia