



COMPARATIVE STUDIES ON THE BRAINS OF *CRICETOMYS GAMBIANUS* AND *THRYONOMYS SWINDERIANUS*

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ABSTRACT

Scientific research in the biomedical sciences requires research animals as laboratory models for these purposes. The development of indigenous research animals such as Grasscutter (GC) and African Giant Rat (AGR) which do not need to be placed in rigidly controlled facilities and are readily available is essential for research in an environment with limited resources for carrying out scientific investigations. It is crucial to understand the neuroanatomical basis of the different behaviors of these animals. Therefore, this work compared the gross brain morphology and morphometry, prefrontal cortex, olfactory bulb, hippocampus, and flocculonodular lobe of the cerebellum of AGR and GC, and related the findings to their behavioural/functional differences. Five adult AGR and GC each were used for this study. Gross morphometry and morphology were carried out on fixed tissues of whole brain specimens of AGR and GC. Histological analysis was done on some brain structures. Both brains of AGR and GC were lissencephalic. The brain of AGR was oval in shape while that of GC was globe-like. There was a Y-shaped posterior cerebral artery emerging from the dorsal surface of each cerebral hemisphere of the GC, making a prominent depression on the caudal aspect of the cerebrum. Also, the anterior cerebral artery could be seen radiating from the parietofrontal aspect of the medial longitudinal fissure of each cerebral hemisphere. There were differences in the gross morphology and morphometry, and histology of brain structures of AGR and GC which could be related to their behavioral differences.

KEYWORDS: Morphology, Morphometry, Histology, African Giant Rat, Grasscutter.

INTRODUCTION

Cricetomys gambianus is known as the African Giant Rat (AGR) due to its location. In Nigeria, it is called *Oke-ohia* by the Igbo-speaking ethnic group, *okete* in Yoruba language, and *Gafiya* in Hausa language. It is a nocturnal in habit (Kingdon, 1997). The Grasscutter (GC) is a rodent of the specie *Thryonomys swinderianus*. In west Africa, Nigeria, the three main ethnic groups call it *Nchi* (Igbo), *Oya* (Yoruba), and *Gyabji* or *Gyandi* (Hausa) respectively. They are semi nocturnal. It burrows into the ground, but can temporarily shelter in hollow made by other animals (Fitzinger, 1995).

Nigeria spent N1.65 trillion importing live animals, hides and skin in the last four years despite the country's comparative advantage to locally source them (Daily Trust, 2018). Research animals for scientific investigations have been imported into Africa for scientific research in general and biomedical research in particular. However, these research animals often require facilities whose environment is controlled with

electricity and utility service for their maintenance which is usually expensive in many parts of Africa. When these imported laboratory animals are not adequately maintained in special facilities in Nigeria, it often results in their death, or the generation of incorrect research data, which makes the resources, energy, and time invested into the research futile. Therefore, the development of indigenous research animals such as GC and AGR which do not need to be placed in rigidly controlled facilities would help eliminate these challenges. Also, knowledge about the differences in the neuroanatomy of some brain structures such as the prefrontal cortex, hippocampus, olfactory bulb, and the flocculonodular lobe of the cerebellum will provide more insight into the emotional behaviour of GC and AGR. This will improve the handling of these animals and increase their productivity.

EXPERIMENTAL PROCEDURES

MATERIALS

Experimental Animals: Five (5) adult AGRs and Five (5) adult GCs were used for the experiment.

Equipments and Instruments: Dissection kit, specimen containers, glass slides, cover slips, buckets with cover, meter rule, graduated measuring cylinder and beaker, digital weighing balance, plane sheath, syringe and needle, hand gloves, vernier caliper, etc.

Reagents: 10% Buffered formal saline solution, Heamatoxylin and Eosin stain, Nissl stain (Toluidine blue), Graded Alcohol solution (70%, 80%, 95%, 100%), DPX (distyrene, plasticizer, and xylene).

METHOD

Ethical approval for this research was sought and obtained from the Research Ethics Committee, University of Port Harcourt, Choba, Rivers State, with reference number UPH/CEREMAD/REC/MM54/004. The guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) were adhered to. A total number of ten (10) mammals (5 AGRs and 5 GCs) was captured from the wild and delivered to Alex Ekwueme Federal University Ndufu-Alike Ikwo (AE-FUNAI) Animal House. Animals was physically observed and vital signs was checked to see if the animals were apparently healthy. The animals were weighed and allowed to acclimatize for two weeks and the weights were taken again before the commencement of the experiment. They were properly fed *ad libitum* with vital feeds and water. They were sacrificed by cervical dislocation, and decapitated at the occipito-atlantal junction. The scalp tissue layers were removed using forceps thereby exposing the skull. The skulls were opened up in order expose the brain tissues and to facilitate penetration of the fixative into it. Then, the open skulls were immersion-fixed in 10% buffered formal saline solution and transported to Histology Laboratory, Department of Anatomy, Faculty of Basic Medical Science, Alex Ekwueme Federal University, Ndufu Alike Ikwo, Ebonyi State. The tissues were washed with the fixative so as to remove debris and blood, and allow to fix for one (1) week in freshly prepared 10% buffered formal saline solution. All the tissues were subjected to the same condition of fixation to reduce differences in tissue shrinkages resulting from the use of fixatives. Afterwards, the fixed brain tissues were carefully removed from the skull using forceps using the method of Ibe et al., 2017. The dural covering of the intact brains were removed completely, thereby exposing the brains. Gross morphological and morphometric studies involved observing of differences and similarities on brain shapes and sizes, measurement of brain weight and volume, olfactory bulb length and width, cerebral length and width measurement. Also, photographs of the brains were taken. Using a scalpel, the brain structures to be studied were dissected out. All the dissected sections were kept

in a well labeled separate specimen container containing 10% buffered formal saline solution. The tissues were processed histologically and stained using haematoxylin and eosin stain or toluidine blue stain.

RESULTS

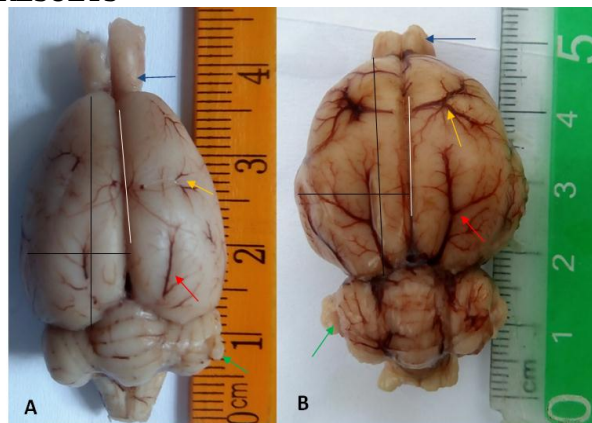


Figure 1: Dorsal view of AGR brain (A) and GC brain (B). AGR cerebrum is oval in shape while GC is globe-shaped. GC cerebrum showed a 'Y' shaped posterior cerebral artery emerging from the occipital lobe of the cerebrum (Red arrow) which appeared slightly V-shaped or straight with smaller branches in AGR (Red arrow). Yellow arrow on the frontal lobe shows the anterior cerebral artery, while the olfactory bulb is shown by the blue arrow. The flocculonodular lobe is seen protruding from the lateral aspect of the cerebellum (Green arrow). Both brains present a median fissure (white line). + on each cerebrum represents cerebral length and width measurement.



Figure 2: Ventral view of AGR brain (A) and GC brain (B). Some features seen on the ventral aspect are: Olfactory bulb (Blue arrow), Flocculonodular Lobe (Green arrow), Optic tract (Red arrow), Middle Cerebral Artery (Yellow arrow).

GROSS MORPHOMETRY OF AGR AND GC BRAINS**Table 1: Gross Morphometric Differences In Variables Obtained From Agr and Gc Brains.**

Variables	Grass cutter	African giant rat
Brain Weight (g)	12.186±0.539 *	5.240±0.311
Brain Volume (cm ³)	18.333±1.666*	5.333±0.600
Olfactory bulb length (cm)	0.318±0.093	0.505±0.041
Total olfactory bulb width (Right+left) (cm)	0.623±0.01*	0.506±0.012
Full cerebral width (cm)	2.766±0.066*	1.878±0.062
Cerebral length (right) (cm)	2.606±0.013	1.900±0.055

Data were expressed as mean ± standard error of mean (Mean ± SEM). T test was done to compare the mean of the groups and determine the level of statistical significance. *= $p \leq 0.05$.

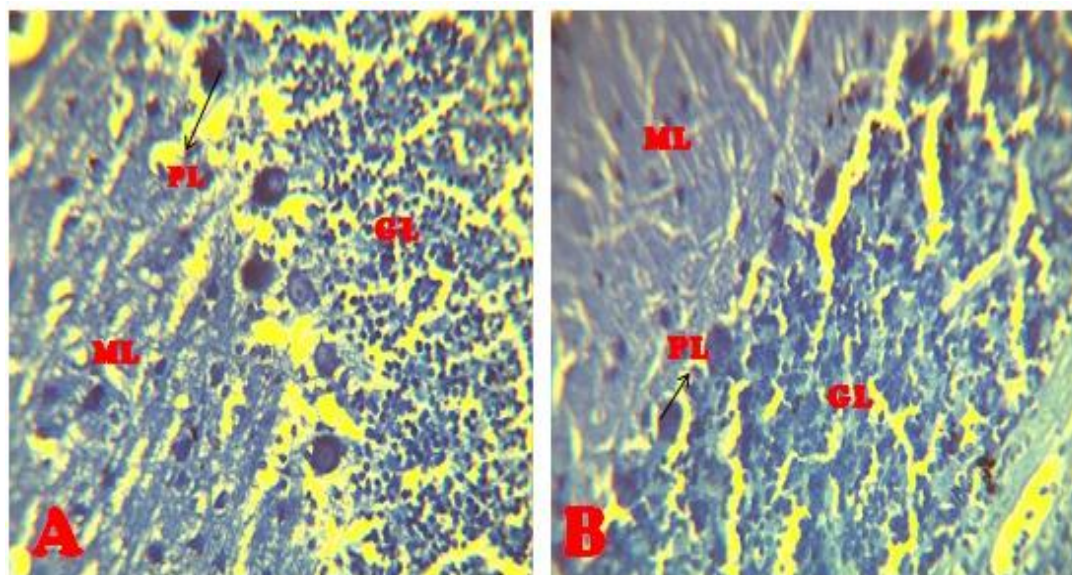
HISTOMORPHOLOGY OF FLOCCULONODULAR LOBE OF GC AND AGR

PLATE 1: Photomicrographs showing the purkinje layer (PL), molecular layer (ML), and granular layer (GL) of the flocculonodular lobe of GC (A) and AGR (B). Toluidine Blue stain X400

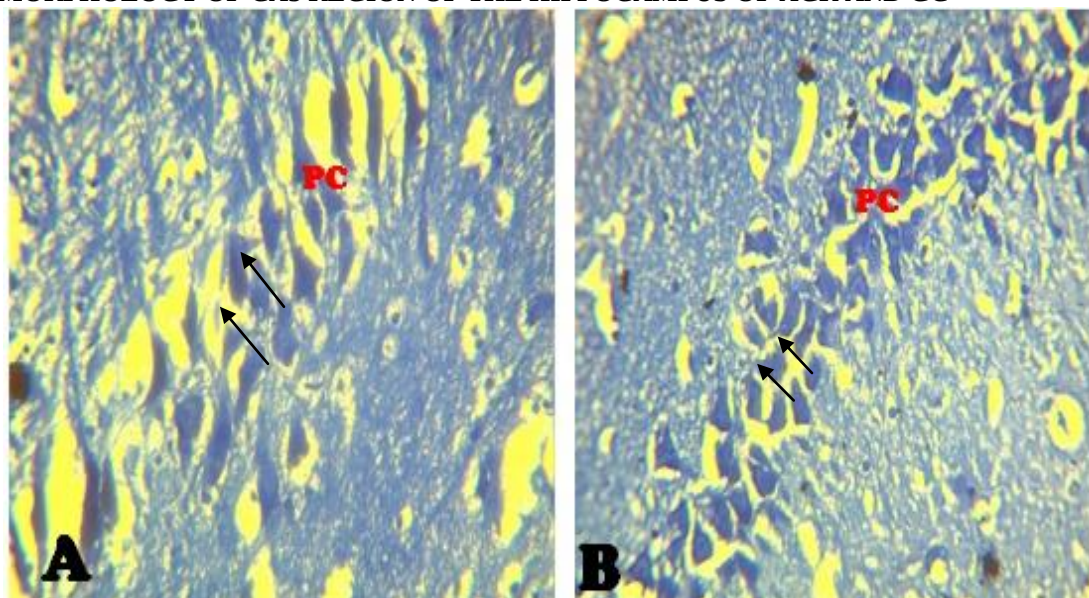
HISTOMORPHOLOGY OF CA3 REGION OF THE HIPPOCAMPUS OF AGR AND GC

PLATE 2: Photomicrograph Showing the Pyramidal Cells (pc) of the ca3 Region of the Hippocampus in gc (a) and Agr (b) Represented by the Black Arrow. Toluidine Blue stain x400.

HISTOMORPHOLOGY OF THE OLFACTORY BULB OF AGR AND GC

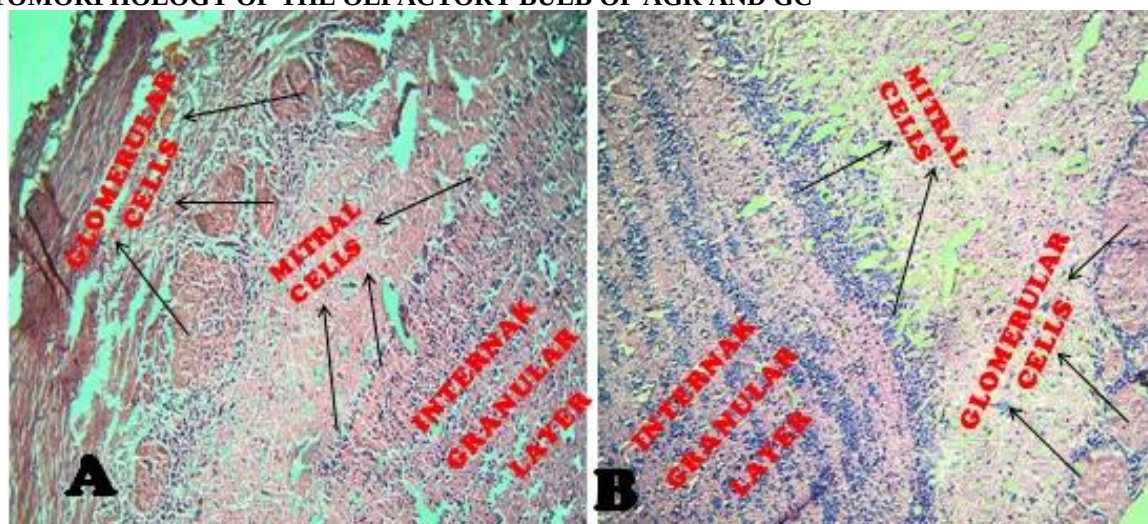


PLATE 3: Photomicrograph Showing the Glomerular Cells, Internal Granule Cells and the Mitral Cells Layers of the Olfactory Bulb of gc (a) and agr (b). H and e stain x100.

HISTOMORPHOLOGY OF THE PREFRONTAL CORTEX OF AGR AND GC

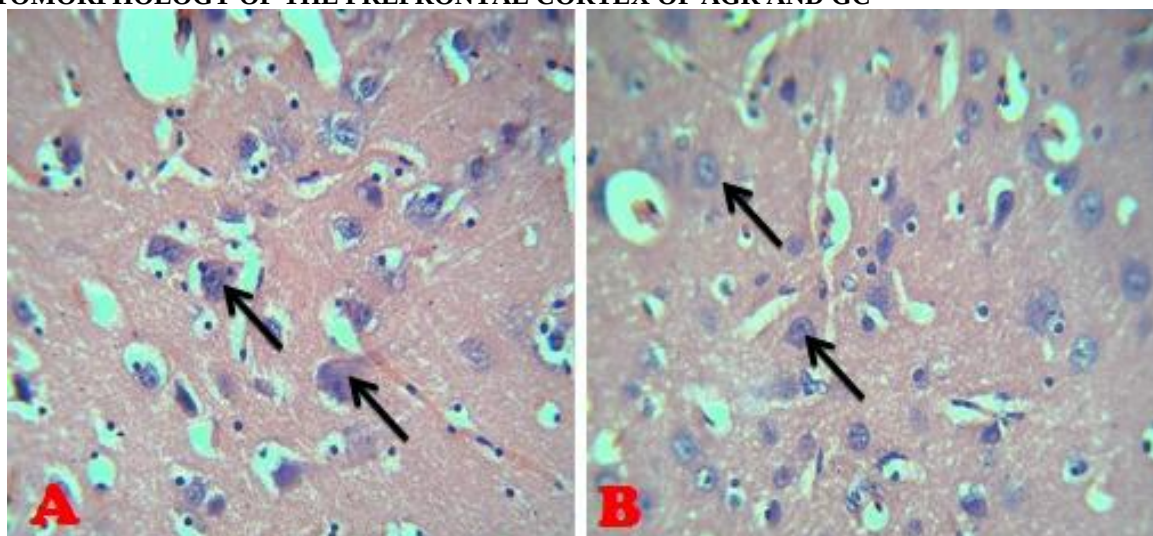


PLATE 4: Photomicrograph showing layer V cells of the prefrontal cortex (Black arrow) of GC (A) and AGR (B). H and E stain X400

5.0 DISCUSSION

5.1 GROSS MORRPHOLOGY

In this study, the brain of AGR was oval or somewhat pyramidal/ triangular in shape (when viewed without the cerebellum). It is narrowed rostrally and widened caudally. This shape was reported earlier by Ibe *et al.* (2014). The right and left cerebral hemispheres of the GC was observed to have a globe- like shape when both hemispheres were viewed together. It showed a rounded frontal lobe with the olfactory bulbs protruding anteriorly. At the caudal end, the occipital lobe appeared somewhat semi-circular. The largest diameter of the cerebrum was at the middle part of the cerebrum. The cerebral hemispheres of GC and AGR were lissencephalic. However, GC cerebral cortex indicated two depressions (minor sulci) located laterally on the posterior aspect of each hemisphere while that of AGR appeared to be void of any gyri or sulci. Lissencephaly is

normal in rodents such as rats, AGR, and GC. This rarely happen in larger mammals but a case of congenital lissencephaly has been reported in a 30-day-old male cross-breed goat and it seems to be the first case of lissencephally in larger mammals (Lemos dos Santos, *et al.*, 2013).

Still on the dorsal surface of the cerebrum, GC showed a 'Y' shaped artery emerging posteriorly from the occipital lobe (Figure 1). This artery is the posterior cerebral artery. This vessel was seen on both halves of the cerebral hemisphere. On the cerebrum of the AGR, the posterior cerebral artery appeared slightly V-shaped with smaller branches. Relatively anterior to the parietal lobe, a medial artery was also seen to be arising from the region of the medial longitudinal fissure and branching approximately on the parietofrontal region of the cerebral cortex of GC and AGR. This is the anterior cerebral

artery. These mentioned arteries were very prominent and well defined in both animals.

Flocculonodular lobes were identified on the cerebrum of GC and AGR (Figure 2). The lobes were of relatively similar size, colour and shape. The flocculonodular lobe is a lobe of the cerebellum and was seen as a protrusion from the paraflocculus, positioned beside the paraflocculus. This is different in the case of larger mammals and man where the flocculonodular lobe is embedded in the cerebellum (Ghez and Fahn, 1985).

The ventral surface of GC showed well-defined and prominent olfactory bulbs in both animals. These structures were located anteriorly in the intact brain and beneath the prefrontal cortex. This structure in AGR was longer in length compared to that of GC which was shorter and blunt. This could indicate better olfactory function in the AGR when compared with the GC. This may be a reason why AGR is considered as good sniffers useful in detecting individual with tuberculosis (Poling, 2011), and also in detecting land mines (Poling *et al.*, 2010). The persistence in its sense of smell may be as a result of high dependency on olfaction due to its poor sight (nocturnal nature) (Alawa *et al.*, 2018). Ventrally, the middle cerebral artery was seen running transversely and laterally from the optic chiasma in both AGR and GC. It formed part of the Circle of Willis (see figure.2).

5.2 GROSS MORPHOMETRY

In this present study, the mean weight and volume of the brains of AGR and GC are shown in table 1. The volume and weight of the brain of GC was statistical higher than that of AGR. Luder *et al.*, (2009) established that higher intelligence, cognition, and behaviour could be related brain size since in vivo assessments have confirmed mainly positive correlations, suggesting that optimally increased brain regions are associated with better cognitive performance (Luders *et al.*, 2009). Witelson *et al.* (2006) studied intelligence in relation to postmortem cerebral volume. In their study, intellectual ability correlated with cerebral volume, but the relationship depended on the realm of intelligence studied, as well as the sex and hemispheric functional lateralization of the subject. Higher animal like Llama (*Lama Glama*) have shown higher intelligence (Tansley, 2011). Therefore, as the brain size increases, the level of intelligence increases (Kaas, 2000). The mean body weight of adult AGR and GC were recorded as 891.7 ± 100.3 g and 1181 ± 210.2 g respectively by Nzalak *et al.* (2008). However, the average brain weight to body weight ratio of AGR is 1:180 (Olude *et al.*, 2016), while for the GC it was 1:214 (Ajayi *et al.*, 2011). These variations in brain weight to body weight ratio could be as a result of specie differences. These values can be related to the morphometric indices adopted for brain size and body weight comparison in relation to intelligence and behaviour. Byanet and Dzenda (2014) however established that brain size can be used to predict body weight. Relationships between the brain and body

weights were quantitatively examined in AGR and GC to determine how accurate to estimate the former from the latter. From their studies, brain weight may be estimated accurately from body mass in the GC. Although both animals are intelligent, GC was observe to possess higher intelligent behaviour due to its body weight to brain weight ratio (1:144) when compared to AGR (1:292) (Byanet and Dzenda, 2014). Therefore based on these findings, the grass cutter is said to exhibit higher cognitive characteristics than AGR (Byanet and Dzenda, 2014).

The present study showed the length of the olfactory bulb in the AGR morphometrically, though not statistically significant, was higher when compared to that of the GC. This may be a reason for the greater olfaction of AGR coupled with its nocturnal nature. The relatively longer size of this bulb of the AGR made it useful in detecting land mines, land explosives, and tuberculosis (Poling, 2011; Poling, Weetjens, Cox, Beyene, & Sully, 2010, 2011; Poling, Weetjens, Cox, Mgode, *et al.*, 2010). The mean width of the olfactory bulb was higher in GC when compared to that of the African giant rat. This may be responsible for the poor sense of olfaction in the grass cutter, but this is not the case in dogs that have both higher length and wider diameter which could be linked to their higher olfactory function in comparison. Length proportion of the olfactory bulb, tract and stria to that of the hemisphere increases in the order of human, goat and dog. (Kavoi *et al.*, 2011).

This present study showed significant differences in the cerebral lengths of AGR and GC. This could be a reason for the different cognitive functions of these animals. Kaas (2002) reported that differences in certain brain parameters could be attributed to size, density, and dendritic arborization of neurons in those regions of the brain. There exists a relationship between brain size, intelligence, and gyrification. As the brain size increases, the degree of gyrification and intelligence also increase. The relative larger size of the brain of the GC compare to AGR allows for studies on the lissencephalic cerebrum. It was concluded that the juvenile African grasscutter may have higher cognitive ability than the adult rodent, thus, juveniles should be preferred in physiological studies of memory and cognition (Ibe *et al.*, 2017)

5.3 HISTOMORPHOLOGY

The flocculonodular lobe examined in the two mammalian species were similar in terms of morphology and structural composition as they consist of three layers each (the molecular layer, the purkinje layer, and the granular layer) which are similar to that of humans. The purkinje cells of the GC appeared larger when compared to that of the AGR. Cells of the CA3 region of the hippocampus were both pyramidal and oval in shape when compared in both animals. However, cell of the AGR were densely packed and smaller-sized in comparison with the GC. This was also observed in the

prefrontal cortex of both animals. The olfactory bulb studied in both animals had similar layers of cells histologically. The prefrontal cortex, when examined in these animals, consisted of 6 layers. The layer V cells of the prefrontal cortex were similar in the two mammals. However, the Betz cells of the GC were larger in size when compared to that of the AGR.

CONCLUSION

Structural differences exist in the brains of the GC and AGR. These variations could be related to the behavioral differences of these animals. Understanding these differences is vital for effective handling of the animals and increased productivity since they serve as indigenous research models for scientific investigations..

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REFERENCES

1. Ajayi, I.E., Ojo, S.A. Onyeansi, B.I. George, B.D.J., Ayo, J.O., Salami, S.O., and Ibe, C.S. Gross Observations and Morphometry of the Medulla Oblongata of the African Grasscutter (*Thryonomys swinderianus*). *Veterinary Research*, 2011; 4: 5-8. DOI: 10.3923/vr.2011.5.8
2. Alawa J.N., George I. O., Hamman W.O., Ibrahim D.I., Akpulu P., Dalhatu S. and Alawa C.B.I. Comparative Neuroanatomical study of the Amygdala and Fear Conditioning in Nigerian Breeds of Artiodactyla: Sheep (Uda) and Goats (Red Sokoto). A conference paper presented orally at American Association of Anatomists (AAA) Postdoctoral Poster Competition, Experimental Biology Meeting, San Diego, California, U.S.A. April, 2018.
3. Asibey, E. O. A and Addo, P. G. (2000). The grasscutter, a promising animal for meat production. In: African perspectives. Practices and policies supporting sustainable development (Turnham D ed.) Scandinavian Seminar College, Denmark, in association with Weaver Press, Harare, Zimbabwe. www.cdr.dk/sscafrica/as&ad-gh.htm
4. Byanet, O. and Dzenda, T. Quantitative Biometry of Body and Brain in the Grasscutter (*Thryonomys swinderianus*) and African Giant Rat (*Cricetomys gambianus*): Encephalization Quotient Implication. *Research in Neuroscience*, 2014; 3(1): 1-6. DOI: 10.5923/j.neuroscience.20140301.01
5. Dwarika, S., Maseko, B.C., Ihunwo, A.O., Fuxe, K. and Manger, P.R. Distribution and morphology of putative catecholaminergic and serotonergic neurons in the brain of the greater cane rat, *Thryonomys swinderianus*. *Journal of Chemical. Neuroanatomy*, 2008; 35: 108-122.
6. Fitzinger, F., Cane Rats. In: Walker's Mammals of the World, Nowak, R. (Ed.). The John Hopkins University Press, Maryland, 1995; 1650-1651.
7. Francis A. I. (2018). Nigeria Spent N1.65 Trillion On Import of Live Animals. 4th of April, 2019. <https://allafrica.com/stories/201804040332.html>. Accessed 07/12/2019
8. Ibe, C. S., Onyeansi, B. I., Hambolu, J. O. Functional morphology of the brain of the African giant pouched rat (*Cricetomys gambianus*, Waterhouse, 1840). *Onderstepoort Journal of Veterinary Research*, 2014; 81(1): Art. #644, 7 page. <http://dx.doi.org/10.4102/ojvr.v81i1.644>
9. Ibe, C. S, Salami, S. O., Wanmi, N. Brain size of the African Grasscutter (*Thryonomys swinderianus*, Temminck, 1827) at defined postnatal periods. *Folia Veterinaria*, 2017; 61, 4: 5—11. DOI: 10.1515/fv-2017-0031
10. Kavoi B. M., Jameela, H. Comparative Morphometry of the Olfactory Bulb, Tract and Stria in the Human, Dog and Goat. *International Journal of Morphology*, 2011; 29(3): 939-946.
11. Kingdon, J. The Kingdon field guide to African mammals. *Academic Press*. London, United Kingdom, 1997.
12. Lemos dos Santos, B., Dame M. C. F., Coelho A. C. B., Oliveira P.A, Marcolongo-Pereira C., Schil d, A. L. Lissencephaly-pachygyria and cerebellar hypoplasia in a calf. *Ciencia. Rural*, 2016; 46: 9. Santa Maria. <http://dx.doi.org/10.1590/0103-8478cr20151572>
13. Luders, E., Narrs K. L, Thompson, P., Toga, A.W. Neuroanatomical correlates of Intelligence. *Intelligence*, 2009; 37(2): 156-163. DOI: 10.1016/J.Intell.2008.07.002
14. Nzalak, J. O., Ayo, J. O., Neils, J.S., Okpara, J. O., Onyeansi, B. I., Ghaji, A., Ojo, S. A. Morphometric studies of the cerebellum and forebrain of the African giant rat (*Cricetomys gambianus*, Waterhouse). *Tropical Veterinarian*, 2005; 23(3&4): 87-92.
15. Nzalak, J.O., Byanet, O., Salami, S.O., Umosen, A.D., Maidawa, S.M., Ali, M.N. (2008). 'Comparative morphometric studies of the cerebellum and forebrain of the African Giant rat (*Cricetomys gambianus*, Waterhouse) and that of grasscutter (*Thryonomys swinderianus*), *Journal of Animal and Veterinary Advances* 7, 1090-1092.
16. Olude, M. A., Olopade, J. O., Ihunwo, A. O. (2014b). Adult neurogenesis in the African giant rat (*Cricetomys gambianus*, Waterhouse). *Metabolic Brain Disease*. 29(3): 857-66.
17. Olude, M. A., Ogunbunmi, T. K., Olopade, J. O., Ihunwo, A. O. (2014a). The olfactory bulb structure of African giant rat (*Cricetomys gambianus*, Waterhouse 1840) I: Cytoarchitecture. *Anatomical Science International*. 89(4): 224-31.
18. Poling A, Weetjens B, Cox C, Beyene N, Sully A (2010). Using giant African pouched rats

- (*Cricetomys gambianus*) to detect land mines. *The Psychological Record*. 60:715–727.
19. Poling A, Weetjens B, Cox C, Mgode G, Jubitana M, Kazwala R, (2010). Using giant African rats to detect tuberculosis: 2009 findings. *American Journal of Tropical Medicine and Hygiene*. 83:1308–1310.
 20. Poling A, Weetjens B.J, Cox C, Beyene N, Bach H, Sully A (2010). Teaching giant African pouched rats to find land mines: Operant conditioning with real consequences. *Behavior Analysis in Practice*. 3:19–25
 21. Poling A, Weetjens B.J, Cox C, Beyene N.W, Bach H, Sully A (2011). Using trained pouched rats to detect land mines: Another victory for operant conditioning. *Journal of Applied Behavior Analysis*. 44:351–355.
 22. Poling A. Looking to the future: Will behavior analysis survive and prosper? *The Behavior Analyst*, 2010; 33: 7–17.
 23. Poling A. Tuberculosis Detection by Giant African Pouched Rats. *Behaviour Analysis in Practice*. Spring, 2011; 34(1): 47–54. doi: 10.1007/bf03392234
 24. Semple, B. D Klas Blomgren, Kayleen Gimlin, Donna M. Ferriero, and Linda J. Noble-Haeusslein. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology*, Jul-Aug Apr, 11; 0: 1–16. doi: 10.1016/j.pneurobio.2013.04.001
 25. Tansley, A. (2011). Comparative Intelligence of the Llama (*Lama glama*): Implications for Welfare. Burn 2011. Biosciences Undergraduate Research at Nottingham © School of Biosciences, UoN
 26. Witelson, S. F., Beresh, H., Kigar, D. L. Intelligence and brain size in 100 postmortem brains: sex, lateralization and age factors. *Brain*, Feb, 2006; 129(Pt 2): 386-98. Epub 2005 Dec 9.
 27. Ghez, C., & Fahn, S. The cerebellum. In E. R. Kandel & J. H. Schwartz (Eds.), *Principles of neural science*, 1985; 502{522). Elsevier Science Publishing.