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Morphological Differences in the Cerebrum of Adult and Juvenile *Thryonomys swinderianus* (African Grasscutter)

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ABSTRACTS

The African grasscutter (AGC; *Thryonomys swinderianus*) has been observed to possess unique physiological and structural features enabling them to survive in challenging environments. This behavior displayed by AGCs across different age groups could be attributed to a well-developed CNS where M1 cerebrum plays a critical role. This study described the morphological (gross and microscopic) characteristics of the cerebrum of adult and juvenile AGCs. Eight AGCs were procured and euthanized, brain was excised for morphological characteristics (gyrification, brain dimensions, and organosomatic index), light microscopic assessment of the cerebral M1 region using H&E stains for histological features, and Cresyl Violet stain for histometric analysis (pyramidal soma size). Results revealed similar gyrification patterns on the dorsal surface of cerebral hemispheres across the age groups. The assessed brain dimensions revealed higher values ($p>0.05$) for the adult, except in the cerebral dorsoventral length, compared to the juvenile AGCs. The organosomatic index was higher ($p<0.05$) in the juvenile compared to adult AGCs. Histologically, layers III and V of the cerebral M1 region presented with pyramidal neurons as the predominant cells across the age groups, and appeared denser in the juvenile AGCs. Histometrically, the juveniles revealed higher ($p>0.05$) pyramidal soma size values in cerebral M1 layers (III and V), compared to the adult AGCs. In conclusion, morphologic features of the AGC's brain are relatively similar to those described in other rodents. Variations in the gross and microscopic features of the AGC cerebrum exist across age groups.

Keywords: gyrification, histology, histometry, laminae, pyramidal neurons

INTRODUCTION

The brain, an integral part of the nervous system of vertebrates, has developed in both size and complexity, with mammals, especially humans, having the largest brain in proportion to their body size[1]. Different parts and regions of the brain are morphologically and functionally unique; critical as a seat of intelligence, interpreter of senses, initiator of body movement, and controller of behavior[2]. The cerebrum is the largest and most developed of the major parts of the brain, composed of the cortical and subcortical structures. The mammalian cerebral cortex presents with unique and distinctive morphologic features, involved in motor activity, memory, language and consciousness[3].

Thryonomys swinderianus (African Grasscutter, AGC) is an African rodent species with unique morphologic and physiological adaptations that enable them to survive in challenging environments. The AGCs have shown wonderful use of the hind limbs by standing upright on their limbs, taking a bunch of grass in their fore limb, sitting upright on their haunches and feeding the grass into their mouth slowly cutting it up into small bits [4]. Additionally, the AGC is a skilled burrower whose well-developed central nervous system may have evolved in response to these behaviors. Certain behavioral patterns including feeding, agility, alertness, response to environmental pressures, and escape from predators have been described in AGCs in their natural habitat across different age groups. Hence, has drawn attention of neurobiologist to consider this species as a potential research tool [5, 6].

Previous studies have described the basic features of AGC nervous system; the brain [6-9] and recently, the spinal cord [10]. However, there are no well-established investigations towards depicting the differences in the cortical neuronal organization and cytoarchitectural features of the cerebral M1 region which is a critical

step towards understanding planned voluntary movement of this species across different age groups. Therefore, this study described and characterized the morphological features of the cerebrum in juveniles and adult AGCs (*Thryonomys swinderianus*) using gross and microscopic approaches.

MATERIALS AND METHODS

Experimental animals

A total of eight (8) apparently healthy AGCs consisting of 4 adults and 4 juveniles were obtained from Okiki Farm, Lokoja, Kogi State, Nigeria. The AGCs were transported in a ventilated wooden cage to the Animal House, Department of Human Anatomy, Faculty of Basic Medical Science, Ahmadu Bello University (ABU), Zaria, housed under standard laboratory conditions, and allowed to acclimatize for a week prior to the commencement of the study. The AGCs were fed with sugar cane during the study period.

Ethical approval

This study was conducted in accordance with global best practices for laboratory research with consent from the Committee for Ethics in Animal Experimentation of ABU, Zaria (ABUCAUC/2023/108).

Experimental design

The AGCs were grouped into two groups, containing adult and juveniles (n= 4), weighed and euthanized using chloroform anesthesia. The brains were carefully dissected out of the cranial cavity, morphological characteristics were measured and immediately fixed in neutral buffered formal saline for subsequent studies (Figure 1).

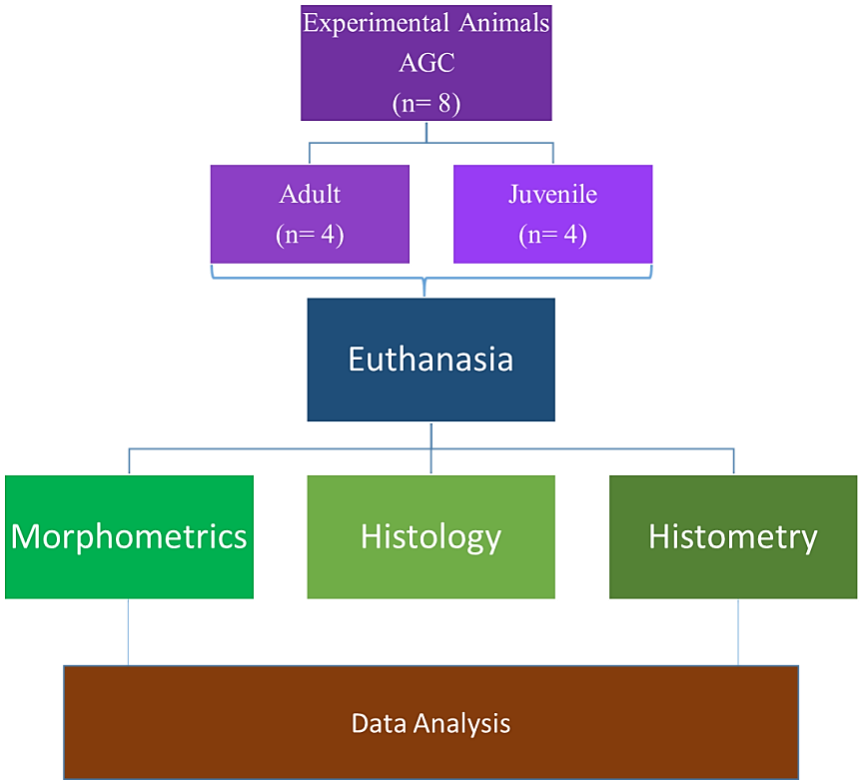

Figure 1: Experimental design

Morphological Studies

Physical observation of gross features was conducted on the dorsal, lateral and ventral surfaces of the harvested whole brain. The brains were weighed using a digital weighing scale (Notebook Series Digital Scale, China; 0.01g) and organosomatic (brain-body weight) index was computed (brain weight/absolute body weight ×100 [11]) Brain dimensions were measured according to the methods described by Ivang *et al.* [12] using an electronic digital Vernier caliper (150 mm, China). The dimensions were: brain length (antero-posterior), cerebral length (antero-posterior most prominent points), cerebral width (right-left most prominent points), and cerebral dorso-ventral length (thickness most prominent points) (Figure 2).

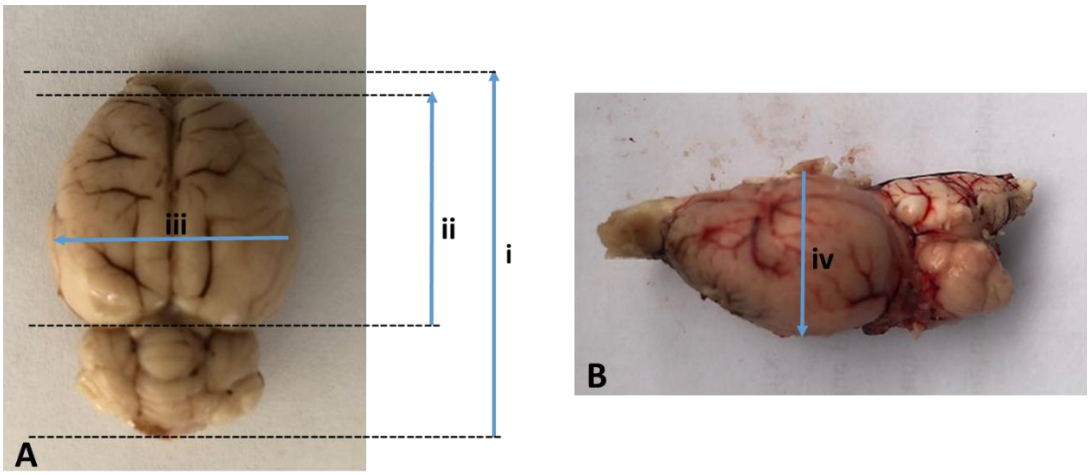

Figure 2: Measurement of brain dimensions of the African grasscutter. **A**: Dorsal surface **B**: Lateral surface; i: Brain length (Rostro-caudal/or anteroposterior); ii: Cerebral length; iii: Cerebral Width or Transverse Dimension; iv: Cerebral dorsoventral length.

Histological assessment

The fixed brain specimens were processed using histological techniques for light microscopic examinations, stained with Hematoxylin and Eosin (H&E) and Cresyl Fast Violet (CFV) stains for the demonstration of cytoachitectural features. The specimens were sectioned coronally to target specific brain region of interest; primary motor cerebral cortex (M1 cerebral region), using prescribed landmarks from the Rat Brain Atlas [13] (Figure 3). The processed paraffin sections were examined at different microscopic magnifications and compared across the different age groups (adult and juvenile). Histological tissue processing and micrography was carried out at the Histological Unit of the Department of Human Anatomy, ABU, Zaria.

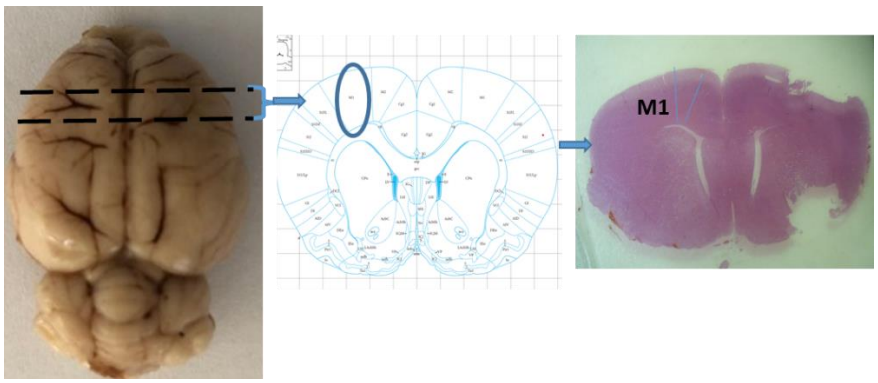

Figure 3: Coronal section of the AGC brain targeting M1 cerebral region. M1: Primary motor cerebral cortex; broken black line: point of brain section.

Histometric analysis

Histometry was conducted according to the method of Huda and Zaid [14] and modified as described by Agbon *et al* [15]. This involved measuring the cell soma area and perimeter of pyramidal cells from CV-stained micrographs of layers III and V (M1 cerebral cortex) of AGCs (juvenile and adult). A light microscope (HM-LUX, LeitzWetzlar, Germany) with a 25/ 0.5 × objective (× 250 magnification), a micrometer slide (1 mm graduated in 0.01 mm units; that is divided 10 into 100 µm units) and a computer running imaging software (AmScope MT *version* 3.0.0.5, USA) according to the manufacturer's instruction was used. Five different micrographic fields were randomly captured in the M1 region (layers III and V) and 7–10 neurons that met the criteria for selection (that is, pyramidal neurons with well-outlined nucleus in the cell profiles were randomly selected); using the AmScope imaging software polygon tool, soma area and perimeter were measured and mean values analyzed. Mean values obtained were statistically compared across age groups.

Data analysis

Data obtained were analyzed using the GraphPad Prism *version* 9.0 and the results were expressed as mean ± S.E.M. Presence of significant differences among means of the groups were determined using independent *t-test*. P-values less than (p<0.05) was considered statistically significant.

RESULTS

Morphological assessments

The AGC brains were observed to be milky in color and presented with two major sulcal depressions on the dorsal surface: a coronal plane-oriented depression and the other a sagittal plane-oriented depression. The coronal plane-oriented depression separates the cerebrum (forebrain) from the cerebellum (hindbrain) and the sagittal plane-oriented depression separates the cerebrum into two hemispherical halves. Minor (sulcal) depressions (grooves) with certain patterns were observed on the dorsal surface of each halve of the cerebral hemispheres of the adults AGCs. These minor depressions, however, were not as prominent in the juveniles compared to the adults. Additionally, the pattern of these depressions varies in the juvenile compared to the adult AGCs.

The brain ventral surfaces in both adult and juvenile AGCs presented with distinct parts of the brain including the optic chiasma just rostral (anterior) to the midbrain. Other brainstem structures (pons and medulla) are delineated by depressions, the medulla continues caudally with the spinal cord. Moreover, vasculatures and other related features were observed (Figure 4).

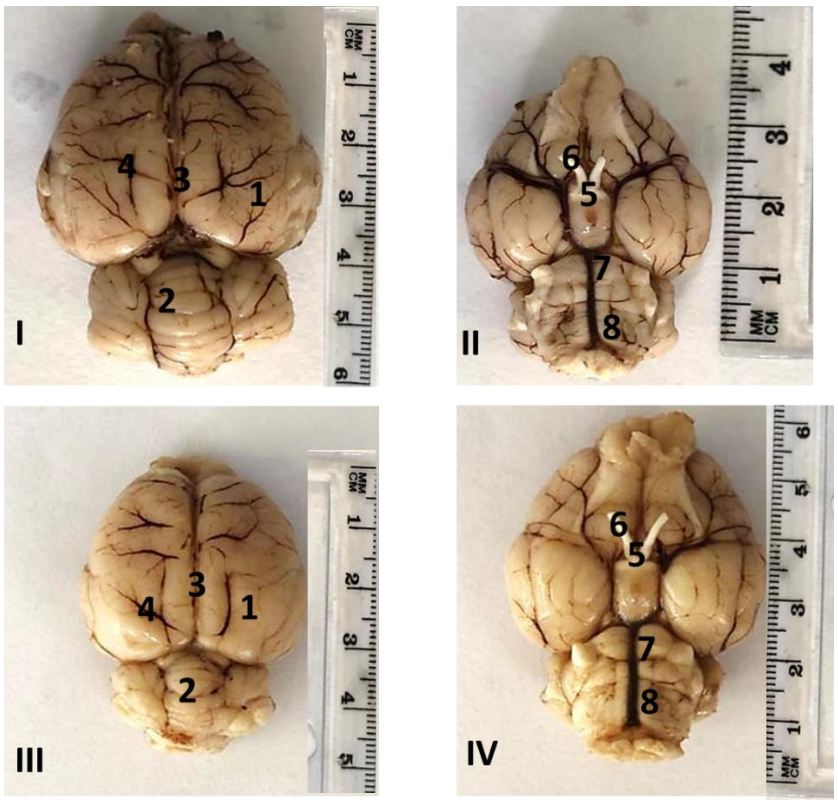

Figure 4: Brain of the African grasscutter. Dorsal View, I: adult; III: juvenile; Ventral view, II: adult; IV: juvenile; 1: Cerebrum; 2: Cerebellum; 3: Sagittal (intercerebral) fissure, separating the cerebral hemispheres; 4: Sulcal depression; 5: Optic chiasma; 6: Optic tract; 7: Pons; 8: Medulla.

Brain weight and dimensions

The mean AGC brain weight for adults was greater than 11 g compared to that of the juvenile which was less than 10 g. A comparison of the brain weights revealed insignificant differences between the male adult and juvenile (Figure 5). The organosomatic index of AGCs revealed significant (p<0.05) differences relative to age; the juveniles had higher mean value than their adult counterparts (Figure 6).

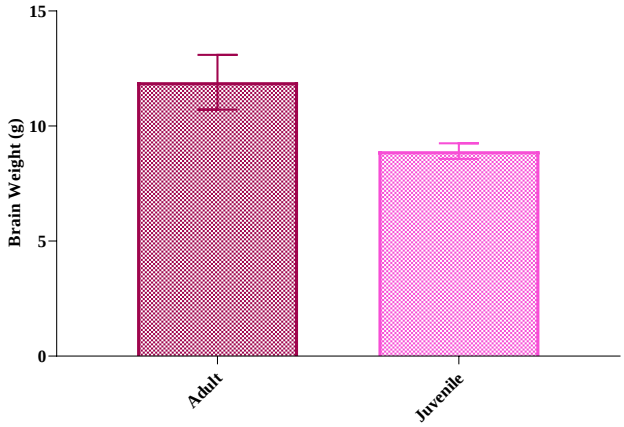

Figure 5: Brain weight comparison between adult and juvenile AGC. n=6, Mean ± SEM, Independent sample t-test, $p>0.05$ when juvenile was compared to adults

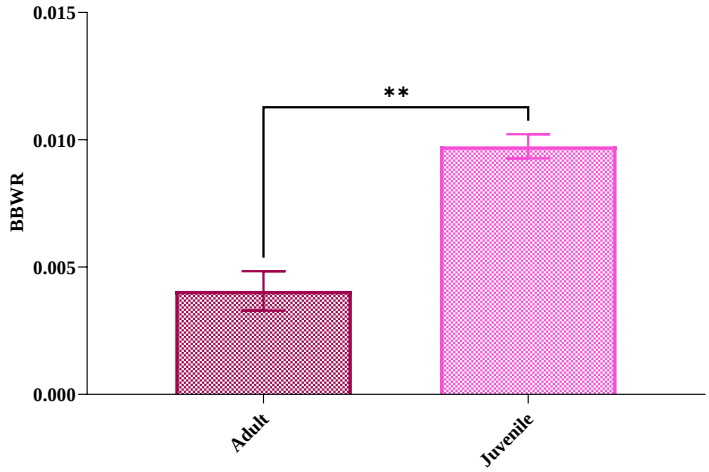

Figure 6: Brain body weight (BBWR) comparison between adult and juvenile AGC. n=6, Mean ± SEM, Independent sample t-test, **=$p<0.005$ when juvenile was compared to adults

Brain dimensions

Relative to brain dimensions, the adult AGCs revealed higher mean values for all the measured dimensions, except cerebral dorsoventral length, compared to their juvenile counterpart. The difference was only significant in the brain length **(Table 1).**

Table 1: Morphometric comparison between Adult and Juvenile AGC

Variables	Adult n=3 (Mean ± SEM)	Juvenile n=3 (Mean ± SEM)	*t*	*P*
CL (mm)	25.73±3.57	21.13±1.62	2.031	0.142
CW (mm)	28.73±0.25	27.83±0.84	1.780	0.198
BL (mm)	44.77±0.45	34.5±1.87*	9.228	0.001
DVL (mm)	16.8±0.79	18.07±2.21	-0.934	0.403

n=6, Mean ± SEM, Independent sample t-test, *=$p<0.05$ when juvenile was compared to adults

CL: Cerebral Length, CW: cerebral width, BL: Brain Length, DVL: Dorsoventral Length.

Histological studies

Coronal section through cerebral cortex at varying microscopic magnifications revealed distinct histoarchitectural features of the adult and juvenile AGCs. At a lower magnifying power, the cortical and subcortical region including their structures were observed (Figure 7).

At a high microscopic power of ×40, dorsoventrally, the presence of pia mater lining the cortex with shallow depressions, cortical region with six lamina (layers), blood vessels and a white mater region (devoid of prominent cell nuclei; the corpus callosum) were observed. There were no observable differences in the histoarchitectural features between the age groups (Figure 8).

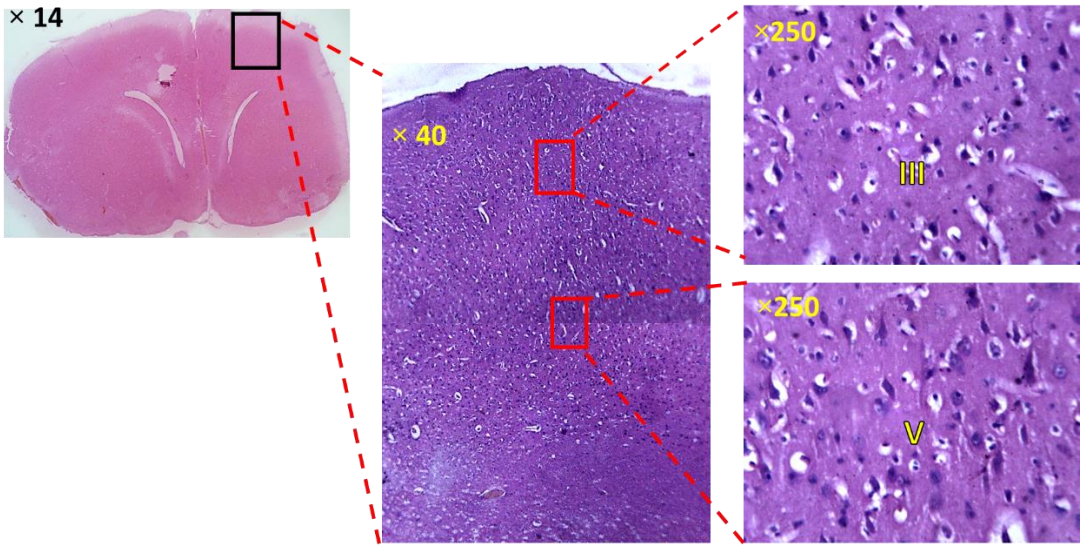

Figure 7: Coronal section of African grasscutter brain. H&E stain. III: layer III of cerebral cortex; IV: layer V of cerebral cortex

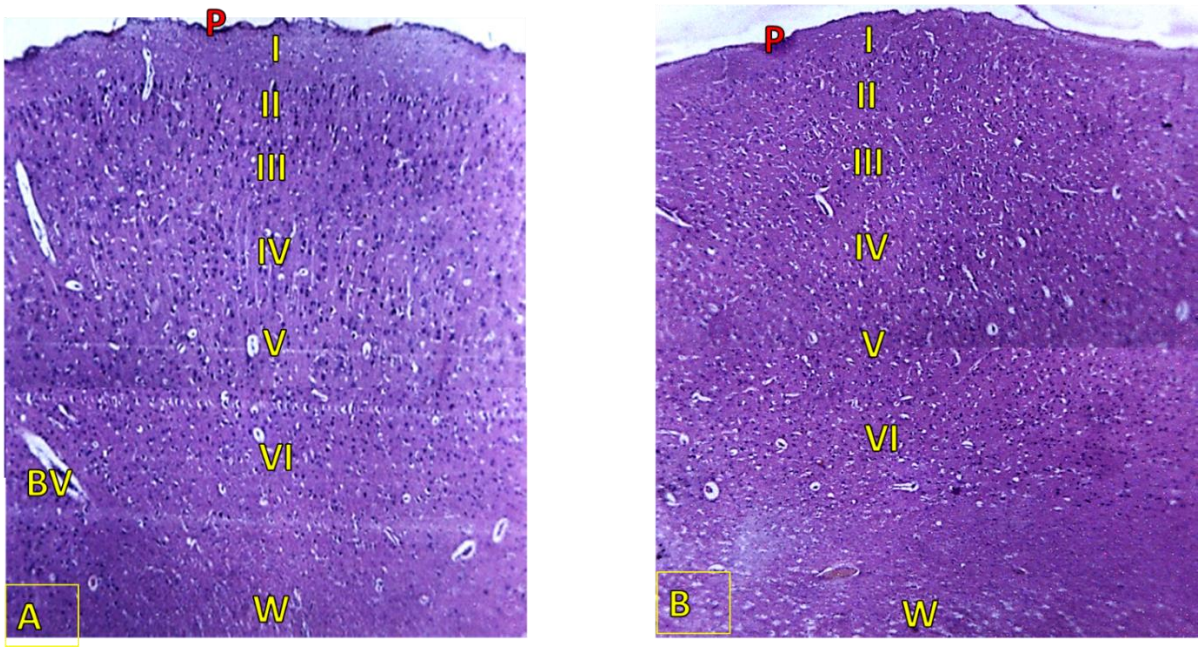

Figure 8: Coronal section of adult and juvenile African grasscutter brain. H&E Stain X40. A: Adult; B: Juvenile; P: Pia mater; I: molecular layer; II: external granular layer; III: external pyramidal layer; IV: Internal granular layer; V: Internal pyramidal layer; VI: Multiform layer; BV: Blood vessels; W: White mater.

Cortical M1 region (layers III and V) at a higher microscopic power of ×250 revealed two major neurons: stellate and pyramidal; neuroglia cells, and blood vessels. The pyramidal neurons in layer V were observed to be denser compared to those in layer III. In comparison, the distribution of cells (neuronal and glial) appeared to be different between the age groups; the juveniles presented with more distinct pyramidal cells when compared to the adults. Staining of the layers III and V sections with Cresyl Fast Violet revealed positive reactivity with distinct cell morphologies; stellate and pyramidal neurons including blood vessels (Figure 9).

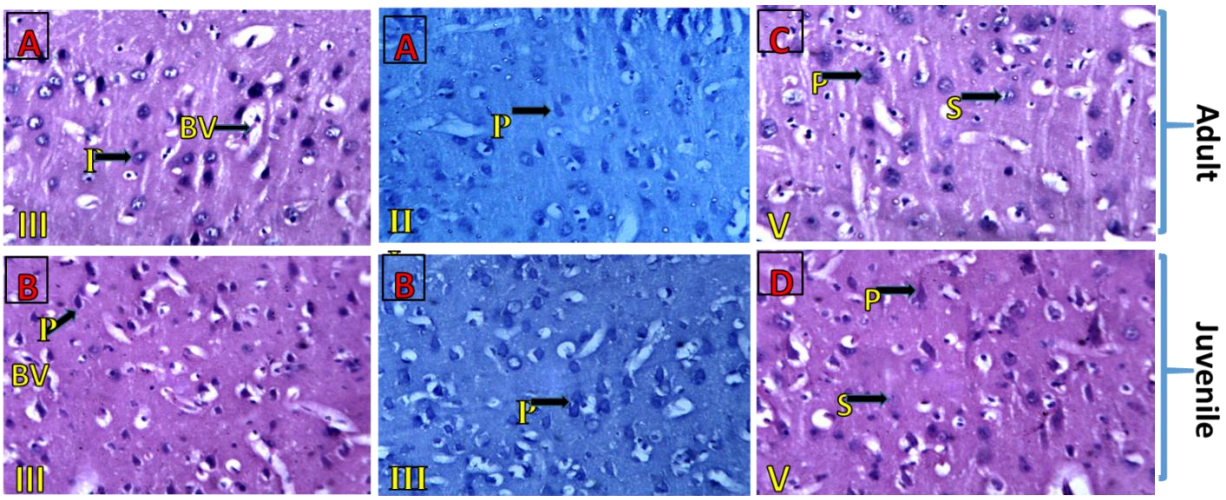

Figure 9: **Coronal section of African grasscutter brain.** H&E and CFV (*micrograph at the middle*) ×250. A and B; layer III of adult and juvenile; C and D: layer V of adult and juvenile; P: Pyramidal neurons; BV: Blood vessels; S: Stalette neurons.

Histometric characteristics

The mean histometric characteristics (soma area and perimeter) of pyramidal neurons in layers III and V revealed higher values in the juvenile compared to the adult AGCs (**Figures 10 and 11).**

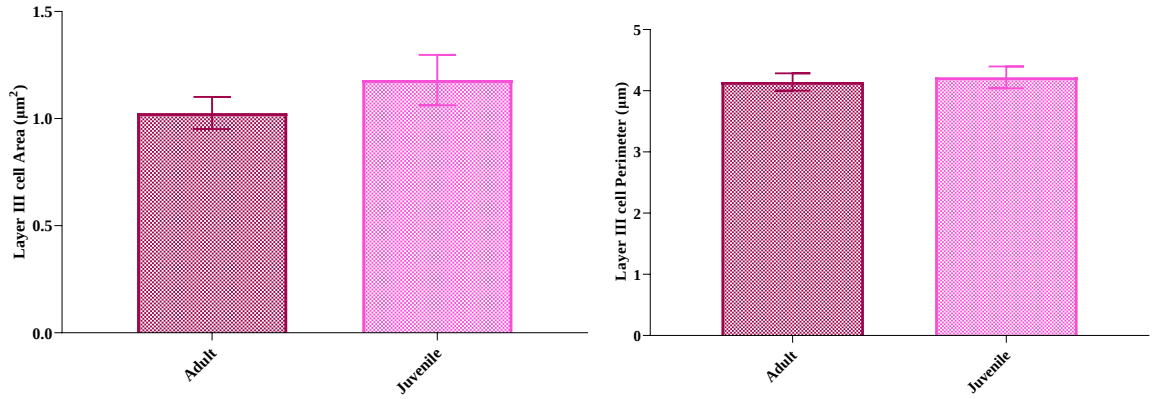

Figure 10: Comparison of Layer III pyramidal cell soma (area and perimeter) between adult and juvenile AGC. n=6, Mean ± SEM, Independent sample t-test, $p > 0.05$ when juvenile was compared to adults

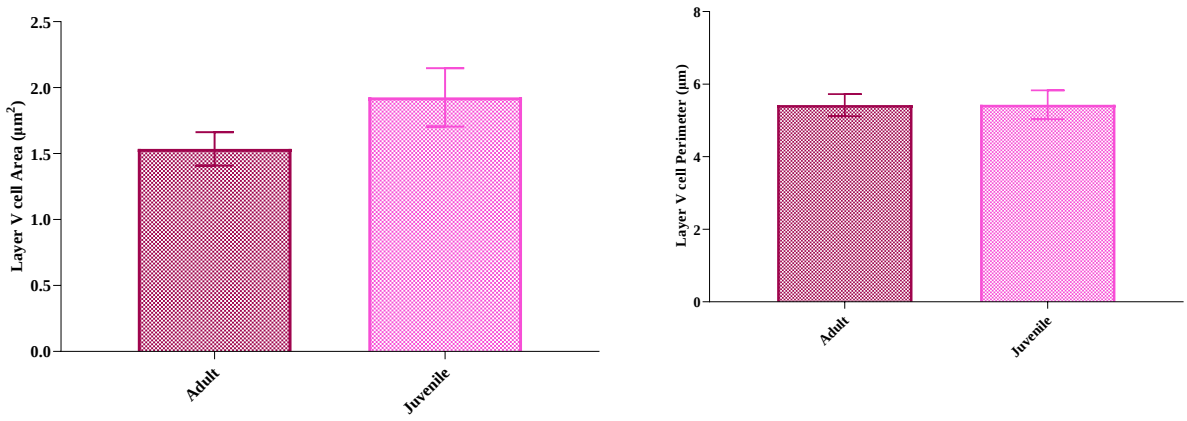

Figure 11: Comparison of layer V pyramidal cell soma (area and perimeter) between adult and juvenile AGCs. n=6, Mean ± SEM, Independent sample t-test, $p > 0.05$ when juvenile was compared to adults

DISCUSSION

Generally, the dorsal and ventral morphological features observed in the AGC brain is in agreement with reported brain characteristics in rodent species [16, 17, 18]. Sulcal depressions observed on the dorsal surface of the cerebrum suggest the species to be gyrencephalic. This finding agrees with reported gyrencephalic cortex in some rodents including the agoutis and guinea pigs having certain patterns of gyri on cortical cerebral surfaces [19, 20]. However, this finding is at variance with the commonly reported lissencephalic cerebral cortex for rodents [21]. This variance could be associated with evolutionary adaptive variation in this species, AGC, or probably influenced by factors including environment and genetics [20].

The observed mean absolute body weight of AGCs; greater than 2 kg is in line with reported values for adults AGCs. This placed the species as a large rodent; larger than the African giant rat (*Cricetomys gambianus*) with reported mean absolute body weight > 1 kg [22] for adults, but less weighty than some rodent species including porcupine with reported mean absolute body weight > 7 kg [23].

Brain size is a measure of its dimension, volume and weight. It varies with species, breed, sex and age. The mean brain weight of AGCs in this study was observed to be > 11 g agreed with reported mean values for adult AGCs [6, 21]. This mean brain weight value is greater than that reported for murine, hamsters, squirrel [24], guinea pigs[18] and African giant rats [9, 22]. However, the brain weight of the juvenile AGC observed to be <10g could be attributed to the size for its age. This finding is in line with the report of [6]; the brain weight increases with the size of the rodent. Changes in brain size are due to changes in the number of neuronal and neuroglial cells in the brain, which is dependent on the extent or rate of neurogenesis [6].

The organosomatic index quantifies the percentage of brain mass relative to the absolute body weight [6] and an integral factor of encephalization quotient, a measure of intelligence of species. This index has been established to differ from one taxon or age group to another [25]. In this study, the juvenile revealed a remarkably higher values for brain-body weight index compared to the adult. This suggests that the juvenile AGC is probably more intelligent than the adult. This finding is in line with the result of encephalization quotient test reported by Ibe *et al.* [6] who pointed out higher cognition in the neonate and juvenile AGCs, compared to the adults. The fact that neurons are lost via apoptosis during neurogenesis as the species advanced in age could possibly be the reason for a lower index value in adult than juvenile AGCs. Additionally, ageing has been established to pose some structural changes on brain cells and by extension the gross morphology of the brain, and cognition [26].

Brain dimensions has been hypothesized in the mammalian species to increase with increasing cranial cavity [6]. In this study, the brain dimensions of the adult AGCs were observed to be higher than that of the juveniles, this could be due to its correlation with the brain weight. This finding agrees with reports that attributed higher brain dimensions to higher brain weights in different species [17, 18]. The mean brain length of the adult AGCs observed in this study is higher than the value reported for the adult African giant pouched rat [27], but less than the value reported for the adult squirrel [21]. These differences could be attributed to species variations, body sizes, and varied cognitive abilities.

Histologically, lamina organization of the AGCs cerebral M1, differentiated by the presence of different cells within the laminae; bounded by pia mater, superiorly and a running corpus callosum, inferiorly is a common characteristic of mammalian species including Wistar rats, Guinea pigs, African giant rat [28, 29, 15]. Neuronal cells are the main cellular components of the nervous tissues, and the glial cells are supporting components [30, 31]. Varying cell morphologies observed in AGCs cerebral M1 (layers III and V) including neuronal cells; stellate and pyramidal cells, and neuroglia cells are typical of nervous tissues presenting with morphologically distinct cell types that are functionally related [17, 32,33,34]. Oorschot [34] reported varying neuronal cell morphologies in mammals including primate and non-primate species, especially rodents; mice and rats.

The presence of pyramidal cells and glial cells in the cerebral M1 region are critically involved in the circuitry of motor related functionality in mammalian species [15] and indicates nutritive, supportive, and other regulatory functions, respectively in the cerebral M1 region of this species (AGCs). The accompanying blood capillaries within the AGCs cerebral M1 layers shows a typical vascularized tissue similarly reported in mammalian species [35, 36].

Pyramidal cells positive reactivity to the histochemical stain, CFV, indicates their involvement in normal physiological and biochemical processes necessary for nervous tissue function [37]. In this study the pyramidal neurons in both layers III and V of adult and juvenile reacted positive to CFV stain; which indicates their involvement in biochemical functions. This is in conformity with the report of [17]Ibegbu *et al*.; histochemical reactivity of pyramidal neurons in cerebral cortex to CFV stain; an excellent neuronal, cell body-specific stain.

Histometric quantification of 2D- histological data is an important tool that provides an objective basis for comparison of histological observation[38, 14]. It improves assessment of certain histological change, which though may be recognizable by eye, are accurately graded and their progression better appreciated by histometric quantification [15, 39]. This study applied histometry to quantify the pyramidal neuronal sizes (area and perimeter) within the layers III and V of the M1 cerebral region and compared across the ages of AGCs. The observed remarkable differences in pyramidal neuronal sizes between adult and juvenile, explained the variation in neuronal sizes amongst the compared age groups with juvenile having a higher value, which could be a reflection of motor functionality of the juveniles being more physically active and adventurous; cells vary greatly in size relative to cellular functionality rather than the size of the organism [40]. This finding is in contrast with the study that reported some cells including neurons are longer and larger in larger animals compared to that of smaller animals of same species [41]. Additionally, finding suggests the AGC pyramidal neuronal sizes are larger than those of Wistar rats reported by Agbon *et al* [15]. This could probably support the notion that neuronal sizes increase with rodents' size. Larger rodents such as African grasscutter and African giant pouched rat may be better models for electrophysiological studies as they have absolutely larger neurons that can easily be used for intracellular electrode recordings than smaller-sized rodents like rat and mouse [23].

Conclusion

Morphologic features of the AGC's brain are relatively similar to those described in other rodents. There exist variations in the gross and histologic features of the AGC cerebrum across age groups. These findings are of potential benefit in understanding the neuroanatomy of this species and their behavior in natural habitat.

Conflict of interests

Authors hereby declare that there is no conflict of interest regarding the publication of this article.

Acknowledgements

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