Cerebral cortex and Hippocampus of the African Striped Ground Squirrel (*Xerus erythropus*) - Cytoarchitectural Studies.

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Abstract

The purpose of this study was to investigate the special neuroanatomical features of the cerebral halves of the African striped ground squirrel (ASGS). The study was conducted by direct observation of ten (10) adult ASGS. They were anaesthetized with an intraperitoneal injection of ketamine Hcl (50mg/kg body weight), Histological sections were prepared after fixation and routine tissue processing. Tissue sections were serially obtained with a microtome and stained using hematoxylin and eosin as routine stain and cresyl fast violet stain. The Cytoarchitecture of the cerebral cortex of the ASGS revealed six (6) layers: Molecular or Plexiform layer, External granular layer, External pyramidal layer, Internal granular layer, Internal pyramidal cells layers and multiform or polymorphic layer. The layers were, however, distinguishable on the basis of predominance of cell types. Interestingly was the numerous and large pyramidal neurons seen in the internal pyramidal layer which could be responsible for the high cognitive and motor function ability of the rodent in the wild. The hippocampus observed in this study, with respect to stratification, subfields and cell types, was similar to those reported in the African giant rats and laboratory rats. Cell types

identified in the hippocampus of the squirrel include pyramidal cells, granule cells and mossy cells. Hippocampal proper subfields *Cornu Ammonus* 1- 4 (CA1 to CA4) were identified. Together, these results provide essential base-line information on the cerebral cortex and Hippocampus of the ASGS that will enable more accurate comparison to be made between members of the rodent family.

Keywords: African striped ground squirrel, cerebral cortex, cytoarchitecture.

Introduction

African striped ground squirrel is a diurnal rodent which belongs to the order Rodentia, family Sciuridae, genus Xerus, and specie erythropus (Thorington and Hoffmann, 2005). They have sandy-brown to dark brown fur and whitish underparts with a lateral stripe of pure white fur which runs from the shoulders to the hip (Herron and Waterman, 2004). Reports have shown that the African striped ground squirrel bury food (caches) in winter using a method called scatter hoarding and locate these caches using both memory and smell (Joanna *et al.,* 2005). The brain controls vital activities for survival (Ibe *et al.,* 2014). Sensory impulses are received from sensory organs through the spinal cord and cranial nerves and then processed for initiation of motor output to effector organs. Specifically, the brain coordinates activities in relation to changes in the external and internal environment (Ibe *et al.,* 2014). The brains of all species are composed primarily of two broad classes of cells: neurons and glial cells. Glial cells (also known as glia or neuroglia) come in several types, and perform a number of critical functions, including structural support, metabolic support, insulation, and guidance of development. Neurons, however, are usually considered the most important cells in the brain (Kandel *et al.,* 2000). The property that makes neurons unique is their ability to send signals to specific target cells over long distances (Kandel *et al.,* 2000). The cerebral cortex is the outermost sheet of neural tissue of the cerebrum of the brain in some vertebrates (Nolte, 2007). It covers the cerebrum and is divided into left and right halves. The cerebral cortex plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. It is composed of many structurally and functionally-unique subunits that perform a wide range of sensory, motor, and mnemonic processes associated with cognition (Nolte, 2007). Abiyere *et al.* (2022) reported the morphology and morphometrics of the cerebral cortex however there is no information on the Neuronal architecture of the cerebral cortex of this rodent. Therefore, this research was to study the Cytoarchitecture of the African striped ground squirrel to

serve as a fulcrum for future clinical and research application and to elucidate some of it cognitive behavior.

Materials and Methods

Research animals

Ten captive and clinically healthy adult African Striped Ground Squirrel (*Xerus erythropus*), consisting of five males and five females were used for this study. The animals were captured live from the wild in Zaria, Kaduna State, Nigeria using food trap cages which do not cause any injury. The traps were made of galvanised metal and were 1.4 m x 0.3 m x 0.2 m in dimension and were meshed. The African Striped Ground Squirrel were transported by road in ventilated cages to a well-ventilated animal house in the Department of Veterinary PubliHealth, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, where they were acclimatized for one month before the commencement of the experiment. During this period of acclimatization, all animals were physically examined in the cage under careful restraint but not with anaesthesia. Only apparently clinically healthy African Striped Ground Squirrel were utilized for this study. The animals were given access to food and drinking water *ad libitum* throughout the experimental period. The animals were fed with commercial rat pellets and fresh sweet potatoes, carrot and maize. This choice of fresh sweet potatoes, carrot and maize was to mimic the natural tuber choice of this rodent in the wild (Usende *et al.,* 2020). The experimental protocol received approval by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC /2021/070), and in conformity with the ethical standards of the 1964 Declaration of Helsinki; National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Brain extraction

Gross appearance of all African Striped Ground Squirrels used for the present study was described. The Squirrels were then anaesthetized with an intraperitoneal injection of ketamine HCL (50mg/kg body weight), lateral to the midline next to the umbilicus, as a single dose (Molina *et al.,* 2015). The body weight of each squirrel was measured with a digital electronic weighing balance (G & G Brothers Group Inc., USA) with a capacity of 2kg and sensitivity of 0.01g. The animals were placed on a dorsal recumbency and an incision was made on the ventral midline from the base of the neck down to the xyphoid to open the thorax. A cut was made on the right atrial wall with a scissors to allow drainage of blood. A 22 gauge needle was inserted into the left ventricle and physiological saline was used to wash

out the blood cells and immediately followed by 10% buffered formalin fixative solution to fix the brain. The African Striped Ground Squirrel was then decapitated at the atlanto-occipital joint and the head of each animal was weighed immediately and recorded. The brains were then carefully dissected out following the protocol described by Ibe *et al.* (2014). The extracted brain was weighed with the digital electronic weighing balance and recorded. The telencephalon comprising of the olfactory bulb and cerebral hemispheres were carefully detached from the brainstem as described by Fletcher 2006 with some modifications. Briefly, the cerebral hemispheres were lifted at the occipital lobe (caudal end), then a cut was made across the cerebral peduncles using a scalpel and a surgical blade to free the hemispheres.

Preparation of Histological Sections and Staining.

The fixative from the tissues was washed off with distilled water. Dehydration of the tissues was done by immersion in series of ascending concentration of ethanol (70%, 80%, 90% and 100%) with a time interval of 1 hour for each stage of dehydration. The tissues were cleared in xylene, infiltrated with molten paraffin wax, according to the standard procedure (Kiernan, 2007) and labelled. Coronal sections of 10µm (Carson *et al.,* 2005) were made using a microtome (Model 42339, Berlin, Germany) after which the sections were floated on lukewarm water in a floatation bath for stretching. The sections were mounted on glass slides, and then dried, deparaffinized, stained and cover-slipped using dibutyl phthalate and xylene (DPX) as the mountant. The sections were stained using hematoxylin and eosin stain (H & E) as routine stain and cresyl violet stain for Nissl substance of neuronal cell bodies (Drury, 1967). The Cerebral cortex Nissl photomicrograph cyto-architecture were studied in comparison with the brain anatomy atlas of the rat (Paxinos and Watson, 1998) as a guide for location of nuclei. The location, cortical organization, arrangement of cell bodies in the nuclei and their neuronal cell types were described. Images of the nuclei and their constituent neuronal cells were photographed with a digital eyepiece (SCOPETEK® DCM500, Resolution 16M pixels) mounted on a light microscope (OLYMPUS® CH23, Germany) at various resolutions.

Results

Cerebral cortex

Grossly, as reported by Abiyere *et al*. (2022) the cerebrum was cone-shaped and lack prominent neocortical sulci. The lobes of the cerebral hemisphere in the present study were not clearly demarcated, but consist of a relatively large occipital lobe, temporal lobe, parietal lobe and an ill-formed frontal lobe (Abiyere *et al.,* 2022)

The result from this present study indicates that the somatosensory cortex of the African striped ground squirrel was characterized by six distinct layers with no definite frontiers separating one layer from the other (Figure 1a and 1b). However, each layer was identified from the other on the basis of one or more cell types that were predominant. The outer layer was covered by the pia matter.

Layer I: is called the Molecular or Plexiform layer, contains few stellate and pyramidal cells (Fig 2)
Layer II: is called the external granular layer. This layer was densely populated by pyramidal cells and granule cells (Fig 3)
Layer III: is called the external pyramidal layer. This layer has similar pyramidal cell as in layer II, however the cells found here were larger, hence they were called medium-sized pyramidal cell (Fig 4)
Layer IV: is called the internal granular layer having large granule cells and also small pyramidal cells (Fig 5)
Layer V: is called the internal pyramidal cells and consists of very large pyramidal cells. The cells found here were the largest with also few granular cells (Fig 6).
Layer VI: is called the multiform or polymorphic layer having granule cells, pyramidal cells and spindle-shaped cell (fusiform cells) all inter-mingled together (Fig 7).

Hippocampus
From all coronal sections in this study, the following was observed as parts of the hippocampal formation: the dentate gyrus, the hippocampus proper (CA1-CA4), and the subiculum. (Fig 8)

Dentate Gyrus
We observed morphological plasticity in the dentate gyrus, it appeared as C-shaped in some sections (Fig 8) and V-shape in others (Fig 9). Dentate gyrus (DG) was seen surrounding CA4 by its upper & lower limbs. The rodent dentate gyrus had 3 layers: ***polymorphic layer*** was the innermost layer containing polymorphic nerve cells. Cell populations identified included the mossy cells, glial cells and pyramidal cells. ***The granule cell layer*** of the dentate gyrus was the middle layer that contained oval shaped dentate granule cells. ***The molecular layer*** of the dentate gyrus of the African striped ground squirrel with H and E stains revealed scanty cellular populations. (Fig 11, 12 and 13).

Hippocampus Proper
The hippocampus proper of the African striped ground squirrel had four subfields namely: *Cornu Ammonis* 1 (CA1); *Cornu Ammonis* 2 (CA2); *Cornu Ammonis* 3 (CA3) and *Cornu Ammonis* 4 (CA4) (Fig 8). The cell types seen in CA 1-4 were pyramidal neurons (Fig 10).

Subiculum

The subiculum was observed to be a continuation of the CA1 (Fig 11)

Discussion

Cytoarchitecture refers to the typical pattern of cellular arrangement in the cerebrum. The size and packing densities of neurons are not uniform in the cerebral cortex. The underlying tenet of this approach to neuroscience is that structural differences in the cerebral cortex are associated with functionally unique areas. The differentiation of cortical layers is largely based on the distinctive population of projection neurons in each layer. A neuron is considered to be in a layer according to its cell body. A pyramidal neuron with a cell body in layer V may be referred to as a layer V neuron, or a layer may be described as having pyramidal neurons (Peter and Jones, 1994; Conn, 1995).

The cerebral cortex of the adult African striped ground squirrel used in this study differed in neuronal morphology, size and population density. There were six (6) distinguishable layers with no sharp boundaries separating them. The layers were, however, distinguishable on the basis of predominance of cell types. The common cell types observed in this study include: the pyramidal cells and granule cells. These finding were similar to those observed in other mammals in different studies by many authors (Conn, 1995; Victor, 2000).

The molecular or plexiform layer, was the most superficial layer composed of many apical tuft dendrites of pyramidal neurons and afferent axons. Sparse nuclei were observed belonging to neuroglia. It was devoid of neuronal cell bodies; this finding was in agreement with the report of Musa, (2015) who reported that the molecular layer of the cerebral cortex of the African giant rat was composed of dendrites of pyramidal cells. The External granular layer and External pyramidal layer had a granular appearance; it was densely populated by the cell bodies of small, medium and large, pyramidal neurons, and stellate cells. The pyramidal cells are the principal output neurons and as their names suggested they are pyramidal in shape, and a have long apical dendrite leaves the top of each pyramidal cell to ascend vertically to the cortical surface (Conn, 1995). The stellate (granule) cells in this present study are small and showed short axons. They are reported to be the principal interneurons of the neocortex (Conn, 1995; Victor, 2000). The Internal granular layer had a granular appearance, and it was composed of small, round bodies of stellate projection neurons and pyramidal neurons. The Internal pyramidal layer had few very large pyramidal neurons. The cell packing density in this layer is the lowest of all the cortical layers and this corroborate with the finding of Victor

(2000). The Multiform or polymorphic layer had all morphological forms of cells, ranging from stellate (granule) cells, granule cells, and fusiform cells. This layer is called multiform or polymorphic layer because of the varying cell type with their different sizes.

The hippocampal formation is thought to play a role in memory, spatial navigation and control of attention (Andersen *et al.,* 2007). The hippocampal formation is generally accepted to consist of the dentate gyrus, hippocampus proper and subiculum (Amara and Lavenex, 2006). All these structures were identified in the African striped ground squirrel (ASGS) hippocampal formation from this study. The basic cytoarchitecture of the ASGS hippocampus observed in this study, with respect to subfields and cell types, was similar to those reported in the African giant rat (AGR) (Mustapha *et al.,* 2019). Cell types identified in the AGR hippocampus include pyramidal cells, granule cells and mossy cells. Pyramidal cells which are the principal cells of the hippo-campus proper were found in the polymorphic layer of the dentate gyrus. This corroborate with observations in the rat hippocampus (Hussein and George, 2009). The oval-shaped granule dentate cells of the ASGS hippo-campus were found in the distinct granule cell layer of the dentate gyrus where they send their dendrites to the molecular layer of the dentate gyrus (Hargreaves, 2017). Mossy cells were found at the polymorphic layer of the dentate gyrus, where they have been recognized as the second principal gluta-matergic (excitatory) cells of the dentate gyrus after the granule dentate cells. They are thought to have intrinsic and circuitry properties that make them suitable to activate granule dentate cells (Scharfman and Myers, 2013). The hippocampus proper of ASGS was also observed to have four regions (CA1-CA4). These four regions had also been reported in rat by Falougy *et al.* (2008) and in AGR by Mustapha *et al.* (2019). Morphological plasticity was observed in the dentate gyrus of the ASGS showing a C-shaped and V-shaped dentate gyrus. Mustapha *et al.* (2019) also reported similar morphological plasticity (C, V and Wedged shaped) in the dentate gyrus of AGR. The granule cell layer of the dentate gyrus has been well established as one of the sites for adult neurogenesis in the AGR (Olude *et al.*, 2014. This noted neural plasticity may account for the different shapes of the dentate gyrus seen in AGR by Mustapha *et al.* (2019) and in this present study.

Conclusion

In this present study we have been able to provide for the first-time baseline data on the histological features of the cerebral hemi-spheres of the adult African striped ground squirrel in relation to its functions. The cytoarchitecture of the telencephalon revealed six

layers in the cerebral cortex with each layer having different cell types. The following cells were identified in the African striped ground squirrel hippocampus: pyramidal cells, granule cells and mossy cells. The different shapes of dentate gyrus observed in the study are indicative of neural plasticity.

Acknowledgment

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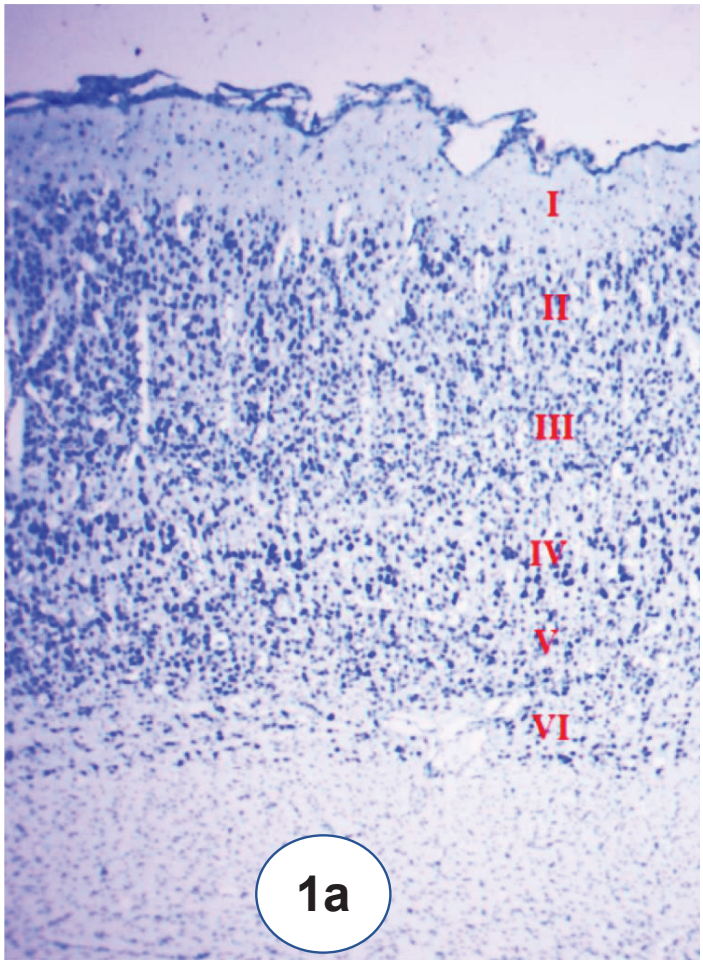

Fig (1a): Coronal section of the somatosensory cortex of adult African striped ground squirrel showing:
I, Molecular layer; II, External granular layer; III, External pyramidal layer; IV, Internal granular layer; V, Internal pyramidal layer and VI, Multiform or polymorphic layer. Cresyl violet stain (x 40).

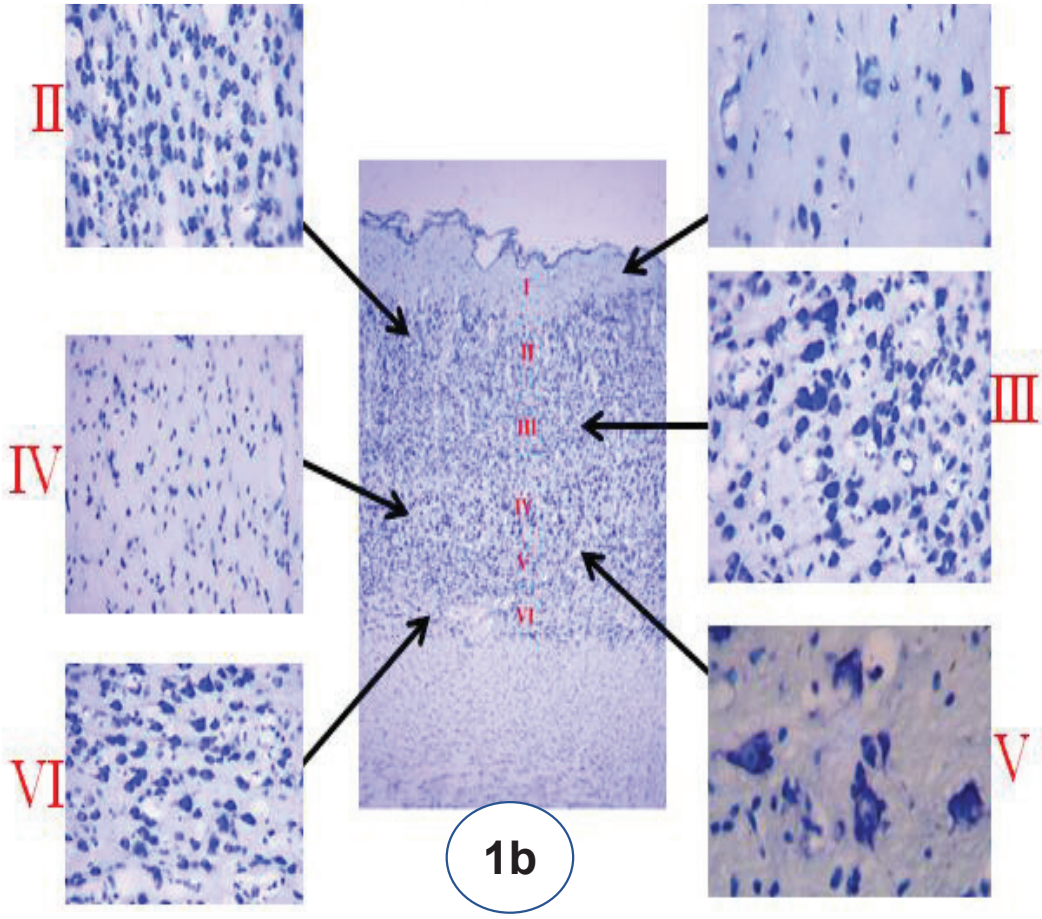

Fig (1b): Coronal section of the somatosensory cortex of adult African striped ground squirrel showing:
I, Molecular layer; II, External granular layer; III, External pyramidal layer; IV, Internal granular layer; V, Internal pyramidal layer and VI, Multiform or polymorphic layer. Cresyl violet stain.

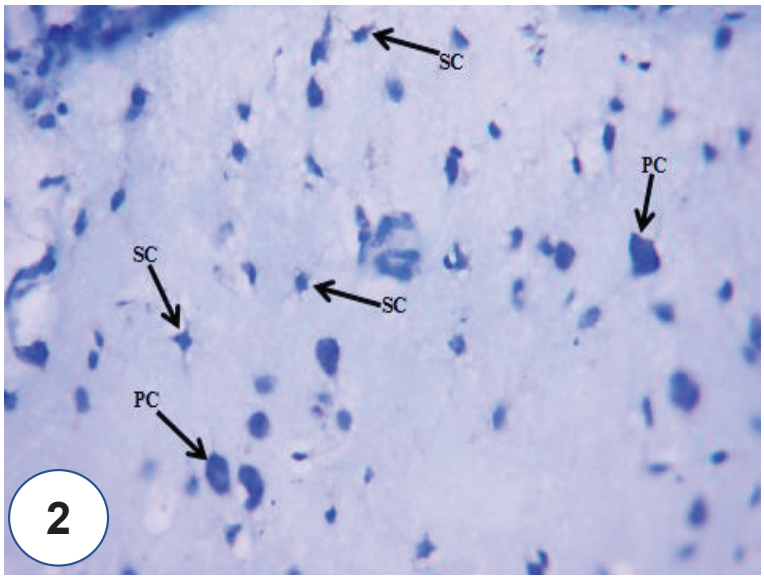

Fig (2): Coronal section of the somatosensory cortex layer I (molecular layer) of the adult African striped ground squirrel showing PC, Pyramidal cells and SC, Stellate cells. Cresyl violet stain (x 400).

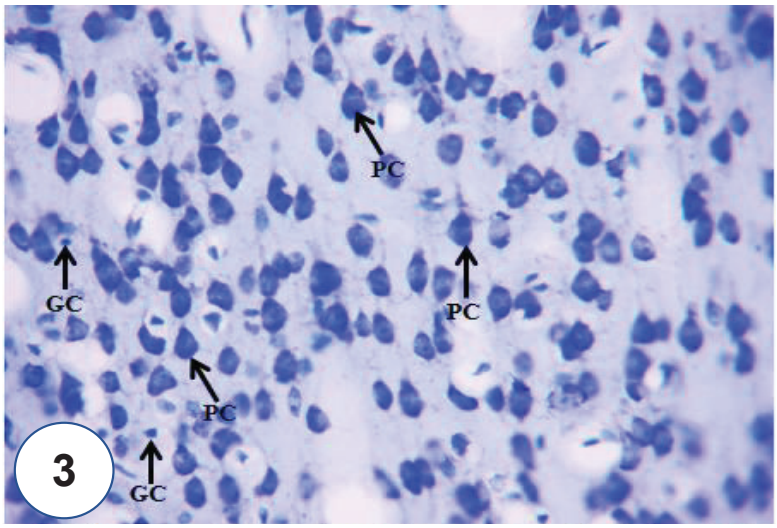

Fig (3): Coronal section of the somatosensory cortex layer II (external granular layer) of the adult African striped ground squirrel showing PC, Pyramidal cells and GC, Granule cells. Cresyl violet stain (x 400).

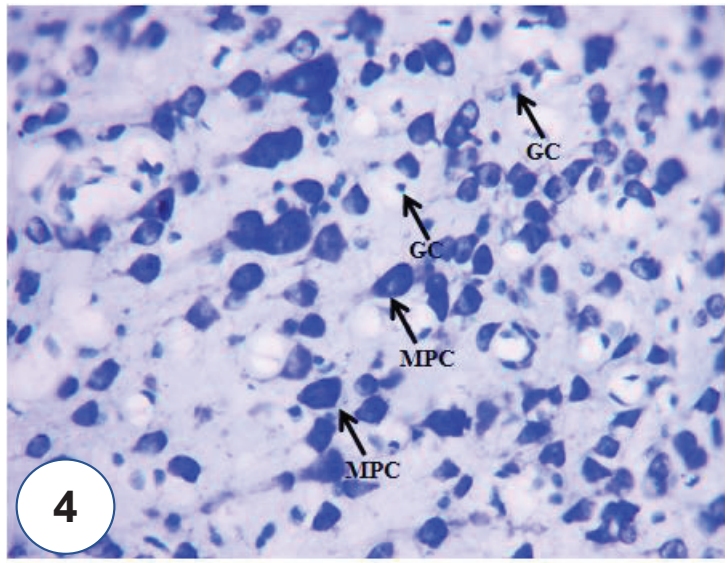

Fig (4): Coronal section of the somatosensory cortex layer III (external pyramidal layer) of the adult African striped ground squirrel showing MPC, Medium pyramidal cells and GC, Granule cells. Cresyl violet stain (x 400).

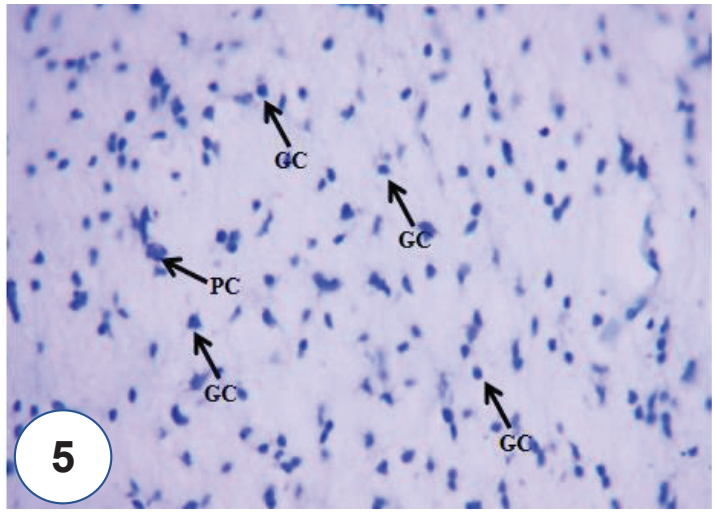

Fig (5): Coronal section of the somatosensory cortex layer IV (internal granular layer) of the adult African striped ground squirrel showing GC, Granule cells and PC, Pyramidal cells. Cresyl violet stain (x 100)

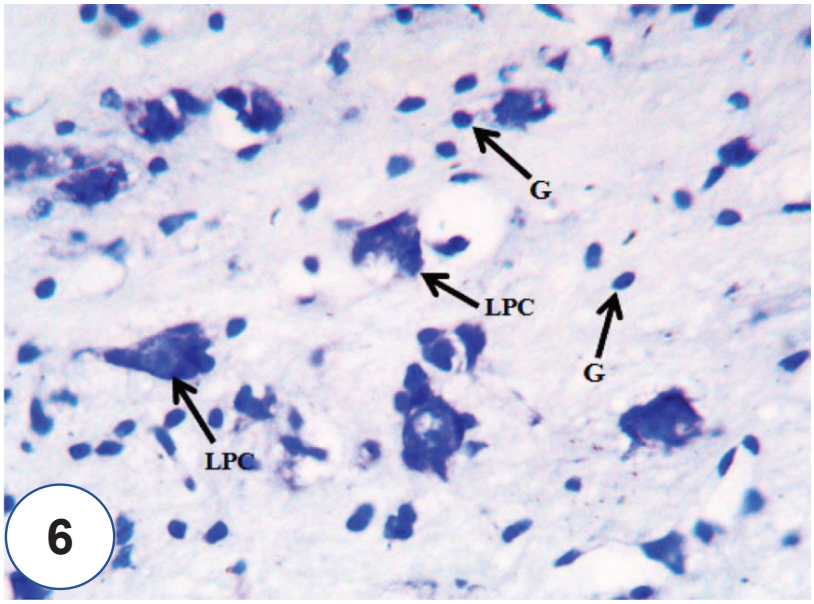

Fig (6): Coronal section of the somatosensory cortex layer V (internal pyramidal layer) of the adult African striped ground squirrel showing GC, Granule cells and LPC, Large pyramidal cells. Cresyl violet stain (x 400).

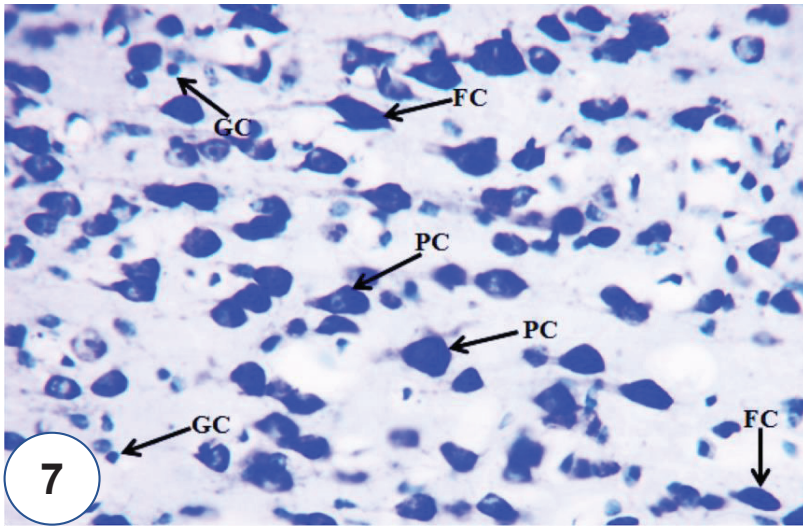

Fig (7): Coronal section of the somatosensory cortex layer VI (polymorphic/multiform layer) of the adult African striped ground squirrel showing: GC, Granule cells; PC, Pyramidal cells and FC, Fusifrom cells. Cresyl violet stain (x 400).

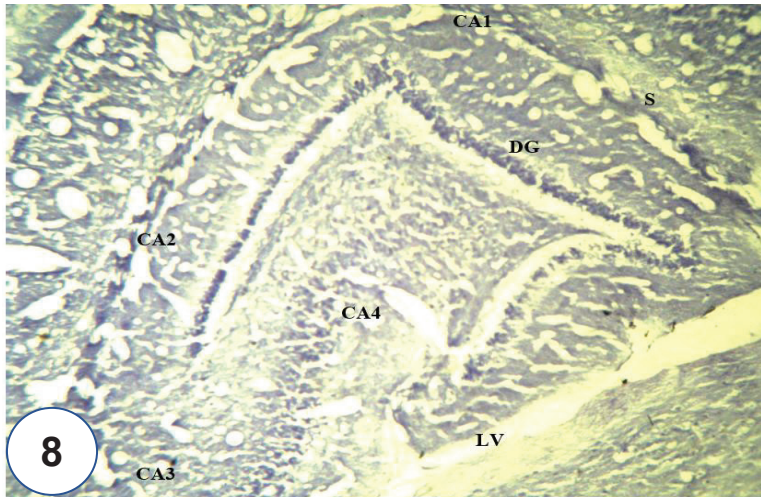

Fig (8): Hippocampus of the adult African striped ground squirrel showing CA1-CA4, Hippocampus proper; DG, Dentate gyrus; S, Subiculum and LV, Lateral ventricle. Cresyl violet stain. (x 40).
***Note the C-Shaped DG**

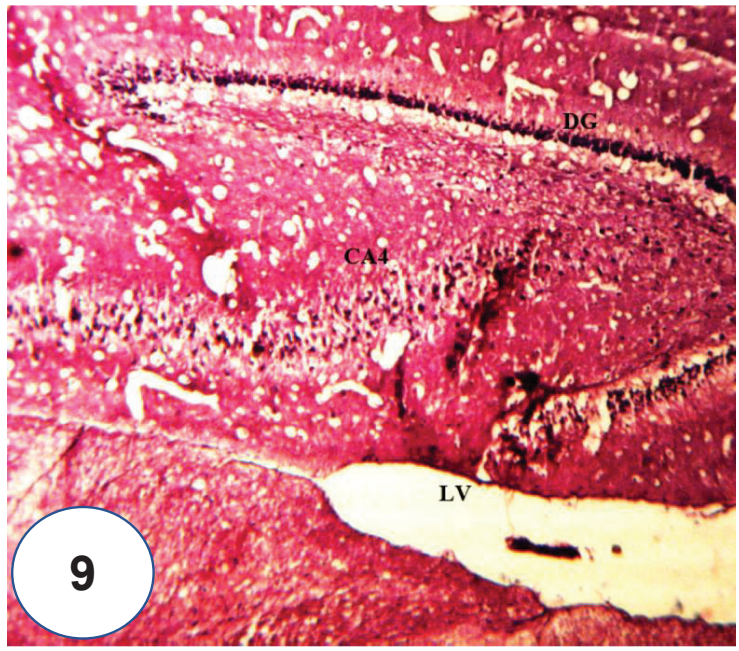

Fig (9): Hippocampus of the adult African striped ground squirrel showing CA4, *Cornu ammonis* 4; DG, Dentate gyrus and LV, Lateral ventricle. Haematoxylin and Eosin Stain. (x 40)
***Note the V-Shaped DG**

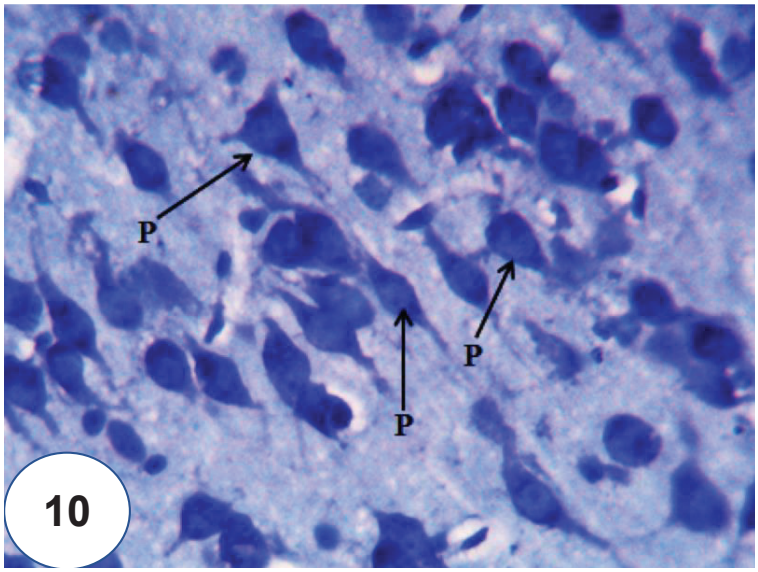

Fig (10): Hippocampus proper (*Cornu ammonis* 1) of adult African striped ground squirrel showing:
P, Pyramidal neuron. Cresyl violet stain. (x 400)

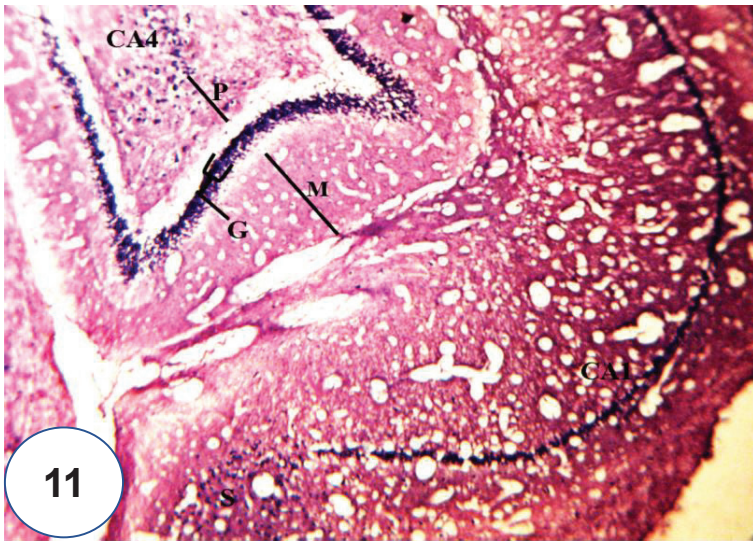

Fig (11): Hippocampus of the adult African striped ground squirrel showing:
M, Molecular layer; G, Granule cells layer; P, Polymorphic layer; CA1, *Cornu ammonis* 1; CA4, *Cornu ammonis* 4 and S, Subiculum. Haematoxylin and Eosin Stain. (x 40)

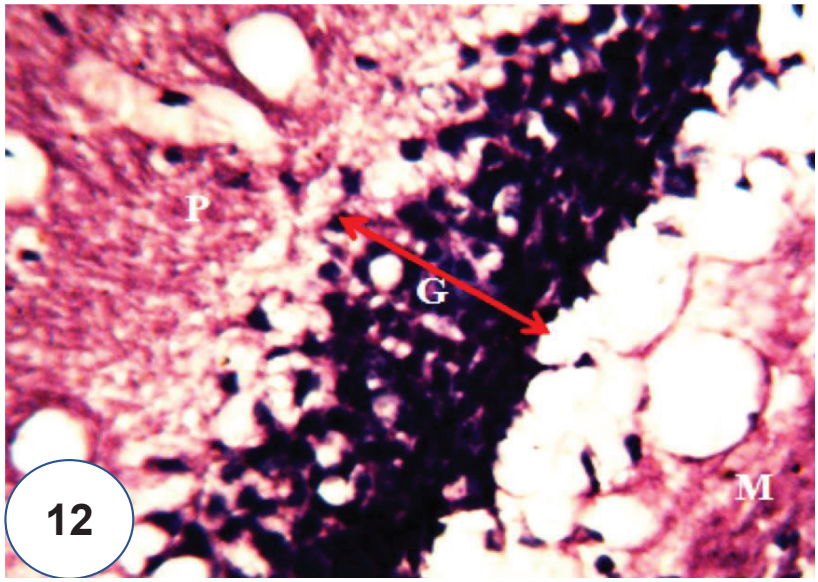

Fig (12): Dentate gyrus of the adult African striped ground squirrel showing:
M, Outer molecular layer; G, Middle granular cell layer with oval shape granule cells and P, Inner polymorphic layer. Haematoxylin and Eosin Stain. (x 250)

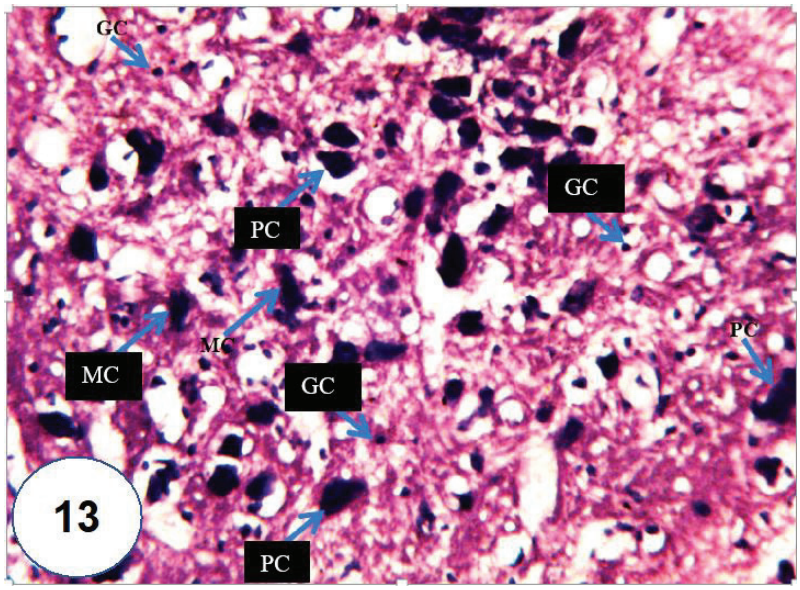

Fig (13): Dentate gyrus (Polymorphic layer) of the adult African striped ground squirrel showing PC, Pyramidal cells; MC, Mossy cells and GC, Glial cells (oligodendrocyte). Haematoxylin and Eosin. (x 400)

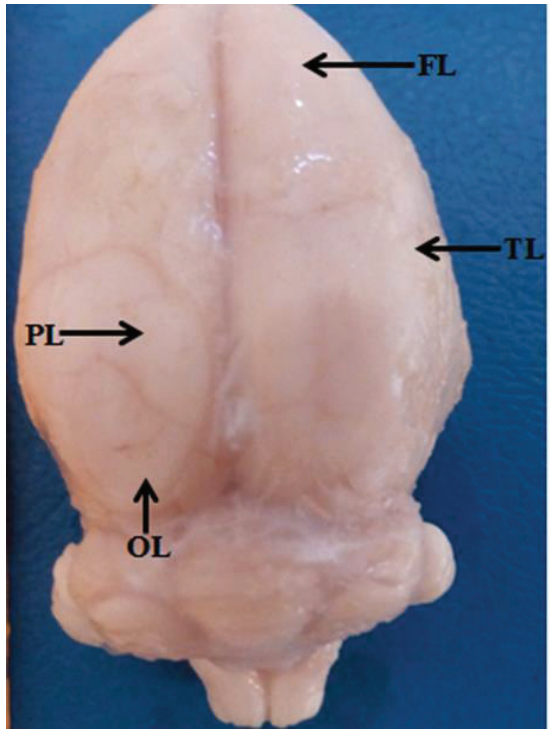

Fig (14): Gross picture of adult African striped ground squirrel cerebrum (Dorsal view) FL, Frontal lobe; TL, Temporal lobe; PL, Parietal lobe and OL, Occipital lobe

Source: Abiyere et.al (2022). J. Vet. Anat. Vol. 15, No. 1, (2022) 17 - 33

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