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Histomorphological Characterization of the Visual Pathway Structures of an African Rodent Species (*Cricetomys gambianus* - Waterhouse 1840; African Giant Rats)

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ABSTRACT

Sensory information for vision is transmitted from the eye (retina) through the optic nerves to other brain regions, in mammalian species including rodents. The *Cricetomys gambianus* (African giant rat, AGRs) share several structural morphological similarities with higher mammals including humans, particularly in their nervous system and sensory processing mechanisms. This study assessed the histomorphological features of visual pathway structures in AGR. Four male AGRs (926 ± 133.0 g) were captured from the wild and used for this study. The AGRs were sedated, perfused transcardially, decapitated, and dissected to expose the intact eyeball and brain for histological processing and assessment. The AGR eyeball and brain sections were stained with Hematoxylin and Eosin (H & E) and assessed at varying magnifying powers using a light microscope. Sections of AGR eyeball demonstrated three distinct layers: the sclera-corneal, uvea, and retina layers. The retina revealed laminarizations with different cellular orientations and densities. Brain sections revealed a tear-shaped, pseudo-laminarized structure, the dorsal lateral geniculate nucleus, with transversing fasciculi through its parenchyma oriented mediolaterally. The midbrain section revealed a rostrally-situated superior colliculus (SC) that relates caudally with the inferior colliculus. The SC parenchyma revealed two distinct layers; narrow superficial (dorsal) and wide deep (ventral) layers. The primary visual cortex revealed a laminar organization having six cellular layers. Histomorphological features of structures of the visual pathway showed similarity with that of other rodent species providing evolutionary advantage for survival in its natural habitat. Hence a potential neuroscience research tool.

KEYWORDS: African giant rats; Brain; Laminarization; Retina; Optic nerve

INTRODUCTION

The mammalian species possess several specialized sensory organs, highly developed and adapted to their specific ecological niches. The sense of vision is critical for a species' behavioral pattern and survival in its natural habitat[1-4]. The structural and physiological features of the visual system differ across different mammalian species. These differences reflect evolutionary trends and adaptation to ecological needs related to their visual environments[5,6]. The visual pathway structures perform the function of receiving external stimuli from the light environment, relaying, and ultimately processing visual information. These structures include the eye (retina), optic nerves, chiasm, tracts, dorsal lateral geniculate nucleus (dLGN) of the thalamus, radiations and primary visual cortex[7,8]. The primary sensory organ, the eye, is responsible for receiving light stimuli, focusing them, and encoding the first neural signals of the visual pathway. The brain, via retinal ganglion cells (RGC) axons in the retina, appropriates these stimuli and conveys them to the optic nerve[9,10]. The optic nerve is an extension of the central nervous system (brain). The optic nerves from the two eyes decussate at the optic chiasma located at the base of the brain[11]. The dLGN is highly organized and represents the main thalamic relay station for visual information in mammals, which transmits and processes sensory information from the retinal ganglion cells to the primary visual cortex[12-14]. Variations in the retinal organization, optic pathway, and visual cortical processing allow for diverse visual capabilities across different mammalian species.

Empirical studies are beneficial in elucidating certain species variations that provide advantages for survival and response to ecological needs. The use of animal models is imperative in such experimental studies. Nocturnal mammalian species like rodents have unique characteristics in their visual system that aid survival in their environment[15-17]. The *Cricetomys gambianus* (African giant rat, AGR) is an under-explored rodent species and has recently been a choice model for several areas of neuroscience research. This could be due to shared anatomical similarities with other mammalian species, including humans, particularly in their nervous system and sensory processing mechanisms[12,18].

The AGRs are nocturnal rodents, mostly because they have little or no tolerance for the intense heat of a typical African day; mostly inactive during the day, but come out at night in search of food[19]. The AGR belongs to the family *Nesomyidae*, order *Rodentia*, and lives in habitats ranging from arid to temperate areas, feeding on vegetables, insects, crabs, snails, and other items [20-22]. The AGRs display a wide range of behavioral patterns in their natural habitat, such as burrowing, foraging, and social interactions[20,23,24]. Elucidating the structural organization of the visual system of this species, AGR, could be beneficial in providing insights into the evolutionary adaptations and the functional capabilities of the visual sense of this species. Thus, this study describes the histomorphological characteristics of the visual pathway structures of the AGR.

MATERIALS AND METHODS

Experimental animals

Four (4) adult male AGRs were captured alive from the wild around Samaru, Zaria, Kaduna State, Nigeria. The AGRs were transported in metal cages to the Neuroanatomy Laboratory, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, and allowed to acclimatize for 3 days before the commencement of the study. The AGRs were given food (water melon, bean cake and ground nuts) and water *ad libitum.*

Animal euthanasia and sample collection

The AGRs were euthanized under chloroform anesthesia, and the body weights were measured using a digital weighing scale (Citizen Scales (1) PVT Ltd., U.S.A., sensitivity: 0.01 g). Thereafter, the AGRs were perfused transcardially; first, with normal saline to do a vascular rinsing, followed by 10% Buffered Formal Saline as described by Gage *et al.* [25] and Ivang *et al*. [26].

The skin and muscles of the AGR scalp were dissected to expose the cranial and facial bones. The cranium was carefully dissected to expose the whole brain, eyeballs and other structures of the visual pathway (optic nerves, chiasma, and tracts). The eyeballs were enucleated by adopting the method described by Mahajan *et al.* [27]. The harvested brain, including structures of the visual pathway, was observed for gross morphologic features and subsequently post-fixed (in the same fixative for 72 hours) for histological assessments (Figure 1).

Histological assessments

The post-fixed brain and structures of the visual pathway specimens were processed for light microscopic examination using histological techniques. The brain specimens of the AGRs were sectioned in coronal and sagittal planes to expose the dorsal lateral geniculate nucleus (dLGN) of the thalamus, superior colliculus (SC) of the midbrain and visual cortex (particularly the primary visual cortex, V1), respectively. Equally, the eyeball (EB), optic nerve (ON) and Optic chiasma (OC) were sectioned in sagittal and coronal planes to aid histological examination.

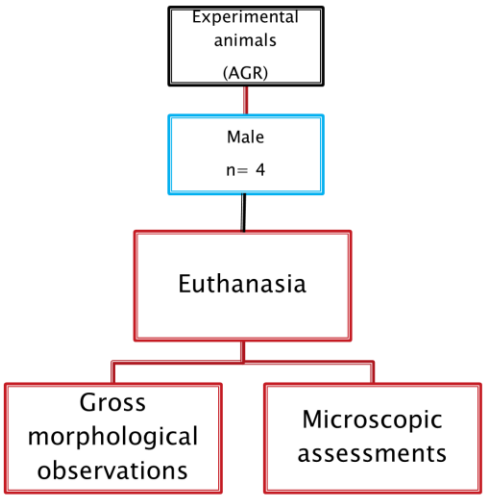

Figure 1: Experimental design

Paraffin histological sections were processed and stained with Hematoxylin and Eosin (H & E) stains to demonstrate general histoarchitectural features of the visual pathway rostro-caudally (antero-posteriorly): EB, ON, OC, dLGN, SC and V1.

The histological tissue processing was carried out in the Histology Unit of the Department of Human Anatomy, ABU, Zaria. Processed tissue slides were examined using a dissecting microscope (AmScope- Stereomicroscope, China) and a bright field microscope (HX-LUX Leitz Wetzlar, Germany) at different microscopic magnifications. The dissecting microscope at ×15 magnification was used to reveal the entire structure of the visual pathway on the coronal section (Figure 2).

Data analysis

Data on the measured body weight were expressed as mean ± SEM using a statistical software -Statistical Package for Social Sciences (SPSS) *version* 23.0.

RESULTS

Gross Morphological Observation

The average absolute body weight of AGR males was 787 ± 208.6 g. The brain dorsal surface presented with distinct parts, including the cerebrum, the largest part of the brain, which lies immediately caudal to the olfactory bulb, rostral to the cerebellum and dorsal to the brain stem. On the ventral surface, the olfactory bulbs presented as rostral outgrowths of the brain, with the eyeballs (spherical and pigmented) laterally placed. Caudally, the paired optic nerves emerged from the eyeball, decussated at the optic chiasma and gave off optic tracts. Caudal to the optic tracts is the hindbrain comprising the medulla oblongata, pons and cerebellum. The pons and medulla formed portions of the brain stem (Figure 3).

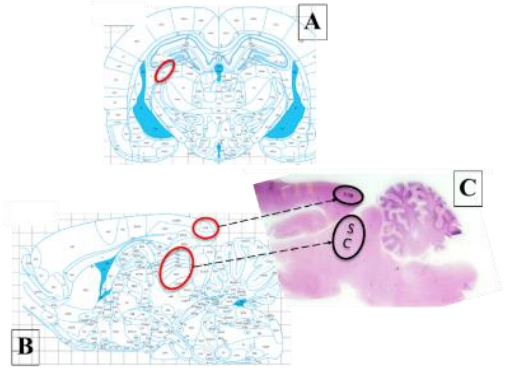

Figure 2: Identification of brain regions: (A) Coronal section (red circle), revealing the dorsal lateral geniculate body of the thalamus. (B) Sagittal section (red circles), indicating the primary visual cortex (V1) and superior colliculus of the cerebral cortex and midbrain, respectively. Adopted from George Paxinos and Charles Watson Rat Atlas, 6th edition, 2007.

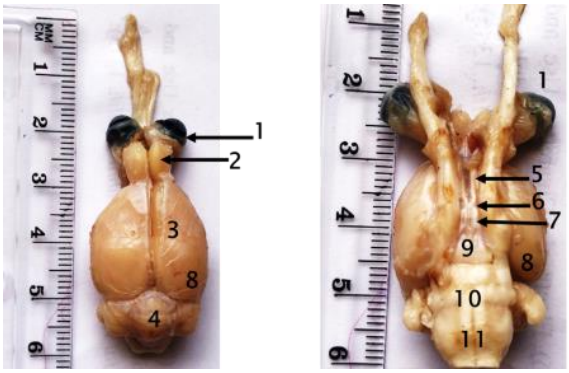

Figure 3: Gross morphology of the African Giant rat brain; A = Dorsal, B = Ventral views.

1= Eyeball, 2= Olfactory bulb, 3= Cerebrum, 4= Cerebellum, 5= Optic Nerve, 6= Optic Chiasma, 7= Optic Tract, 8= Visual Cortex, 9= Midbrain, 10= Pons, 11= Medulla

Histological observation

A sagittal section through the intact AGR brain, with the eyeball in place at lower magnifying power, demonstrated the histoarchitectural features of the eyeball as follows: The eyeball presented three distinct layers: the sclera-corneal layer (outer layer), choroid or uvea (intermediate layer) and retina (inner layer). Rostro-caudally, the cornea surface was curved convexly and makes up about two-thirds of the entire circumference of the eyeball, which is continuous with the sclera at the limbus (sclera-corneal junction). The lens is a large and spherical structure that occupies most of the eye. The lens is moderately transparent with a distinct lens capsule on the periphery, demonstrating varying thickness; this lens capsule was observed to be thicker posteriorly. The iris appeared as a pigmented, thin, cylindrically shaped structure with a central opening – the pupil, located anterior to the lens, that divides the eyeball into anterior and posterior chambers.

The ciliary body demonstrated a triangular-like thickening located between the anterior aspect of the choroid and the posterior aspect of the iris, having a base apposed to the iris and the apex juxtaposed to the choroid. The ciliary process is a

cylindrical pigmented tree-like structure which is continuous with the choroid posteriorly. Posterior to the limbus is the choroid and retina. Lying between the posterior aspect of the lens and the choroid, and a distinct, densely pigmented structure, is the retina. Emerging from the posterior aspect of the sclera is the distinct optic nerve head (Figure 4).

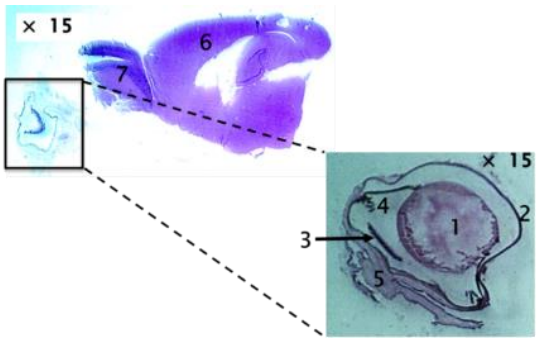

Figure 4: Sagittal section of the brain and eyeball of African Giant rat (H & E)

1= Lens, 2= Cornea, 3= Retina, 4= Vitreous Body, 5 Optic Nerve, 6= Cerebrum,
7= Olfactory Bulb

At higher magnifying power, the histological features of the AGR retina revealed laminar organization (laminarization) with different cellular orientations and densities. Ten (1-10) distinct retinal laminae (layers) were observed from anterior (vitreous body) to posterior (choroid). These layers are:

Layer 1 (Inner Limiting Membrane, ILM), which forms the basement membrane for Layer 2 (Nerve Fiber Layer, NFL), where the neuronal processes (axons) of ganglionic cells congregate. Layer 3 (Ganglion Cell Layer, GCL): contains predominantly the nuclei of large oval-shaped (ganglion) cells, axons of the optic nerve fibers and some cell types. Layer 4 (Inner Plexiform Layer, IPL) appeared with a reticular architecture containing neuronal synapses of certain cells. Layer 5 (Inner Nuclear Layer, INL) is pigmented and contains densely packed cell bodies. Layer 6 (Outer Plexiform Layer, OPL) is lightly pigmented, having a similar architecture to that of layer 4, but with less thickness. Layer 7 (Outer Nuclear Layer, ONL) has a similar cellular density to layer 5, with more than twice the thickness. Layer 8 (Outer Limiting Membrane, OLM); this layer separates the inner segment portions of the photoreceptors from their cell nucleus. Layer 9 (Photoreceptor Layer, PL) is deeply stained and contains specialised cells (photoreceptor cells - rods and cones) with ciliary-like projections oriented towards the choroid. Layer 10 (Retinal Pigment Epithelium, RPE) - single layer of cuboidal cells (Figure 5).

Coronal sections of the optic nerve and chiasma at lower magnifying power revealed the meninges that ensheath the nerve. A thick-walled spherical structure (central retinal artery) was observed lateral to the meninges. At higher magnification, the cytoarchitectural features of the optic nerve revealed distinct cell morphologies, including stellate and horizontal cells.

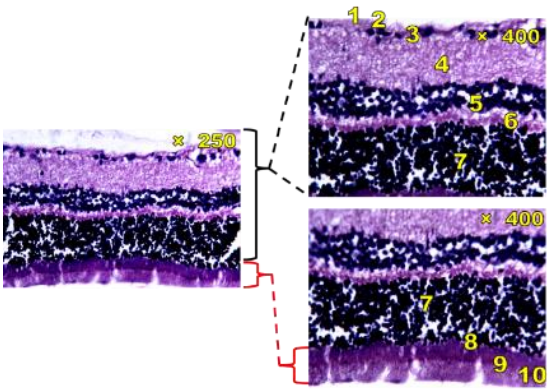

Figure 5: Sagittal section of the retina of African Giant rat

1: Inner limiting membrane, 2: Nerve Fiber Layer, 3: Ganglion cell layer, 4: Inner plexiform layer, 5: Inner nuclear layer, 6: Outer plexiform layer, 7: Outer nuclear layer, 8: Outer limiting layer, 9: Photoreceptor layer, 10: Retinal Pigmented epithelium. (H & E)

Glial cells were observed to be related to the optic nerve fascicle within the parenchyma and periphery of the fascicle (Figure 6). There is a confluence (decussation) of prominent fusiform cells at the optic chiasma. Other cell types were observed with unique patterns lateral to the decussation of the nerve fibers (Figure 7).

Coronal sections through the brain of AGR at lower magnifying power showed the dLGN, which is bordered superiorly by the CA3 area of the hippocampus. The dLGN appeared as a tear-shaped structure with no observable cytoarchitectonic lamination. Although at a higher magnification, nerve fibers were observed transversing through the dLGN parenchyma in a medio-lateral (right to left) orientation, presenting a pseudo-laminarization. The dLGN demonstrated typical features of nervous tissues, including neuronal and glial cells with related vasculatures (blood capillaries). Additionally, pockets of clustering neuronal cells were observed (Figure 8).

A sagittal section through the midbrain of AGR revealed the superior and inferior colliculi. A rostrally located superior colliculus (SC) is separated from a caudally situated inferior colliculus (IC) by a depression (intercollicular sulcus). The SC appeared to be lower than the IC and bordered superiorly (dorsally) by the caudal aspect of the cerebral hemisphere, where which lies the visual cortex (V1M) lies (Figure 9).

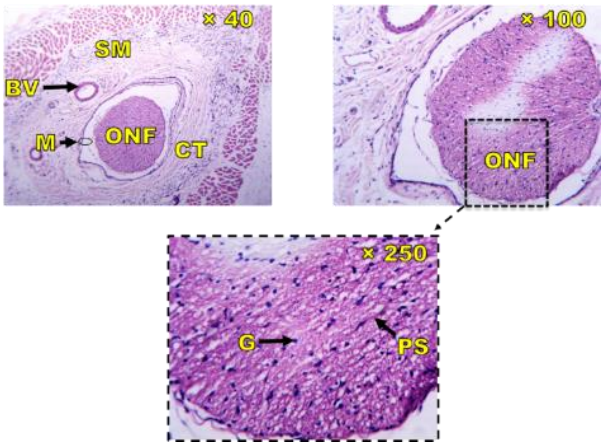

Figure 6: Coronal section of the optic nerve of African Giant rat

BV = Blood Vessels, CT = Connective Tissue, M = Meninges, ON = Optic Nerve; ONF = Optic Nerve Fascicle, G = Glial cell, S = Stellate cells, PS = Pial Septum; SM = Skeletal Muscles. (H & E)

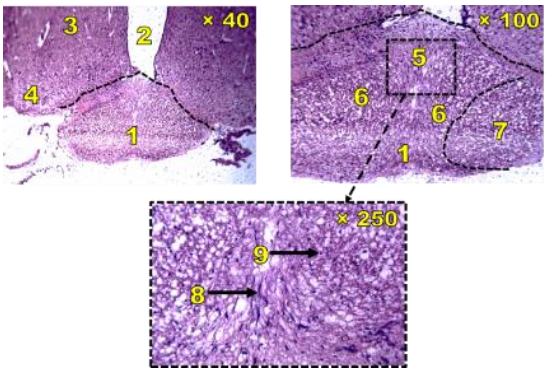

Figure 7: Coronal section of the Optic Chiasma of African Giant rat

1 = Optic Chiasma; 2 = 3rd Ventricle; 3 = Medial preoptic area Decussation; 4 = Ventrolateral preoptic nuclei; 5 = Decussation; 6 = Nasal retinal nerves; 7 = Temporal retinal nerves; 8 = Fusiform cells; 9 = Oligodendrocytes. (H & E)

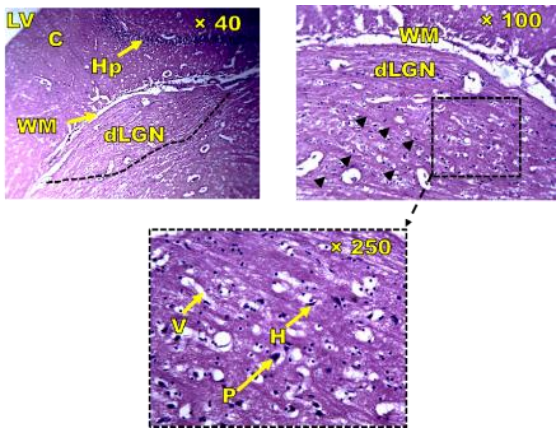

Figure 8: Coronal section of the Dorsal Lateral Geniculate Nucelus of African Giant rat

C = Cerebrum; HP = Hippocampus; LV = Lateral ventricle; WM = White matter; V= Blood vessels; H = Horizontal cells; P = Pyramidal cells; Arrow heads = Nerve fibers (H & E)

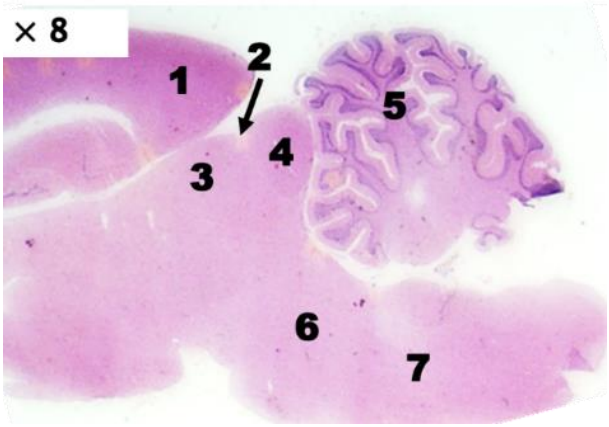

Figure 9: Sagittal section of the brain of African Giant rat

1= Cerebral cortex; 2 = Intercollicular sulcus; 3 = Superior colliculus; 4 = Inferior colliculus; 5 = Cerebellum; 6 = Pons; 7 = Medulla Oblongata (H & E)

The SC parenchyma revealed two majorly distinct layers, presenting the SC with an outer (dorsal or superficial), narrow layer and an inner (ventral or deep), wide layer orientation. The cell distribution at the outer layer could be likened to the 'outer granular layer' of the cerebral cortex, manifesting with densely packed cells of different types. The inner layer demonstrated with variety of cells with different shapes and sizes, which included pyramidal, stellate and horizontal cells. The superficial layer consists of two layers (Laminae 1 and 2), while the deep layer consists of 5 layers (Laminae 3-7). These layers, from dorsal (superficial) to ventral (deep), are:

Lamina I (*stratum zonale* or Zonal layer) is a thin layer consisting of small myelinated axons together with marginal and horizontal cells, and forms the basement for Lamina II (*stratum griseum superficiale* or Superficial grey layer), which contains numerous neurons of varying shapes and sizes. The Lamina III (*stratum opticum or* Optic layer) consists mainly of axons coming from the optic tract. Lamina IV (*stratum griseum intermedium* or Intermediate grey layer) is the

thickest layer and is filled with neurons of various sizes. Lamina V (*stratum album intermedium* or Intermediate white layer) consists mainly of fibers from various sources. Lamina VI (*stratum griseum profundum* or Deep grey layer), which consists of loosely packed neurons and myelinated fibers and the Lamina VII (*stratum album profundum* or Deep white layer), consisting entirely of fibers (Figures 10 and 11).

The AGR V1 cytoarchitecture revealed a variety of cells ranging from neurons to neuroglia, organized into cellular layers. Dorso-ventrally, the V1 presented with six layers (1 - 6): Layer 1(the molecular layer) consists of a few cells. A dense population of stellate and other cells make up the Layer 2 (the outer granular layer); Layer 3 (outer pyramidal layer) consists of pyramidal cells; prominent stellate cells were observed in the fourth layer (Layer 4, inner granular layer).

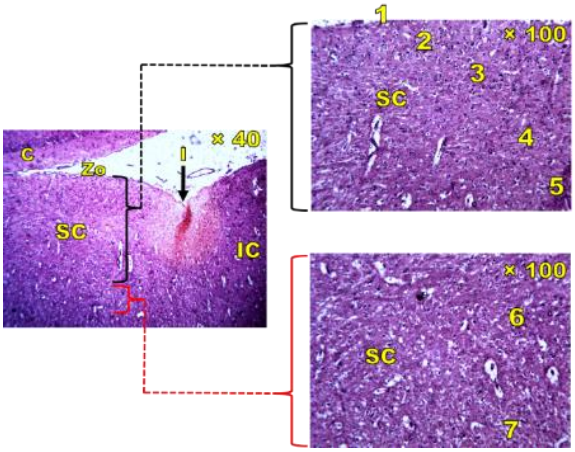

Figure 10: Sagittal section of the Superior colliculus (SC) of African Giant rat

C = Cerebral cortex; IC = Inferior colliculus; I = Intercollicular sulcus; Zo = Zonal layer; 1 = Zonal Layer, 2 = Superficial Grey layer; 3 = Optic Nerve layer; 4 = Intermediate Grey layer; 5 = Intermediate White layer; 6 =Deep Grey layer; 7 = Deep White layer. (H & E)

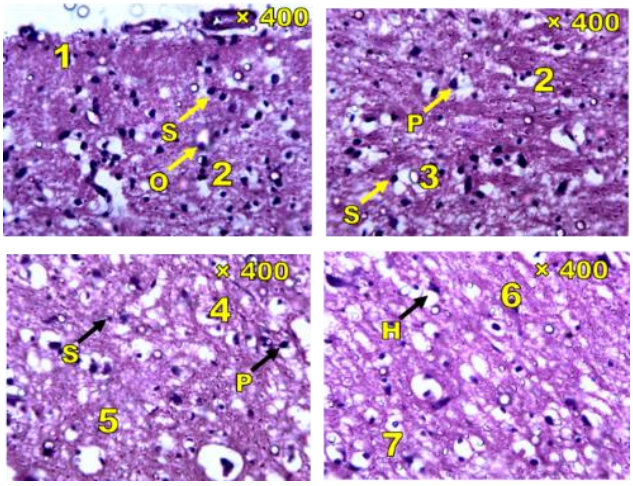

Figure 11: Sagittal section of the Superior colliculus (SC) of African Giant rat; P = Pyramidal cells; S = Stellate cells; H = Horizontal cells; O = Oligodendrocytes. (H & E)

The stellate cells in this layer appeared to be denser compared to those observed in layer 2. The inner pyramidal layer (Layer 5) consists predominantly of pyramidal cells, while Layer 6 (the polymorphic layer) consists of numerous cell types, including pyramidal and stellate cells, amongst other cells. The dorsal three layers (layers 1-3) revealed distinct columnar organization and varying cell types compared to the ventral three layers (layers 4-6) (Figures 12 and 13).

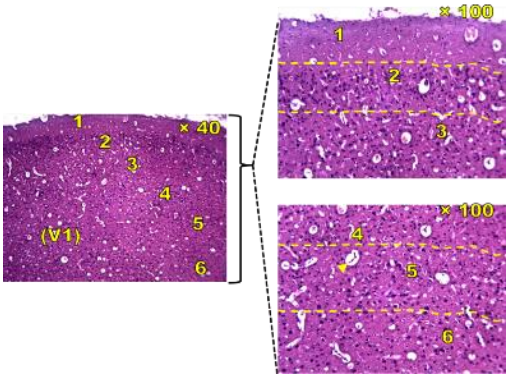

Figure 12: Sagittal section of the Primary visual cortex (V1) of African Giant rat

A=Astrocytes; O= Oligodendrocytes; V= Blood vessels;1= Molecular layer; 2= Outer granular layer; 3= Outer pyramidal layer; 4= Inner granular layer; 5= Inner pyramidal layer; 6= Polymorphic layer. (H & E)

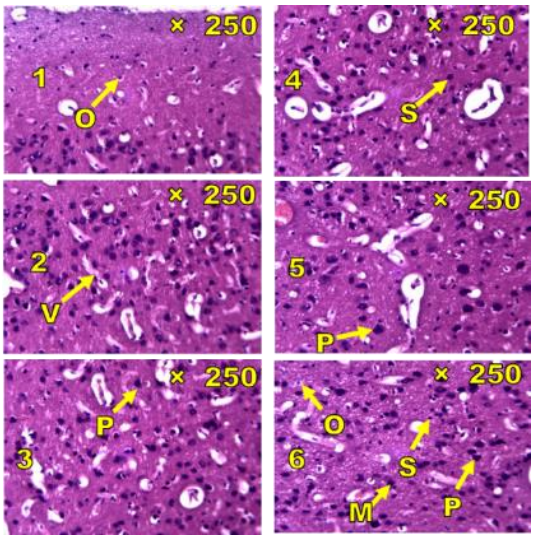

Figure 13: Sagittal section of the Primary visual cortex (V1) of African Giant rat

M=Multiform cells; P= Pyramidal cells; S=Stellate cells; O=Oligodendrocytes; V=Blood Vessels (H & E)

DISCUSSION

In this study, the visual pathway structures of AGR were described using a histomorphological approach. The mean absolute body weight of AGRs measured to be greater than 500 g for adults agrees with reported range values for adult AGR[24]. Studies have reported mean absolute body weight values for smaller rodents such as rats and mice to be lower than values for larger rodents, including *Cricetomys gambianus*, *Thryonomys swinderianus* (grasscutter), > 2 kg[26] and porcupine, > 7 kg[28]. The observed gross morphological features of the AGR brain on the dorsal and ventral surfaces, including components of the visual pathway, are generally in line with the morphological characteristics reported for rodents and other mammalian species [29-34].

Histologically, sections of AGR brain and eyeball were examined to demonstrate the histoarchitectural features of visual pathway structures (EB, optic nerve, chiasma, tracts, dLGN, SC and V1). The AGR eyeball demonstrating three distinct layers: sclera-corneal, vascular and retinal layers is in line with reported morphology for rodents and other mammalian species[35-38]. The shape of the cornea reflects visual adaptation in relation to an animal's particular lifestyle[39].

The convexity of the cornea observed in this species, AGR, agrees with reported morphology for nocturnal mammals[40]. However, this finding is in contrast to the shape reported for diurnal animals; a dome-shaped cornea[41]. The lens morphology observed in AGR is suggestive of an evolutionary adaptation that allows this species to effectively gather and focus light in low-light environments, enhancing visual abilities during nocturnal or crepuscular activity. This is in line with characteristics lens morphology reported for rodents[42-44,37].

The pigmented and cylindrically-shaped AGR iris is in line with documented reports for other rodent species[45]. Junqueira *et al*. [46] and Cvekl and Ashery-Padan [37] postulated that heavy iridial pigmentation in the stroma, anterior and posterior epithelia, prevents passage of the light into the interior of the eye except through the pupil.

The pigmented and convoluted (tree-like) ciliary processes of the AGR eyeball are similar to those described by Patra [47] in small laboratory mammals (hamsters, guinea-pigs and mice). These microanatomical features are present to enlarge the total epithelial surface area for adequate production of aqueous humor. It is commonly accepted that the ciliary epithelium is engaged in the production of the aqueous humor[48-50].

A well-organised (laminarization) histoarchitectural feature of the AGR retina at a closer observation demonstrating distinct cellular layers is in line with reports on rodent species, including mice and rats, exhibiting laminarization

with discrete nuclear and plexiform laminae[36,37]. Specialized retinal cells, especially the ganglionic cells in the ganglionic cell layer and cells (rods and cones) of the photoreceptor layer, are critical in conveying photo-stimuli from the environment to specific brain parts involved in the visual pathway[2,51,52].

The dLGN in rodents is a crucial component of the visual pathway, playing a vital role in the thalamic processing and relay of visual information from the retina to the visual cortex. The unique tear-shaped morphology observed in AGR dLGN with a clustered arrangement of neurons suggests adaptation that allows for the efficient transmission and integration of visual information. This finding agrees with reported dLGN morphology for rodents, including mice and rats[53-55]. Pseudo-laminarization of the dLGN observed in AGR is in line with the reported organization for rodent species (e.g., mouse and rat)[53,56]. This architecture is in contrast with laminar organization frequently reported for higher mammalian species, including cat and monkey[57,58].

The AGR SC appeared as a laminated structure located in the dorsal midbrain from a sagittal section. This aligns with reports by Stein and Meredith [59] who described a laminar organization of SC in rodents, cats and other mammals. Ito *et al.* [60] reported that the superficial layers of the SC are primarily involved in visual processing, while the deeper layers are associated with multimodal sensory integration and motor control [59,61,60].

In mammalian species, the V1 serves as the main recipient of thalamic inputs and presents with an organized laminar morphology. In this study, the AGR V1 exhibited a six-layer organization with prominent cell types in the granular layer (layer IV). This finding is in line with reported V1 laminarization for rodents and higher mammals[62]. However, this organization is less distinct from the laminar architecture described for carnivores and primates[63]. The characteristic organization of the AGR V1 reflect the evolutionary adaptive abilities for survival in its natural habitat as a nocturnal mammal.

CONCLUSION

The structures of the visual pathway of the African giant rat presented with histomorphological characteristics similar to those of other rodent species, providing an evolutionary advantage for survival in its natural habitat. Thus, findings further validate the claim of the rodent species as a potential tool in neurosciences and related research fields.

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