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ULTRASTRUCTURAL MORPHOLOGY OF THE EPENDYMA AND CHOROID PLEXUS IN THE AFRICAN GIANT RAT (*CRICETOMYS GAMBIANUS*)

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ABSTRACT

Ependymal cells line the interface between the ventricular surfaces and the brain parenchyma. These cells, in addition to the choroid plexus, form the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) and serve important functions in the protection and regulation of brain metabolism. The African giant rat (AGR) has been used as sentinels to detect potential neuropathology arising from ecotoxicological pollutions. This study examined the lateral ventricular lining by using histology, immunohistochemistry and electron microscopy. Marked variations were observed in some regions of the ventricles which showed multi-layering of ependymal cells that differed from the typical single layered ependymal cells at the apical surface, while subependymal structures revealed indistinctive neuropil and glia following histological examinations. The ependymal cells which form the epithelial lining of the ventricles were comprised of cuboidal or low columnar cells, with the plasmalemma of abutting cells forming intercellular bridge appearing links by: tight junctions (*zonula occludens*), intermediate junctions (*zonula adherens*), desmosomes (*macula adherens*) and infrequent gap junctions. The choroid plexus revealed cells of Kolmer with several cilia and microvilli. The possible functional components of the ependyma and choroid plexus morphology of the AGR are discussed and thus provide a baseline for further research on the AGR brain.

Key words: African Giant Rat; choroid plexus; ependymal; tight junctions; ultrastructure

INTRODUCTION

The African giant rat (*Cricetomys gambianus*) is a nocturnal, sub-Saharan African rodent which shares close dwellings with human societies. For this reason, they are currently being utilized as sentinel models to study ecotoxicological pollutions and disease burdens [37]. This continued use as a research model for biomedical research has also spurred studies into their morphophysiology and especially that of the nervous system [15, 24, 25, 27]. The information generated has been progressively helpful in proposing better concepts in the adoption of the AGR as a convenient laboratory animal for investigating diseases of great significance to humans and their domestic animals [39].

One of the more recent uses of the AGR has been the use of their olfactory prowess to sniff out landmines and diagnose tuberculosis infected sputum samples [30, 39]. This attribute therefore requires the proper understanding of the neural systems in the AGR. The gross and cellular central nervous system (CNS) profile of the African giant rat have been elucidated with various studies covering: adult neurogenesis, astrocyte heterogeneity and oligodendrocyte morphology [25—29]. The ependyma and choroid plexus are yet to be described for this rodent.

Morphologically, the ependyma is typically known as a single-layered, cuboidal to columnar-celled, ciliated epithelium on the surface of the ventricles of the brain and central canal of the spinal cord [10] and is responsible for important functions related to: morphogenesis, brain physiology, neuro-protection and water transport [9, 16]. The typical rodent ependyma morphology has been shown to consist of multi-layering populations, sub-ependymal cells and the existence of abundant gap junctions [12, 21]. The choroid plexus meanwhile, is an epithelial–endothelial vascular convolute within the ventricular system of the vertebrate brain which consists of fenestrated blood vessels and synthesizes the cerebrospinal fluid [7, 40]. Adjoining choroid plexus cells have tight junctions that form the blood- cerebrospinal fluid barrier (BCSFB). This barrier along with the blood-brain barrier (BBB), play a major role in the homeostatic regulation of the brain by protecting the CNS from the inflammatory molecules, pathogens, and toxins which may be circulating in the blood stream [8, 31].

This paper therefore seeks to add to the body of knowledge on the neuro-cellular profile of the African giant rat by describing the morphology of ependymocytes and choroid plexus using the electron microscopy and allude to the functional basis of maintaining the integrity of the BBB and BCSFB.

MATERIALS AND METHODS

A total of 6 male African giant rats (*Cricetomys gambianus*) randomly assigned into two groups (A and B; n = 3/group) were used for this study. All rats were anaesthetized with intraperitoneal injection of Ketamine/Xylazine (90/10 $mg.kg^{-1}$) and were subsequently perfused transcardially with 4 % paraformaldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4. (for animals in group A) and freshly prepared Karnovsky fixative (for animals in group B). The brains harvested from group A were processed for histology (with H&E and Cresyl violet stain for anatomical orientation) while immunohistochemistry (IHC) with anti-glial fibrillary acidic protein (anti-GFAP) was performed using the free-floating method for tanycytic astrocytes of the subependyma in accordance with the methods described by Olude et al. [27] and Mustapha et al. [24]. The brain samples from group B were prepared for both scanning and transmission electron microscopy as described by Olude et al. [25]. The images were captured using Zeiss Axioskop (Carl Zeiss Microscopy GmbH, Jena, Germany) and Axiovision software (Carl Zeiss Microscopy GmbH, Jena, Germany).

RESULTS

The ependymal cells with variable numbers of cilia, formed the epithelial lining of the lateral ventricles comprised of cuboidal or low columnar cells in shape. Regional variations were observed in some regions of the ventricles with multi-layering of ependymal cells which differed from the typical single layered ependymal cells at the apical surface. The sub-ependymal structures reveal indistinctive neuropil and glia following histological examination (Fig. 1a, b). Anti-GFAP immunohistochemistry revealed a clear zone of ependymal cells and brush borders (arrowhead in Fig. 1c) and a sub-ependymal zone with sequential extensions of processes (black arrows in Fig. 1d) into the subependymal hypocellular level (Fig. 1c, d).

The examination of the ultrastructure revealed that the

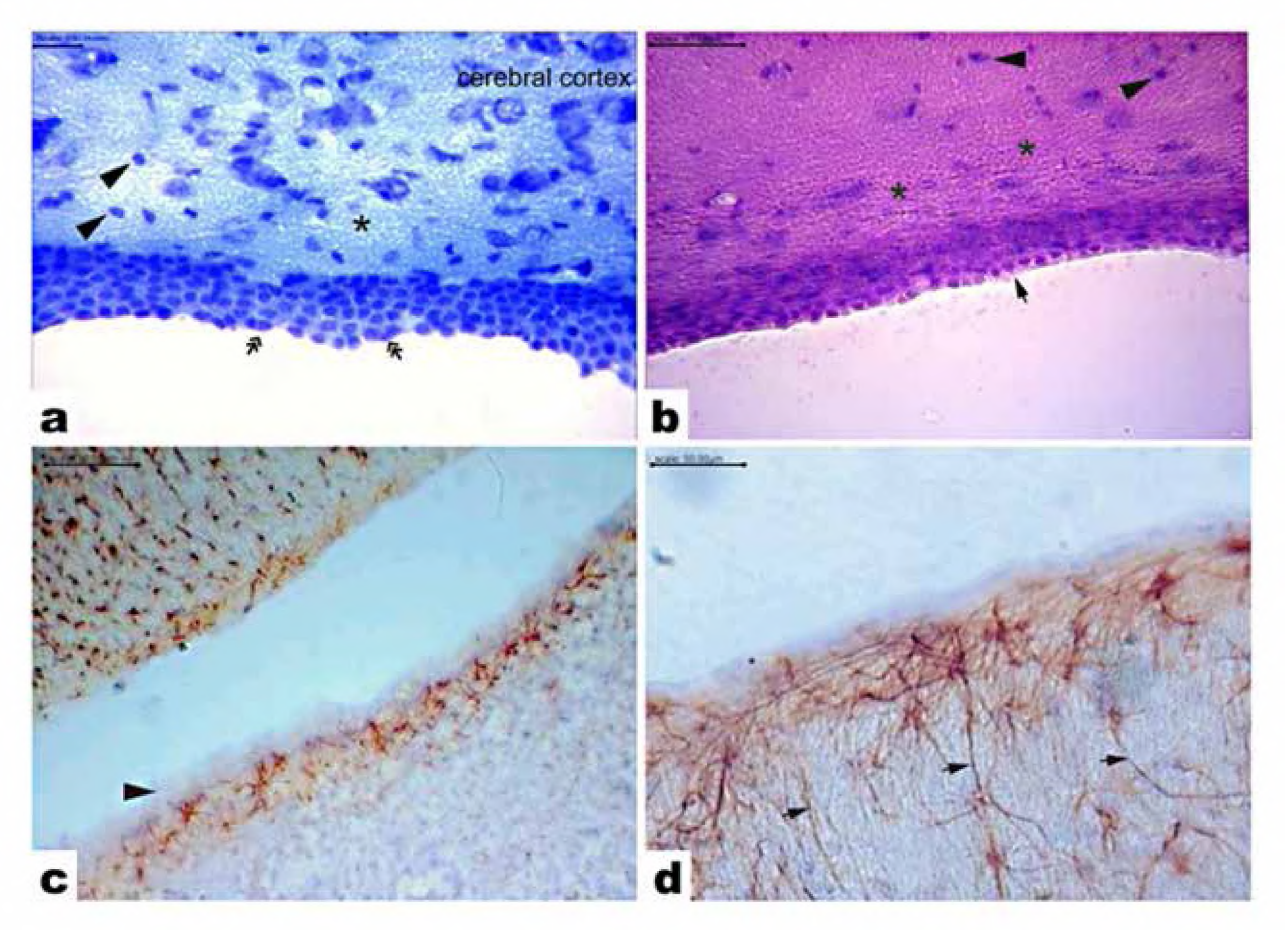

Fig. 1. Histology of the ependymal and sub-ependymal structures: [a] Nissl stain and [b] H&E stain

Note the multi-layered ependymocytes (double arrowheads, **a)** compared to the single-layered ependymocytes (black arrow; **b)** and also the indistinct neuropil (asterisks) and glia (arrowheads, a & b) in the subependymal layer; **c)** Anti-GFAP showing distinguishable ependymal cell and brush borders (arrowhead). **d)** Anti-GFAP showing zone of extensive processes projecting to the sub-ependymal hypocellular layer (arrows). Scale bars: 100 µm (a); 50 µm (b—d)

epithelial surface presented broad ridges with finger-like projections of ciliary shafts and numerous microvilli. The nucleus was simple, regularly oval and occupied large portions of the cell close to the wall while the nuclear envelope had patches of ribosomes on the outer cytoplasmic surface (Fig. 2a). Several mitochondria were seen in the supranuclear region and fewer in the basal aspects. They were typically elongated with internal transverse cristae structure (Fig. 2a, 2b). The endoplasmic reticulum was widely spread, presenting smooth surfaced vesicles with short canaliculi (Fig. 2b), while the Golgi complexes which were also found supranuclear in the cytoplasmic region with dense cluster of vesicles and flattened cisternae in a stack arrangement (Fig. 2b). These were very closely associated with mitochondria and endoplasmic reticulum while within the cytoplasm of the ependyma cells (Fig. 2b). Few supraependymal intraventricular macrophages were sighted at the apical surface of non-ciliated ventricular ependymocytes, with few microvilli (Fig. 2c). Cilia were observed as typical as in protozoans with 9 + 2 arrangement (nine peripheral doublets and a central pair) enclosed within the ciliary membrane (Fig. 2d). The structural layout of the crowning ependymal cilia was built on a typical ciliary complex with basal body, rootlets within the surrounding granular zones in the subapical zone. Cone-shaped striated basal feet were noted to be in contact with the rootlets of the basal bodies (Fig. 2e). Tight junctions (*zonula occludens*), intermediate junctions (*zonula adherens*) and desmosomes (*macula adherens*) and infrequently gap junctions were identified along the adjoining borders of neighbouring cells. *Zonula occludens* and *zonula adherens* were mostly found close to the luminal junctions while desmosomes were seen largely on the distal adjoining lateral portions of the plasmalemma of contiguous ependymocytes (Fig. 2b).

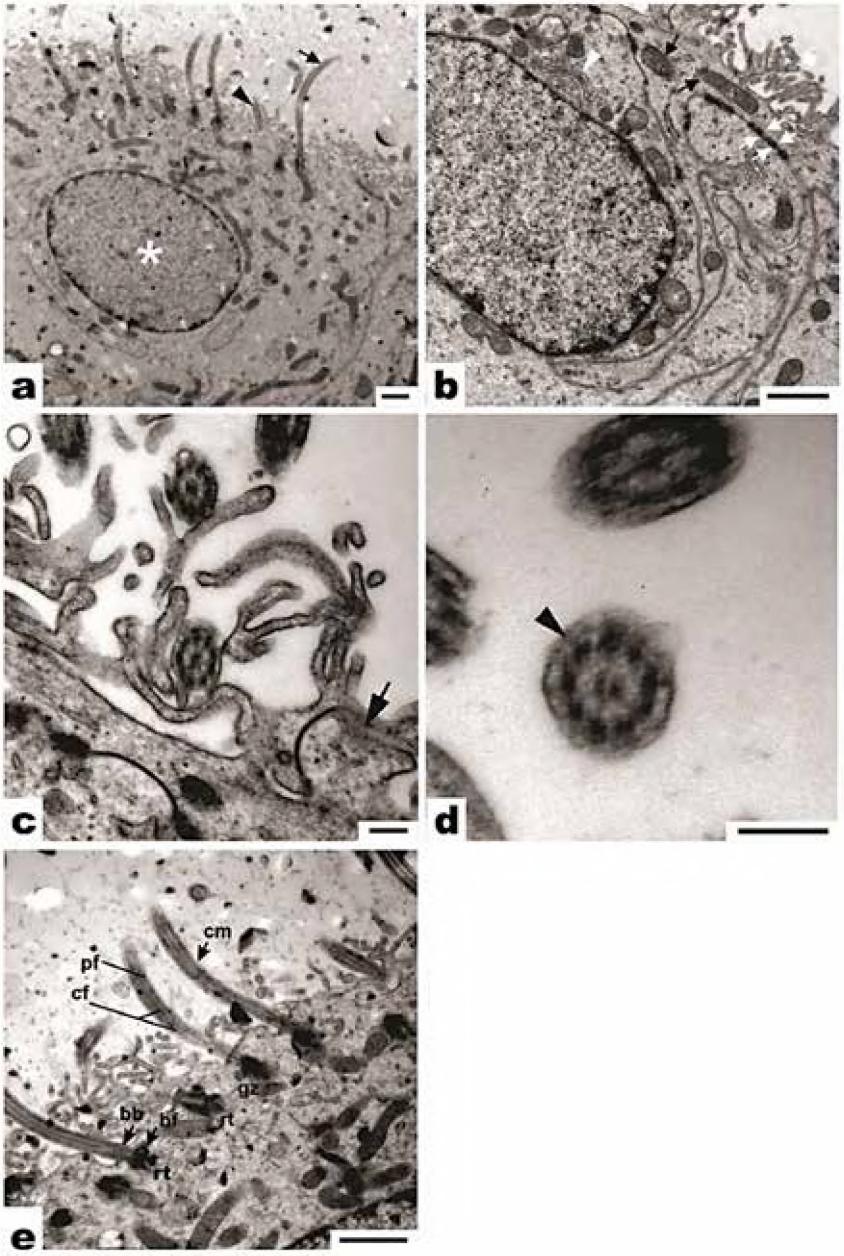

Fig. 2. Transmission electron micrograph of the AGR ependymal layer showing: a) distinct intracellular details of an ependymocyte

Note the oval shaped nucleus (white asterisk), apical cilia (black arrow) and microvilli (black arrowhead); **b)** cellular junctional complexes formed by two abutting plasmalemmae (white arrows) with numerous mitochondria (black arrows) at the apical membrane and Golgi complexes (white arrowhead) within the cytoplasm; **c)** A non-ciliated portion of the ependymal layer with a supra-ependymal intraventricular macrophage (black arrow) at the apical surface with scanty microvilli; **d)** Cross section of the ciliary shafts showing the typical 9+2 arrangement of ependymal cilia (black arrowhead); **e)** The ciliary complex of the AGR ependyma highlighting the basal body (bb), rootlets (rt), basal foot (bf), and granular zone (gz). cf, central fibres; cm, ciliary membrane; pf, peripheral subfibre. Scale bars: 500 nm (a—b, e); 200 nm (c—d)

The scanning electron micrographs of the roof and floor of the lateral ventricles were viewed and presented minimal differences with slight morphological patterns. The surface appearance and modifications of the roof were almost always more populated and denser while the floor showed areas of patches with the cilia slightly thicker and less clumped than those of the roof (Fig. 3a—d). The wall epithelium looked matted and slightly scantier with domains of "baldness" with regular patterns.

The surface of the choroid plexus of the lateral ventricles showed varied folds with homogeneous vesicle-like projections which represent choroid cells (Fig. 4a, 4b).

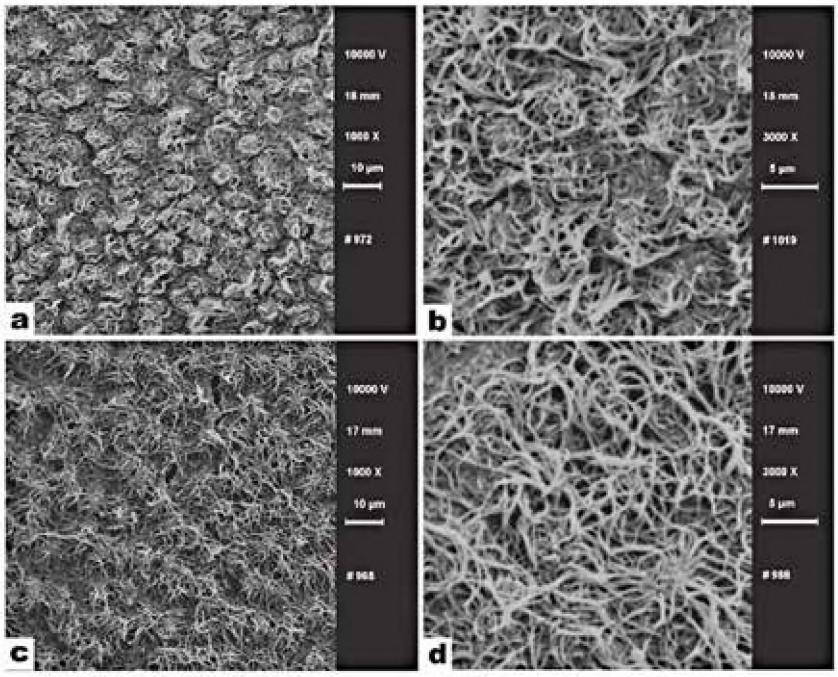

Fig. 3. Scanning electron micrographs of the lateral ventricles showing slight variations in the morphology in the walls of the lateral ventricles at a) low magnification; b) high magnification and in the roof of the lateral ventricles at c) low and d) high magnification

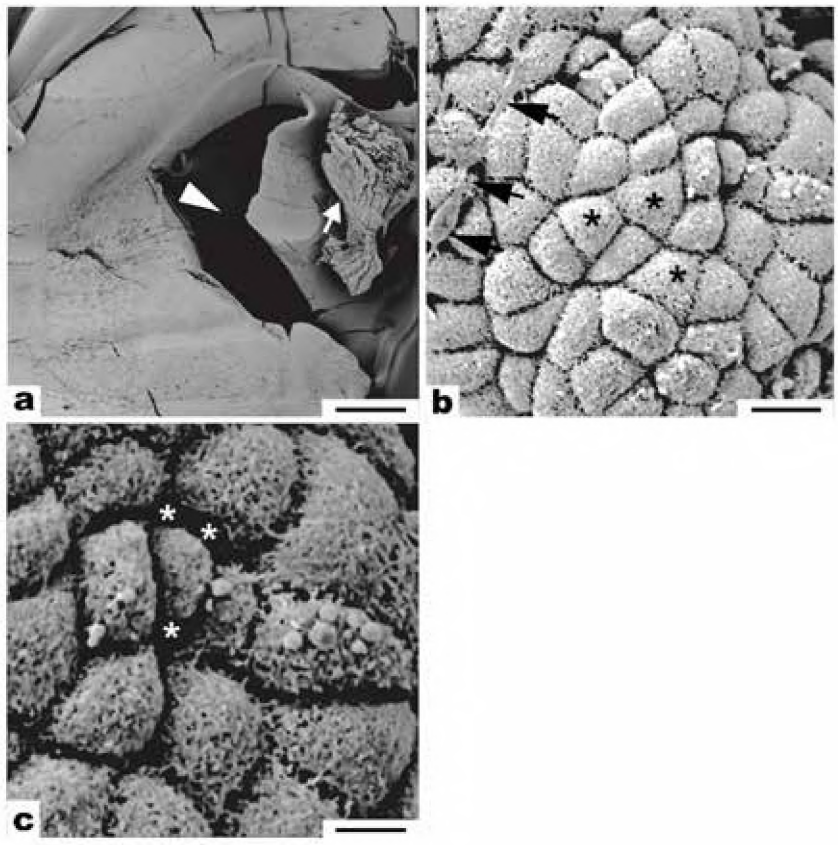

Fig. 4. Scanning electron micrograph showing: a) the choroid plexus (white arrow) at lower magnification projecting into the lateral ventricle (white arrowhead); b) Epiplexus cell of Kolmer with distinct protoplasmic extensions (black arrows) as well as choroid cells (black asterisks) and c) junction sites separating one choroid cell from another (white asterisks). Scale bars: a) 1000 µm; b) 10 µm; c) 5 µm

There were electron-dense areas surrounding each cell which were the junction sites from one choroid to another (Fig. 4c.). The surfaces of the choroid cells had cilia and microvilli. Epiplexus cells of Kolmer were observed with their protoplasmic extensions (Fig. 4b). The lateral ventricle is demonstrated by the white arrowhead (Fig. 4a).

DISCUSSION

The basic morphology of ependymal cells ranges from flattened, cuboidal to columnar cells. In the AGR, we identified cuboidal or low columnar cells in shape. Other features presented in the AGR, including the regional multi-layering of ependymal cells are reported as typical rodent features [10, 21]. The abundant occurrence and arrangement of mitochondria, Golgi complex and perinuclear ribosomes indicates active energy metabolism in the production and circulation of cerebral spinal fluid (CSF) in the ventricles along with the various functions of the ependymal cells in water regulation, and disease monitoring [38].

The lateral ventricles host the greater part of the subependymal layer [35], and our anti-GFAP staining revealed positive cells which appear as astrocytes but have extensions into the subependymal neuropil. Astrocytic heterogeneity has been described in the AGR with subependymal astrocytes identified as periventricular astrocytes [27]. Although this study did not utilize special immunostaining antibodies to delineate tanycytes, the described cells identified resemble tanycytes [23]. In the lateral ventricles, stem cells from the neuroepithelium are retained between the ependymal cells and subventricular zone, thus, regions of multi-ciliated ependyma have been reported to play an important role in neurogenic and gliogenic activities [16]. This function is alluded to in the juvenile AGR, which has been reported in one of our previous studies as having the most active adult neurogenesis profile in the AGR subventricular zone [29]. The proliferative potential of the adult forebrain ependyma and sub-ependyma has been reported [6]. However, only subependymal cells have neural stem cell characteristics as determined by their self-renewing capability (generating secondary spheres) and their ability to clonally give rise to neurons and glia [22, 32]. The existence of indistinct neuropil and glia seen with histology in the AGR may indicate AGR sub-ependymal clonal ability. The multi-layered ependyma most of the time contained tanycytes whose basal processes extend into the adjacent subependymal layer and neuropil [21]. This is believed to be in consonance with the findings in the AGR. Multilayering has also been reported as ependymal tissue response to injury [4]. It is hereby suggested that the glial populations of the subependyma be studied along ependymal studies in toxicity and neurodegenerative processes.

Tight junctions are widely demonstrated in ependymal cells and are seen as early as in the development in the neuroepithelium where they present *zonula occludens* apically and *zonula adherens* and gap junctions in the lateral plasma membrane domains [1, 17]. The mRNA coding connexins have been indicated as being important in this respect [3]. This gap junction pattern has been suggested to function in the reception of extrinsic growth factors from CSF likely through sensory apical cilia [14]. They function in electrical and metabolic couplings integrating the functioning of the cell layer. Furthermore, they are known to play a role in cilia beating synchronization and consequently in the circulation of CSF. They also integrate ependymal function with underlying astroglia, thus regulating water and ion transport [2, 16, 34].

Ependymal tight junctions in the AGR brain were tortuous and had regions of occlusion and adhesion. The ciliary arrangement 9+0 shows that the non-motile cilia (primary cilia) are composed of nine radially organized microtubule doublets, whereas the other 9+2 arrangement shows that the motile cilia contain an additional central microtubule doublet [20]. The works of Marshall and Kintner [20], has shown the existence of Dynein arms in the 9+2 arrangement that generate the shearing force needed for motility. Also, these types of cilia differ by the kinds of receptors on their membrane and the posttranslational modifications of their microtubules [11].

The choroid plexus cells contribute CSF secretion and serve as integral parts of the CNS barrier system which separate blood, CSF and CNS interstitial space via the BCSFB in conjunction with the BBB [33]. The morphology of individual choroid plexus in the lateral ventricles, third and fourth ventricles has been reported [36]. In addition to their protective role, the cells of the choroid plexus have been reported to act as a defence system by protecting the brain against acute neurotoxic insults, using a complex, multi-layered detoxification system [13]. The Kolmer cells, present on the AGR choroid plexus play this defensive role.

Epiplexus or Kolmer cells have been described as intraventricular macrophages associated with the choroid plexus. These intraventricular macrophages are referred to as "supraependymal" cells when found on the ependyma [17]. Entering by the transcellular pathway or the paracellular [18], these cells express complement type 3 (CR3) receptors, major histocompatibility class I and II (MHC I and II) antigens, and leukocyte common antigens under normal conditions. Thus, indicating their involvement in endocytosis similar to other macrophages and microglial cells in the brain (CR3) and antigen processing and presenting capacities for lymphocytes in case of MHC I and II [19]. These intraventricular macrophages may also be involved in sequestration of iron in certain neuropathological conditions that cause an increase in iron content in the CSF [5].

This paper has described the ultrastructure of the AGR morphology and shown possible functional components of the ependymocytes, the choroid plexus, tight junctions and subependymal tissues in view of its usefulness in research.

CONCLUSIONS

The ependymal layering of the brain of African Giant Rat showed marked variations in some regions of the ventricles with multi-layering of ependymal cells that differ from the typical single layered ependymal while subependymal structures reveal indistinct neuropil and glia with histological examination. This existence of indistinct neuropil and glia seen in this rodent may indicate sub-ependymal clonal ability. The ultrastructure of the ependymal cells and choroid seems typical depicting high secretory activity, but also a protective role against brain insults.

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