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Analysis of Histological Features of the Cerebral Cortex and Hippocampus of Albino Rats Using Haematoxylin & Eosin Stain- An Observational Study

M. V. Shreejha [a‡], R. Priyadharshini [a#†*], Palati Sinduja [a#†] and V. Meghashree [a#†]

[a] *Department of Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 77, Tamil Nadu, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: The study determined the histological layers of the cerebral cortex and hippocampus of the albino rat brain samples has been used in the study. The Cerebral cortex is composed of the Molecular layer, external granular, external pyramidal layer, internal granular layer and interior pyramidal layer. The layers of the hippocampus are alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare. The aim of the study is to analyze the detailed histological features of the cerebral cortex and hippocampus layers of albino rats at the magnification of 10X,100X,40X. By using haematoxylin and eosin stain as an observational study.
Materials and Methods: The samples were preserved and fixed with the formalin and stained by haematoxylin and eosin and observed with a light microscope.
Results: The molecular layer is the superficial layer containing neurons. The outer granular layer

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[‡] *Undergraduate;*
[†] *Dr.;*
[#] *Assistant Professor;*
**Corresponding author: E-mail: priyadharshinir.sdc@saveetha.com;*

of the cells are densely packed. Outer pyramidal layer contains rich pyramidal cells, Inner granular layer contains stellate cells, Inner pyramidal layer contains glial cells and the deeper multiform layer is composed of pyramidal cells. The hippocampus contains three layers of cornu Ammonia CA1, CA2, CA3. CA1 responds to memory and is covered by the choroid plexus. CA2 contains 3 major cell dentate gyrus, pyramidal cells, pyramidal neurons and CA3 composed of stratum lucidum.

Conclusion: The study of brain analysis of histological features of the cerebral cortex and hippocampus of the brain adds a greater insight in understanding the histology of various types of layers in rat brain and morphology of brain cells.

Keywords: Rat brain; H and E strain;t; Hippocampus; cerebral cortex.

1. INTRODUCTION

The acquisition of a working knowledge of the anatomy of the rat central nervous system (CNS) provides a great challenge to anybody who has not undertaken some training in mammals or the human body and specifically in any other mammalian or human neurostructures [1]. The major features of the surface of the rat brain are the olfactory bulbs which are labelled as the olfactory lobes; the term olfactory bulbs has been used nowadays. In comparison to man, the rat has large olfactory bulbs at the anterior pole of the brain and the surface of the cerebral hemispheres is not thrown into a mass of ridges (gyri) separated by grooves (sulci) [2]. Only the horizontal rhinal fissure will be separated from the older parts of the cerebrum from the newer parts. This fissure can be seen better from the ventral or lateral aspects of the brain [3].

The posterior poles of the hemispheres in the rat are rounded and wider than the anterior poles but do not overlap the cerebellum as they are seen in man [4]. The surface of the cerebellum is fissured. The structures of the cerebral mantle are the outer covering of the cerebral hemispheres and are folded into some peaks like structures called gyri and grooves called sulci lobes and folds [5]. The layers are neocortex which consist of 6 layers: the Layer I Molecular layer contains few scattered neurons and consists of extensions of apical dendritic tufts, neurons and horizontally oriented axons also as glial cells. Layer II, the external granular layer and small pyramidal cells. Layer III contains the external pyramidal layer and small medium-size pyramidal neurons and they are also called non-pyramidal neurons. In Layer IV, the structures are internal granular layers containing various types of stellate cells and pyramidal cells. Layer V, the interior pyramidal layer, contains a large type of pyramidal neuron. Layer VI, the polymorphic or multiform layer, contains few large pyramidal neurons and lots of small spindle-like pyramidal structures and multiform neurons [6].

The hippocampal formation may be a bilateral structure sandwiched between the cerebral mantle and therefore the thalamus. The hippocampus was performed by various layers of cornu ammonis and dentate gyrus. The different layers of the cornu ammonis (alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum, stratum moleculare) and the dentate gyrus (stratum moleculare, stratum granulosum, polymorphic layer. The hippocampus is in the form of a curved tube, which has been compared to a seahorse, and a common unicorn plant (CornuAmmonis). Its abbreviation CA is employed in naming the hippocampal subfields CA1, CA2, CA3, CA4. The Pyramidal cells contain large amounts of the conical soma, prominently developed apical dendrite with tuft, and full development of the basal dendritic arbor [7,8].

H and E stain is the combination of two histological stains: Hematoxylin and eosin. The hematoxylin stains cell nuclei purplish blue, and eosin stains the extracellular matrix and cytoplasm, which is in pink colour and is routinely used for the detailed view of tissues in Histopathology.[9]. The aim of the study is to identify and observe the cerebral cortex and hippocampus structure in H and E stains in rat brain samples [10].

2. MATERIALS AND METHODS

The research was undertaken in the Pink lab of the Department of General Pathology, at Saveetha dental college, Saveetha institute of medical and technical science, Chennai.

The fresh whole brain of the albino rat was used in the study. 20 Albino rats have been used in this study 10 normal and 10 fully fat induced rats. 10 males and 10 females rat at the weeks of 2

has been used in this study. Samples were preserved and fixed in the 10% neutral buffered formalin. Each brain was carefully dissected to expose the cerebral cortex and hippocampus. The samples were processed and embedded. Tissue sections of 4-5 thickness were obtained by a rotary microtome. The validation of the procedure was given by a research guide. Sections of healthy rat brains with cerebral cortex and hippocampus regions were taken for the study. The inclusion criteria of the study are normal, healthy rat brain samples, without any drug induced, and only the layers of the cerebral cortex and hippocampus have been observed.

The sections were then stained by the following staining methods.

Hematoxylin and Eosin stain has been brought from the local shop present near by area.The first step in performing an H&E stain, the slide has been kept in the suitable adhering agents, then slightly warm to melt the wax off, remove the wax with xylene by 3 changes and also with propanol for 3 changes, bring the smear to the water for 3 minutes, dip the smear in rapid nuclear strain for 60 seconds then add 3 drops of scott's tap water buffer and wash after 10 seconds, dip the smear in eosin stain for 60 seconds and wash the smear for 15 sec to remove the excess water, final clearance with propanol and with xylene followed by DPX mount, it is a mixture of distyrene, a plasticizer, and xylene used as a synthetic resin mounting media (3).

3. RESULTS

Microscopic explanations of (H&E) hematoxylin and eosin stained slides of the cerebral cortex and hippocampus of the rat brain samples used in the study. The cerebral cortex is responsible for sensation, perception, memory, association, thought, and voluntary physical action. There are six layers present in the cerebral cortex: Layer 1 Molecular Layer, Layer 2 outer granular layer, layer 3 outer pyramidal Layer, layer 4 inner granular layer, Layer 5 inner pyramidal layer, layer 6 multiform layer explained in (figure 1). The molecular layer is the first and superficial layer consisting of neurons which are in round or ovoid shape. The layer is composed of unmyelinated fibrous cells with few cells and a small number of purkinje cells. The purkinje cells are present at the distal part of the molecular layer explained in (Figure 2). The outer granular layer is the second most layer in which the cells are densely packed, these layers are deeply stained and contain small pyramidal cells and numerous stellate neurons and also it contains small chromatin with a thin rim of cytoplasm. Outer pyramidal layer is the third layer containing rich pyramidal cells, (figure 3) they have basophilic characters and stellate cells (figure 4) can also be seen. Cells specifically in the outer granular layer were less crowded. Glial cells can also be seen in this layer. Most of the glial cells in this layer seem to look shrunken . Inner granular layer (figure 5) is the fourth layer containing densely packed stellate cells. The (figure 6) shows the pyramidal cells and glial cells ; the pyramidal cells in this layer are less but cells are in pyramidal shape. The Multiform layer is the fifth layer and deepest layer compared to all the five layers, the fusiform cells are seen maximum in this layer. The Hippocampus is supplied by, posterior cerebral artery, which is divided into three branches: Anterior, middle, and posterior. Supply is by the anterior choroidal artery. Veins of the hippocampus will drain at the basal vein. Figure 7a and 7b, 9, 10 shows the cornu ammonis CA1 is the subiculum connects the hippocampus with entorhinal cortex in the ventricles, hippocampus is covered by choroid plexus,which is responsible for sensory and memory, CA2 is called as small zone layer in which the pyramidal cells may reduce, the layer is made up of 3 major cells dentate gyrus, pyramidal cells and pyramidal neurons, CA3 is the second most layer contains synapses from the mossy fibres through stratum lucidum and also contains cell bodies, axo axonic cells, bistratified cells, and radical trilaminar cells. The (figure 8) shows the axons and dendrites (H&E 40X) explains numerous nerve cells and oligodendrocytes The (Figure11) explains about the Purkinje cells are large neurons, with a large eosinophilic cell bodies that also contains nissl substance. (Figure12) explains about the Neurons are called small neurons which are responsible for receiving sensory input, for sending motor commands, and for transforming electrical signals. (Figure13) explains about the astrocytes in star-shaped and glial cells in the brain and support of the endothelial cells form the blood brain barrier. (Figure14) explains that Oligodendrocytes are a type of neuroglia, whose main function is to provide and support the axons in the central nervous system. (Figure15) explains that the Microglia are the immune cells of the CNS, similarly to the macrophages which respond to the pathogens and injury by changing the morphology migrating the injury site. (Figure16) The endothelium cells represent an active interface between blood and the central nervous system.

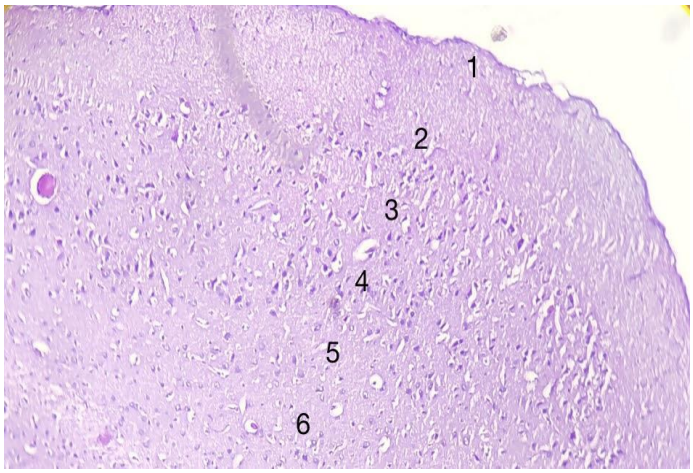

Fig. 1. Explains the photograph of a section in the cerebral cortex of control adult rats showing the general histological structure of the cerebral cortex. Molecular layer (1), outer granular layer (2), outer pyramidal layer (3), inner granular layer (4), inner pyramidal layer (5) and the multiform layer (6) (H&E 10X)

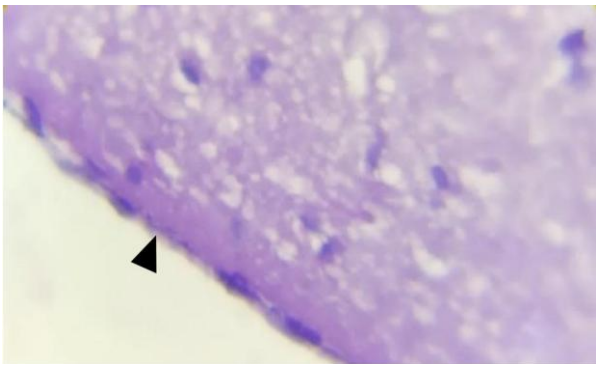

Fig. 2. Explains the photograph of a section in the cerebral cortex of control adult rats showing the general histological structure of pia mater and molecular layer, contains two main types of neurons stellate cells and basket cells which are seen in scattered form (H and E stains at 100X)

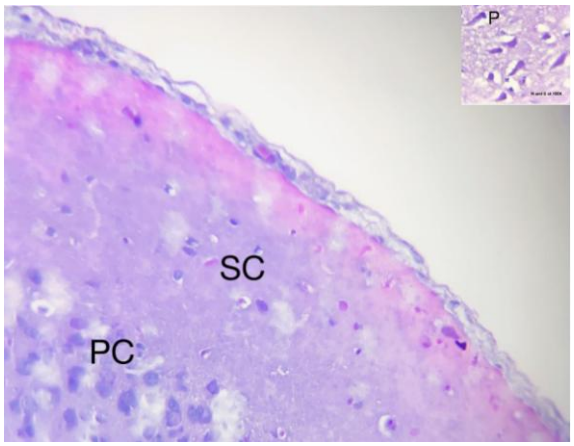

Fig. 3. Explains the photographic explanation of granular layers in H and E stains at 40X and contains small pyramidal cells (PC) and stellate cells (SC), containing various axons and dendritic connections. Pyramidal cells at 100X in H and E stains

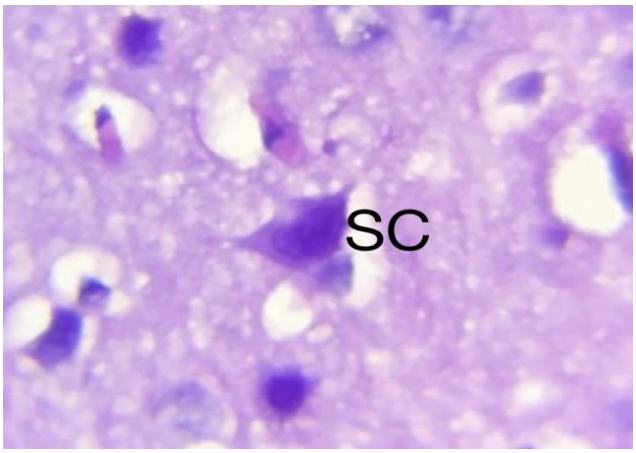

Fig. 4. Explains the photographic explanation about stellate cells in H and E stain present in the granular layer

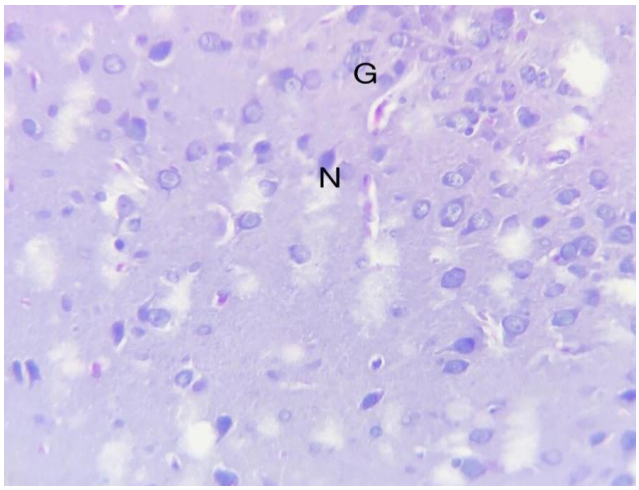

Fig. 5. The photograph explains the granule cell (G) with pale open face nucleus and neuroglia (N) with dense nuclei in (H and E10X)

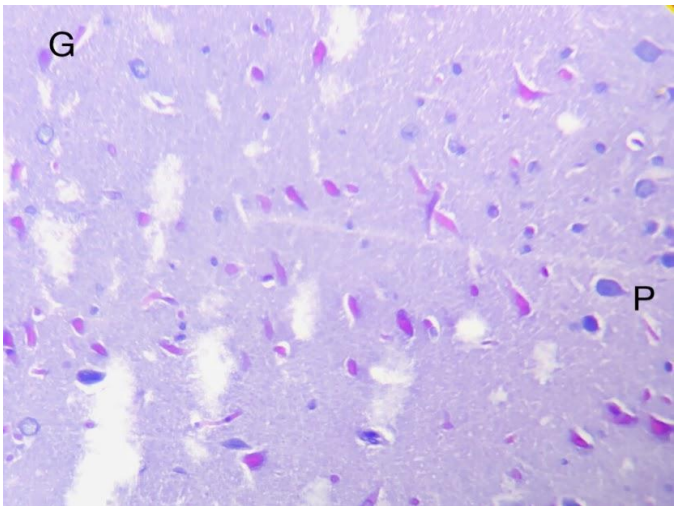

Fig. 6. The photograph explains the pyramidal cell (P) and glial cell (G) (H&E 40X) The cells in this layer are seen as shrunken

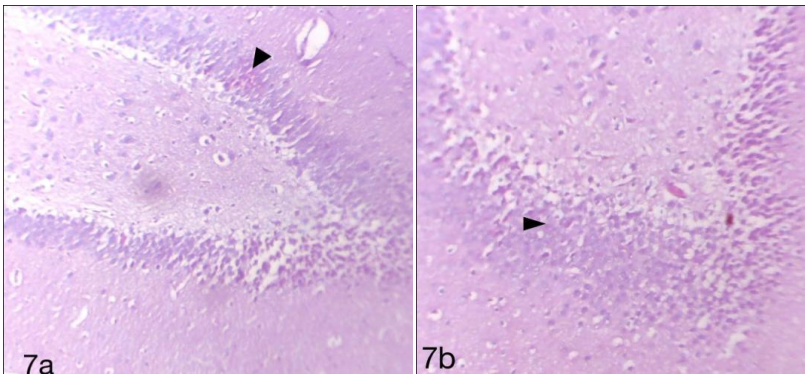

Fig. 7a. Explains the photographic sections of the CA2 pyramidal layer lying within Ammon's horn and is most often interposed between CA1 and CA3 pyramidal layers. The pyramidal cells of the CA2 region are large and densely packed. Fig. 7b. explains the photographic section of CA3 is the second most layer contains synapses from the mossy fibres through stratum lucidum and also contains cell bodies, axo axonic cells, bistratified cells, and radical trilaminar cells at 40 X in (H and E stains)

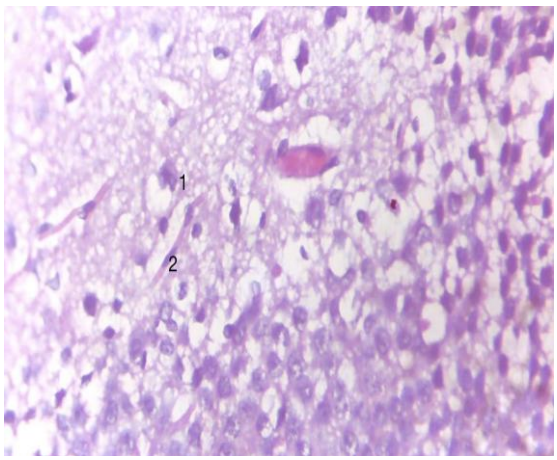

Fig. 8. The photograph explains the axons and dendrites (H&E 40X) explains numerous nerve cells (1) and oligodendrocytes (2)

Fig. 9. Explains the photographic section of cornuammonisCA1 is the subiculum connects the hippocampus with entorhinal cortex in the ventricles, CA2 is called as small zone layer which contain pyramidal cells, CA3 is the second most layer contains cell bodies, axo axonic cells, bistratified cells, and radical trilaminar cells at 4X in (H and E stain)

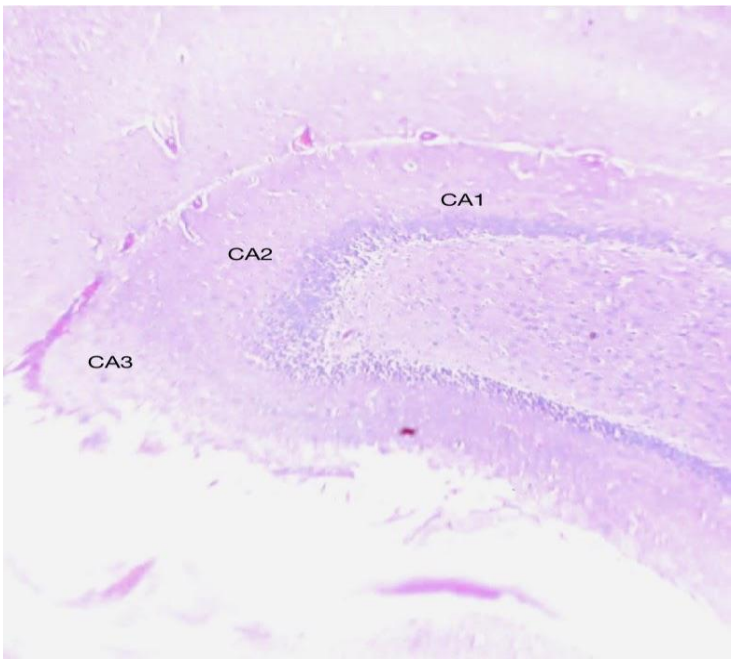

Fig. 10. Explains the photographic section of the layer cornu ammonis which contains pyramidal cells and is basophilic in nature at 10X in (H and E stain)

Table 1. Observational diagram of the histological diagram of rat brain cells

CELL TYPE	LOCATION	SHAPE
PYRAMIDAL CELL	MAINLY IN LAYER 3 AND 5	
FUSIFORM CELL	4 LAYER	
RETZIUS CAJAL CELL	1 LAYER	
BASKET CELL	4 LAYER	
STELLATE CELL	5,6 LAYER	

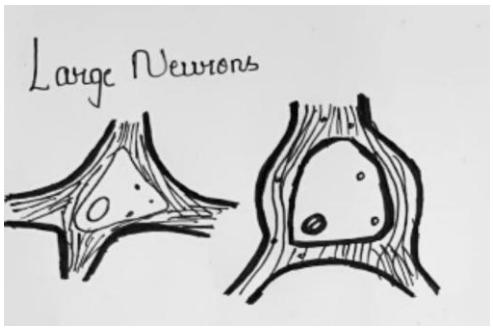

Fig. 11. Purkinje cells are large neurons, with a large eosinophilic cell bodies that also contains nissl substance

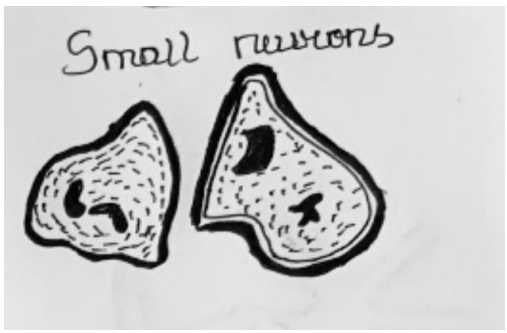

Fig. 12. Neurons are called small neurons which are responsible for receiving sensory input, for sending motor commands, and for transforming the electrical signals

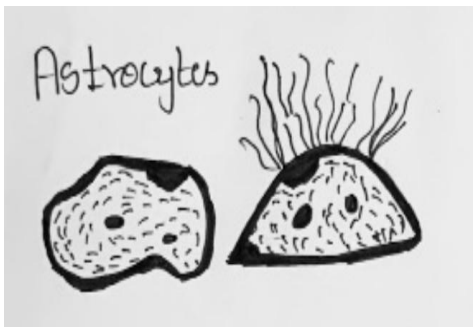

Fig. 13. The astrocytes in star-shaped and glial cells in the brain and support of the endothelial cells form the blood brain barrier

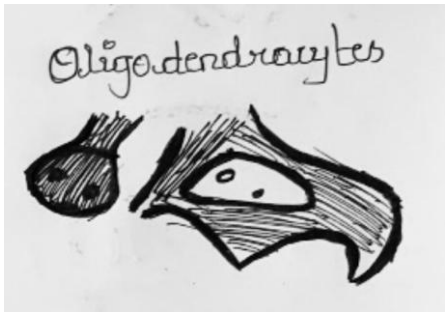

Fig. 14. Oligodendrocytes are a type of neuroglia whose main functions is to provide and support to the axons in the central nervous system

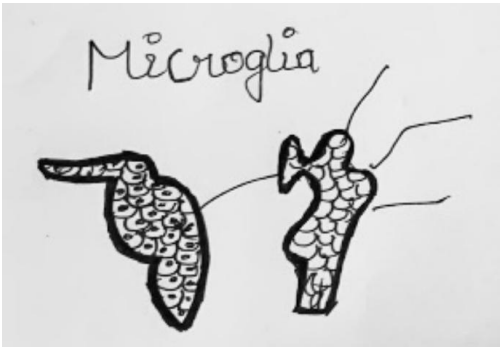

Fig. 15. Microglia are the immune cells of the CNS, similarly to the macrophages which respond to the pathogens and injury by changing the morphology migrating the injury site

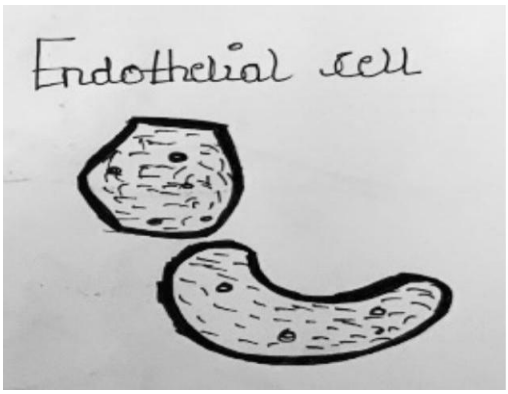

Fig. 16. The endothelium cells represent an active interface between blood and the central nervous system

4. DISCUSSION

In our study we have observed the layer present in the cerebral cortex and hippocampus in H and E stain, cerebral cortex contains six molecular layers and has horizontal connections. External granular layer contains small granule cells and intracortical connections, External pyramidal layer contains small pyramidal shape cells, Internal granular layer contains large granular cells, Internal pyramidal layer contains large pyramids structure compare to external pyramidal layer, the multiform layer contains fusiform cell and pyramidal cell, which is present every layer. The histology of the Cerebral Cortex and neurons and also hippocampus have been described in both H and E.

In previous research the detailed explanation about the cerebral cortex and hippocampus, Palladium is the part of the basal ganglia of the brain which consist of the globus pallidus and the ventral pallidum. The globus pallidus appears in a pale and present spherical area of the brain. There are two types of pallidum present in the cerebral cortex. Neopallium are present at any stage of development, Archipallium and paleopallium; they are not present in any 6 layers but can be usually seen in 3 layers [11,12]. The major cells are Pyramidal cell, fusiform cell, Retziuscajal cell, basket cell, stellate cell, cells of martinotti, the location of the pyramidal cell is mainly present at layer 3 and 5 , the location of fusiform layer is located at layer 6 and shape of the cell is spindle, the cajal cell present in layer 1 and shape of the cell is elliptical, Basket cell present in layer 6 the shape of the cell is angulated, stellate cell present in layer 3 and shape of the cell is angulated, cells of the martinotti present in both 5 and 6 and shape of the cell is angulated [13]. CornuAmmonis in this area abuts the dentate gyrus in one end and the subiculum on the other end and this is divided into three (CA1, CA2 and CA3) and the surface is covered by the layer of nerve fibres and described as alveus. The axons of the pyramidal cells of the CA will run to the alveus. Five layers have been seen in the CA, but in our study we described three layers: the superficial molecular layer, the layer of pyramidal cells and the deeper

layer of polymorphic cells [14] and it also consists of two systems olfactory and limbic system, the olfactory System includes structures like, pathway from the olfactory epithelium, cerebral cortex. The olfactory bulbs of the rat are large and projections from the anterior ends of the cerebral hemispheres. The bulbs have a laminated structure by an important layer of mitral cells in which they block the axon cells and bring them back again to the brain. The axons which are back from the bulb can be grouped as lateral, medial and intermediate olfactory tracts. The lateral tract is the largest, carrying the majority of the information [15]. In the Limbic System there are Four important pathways [16], The Purkinje Cells are most important in the cerebellum. Purkinje cells contain large neurons, with large eosinophilic cells. The outer feature of Purkinje cells is a two-dimensional, dendritic fan appearance that extends upwards and crosses the axis of the lobules of the cerebellum [17], The Granular Layer contains small nuclei that stain darkly, and cytoplasm can be seen. Axons and dendrites have also been seen. The dentrices cluster around the terminal fibres, forming little islands that stand out as pale pink-stained spaces in H&E-stained sections [18-20]. Study was limited only by observing the layers of the cerebral cortex and hippocampus, not all the structures present in the brain sample. The futurescope of the study, we have planned to inject a drug (Ketamine hydrochloride with saline) into the rat and absorb the layers of the cerebral cortex and hippocampus. Our team has extensive knowledge and research experience that has translated into high quality publications [21,22-35,36-40].

5. CONCLUSION

Our study of brain analysis of histological features of the cerebral cortex and hippocampus of the brain adds a greater insight in understanding the histology of various types of layers in rat brain and morphology of brain cells.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical approval number for the study is IHEC/SDC/UG-1962/21/212.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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