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Original Research Article

CYTOARCHITECTURE OF HUMAN ADULT HIPPOCAMPUS

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ABSTRACT

Background: Hippocampus assumes the most amazing part of brain of all mammals. It is located in the floor of inferior horn of lateral ventricle. It belongs to archipallium .

Materials and Methods: twenty human adult brains from cadavers ages 30 to 70 years are used. Hippocampus dissected out of Brain, specimens were processed into paraffin blocks, sectioned (10 µm thick), and stained using Haematoxylin and Eosin, cytometric analysis was done under light microscope with eyepiece micrometers.

Results: The lamellae of the hippocampus were morpho and cytometrically evaluated for thickness of various layers and neuronal sizes , form and density in different gestational period.The neurons that are present are pyramidal in shape and their size and density varied in different Cornu Ammonis sub fields of hippocampus. The Plexiform layer consisted a thickness of 920 µ to the maximum and 276 µ to the minimum. The thickness of polymorph layer ranged from 1288 µ to 920µ. Dentate gyrus showed a trilaminar normal pattern. The granule cells are conspicuous by their small size and their diameter is 9.2 µ. The indices almost tallied with the observations of earlier authors.

Conclusion: The above indices almost tallied with the observations of D.W. Zaidel (1999).

KEY WORDS: Hippocampus, Cornu ammonis (CA), Dentate gyrus, Adult, Cytometric.

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INTRODUCTION

The name hippocampus stems from the supposed resemblance of the complex, in coronal section, to the profile of a seahorse [1]. The hippocampus assumes the most amazing part of brain of all mammals. It is located in the inferior horn of lateral ventricle of brain Fig.: 1. Hippocampal formation is composed of hippocampus, dentate gyrus and subiculum [2]. Hippocampus consists of V/U shaped band of deeply stained cells the gyrus dentate and similar V/U shaped Cornu Ammonis (CA). It arranged that one limb of Cornu Ammonis (CA) lies between divergent limbs of gyrus dentate. Fig.5. The hippocampus remains structurally plastic throughout life [3,4] with properties of undergoing consistent changes depending on the past experience of organism. Papez (1937) related the emotional disturbances to the damage of hippocampus [5]. Many authors have observed that hippocampus is organized into lamellar portion and the orientation of lamellae differs among species [6]. Silva et. al. observed that cytoarchitecture of hippocampus proper of mammelia follows a general pattern [7]. The trilaminar cortex of the dentate gyrus is the least

complex of the hippocampal fields, and its major cell type is the granule cell, found in the dense granule cell layer. The Gyrus dentatus consists of three layers. A Plexiform or Molecular Layer, A granular layer, A Polymorph layer. The granule cell and molecular layers are sometimes referred to as the fascia dentate. The polymorphic layer or hilus of the dentate gyrus, contains cells that give rise primarily to ipsilateral association fibres that remain within the dentate gyrus and do not extend into other hippocampal fields. The granule cell layer encloses a portion of the pyramidal cell layer of the cornu ammonis [8]. The hippocampus is trilaminar archicortex with an outer molecular layer, a middle pyramidal layer and an inner polymorphic layer (Fig. 2). The thin layer of fibers adjacent to the polymorphic layer of the hippocampus is known as the *alveus*.

These fibres coalesce to form the fimbria and subsequently, the crura of the fornix (main efferent pathway of the hippocampal formation). The crura of the fornix converge to form the body of the fornix, which later forms the columns of the fornix and pass through the hypothalamus into the mammillary bodies [9]. It may best be divided into three distinct fields, following the nomenclature of Lorente de Nó (1934) [10], namely CA1, CA2 and CA3. Field CA3 borders the hilus of the dentate gyrus at one end, and field CA2 at the other. In early accounts a field CA4 was also identified. CA4 has been dropped from most contemporary accounts of the hippocampal formation [11]. Field CA3 pyramidal cells are the largest in the hippocampus and the whole pyramidal cell layer in this field is about 10 cells thick. The border between CA3 and CA2 is not well marked as the pyramidal cells of the former appear to extend under the border of the latter for some distance. The CA2 field has the most compact layer of pyramidal cells. Field CA1 is usually described as the most complex of the hippocampal subdivisions and its appearance varies along its transverse and rostrocaudal axes. The CA1/CA2 border is not sharp and at its other end CA1 overlaps the subiculum for some distance. The thickness of the pyramidal cell layer varies from about 10 to more than 30 cells and about 10% of neurons in this field are interneurons [8]. CA1 is considered the main output of the hippocampus with fibers extending to the alveus, fimbria and then fornix. Internal circuit. The three primary pathways of this area are called the perforant pathway, mossy fibers and Schaffer collaterals. CA1 or the Sommer sector, is the region most vulnerable to hypoxia. CA4, sometimes described as the endfolium, has intermediate vulnerability to insults, whereas CA3, which enters the concavity of the gyrus dentate, is only slightly vulnerable. CA2 is the most resistant and well-preserved sector [9]. The CA regions are also structured depthwise in clearly defined strata (or layers): Stratum oriens (str. oriens), Stratum pyramidale (str. pyr.) one of the more visible strata to the naked eye. Stratum lucidum (str. luc.) thinnest strata only found in the CA3 region. Stratum radiatum (str. rad.) Stratum moleculare (str. mol.).

The hippocampal sulcus (sulc.) or fissure is a cell-free region that separates the CA1 field from the dentate gyrus. Because the phase of recorded theta rhythm varies systematically through the strata, the fissure is often used as a fixed reference point for recording EEG as it is easily identifiable [12]. The different subfields of the hippocampus, whether of cornu ammonis or the dentate gyrus, were found to differ in their criteria, basically because of the density of the afferent or efferent fibers of each layer. The discrepancy in the thickness of the different subfields of the hippocampus merits the search for factors such as the composition of the extracellular matrix and the capillary density, and other factors which may play a role in the distinct hippocampal lamellar organization [12] In the individual brain there was a positive correlation found respectively between high and low cell density in the different CA fields. The pyramidal cell density decreased in persons more than 68 years old. No difference was found between neruonal cell density in the two sides of the brain. Sex did not influence the neuronal cell density [13]. Hippocampus is now known not just to be important in learning and memory but also in: Spatial navigation, Emotional Behavior, Regulation of hypothalamic functions and its involvement in ageing, Alzheirmer and schizophrenia studied by many However lacunae in cytoarchitectural studies in human

hippocampus have prompted me to undertake the study adult human hippocampus by light microscopy.

MATERIALS AND METHODS

The present work is the result of study of human adult brains. The human material of this work comprises of the specimen of 20 adult obtained from The Department of Anatomy from 2000 to 2002 AMC Visakhapatnam. Adult brains were obtained from dissecting cadavers. The age ranged from 30 to 70 years, the brains were removed as per dissection procedure and stored in formaline. The hippocampus has been identified in the inferior horn of lateral ventricle and hippocampus along with the fornix were removed and stored in 10%formaline for further processing. Parts of hippocampus cut and processed.

The tissue after washing with water is fixed in 10% solution of formaldehyde for 24 hours then it is passed through graded alcohols 50%, 70%, 80%, 90% and 100%. It is cleared in cedar wood oil and embedded in paraffin. The blocks were kept in refrigerator 2 to 3 hours before sectioning and sections of 6 microns thickness are cut with Spencer's rotary microtome. They are later stained with Haematoxylin and Eosin.

Staining procedure: Paraffin was removed with Xylol (3-5 minutes) and passed through graded alcohols of 100%, 90%, 80%, 70% and 50%. They were then washed with water and stained with Haematoxylin for 5 minutes. Sections were kept in running tap water till light blue colour appeared. They were then stained with Eosin for 1 to 2 minutes. They were then washed with water, dehydrated with alcohol cleared with xylol and mounted with Canada Balsam.To demonstrate nerve fibres, nerve cells, the hippocampus is stained by Nissl stain for nissl body.

Micro Photography: The micro photographs of Haematoxylin and Eosin sections were studied on a Pentium 4 computer having a closed circuit camera and an adopter fixed to Labomed binocular research microscope. HD TV software with capturing card utilized for projection of good resolution pictures. The H & E stained sections were examined with 40, 100, 400 magnifications the CC camera with adapter is attached to one of the eyepieces of the binocular microscope. With the manipulation of fine adjustment of the camera, and with the fine adjustment of the microscope up to 400 magnification pictures have been obtained with good resolution on the computer screen and this has been utilized for the taking microphotographs of various sections. The Hippocampus of adult brains after taking the morphometric measurements has been subjected to histological staining procedure. As described above and studied by light microscope for the following features:

Various stratification's in Hippocampus, Thickness of each layer and architecture of the cells present in each layer, Cell count in each layer.

For various measurements the eye piece and micrometer scale were used. FIG. 5

STANDARDISATION OF MICROMETER SCALE:

a. In low power 10X10 magnification: 100 Divisions of eye piece scale are equal to 92 divisions of micrometer scale i.e. 0.92mm.

1 division is equal to 9.2 microns or .0092mm.

b. In High power 10X40 magnification: 100 Divisions of eye piece scale is equal to 24 divisions of micrometer scale i.e. 0.24mm.

1 division equal to .0024mm or 2.4 microns

RESULTS

The section of the hippocampus of adult has been processed with haematoxylin and eosin staining and it has been found that hippocampus is a trilaminar cortex (Fig. 2) having a cellular layer that is the Pyramidal cell Layer with Plexiform Layer outside and Polymorphic Layer inside. The pyramidal cells constitute multicellular layer, and the number of cell lines of the hippocampus in different sub fields varied. The Pyramidal cell layer thickness varied from 368 µ to a maximum 552 µ. The neurons that are present are mostly pyramidal in shape and their size varied, from minimum of 9.6 u to a maximum of 12 µ in CA3 sub field, 9.6 µ to 14.4µ in CA 2 sub field, 9.6 ì to 14.4 ì in CA1 sub field (Table 1 and Fig. 3). The density of the cells per 1 mm in the CA3 accounted for 100 cell, andCA2 also 100 cells where as in CA1 it are 90 cells. The cells are having large and rounded nucleus, dendrites

from apical part of neuron are clearly seen and the length of dendrite is longer than the dendrites that are from basal angles. Interspersed in between the pyramidal cells are small granule cells (Table 1).The Plexiform layer consisted a thickness of 920 ì to the maximum and 276 µ to the minimum. The polymorphic layer consisted of a thickness of 1288 µ to the maximum and minimum of 730 µ. The polymorphic layer consisted of the stratum radiatum having the plexiform network of apical dendrites of pyramidal cells, collateral schaeffer cells derived from basal axons of pyramidal cells and dendrites of granule cells. The dentate gyrus showed also a trilaminar pattern having granular cells having outer molecular layer middle granular layer and inner polymorphic layer (Fig. 4). Molecular layer continued with the hippocampus around the hippocampal fissure. The granular cells consisted of small cells whose diameter ranged from 92µ To 138µ . The neuron size is uniform and it constituted around 12µ. (Table 2 and Fig. 4).

Fig. 1: Gross dissection of Hippocampus in human adult brain.

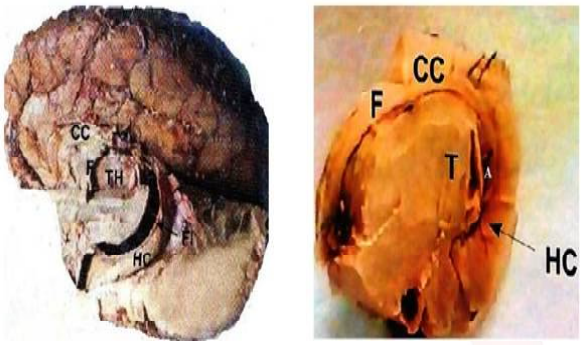


Fig. 2: v/v of cornu ammonis in dentate of hippocampus.

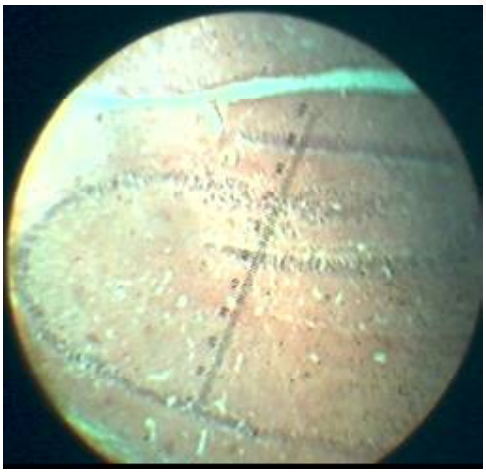


Fig. 3: Neurons in (CA3) Cornu Ammonis 3 subfield.

Fig. 4: Neurons in (CA2) Cornu Ammonis 2 subfield.

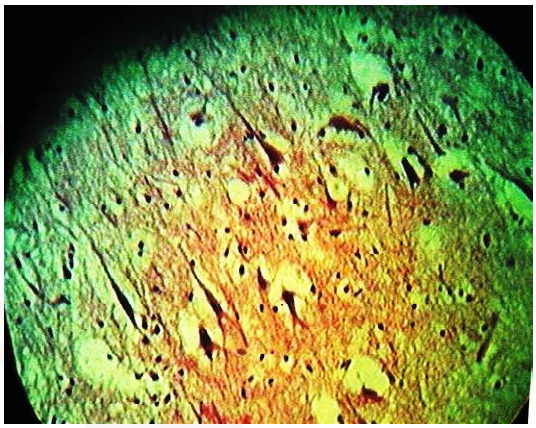


Fig. 5: Neurons in (CA1) Cornu Ammonis 1 subfield.

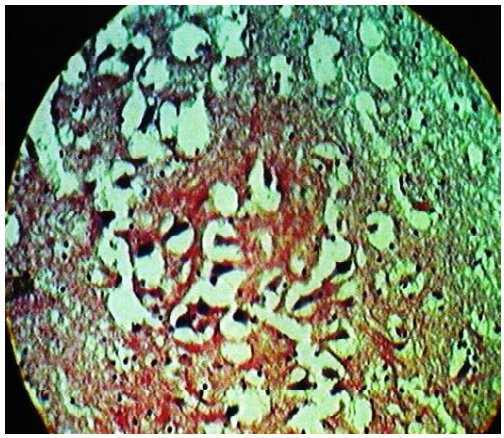


Fig. 6: Neurons in Dentate Gyrus G : Granular cells.

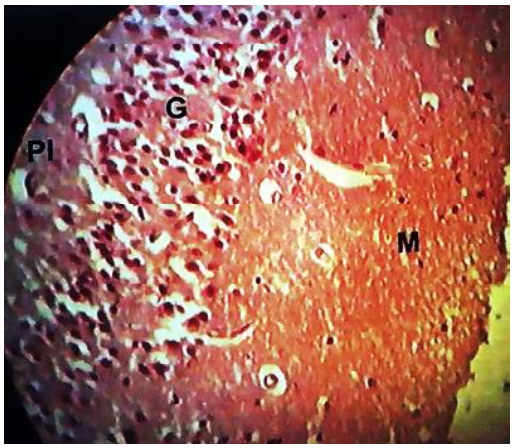
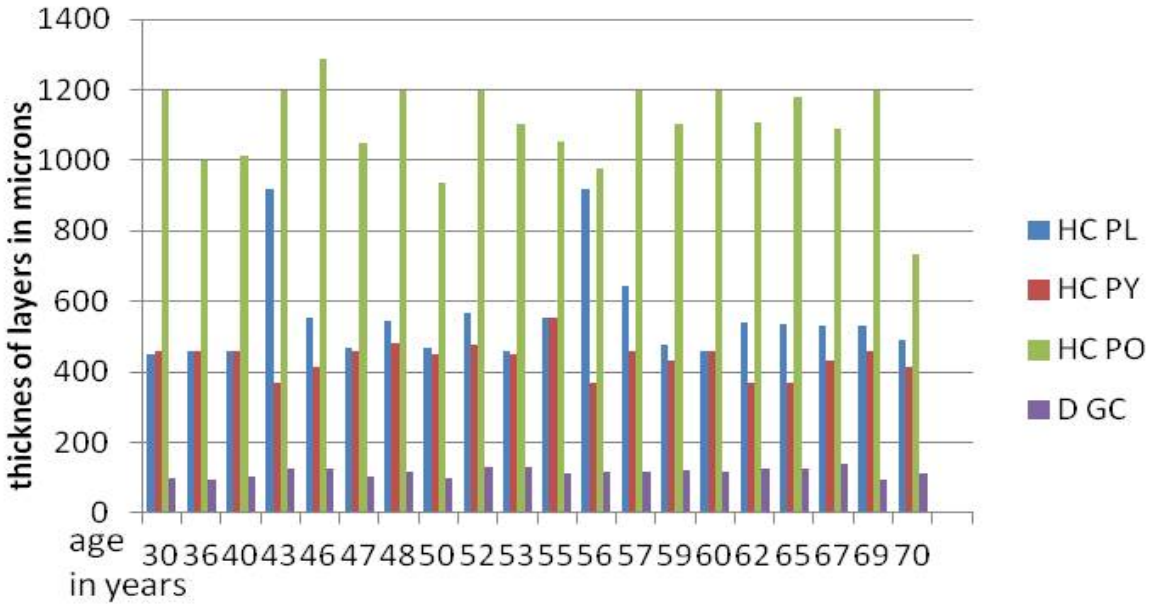


Fig. 7: Histobar Hc. PI : Hippocampus Plexiform Layer.



Hc. PY: Hippocampus Pyramidal Layer.
Hc. PO: Hippocampus Polymorphic Layer.
D.GC: Dentate Granular Cell Layer

Table 1: Thickness of various layers of adult hippocampus in microns.

AGE	HC PL	HC PY	HC PO	D GC
30	452	458	1196	98
36	458	457	1000	92
40	459	458	1012	104
43	920	368	1196	127
46	552	414	1288	126
47	468	460	1050	102
48	543	482	1196	115
50	466	450	936	100
52	567	478	1196	129
53	460	449	1104	131
55	552	562	1054	112
56	920	368	978	116
57	644	460	1196	118
59	476	434	1104	122
60	460	460	1196	118
62	542	368	1106	124
65	534	368	1178	126
67	529	431	1088	138
69	532	460	1196	96
70	489	414	736	110

Note: Plexiform Layer: Stratum Oriens. Polymorphic Layer: Radiatum Lacunosum Moleculare.
Hc. PI: Hippocampus Plexiform Layer. Hc. PY: Hippocampus Pyramidal Layer.
Hc. PO: Hippocampus Polymorphic Layer. D.GC: Dentate Granular Cell Layer.

Table 2: Neuron size in (ca) cornu ammonis of hippocampus subfields in microns.

Age in Years	CA3	CA2	CA1
30	12	14.4	14.4
36	9.6	9.6	9.6
40	12	9.6	12
46	9.6	12	12
47	12	12	14.4
48	9.6	12	12
50	9.6	12	12
52	12	12	14.4
53	12	12	14.4
55	9.6	12	12
56	12	9.6	12
57	9.6	12	12
59	12	12	14.4
60	9.6	9.6	9.6
62	12	14.4	14.4
65	12	14.4	14.4
67	12	14.4	14.4
69	9.6	12	12
70	9.6	12	12

DISCUSSION

In the hippocampus as in the rest of the brain, neurons populate, region differentiated accordingly their morphology dendritic arborization or synaptic, histo chemical properties. Regional differentiation based on

neuronal parameters of size formed and density and thickness was examined in correlation values between hippocampus sub fields in each size separately. This has been substantiated in Table 2. The different subfields of the hippocampus, whether of cornu ammonis or the dentate gyrus, were found to differ in their criteria, basically because of the density of the afferent or efferent fibers of each layer. The discrepancy in the thickness of the different subfields of the hippocampus merits the search for factors such as the composition of the extracellular matrix and the capillary density, and other factors which may play a role in the distinct hippocampal lamellar organization.[12] A low correlation value show neuronal maturation rate or the cell combination of cell proliferation, death and maturation. Such a value is obtained in CA3 and CA2 sub fields of hippocampus. CA2 neurons In adult hippocampus are only 9.2% larger than CA3 neurons. Slight variations were observed in the present study. Zeidal et al (1996) [3] recorded 11% increase in the neuronal size of CA2 over CA3. CA2 neurons are large sized in early development could reflect long range axons activity as per Zeidal (1999) [14].

Neuron size, shape increased with brain weight bilaterally, the regularity in neuronal orientation was same bilaterally and the CA2 is greater than CA3 neuron. Increased in the neuron size affect happened to be left and right symmetrical. This will have implication for the development of functional asymmetry observed in adulthood, to the periods of vulnerability to disease or plasticity in normal growth.The thickness of various layers of adult hippocampus has been projected in histobar diagram to show the relation between various layers of hippocampus. (Fig. 8) Table 1 showed the maximum thickness of 552 µ of pyramidal cell layer in the age groups of 55 years and plexiform layer also incidentally showed an equal thickness of 552 µ which is a variation as the thickness of the plexiform layer is proportionally higher than that of the pyramidal cell thickness, since the plexiform layer is constituted by the synaptic network of dendrites, axons of pyramidal cells and granule cells. The polymorph layer is maximum in the age group of 67 years and its thickness is 1288 µ, which is normal. The pyramidal cell layer accounted for the thickness of 431 µ and plexiform layer accounted 552µ, which substantiated the earlier observations that the plexiform layer is always higher than that of pyramidal cell layer.

CONCLUSION

Light microscopic study of sections of hippocampus revealed a trilaminar cortex, having pyramidal cell layer in the middle a plexiform, polymorph layer on outside and inside of the pyramidal cell layer. The neurons that are present are pyramidal in shape and their size varied in different Cornu Ammonis sub fields of hippocampus.CA3 sub field has pyramidal cells 12 microns (maximum) to 9.6 microns (minimum).CA2 sub field has pyramidal cells 14.4 microns (maximum) to 9.6 microns (minimum).CA1 sub field has pyramidal cells 14.4 microns (maximum) to 9.6 microns (minimum).The density of cells in CAS per 1mm area is 100 where as in CA2 it is 100 and in CA1 the density is 90 cells. The thickness of polymorph layer ranged from 1288 microns to 920 microns. Dentate gyrus showed a trilaminar normal pattern. The granule cells are conspicuous by their small size and their diameter is 9.2 microns. The above indices almost tallied with the observations of D.W. Zaidel (1999) [14].

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Conflicts of Interests: None

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