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Cytoarchitecture of Human Foetal Hippocampus

Medical Science

KEYWORDS : Hippocampus, Dentate gyrus, Cornu Ammonis(CA), Cyto-architecture, Foetal.

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ABSTRACT

Background: *The hippocampus is empirically assumed to have different functions, of which the best known are: the representation of self-location in cognitive dimensions, and the storage and retrieval of memory.****Materials and methods****:Eighteen human foetal brains from 15 to 39 weeks 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 through binocular microscope using micrometers.* ***Results****: The lamellae of the hippocampus were cytometrically evaluated for thickness of various layers and neuronal sizes , form and density in different gestational periods .****Conclusions****: This study confirmed the existence of differences in nerve fiber distribution among the subfields of the hippocampus which is attributed to differences in the embryonic development processes and to the selectivity of the different connections. 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 form and density and thickness was examined. In correlation values between hippocampus sub fields in each size separately. For unknown reason in human neonates and in very young infants, hippocampal sub field neurons are qualitatively most differentiated form. CA3 neurons qualitatively close behind CA2 in form differentiation. CA1 neurons appear immature with poor differentiation rendering them difficult quantitatively.As explained in material and methods foetus brains processed and slides prepared. they were examined under microscopewith micrometer.At 15 weeks gestation showed a uniform cellular pattern, There is no distinction of the other layers. in 18 weeks gestation the hippocampus showed 3 zones i.e. ependymal, mantle, marginal zones. At full term it reaches adult hippocampus appearance with pyramidal layer molecular and polymorphic above and below. In the pyramidal layer there is a variation in the neuron size in CA3, CA2, CA1 sub fields. The neuron size in CA1 sub field showed 9.6 µ at 22 weeks, 14.4µ at full term. Dentate gyrusby full term 8 cell thick layer measuring 110.4µ has been observed.The plexiform and molecular layer though noticed at 20 weeks but their definite configuration is observed only at full term. The cells in the plexiform layer are scanty and small,*

INTRODUCTION

Hippocampus is phylogenetically one of the oldest parts of the brain; it present in floor of lateral ventricle in the temporal lobeof cerebrum and forms part of the limbic system. It consists of the complex interfolded layers of the dentate gyrus and cornu ammonis,[1] develops from morphogenetically discrete components that differ in their proliferative activity: the ammonic neuroepithelium and the primary dentate neuroepithelium.[2] Embryologically the hippocampus belongs to archicortex of cerebrum , phylogenetically allocortex of cerebrum undergoes slower organization and maturation than the cerebrum. [3] The prenatal development of human hippocampus According to Humphrey [4] before the 10th week of development, the dentate gyrus and cornu ammonis are thin rudimentary structures positioned successively along the posterolateral aspect of the diencephalon (the posteromedial wall of the lateral ventricle). The hippocampal sulcus appear by 10wk of gestational age , at 13-14 weeks unfolded hippocampus at medial wall of temporal lobe . Due to faster growth of neocotex results medial displacement and internal inversion of dentate gyrus , hippocampus and subicum regions.[5,6] At 15-16 weeks dentate and cornu ammonis start to infold by 18-20 weeks the dentate and cornu ammonis folded into temporal lobe projecting into lateral ventricle , the hippocampus and subiculum approximate each other across hippocampal sulcus , the hippocampal sulcus reoriented from vertical to horizontal.[6] The layers of hippocampus formed by 24-25 weeks,[7]at this age the developing hippocampus has acquired most characterstics of adult hippocampus but fusion of hippocampal sulcus continues until the 30th week [4,8] 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 layer encloses a portion of the pyramidal cell layer of the cornu ammonis [9].The hippocampus is trilaminar archicortex. It consists of a single pyramidal cell layer, with plexiform layers above and below it. It may best be divided into three distinct fields, CA1, CA2 and CA3 [10]. Field CA3 borders the hilus of the dentate gyrus at one end, and field CA2 at the other. Field CA3 pyramidal cells are the largest in the hippocampus and receive the mossy fibre input from dentate granule cells on their proximal dendrites. The whole pyramidal cell layer in this field is about 10 cells thick. The border between CA3 and CA2 is not well marked. The CA2 field has the most compact layer of pyramidal cells. It completely lacks a mossy fibre input from dentate granule cells and receives a major input from the supra mammillary region of the hypothalamus. By 8th month of intra uterine life double pyramidal cells are characteristically present in "Stratum pyramidale [11].The dendritic patterns of plexiform layer are not clearly seen. Stratum radiatum, Stratum lacuno - moleculare are having paucity of fibers and completion of the layers is seen only in post natal life. Paucity of cells of oriens also been observed. Hippocampal formation is composed of hippocampus dentate gyrus and subiculum. It is common to describe several strata within the layers of the hippocampus. Starting from the ventricular aspect, these are the alveus (contains subicular and hippocampal pyramidal cell axons converging on the fimbria of the fornix); stratum oriens (mainly the basal dendrites of pyramidal cells and some interneurones); stratum pyramidalis; stratum lucidum (contains mossy fibres which make contact with the proximal dendrites of pyramidal cells in field CA3); and the stratum radiatum and stratum lacunosum-moleculare. The stratum lucidum is not as prominent in man as it is in other primates, and is not present in fields CA1 and CA2.In the stratum radiatum and stratum oriens, CA3 and CA2 cells receive associational connections from other rostrocaudal levels of the hippocampus, as well as afferents from subcortical structures, e.g. the septal nuclei and supramammillary region. The projections from pyramidal cells of fields CA3 and CA2 to CA1, often called Schaffer collaterals, also terminate in the stratum radiatum and stratum oriens. The projections from the entorhinal cortex to the dentate gyrus (the perforant pathway) travel in the stratum lacunosum-moleculare, where they make synaptic contact *en passant* with

the distal apical dendrites of hippocampal pyramidal cells. Number of principal neurons in the subdivisions of human hippocampal formation are granular cells in dentae gyrus 18 millions,at hilus 1.72 millions,at CA32.83 milllions , CA1 14 miilions and subuculum 5.95millions, west et al 1994 [12]. At entorhinal cortex 8.1 miilion west and slomianka 1998 [14] .The functions of hippocampus kept on getting modified till recently . neuronal structure of hippocampus essentially is plastic with properties of undergoing consistent changes depending on the past experience of organism.[15] The hippocampus is largely epileptogenic[16] . This type of different cytoarchitectural constitution by the hippocampus coupled with the different functional aspects and neuronal mechanisms that are present within the hippocampus in different mammals and sub mammalian forms are available plenty in the literature. However lacunae in the study of human hippocampus and its cytoarchitectural studies have prompted this author to undertake the study of microscopic structure of foetal hippocampus.

MATERIAL AND METHODS

The present work is the result of study of human foetal brains. The human material of this work comprises of the specimen of 18 fetuses obtained from The Department of gynecology from 1999 to 2002 AMC Visakhapatnam. fetus brains were obtained from 1999 dissecting fetusus. The the gestational age ranged from 15 weeks to full term as judged by the Crown Rump length, the dead foetuses obtained from department of Obstetrics were perfused with 10% formalin and foetal brains were fixed by the injection of 10% formalin through the anterior frontanellae. Perfect hardening of human foetal brains took a little longer time inspite of taking all precautions most probably due to the non-availability of sufficient of neuroglial tissue. The brains were removed as per dissection procedure and stored in 10%formalin.The hippocampus has been identified in the inferior horn of lateral ventricle and hippocampus along with the fornix were removed and stored in 10% formalin 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 cederwood 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 haemtoxyline and eosine 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 foetal brains after taking the morphometric measurements has been subjected to histological staining procedure, described above and studied for the following features :

Various stratification's in Hippocampus

1. Thickness of each layer and architecture of the cells present in each layer.
2. Cell count in each layer.
3. For various measurements the eye piece and micrometer scale were used.

STANDARDISATION OF MICROMETER SCALE :

a) In low power 10X10 magnification

Hundred divisions of eye piece scale are equal to 92 divisions of micrometer scale i.e. 0.92mm.
1 division is equal to or 9.2 microns.0092mm.

b) In High power 10X40 magnification

Hundred 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

OBSERVATIONS

Sections of foetal hippocampus have been subjected to microscopic study and the following observations have been made. Foetal hippocampus when examined under microscope at 15 weeks gestation showed a uniform cellular pattern, which is very dens, and, a part showed ependymal zone. There is no distinction of the other layers.(Fig. No.2) in 18 weeks gestation the hippocampus showed 3 zones i.e. ependymal, mantle, marginal zones. The configuration of the mantle zone does not show any differentiation. The marginal zone is having scanty cell having more of fibres. Thickness of these zones could not be made out due to irregularity and dis uniformity of mantle zone (Fig.No.3).The individual layers namely pyramidal cell layer, plexiform layer polymorphic layer could be made out from layer at 20 weeks onwards. The plexiform layer at 20 weeks is 140 μ, at 24 weeks 150 μ,32 weeks750 μ and at full term it is1024μ. The pyramidal cell layer in thickness at 20 weeks is 184 μ, at 24weeks 276 μ, at 32weeks 428 μ and at full term534 μ. The polymorphic layer at 20 weeks is 264 μ, at 24 weeks 322 μ, at 32 weeks 782 μ and at full term736 μ.(Table.1).In the pyramidal layer there is a variation in the neuron size in CA3, CA2, CA1 sub fields.The neuron size in CA3 is 7.2 μ at 22 weeks (Fig. No.14), 9.6 μ at full term. The neuron size in CA2 sub field showed 7.2 μ at 20 weeks, 12 μ at full tern. The neuron size in CA1 sub field showed 9.6 μ at 22 weeks, 14.4μ at full term.(Table.2) (Fig.2 (1.2.3). The pyramidal cells present in sub field of cornu Ammonis in different age groups of foetusus is as follows, at 22 weeks field showed density of 170 cells per 1mm, CA2 field showed density of 180 cells per 1mm, CA1 field showed density of 140 cells per 1mm (fig.13).At 24 weeks CA3 field showed density of 200 cells per 1mm, CA2 field showed density of 240 cells per 1mm, CA1 field showed density of 160 cells per 1mm (Fig. No15).At 32 weeks CA3 field showed density of 130 cells per 1mm, CA2 field showed density of 140 cells per 1mm, CA1 field showed density of 150 cells per 1mm (Fig). 20 weeks onwards. The At full term CA3 field showed density of 100 cells per 1mm, CA2 field showed density of 100 cells per 1mm, CA1 field showed density of 130 cells per

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1mm (Fig.). There are no double pyramidal cells any of these sub field hippocampus noticed. Dentate gyrus does not show any differentiation up to 20 weeks; at 20 weeks the granular layer is fully differentiated; the thickness the thickness of granular cell layer is 18.4µ. The thickness is increaseed in size gradually, at full term the thickness 110.4µ in size. At 20 weeks, 2-3 cell thick cellular layer has been observed at commencement of 20th week whereas by full term 8 cell thick layer measuring 110.4µ has been observed. (Table No I).The plexiform and molecular layer though noticed at 20 weeks but their definite configuration is observed only at full term.The cells in the plexiform layer are scanty and small, no attempt has been made for numerical counting.

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. For unknown reason in human neonates and in very young infants, hippocampal sub field neurons are qualitatively most differentiated form. CA3 neurons qualitatively close behind CA2 in form differentiation. CA1 neurons appear immature with poor differentiation [14]rendering them difficult quantitatively. It has been established by Zeidal et al (1997)[16] that asymmetric neuronal somata are present particularly in sub field CA2 of developing hippocampus.As the neuron in CA3 sub field grow larger the density declines an outcome that suggest the maturation of neurons.This has been substantiated in Table II, Fig No l. The thickness of CA3 9.6µ whereas the density of cells is 100 per 1mm. The density of cells has been decreased as the foetus grows in age because of maturation of neuron. Zeidal D.W[17]. (Anatomical record 254-87-91 1999). It is widely accepted proliferation and programmed cell death precede all in early development the cell maturation.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 variation that is observed in the present study. Zeidal et a! (1996) recorded 11% increase in the neuronal size of CA2 over CA3. A comparative study of various layer of foetal hippocampus of various gestational age groups (Table.1.) showed that plexiform layer has attained maximum thickness at 28 weeks onwards and it remained stationary at full term also. There is a slow ascendancy in the thickness of pyramidal cell layer from 23 weeks gestation onwards and 33 weeks and the plexiform and pyramidal cell layer remained stationary almost 33 weeks have seen the maximum. At full term the plexiform layer has outgrown the pyramidal cell layer. This is in conformity with observations of D.W. Zeidal (1997).[16]

CA2 neurons are large sized in early development could reflect long range axons activity as per Zeidal (1999)[17].

SUMMARY AND CONCLUSION

The light microscopic study of hippocampus at 15 weeks gestation showed a uniform cellular pattern without any distinctions of layers.At 18 weeks gestation the hippocampus showed ependymal, mantle and marginal zones. It is trilaminar cortex having plexiform, pyramidal and polymorph layers can be made out from 20 weeks on wards. At 20 weeks of gestation the plexiform layer is 140 microns and full term it is 1024 microns. The pyramidal cell layer is 184 microns thicker at 20 weeks and 534 microns at full term. The polymorph layer is 264 microns thickness at 20 weeks and 900 microns at full term. The neuron size in pyramidal layer varied in CA3, CA2 and CA1 sub fields of hippocampus.The neuron size of CA3 sub field at 20 weeks is 7.2 microns and at full term it is 9.6 microns. The neuron size of CA2 sub field at 20 weeks is 7.2 microns and at full term it is 12 microns.The neuron size of CA1 sub field at 20 weeks is 7.2 microns and at full term it is 14.4 microns. The pyramidal cell density in CAS sub field at 22 weeks is 170 cells per 1 mm; CA2 field showed density of 180 cells per 1 mm, CA1 field showed density of 140 cells per 1m.m.There is a decrease in cell density in CA3 and CA2 sub fields at full term that is because of maturation cells at full term.Dentate gyrus does not show any differentiation upto 18 weeks of gestation. At 20 weeks the granular cell layer is fully differentiated and it is 18.4 microns in thickness.The thick ness of granular layer is increased abruptly by 23 weeks of gestation and at full term the thick ness is 110 .4 microns in size.The above conclusions have been in agreement with the works taken up by the earlier authors.

FIG. 1 : Dissected human foetuses Hippocampus in brain at 20 weeks and fullterm

FIG. 2 : MICROPIC PICTURES OF HIPPOCAMPUS AT 15, 18, & 22 WEEKS GESTATION PERIOD AS FIG 1, 2, 3

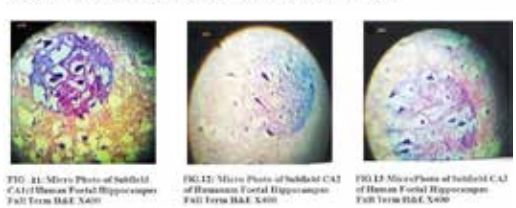

Table .1. Thickness of various layers of foetal hippocampus

Age in weeks	Hc. Pl.	Hc. Py.	Hc. Po.	D. Gc.
20	140	184	264	18
22	148	230	270	18
24	150	276	322	36
26	188	340	380	74
27	214	368	456	74
28	300	380	756	74
30	664	396	764	74
31	680	414	776	74
32	750	428	782	90

33	768	442	800	92
34	800	460	840	92
35	940	484	850	96
36	950	500	856	100
37	962	515	860	110
38	980	512	882	110
39	1024	534	900	110

Hc .Pl. Hippocampus plexiform layer: Hc .Pl. Hippocampus pyramidal layer
Hc .Po. Hippocampus polymorphic layer: D. Gc Dentate granular cells

Table. 2: Neuron size in Cornu Ammonis (CA)

Age in weeks	CA3	CA2	CA1
20	7.2	7.2	7.2
22	7.2	7.2	9.6
24	9.6	12	12
26	9.6	12	12
27	9.6	12	12
28	7.2	9.6	12
30	7.2	9.6	12
31	9.6	12	12
32	12	12	12
33	9.6	12	14.4
34	9.6	12	14.4
36	9.6	12	14.4
37	9.6	12	14.4
38	9.6	12	14.4
39	9.6	12	14.4

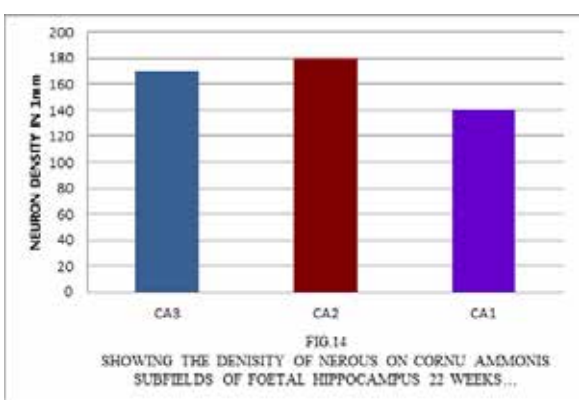

FIG.14
SHOWING THE DENISITY OF NEROUS ON CORNU AMMONIS SUBFIELDS OF FOETAL HIPPOCAMPUS 22 WEEKS...

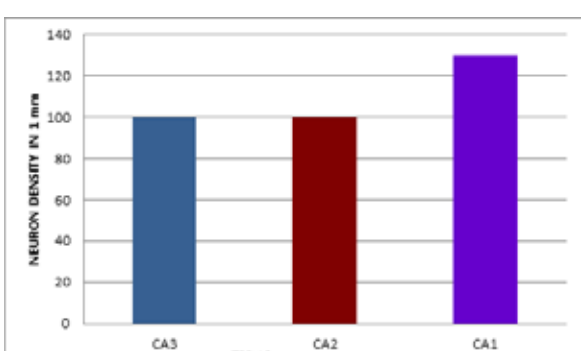

FIG.15.
SHOWING THE DENISITY OF NEROUS ON CORNU AMMONIS SUBFIELDS OF FOETAL HIPPOCAMPUS 39 WEEKS GESTATIONAL AGE

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