Research Article

Prenatal and Postnatal Development of the Rat Hippocampus (Histological Study)

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Abstract
The differentiation of the hippocampus starts in the subiculum and proceeds towards the Ammon's horn and the dentate gyrus is the last structure to be formed. Lamination of the subiculum starts on E17 and progress to the Ammon's horn on E18; CA1 is formed, while the CA3 is not completely developed. On E19, the CA3 is completed with formation the ectal limb of dentate gyrus while the endal limb is still unformed and appears as a ball-like mass of cells. Between E20-P1, Ammon's horn shows agreat increase in length as acurved C-shape structure. The endal limb of dentate gyrus appears prenatally and develops rapidly to become morphologically similar to the ectal limb by P7. During the first month of development, there are increases in length of Ammon's horn, dentate gyrus size and size of both pyramidal cells and granule cells. In adult, the length of Ammon's horn is markedly large and the hippocampal subfields are prominent.
Key Words: Development, Hippocampus, Ammon's horn and dentate gyrus

Introduction
Pallium (the cerebral cortex) coats the surface of cerebral hemispheres. The cerebral cortex is divided according the development level, structure and function into six- layered neocortex and three-layered allocortex. The hippocampus is the best example for allocortex. The periallocortex or mesocortex are used to mark the transitional zone between the neocortex and allocrtex[8]**.**

The hippocampus was first described by Arantius (1587) in his book "De HumanoFoetu" the structure in the temporal horn of the lateral ventricles that looks likehippocampus (sea horse) or bombycinusvermiscandidus (white silk worm),[15].

In **1911**, **RamónyCajal** and **lorente de Nó**used Gogi's technique in studying the cellular architecture of hippocampal formation[9,14].

In **1937, Papez** described his circuit and the hippocampus was one of the structures integrated in[12]. He suggested that it might constitute the circuit of emotion. In 1952 Maclean named this circuit the "limbic system"**[10].**

This study aimed to identify the morphological changes of both cornuAmmonis and dentate gyrus in consequent ages during embryological and postnatal development and analyze these differences in a functional context. It is necessary to have the anatomical base of this region by simple microscopic study of the rat hippocampus using rapid and easy method (H & E).This method can identify layers, pyramidal cells of cornuammonis and granule cells of dentate gyrus.

Materials and methods
Twenty four pairs of male and female albino rats were used in the present study. The rats were put in separate cages, each contained one male and one female rat. They were kept for mating for 24 hours. The next day, female rats were examined for vaginal plug of semen.The first day of conception was determined if the vaginal plug is positive and considered to be E1.Pregnant female rats were kept separate in cages and were followed up for pregnancy dating. From E16-E21, two or

more pregnant rats were taken for each day of age, and then sacrificed by decapitation. The abdomen and the uterus were opened up, and then the embryos were removed, heads were separated and injected by Bouin fixative through making pores at the margin of skull cap.From postnatal ages P1, P7, P14, P21, P30 and >P90 (adult), three rats were taken for each age and sacrificed by decapitation. The skulls were opened and the brains were extracted for removal of hippocampus in the following steps:

1) In order to expose the hippocampus we need to remove the cerebral cortex covering it. The first incision is at the end of the hemisphere; the incision should be about 0.7mm deep for adult mouse that not to hurt the hippocampus while to expose it (the incision depth is reduced to 0.2 mm in one day old rat). The 2nd incision is about 1.5-2mm in front of the first one, this incision we cut into the lateral ventricle, both of the incisions go to the ventral of the brain and meet there. This piece of cortex is free, after pulling it up, we will see the hippocampus.
2) On the other side of the brain, both the sides of the cortex arepulled up that covering the hippocampus along the ventricle. Now we can see the dorsal part of the hippocampus.
3) The rest of the hippocampus is separated from the cortex covering it along the surface of the hippocampus towards the ventral part of the hippocampus.
4) The hippocampus picked out from the brain.
   Either the head (E16-E21), or the hippocampus (P1-adult), was kept in Bouin's fixative for 24 hours, and then transferred to 10% neutral formalin. All specimens were embedded in paraffin. Serial sections (6 µm) were prepared in the coronal plane for Haematoxylin and Eosin staining.

Results

1) Prenatal ages: (Fig. 1- 4)

The hippocampus proper starts to appear by the 16th day of intra-utrine life and its development would be following up to reach the final appearance.It is formed of two major parts, the cornuammonis and the dentate gyrus. Both structures are curved; the terminal part of cornuammonis is directed into the concavity of dentate gyrus; (the hilum).

The coruammonis appears as a column of less densely packed, randomly oriented cells lie adjacent to the tightly packed, radially oriented neuro-epithelium. The subfields of cornuammonisare difficult to be identified. The boundary between the subiculum and conruammonis is not recognizable. The cells of cornuammonis appear small with small deeply pigmented nuclei.

The dentate gyrus starts to appear as a collection of young nerve cells migrating from the neuro-epithelium near the outgrowth of the chorid plexus to a subpial area in the concavity formed by the neuro-epithelium of cornuammonis at its ventral end.

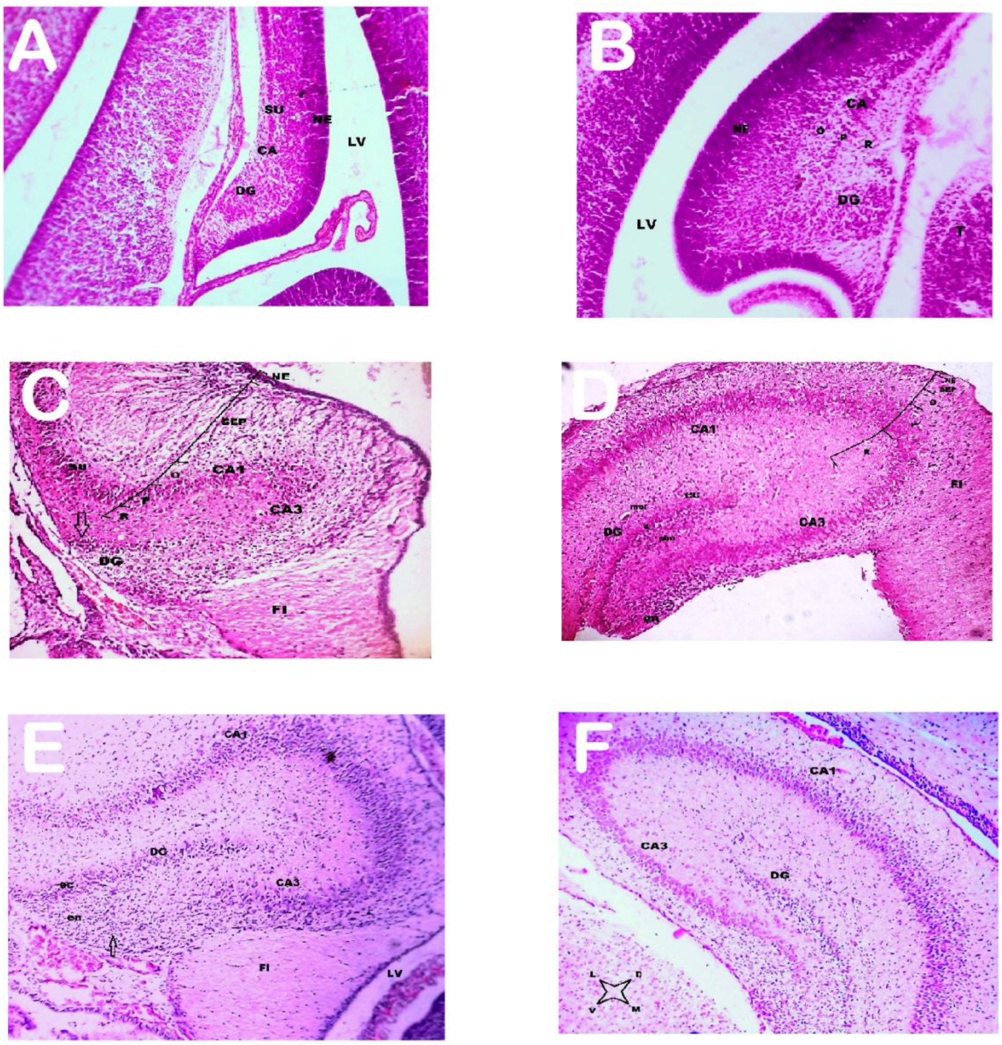
On E17, the coruammonis is seen to make more arching towards the lateral ventricle and temporal cortex, the stratum oriens and stratum radiatum appear to be formed but they are thin. The cells appear small and rounded with deeply pigmented nuclei.

The dentate gyrusarea appeared larger than the previous age. Cells of the dentate gyrus are closely packed in a ball- like subpial mass. The limbs are not formed yet.

On E18, the cornuammonis appears as a small C- shaped structure, bulging laterally, towards the cavity of lateral ventricle, making two limbs, the prominent superior limb is continuous with the cortex is called CA1 and faint, uncompleted inferior limb growing towards the hilum of dentate gyrus is called CA3. The fimbria rises below the point of meeting between the two limbs of CA. The hippocampal subfields are well apparent, from ventricular side outwards; neuro-epithelium, large sub-ependymal zone, stratum oriens, pyramidal layer and stratum radiatum. The thickness of neuro-epithelium is apparently diminished than the previous age with appearance of large sub-ependimal zone containing radially oriented neuroblasts towards the CornuAmmonis. The sub-ependymal zone is present throughout the length of hippocampus, larger towards the subiculum than towards Ammon's horn. The cells acquire the characteristic pyramidal appearance starting by this age, with apical dendrites towards the stratum radiatum. Cells of CA1 are small, forming more layers than CA3, nuclei are vesicular. Cells of CA3 are larger than CA1. The dentate gyrusappears as larger mass of cells with starting formation of ectal limb of the granular cell layer that appear superficial to the dentate hilus, less dense with deep nuclei and their apical dendrites extending towards the molecular layer. The remaining cells of the dentate gyrus below the hilus are densely packed with deep nuclei.

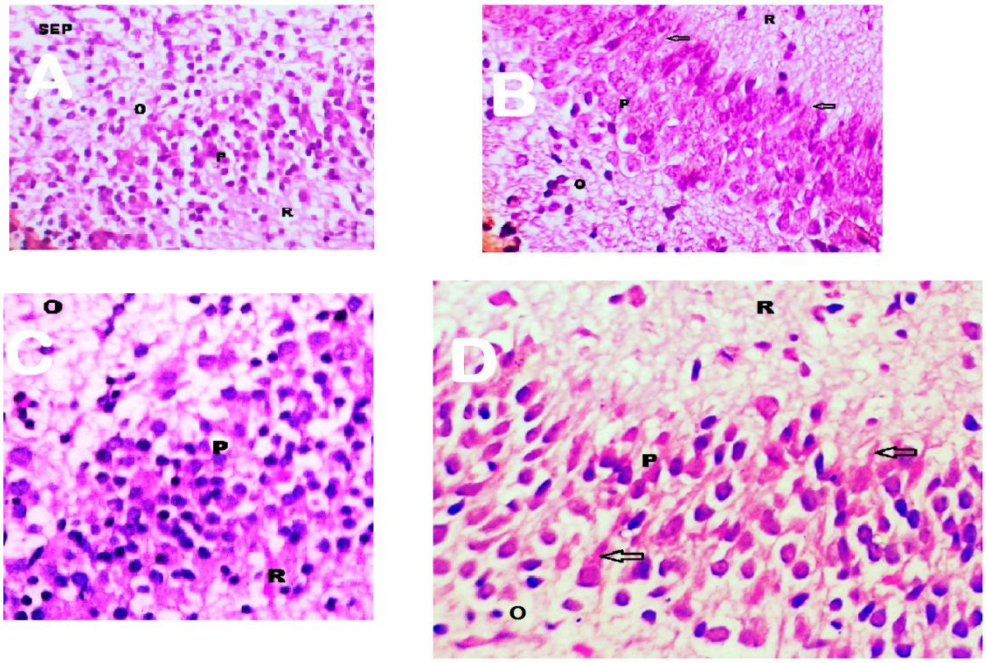
Fig. 1

PRENATAL AGES



light micrograph of coronal sections of embryos of albino rat. A: 16 days , B: 17 days, C: 18 days, D: 19 days, E: 20 days, F: 21 days, Cornu Ammonis: CA1 & CA3, dentate gyrus: DG, subiculum: SU, neuroepithelium: NE, subependymal zone: SEP, lateral ventricle: LV, fimbria: FI, stratum oriens: O, stratum radiatum: R, stratum pyramidale: P, ectal limb: ec, endal limb: en, polymorphic layer: plm, granular layer: g, molecular layer: mol. (H & E×100)

Fig. 2



light micrograph of CA1, showing the prenatal changes, A: 18 days, B: 19 days, C: 20 days, E: 21 days. Stratum oriens: O, stratum pyramidale: P, stratum radiatum: R.
Apical dendrites are marked by arrows (H & E ×400)

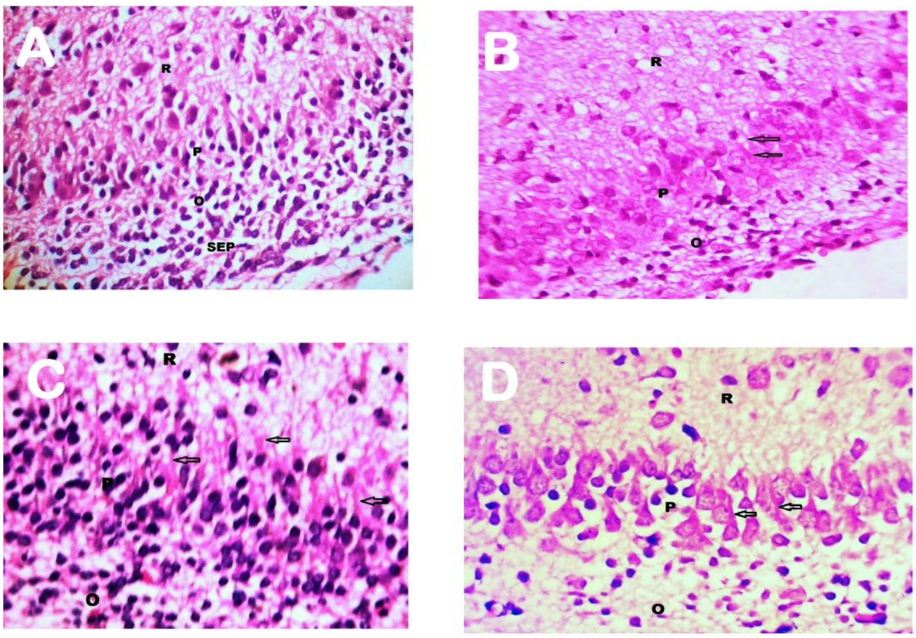
On E19, the cornuammonis appears larger C- shaped structure. The CA3 part is completed towards the dentate hilus. The subfields of CA could be seen with great apparent change in thickness, the neuro-epithelium layer is thin, sub-ependymal layer is contracted, pyramidal layer is thickened and stratum radiatum is markedly enlarged. The dominant neurons in the hippocampus proprius are the pyramidal cells. The size and the density of these neurons are variable throughout the CA. pyramidal cells show marked increase in apparent size than the previous age. Cells of the pyramidal layer of CA1 arranged from 5 to 7 layers, with prominent apical dendrites that extended to stratum radiatum and stratum lacunosum. The nuclei are vesicular with prominent nucleoli. Cells of CA3 are arranged from 3 to 5 layers, larger in size with thicker apical dendrites. The dentate gyrus is formed of two limbs; the supra-pyramidal (ectal) limb, which appears more developed; cells are arranged in layers and larger than the newly formed infra-pyramidal (endal) limb which appears as a collection of densely packed cells. The chief cells of the dentate gyrus are the granular neurons and so, the 3 layers of dentate gyrus are apparent in the supra-pyramidal limb, the most superficial layer is the molecular layer; the next granule cell layer in which granule cells are rounded with pale nuclei, and the polymorphic layer that related to dentate gyrus hilum and characterized by presence of large Mossy cells. The cells of infra-pyramidal limb are smaller, dispersed and have deeply pigmented nuclei, the part of CA3 grows towards the dentate gyrus hilum, pyramidal cells appeared to have indistinct outlines of cells with very pale nuclei (may be a sign of cell division activity).

On E20, the cornuammonis increased in its length that made more space between two limbs of its C- shape and made more lateral bulging and another new superior bulge at CA1. The pyramidal cells of CA1 are small with prominent apical dendrites directed to stratum radiatum. The nuclei are vesicular with prominent nucleoli. The cells of CA3 are larger in size with thicker apical dendrites than CA1 cells. The dentate gyrus is more developed, the infra-pyramidal limb of dentate gyrus shows slight increase in its length, but it is not fully developed. The outer molecular layer of supra-pyramidal (ectal) limb of dentate gyrus is apparent well, and formed mainly by dendrites of the granule cells. The granule cells are small, rounded with little cytoplasm and have deeply pigmented nuclei, while the cells of infra-pyramidal (endal) limb are smaller in size and dispersed.

On E 21, the cornuammonis shows more increase in its length that made more separation between two limbs of C- shape. The cells of the pyramidal layer of CA1 have prominent apical dendrites. The nuclei are vesicular with prominent nucleoli.The cells of CA3 are arranged in less layering than in CA1, they are larger in size with thicker apical dendrites.

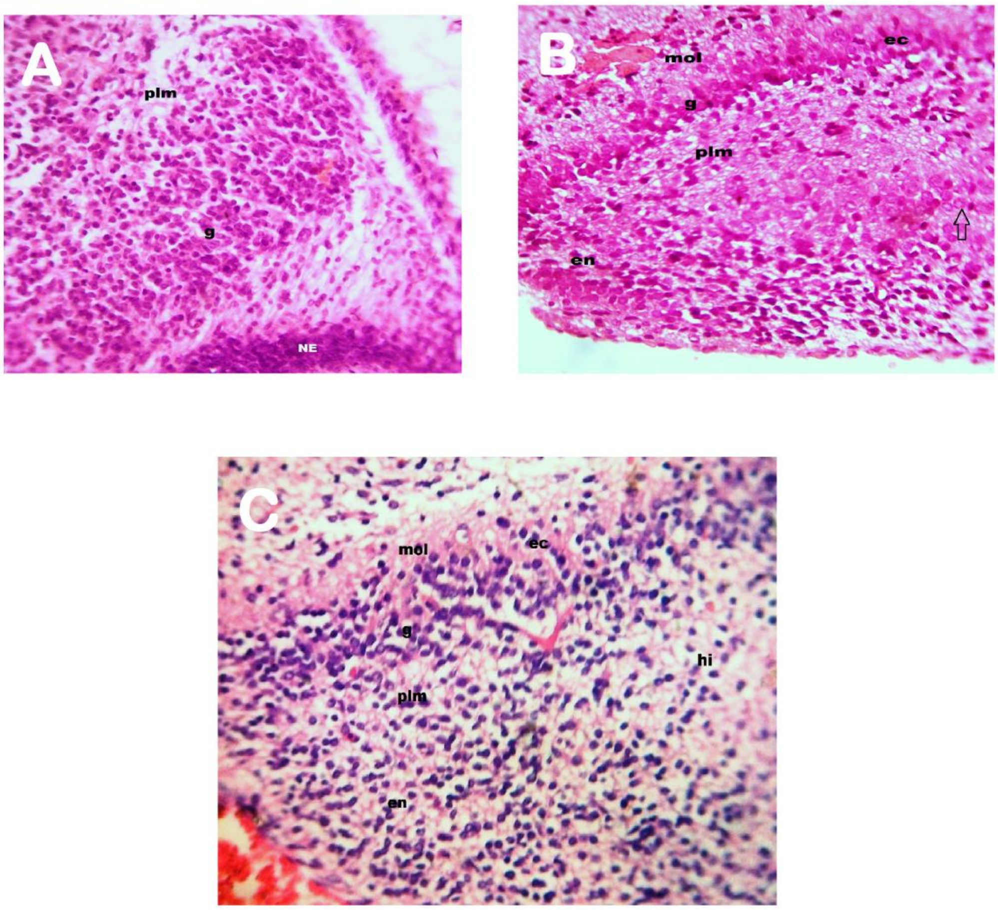
The infra-pyramidal limb of dentate gyrus shows slight increase in its length. The cells of supra-pyramidal (ectal) limb appear to have vesicular nuclei with more prominent apical extensions towards the molecular layer. The cells of the infra-pyramidal (endal) limb appear smaller in size with deeply pigmented nuclei.

Fig. 3



light micrograph of CA3, showing the prenatal changes. A: 18 days, B: 19 days, C: 20 days, D: 21 days. Stratum oriens: O, stratum pyramidale: P, stratum radiatum: R. Apical dendrites are marked by arrows (H & E × 400)

Fig. 4



Light micrograph of dentate gyrus. Prenatal ages: A: 18 days, B: 19 days, C: 21 days. ectal limb: ec, endal limb: en, molecular layer: mol, granular layer: g, polymorphic layer: plm. (H & E × 400)

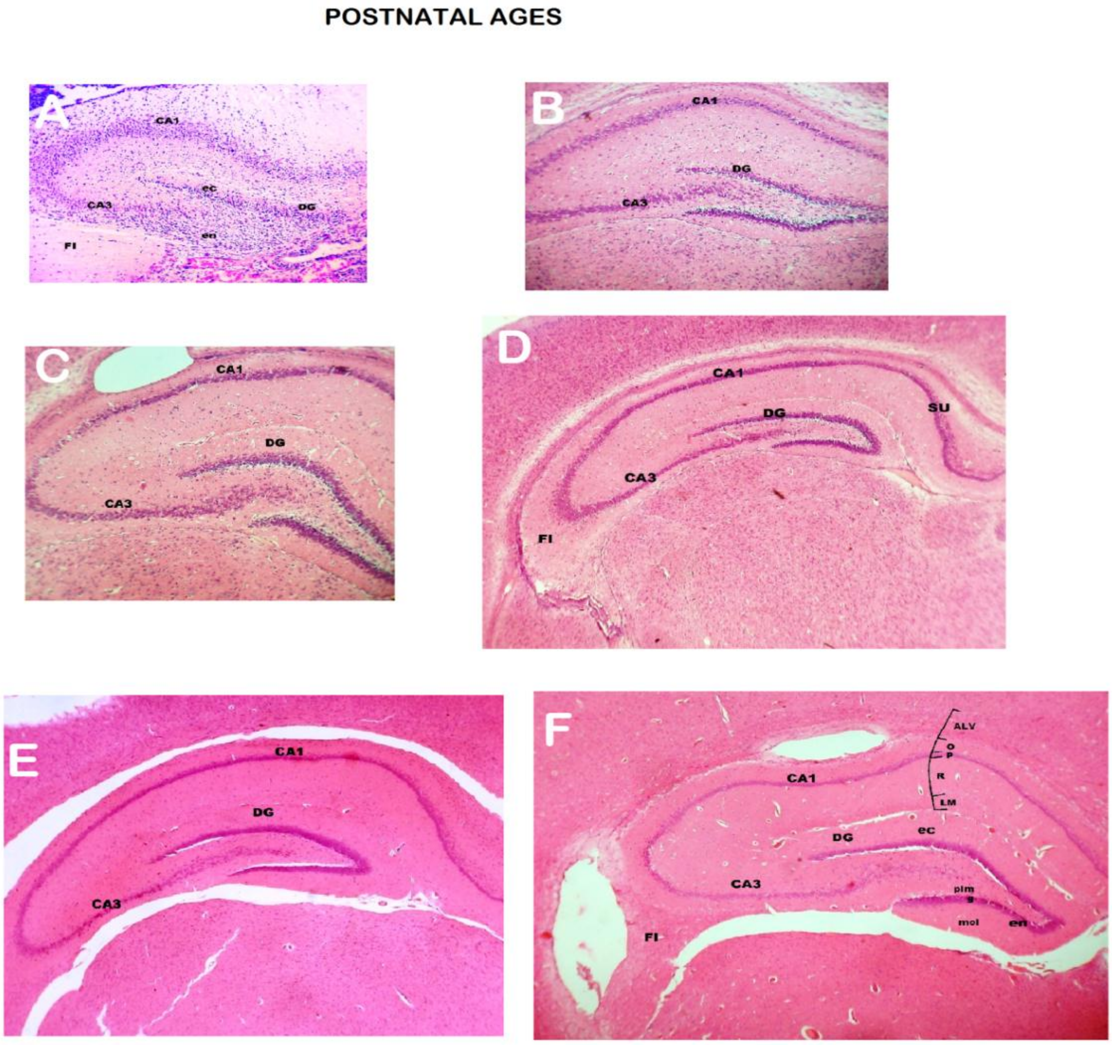
2) Postnatal ages:

On the first day of life, the cornuammonis shows more increase in its length than the age of the last day of intra-uterine life that made more bulge into the cavity of lateral ventricle. The pyramidal cells of CA1 are smaller in size than CA3 cells with prominent apical dendrites. The nuclei are vesicular. The pyramidal cells of CA3 are larger in size with thicker apical dendrites. The infra-pyramidal limb of dentate gyrus is well formed than the previous age. Granule cells of supra-pyramidal limb are rounded with little cytoplasm with rounded nucleus and dendrites directed towards the superficial molecular layer of dentate gyrus. Cells of infra-pyramidal limb appear more dispersed, with more vesicular nuclei.

During the first week of life, the cornuammonis shows great increase in its length and more lateral bulging into the cavity of lateral ventricle. The pyramidal cells of CA1 show apparent increase in size than the previous age with more prominent apical dendrites. The nuclei were vesicular with prominent nucleoli. The pyramidal cells of CA3 near to the hilum of dentate gyrus show more increase in size. Most of cells had large vesicular nuclei with prominent nucleoli and thicker apical extensions. The infra-pyramidal of dentate gyruslimb is well developed. Granule cells appear crowded. The cells towards the outer molecular layer are larger with vesicular nuclei and double nucleoli appearance, while the basal cells towards the polymorphic layer appear small in size with deeply stained nuclei. Large, fusiform basket cells are present at the polymorphic layer, which are more numerous at the ectal limb than the endal one.

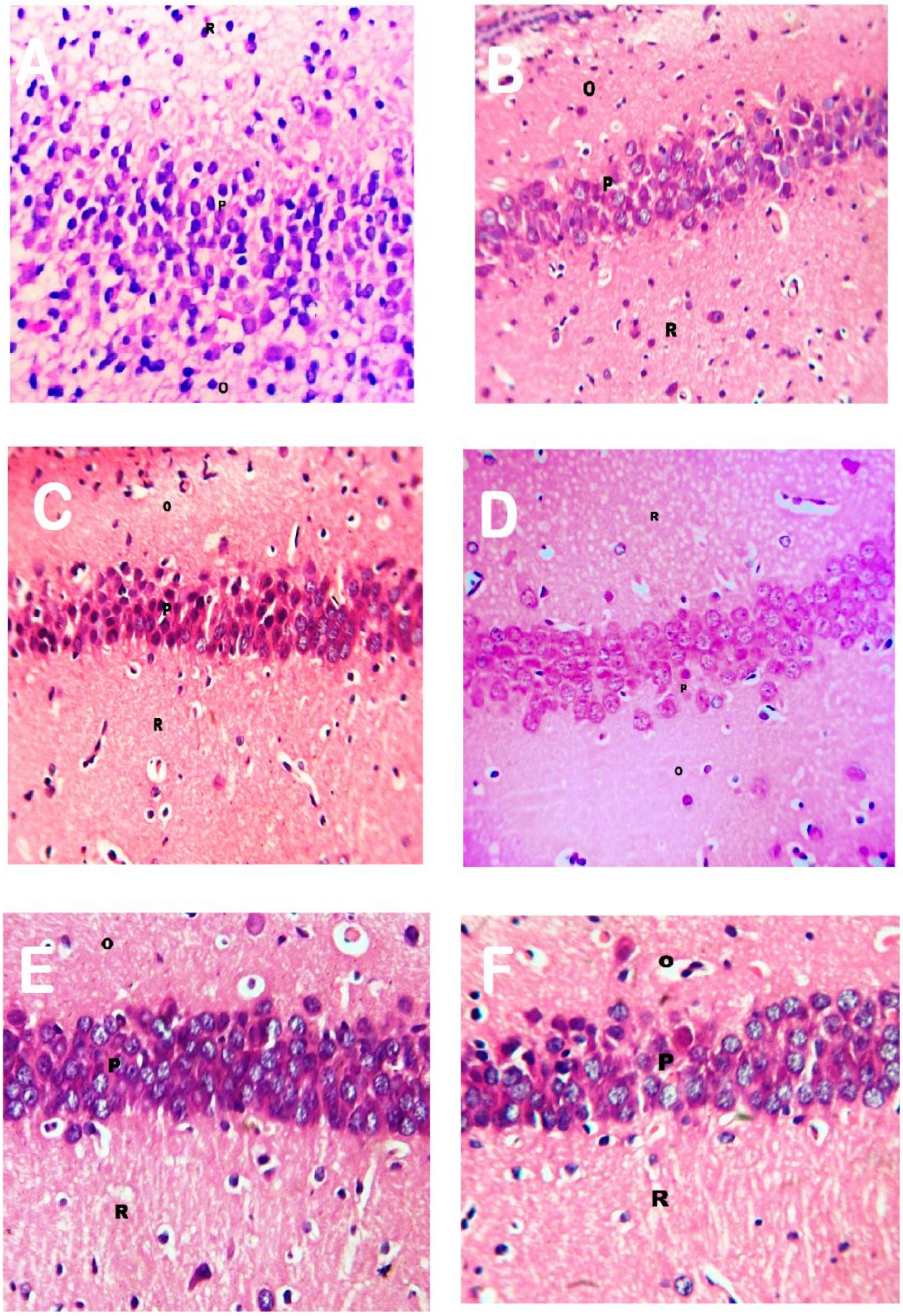
apparently increased size than the previous age and appeared to be more spherical in shape. The nuclei are vesicular with prominent nucleoli. Cells of CA3 are arranged from 3 to 5 layers, show more increase in size with large vesicular nuclei, prominent nucleoli and thicker apical extensions. Most of nuclei of these cells showed deeply pigmented chromatin is lets. The dentate gyrus cells show an apparent increase in size than the previous age, the

Fig. 5



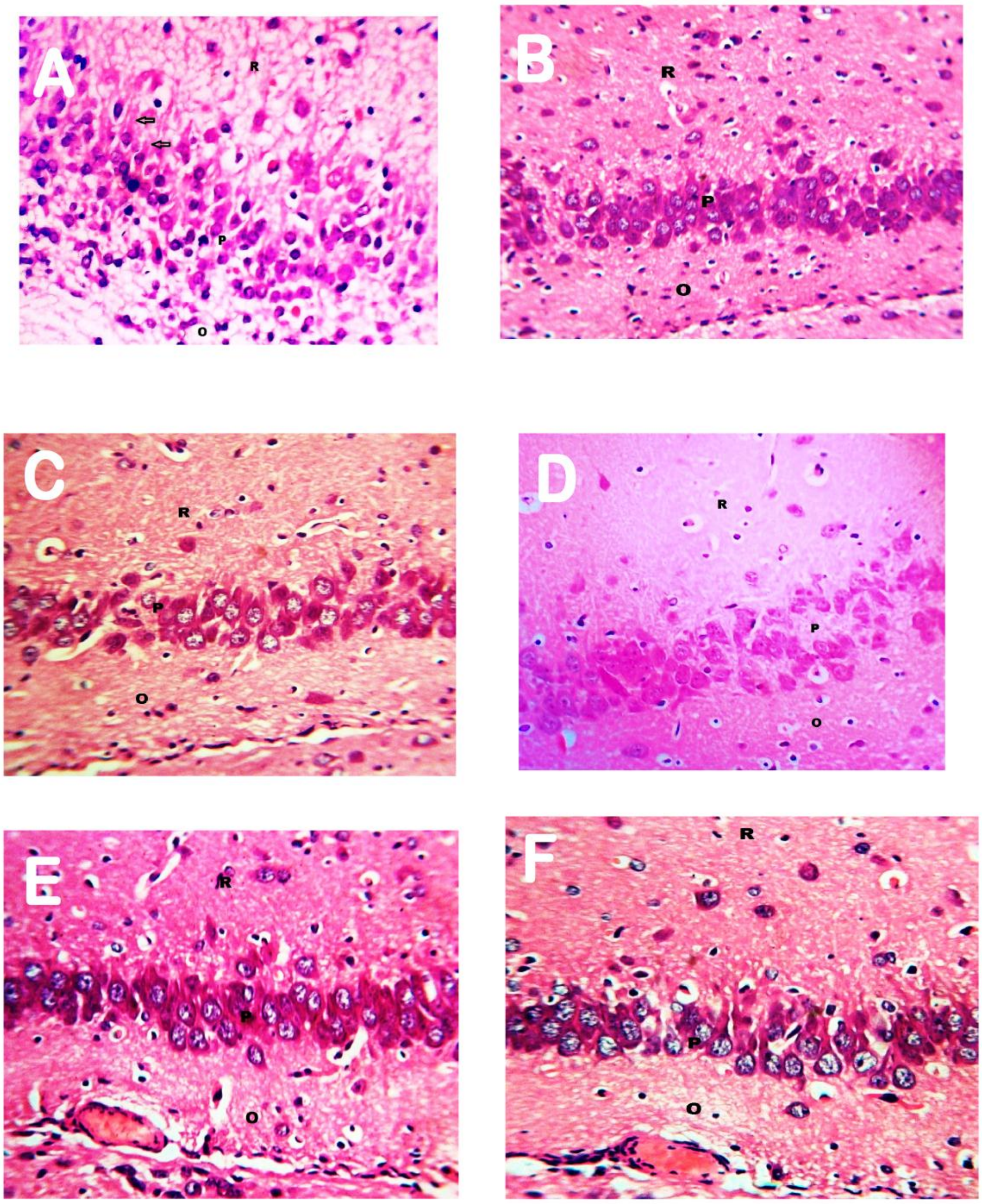
light micrograph of coronal sections of hippocampus. A: one day, B: one week, C: two weeks, D: three weeks, E: one month, F: adult age. Cornu Ammonis: CA1 & CA3, dentate gyrus: DG, subiculum: SU, fimbria: FI, alveus: ALV, stratum oriens:O, stratum pyramidale: P, stratum radiatum: R, stratum lacunosum moleculare: LM, ectal limb: ec, endal limb: en, polymorphic layer: plm, granular layer: g, molecular layer: mol. (H & E × 100)

Fig. 6



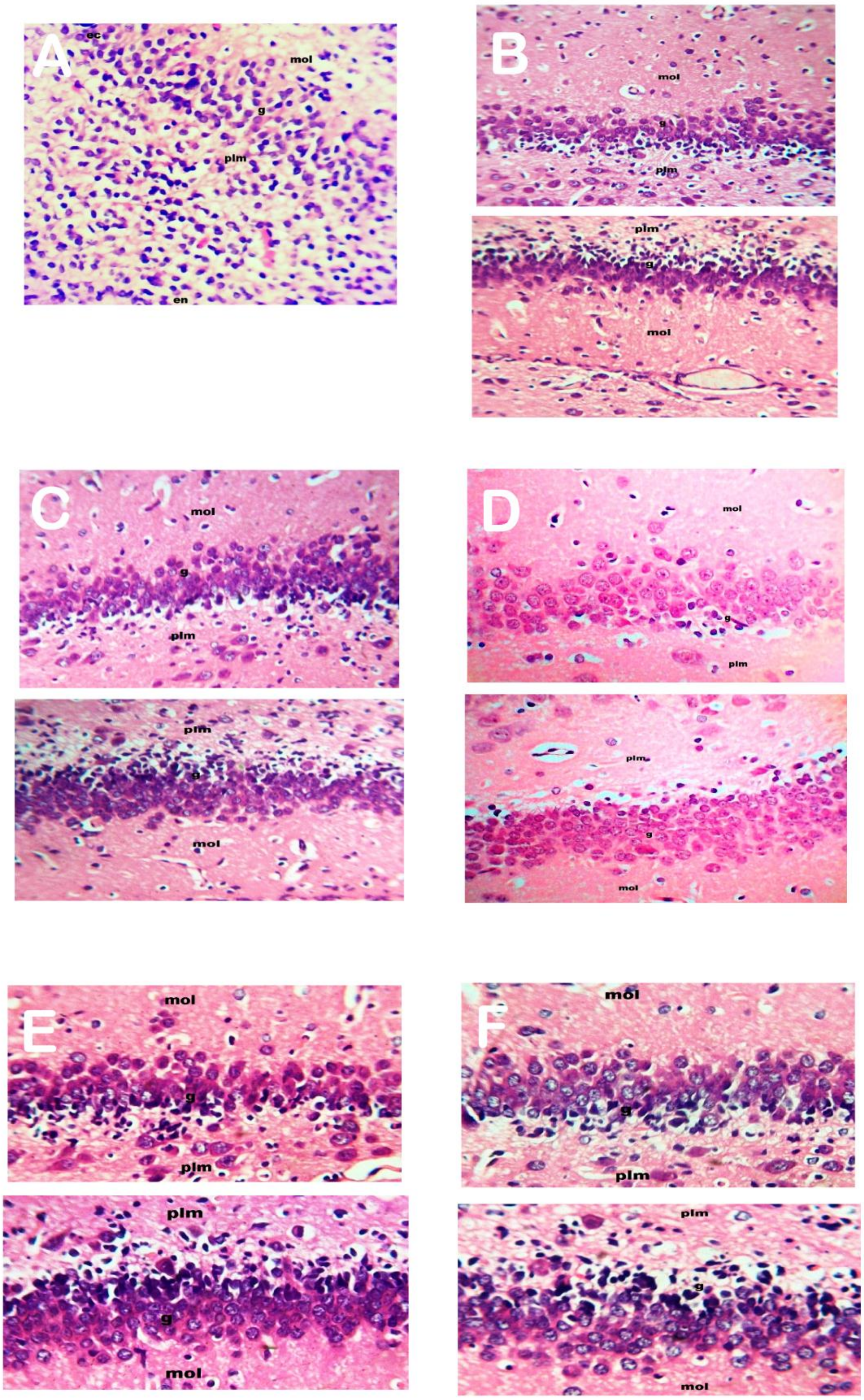
light micrograph of CA1,showing the postnatal changes; A: one day of age, B: one week of age, C: two weeks of age, D: three weeks of age, E: one month of age, F: adult age. Stratum oriens: O, stratum pyramidale: P, stratum radiatum: R. (H & E×400)

Fig. 7



Light micrograph of CA3, showing the postnatal changes, A: one day of age, B: one week of age, C: two weeks of age, D: three weeks of age, E: one month of age, F: adult age. Stratum oriens: O, stratum pyramidale: P, stratum radiatum: R. (H & E ×400)

Fig. 8



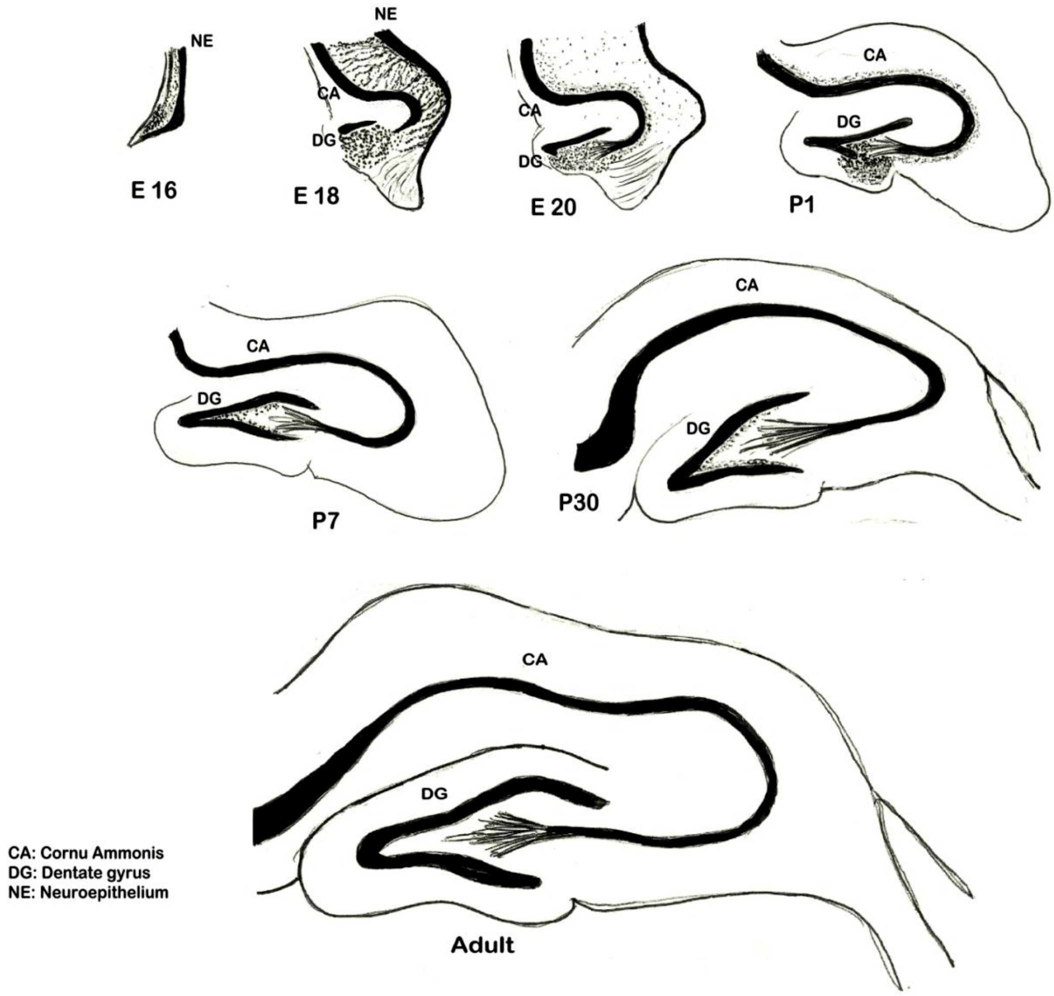
Light micrograph of dentate gyrus, postnatal changes, A: one day, B: one week, C: two weeks, D: three weeks, E: one month, F: adult age. The upper figures for ectal limb & the lower figures for the endal limb. molecular layer: mol, granular layer: g, polymorphic layer: plm

(H & E ×400)

On the third week of life, the cornuammonis increases in its length more than the previous age that made more external bulging. The pyramidal cells of CA1 part are rounded in shape. The nuclei are vesicular with prominent nucleoli. The pyramidal cells of CA3 show apparently more increase in size with large vesicular nuclei, prominent nucleoli and chromatin islets. The dentate gyrus with its both limbs showed more increase in length, granule cells of endal limb were apparently smaller and more crowded than ectal limb. Number of granule cell layers in endal limb are from 7 to 10 while in ectal limb are from 4 to 7 layers with appearance of multiple chromatin islets in their nuclei giving the double nucleoli appearance.

By the first month of age, the cornuammonis greatly increase in size. Cells of the pyramidal layer of CA1 part are crowded and rounded in shape. The nuclei were vesicular with prominent nucleoli. Pyramidal cells of CA3 show apparently more increase in size with large vesicular nuclei, prominent nucleoli and chromatin islets. The dentate gyrus with its both limbs show more increase in length. Granular cells are apparently larger.

The cornuammonis in Adult ageis seen as a large and more curved C- shaped structure with the fimbria close to the most curved part near the dentate gyrus. The pyramidal cells of CA1 are not crowded; the development of cell dendrites that push cells away from each other formed from 5 to 7 layers, with prominent apical dendrites that extending to stratum radiatum and stratum lacunosum. The nuclei are vesicular with prominent nucleoli. The cells of CA3 part are formed of 3 to 5 laers, larger in size with thicker apical dendrites. The pyramidal cells closer to the dentate gyrus hilum are fewer in layers with increasing in their size, paler cytoplasm and larger nucleus. The two limbs of dentate gyrus show apparent increase in size with slight increase in cellular size.



Discussion

The present study of rat hippocampus shows the development of inter-digitating structure of the C-shaped Ammon's horn and the V-shape dentate gyrus by using simple H&E method. These two components show a three layered structure containing a single principle layer of densely packed cells named pyramidal cell layer in the Ammon's horn and granular cell layer in the dentate gyrus, with superficial fiber layers above and deep layers below.**[14, 9, 2]** In this experiment, better results were noticed by using the formalin fixed sections, where the structure of the developing hippocampus proprius and dentate gyrus is observable.As previously mentioned, the hippocampus is subdivided into various areas. Many studies addressed the question of when and how the developing hippocampus is subdivided into fields. There is strong evidence that cell fate is pre-programmed at the time of neurogenesis, [5,11, 18]**.**

Hippocampal development is composed of multiple steps occur at the level of neuroblat cells: cell proliferation, migration, differen-tiation and survival.[21,4] The neuro-epithelium is prominent as long as active neurogenesis occurs throughout the hippocampal region. In the subicular complex, Ammon's horn and dentate hilus, neurons originate mainly between E16-E19.**[19]** The dorsal neuro-epithelium may produce more neurons than the ventral neuro-epithelium closer to the fimbria.[5] Hippocampal neurons migrate from hippocampal neuro-epithelium, which consists of three components: Ammonic neuro-epithelium, dentate neuro-epithelium and glio-epithelium.[3] The Ammonic neuro-epithelium gives rise to pyramidal cells of Ammon's horn, the dentate neuro-epithelium is the origin of granule cells of dentate gyrus and glio-epithelium generate the glial cells.[1,7] The sub-ependymal zone is a transitional zone during the prenatal development of hippocampal region that present between the layer of neuro-epithelium and stratum oriens of the developing cornu Ammonis. Mitotic figures are always sparse, and probably most of the densely packed cells are primitive neuroblasts migrating to their respective location.[6]

The stratum pyramidale is starting to appear between E17- E19. The pyramidal cells of CA1 are formed earlier than CA3.[7,4] Between E21 and P1, the pyramidal layer expands rapidly more in length than in depth. CornuAmmonis grows towards the fimbria, with further forward linear expansion making a sharp deflection towards the ball- like dentate gyrus.[17] Pyramidal cells of Ammon's horn are proceeded in its development by large and medium sized cells which are formed earlier between E16- E18 in the strata oriens and radiatum.**[7,20]**Although the dentate gyrus is the last hippocampal structure to appear, the neuroblast cells migrating towards the dentate gyrus are noticed during early development (E16 and E17) as a ball-like mass of neuroblastswith deeply stained nuclei. From E18 on, fimbrial growth forces migrating cells to move across it and through the stratum oriens to get to the dentate gyrus. The dentate gyrus has a rapid expansion in size between (E18 and E19),[16] The granular layer first appears on E18- E19; initially, the ectal limb is more prominent than the endal limb. The layer thickens throughout its extent postnatally, and by P7, both limbs are homogenous. As the granule cells migrate from the hilus, the hilus itself is pushed into a V-shape by the rapidly invaginating Ammon's horn on E19-P1,**[6]** The sharp fold of the granular layer may simply explain this distortion.[7]There are two criteria for development of dentate gyrus; first, neurons of granule cell layer located in ectal limb are developed earlier than those of endal limb; second, neurons in the outer granule cells are developed earlier than the inner cells.[13]

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