

# Postnatal Development of the Hippocampus in Male Albino Rats: A Histomorphometric Study

Original  
Article

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## ABSTRACT

**Introduction:** In recent years, deterioration in cognitive, learning, and memory become one of the significant problems in human life. Hippocampus is a pivotal part of the brain's limbic system which serves a critical role in memory, learning process and regulating the emotions. In most regions of the brain, neurons are generated only at specific periods of early development, and not born in the adulthood. In contrast, hippocampal neurons are generated throughout development and adult life.

**Aim of the Study:** Was to assess the postnatal development of the hippocampal formation.

**Material and Methods:** Twenty-four male albino pups were divided into four groups. Ages day 1, week 1, week 2 and week3, all the pups were sacrificed; the brain were dissected and processed for histological and morphometric methods.

**Results:** The general architecture of the hippocampus proper with its polymorphic, pyramidal, and molecular layers was present at day1, whereas the details of the adult structure appeared at week 2. There was a statistically significant increase in the thickness of the PL of the CA1 in group II compared to group I. There was a statistically non-significant increase in the number of pyramidal cells of CA1 in group II compared to group I, while in group IV there was a statistically non-significant decrease in number of cells compared to group III.

**Conclusion:** The important sequences of events in the growth and maturation of the hippocampal formation in male albino rat occurred in the first 2 postnatal weeks. In addition, the period of weaning in group IV(week3) and mother separation could affect negatively the maturity of the hippocampus at this period in a form of increase apoptosis of pyramidal cells.

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**Key Words:** Development, hippocampus, histomorphometry, post-natal, pyramidal cells.

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## INTRODUCTION

The hippocampus has an important function. It is a part of the limbic system that plays vital role in the process of learning and memory. It also regulates the sexual and emotional behaviors<sup>[1]</sup>.

The hippocampus is a well-known brain curved structure of grey matter that covers the whole length of the floor of the inferior horn in the lateral ventricle. Its anterior end is extended to form the pes hippocampus. Since the view of coronal cut resembles a sea horse, the structure is known as the hippocampus<sup>[2]</sup>.

The hippocampus proper, the dentate gyrus, and the subiculum make up the hippocampal formation. The main component, the hippocampus proper, is divided into areas known as Cornu Ammonis (CA), or Ammon's horn, numbered CA1 through CA4. The subiculum, which serves as the hippocampal formation's primary output source, is continuous with Ammon's horn. The parahippocampal gyrus, a section of the cerebral cortex that encircles the hippocampus, is connected to the subiculum. Memory recall and storage are both facilitated by the parahippocampal gyrus<sup>[3]</sup>.

The three layers of the hippocampus proper are called the polymorphic (OL), pyramidal (PL), and molecular (ML) layers<sup>[4]</sup>.

The majority of the brain's areas develop neurons throughout certain stages of early development rather than at birth. Hippocampal neurons, on the other hand, are created throughout development and adulthood<sup>[5]</sup>. The work is aimed to evaluate the postnatal structural alterations of the hippocampus in male albino rats.

## MATERIAL AND METHODS

### *Experimental Animals*

Twenty female adult albino rats Sprague Dawly strain (weighing 200-250 gm) were procured from animal house of Medical Research Center (MRC) of Faculty of Medicine, Ain Shams University. They were maintained in the same settings throughout the trial, fed regular rat food, and given unlimited access to water (ad libitum). Animals were not exposed to painful treatment. Each two females at pro-estrous phase were caged with one male for a day and next day a vaginal smear was examined for sperms which indicated positive conception (GD zero) and was

followed till delivery<sup>[6]</sup>. The experiment was done in the animal house of Medical Ain Shams Research Institute (MASRI) of Faculty of Medicine, Ain Shams University. The duration of the experiment was 3weeks.

### **Experimental design**

Twenty-four male albino pups were used in this work. They were distributed into 4 groups, (six rats each); according to their age<sup>[7,8]</sup>.

- Group I: one day old rats.
- Group II: one-week old rats.
- Group III: two weeks old rats.
- Group IV: three weeks old rats.

Male pups were used in the study to avoid the female hormonal effect<sup>[9]</sup>. The experiment was done in the animal house of Medical Ain Shams Research Institute (MASRI) of Faculty of Medicine, Ain Shams University. The duration of the experiment was 3weeks.

### **Ethical Consideration**

The current experiment was held according to the Committee of Animal Research Ethics "CARE," Faculty of Medicine, Ain Shams University, Cairo, Egypt. All animal procedures were performed in accordance with the recommendations of the "CARE" guidelines.

### **Sample collection**

#### **Retrieval of the hippocampi**

At the end of the experiment, six male pups from each group were sacrificed on the planned days by a lethal dose of anesthesia according to the protocol of the Animal Care of Ain Shams University. An intra-cardiac injection of Bouin's solution was done before the removal of the brains for better fixation<sup>[10]</sup>.

The brains were removed rapidly from the skulls; two cerebral hemispheres were separated. The 1<sup>st</sup> (1mm) medially was excluded and the specimen was taken from the next (5mm) and the remaining lateral portion of each cerebral hemisphere was discarded. From the (5mm) specimen the hippocampus retrieved mainly from its inferomedial portion through parasagittal sections. Specimens were kept in the fixative (Bouin's solutions) for at least ten days.

In group I, due to the small sized brain, it was difficult to discriminate the hippocampus, the whole cerebral hemisphere was taken, and the identification of the hippocampus occurred under a light microscope.

### **Light microscopic studies**

Half of the specimens were processed for paraffin sections and stained with H&E and then examined by light microscopy, and the other half of specimens were fixed in 3% phosphate buffered glutaraldehyde and small pieces (1x1mm<sup>3</sup>) were processed to prepare blocks for semi thin.

### **Sections with 1% toluidine blue**

#### **Histomorphometric study and image analysis**

In order to determine the thickness of the pyramidal layer and the number of pyramidal cells, six non-overlapping areas from six stained slices of six distinct rats were analyzed for each group. Leica's image analyzer was used to take measurements (Q 500 MC program, Wetzlar, Germany). Measurements were done at  $\times 400$  magnification at Faculty of Medicine, Cairo University.

#### **Statistical analysis**

The statistical tool SPSS version 17 was used to calculate the mean values and standard deviations (IBM Corporation, New York, USA). To compare groups, one-way analysis of variance (ANOVA) and a post hoc test were utilized. A *P* value of 0.05 or higher was regarded as significant, and a value of 0.001 was regarded as highly significant. All photos were examined by a person who was blind to the study groups' coding in order to avoid bias; analyzing digital images.

## **RESULTS**

### **Light microscopic examination**

**Group I** (one day old rats): Examination of Hematoxylin and Eosin stained sections revealed that the hippocampus proper (HP) was formed of C-shaped area cornu ammonis (CA). The (CA) was divided into superior part contained CA1 and CA2 and inferior part contained CA3 and CA4. CA1 expanded laterally to the subiculum (S). At this age CA2 can't be differentiated from CA1. CA1 presented the distal superior part of (CA), unlike CA3 which represented the proximal inferior part of (CA). CA4 represented the continuation of CA3 in the concavity (hilus) of the dentate gyrus (DG) (Figure 1).

The CA areas involved three layers; an outer (OL), a middle (PL) and an inner (ML) (Figure 2).

The polymorphic layer (OL) included axons of pyramidal neurons, cell bodies of inhibitory basket cells, horizontal trilaminar interneurons, basal dendrites of pyramidal cells and some glial cells. The pyramidal layer (PL) was the principal layer of CA. It revealed closely packed small pyramidal cells that systemically aligned in many rows. They were spindle or fusiform in shape with thin rim of cytoplasm and big oval nuclei with obvious nucleoli. Few cells revealed expanded appendages or dendrites (Figures 2,3).

The molecular layer (ML) was the least cellular layer. It contained little number of interneurons (Figures 2,3). In semi thin sections, the PL seemed with multiple rows of pyramidal cells. The pyramidal cells were fusiform in shape with big nuclei with obvious nucleoli and thin rim of cytoplasm. Few pyramidal cells showed extended appendages or dendrites (Figure 4)

**Group II** (one-week old rats): The CA and subiculum regions comprised of three layers; (OL), (PL) and (ML) (Fig.5). ML appeared thicker but less cellular (Figure 5) than the previous age (Figure 1).

In the polymorphic layer (OL); the horizontal trilaminar interneurons with its bipolar dendrites and some glial cells with its characteristic perinuclear halo were obvious in OL (Figure 6).

In the pyramidal layer (PL); the pyramidal cells of CA1 were closely packed and regularly arranged in many rows. Some pyramidal cells appeared with apical dendrites (Figure 6). The CA3 showed large, widely separated pyramidal cells and most of them exhibited apical dendrites (Figure 7)

The molecular layer (ML) presented with few interneurons and glial cells with their characteristic perinuclear halos (Figure 6).

In semi thin sections; the pyramidal cells were fusiform in shape with large nuclei, prominent nucleoli and thin rim of cytoplasm (Figure 8).

**Group III** (two weeks old rats): Examination of sections of the hippocampus revealed an apparent increase in the size at this age. The alveus appeared bordering the inferior horn of lateral ventricle and the fimbria seemed at its end (Figure 9). The hippocampal sulcus between DG and CA was well detected (Figure 9) compared to group II (Figure 5). The difference between CA1& CA2 was demarcated, as the pyramidal cells in CA1 were closely packed together while in CA2 they were less arranged which indicated the site of the transition (Figure 9).

The polymorphic layer (OL), showed an apparent decrease in the cellularity (Figure 10) compared to group II (Figure 5).

The pyramidal layer (PL), showed closely packed pyramidal cells in CA1, apical dendrites directed to ML and formed longitudinal arrays (Figure 11). In CA3; pyramidal cells of PL appear widely separated, large with their apical dendrites (Figure 12).

The molecular layer (ML), showed some glial cells with their characteristic perinuclear halo (Figures 11,12).

In semi thin sections, PL contained multiple rows of pyramidal cells that appeared oval with large nuclei, prominent nucleoli, thin rim of cytoplasm and well-defined apical dendrites (Figure 13)

**Group IV** (Three weeks old rats): Examination of sections of the hippocampus revealed an apparent increase in size (Figure 14) compared to group III (Figure 9). The alveus seemed bordering the lower horn of lateral ventricle and the fimbria appeared at its end (Figures 14,17). The hippocampal sulcus (Figure 14) looked narrower than in group III (Figure 9).

The hippocampus proper (HP): The (CA1) and (S) were obvious with their three layers; (OL), (PL) and (ML)

(Figure 15). The polymorphic layer appeared with few cells (Figure 15). Parasagittal section in the hippocampus showed the (CA2 &CA3) with their three layers; OL, PL and ML. The alveus appeared bordering the lateral ventricle and the fimbria was apparent (Figure 16).

The pyramidal layer showed pyramidal cells that were more differentiated and well developed (Figures 17,18) than in group III (Figures 11,12). Most of the pyramidal cells presented with apical dendrites (Figure 17). These dendrites were numerous and well-defined in CA3 (Figure 18). Few darkly stained pyramidal cells were detected in CA1 and in CA3 (Figures 17,18). The molecular layer appeared thicker (Figure 15) than group III (Figure 9).

In semi thin sections, the pyramidal cells were fusiform in shape with large nuclei, prominent nucleoli and thin rim of cytoplasm. Darkly stained pyramidal cells with pyknotic nuclei were apparent (Figure 19).

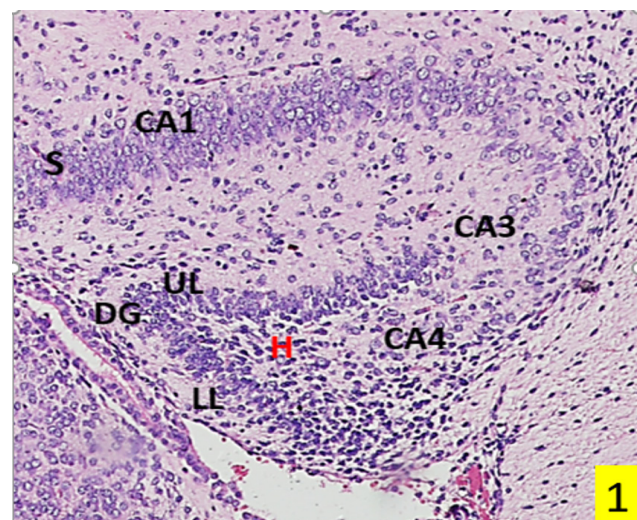
### Statistical Results

#### Thickness of the pyramidal layer of the CA 1

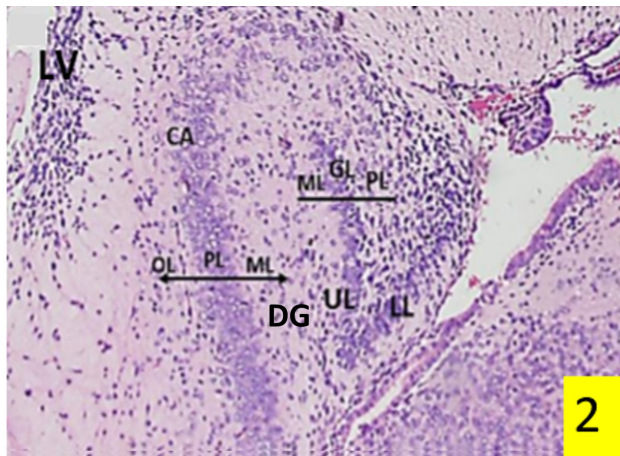
There was a statistically significant increase in the thickness of the PL of the CA1 in group II relative to group I with ( $P=0.0049$ ), a highly statistically significant decrease in group III relative to group II with ( $P=0.003401$ ), meanwhile a non-significant changes in the thickness in group IV relative to group III (Table 1, Histogram 1).

#### Number of pyramidal cells in CA1

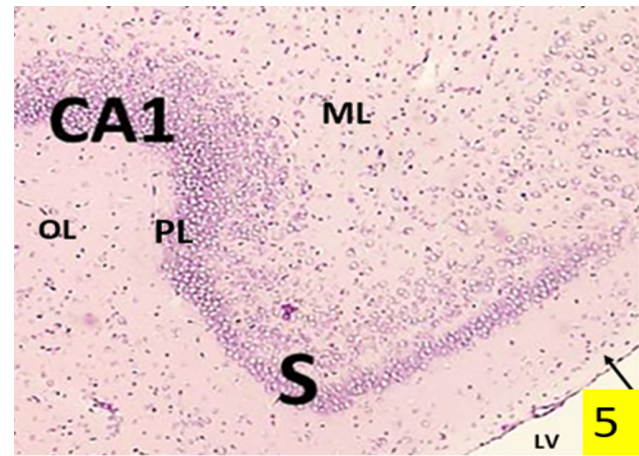
There was a statistically non-significant increase in the number of pyramidal cells of CA1 in group II relative to group I ( $P= 0.878$ ), also there was a statistically non-significant increase in number of cells in group III relative to the group II ( $P=0.599$ ), while in group IV there was a statistically non-significant decrease in number of cells relative to group III ( $P=0.431$ ) (Table 2, Histogram 2).



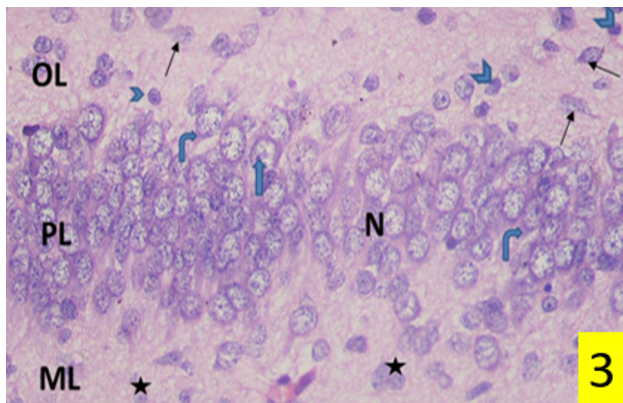
**Fig. 1:** A photomicrograph of a parasagittal section in the hippocampus of rat (group I), showing the different parts of the hippocampus; (S), (CA1) and (CA3). The (DG) appears as V-shaped capping of the free border of (CA4), it has two limbs; upper (UL) and lower limbs (LL) met at the crest with a wide area that includes the hilus (H). (Hx. &E X100)



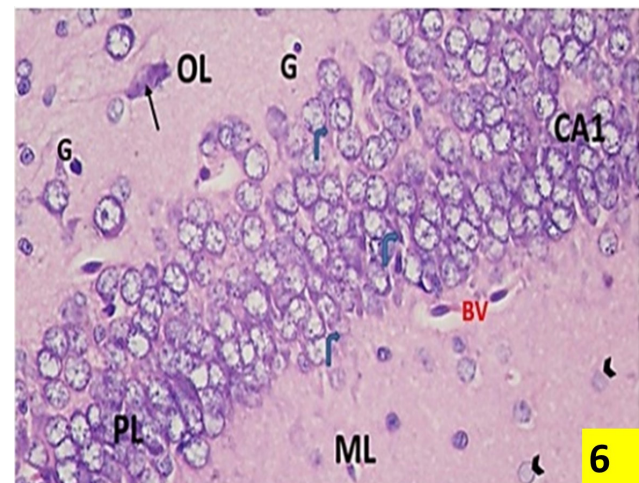
**Fig. 2:** A photomicrograph of a parasagittal section in the hippocampus of rat (group I), showing the CA regions consisting of three layers (double head arrow); (OL), (PL) and (ML). The DG appears with its two limbs UL and LL, it consists of three layers; (ML), (GL) and (PL). DG layers seem more differentiated in the upper limb than the lower limb. LV= Lateral ventricle. (Hx. &E X100)



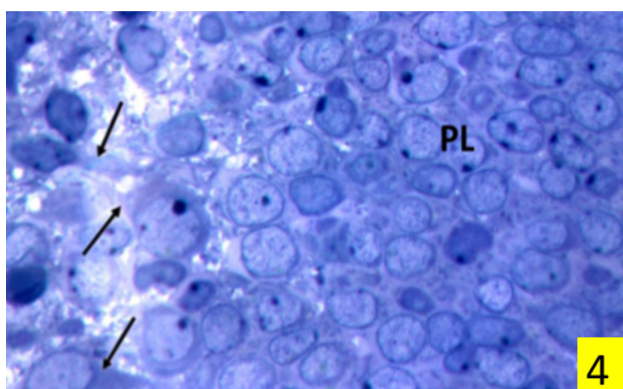
**Fig. 5:** A photomicrograph of a parasagittal section in the hippocampus of rat (group I), shows the CA1 and subiculum (S) regions consist of three layers; (OL), (PL) and (ML), there is an apparent increase in the thickness of (ML). LV= Lateral ventricle (Hx. &E X 100)



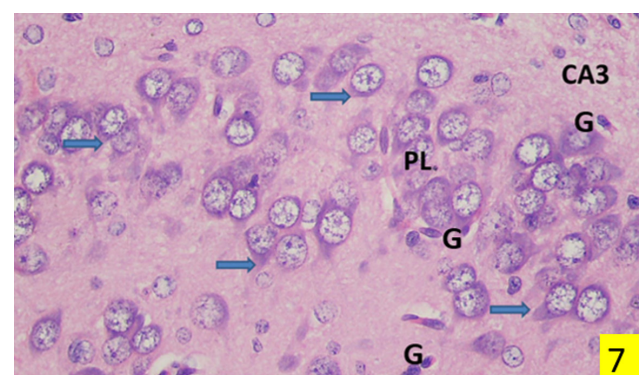
**Fig. 3:** A photomicrograph of a parasagittal section in the hippocampus of rat (group I), showing the (OL) with some glial cells (arrowheads) and horizontal trilaminar interneurons with its bipolar dendrites (arrows). The (PL) seems with its closely packed pyramidal cells arranged in many rows, they are fusiform in shape with large oval nuclei (N), prominent nucleoli (thick arrow) and thin rim of cytoplasm, some pyramidal cells show extending processes (curved arrows). The (ML) seems with little number of interneurons (\*). (Hx. &E X400)



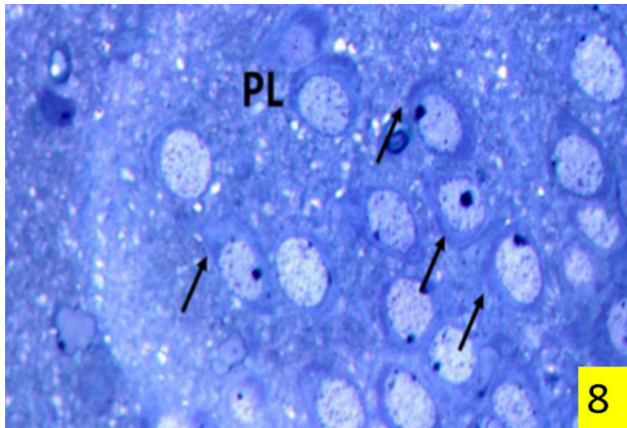
**Fig. 6:** A photomicrograph of a parasagittal section in the hippocampus of rat (group II) showing (CA1); its OL including horizontal trilaminar interneuron (arrow) and glial cells (G) with their characteristic perinuclear halos. The (PL) show closely packed pyramidal cells arranged in many rows. Some pyramidal cells with apical dendrites (bending arrows) are apparent. The (ML) appears with few interneurons (arrowheads). BV= blood vessels. (Hx. &E x400)



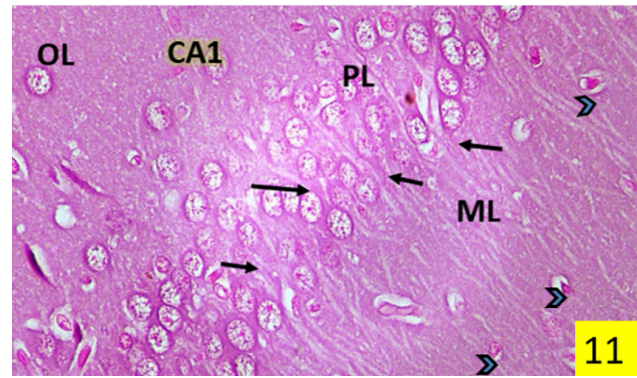
**Fig. 4:** A photomicrograph of a semi thin section in the hippocampus of rat (group I) showing the PL containing multiple rows of pyramidal cells that appear fusiform in shape with large nuclei and prominent nucleoli and thin rim of cytoplasm. Few pyramidal cells show extended dendrites (arrows). (Toluidine Blue x 1000)



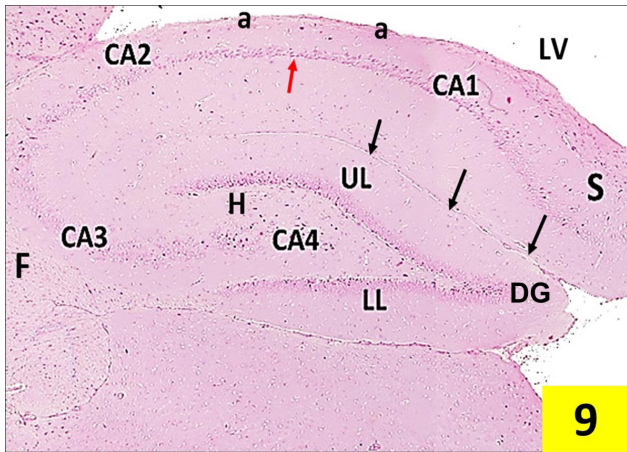
**Fig. 7:** A photomicrograph of a parasagittal section in the hippocampus of rat (group II) showing the PL of CA3 with large, widely separated pyramidal cells. Most of them exhibited apical dendrites (arrows). Some glial cells (G) are obvious. (Hx. &E X 400)



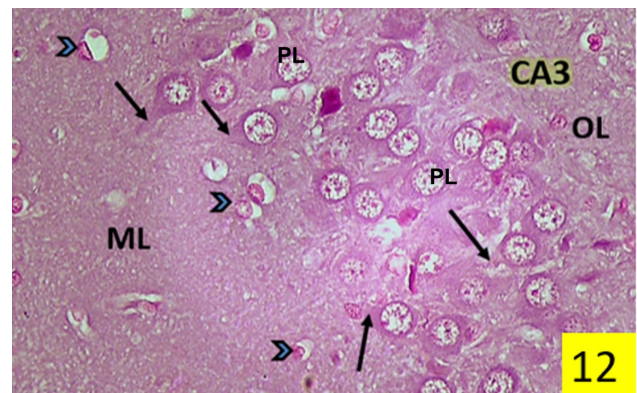
**Fig. 8:** A photomicrograph of a semi thin section in the hippocampus of rat (group II) showing the pyramidal layer (PL) with fusiform pyramidal cells. They appear with large nuclei, prominent nucleoli and thin rim of cytoplasm (arrows). (Toluidine Blue X 1000)



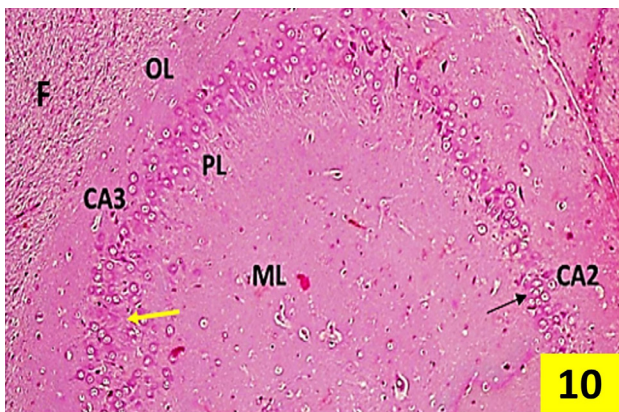
**Fig. 11:** A photomicrograph of a parasagittal section in the hippocampus of rat (group III) showing the (CA1); the pyramidal cells of PL appear closely packed with apical dendrites passing to ML (arrows) and forming longitudinal arrays. In the (ML), lightly stained glial cells are detected with their characteristic perinuclear halo (arrowheads). (OL & ML) seem with few cells. (Hx. & E X400)



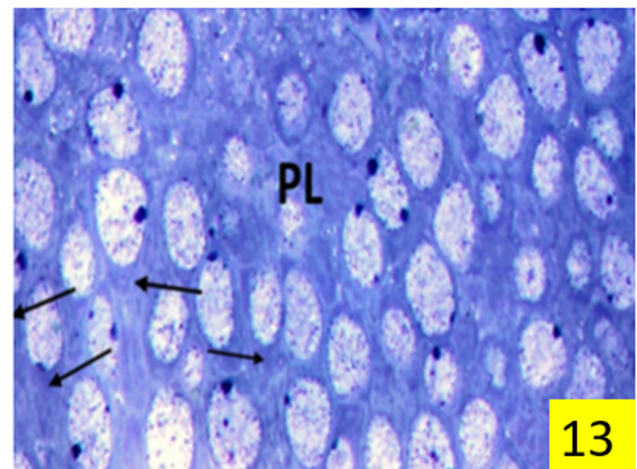
**Fig. 9:** A photomicrograph of a parasagittal section in the hippocampus of two weeks old rat (group III) showing the different parts of the hippocampus; (S) and CA areas (CA1-4). The DG seems thick, its upper (UL) and lower (LL) limbs and the hilus (H) are detected. The line of transition between CA1 and CA2 is obvious (red arrow). The alveus (a) and the fimbria (F) are apparent. The hippocampal sulcus (arrows) is well noticed between CA and DG. LV= lateral ventricle. (Hx. & E X40)



**Fig. 12:** A photomicrograph of a parasagittal section in the hippocampus of rat (group III) showing the (CA3); pyramidal cells of PL appear widely separated, large with their apical dendrites (arrows). The molecular layer (ML) appears with few cells, lightly stained glial cells (arrowheads). OL= polymorphic layer. (Hx. & E X400)



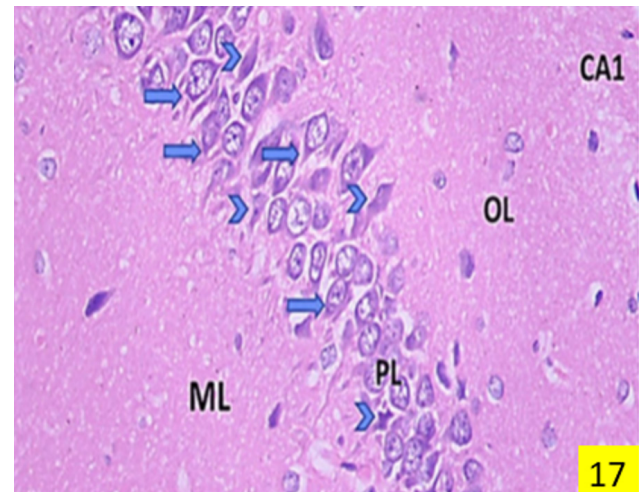
**Fig. 10:** A photomicrograph of a parasagittal section in the hippocampus of rat (group III) showing the three layers of (CA2 & CA3); the (OL), (PL) and (ML). The pyramidal cells of (CA2) (arrow) seem arranged in rows. The pyramidal cells of (CA3) (yellow arrow) appear large and widely separated. The fimbria (F) is apparent. (Hx. & E X100)



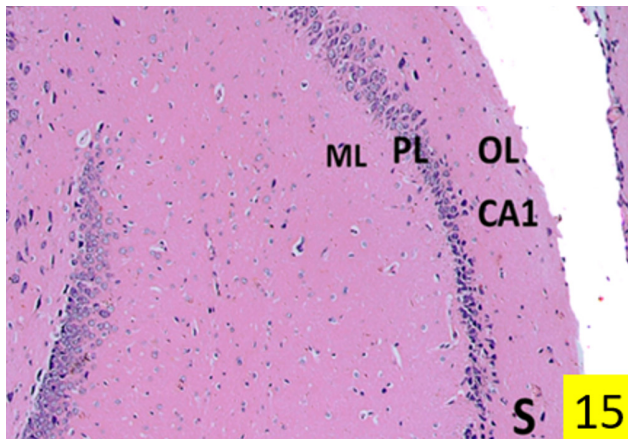
**Fig. 13:** A photomicrograph of semithin section in hippocampus of rat (group III) showing the pyramidal layer (PL) containing multiple rows of pyramidal cells that appear oval with large nuclei, prominent nucleoli, thin rim of cytoplasm and welldefined apical dendrites (arrows). (Toluidine Blue X 1000)



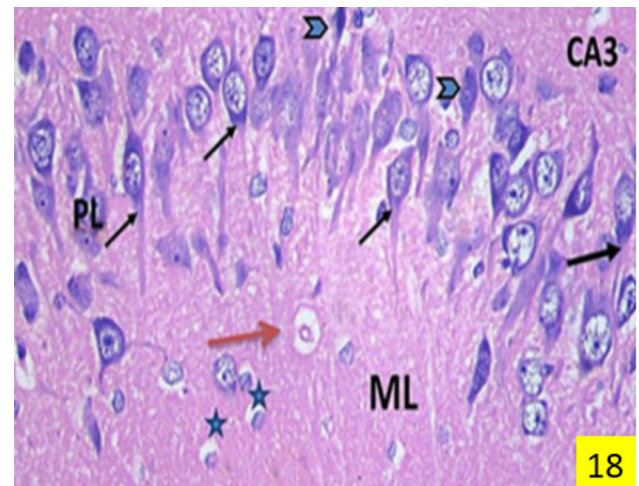
**Fig. 14:** A photomicrograph of a parasagittal section in the hippocampus of rat (group IV) showing the different parts of the hippocampus; (S), (CA) and (DG). The alveus (a) appears bordering the lateral ventricle (LV) and the fimbria (F) seems at its end. The hippocampal sulcus (arrows) looks narrow. (Hx. & E X40)



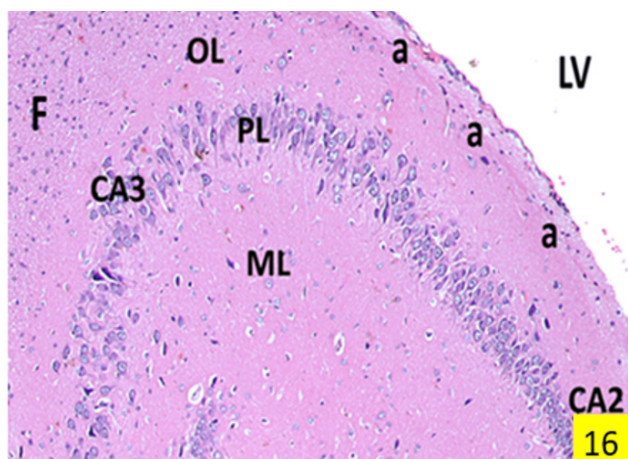
**Fig. 17:** A photomicrograph of a parasagittal section in the hippocampus of rat (group IV) showing the CA1 with its three layers (OL), (PL) and (ML). Most of the pyramidal cells show numerous well-defined apical dendrites (arrows). Few darkly stained pyramidal cells (arrowheads) are detected. (Hx. & E X400)



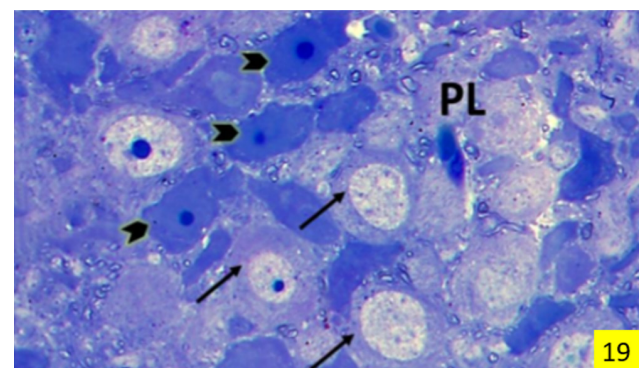
**Fig. 15:** Higher magnification of the previous figure showing the subiculum (S) and (CA1) with their three layers; (OL), (PL) and (ML). The pyramidal cells of PL appear closely packed and arranged in many rows. (Hx. & E X100)



**Fig. 18:** A photomicrograph of a parasagittal section in the hippocampus of rat (group IV) showing the PL of CA3. The pyramidal cells appear large with (arrowheads). Glial cells (\*) are detected in the ML. Red arrow= Open face nerve cell. (Hx. & E X400)



**Fig. 16:** A photomicrograph of a parasagittal section in the hippocampus of rat (group IV) showing the (CA2 & CA3) with their three layers; (OL), (PL) and (ML). The alveus (a) appears bordering the lateral ventricle (LV) and the fimbria (F) is apparent. (Hx. & E X100)



**Fig. 19:** A photomicrograph of a semi thin section in the hippocampus of rat (group IV) showing the pyramidal layer (PL) with pyramidal cells that appear fusiform in shape with large nuclei, prominent nucleoli and thin rim of cytoplasm (arrows). Some pyramidal cells with darkly stained nuclei are apparent (arrowheads). (Toluidine Blue X 1000)

**Table 1:** Means ± SD of the thickness of the PL of the CA1 in um/ HPF in all groups

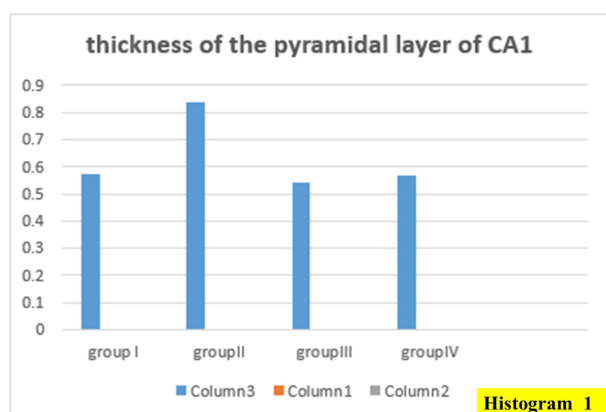
Group	Group I	Group II	Group III	Group IV
Means ± SD thickness of the pyramidal layer (um)	0.575±0.115	0.8382±0.138	0.548±0.125	0.574±0.096
<i>P value</i>	0.0049	0.003401	0.74	

The level  $P \leq 0.05$  was considered the cut-off value for significance. Results were considered statistically significant when  $P value \leq 0.05$  and highly significant when  $P value \leq 0.001$

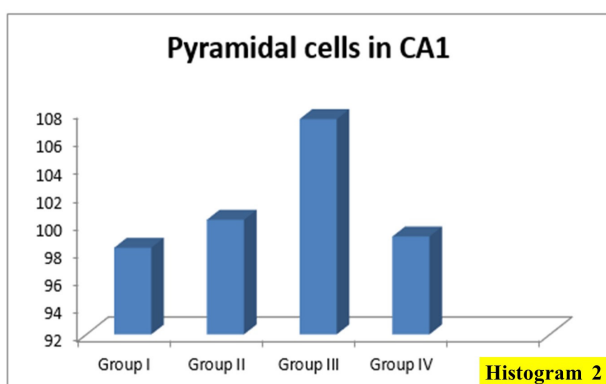
**Table 2:** Means ± SD of the number of pyramidal cells in CA1/ HPF in all groups

	Group I	Group II	Group III	Group IV
Means ± SD of number of pyramidal cells in CA1	98.2±21.00476136	100.2±18.7136314	107.4±21.69792617	99±6.519202405
<i>P value</i>	0.878	0.599	0.431	

The level  $P \leq 0.05$  was considered the cut-off value for significance. Results were considered statistically significant when  $P value \leq 0.05$  and highly significant when  $P value \leq 0.001$



**Histogram 1:** Means of the thickness of the pyramidal layer of CA1/ HPF in all groups.



**Histogram 2:** Means of the number of pyramidal cells in CA1/ HPF in all groups.

## DISCUSSION

Recently, deterioration in memory, cognition and learning became one of the great obstacles in human life. Hippocampus is an important part of the brain's limbic system which plays a major role in emotion regulation, memory and learning process<sup>[11]</sup>.

The hippocampal formation is a multi-compartmental zones in the medial temporal lobe specifically included in declarative and relational memory processing. Behavioral studies have supposed a protracted functional maturation of these components in primates and postnatal developmental disorders in the hippocampal formation are suggested to be involved in neurodevelopmental disorders, such as Down syndrome, schizophrenia, ADHD, autism, and epilepsy<sup>[12]</sup>.

The goal of this work was to study the postnatal development of the hippocampus of male albino rats. So, the rats were classified into four groups according to their age; group I (one day old rats), group II (one-week old rats), group III (two weeks old rats), and finally group IV (three weeks old rats). In the existing work, male albino rats were utilized to avoid the female hormonal effect that was supported by previous investigators<sup>[9]</sup>.

In the present work, the hippocampus was found in the brain exactly in the temporal lobe related to the inferior corn of the lateral ventricle. The hippocampus was covered by the alveus that delineated the inferior horn of the lateral ventricle, medially the alveus forms the fimbria. The alveus was first detected in group II. It was previously reported that alveus of the rat hippocampus began at the end of embryonic life 18 GD<sup>[8]</sup> and turned to the adult form at 10 PND<sup>[4]</sup>.

In the present work, the obvious architecture of the hippocampus proper was exist in group I, the hippocampus was formed of CA, DG, and the subiculum. The subiculum is the extension of the para hippocampal gyrus, but it is formed of a trilaminar layer as the CA. Many other works on the rat stated that cells of the hippocampal formation were evolved from GD15 into adulthood.

Whereas, the pyramidal cells of the HP were grown before birth or prenatally from the ventricular region<sup>[13]</sup>. On the other hand, other studies reported that the granule cells of the rat's cerebellar cortex, developed mainly after birth or postnatally<sup>[14]</sup>.

The CA was built of four zones: CA1, CA2, CA3 and CA4. Moreover, CA4 regarded as the extension of CA3 between the two limbs of the DG. CA4 became clearly defined in group II. Some authors studied the after birth or postnatal development of the hippocampus in rats and reported similar results<sup>[4]</sup>.

The current work revealed that the differentiation between the four areas of the hippocampus was not clear in group I (day 1). In group II (week1) CA3 was well detected, with the starting of the second week (group III), the line of transition between CA1 and CA2 was distinguished.

This was in accordance with other studies who studied the postnatal development of the hippocampus in Macaque monkeys and concluded that the CA1 region developed first then CA3 while CA2 developed later<sup>[7]</sup> and also seen clearly in rats<sup>[4]</sup> who demonstrated similar findings

In the current study, the CA was consisted of 3 laminae, an outer (OL), a middle principle (PL) and an inner (ML), all these layers were presented in group I. The same results were described by other workers, who studied the postnatal development of the hippocampus in rats and described the CA as a trilaminar structure consisted of 3 laminae (OL), (PL) and (ML)<sup>[8]</sup>.

Some researchers studied the human hippocampal volume in childhood, adolescence, and adulthood in healthy controls versus patients with bipolar depression and revealed that the hippocampus of healthy controls significantly increased in volume in young ages then there was a non-significant increase in old ages<sup>[15]</sup>. Some scientist stated that the hippocampus formation grew and matured most quickly and significantly during the first two postnatal weeks, with subsequent growth being slower<sup>[4]</sup>.

In the current study, the pyramidal layer revealed closely packed pyramidal neurons systematically sorted in many rows, they were spindle shaped with thin rim of cytoplasm and big oval nuclei with prominent nucleoli. The difference between the regions of the CA began to appear in the first week (group II) as the pyramidal cells of CA3 were loosely arranged and larger in size than those of CA1 and CA2. In the second week (group III), the difference between CA1& CA2 was demarcated, as the PL in CA1 was closely packed together while those in CA2 was less arranged which was a site of the transition. These findings agreed with the study on albino rats of other authors who concluded that the initiation of discrimination between the small compact closely and firmly united pyramidal cells at CA1 and the less arranged pyramidal neurons at CA2 was at the age of two weeks postnatally<sup>[4]</sup>.

The present work revealed a gradual decrease in the thickness of PL in the four groups, began with a statistically non-significant reduction in group II relative to group I and ended by a highly statistically significant decrease compared to group IV. These results were matching with those of some scientists, who studied the prenatal and postnatal growth of the hippocampus in rats and detected reduction in the thickness of PL with an enlargement in the size of the pyramidal cells till the fourth week postnatally<sup>[8]</sup>.

In this study, the apical dendrites of the pyramidal cells began to be revealed in group I and grown continually in the following groups. Other studies reported that the maturation of axonal and apical appendages or dendrites of the pyramidal cells was responsible for the increment in the width or thickness of the OL and ML<sup>[8]</sup>.

In the present study, some darkly stained pyramidal cells appeared in group IV. Many workers studied the effect of community segregation from the age of substituting

other food for the mother's milk in the diet ) or weaning on the hippocampal composition and function in the rat in the third and fourth weeks postnatally and reported a group of structural alterations in the hippocampus including decreased expression of the synaptic protein synaptophysin, decrease in the whole number of newborn nerve cells and reduction of the appendage or dendritic length and dendritic spine bulk of pyramidal cells<sup>[16]</sup>.

These darkly stained pyramidal cells were seen in CA1 and in CA3, but they rarely appeared in CA2. Other authors studied the different areas of the rat HP and concluded different vulnerabilities in the four hippocampal areas. They added that CA1 was markedly liable to blood and oxygen deficiency and some types of temporal lobe epilepsy. CA2 and CA3 were resistant sectors, whereas CA4 exhibited a medium vulnerability to anoxia, ischemia,<sup>[3]</sup>.

Similarly, other studies observed dark pyramidal cells with small dark stained nuclei in CA1 of rats after brain insult<sup>[17]</sup>. Some reported that the dark neurons represented the early stages of neuronal histopathology following various brain injuries. Depending on how much of their dendrites' cytoskeleton was destroyed, some of them perished and the others lived. Age-related increases in the number of darkly colored cells, which are thought to be a sign of neuronal damage<sup>[14]</sup>.

In this work, a fluctuating change in the thickness of the ML was noticed. These results were clarified by<sup>[18]</sup>. They stated that the growth of granule cell axons, or Mossy fibers, which occupied the inner ML of the hippocampus and terminated on the proximal part of the apical and basal dendrites of the pyramidal cells of the inferior region of the hippocampus proper, was most likely the cause of the increase in the molecular layer's thickness. Additionally, some scientists claimed that the decline in the thickness of the CA layers and the number of pyramidal neurons in the various CA locations may be explained by the apoptosis of the pyramidal cells<sup>[4,7]</sup>.

If there were enriched habitat for animals included a variety of elements, including more possibilities for gaining knowledge, learning, more community activities, more body or physical activity, and larger housing, this will improve the development of all components of hippocampus. Moreover, this also happened to people. Early detection of some disorders, like autism, that is characterized by under development of the DG and CA4. The best treatment is the frequent sustainability to cultivated learning environments, that may encourage improved growth of this structure in those kids. Similar to this, existence in a supplemented environment may upgrade spatial memory function in people with Alzheimer's disease, Parkinson's disease, and cerebral stroke, these all achievements were produced by remarkable accelerations in the process of neurogenesis in the hippocampus<sup>[19]</sup>.

In the current work, in group IV "the time of weaning of the rat's pups" there was a statistically non-significant reduction in the number of pyramidal cells of CA1



compared to group III and a statistically significant decrease in the pyramidal cells of CA3 relative to group III. This agreed with the results of some workers, who studied the postnatal development of the hippocampal formation of Wistar rats and reported a reduction in its volume with increasing anxiety. So, they concluded an inverse relationship between hippocampal volume and anxiety<sup>[20]</sup>.

### CONCLUSION

Finally, it could be concluded that there were major series of events that occurred in the growth and maturation of the cells of the CA in the male albino rats. Also the full development of the alveus and fimbria happened in the initial 2 weeks after birth or postnatally. In addition, the period of weaning and mother separation could affect negatively the maturity of the hippocampus at this stage.

### CONFLICT OF INTERESTS

There are no conflicts of interest.

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## الملخص العربي

### نمو الحصين في فترة ما بعد الولادة في ذكور الفئران البيضاء: دراسة نسيجية قياسية

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**المقدمة:** أصبح التدهور المعرفي والتعلم والذاكرة إحدى المشكلات المهمة في حياة الإنسان. يعد الحصين جزءًا محوريًا من الجهاز الحوفي للدماغ والذي يلعب دورًا مهمًا في الذاكرة وعملية التعلم وتنظيم المشاعر. في معظم مناطق الدماغ، تتولد الخلايا العصبية فقط في فترات محددة من النمو المبكر، ولا تولد في مرحلة البلوغ. في المقابل، يتم إنشاء الخلايا العصبية في الحصين طوال فترة النمو وحياة البلوغ.

**الهدف من الدراسة:** كان تقييم تطور تكوين الحصين بعد الولادة.

**المواد والطرق:** تم تقسيم أربعة وعشرين من ذكور جرو ألبينو إلى أربع مجموعات. الأعمار: اليوم الأول والأسبوع الأول والثاني والأسبوع الثالث، تم التضحية بجميع الجراء؛ تم تشريح الدماغ ومعالجته بالطرق النسيجية والمورفومترية. **النتائج:** كان الهيكل العام للحصين المناسب بطبقاته متعددة الأشكال والهرمية والجزئية موجودًا في اليوم الأول، بينما ظهرت تفاصيل التركيب البالغ في الأسبوع ٢. علاوة على ذلك، زاد عدد طبقات الخلايا الحبيبية بالتزامن مع زيادة السماكة من الطبقات الجزئية ومتعددة الأشكال لكل من الحصين السليم.

**الخلاصة:** حدثت التسلسلات الهامة للأحداث في نمو ونضج تكوين الحصين في ذكور الجرذان البيضاء في أول أسبوعين بعد الولادة.