Original Article

Effect of Enriched Environment on Neurogenesis in the Subventricular Zone of Male Albino Rat

Azza Mostafa Mohammed Aboshanady, Mona Mohammed Mousa Zoair, Samia abdelmessieh Youssef and Mamdouh Abdelaziz Mahmoud

Department of Anatomy and Embryology, Faculty of Medicine Tanta University, Egypt

ABSTRACT

Introduction: Aging results in disturbance of neurogenesis in the subventricular zone (SVZ). Living in enriched environment (EE) has been proved to stimulate neurogenesis in different brain areas. It reduces emotional stress, ameliorates behavior and improves cognitive functions. This work focuses on outlining the effect of EE on SVZ neurogenesis in albino rat and its restorative effect on age related changes.

Material and Methods: Sixty male albino rats were used and were divided into two groups; control group and enriched environment group (n=30 each). Control group were put in standard cages all over the experiment and was subdivided according to their ages into three subgroups (n=10 each). Rats of postnatal control subgroup (IA; one-day age) were put in standard cages for three weeks. Adult control rats (subgroup IB; three months' age) were put in standard cages for another three months. Old age control rats (subgroup IC; ten months' age) were put in standard cages for another ten months. Enriched environment group was subdivided according to their ages into three subgroups IIA, IIB, IIC (n=10 each) with the same ages of control subgroups and were put in enriched environment cages with larger dimensions for the same periods as the control ones. Rats were injected 2 hours before scarification by BrdU labeling to detect proliferating cells in SVZ. Brain specimens were processed for histological and immunohistochemical studies and statistically analyzed.

Results: This study revealed highly significant increase of neurogenesis in enriched subgroups when compared with control ones evidenced by tracing of proliferating neuroblasts by BrdU labelling. SVZ exhibited histopathological changes in in both adult and old age control subgroups when compared to the postnatal one which were improved in the corresponding enriched subgroups.

Conclusion: Enriched environment has stimulatory effect on SVZ neurogenesis in different ages.

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Key Words: BrdU, enriched environment, GFAP, SVZ.

Corresponding Author: Azza Mostafa Mohammed Aboshanady, PhD, Department of Anatomy and Embryology, Faculty of Medicine Tanta University, Egypt, **Tel.**: +20 10 0681 5386, **E-mail:** azzaaboshanady@yahoo.com

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INTRODUCTION

Neurogenesis is the capability of adult brain to produce new neurons throughout life[1]. In mammals it persists in two main brain areas: the subgranular zone (SGZ) of hippocampus and the subventricular zone (SVZ) of lateral ventricle[2]. Production of new nerve cells is essential for integrated brain function, repair, memory and learning[3].

SVZ is the largest site of new neuron formation[4]. It is composed of four cell types; type E ependymal cells, type B astrocytic neural stem cells, type C transit-amplifying cells and type A neuroblasts[5]. Newly formed neuroblasts of the SVZ migrate toward the olfactory bulb (OB) along a specific pathway called the rostral migratory stream (RMS)[6]. Most of the newly formed neurons differentiate after reaching the OB into granule cells of the granule cell layer and few cells differentiate into periglomerular cells in the glomerular layer[7].

BrdU labeling is commonly used for detection of proliferating cells in living tissues as it incorporates DNA of dividing cells during the S-phase of the cell cycle[8]. BrdU immunohistochemistry is used to study of the development of the CNS and confirm that adult neurogenesis occurs in postnatal mammalian brain, including humans[9].

During aging, neural stem cells of SVZ decrease in their number and function with reduction of the newly formed neurons and decline in the olfactory memory[10;11].

Enriched Environment (EE) is a non-invasive therapy that exerts pronounced effects on the normal and diseased brain from the molecular to the structural level as it increases neurogenesis, provides sensory, social and motor stimulation and improve the behavioral performance and memory[12;13]. Therefore, this research aimed to evaluate the effect of enriched environment on SVZ neurogenesis in three different age groups.

MATERIALS AND METHODS

Animals

Sixty male albino rats were used in the present study. They were obtained from the animal house of Faculty of Medicine, Tanta University. All animals were housed

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in clean properly ventilated cages and were bedded with wood shavings and fed the same laboratory diet. This study was approved by the research ethics committee of Tanta Faculty of medicine (Number:33275/08/19)

Experimental groups

The rats were divided into two groups; control group and enriched environment group.

Control Group I: It included 30 rats which were put in cages measured 30×20×15 cm all over the experiment. This group was subdivided according to their ages into three subgroups:

- Subgroup IA (Postnatal Control Subgroup)[14]: Consisted of 10 rats at the age of one day and were put in standard cages for three weeks.
- Subgroup IB (Adult Control Subgroup)[15]: Consisted of 10 rats at the age of three months and were put in standard cages for another three months.
- Subgroup IC (Old age Control Subgroup)[16]: Consisted of 10 rats at the age of ten months and were put in standard cages for another ten months.

Enriched environment Group II: It included 30 rats which were put in enriched environment cages all over the experiment. The cages measured 100×65×35cm[17]. This group was subdivided according to their ages into three subgroups:

- Subgroup IIA (postnatal Enriched Environment Subgroup)[14]: Consisted of 10 rats at the age of one day and were put in enriched environment cages for three weeks.
- Subgroup IIB (Adult Enriched Environment Subgroup)[14]: Consisted of 10 rats at the age of three months and were put in enriched environment cages for three months.
- Subgroup IIC (Old age Enriched Environment Subgroup)[16]: Consisted of 10 rats at the age of ten months and were put in enriched environment cages for ten months.

Rats of enriched environment were exposed to physical and social enrichment. Physical enrichment included toys, elevated platforms, exercise wheels and novel objects that were changed every week. The cages contained plastic tubes that rearranged every week. The cages were cleaned every week and the site of food, water and toys were changed every week. Social enrichment involved the housing of 5 rats in one large cage in order to promote social interactions and play[12,16].

BrdU labeling

To assess cell proliferation at the subventricular zone (SVZ), rats were injected with 50 mg / kg BrdU intraperitoneal then; they were sacrificed 2 hours after BrdU injection[14]. Their brains were fixed overnight, and then coronal sections were taken at the level of optic chiasma to show SVZ.

Histological and Immunohistochemical studies

Some specimens were fixed in 10% buffered formalin solution and processed to be stained by:

a. Hematoxylin and eosin stain to study the general histological picture of SVZ[18].
b. Immunohistochemical GFAP stain for analysis of type B astrocytes and their processes and Anti-BrdU for tracing proliferating cells.

Procedure was as follows

Brain sections were deparaffinized and dipped in phosphate buffered saline (PBS, pH 7.4) (Sigma Chemical Co.).

For GFAP; Sections were washed in PBS for several times and treated for 30 min with 3% (dilution from 30%) H_2O_2 then washed with PBS before applying the primary antibody. The primary antibody rat anti-GFAP was diluted 1:20 in PBS and added and incubated for 1 hr. The primary antibody was omitted before adding the secondary antibody biotin anti-rat, which was diluted 1:20 in PBS containing 1% bovine serum albumin (BSA; Sigma Chemical Co.) for 30 min[19].

For Anti BrdU; sections were treated with 2 N HCl for 30 min at 60 °C in order to fragment DNA then, they were neutralized in 0.1 M borate buffer (pH 8.4). Tissue sections were incubated in 20% methanol containing 0.3% H2O2 for 30 min to suppress the activity of endogenous peroxidase. Then, sections were exposed to 1:500 diluted anti-BrdU primary monoclonal antibodies and incubated overnight at room temperature. After that Sections were incubated with biotinylated goat anti-rat IgG (dilution 1:200) (the secondary antibody) and AB complex[20].

Finally, the slides of both stains were washed in distilled water, dried and placed in xylene for 5 min and mounted with a mixture of distyrene, a plasticizer and xylene (DPX) to preserve stain. GFAP stain revealed brownish coloration of the processes of astrocytes (dendrites and spines) whereas, Anti BrdU stain showed brown colored nuclei in S- phase of cell cycle

Other specimens were fixed in 2.5% glutaraldehyde in 0.1 phosphate buffer saline and processed for transmission electron microscopic examination (EM)[21].

Morphometric study[22]

The mean number ± SD of the following cells were counted each in 10 different fields at magnification of 400/ slide in each group using an image J analyzer software program:

1. GFAP +ve type B astrocytes in SVZ in coronal sections of albino rats' brains.
2. BrdU +ve proliferating cells in SVZ in coronal sections of albino rats' brains.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and analysed by using SPSS program version 20 (simple t-test). A difference among the means of a *P value* ≤ 0.05 was considered statistically significant and a *P value* ≤ 0.001 was considered statistically highly significant. Whereas, a *P value* > 0.05 was considered insignificant[23].

RESULTS

Hx.&E.

Hx. &E. stained coronal sections of rats' forebrain of postnatal control subgroup (IA) showed the site and structure of the SVZ. It was seen along the lateral side of the lateral ventricle, darker than the rest of the brain tissue. SVZ was composed of different cell layers. The first (ependymal) layer was lining the lateral ventricle. It was a single layer of cuboidal or columnar epithelium with large basal rounded nuclei and apical cytoplasm and exhibited several cilia. Latral to ependyma, there was thick layer of lightly and darkly stained cells. The darkly stained cells were slightly elongated and arranged in groups. The lightly stained cells were rounded in shape and present between darkly stained groups of cells (Figure 1 IA).

In adult control subgroup (IB), SVZ appeared occuppying smaller area compared with postnatal subgroups. The ependymal cells became separated from rest of cells of SVZ. They revealed small sized basal nuclei and some of them appeared shrunken, irregular and darkly stained. Decreased density of the darkly and lightly stained cells was noticed with increased distance between them as compared with that in the postnatal subgroups (Figure 1 IB).

In old age control subgroup (IC) reveled SVZ as a faint narrow area lateral to the lateral ventricle with fewer number of cells as compared with postnatal and adult subgroups. Most ependymal cells were flattened with darkly stained irregular nuclei. Decreased cellular density of darkly and lightly stained cells was noticed with wide separation between them (Figure 1 IC).

After exposure to EE, SVZ exhibited larger area with increased cellular denisty compared to their control subgrogroup (Figure 1 C). Groups of large number of darkly stained type A neuroblasts were observed surrounded by type B astrocytes with vesicular nuclei and lightly stained cytoplasm as compared with those of control subgroups (Figures 1 IIA, IIB,IIC).

GFAP immunostained coronal sections

In postnatal enriched subgroup (IIA), the reaction was strongly +ve and the dendrties of astrocytes appeared longer and thicker than that of control subgroup (IA) and sometimes showed spines. Adult enriched subgroup (IIB) showed strongly stained type B astrocytes with increased thickness of their dendrities as compared to control subgroup (IB). Old age control subgroup (IC) showed very few type B astrocytes with mild reaction but in old age enriched subgroup (IIC) they exhibited strong reaction with increased thickness of their dendrities (Figure 2).

Anti- BrdU immunostained coronal sections

Anti-BrdU stained coronal sections of rat forebrain showed increased number of BrdU positive proliferating cells (brown colouration of nuclei) of SVZ in postnatal group compared with other groups. The number of BrdU positive cells in adult group was larger than that in old aged group. In each age group, there was increase in the number of BrdU positive cells in the enriched subgroup compared with the control one (Figure 3).

Statistical analysis of immunohistochemical stains

Number of GFAP +ve cells

The present study revealed highly significant decrease in the number of GFAP +ve cells in old age control subgroup (26.50±3.21) when compared with postnatal control subgroup and adult control subgroup (45.50±3.06 and 38.42±5.18 respectively). Whereas, adult control subgroup revealed significantly decreased number of GFAP +ve cells in comparison to postnatal control one (Histogram 1).

Furthermore, There was highly significant increase in the number of GFAP +ve cells in postnatal (53.67±5.71), adult (46.58±2.35) and old age (37.75±4.81) enriched subgroups as compared to control ones (45.50±3.06, 38.42±5.18 and 26.50±3.21 respectively) (Histogram 1).

Number of BrdU +ve proliferating cells

There was highly significant decrease in the number of BrdU +ve cells in old age control subgroup (9.18±2.18) when compared with postnatal control subgroup and adult control subgroup (31.72±1.42 and 15.73±2.20 respectively). Also, there was a highly significant decrease in the number of BrdU +ve cells in adult control subgroup when compared with postnatal control subgroup (Histogram 2).

There was highly significant increase in the number of BrdU +ve proliferating cells in postnatal (34.90 ± 1.22), adult (20.64±4.34) and old age (13.55±3.62) enriched subgroups as compared to control ones (31.72±1.42, 15.73±2.20 and 9.18±2.18 respectively) (Histogram 2).

Electron microscopic examination of SVZ

In postnatal control and enriched subgroups, epedymal cells (E cells) were columnar in shape. They had large rounded or oval basal euchromatic nuclei with regular contour and dispersed chromatin. Its apical surface showed cilia toward the ventricular cavity. The cytoplasm contained many mitochondria with well defined electron dense cirstae. Rough endoplasmic reticulum and free ribosomes were seen in the cytoplasm in both subgroups (Figures 4 IA,IIA). With the advance of age, the E cells appeared more cuboidal (Figure 4 IB) or even flattened in old age (Figure 4 IC). Degenerative changes as destructed

mitochondria, reduced cytoplasmic volume and number of cilia (Figures 4 IB,IC) were noticed in control subgroups versus enriched ones (Figures 4 IIB,IIC).

Type B astrocytes had rounded or oval nuclei with regular bounderies and dispersed chromatin. The cytoplasm had cytoplasmic processes and showed many mitochondria with cristae (Figures 5 IA,IIA). In adult group, Type B cells revealed dense heterochromatic nuclei with perinuclear halo in control subgroup but exhibit oval euochromatic nuclei in enriched subgroup (Figures 5 IB,IIB). With the advance of age, type B cells showed degenerative changes as destructed mitochondria with absent cristae specially in control subgroup (Figures 5 IC,IIC).

Type A neuroblasts were arranged in clusters, separated from each others by open extracellular spaces. They had scanty dark cytoplasm and their nuclei showed dense heterochromatin (Figures 6 IA,IIA). In adult control subgroup, type A neuroblasts showed relatively irregular smaller nuclei with increased spaces between them compared to enriched subgroup (Figure 6 IB). With aging, increased extracellular spaces were observed between type A cells compared with postnatal and adult groups and some pyknotic changes of their nuclei were noticed in control subgroup (Figure 6 IC, IIB, IIC).

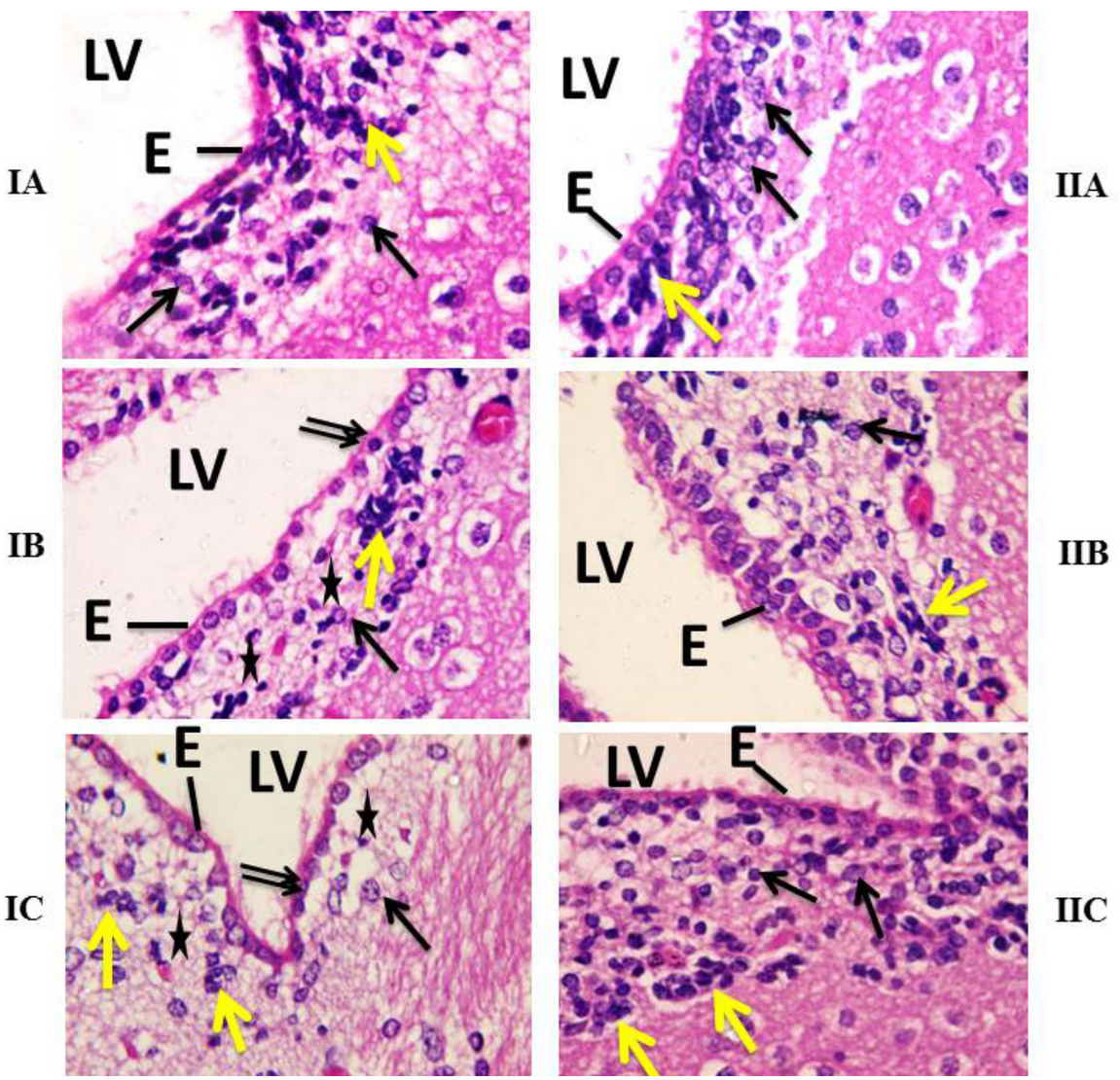


Fig. 1: Photomicrographs of coronal sections of albino rat brain: {postnatal control subgroup (IA), postnatal enriched subgroup (IIA), adult control subgroup (IB), Adult enriched subgroup (IIB), old age control subgroup (IC) and old age enriched subgroup (IIC)} showing the layers of SVZ. In IA subgroup, lateral ventricle is lined by single layer of cuboidal ependymal cells with cilia (E). Some ependymal cells appeared flattened with small sized irregular darkly stained nuclei (double arrows) in adult and old age control subgroups (IB & IC). Lateral to ependymal, groups of lightly (black arrows) and darkly stained cells (yellow arrows) are seen. Lightly stained cells are rounded with vesicular nuclei are seen between darkly stained cells. The darkly stained cells are elongated and arranged in groups. Apparent decreased cellular density of lightly &darkly stained is observed in adult and old age control subgroups with increased distance between cells (star). Great reduction in darkly stained cells which become arranged in group of 2 or 3 is observed in IC. In enriched subgroups, ependymal cells appear columnar in IIA & IIB subgroups and cuboidal in IIC with increased apical cilia. Darkly & lightly stained cells appear larger and more prominent with increased cellular density and reduced distance between cells (IIA, IIB & IIC). Hx. &E. × 1000

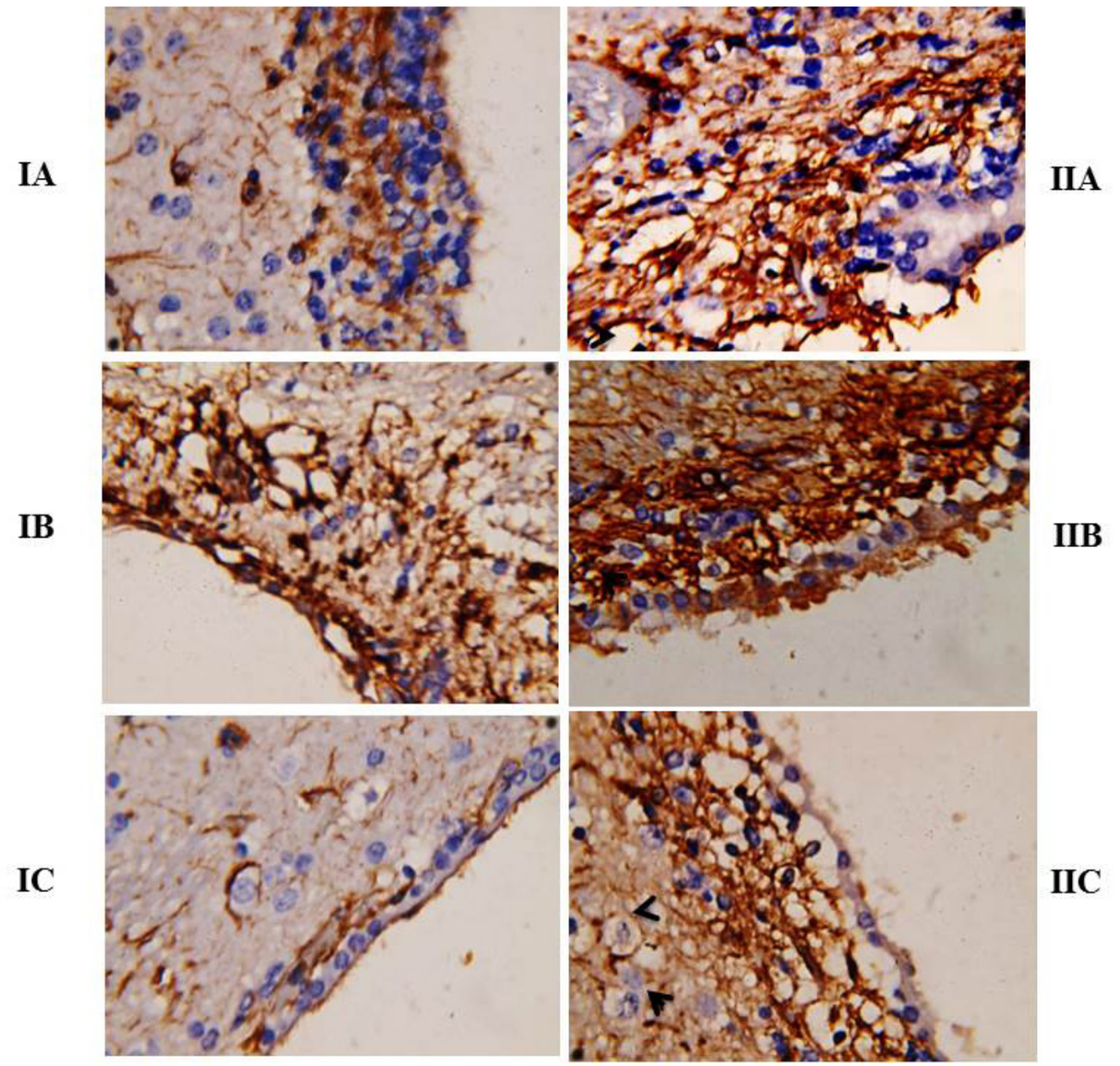


Fig. 2: Photomicrographs of coronal sections of albino rat brain: {postnatal control subgroup (IA), postnatal enriched subgroup (IIA), adult control subgroup (IB), Adult enriched subgroup (IIB), old age control subgroup (IC) and old age enriched subgroup (IIC)} showing positive reaction (brown coloration) of type B astrocytes which are the main component of SVZ. Strong positive reactions are observed in astrocytes of postnatal and adult enriched subgroups in comparison with postnatal and adult control ones (moderate reaction). Their dendrities appear longer and thicker than those of the control subgroups and sometimes showed spines. Very few type B astrocytes with mild reaction are noticed in old age control subgroup but in enriched subgroup the reaction is strong with increased thickness of their dendrites and show spines. GFAP × 1000

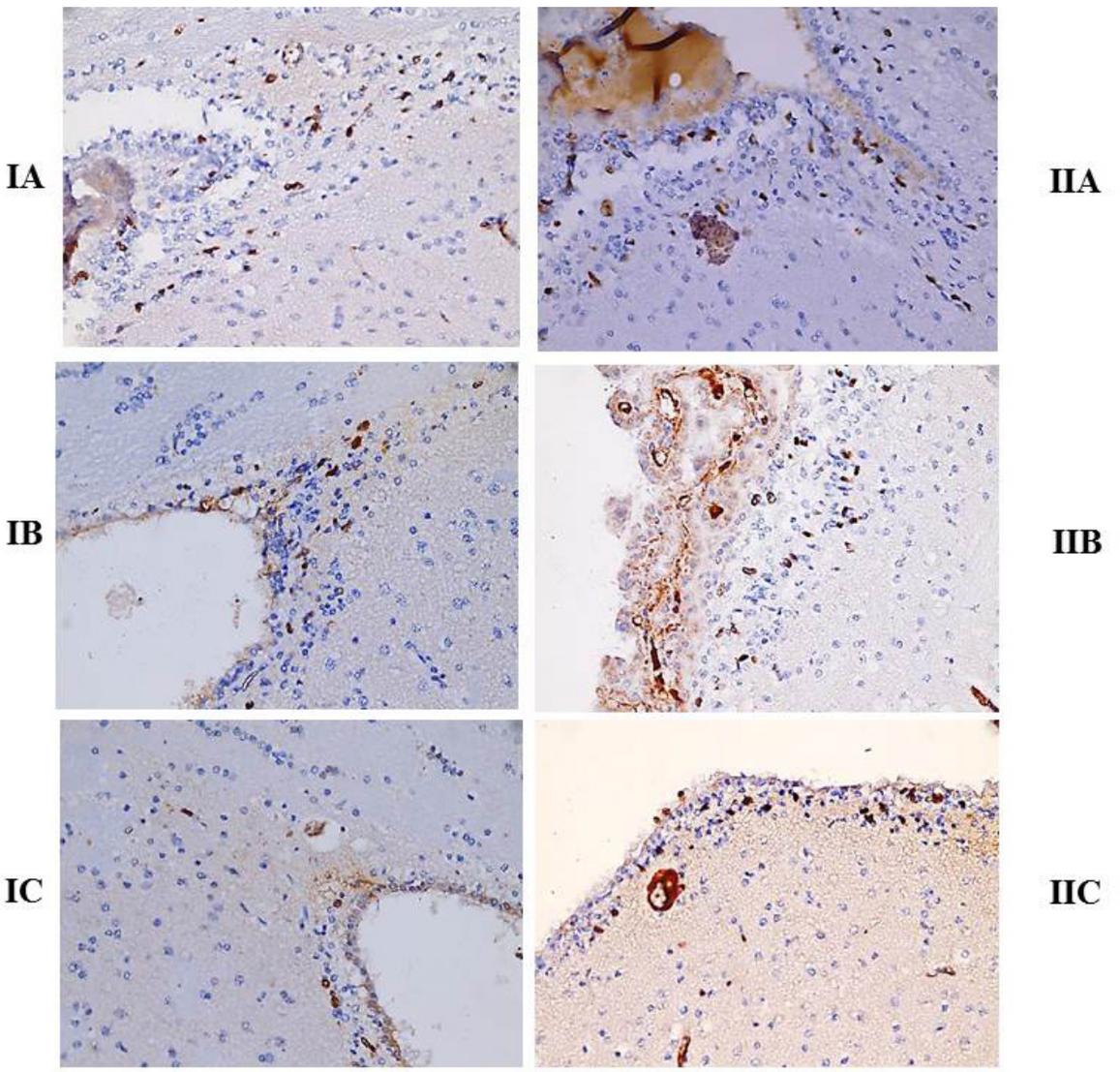


Fig. 3: Photomicrographs of coronal sections of albino rat brain {postnatal control subgroup (IA), postnatal enriched subgroup (IIA), adult control subgroup (IB), Adult enriched subgroup (IIB), old age control subgroup (IC) and old age enriched subgroup (IIC)} showing BrdU positively stained proliferating cells (brown colored nuclei) in SVZ of different age groups. There is an increase in the number of BrdU +ve cells in postnatal group compared with other groups. The number of BrdU +ve cells in adult group is larger than that in old aged group. In each age group, there is increase in the number of BrdU +ve cells in the enriched subgroup compared with control one. Anti-BrdU × 400

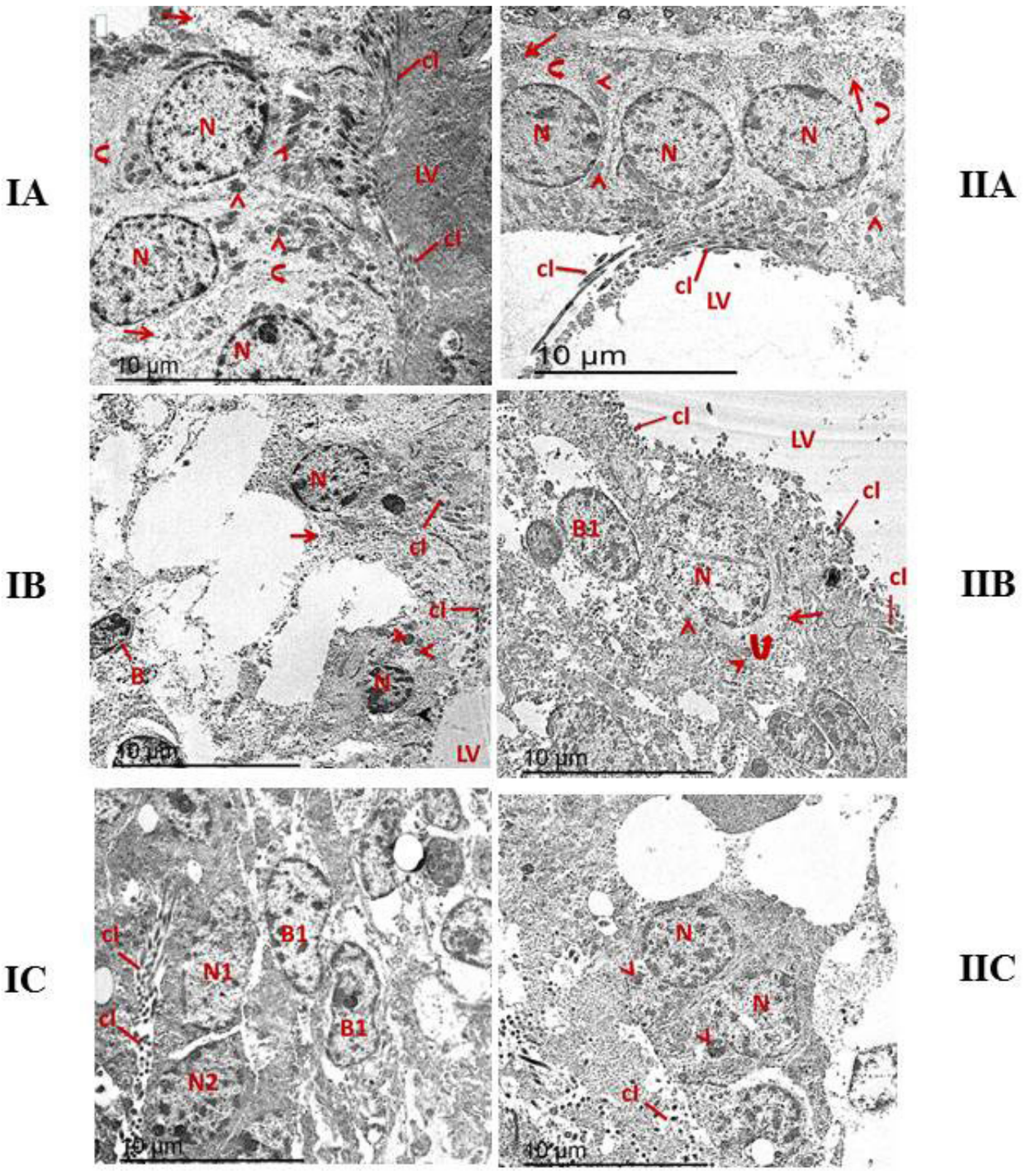


Fig. 4: Ultrathin sections of albino rat brain: {postnatal control subgroup (IA), postnatal enriched subgroup (IIA), adult control subgroup (IB), Adult enriched subgroup (IIB), old age control subgroup (IC) and old age enriched subgroup (IIC)} showing ependymal cells. In postnatal subgroups, they show large rounded or oval nuclei with condensed chromatin (N), the cytoplasm contain mitochondria (arrow heads), free ribosomes (arrows), RER (curved arrows) and exhibit cilia (cI) towards the cavity of lateral ventricle (LV). In IB subgroup, they show irregular nuclei with dispersed chromatin (N) and widely separated from irregular type B cells. While, in IIB subgroup they appear cuboidal with oval nuclei (N). IC subgroup show flattened cells with flattened nuclei (N1) and some show pyknotic changes (N2) and reduced cytoplasmic volume while it's enriched subgroup (IIC) show cuboidal ependymal cells with rounded nuclei (N) and increased cytoplasmic volume with apparent cellular organelles (arrow heads).

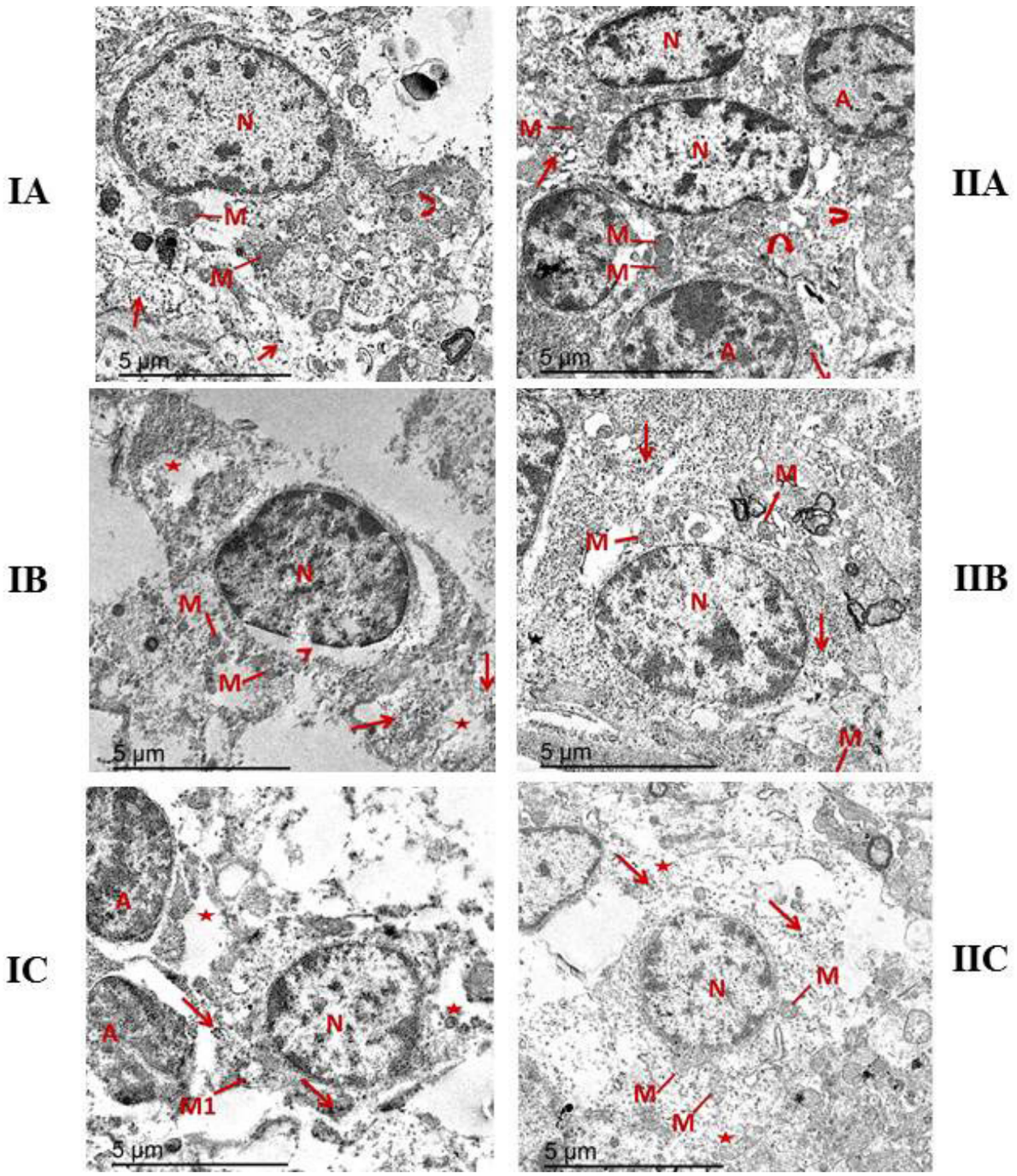


Fig. 5: Ultrathin sections of albino rat brain: {postnatal control subgroup (IA), postnatal enriched subgroup (IIA), adult control subgroup (IB), Adult enriched subgroup (IIB), old age control subgroup (IC) and old age enriched subgroup (IIC)} showing astrocytes. In postnatal subgroups, they have large nuclei with dispersed chromatin (N). The cytoplasm contains many mitochondria with well-defined cristae (M), free ribosomes and RER. IIA subgroup show increased neuroblasts (A) nearer to astrocytes. IB subgroup show perinuclear halo (arrow heads) and vacuolations in their cytoplasm (stars) which are reduced in IIB. IC the cytoplasm contains destructed mitochondria with absent cristae while IIB reveals some mitochondria with electron dense cristae.

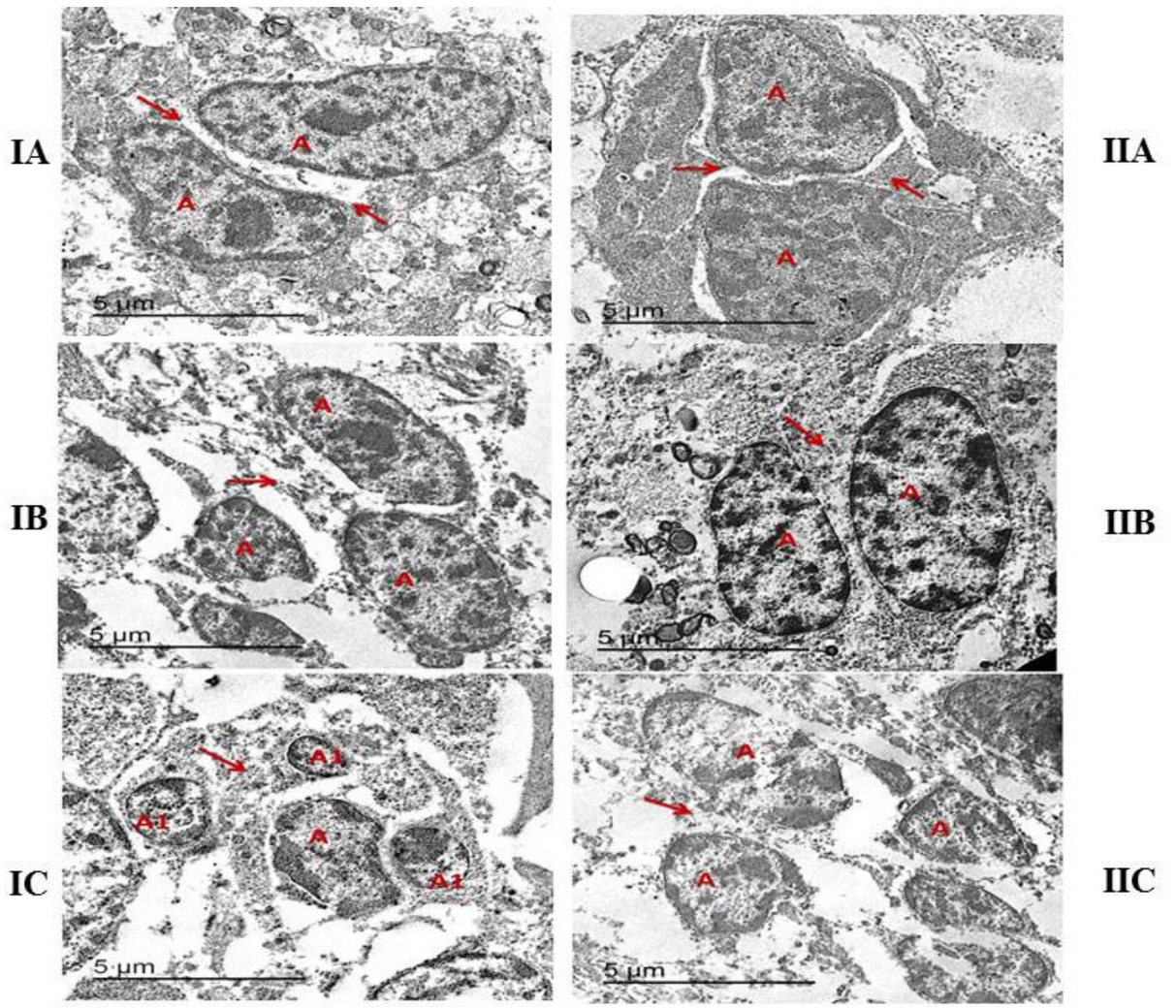
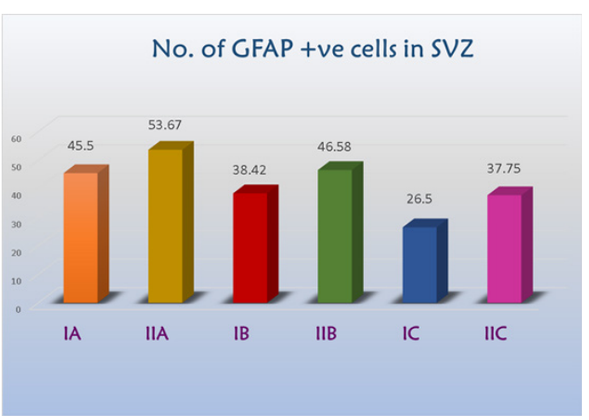
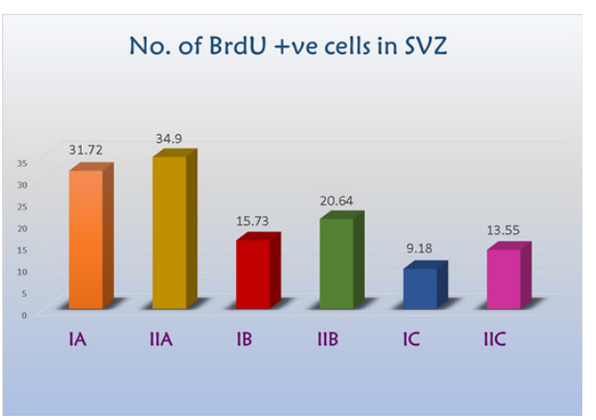


Fig. 6: Ultrathin sections of albino rat brain: {postnatal control subgroup (IA), postnatal enriched subgroup (IIA), adult control subgroup (IB), Adult enriched subgroup (IIB), old age control subgroup (IC) and old age enriched subgroup (IIC)} showing neuroblasts. In postnatal subgroups, they have dense heterochromatic nuclei (A) and scanty cytoplasm. They are arranged in groups separated from each other's by open extracellular spaces (arrows). IB they show relatively irregular smaller nuclei and larger extracellular spaces while appear larger with regular larger heterochromatic nulei and reduced spaces in IIB. In IC, some nuclei exhibit pyknotic changes (A1) which are improved in IIC.



Histogram 1: Number (No.) of GFAP +ve cells in the SVZ of all subgroups



Histogram 2: Number (No.) of BrdU +ve cells in the SVZ of all subgroups

DISCUSSION

Neurogenesis in SVZ is essential for integrated structure of olfactory bulb and has a major role in continuous production of new neurons migrating through RMS to OB and function in brain repair during adulthood[24,25].

With aging, some researchers explained the impaired brain ability to generate new neurons as a repair mechanisms following injury or disease or even in healthy subjects due to neural stem cell loss or dysfunction[24,26]. However, brain function and cognitive ability may be improved by changing the quality of lifestyle. Enriched environment has great effects on intact and diseased brain and it can improve brain function and cognitive ability[27;28]. Therefore, the present experimental study aimed to throw a light on the effect of enriched environment on neurogenesis in subventricular zone (SVZ) in three different age groups and if it could improve decreased neurogenesis with aging.

Subventricular zone (SVZ) in the present work was seen along the lateral side of the lateral ventricle, darker than the rest of the brain tissue. It became thinner and occupied smaller area in adult control subgroup than that in postnatal control subgroup. It even became faint narrow area in old age control subgroup. Similarly, previous researcher[29] confirmed the changes in human SVZ neurogenesis during life, where proliferation and migration of RMS neurons is active during infancy but exhibit postnatal decline to adulthood. Moreover, previous study[10] found marked changes in the structure of SVZ of aged rodent as it became thinner and the morphology and functions of its cells changed greatly.

Furthermore, this work revealed marked reduction in the cellular density of all types of cells in the SVZ with increased distance between them in both adult control subgroup and old age control subgroup when compared with postnatal subgroup. This result agrees with other researcher[30] who found that the number of neural stem cells in the SVZ was reduced by two folds in old mice (24-26 months) when compared with young mice (2-4months). However, previous study[31] found significant decrease in numbers of type A with aging while type B and type E cells were relatively constant. Other study[32] explained the decline of SVZ neurogenesis as a result of decrease of its cells by reduction of the neural stem cells by 38% and neuroblasts by 58%.

In the present study, some ependymal cells appeared flattened, shrunken, and irregular with darkly stained irregular nuclei in control group. Previous researches[10;24] reported ventral stenosis of the lateral ventricular wall and restriction of the neurogenic area to dorsolateral region of SVZ with flattening of ependymal monolayer and dispersion of cilia.

Enriched environment can reduce the anxiety and improve brain function and elasticity[33]. The duration of housing in enriched environment is very important factor. Some studies showed that exposure to enriched environment for 40 minutes produced significant changes in the RNA and in weight of samples of cerebral cortex, while, longer exposure increases entire thickness of cerebral cortex[34].

In our study, we put the animals in enriched cages for 3 weeks in postnatal enriched subgroup, 3 months in adult enriched subgroup and 10 months in old age enriched subgroup. In all enriched subgroups, we observed that the SVZ occupied larger area as compared with control subgroups with increased cellular density of all types of cells. The cells appeared larger in size and more prominent in comparison with the control subgroups in the different age groups. Previous researcher[35] explained these findings that enriched environment normalized levels of proliferation in SVZ and increased number of neural stem cells in post ischemic rats when compared with control non ischemic rats. On the other side, other researcher[36] stated that enriched environment has no significant increase on SVZ neurogenesis.

In agreement with the present study, other researcher[37] reported that exposure to enriched environment every day in aged animals stimulates neurogenesis through increasing the probability of new neuron survival till maturity. Also, other researchers[38] found on their study on young (2-8 months) and old aged (20-22 months) rats that neurogenesis decline with aging but enriched environment was able to enhance survival of newly formed neurons regardless of the age and partially restored their ability for rapid acquisition greatly reduced in old rats.

We used GFAP stain for analysis of astrocytes (Type B cells) and their processes in the SVZ. The astrocytic cells markedly decreased with aging and their processes became thinner and shorter. There was a significant decrease in the number of GFAP +ve cells in adult control subgroup when compared with postnatal control subgroup. Furthermore, there was highly significant decrease in GFAP +ve cells in old age control subgroup when compared with adult and postnatal control subgroups. Previous study[24] also, observed a significant reduction of astrocytes with their processes contacting the wall of lateral ventricle, neuroblasts and type C cells with age. They explained the reduction in cell number due to cell death or inability of the neural stem cell to maintain self-renewal capability with each division cycle.

The GFAP stained sections of enriched subgroups in this work showed stronger reaction and the dendrites of astrocytes appeared longer and thicker than that of control subgroups and sometimes showed spines. There was highly significant increase in the number of GFAP +ve cells in postnatal, adult and old age enriched subgroups as compared to control ones. These results could be explained by increased brain connections, densities and number of synapses, dendritic arborization and length of dendritic spines with enriched environment. Moreover, enriched environment has a positive effect on neurogenesis and may improve brain function greatly in normal animals and in

animals with brain disorders. Furthermore, it increases the possibilities of integration of grafted cells in the host brain after mechanical, neurochemical and ischemic lesions[39;40].

For tracing the proliferating cells in SVZ in the present study, BrdU was injected in rats 2 hours before scarification. The number of BrdU +ve proliferating cells in SVZ exhibited highly significant decrease in adult control subgroup when compared with postnatal control subgroup and in old age control subgroup when compared with postnatal and adult control subgroups. Previous researcher[31] explained the significant decline in BrdU +ve cells in 10 months and 22 months' mice by two mechanisms; lengthening of cell cycle in elderly mice and ⁄or increased cell death that led to decrease in the number of cells in S phase. Moreover, decline in the number of BrdU +ve proliferating cells and Ki 67 +ve cells in SVZ and RMS were noticed in age dependent manner in 6, 12, 18 and 23 months' mice[41] or in 2,6,18 months' mice[26].

Previous studies[42-44] reported accumulating DNA damage in aged SVZ cells from various insults. They revealed increased expression of cyclin dependant kinase (Cdk) inhibitors which is responsible for delaying cell cycle re-entry and explained that lengthening of the cell cycle allow DNA repair and hence decreasing the rate of proliferation.

Many other researches using label-retention strategies and immunohistochemistry confirmed this result and demonstrated 50 % decline in neurogenesis in old mice[32;45]. That decline might be due to reduced epidermal growth factor receptor signaling[30] or to alterations in cycling progenitor cells with decreased proliferative activity of aged SVZ[32].

In the present study after exposure to enriched environment, there was highly significant increase in the number of BrdU +ve proliferating cells in SVZ in postnatal, adult and old age enriched subgroups as compared to control ones. Previous researcher[26] found significant increase in neurogenesis in mice housed in enriched environment for 20 days. They explained that enriched environment might contribute to neuroprotection and enhance survival of new born neurons and restoration of neural stem cell proliferation rate.

Ultrathin examination of SVZ in the present research revealed degenerative changes in its cells with aging. Ependymal cells in postnatal control subgroup appeared columnar with large rounded or oval basal euchromatic nuclei with regular contour and dispersed chromatin. Their apical surfaces showed cilia toward the ventricular cavity. The cytoplasm contained many mitochondria with well-defined electron dense cirstae. The cells appeared more regularly arranged in the enriched subgroup with more projecting cilia in the ventricular cavity. The E cells appeared more cuboidal in adult group with advance of age or even flattened in old age. Also, destructed mitochondria reduced cytoplasmic volume and number of cilia was noticed in adult and old age control subgroups. With aging, type A neuroblasts showed relatively irregular smaller nuclei with increased spaces between them as compared to enriched subgroups and even some pyknotic changes of their nuclei were noticed in the old age control subgroup. With the advance of age, type B cells showed degenerative changes as destructed mitochondria with absent cristae or dissociated cytoplasm especially in adult and old age control subgroups when compared with enriched subgroups. The changes of the mitochondria observed in the present study agree with[46] as they reported the importance of adult neurogenesis as a mechanism of brain plasticity and the role of mitochondria in SVZ cells due to stimulation of self-renewal capacity of neural stem cells, and neuronal differentiation.

Previous researcher[47] clarified the restoration of SVZ neurogenesis in aged mice by intracerebro-ventricular infusion of fibroblast growth factor -2 and heparin-binding epidermal growth factor-like growth factor (HB-EGF) which suggests that aged SVZ can retain its capacity for increase neurogenesis if properly stimulated. Therefore, enriched environment can modulate brain plasticity regardless of age by stimulating production of neurogenic factors that are normally reduced with aging[26]. Moreover, enriched environment has remarkable effects on cell morphology. It increases thickness and weight of cerebral cortex, increases cell size and nuclear size. The changes in brain structure in enriched animals are due to release of neurotransmitters, growth factors and vascular arborization which are associated with neuronal structure, plasticity, neuronal excitability, synaptic transmission and neuroprotection[48;49]. Also, enriched environment significantly increases survival, proliferation, migration and differentiation of newly formed neurons with decreased apoptotic death of immature neurons and increased expression of VEGF signaling[50].

Mechanisms of enriched environment on the neurogenesis in SVZ in both healthy and pathological brain are many. It may increase myelination of nerves, astrocytic material and their contact synapses with increased degree of perfusion of brain capillaries[51]. Also, it may release neurotrophic factors that can act on various types of cells including neurons, astrocytes, microglia and endothelial cells[52;53]. Moreover, it causes changes in adipokine levels in blood and CSF which have anti-inflammatory effects in the brain[54].

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير البيئة المخصبة على التكون العصبي في المنطقة تحت البطينية للبطين الجانبى فى ذكر الجرذ الأبيض

عزة مصطفى محمد أبوشنادى، منى محمد موسى زعير، سامية عبدالمسيح يوسف، ممدوح عبدالعزيز محمود

قسم التشريح والأجنة، كلية الطب جامعة طنطا، مصر

الخلفية: تؤدى الشيخوخة الى حدوث اضطرابات فى التكون العصبى للمنطقة تحت البطينية فى البطين الجانبى . وقد ثبت أن العيش فى البيئة المخصبة يحفز التكون العصبى فى مناطق المخ المختلفة. فهو يقلل من الضغط العاطفى و يحسن السلوك ويطور الوظائف الإدراكية. يركز هذا العمل على دراسة تاثير البيئة المخصبه على التكون العصبى فى المنطقة تحت البطينية فى ذكور الجرذان البيضاء وتاثيرها التعويضى على التغييرات المرتبطة بالشيخوخه.

المواد وطرق البحث: تم استخدام ستين من ذكور الجرذان البيضاء من مختلف الأعمار فى هذه الدراسة وتم تقسيمهم إلى مجموعتين. المجموعة الضابطة ومجموعة البيئة المخصبة(٣٠ لكل منها). المجموعة الضابطة تم وضعها في أقفاص قياسية خلال التجربة وتم تقسيمها وفقًا لأعمارها إلى ثلاث مجموعات فرعية (١٠ لكل منها).

تم وضع جرذان المجموعة الفرعية الضابطة بعد الولادة (IA) ذات اليوم الواحد في أقفاص قياسية لمدة ثلاثة أسابيع ، وتم وضع المجموعة الفرعية الضابطة للبالغين (IB) ذات عمر الثلاثة أشهر في أقفاص قياسية لمدة ثلاثة أشهر أخرى. أما المجموعة الفرعية الضابطة لكبار السن (IC) بعمر عشرة أشهر فقد وضعت في أقفاص قياسية لمدة عشرة أشهر أخرى.

وقد تم تقسيم مجموعة البيئة المخصبة وفقًا لأعمارها إلى ثلاث مجموعات فرعية (IIA)و(IIB) و (IIC) (١٠ لكل منها) بنفس أعمار المجموعات الفرعية الضابطة وتم وضعها فى أقفاص البيئة المخصبة ذات الأبعاد الأكبر ولنفس المدد الزمنية مثل المجموعات الفرعية الضابطة.

تم حقن الجرذان ساعتين قبل التضحية بها بواسطة المتتبع BrdU وذلك لتتبع الخلايا المتكاثرة .و في الوقت المحدد لكل مجموعة فرعية ، تم التضحية بالجرذان وتم تجهيز عينات من المخ للدراسات النسيجية والهستوكيميائية المناعية والتحليل الإحصائي.

نتائج البحث: كشفت هذه الدراسة عن انخفاض ذو دلالة احصائية فى التكون العصبى فى المجموعات الفرعية الضابطة فى البالغين و كبار السن بالمقارنة بمجموعة بعد الولادة و زيادة ذو دلالة احصائية فى التكون العصبى فى المجموعات الفرعية المخصبة عند مقارنتها بالمجموعات الضابطة. كما لوحظ تغييرات نسيجية فى المنطقة تحت البطينية فى المجموعات الفرعية الضابطة فى البالغين و كبار السن بالمقارنة بمجموعة بعد الولادة و قد تحسنت هذه التغيرات فى المجموعات الفرعية للبيئة المخصبة .

الاستنتاج: البيئة المخصبة لها تأثير ايجابى على التكون العصبى فى مختلف الأعمار.