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# THE CELLULAR COMPOSITION OF NEUROGENIC PERIVENTRICULAR ZONES IN THE ADULT TILAPIA BRAIN

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## ABSTRACT

***The cellular organization and constituents of the proliferating periventricular zones (PVZs) had been described in the adult brain of Nile Tilapia (Oreochromis Niloticus), a delegate of one of the tremendous vertebrate ordered requests, the perciform fish. To induce this Patten, light and electron microscopic studies were applied to examine the anatomical and histological organization of the PVZs. These examinations were confirmed by using nestin analog labeling of the undifferentiated young cells which is considered as a protein marker of undifferentiated neural cells.***

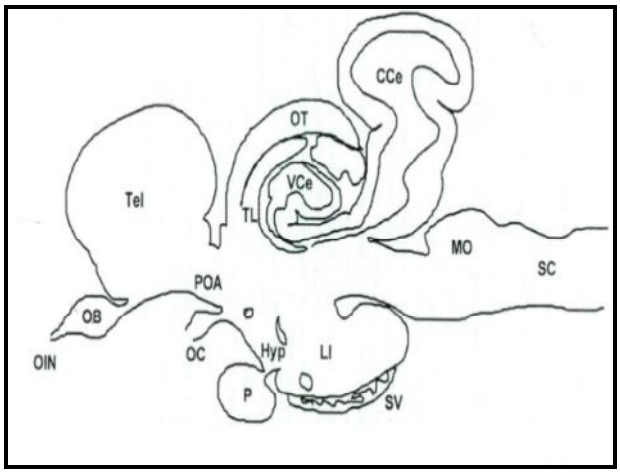
## INTRODUCTION

Neurogenesis and Neural differentiation had been inspected in early stages of embryo growth and after birth in the brain of several vertebrate species **(Gage et al., 2008; Kempermann, 2011).** Researchers included about that point demonstrated that there is a broad contrast between mammals and fishes. In adult mammals, neurogenesis is limited to two regions in the brain, the sub-ventricular zone (SVZ) of the lateral ventricles, and the sub granular zone of the hippocampal dentate gyrus **(Altman, 2011).** In adult fish brain**,** there are several brain areas that can deliver new neural cells and the quantity of recently born cells are considerably more than that produced in the mature brain of mammals **(Zupanc, 2011)**

Recently, the Nile tilapia has approved as a model design for mature neural proliferation due to its powerful neurogenic ability of the developed brain. Previous investigations illustrated that various distinctive neuroanatomical zones over the central nervous system (CNS) retaining mitotic active cell populaces **(Zupanc et al., 2005).** Moreover, about half of these cells proliferate to offer ascent to the recently produced neural cells **(Hinsch and Zupanc, 2007).** Some of these neurogenic districts are confined inside particular neuroanatomical areas neighboring the ventricles of the brain [periventricular zone] like as rodent, bird, and reptile **(Garcia-Verdugo et al., 2002**).

The brain of Nile Tilapia like any other actinopterygian fish is composed of five main parts (telencephalon, diencephalon,

mesencephalon, metencephalon ,and myelencephalon).The anatomical features are described as the following**:** a) two small olfactory lobes situated front and beneath to the 2 halves of the cerebrum. b) A slight minor cerebrum. c) Diencephalon with vast matched substandard flaps of hypothalamus jutted out on the ventral perspective of the brain [Inferior lobes]. d) Two bulged projections of the optic tectum situated above the diencephalon e) Cerebellum which is well prominent above medulla oblongata. f) Large medulla oblongata **(Bulter, 2011 b).**




*Sagittal section of the brain of the Nile Tilapia, Oreochromis Niloticus, Showing the levels of orientative transverse sections*. **(Bulter b,2011)**

**OT:** Optic Tectum **TL:** Torus longitudinalis **VCe:** Valvula Cerebellum **OIN:** Olfactory nerve **POA:** Preoptic area **P:** Pituitary gland **LI:** Lobe inferioris **SV:** Saccus Vasculosus

**MO:** Medulla Oblongata **OC:** Optic chiasm **CCe:** Corpus cerebellum **SC:** Spinal cord

**Hyp:** Hypothalamus

The proliferation zones in adult teleost fish are located at three main sites either adjacent to the ventricular luminal surface (periventricular neurogenic zones), Para ventricular or far from the ventricle-cisternae systems (extra ventricular neurogenic zones) **(Zupanc and Horschke, 1995; Ekström et al., 2001).**

The periventricular zones (PVZ) of the adult fish brain incorporates the main dynamic sites of neurogenesis all over the brain. Several researches have highlighted a well difference among the various neurogenic niches in the adult fish brain in both glial phenotypes and the declaration of various transcription factors of incorporated in development. These examinations demonstrated ,that the PVZs are supplied with both slow and fast cycling cell populations, originating from a radial glia-like adult neural stem/progenitor cells **(Adolf et al., 2006*).*** Recently, a classifying pattern depending on a comprehensive study of immunohistochemistry has illustrated three types of cycling progenitor cells; class II, III, IV **(Marz et al., 2010; Rothenaigner et al., 2011).** Furthermore, the pallial–subpallial divisions of various PVZs have revealed that progenitors express, and might be organized by various transcription and growth factors such as fibroblast growth factors **(Adolf et al., 2006; Ganz et al., 2010)**.

There are four periventricular zones in the adult teleost brain classified according to the four ventricles existed. They are telencephalic ($1^{st}$V), mesencephalic ($2^{nd}$V), diencephalic ($3^{rd}$V) and rhombencephalic ($4^{th}$V) periventricular zones **(Zupanc, 2011)**.

The aim of our work was to shed light on the cellular configuration of the periventricular zones in the brain of adult tilapia using light and electron microscope as well as immunohistochemistry methods

## MATERIAL AND METHODS

### Tissue collection for Light microscopy:

This study was carried out on the brain of 100 adult Nile Tilapia (*Oreochromis Niloticus*) of both sexes, about 4 month each and 200-250 gm weight per each fish. Samples were taken at various seasonal periods for light microscopy, electron microscopy, and immunohistochemistry studies. All samples were obtained from fish farms in Manzala at dakahlia governorate. The fishes were transported in water boxes to the histology and cytology department, faculty of veterinary medicine, Mansoura University.

### Tissue Preparation:

Adult Nile tilapias were anesthetized with a solution of distilled water and 0.02% tricaine methanesulfonate solution. Fishes were fixed with insect pins in a sylgard- covered petri dish containing artificial cerebrospinal fluid (aCSF).

The aCSF was kept at room temperature and a PH of 7.4. Firstly, remove skull bones, then the brain was observed and cut the cranial nerves, and the brain was removed using micro-dissecting tweezers and the tips of insect pins.

Intensive precaution was applied to avoid any injury or harm to olfactory lobes and cerebrum due to they could be easily detached. To achieve this, we induce well dissection at the level of the connection between the spinal cord and brainstem, then extract the brainstem with an insect pin, and cut the ventral roots of the cranial nerves. Cut the optic nerve with small scissors then remove the whole brain. Immediately, immerse and rinse the brain in aCSF for 2-3 mins.

### Tissue fixation and processing:

Small pieces (0.5 $cm^3$) of the brain were kept in both Bouin's and 10% neutrally buffered formalin solutions for 18 hrs and 72 hrs respectively. The bouin's maintained samples were intensely rinsed in 70% ethanol (3×24hrs) to get rid of the fixative before the subsequent steps of tissue processing. Formalin-fixed samples were washed for 2hr under the running tap water before ethanol immersion. The tissue samples were dehydrated in ascending grades of ethanol (80%, 95% and absolute), cleared in xylene and embedded in paraffin wax. 5 micron thick sections were cut by microtome and mounted on a glass slide for ordinary stain.

The brain sections were dewaxed in xylene then rehydrated in descending grades of ethanol (absolute, 95%, 80% and 70%) and distilled water. The subsequent stained sections were dehydrated again in ascending series of ethanol (70%, 80%, 95% and absolute) and cleared in xylene. The histological processing and preparation of the brain slides were done according to the standard histological techniques (**Bancroft, 2015**). The used stain is Harris Hematoxylin and Eosin: for general histological examination.

### Specimen Preparation for Transmission Electron Microscopy:

Ultrastructure of the brain of *Oreochromis Niloticus* was examined using transmission electron microscope. To attain this work, small samples (about 1$mm^3$) from various periventricular zones in the brain, were

directly collected from the fish and immediately fixed in 2.5% buffered glutaraldehyde + 2% paraformaldehyde in 0.1 M sodium phosphate buffer PH 7.4 then leave tissue overnight at 4°C.Subsequently, the specimens were washed (4x15 min) in 0.1 M sodium phosphate buffer + 0.1M sucrose, and then post-fixed for 2hr at 4°C in 2% osmium tetroxide sodium phosphate buffered PH 7.4.

Then wash (3x15min) in 0.1M sodium phosphate buffer PH 7.4. Dehydrate (2x15min) in graded series of ethanol (50%, 70%, 90%, and 100%).The specimens are gradually embedded in Epon, where they are firstly dipped in propylene oxide (2x15 min), then in acetone- Epon mixture as the following: 30 min- 2:1 acetone : Epon, 30 min 1:1 acetone : Epon mixture, 30 min 1:2 acetone : Epon mixture, then finally in pure Epon solution overnight at 4°C.

After this, the specimens are embedded in gelatin capsules and incubated for 24 hrs at 4°C for polymerization.For a general view, semithin sections (1 μm) were cut using an ultramicrotome (Ultracut) and stained with methylene blue to be observed by LM .Ultrathin sections (60 nm) were cut from selected blocks, mounted on uncoated copper grids and routinely contrasted with urany1-acetate (80%) and lead citrate (1%).

After drying for 15 min, the sections could be prior to the examination. Ultrathin sections were examined at 80kv using a JEOL electron microscope at EM unit, Faculty of Agriculture, Mansoura University, Egypt.

The protocol of this technique was carried out according to **Karnovsky, (1965).**

**Nestin Immunofluorescence Protocol-Indirect-Single Labelling:**

**1]Slide Preparation (Formalin-fixed-Paraffin-tissue sections)**

- Deparaffinize tissue sections in xylene (2 times x 10 min).
- Hydrate with absolute alcohol (2 times x 5 min) and then immerse slides through descending series of ethanol (95%, 90%, 80%, 70%, and 50%), each for (2 times x 5 min).
- Rinse the slides with distilled water.

**2] Permeabilization – Inactivation**

- This step is induced to destroy the cell membrane for exposure of antibody binding sites by using a detergent solution – Triton x-100 diluted in PBS to about 0.1 – 0.25%, and then the slides kept incubated for 10 min in a humid chamber.
- Rinse with PBS (3 times x 5 min).

**3] Blocking**

- To block unspecific binding of the antibodies, tissue sections were incubated with a blocking buffer solution-Bovine Serum Albumin (BSA) diluted to about 1% in PBS.
- The cells incubated for 30-60 mins at room temp in a humid chamber
- Wash the applied serum by water aspirator not by 1xPBS solution.

**4] Primary Antibody Incubation**

- Add primary antibody (1:200 dilution of mouse-antinestin) for each slide or coverslip. Incubate at $4^0$C over night or for 1 hr at room temp in a humid chamber.

After incubation, wash slides with 1xPBS + 0.5% Triton x-100 solution (3 times x 5 min).

**5] Secondary Antibody Incubation**

- Apply secondary antibody (Goat-Anti Mouse-FITC-Conjugated-Ab- 1:200) to each section. Dilute the secondary antibody in 1xPBS + 0.5% Triton x-100 +1% BSA then incubate for 1 hr at room temp in dark.
- Drain off excess antibody then wash 3 times with PBS 3 times for 5 min each in dark.

**6] Counter Staining**

Add counter stain DAPI for 1 min then rinse with PBS.

**7] Mounting**

- Mount coverslips with a drop of mounting media and then seal with clear nail polish to prevent drying and movement under the microscope. Store in dark at -20$^{o}$c or 4$^{o}$c and finally examine with Zeiss Axioskop Fluorescence Microscope at cytology and histology department, faculty of vet medicine, Mansoura University.

This protocol is carried out according *to* **(Leung and Chou, 2011**)

## RESULTS

**Brain Anatomy:**

The brain of adult Nile Tilapia from the dorsal view was composed of olfactory lobes, cerebrum (2 cerebral hemispheres), cerebellum and medulla oblongata. Olfactory lobes were in pairs (one on each side), and attached to the olfactory tract. The cerebral hemispheres were located behind the olfactory lobes and constitute the anterior part of the telencephalon. The telencephalic ventricular space (1$^{st}$ v) could be seen in the frontal groove of cerebrum **.**The mesencephalon including the optic lobes which appeared as a pair of round lobes attached to the cerebrum. The cerebellum was the most prominent part in the brain of fish showing a crest on top (Corpus Cerebelli) laying on a stem (Valvula cerebelli). The medulla oblongata constituted the mass of the hind brain which continue to form the spinal cord **(Fig. 1).**

At the ventral view, the brain revealed diencephalon, which was composed of 2 inferior lobes, they were two large lobes, in pairs one on each side and contained the major part of the infundibulum. The pituitary gland was a round mass located in between the inferior lobes. Saccus vasculosis was a thin and long sac located in between the inferior lobes under the pituitary gland. Medulla oblongata appeared as a long stem extending from the brain **(Fig. 2).**

**Light Microscopy study:**

The light microscopic structure of the four types of brain ventricles demonstrated that they were lined with simple cuboidal to squamous epithelium (Ependymal cell lining). In certain areas of the four ventricles under the epithelial lining, there were proliferating areas of small cells with small rounded or longated nuclei with some signs of mitosis.

**Telencephalic periventricular zone (First Ventricle)**

This area was situated at the frontal groove of telencephalon inserted in between the olfactory lobe and the 2 cerebral hemisphere **(Fig. 3, 4)**.Cerebrum was subdivided into dorsal and ventral part. The mitotic active young cells were situated along the ventricular surface especially at a narrow zone between the dorsal (pallium) and ventral (supallium) parts. The cells in this area mainly revealed rounded nuclei **(Fig. 5, 6)**

**Mesencephalic periventricular zone (Second Ventricle)**

This zone was located in between the optic tectum (Teo), torus longitudinalis (TL) and torus semicircularis (TS) **(Fig. 7)**. The existence of young proliferating cells along the tectal ventricle was examined at distinct sites .At the middle part of the internal optic tectum layer **(Fig. 8),** the caudal pole of the tectum **(Fig. 9),** the torus longitudinalis where a dense cluster of proliferating young cells were located at its dorsolateral aspect facing the ventricular surface **(Fig. 10)** and the torus semicircularis where the newly born cells were present at the dorsomedial border facing the tectal ventricle **(Fig. 11)**.

**Diencephalic periventricular zone (Third Ventricle)**

From rostral to caudal, this zone extended from the central and posterior thalamic nucleus, periventricular nucleus of the posterior tuberculum(TPP) extending along the entire rostral-caudal extension of the hypothalamic ventricular recess nucleus (Nucleus recess lateralis (nRL) **(Fig. 12,13).** The proliferating young cells were more predominant at the medial part of the nucleus recess lateralis, the cells in this area mainly displayed rounded nuclei **(Fig. 14)**.

**Metencephalic periventricular zone (Fourth Ventricle)**

The fourth ventricle was situated beneath the corpus cerebellum, surrounded by the valvula cerebellum and extended caudally into the medullary oblongata parenchyma **(Fig. 15).** The cells located at the anterior part of the molecular layer of corpus cerebellum showed rounded nuclei **(Fig. 16).**

**Electron Microscopy Study:**

The periventricular zones of dorsal telencephalon (D) comprised the wide surface region and course in a medial pattern along the dorsal ventral axis of the telencephalic ventricle, ending at the ventral telencephalon (Vd).

**Ependymal lining of the Telencephalic Ventricle (Dorsal Portion)**:

These ependymal cells were mainly cuboidal to low cuboidal**,** revealing clusters of heterochromatin within an elliptical to elongated nuclei. The cytoplasm was electron lucent and contained various mitochondria. These cells also showed microvilli projecting into the lumen at the apical surface**.** The ependymal cells were found at the top of the telencephalic ventricle and not recognized anywhere over the PVZs **(Fig. 17)**.

The main cell patterns were distinguished in the middle portion of the dorsal telencephalon. At the dorsal subdivision of the dorsal area of the telencephalon, there was a single cell layer delighting an altered ependymal like appearance (class II cells), these cells had an ovoid or asymmetrical nuclear shape with generally uniformly arranged, electron lucent chromatin. They demonstrated one to two large nucleoli effectively recognized inside the nucleus. The cytoplasm was plenteous, delicately stained and frequently reach out along the luminal surface **(Fig. 18).**

Class III cells were characterized by their elongated or uneven shaped nucleus one to two conspicuous nucleoli. The chromatin of these cells was regularly distributed and electron dense. These cells contain slight electro lucent cytoplasm and small amount of detectable organelles **(Fig. 19).**

Class IV cells were easily demarcated from class III by their densely stained, extremely reticulated chromatin structure of various levels of aggregated heterochromatin and the uncommon existence of obvious nucleoli**.** The cytoplasm of class IV cells was more electron dense than that of class III and contain less organelles **(Fig. 20).**

**Nestin ImmunoFluoresence Examinations:**

**1] First Ventricle (Telencephalic Ventricle)**

Nestin labeled cells were observed at distinct sites along the ventricular wall. The main significant sites were at the anterior region of the dorsomedian telencephalon **(Fig. 21),** where they were present in large quantities than that observed at the ventral portion of the dorsomedian telencephalon **(Fig. 22).**

At the ventral telencephalon, these nestin expressing cells were situated at the dorsal part of the ventral subdivision **(Fig. 23),** and at the supracommissural part of the ventral telencephalon **(Fig. 24).**

**2]Second Ventricle (Mesencephalic Ventricle)**

At this zone, the Nestin-labeled cells were examined at the dorsomedial aspect of the internal layer of optic tectum **(Fig. 25),** and at the dorsolateral border of the torus Longitudinalis **(Fig. 26).**

**3] Third Ventricle (Diencephalic Ventricle**)

In this region, nestin positive cells were seen at various sites along the entire ventricular surface, at the preoptic area where they were distributed at the dorsoventral aspect of the nucleus. The cells at this area displayed rounded nuclei **(Fig. 27),** at the lining of the medial aspect of the hypothalamic nucleus. The cells mainly displayed round to ovoid nuclear counter **(Fig. 28).** Another location of the proliferative young cells was at the periventricular posterior tuberculum nucleus distributed along the immediate vicinity of the third ventricular wall **(Fig. 29)**.

**4]Fourth ventricle (Metencephalic Ventricle)**

In this area, nestin expressed cells were demonstrated at the dorsal molecular layer of corpus cerebellum, where they were distributed over large areas within this area. The cells mainly revealed elongated nuclear shape **(Fig. 30).**

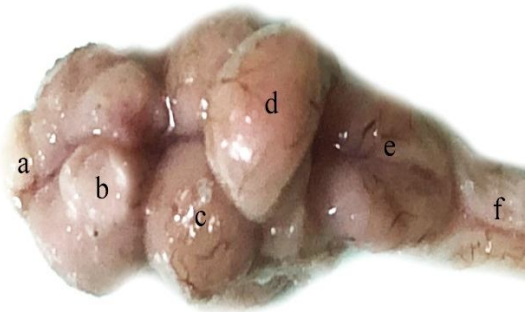



**Figure 1:** Over view of the brain anatomy in adult Nile Tilapia "dorsal view". a) Olfactory lobe. b) Cerebrum. c) Optic lobe. d) Cerebellum. e) Medulla oblongata. f) Spinal cord

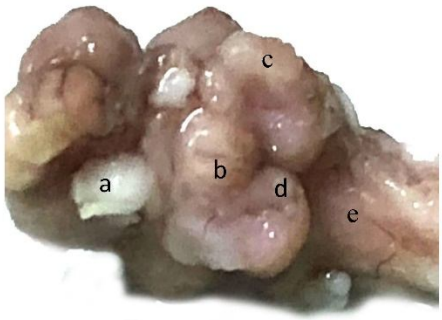



**Figure 2:** Over view of the brain anatomy in adult Nile Tilapia "ventral view". a) Optic chiasm. b) Pituitary gland. c) Inferior lobes. d) Saccus vasculosis e) Medulla oblongata

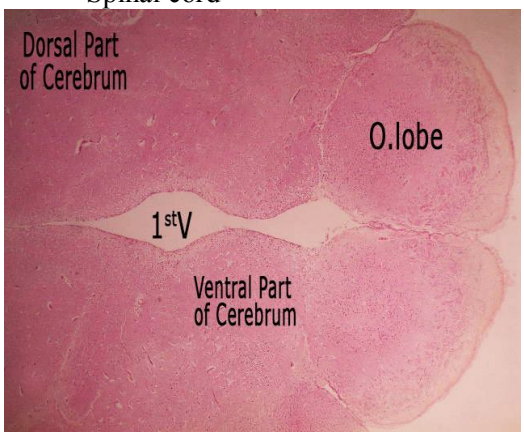



**Fig. 3:** Photomicrograph showing the olfactory lobe, cerebrum and in between the telencephalic ventricle ($1^{st}v$) (H&E stain, x4).

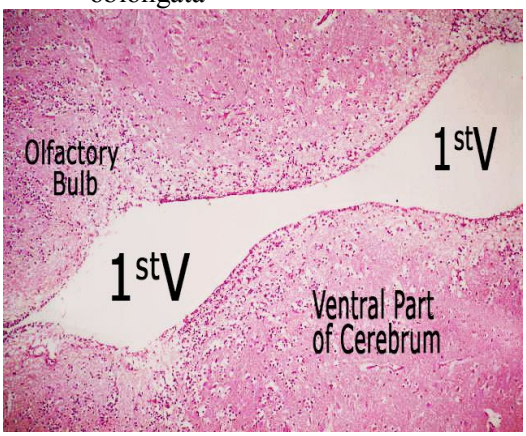



**Fig. 4:** Transverse section showing the distribution of young mitotic cells along the ventricular surface within the ventral telencephalon,(H&E stain x10).

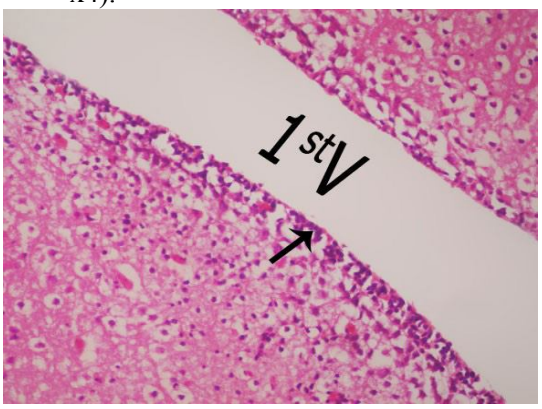



**Fig. 5:** Photomicrograph showing the narrow zone of the dorsal telencephalon. Proliferating cells are highly distributed near the ventricular wall. (Notice the arrow), (H&E stain, x40).

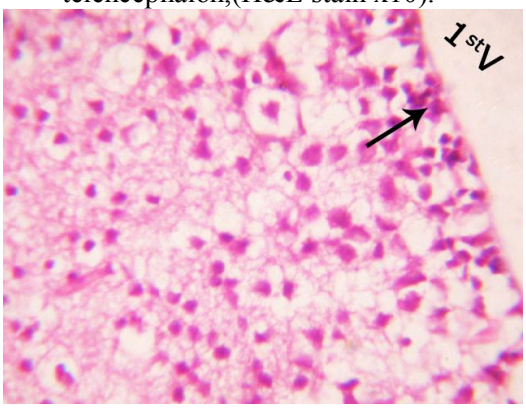



**Fig. 6:** A higher magnification photo of the narrow zone showing the 1st ventricle and round nuclei of mitotic cells. (Notice the arrow), (H&E stain x100).

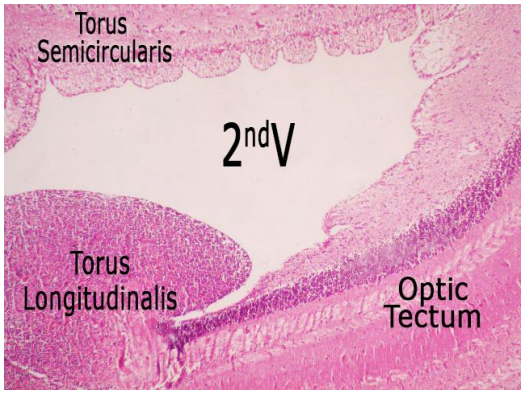



**Fig.7**:photomicrograph revealing the mesencephalic ventricle with its associated structures.(H&E stain , x10)

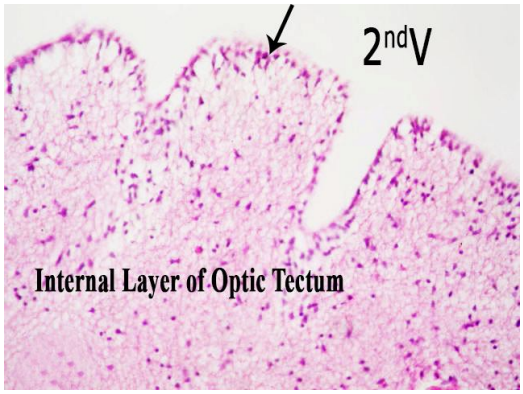



**Fig. 8:** A high proliferation activity at the middle part of the inner layer of optic tectum (arrow). (H&E stain, x40).

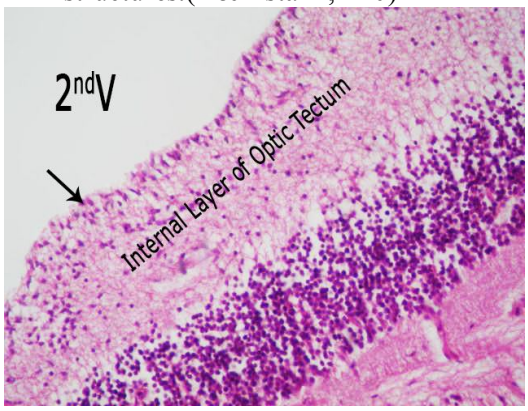



**Fig. 9:** Transverse section revealing large amounts of proliferating cells within the caudal end of the optic tectal internal layer. (Arrow). (Notice the round nuclei of cells). (H&E stain, x40).

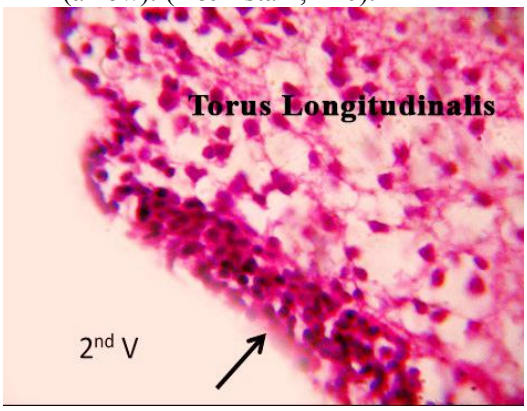



**Fig. 10:** Torus Longitudinalis (TL). The site of intrusion is characterized by the presence of a dense cluster of dividing cells. (Arrow) (H&E stain, x100).

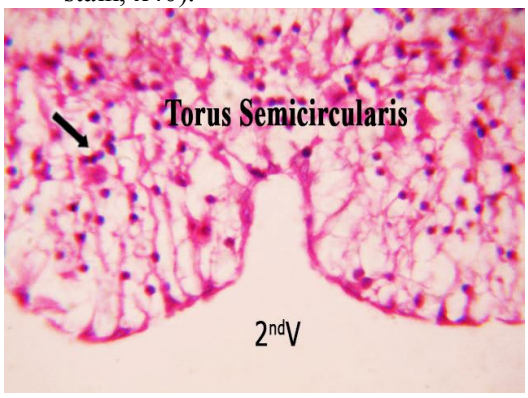



**Fig.11**: Transverse section through the tours semicircularis revealing mitotic young cells extending along the ventricular surface.(Notice the arrows),(H&E stain,x100).

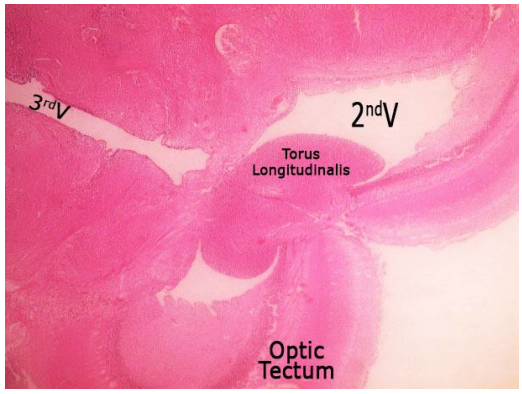



**Fig.12**: Transverse section through the optic tectum and diencephalon illustrating the organization of second and third ventricle.(H&E stain,x4).

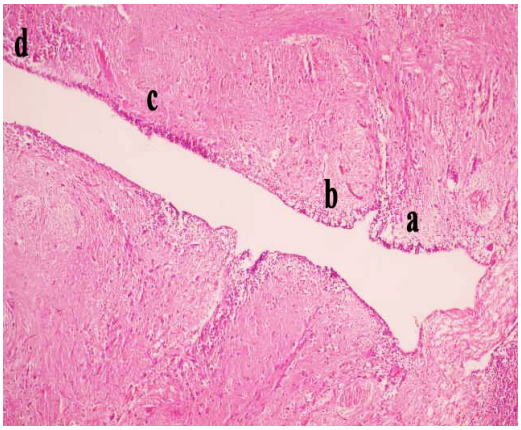



**Fig.13**: Photomicrograph revealing third ventricle with its assocated structures.
a)Dorsal Posterior Thalamic Nucleus (DP)
b)Cental Posterior Thalamic Nucleus (CP)
c)Periventricular Nucleus of the Posterior Tuberculum (TPP) d)Medial part of the Nucleus Recess Lateralis(nRLm)

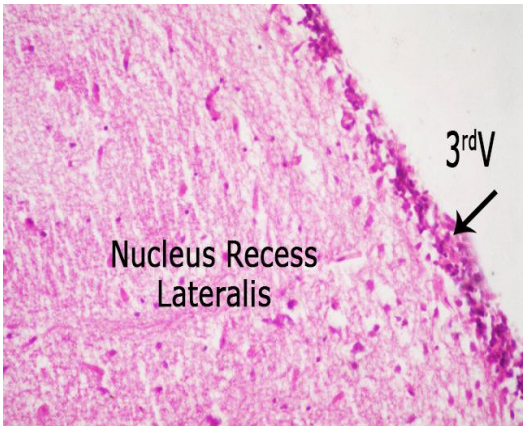



**Fig.14:** A dense areal density of active young cells placed at the medial part of the nucleus recessi lateralis (nRLm) (H&E stain, x40).

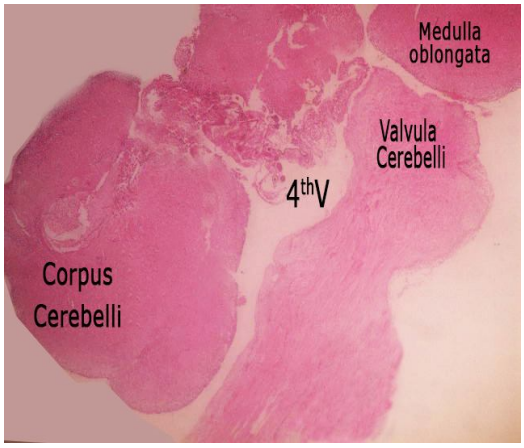



**Fig.15:**Photomicrograph revealing the rhombencephalic ventricle (4th V), situated beneath the corpus cerebelli. (H & E stain, x4).

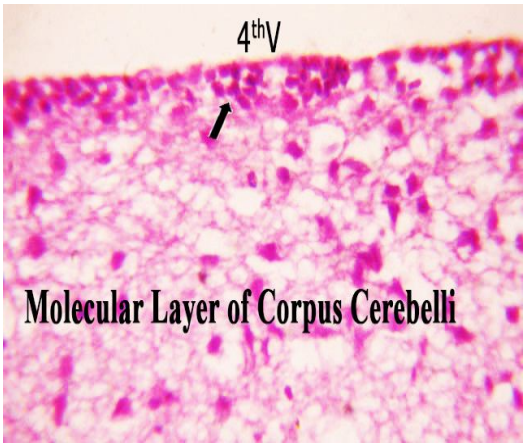



**Fig.16**:Existence of proliferating young cells along the ventricular lumen.The cells reveal elongated nuclei.(arrows).(H&E stain,x 100)

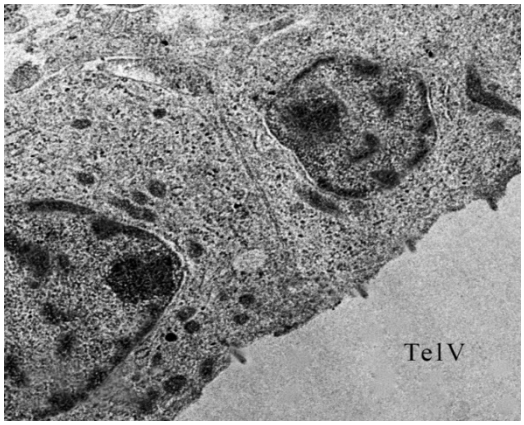



**Fig .17:** TEM micrograph of the dorsal ependymal lining (DEL).A one row of cuboidal epithelial cells enfolding the dorsal of the telencephalic ventricle (TelV). The cells reveal aggregated heterochromatin, bulbus microvilli,vacuoles and many mitochondria, darkly stained lipid droplets scattered throughout the cytoplasm. Junctional complexes could be examined binding the membranes of the adjacent ependymal cells (TEM, X 2000).

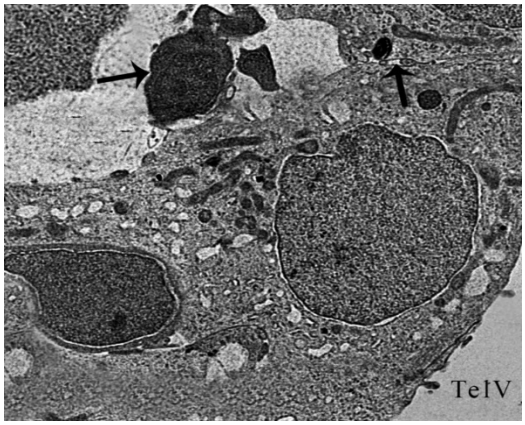



**Fig .18**: TEM micrograph revealing class II cells.They are confined to the neighboring cells by the use of close-fitting and intermediate junctions including desmosomes (arrow).An active dividing cell noticed close to the TelV that seem to be in later stages of division (TEM, X 1500).(arrow).

Nucleus: Ovoid or asymmetrical in shape,chromatin evenly distributed, non clumped and medium stained.Nucleoli range from one to two.

Cytoplasm: predominant and electron lucent revealing many mitochondria, few to intermediate microvilli, lipid droplets, small vacuoles and dense bodies.

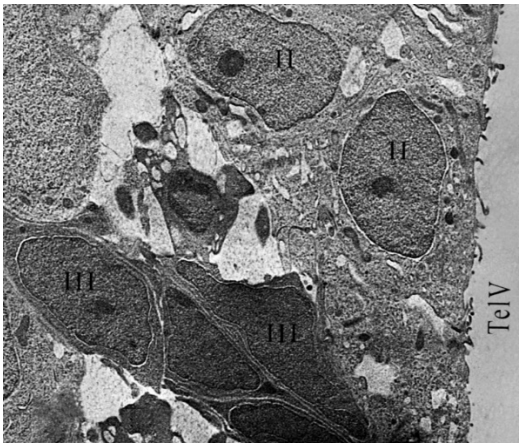



**Fig.19:** Ultrastructure identification of class III cells. They are featured by their elongated to irregular nucleus with equally distributed chromatin, one to two obvious nucleoli and little cytoplasm containing few organelles (TEM, X 1000).

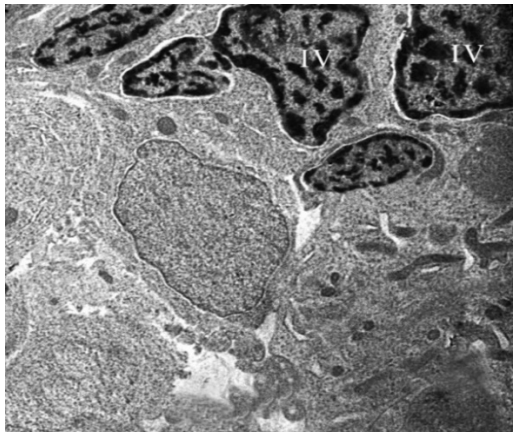



**Fig .20:** Ultrastructural organization of class IV cells. They are featured by their frequently irregular nucleus with different levels of staining, reticulate chromatin and few cytoplasm. A great portion of heterochromatin located at the borders of the nucleus. These cells mainly present at the ventricular surface of the PVZ, but also may be seen in the deep layers of PVZ (TEM, X 4000).

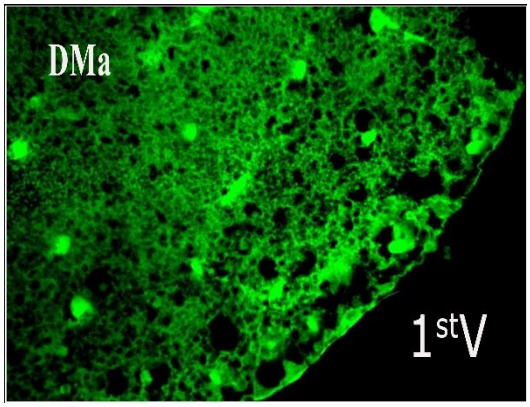



**Fig. 21:** Fluorescent image showing nestin-posiive cells. A large number of these cells are found at the anterior part of the dorso-median telencephalon (DMa). The cells display rounded shape.

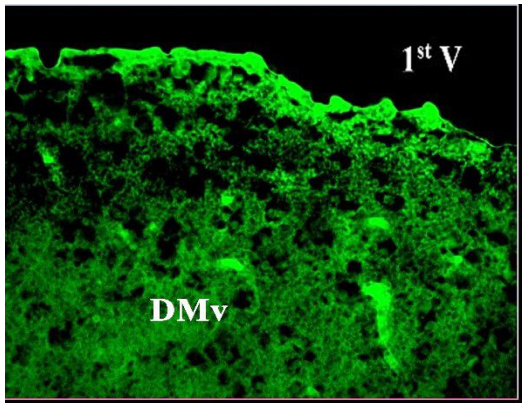



**Fig. 22:** Fluorescent image showing distribution of nestin-expressed cells at the ventral portion of the dorso-median telencephalon (DMv). The number of cells decreases than that in the dorsomedian telencephalon (DMa).

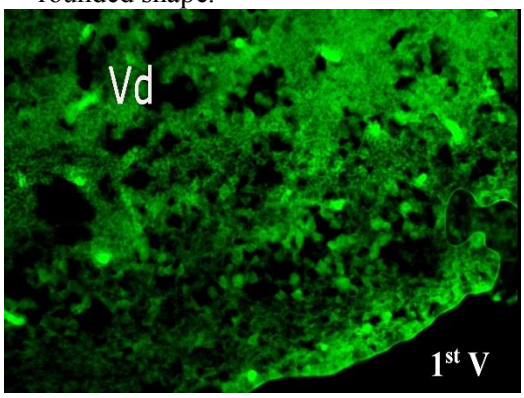



**Fig.23:** Fluorescent image revealing large number of nestin-positive cells at the dorsal part of the ventral telencephalon (Vd).

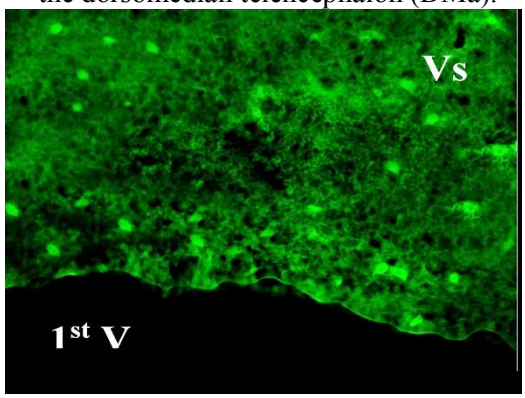



**Fig. 24:** Fluorescent image showing nestin-labeled cells at the supracommissural part of the ventral telencephalon (Vs).

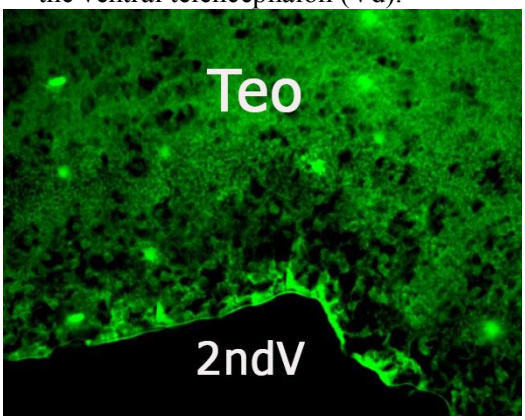



**Fig.25:** Fluorscent image through the tactal ventricle (2ndV) revealing the proliferation of cells within the innermost layer of the optic tectum (Teo).

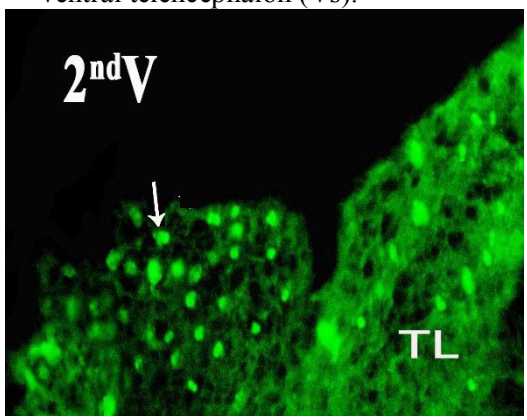



**Fig. 26:** A massive aggregations of nestin-labeled cells within the torus Longitudinalis (TL). (Notice the arrow).

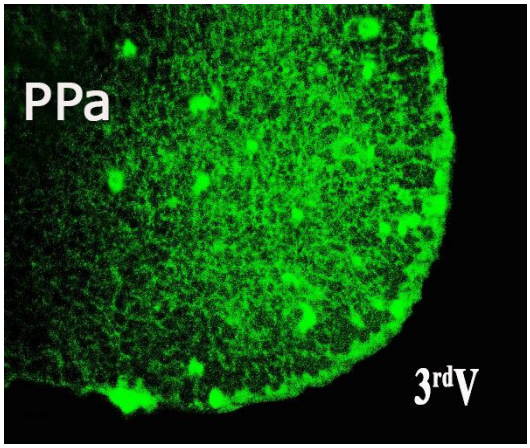



**Fig.27:** Fluroscent image showing persistence of nestin-labeled cells at the periventricular preoptic nucleus zone (PPa). The cells display round to ovoid nuclei.

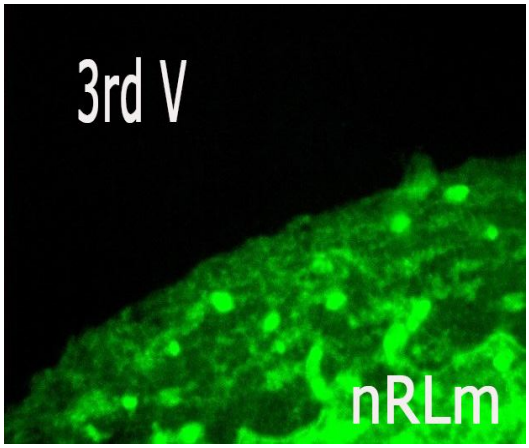



**Fig. 28:** A massive quantity of nestin-positive cells located at the medial boundary of nucleus recess laterals (nRLm).The cells display round to ovoid nuclei.

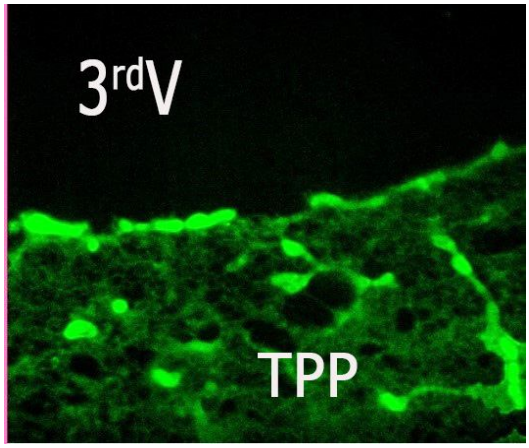



**Fig. 29:** Existence of nestin-labeled proliferating cells at the nucleus of periventricular posterior tuberculum (TPP). The show round to elongated nuclei.

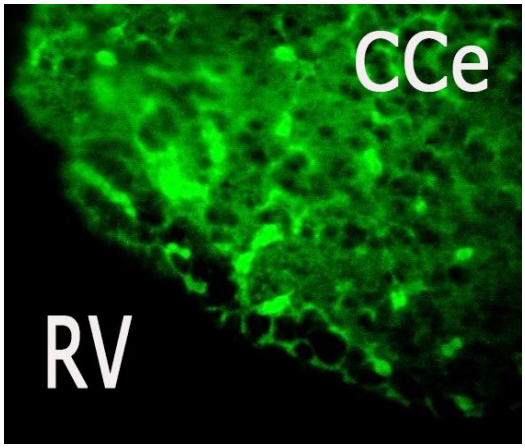



**Fig.30:** Fluroescent image showing positive-nestin-labeled cells throughout the corpus cerebelli. (RV, Rhembencephalic ventricle). (CCe, Corpus Cerebelli). The cells reveal elongated nuclei.

## DISCUSION

The brain in fishes is composed of five various parts: Telencephalon ,Mesencephalon , Diencephalon, Metencephalon and Rhomphencephalon **(Gonzalez and Northcutt, 2011)**. The brain ventricles include the telencephalic, tectal, diencephalic and rhombencephalic ventricles **(Butler, 2011 a, b)**.

In this study, we used light, electron and nestin analog experiments to characterize the cells constituent of the periventricular regions in the mature nile tilapia brain. The results demonstrates that predominant mitotic dividing cells are existed adjacent to the ventricular lining of telencephalic pallium and subpallium,optic tectum, torus longitudinalis,torus semicircularis and diencephalic preoptic nucleus, hypothalamic ventricular nuclei and corpus cerebellum.

The overall organization of nestin-expressing cells along the ventricular lumen were resembling to that observed in another fish species including *Zebrafish* **(Zupanc et al., 2005; Grandel et al., 2006)**, *brown ghost knife fish* **(Zupanc and Horschke, 1995)**,*three spined stickleback* **(Ekström et al., 2001)** and *gilthead sea bream* **(Zikopoules et al., 2000).**

The observations of light microscopic and nestin labeling experiments revealed the presence of young mitotic cells distributed along the extension of the ventricular lining of telencephalon especially at the dorsal and median subdivisions of the dorsal telencephalon.A prominent cluster of newly born cells were located adjacent to the narrow zone of the subpallium telencephalon.The cells at these sites mainly display rounded to ovoid nuclear profile.These findings confirm the conclusions of the previous studies in *Zebrafish* **(Zupanc, 2011)**,*Astatotilapia Burtoni* **(Maruska et al., 2012)** and *Mozambique tilapia* **(Teles et al., 2012).**In contrast to the results obtained in the Brown Ghost ,most of the mitotic active cells were located at the dorsolateral telencephalic area **(Zupanc and Horschke, 1995) .**

In the mesencephalic periventricular zone of nile tilapia,the existence of proliferating young cells were examined at significant sites.At the dorsal,medial and ventral aspects of the caudal third of the internal optic layer,dorsolateral ventricular aspect of the torus longitudinalis and the dorsomedial border of the torus semicicularis.These findings were compatible with the observations reported in *Stickleback* **(Ekström et al., 2001),** *Zebrafish* **(Grandel et al., 2006)** ,*Oryzias Latipes* **(Kuroyanagi et al., 2010),** *Nothobranchius Furzeri* **(Terzibasi et al., 2012)** .Conversely,in the torus semicircularis of mozambique tilapia ,the newly born cells are widely distributed along the ventricular surface **(Teles et al., 2012)** rather than the limited existence of cells as in the nile tilapia.

In the diencephalic periventricular area, the present study has demonstrated that the vast majority of active young cells are situated at the ventricular lining of the preoptic area, dorsal and posterior thalamic nuclei, posterior tuberculum nucleus and the hypothalamic ventricular nucleus. This agrees with the results that were reported in *Stickleback* **(Ekström et al., 2001),** *Zebrafish* **(Grandel et al., 2006),** *Gilthead sea bream* **(Zikopoules et al., 2000)** and *Mozambique tilapia* **(Teles et al., 2012).**

In the cerebellum, the high density of labeled cells was examined at the ventricular lining of the molecular layer in the corpus cerebellum.These results were greatly similar to what reported in the previous species **(Marz et al., 2010)**

Detailed TEM examinations across the

dorsal telencephalic periventricular zone exhibits four morphological different cells, all of which reveal various structures. The basic cell organization of the PVZs of Dd (dorsal region of dorsal telencephalon) and Dm (median region of dorsal telencephalon) are the class II cells. They are commonly most predominant in these regions, in addition to class III and class IV. The DEL(dorsal ependymal lining) could be included to the distinct cell populations of PVZs concerned with the dorsal telencephalon. These results were nearly similar to that reported in Zebrafish **(Lindsey and Tropepe, 2006)** except for the presence of class V in zebrafish but not observed in nile tilapia.

Current examinations in the mature fish brain have shown the closely packed cellular nature of the proliferating constituents in vertical columns between which the developing process, blood vessels and relatively extensive extracellular spaces are found in the dorsomedian telencephalon. This was demonstrated by resin implanted semithin segments where general arrangement and the presence of mitotic forms at the surface of the ventricle, like the area of mitosis found in the neurogenic zone of dorsolateral telencephalon and posterior region of dorsal telencephalon in zebrafish **(Ganz et al., 2010).**

The proliferation activity of cell subtypes of class II and class III cells at the ultrastructural level was analyzed and affirmed by immunohistolabelling for BrdU positive cells. The results revealed that these two cell sorts are more abundant in regions encompassing the surface of ventricular telencephalon in zebrafish, they are immunopositive for BrdU and the common radial glial marker S100β **(Kishimoto et al., 2011).**

## CONCLUSION

This study examined variations in the neural configuration in the mature tilapia brain and demonstrated significant cell morphologies constituting the distinct periventricular zones. The results of this approach revealed that the predominant cell sorts and subtypes concerned with the neurogenic pattern are the cycling mitotic type II cells. They are mainly applicant for the morphological appearance of radial glial stem cells, besides another examined cell types which compose the profound layers of zones. Finally, by examining the distribution and organization of mitotic cells between various neurogenic periventricular regions. The results inspected here provide a background for further exploring the selective role of adult neurogenesis cell proliferation.

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# الملخص العربي

## التكوين الخلوى للمناطق المولدة للخلايا العصبية في مخ اسماك البلطى البالغة

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تم فحص تكوين الخلايا العصبية وانتشارها المكثف في بعض المناطق على طول سطح تجويف المخ ، هذه الظاهرة لاتحدث فقط خلال مرحلة تكوين الجنين ولكن أيضا تستمر الى مرحلة نمو أجزاء المخ بشكل كامل خلال مرحلة البلوغ ، بغض النظر عن الثديات ، فأن الجهاز العصبى في الأسماك يظهر به انتشار واسع النطاق للمناطق العصبية ذات فاعلية تكاثر عالية . كما تم وصف المناطق الخلوية ومكونات المناطق المحيطة بتجويف المخ في اسماك البلطى النيلى البالغة ، والتي تحتل المرتبة الاولى من الأسماك ذات الفقاريات ، لاتمام هذا العمل، تم تطبيق الدراسات المجهرية والاليكترونية لفحص الشكل التشريحى والهستولوجى بالإضافة الى التركيب الخلوى بالمجهر الإليكتروني ، و تم تأكيد النواتج عن طريق nestin immunohistochemistry والذى استدل عن وجود خلايا جزعية وقابلة للانقسام في هذه المناطق .