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# Anatomical and histological features of the brain of adult common carp (Cyprinus Carpio)

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ORIGINAL ARTICLE

# Anatomical and Histological Features of the Brain of Adult Common Carp (*Cyprinus carpio*)

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**Abstract**

**BACKGROUND: The study investigated the anatomical features and histological structures of the brain of adult common carp. In addition, these features were compared with those of other types of fish.**
**METHODS: Adult fish (8–18 months) were collected and their gross anatomical features were inspected; and small specimens from different brain parts were fixed, sectioned, stained, and histologically examined.**
**RESULTS: In the common carp, the olfactory bulb was round, somewhat tiny, and connected to the telencephalon by a very long, thin olfactory tract. Hemispheres of the telencephalon were located rostrally. Only one median ventral telencephalic ventricle was visible. The torus longitudinalis and torus semicircularis connect the paired optic lobes that make up the large midbrain dorsally and ventrally, respectively. The valvula cerebelli protruded forward, whereas the corpus cerebelli was positioned posteriorly. The two vagal lobes encircled the two face lobes of the myelencephalon posteriorly and were intimately linked to one another. Fish telencephalon histology showed a greatly reduced structure consisting of a single layer of linked neurons buried in a large neuropil. Conversely, a laminar histological structure was observed in the optic tectum.**
**CONCLUSIONS: The brain of a common carp is similar to that of many other fish in that it is primarily composed of five structures: telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon. The anatomical characteristics of the common carp brain include the olfactory bulb, olfactory tract, optic tectum, cerebellum, and facial and vegal lobes.**

*Keywords:* Anatomy, Brain, Common carp, Histology, Teleost

## 1. Introduction

The brain and the spinal cord are the primary components of the central nervous system in fish and vertebrates, connecting them to receptors and afferent organs through motor and sensory nerves [1]. In contrast, the brains of fish and mammals differed greatly in their anatomical and histological makeup but shared the same number of brain partitions [2]. According to Nieuwenhuys [3], the forebrain of fish is composed of paired olfactory bulbs that are dorsally covered by the diencephalon, which is composed of the thalamus, hypothalamus, subthalamus, epithalamus, and the pretectum. The paired olfactory bulbs were joined ventrally to the paired telencephalic hemispheres. Butler [4] observed that paired optic lobes connected on the inside by torus longitudinals form the roof of the midbrain, followed by the cerebellum (metencephalon) and large medulla oblongata (myelencephalon).

The rostral portion of the forebrain in fish is called the telencephalon, and it serves important purposes, such as receiving and conducting scent signals. Anatomically, the telencephalon is composed of two parts: paired massive cerebral hemispheres and olfactory bulbs [3]. An extremely long, thin olfactory tract connects the spherical or oval olfactory bulb of *Cyprinus carpio* (Cyprinifors order of Actinopterygii class) to the telencephalon [4]. However, Eleginops have large olfactory bulbs that are approximately

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half the volume of telencephalic lobes [5]. In some taxa, such as elasmobranchs, sarcopterygians, and agnathans, their telencephalon was formed by evaginating (bubbling out) the lateral walls of the neural tube, which contained central lateral ventricles, whereas the telencephalon of the actinopterygian forebrain was formed by bending out (eversion) of the dorsal walls of the embryonic neural tube. Therefore, the forebrain of actinopterygians such as carp has a single, median ventral ventricle [3].

The diencephalon, positioned between the telencephalon and optic lobe and dorsally covered by the posterior bulge of the telencephalic hemispheres, houses the diencephalic ventricle. It mainly consists of three parts: dorsal part (epithalamus), lateral part (thalamus), and the ventral part (hypothalamus) [6]. The relatively large mesencephalon comprises two primary structures: the dorsal optic tectum and the ventral tegmentum. The optic tectum is further divided into two optic lobes with a layered histological structure consisting of five to six principal layers with varying contents of nonmyelinated or myelinated axons and neurons [7]. It is comparable to the cerebral cortex of mammals because of its six layers with a distinct multilayered arrangement of nerve cells [8].

The hindbrain of fish consists mainly of the cerebellum (metencephalon) and medulla oblongata (myelencephalon). The cerebellum is the most prominent structure in the fish brain, especially in the order Cyprinidae. It is located dorsally on the mesencephalon and is primarily composed of corpus cerebelli and valvula cerebelli [9]. In fish, the corpus cerebellum is like that of mammals and is located on top of the rostrum of the rhombencephalon. In contrast, valvula cerebelli was only found in ray-finned fish and bulged forward below the tectum in the tectal ventricle [10]. The vagal lobe consists of paired cylindrical lobes that are located behind the facial lobe and dorsal to the medulla oblongata, which encloses the facial lobe ventrally. The facial lobe was then surrounded anteriorly by the corpus cerebelli and posteriorly by the vagal lobe, despite the appearance of attachment [11].

In fish, the olfactory lobe is a small round-shaped lobe present in all fishes surrounded by a thin epithelium. It is composed of numerous round-shaped neurons that are distributed through the olfactory bulb, which is placed on a wide neuropil [12]. These neurons are larger than those neurons of the cerebrum with their larger nuclei. The olfactory epithelium and bulb contain thin dendrites, axons, and basal and receptor cells [8], and the telencephalon is significantly smaller than the telencephalon of other mammals. As in mammals, the cerebral cortex consists mainly of six layers, known as the neocortex, whereas teleosts only have a single layer containing fields of interlocked neurons rooted in widespread neuropil [7].

In fish, the diencephalon is the lower part of the brain and includes the epithalamus, thalamus, and hypothalamus. The epithalamus, positioned beneath the optic tectum, houses the pineal gland, habenular ganglion, and saccus dorsalis [2]. The thalamus is located between the tegmentum and the hypothalamus, while the hypothalamus situated at the back is crucial and comprises the pituitary gland and saccus vasculosus. The optic lobe, a prominent part of the midbrain, contains two lobes and a tectal ventricle and is made up of six distinct layers: stratum marginale, stratum opticum, stratum fibroetgricialem, stratum album central, stratum griseum central, and stratum periventriculae [13].

The cerebellum in *Epinephelus coioides* fish is made up of the corpus cerebelli and valvula cerebelli. Histologically, it consists of three main layers: an outer molecular layer, an inner granular layer, and an intermediate ganglionic layer [8]. The molecular layer contained a constant sheet of neuropils with oriented dendrites, eurydendroid cell dendrites, and satellite cells. Below the molecular layer, the ganglionic layer contained Purkinje and eurydendroid cells. In mammals, the ganglionic layer does not contain eurydendroid cells and is referred to as the Purkinje cell layer. Purkinje cells have rounded or pear-shaped somata, whereas eurydendroid cells have triangular or rhomboid-shaped somata. Eurydendroid cells were larger than Purkinje cells, with smaller nuclei. The third layer, the granular layer, is situated beneath the ganglionic layer and consists of dark granule cells and small multipolar neurons [14]. The medulla oblongata, which is the stem of the brain, comprises the main mass of the hindbrain and contains the rhombencephalic ventricle. It was composed of neuropil with various cell types, and the structure of the medulla oblongata was significantly influenced by the fish's feeding habits, particularly the facial, vagal, and somatic sensory lobes. The vagal lobe comprises a sensory, fiber, and motor layer [15].

## 2. Material and methods

### 2.1. Specimen collection

Brains of 25 adult common carp (*C. carpio*) of both sexes, weighing ~500–1500 g per fish, are the subjects of the current examination. Samples were collected at various times of the year for light microscopy purposes. All specimens were sourced from fish farms in Kafr A-Shaykh and Manzala in the Dakahlia governorate. Fish were transported in water bags to

the Anatomy and Embryology Department, Faculty of Veterinary Medicine, Mansoura University.

### 2.2. Tissue fixation and preparation

Common carp were rendered unconscious using distilled water and 0.02 % Tricaine Methanesulfonate (Sigma–Aldrich, St Louis, Missouri, USA). Fish were secured with insect pins in a Petri dish covered with sylgard and filled with artificial cerebrospinal fluid. Extensive precautions were taken to ensure the brain's safety during dissection. Specifically, the dissection was initiated at the junction between the spinal cord and the brainstem, and the brainstem was carefully removed using an insect pin. The optic nerves were carefully dissected using small scissors, and the entire brains were subsequently collected for analysis. Following this, all specimens were promptly submerged in cerebrospinal fluid for 2–3 min. Small fragments of the brain, measuring ~0.5 $cm^3$, were then fixed in Bouin's solution for periods of 18 and 72 h, respectively. After fixation, Bouin's samples underwent thorough washing in 70 % ethanol to eliminate any residual fixative before the next stages of tissue processing. In contrast, the samples fixed in formalin were rinsed for 2 h under running tap water before being immersed in ethanol. The tissue samples underwent dehydration using various ethanol concentrations, followed by clearing in xylene and embedding in paraffin wax. Subsequently, sections were cut using a microtome and mounted onto glass slides for staining, following a specific reference [16] (Plate 1).

### 2.3. Tissue staining

Brain segments underwent dewaxing in xylene and were then rehydrated in decreasing ethanol concentrations (100, 95, 90, 80, and 70 %) followed by distilled water. The stained segments were again dehydrated in ascending concentrations of ethanol (70, 80, 95, and 100 %) and then cleared in xylene. Histological processing and preparation of the brain slides were performed according to standard histological techniques [17]. Harris hematoxylin and eosin stain was used.

## 3. Results

The brains of adult common carp were visualized by the naked eye and appeared to be composed of three main subdivisions: forebrain, midbrain, and hindbrain. The forebrain was divided into relatively small spherical olfactory bulbs that were continued by very long and thin olfactory tracts that posteriorly joined the paired telencephalic hemispheres. Telencephalic hemispheres are located rostral to the diencephalon. Diencephalon represents the

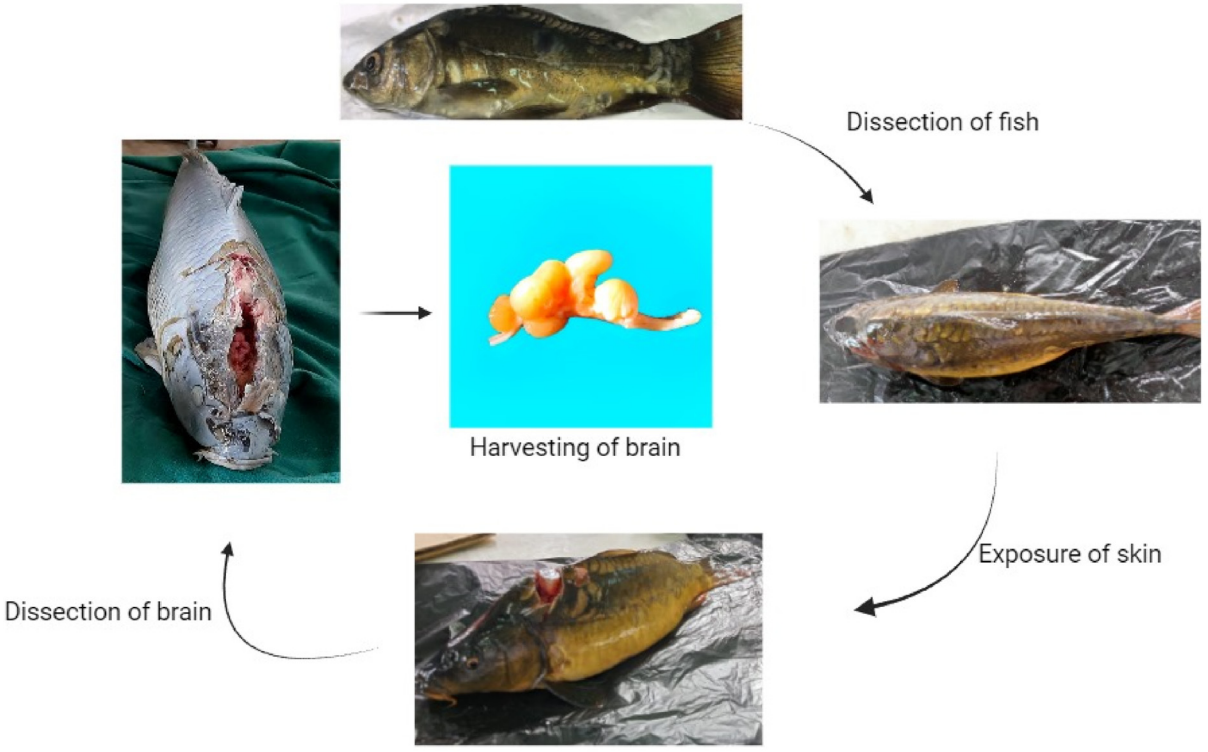


*Plate 1. Photograph of the adult common carp fish showing the drawing for opening and retaining the samples.*

most ventral part of the brain located posteriorly to the telencephalic hemispheres, ventrally to the tectal opticum, and is composed of the epithalamus, thalamus, and the hypothalamus. The midbrain is relatively large and appeared as paired optic lobes that were joined dorsally by the torus longitudinalis and ventrally at the middle with the torus semicircularis. The hindbrain is larger than the forebrain; it is composed of the metencephalon, which included the corpus cerebelli and the valvula cerebelli. The corpus cerebelli was located posterior to the mesencephalon and dorsal to the myelencephalon. Valvula cerebelli bulged forward and below the tectum posterior to the torus semicircularis. Myelencephalon is composed of two facial lobes that appeared closely related to each other; it is surrounded anteriorly by the corpus cerebelli and posteriorly by vagal lobes. The vagal lobes were paired with large cylindrical lobes, located dorsal to the medulla oblongata. The brain of common carp fish presented four ventricles, a single median ventral telencephalic ventricle, a diencephalic ventricle, a tectal ventricle, and a fourth rhombencephalic ventricle (Plate 2).

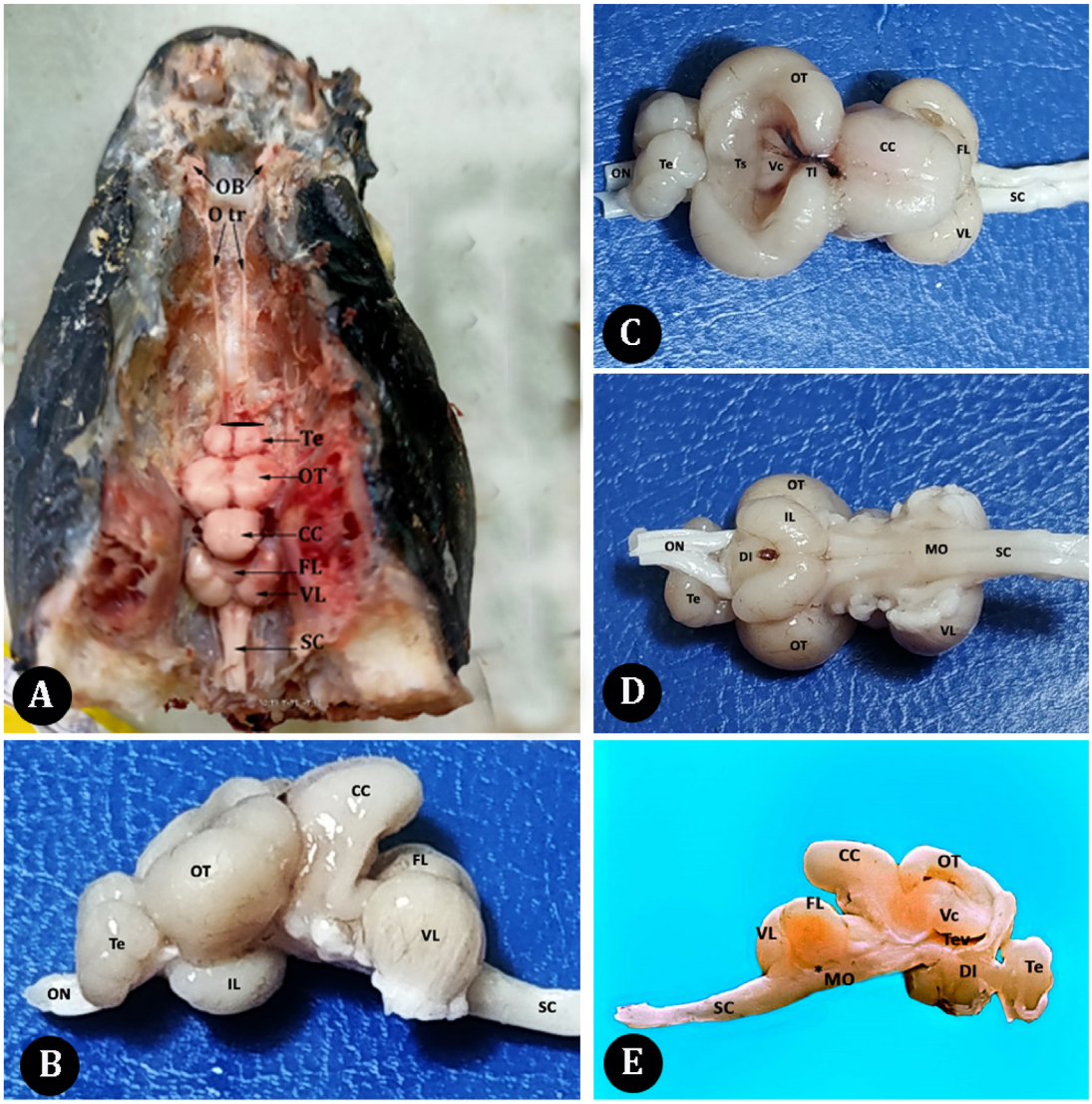


*Plate 2. Photograph of adult common carp brain: (A) in situ showing spinal cord (SC), vagal lobe (VL), facial lobe (FL), corpus cerebelli (CC), telencephalic hemisphere (Te), and olfactory tract (O tr), olfactory bulb (OB). (B) Lateral view, showing the spinal cord (SC), vagal lobe (VL), facial lobe (FL), corpus cerebelli (CC), inferior lobe (IL), optic tectum (OT), telencephalic hemisphere (Te), and the optic nerve (ON). (C) Dorsal view showing the spinal cord (SC), vagal lobe (VL), facial lobe (FL), corpus cerebelli (CC), valvula cerebelli (Vc), optic tectum (OT), torus longitudinalis (Tl), torus semicircularis (Ts), telencephalic hemisphere (Te), and the optic nerve (ON). (D) Ventral view, showing the spinal cord (SC), vagal lobe (VL), medulla oblongata (MO), optic tectum (OT), diencephalon (DI), inferior lobe (IL), telencephalic hemisphere (Te), and optic nerve (ON). (E) Sagittal view, showing the spinal cord (SC), medulla oblongata (MO), vagal lobe (VL), fourth ventricle (Asterix), facial lobe (FL), corpus cerebelli (CC), valvula cerebelli (Vc), optic tectum (OT), tectal ventricle (Tev), diencephalon (DI), and telencephalic hemisphere (Te).*

The olfactory bulb consists of four layers: the olfactory nerve fiber layer, glomerular layer, plexiform layer, and granular layer. The telencephalon of the common carp was of the aversive type, which is composed of two lateral bulbous hemispheres separated by a median single T-shaped common forebrain ventricle, represented by interlocked spherical neurons rooted in widespread neuropil, and the neurons appeared relatively more densely stained with a slightly stained nucleus (Plate 3).

The optic tectum consists of basic six layers that are characterized by a moderate content of nonmyelinated axons, in addition to neurons. These layers, from external to internal, were the stratum marginale, stratum opticum, stratum fibrosum et griseum superficiale, stratum griseum centrale, stratum album centrale, and stratum periventriculare. The mesencephalic periventricular zone (tectal ventricle) is located in between the optic tectum (Teo) and the valvula cerebellum (Plate 3).

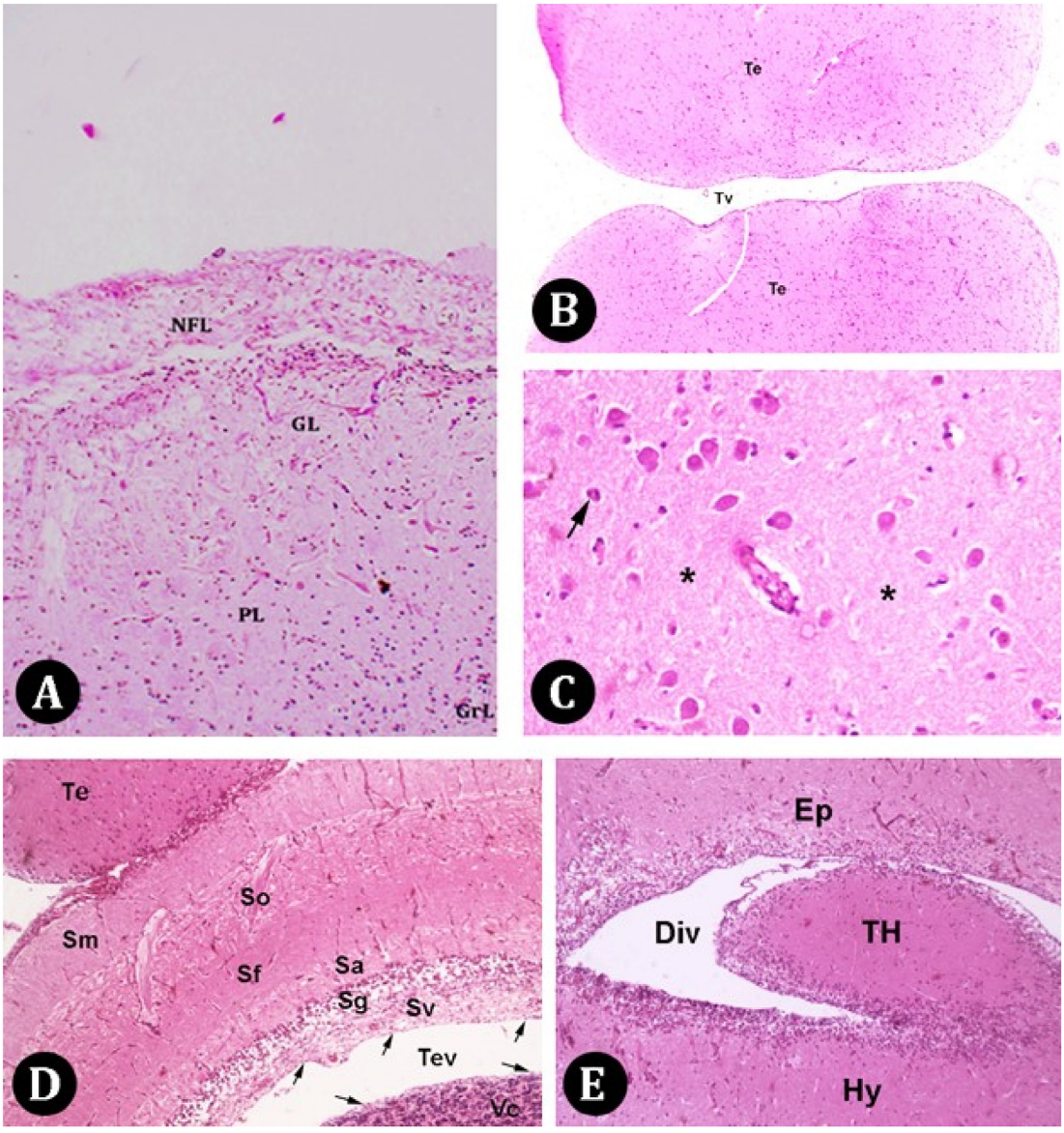


*Plate 3. (A) Photomicrograph of the olfactory bulb showing, the olfactory nerve fiber layer (NFL), glomerular layer (GL), plexiform layer (PL), and granular layer (GrL), hematoxylin and eosin stain ×10. (B) Photomicrograph of the cross-section of the telencephalon showing the telencephalic hemisphere (Te), telencephalic ventricle (Tv), hematoxylin and eosin stain ×10. (C) Photomicrograph of the telencephalon showing neurons (n), neuropil (asterisk), and a progenitor cell with mitotic figure (arrow), hematoxylin and eosin stain ×40. (D) Photomicrograph of optic tectum showing the tectal ventricle (Tev), stratum marginal (Sm), stratum opticum (So), stratum fibrosum (Sf), stratum album (Sa), stratum griesum (Sg), stratum periventricular (Sv), ependymal cell (arrow), telencephalon (T), and Valvula cerebellum (Vc), hematoxylin and eosin stain ×10. (E) Photomicrograph of the diencephalon showing the diencephalic ventricle (Div), epithalamus (Ep), thalamus (TH), and hypothalamus (Hy). Note the presence of many young cells within the ventricular wall, hematoxylin and eosin stain ×10.*

The extension of young proliferating cells along the tectal ventricle is observed in the internal layer of the optic tectum, where a high number of proliferating young cells were condensed along the ventricular surface, especially in the middle region of the internal layer. These cells also showed spherical to elongated nuclei (Plate 4).

Anteriorly to the diencephalic ventricle, we observed many undifferentiated young cells with spherical nuclei, which were distributed throughout the diencephalic periventricular zone (third ventricle). The subventricular zone of the diencephalic ventricle showed abundant and crowded cells with small, densely stained nuclei (Plate 3). The cerebellum is composed of three obvious layers: the external molecular layer, middle ganglionic layer, and the inner granular layer. The molecular layer is represented by a neuropil that contained a significant number of oriented dendrites, eurydendroid cell (efferent neuron) dendrites, and satellite cells (small neurons). The ganglionic layer contained oval-shaped Purkinje cells and eurydendroid cells

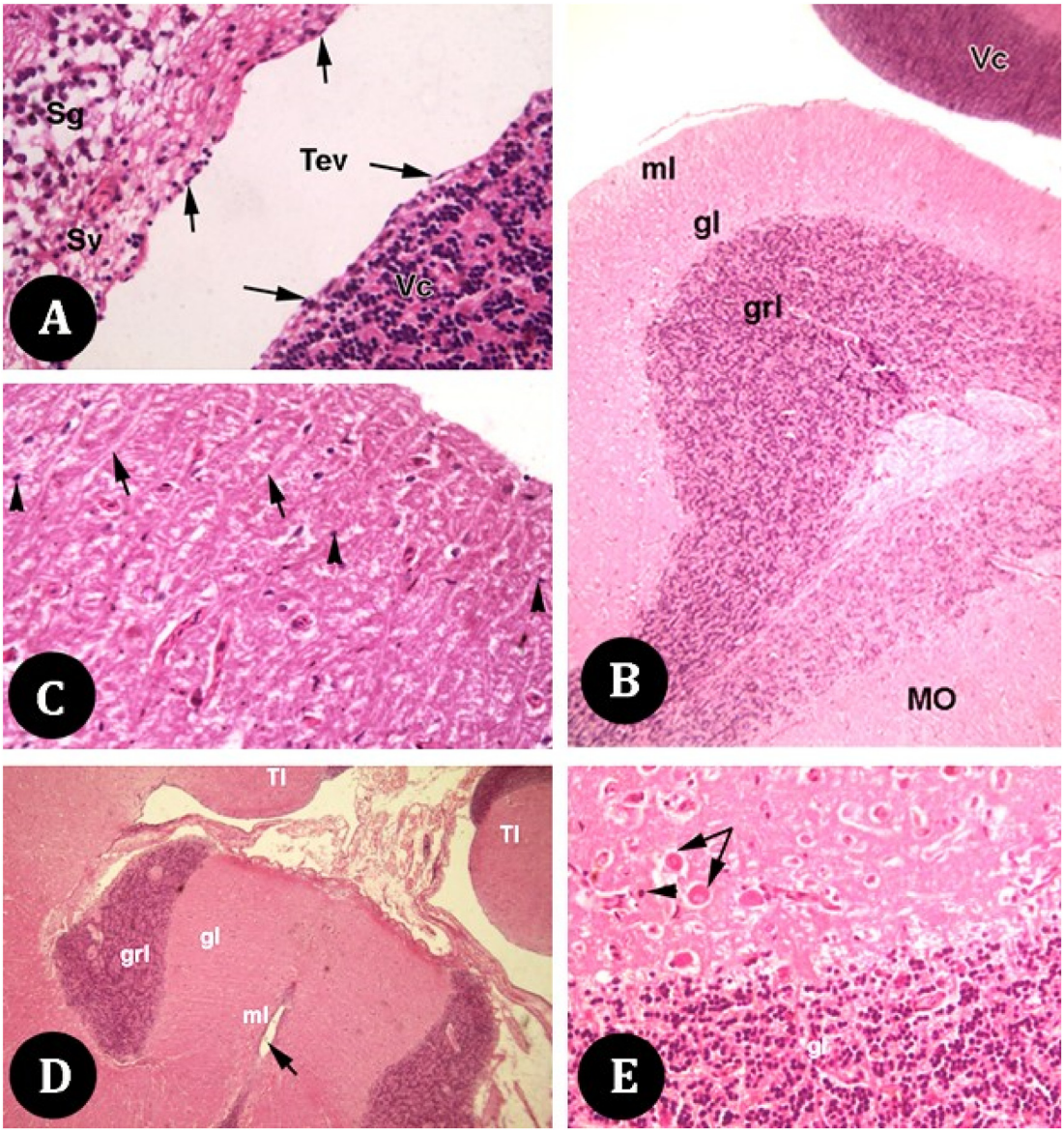


*Plate 4. (A) Magnified view of (E) of Plate 2 showing the diencephalic subventricular zone, microglia (arrow), neurons (arrowhead), and blood vessels (bv), hematoxylin and eosin stain ×40. (B) Photomicrograph of the corpus cerebellum showing the molecular layer (ml), ganglionic layer (gl), granular layer (grl), valvula cerebellum (Vc), and medulla oblongata (MO), hematoxylin and eosin stain ×10. (C) Magnification of (B) showing corpus cerebellum, dendrites (arrow), and small neurons (arrowhead), hematoxylin and eosin stain ×40. (D) Photomicrograph of the cross-section of the valvula cerebellum showing the molecular layer (ml), ganglionic layer (gl), granular layer (grl), and torus longitudinalis (Tl), hematoxylin and eosin stain ×10. (E) Magnification of (D) showing valvula cerebellum, dendrites (arrow), and small neurons (arrowhead), hematoxylin and eosin stain ×40.*

and the granular layer, which was beneath the ganglionic layer and contained dark granule cells and small multipolar neurons with spherical nuclei (Plate 4).

## 4. Discussion

The current research indicated that the telencephalon of the common carp is predominantly made up of two symmetrical hemispheres connected by two small oval olfactory bulbs through a thin olfactory tract. In addition, the diencephalon, which includes the epithalamus, thalamus, and hypothalamus, is also present. The hypothalamus contains the infundibulum and inferior lobes. Between the thalamus and the hypothalamus, the diencephalic ventricle is visible in parasagittal sections, and these findings are consistent with previous studies [6,7]. In our examination of common carp from the Cyprinidae family, we found that the largest lobes of the brain are the optic lobes, which consist of two large optic tecta arched over the valvula cerebellum. Previous studies have indicated that the optic tectum is split into two optic lobes and has a layered histological structure comprising five to six principal layers [7,18]. The optic tectum of lobe-finned fish, like coelacanths and lungfish, was discovered to be less developed than that of ray-finned fishes [19].

Our study also discovered that the cerebellum is the most prominent region of the brain and is located more toward the back of the mesencephalon. It is composed of the corpus cerebelli and valvula cerebelli. Situated on top of the rostrum of the rhombencephalon, the corpus cerebelli is positioned similarly to that of all vertebrates, while the valvula extends forward below the tectum within the tectal ventricle. Although the corpus cerebelli is like that of mammals, the valvula cerebelli was only found in ray-finned fishes [7,14]. Our research showed that the telencephalon in fish is noticeably smaller in comparison to other mammals. Mammals have six layers in the cerebrum cortex, which includes the neocortex. However, teleosts do not have the neocortex and instead have a single layer of interconnected neurons that are present in a widespread neuropil [7]. Distinct specializations were observed in the telencephalic hemispheres. In coelacanths, there was an enlargement of the cerebral cortex, while lungfish exhibited an extensive corpus striatum formed by the ventrolateral wall of each hemisphere. Our studies on common carp do not show these findings [19].

Below the midbrain, the diencephalon is histologically divided into the epithalamus, thalamus, and the hypothalamus. The epithalamus contains the pineal gland and habenular ganglion. The thalamus is positioned between the epithalamus and the hypothalamus and contains numerous nuclei of neurons and neuroglia. The hypothalamus is the most notable region of the diencephalon, containing various cell populations including many neurosecretory cells. However, the pituitary gland and saccus vasculosis extend from the hypothalamus, which is consistent with another study [20]. Our research discovered a layered histological organization in the optic tectum, comprising five to six primary layers differentiated by the proportion of nonmyelinated and myelinated axons as well as the presence or absence of neurons. These layers are arranged from outermost to innermost and include the marginal layer, optic layer, superficial gray fiber layer, central gray layer, central white layer, and periventricular layer. These results are consistent with findings from other studies [7,18].

The ongoing study has revealed that the cerebellar cortex is composed of three separate layers: an outer molecular layer, an inner granular layer, and an intermediate ganglionic layer. Inside the outer molecular layer, there are parallel fibers, dendrites of Purkinje cells, dendrites of eurydendroid cells (efferent neurons), and satellite cells (small neurons). This aligns with earlier research [7,14]. The layer of ganglionic cells is located below the molecular layer and in teleosts'. It contains Purkinje cells and eurydendroid cells. In contrast, eurydendroid cells are not present in mammals. As a result, in teleosts, this layer is called the ganglionic layer, whereas, in mammals, it is referred to as the Purkinje cell layer. Purkinje cell somata are either rounded or pear-shaped, whereas eurydendroid cell somata have a triangular or rhomboid shape. Furthermore, eurydendroid cells are larger than Purkinje cells, and their nuclei were smaller compared with the latter cells. Below the ganglionic layer, the granular layer contains densely packed small multipolar neurons known as granule cells. These observations align with those of previous research [7–14].

## 5. Conclusions

The current study shows that the brain of common carp (*C. carpio*) shows distinctive anatomical and histological features, particularly those of the telencephalic hemispheres, olfactory bulb, optic tectum, and the cerebellum. The present study also showed that the brain of carp only has four principal ventricles: telencephalic, tectal, diencephalic, and rhombencephalic. Meanwhile, the brain ventricles of other fishes consist of the olfactory ventricles,

cerebral ventricle, tectal ventricle, diencephalic ventricle, and rhombencephalic ventricle.

## Ethics approval

The current research work was allowed to be executed according to the standards of the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (M/33).

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## Authors' contributions

This work is part of the MSC thesis performed by E.S. E.S. performed the practical study and wrote the initial draft, and G.Y., S.E., and M.B.S. supervised the findings of this study and approved the definitive version of the manuscript for publication.

## Availability of data and materials

The study data is available on request from the corresponding author.

## Conflicts of interest

There are no conflicts of interest.

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