

Brain Anatomy and Histology of Orange Spotted Grouper (*Epinephelus coioides*)

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Abstract

This research was carried out to identify the different layers and cells of orange spotted grouper brain for further toxicological experiments and defects brought by xenobiotics during exposure periods. The anatomy and histology of the brain of orange spotted grouper was illustrated and compared to mammals and other fishes. The preserved structures of teleosts brain species were different as compared to other orders of teleosts and mammals. The anatomy and histology of the olfactory tract, olfactory lobe, cerebrum, optic lobe, cerebellum, diencephalon and medulla were visualized by the stereoscope and optic microscope using haematoxylin-eosin staining method. Different layers of various parts of the brain were depicted and compared to other species. The telencephalon of *E. coioides* was lobulated. The cerebrum consisted of a single layer unlike the six layered neocortex in mammals. The epiphysis was situated internally within the tectal ventricle. The cerebellum was the most prominent part in this fish, which might not be seen in other orders of teleosts. The cerebellum consisted of corpus cerebelli and valvula cerebelli. The ganglionic layer of cerebellum possessed eurydendroid cells which did not exist in mammals.

Keywords: *Epinephelus coioides*, Brain, Anatomy, Histology.

1. Introduction

The development process of various structures of the brain has occurred through evolution in different vertebrates as well as fishes. From another point of view, regression of parts of the central nervous system, such as olfactory system, has occurred at least 3 times in the course of phylogeny of submammalian vertebrates, namely in teleosts (Mormyridae), in lizards (Chameleon, Anolis), and in birds

(Nieuwenhuys 1966). In fish taxa, such as lobe-finned fishes, coelacanths and lungfishes, some structures of the brain are not as developed as in ray-finned (Actinopterygian) fishes (Gonzalez and Northcutt, 2011). Actinopterygian fish are extremely diverse due to the variation in development and structure of different parts of their brains. However, different structures of brain remain similar across this taxon as well as those in other vertebrates (Butler, 2011^a). The eversion process that the telencephalon undergoes during development in ray-finned fish is not comparable to other vertebrates, but some sensory

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system inputs are similar and some of the functional capabilities are surprisingly like other vertebrates such as mammals (Butler, 2011^a).

The anatomy of different parts of the brain displays a tremendous amount of variation in the both size and degree of differentiation among vertebrates. For example, the anatomy of telencephalon related to the sense of smell, the so called "rhinencephalo" is very different, but one can still recognize a common structural plan related to the morphology of this part of brain among all vertebrates. It seems probable that the early vertebrates possessed a well developed olfactory apparatus and this condition has been preserved in the main line of vertebrate evolution. The neurosensory cells in the nasal epithelium are the primary olfactory elements responsible for the sense of smell in all vertebrates. These elements are provided with extremely fine, unmyelinated axonal offshoots that convey the olfactory impulses to the bulbi (Nieuwenhuys, 1966). The olfactory epithelium contains the olfactory sensory neurons that converge upon their second-order targets in segregated areas of the olfactory bulb. Different odorant groups possess distinct neuron populations of the olfactory bulb in the glomeruli and glomerular plexus (emphasizing on the specialization of the olfactory system in different species). Synchronized oscillations, firing and silence upon stimulation, of the second-order neurons form the olfactory bulb response (Laberge and Hara, 2001).

Many factors are involved in differentiation and specialization of parts of brain for example, some pretectal specializations occur in relation to feeding behaviors in some species (Butler, 2011a). Although, the size and structure of optic lobes vary among speices, the tectal function of the brain appears to be constant among all vertebrates. However, the topographical and laminar organization of the teleostean tectum makes the localization and selection of objects in the environment possible. This task is carried out through different forms of information such as visual, acoustical, lateral line and tactile

information. This operation is performed in consideration to the subsequent orientation of the eyes, head and/or body toward or away from selected objects (Meek, 1983).

This paper explains the anatomy and histology of different parts of the brain of *Epinephelus coioides*, orange spotted grouper, and compares it with mammals and other fishes. This research is important because *E.coioides* is one of the few species living in southern waters of Iran (the Persian Gulf), which has the capability of being cultivated. This fish can be used as a good model system for many research areas, such as toxicology. One of the main pollutants of the environment is mercury and its organic forms, e.g. methylmercury. Since, methylmercury is very toxic and targets the brain more than any other region, it is crucial to study the brain of *E.coioides* for further toxicological analysis. This research will also contribute to the neuroanatomy, histology and evolutionary studies of the brain of teleosts.

2. Materials and Methods

The orange spotted grouper fishes were cultivated in Fisheries Center of Bandar Emam Khomeini, Iran. Fiberglass tanks were setup and filled with water coming from Zangi creek of the Persian Gulf. There were 15 different tanks exposed to different concentrations of methylmercury and 3 control tanks. Each tank contained 15 fish (2 years old, weighing about 60 grs). The water was changed every other day and the fish were fed with fish meal pellet before every water exchange. The fish were acclimated to the new condition for about a week before the experiments onset. One fish from 3 different control tanks was collected for histology experiment. The fishes were collected at days 7, 14, 30 and for depuration studies 7 and 14 days after depuration. The brain of these fishes were dissected and fixed in Bouin's solution comprising of 75 ml saturated water with picric acid, 25 ml formaldehyde and 5 ml glycial

acetic acid. After 24-48 hrs they were stored in 70% ethanol until further use for experiments.

The dehydration step consisted of placing the brains in tissue processor, TISSUE TEK II Rotary model RX-11B, starting from 70% ethanol (two times), 80% ethanol, 90% ethanol, 100% ethanol (2 times) for 2 hrs each, alcohol-xylene for 30', xylene (2 times) for 2 hrs each, paraffin (2 times) for 3hrs each. After dehydration, the tissues were pertained for sectioning using a microtome, LEICA model VRM 2245, to obtain sections of 4-5 μ m. The sections were placed in a 50 °C water bath. Once the paraffin sections were stretched, they were put on the slides for haematoxylin- eosin staining (H & E).

The staining procedure was as follows: xylene (1) 30 min, xylene (2) 15 min, 100% ethanol for 2 min, 90%, 80% and 70 % ethanol each for 1min, haematoxylin for 15 min, running water for 10 min, acid alcohol for 1sec, running water for 5min, Eosin plus a few drops of glacial acetic acid for 5 min followed by washing the slides with ddH₂O. Afterwards, the rehydration step was followed using 70% ethanol for 1 min, 80% and 90% ethanol for 1 min each, 100% and 100% ethanol for 2 min each, xylene for 10 min and another xylene step for 30 min. Finally, the cover slips were placed onto the slides using Canada Balsam glue.

3. Results and Discussion

Figure 1 shows the overview of orange spotted grouper brain (dorsal side) consisting of olfactory tract, olfactory lobes, cerebral hemispheres (cerebrum), cerebellum and medulla oblongata. The olfactory tract contained nerve fibers connected to the smell receptors of olfactory lobes. Attached to the olfactory tract, the olfactory lobes were in pairs (one on each side). The cerebral hemispheres (shaped as 3 lobules) were situated after the olfactory lobes, composing the anterior part of the telencephalon. The telencephalic ventricle space was seen in the frontal

grove of cerebrum. The mesencephalon consisted of the optic lobes which were visible as a pair of round lobes attached to the cerebral hemispheres. The cerebellum was the most prominent part of the brain in this fish with a crest on top (corpus cerebelli) laying on a stem (valvula cerebelli). The medulla oblongata represented the mass of the hindbrain which continued to form the spinal cord.

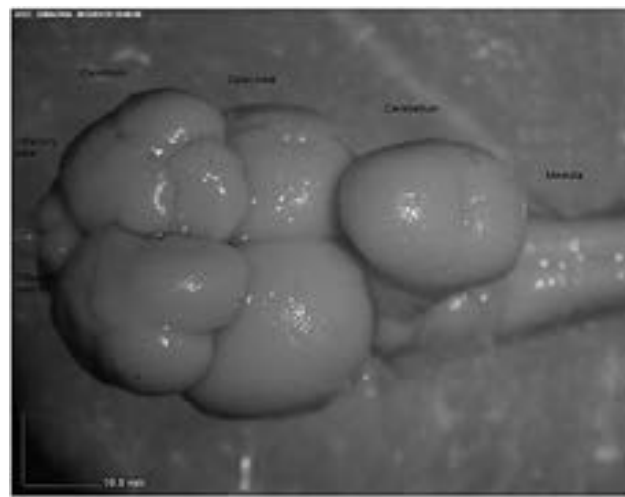


Fig. 1: Photostereomicrograph of the neuroanatomy of a 2 yr old orange spotted grouper brain (dorsal view) fixed in Bouin's solution. From left to right: olfactory tract, olfactory lobe, cereberum, optic lobe, cerebellum and medulla are shown (5X).

The ventral view of the brain is shown in Figure 2. The ventral part of the brain mainly consisted of the diencephalon. The inferior lobes were the biggest lobes in the ventral view. They were in pairs, one on each side which contained the major part of the infundibullum. The pituitary gland was the round mass situated in between the inferior lobes. Saccus vasculosis was a thin and long sac located in between the inferior lobes beneath the pituitary gland. The medulla was the long stem of the brain shown in Figure 2. The ventral view of optic lobes and cerebral hemispheres are seen in this figure due to their large size.

The histology of the telencephalon was studied and similarly showed the olfactory tract containing the nerve fibers. Attached to the olfactory tract was the

olfactory lobe followed by the cerebral hemisphere (shaped as lobules) composing the anterior part of the telencephalon. The cerebrum of this fish was relatively big and the olfactory lobes were smaller as in other fish. The telencephalic ventricle space was seen in the frontal groove of cerebrum between the olfactory lobe and cerebrum. The optic lobe is also visible in Figure 2.

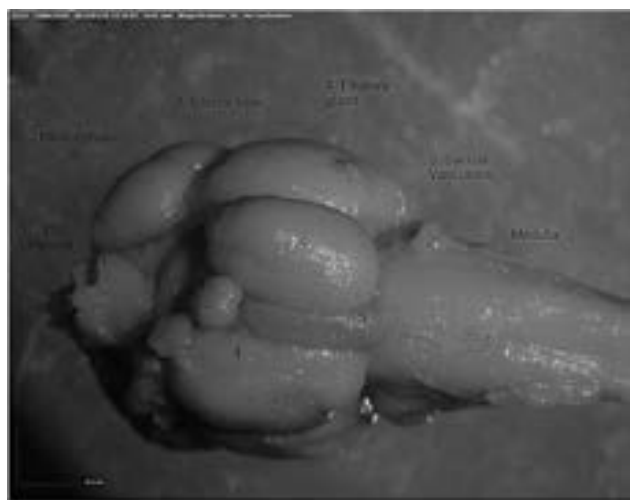


Fig. 2: Photostereomicrograph of the neuroanatomy of a 2 years old orange spotted grouper brain (ventral view) fixed in Bouin's solution. 1. optic chiasma, 2. diencephalon, 3. inferior lobes, 4. pituitary gland, 5. saccus vasculosus and medulla.

The parasagittal sectioning of the whole brain of *E.coioides* is shown in Figures 3 and 4. The cerebrum was fairly big in this species and shaped as lobules rather than a unique part. The optic lobe consisted of two parts, the optic tectum and the tegmentum. The optic lobe in this fish was not the largest part compared to other fishes, such as Cyprinidae family. The tegmentum did not exist in this fish unlike some other teleosts and the torus semicircularis was located in the tectal ventricle. The diencephalon consisted of the epithalamus, thalamus and hypothalamus. The diencephalic ventricle was located between the thalamus and hypothalamus. The most significant part of the brain was the cerebellum, a characteristic of this fish. The cerebellum showed a crest structure on top (corpus cerebelli) lying on a stem (valvula cerebelli).

The cerebellum consisted of three different layers, the dark basophilic layer in the middle which was a granular layer and the light pink around it, was the molecular layer. The other layer (ganglionic layer) was not visible.



Fig. 3: Parasagittal sectioning of telencephalon consisting of the olfactory tract, olfactory lobe and cerebrum attached to the optic lobe of mesencephalon (5X)

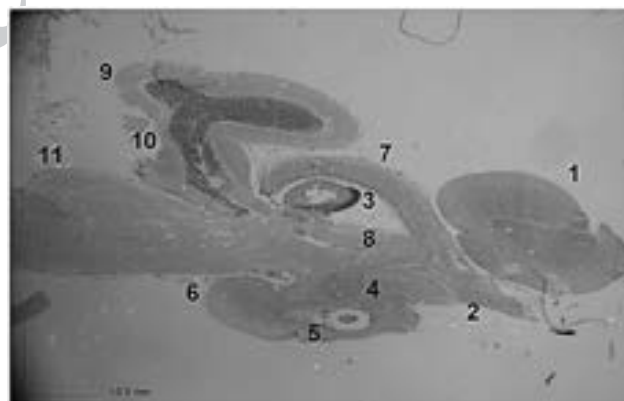


Fig. 4: Parasagittal sectioning of the whole brain of epinephelus coioides showing: 1. Cerebrum, 2. Diencephalon, 3. Torus semicircularis, 4. Thalamus, 5. Hypothalamus, 6. Inferior lobe, 7. Optic tectum, 8. Tegmentum, 9. Corpus cerebelli, 10. Valvula cerebelli, 11. Medulla oblongata (5X).

The olfactory lobe is a round shaped lobe fairly small in all fishes surrounded by a thin epithelium. The olfactory lobe consisted of numerous round shaped neurons scattered through the olfactory bulb which laid on a broad neuropil. These neurons were

bigger than the cerebral neurons and their nucleus were larger. Thin dendrites and axons existed both in the epithelium and the bulb. The epithelium contained basal and receptor cells covered with cilia and microvillia which were not visible with H &E staining in Figure 5.

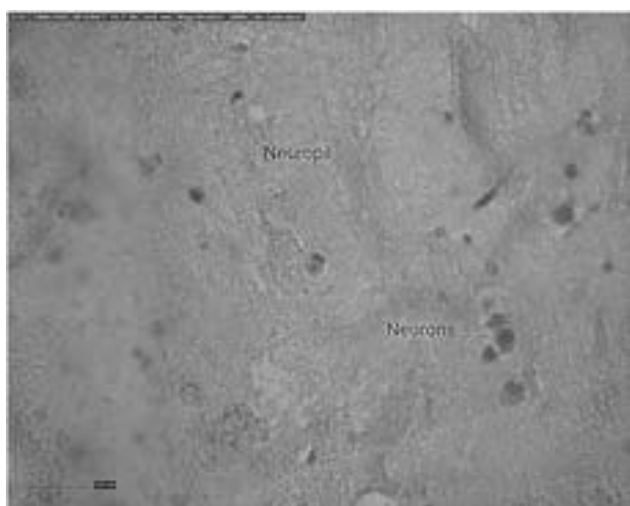


Fig. 5: Photomicrograph of the olfactory lobe of a 2 years old *Epinephelus coioides*. Picture taken by an optical microscope using H&E staining method. The neuropil and neurons are obvious (3000X)

The cerebrum of teleosts were relatively larger than that of mammals and exclusively contained a distinctive layer of neurons embedded on a neuropil. Compared to the olfactory lobe, the cerebrum contained more neurons distributed throughout this lobe, which were smaller in size. The epithelium of cerebrum was thinner compared to the olfactory epithelium. The neuronal processes (axons and dendrites) existed in this layer (Figures 6 and 7).

The ventral part of the brain included the diencephalon. Diencephalon consisted of epithalamus, thalamus and hypothalamus (Figure 8). Right beneath the optic lobe, the epithalamus was situated, which formed the pineal gland. The thalamus was located under the tegmentum and the hypothalamus was beneath the third ventricle (diencephalic ventricle). The hypothalamus consisted of the infundibulum, inferior lobes and the pituitary gland. Saccus

vasculosus was seen more obviously between the inferior lobes in Figure 9.

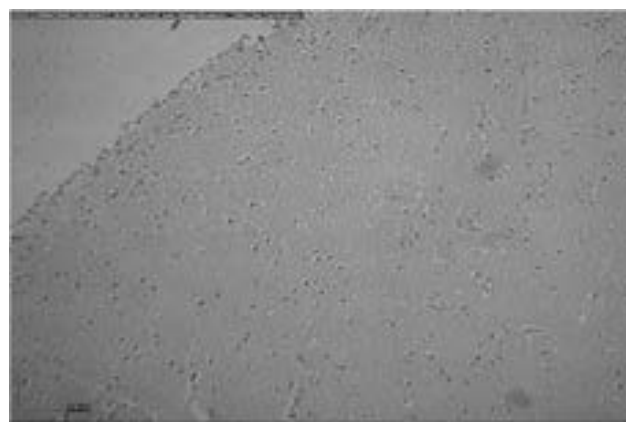


Fig. 6: Photomicrograph of the cerebrum of a 2 years old orange spotted grouper. Picture taken with an optical microscope using H&E staining method (magnification 750X).

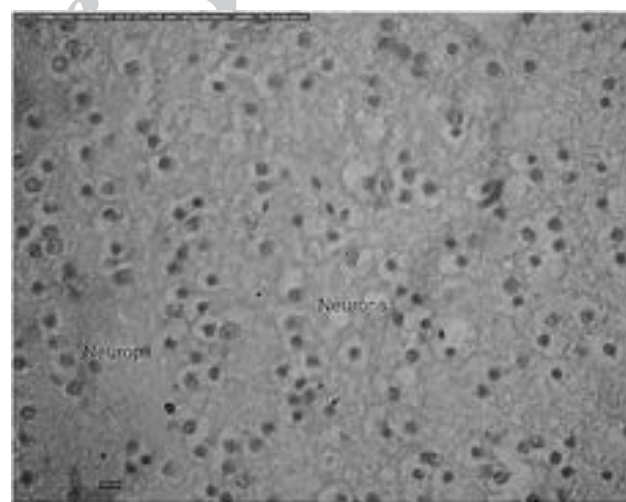


Fig. 7: The cerebrum of *Epinephelus coioides*. The neuropil and neurons are seen. The white area around the neurons are due to shrinkage (3000X).

Saccus vasculosus laid between the inferior lobes and under the pituitary gland (Figure 9). The walls of this organ folded many times and in paraffin embedded preparations, the lumen of the sac seemed to be empty. Blood was seen inside the folds. Saccus vasculosus was a thin-walled sac with cuboidal/columnar neural epithelium, supported by an extensive vascular plexi bathing in connective tissue (Fig. 10). The main functions of saccus vasculosus are sensory, secretory and absorptive.



Fig. 8: The diencephalon of *Epinephelus coioides*. Thalamus and hypothalamus are situated under the optic lobe with the third (diencephalic) ventricle between them, followed by the inferior lobe (infundibulum) and saccus vasculosus (750X).

neurons. It also contained the germinal zone which had a basophilic cytoplasm with elongated nuclei scattered all around it. The adult torus semicircularis (TS) was situated on top of the fish tegmentum. The pineal gland (epiphysis), which is a projection of epithalamus apart of the diencephalon, is also seen in Figure 11.

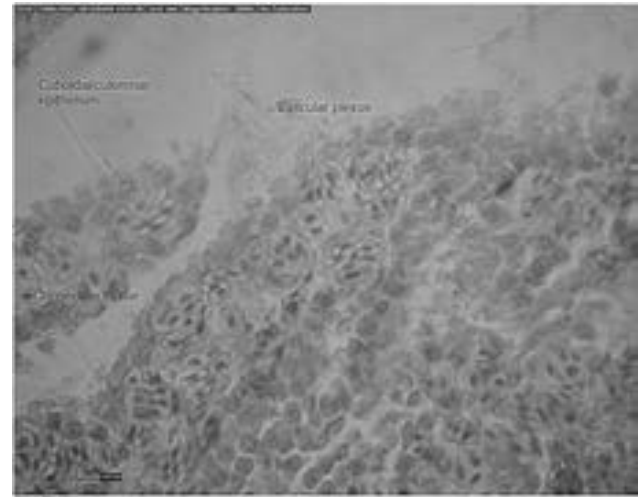


Fig. 10: Saccus vasculosus tissue consisting of cuboidal/columnar epithelium, connective tissue and vascular plexus (3000X).

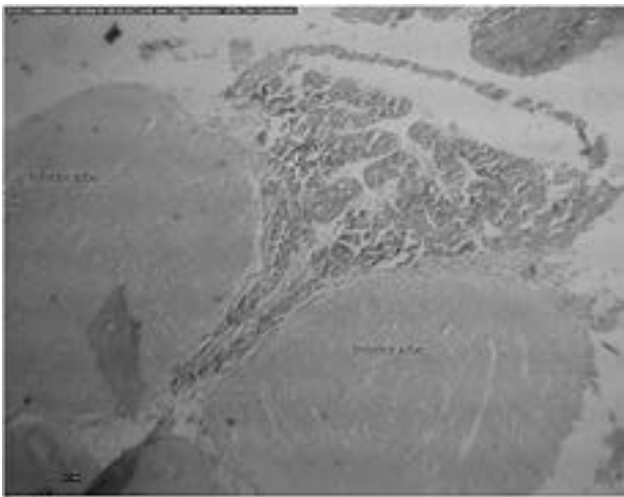


Fig. 9: Saccus vasculosus inserted between the inferior lobes of *Epinephelus coioides* brain. (375X).

The optic tectum was obvious in orange spotted grouper and had 2 lobes which contained the tectal ventricle within them. The mesencephalon was large in all fishes consisting of two different layers. However, the mesencephalon of *E.coioides* was not the biggest part of the brain. It was roofed by the optic tectum and its floor was the tegmentum. The optic tectum had laminar histological architecture that consisted of five principal layers. These layers were distinguished by their relative content of nonmyelinated versus myelinated axons and presence or absence of

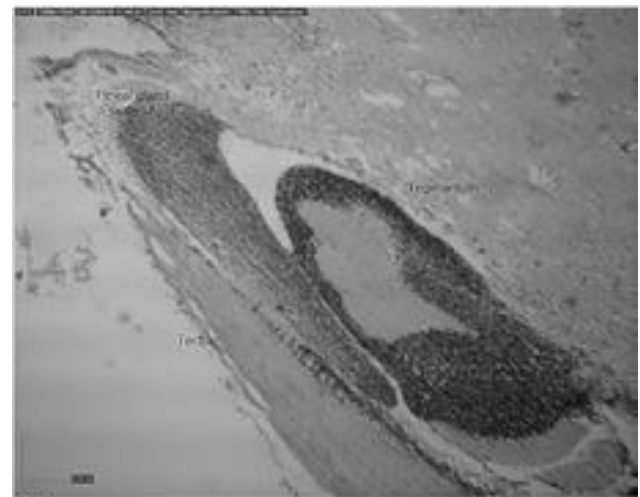


Fig. 11: The optic lobe and its structures consisting of Tectum and Tegmentum beside the epiphysis and Torus semicircularis (750X)

Choroid plexus was covered by simple or psuedostratified columnar epithelium roofing the tectal ventricle which was laid on a loose connective

tissue (lamina propria) covered with thin walled blood vessels (erythrocytes). The cells were beside the capillaries in order to have access to the material the blood circulation brings. This way, the choroid plexus cells could use this material and synthesize the components of the cerebrospinal fluid and secrete it into the lumen of the ventricles (Figures 12 and 13).

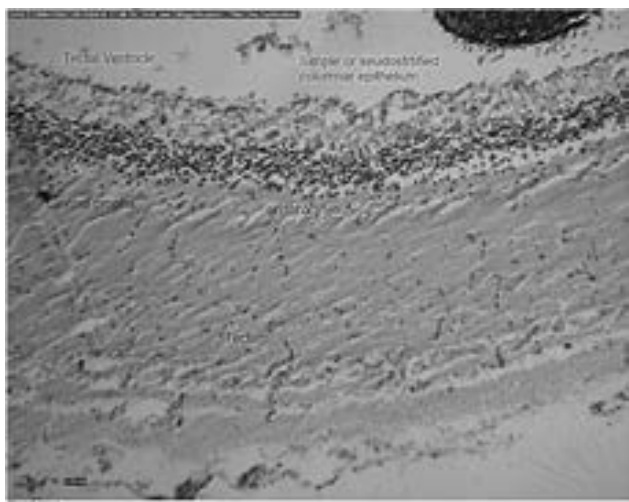


Fig. 12: Choroid plexus of a 2 years old *Epinephalus coioides* brain. The tectum of optic lobe is seen where the lamina propria is attached to it and the simple or pseudostratified columnar epithelial cells are obvious with the erythrocytes among them (750X)

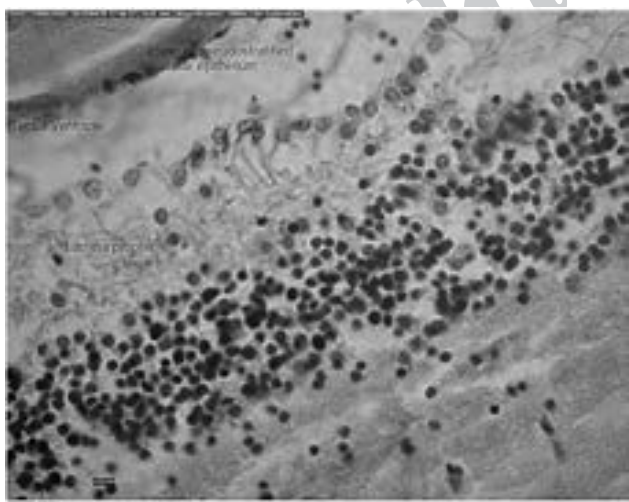


Fig. 13: Choroid plexus at higher magnification. Tectal ventricle and the simple or pseudostratified columnar epithelial cells are seen with the lamina propria as the base (3000X).

The cerebellum consisted of a crest like structure (corpus cerebelli) on a stem (valvula cerebelli). In

Figure 14, the outer molecular layer in light pink and the inner granular layer in dark pink (maroon color) are seen. The middle ganglionic layer with the PURKINJE cells are hard to distinguish.

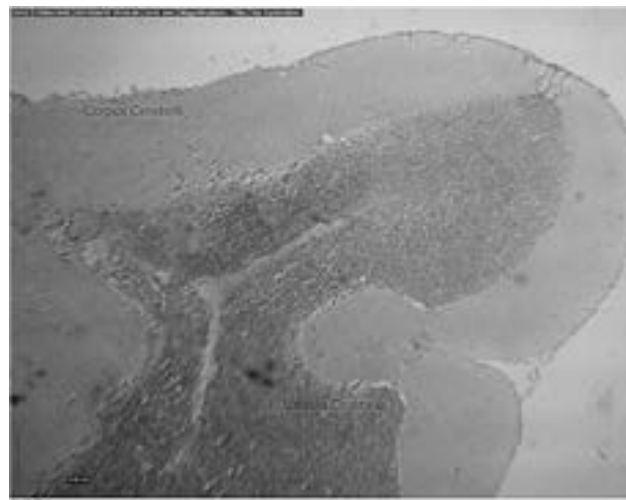


Fig. 14: Photomicrograph of the cerebellum, showing of the corpus cerebelli and the valvula cerebelli (750X).

The Cerebellum consisted of 3 different layers: the granular layer with dark granule cells. Spread among these cells were clear spaces named, "the cerebellar islands" where synapses occurred between axons entering the cerebellum from outside and dendrites of granule cells. Followed by the granular layer, was the ganglionic layer consisting of oval shaped PURKINJE cells, round shaped eurydendroid cells as well as satellite (glial) cells. The outer molecular layer constituted a continuous sheet of neuropil containing a huge amount of oriented dendrites. The satellite and basket cells were obvious in this layer (Figure 15).

The fourth ventricle was situated in the medulla right under the stem of cerebellum (valvula cerebelli). The inferior lobe and saccus vasculosis were positioned around the same longitude (Figure 16).

The medulla oblongata is the stem of the brain which connects the cerebellum to the diencephalon. The medulla oblongata was the major mass of the hind brain and contained the fourth ventricle. Its major body was composed of neuropil which contained different kinds of cells. The somata of Nissl bodies

were basophilic and seen as big triangular cells (Figures 17 and 18). The nucleus of these cells were not visible. The other kind of cells were the neuroglial cells, which their small nuclei are seen in this figure. The neuropil contained the processes of neuroglial and other neuronal cells.

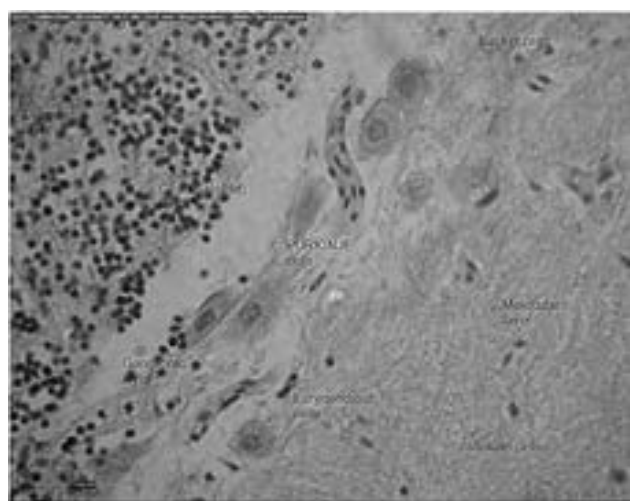


Fig. 15: Photomicrograph of the cerebellum consisting of the granular layer, the ganglionic layer and molecular layer. The eurydendroid cells, PURKINJE cells and glial (satellite) cells are seen in the ganglionic layer (3000X).

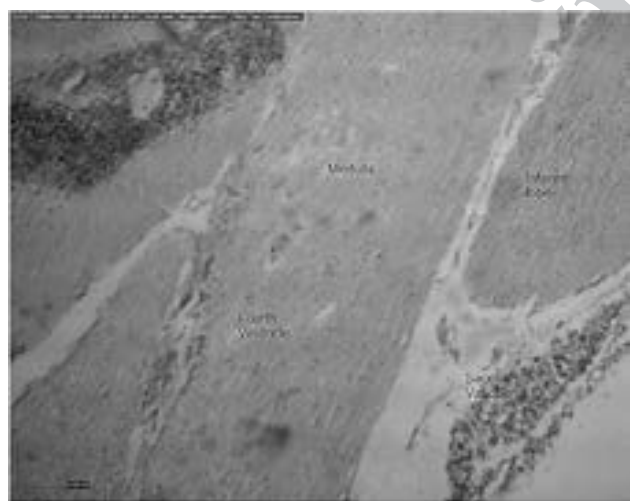


Fig. 16: Photomicrograph of the fourth (rhombencephalic) ventricle and its position (750X)

4. Discussion

The brain of *Epinephelus coioides* consisted of three different parts of prosencephalon,

mesencephalon and rhombencephalon. Prosencephalon was divided into 2 parts: telencephalon and diencephalon. Although, all teleost fishes possess a telencephalon, but this part is composed differently in various species.

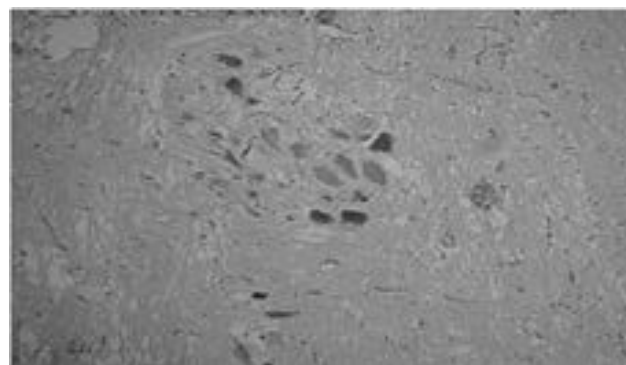


Fig. 17: Photomicrograph of medulla oblongata sagittal section of *E. coioides* (750X)

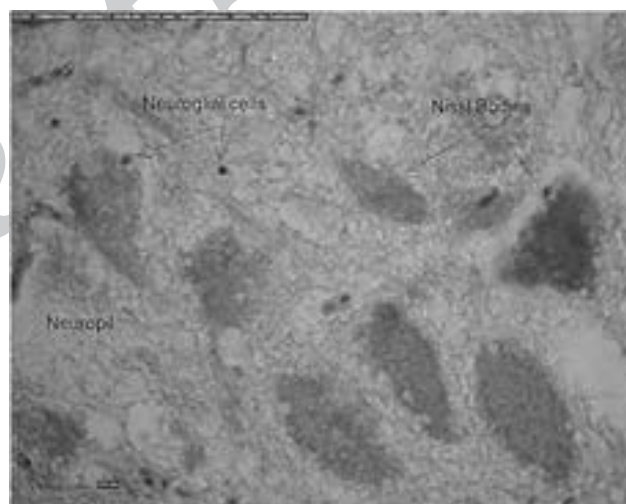


Fig. 18: Photomicrograph of medulla structures showing the Nissl bodies, the neuroglial cells and the neuropil (3000X)

For example, brain of some species, such as brain of salmon from the Salmoniformes order, possess the olfactory bulb and olfactory lobes (Sattari et al, 2002), while others contain the olfactory lobes and cerebrum such as gar (*Lepisosteus osseus*) from Lepisosteiformes order, common carp (*Cyprinus carpio*) from Cypriniformes order of Actinopterygii class (Sattari et al, 2002 and Butler, 2011a), European eel (*Anguilla anguilla*) from Anguilliformes order of Actinopterygii class (Atta, 2013), rainbow trout

(*Oncorhynchus mykiss*) from the Salmoniformes order (Al-Bairuty et al, 2013) and mudskipper (*Periophthalmus barbarous*) from the Perciformes order (Kuciel et al, 2014). In this study, similar to previous studies (Nagarajan et al, 2012), we showed that *E.coioides* from the Perciformes possessed olfactory lobes and cerebral hemispheres.

Telencephalon in fish is much reduced compared to other mammals. The cerebrum ventricles in the transverse sections were sometimes visible, but usually not visible. The telencephalic ventricle was obvious in the parasagittal sectioning of our studies. In mammals, the cerebrum cortex consisted of six layers, the neocortex, while teleosts only possess a single layer containing fields of interconnected neurons embedded on an extensive neuropil (Genten et al, 2009; Mills, 2007 and Bloom and Fawcett, 1968). The brain of lobe-finned fishes, coelacanths and lungfishes, were compared with one another in a study performed by Gonzalez and Northcutt (2011). In both of these lobes –finned fishes, the telencephalic hemispheres revealed unique specializations. In coelacanths, the pallium, the cerebral cortex, was expanded, whereas in lungfishes the ventrolateral wall of each hemisphere forms an extensive corpus striatum (Gonzalez and Northcutt, 2011). These features were not obvious in our studies, due to haematoxylin- eosin staining method. Magnetic resonance imaging is required to obtain further analysis.

The hind brain of lobe-finned fishes, coelacanths and lungfishes were also compared with one another in the study performed by Gonzalez and Northcutt (2011). The hindbrain and cerebellum in coelacanths and lungfishes were comparable to these areas in bony fishes, but the roof of the midbrain, the optic tectum, was not as developed as in ray-finned fishes (Gonzalez and Northcutt, 2011).

A comparison was done between different telencephalic regions of subclasses of Actinopterygii. The bichir *Polypterus senegalus* from the Cladistia

subclass, the sturgeon *Scaphirhynchus platyrhynchus* from Chondrostei subclass, the gar *Lepisosteus osseus* from Neopterygii subclass (*Holeosti infraclass*) and the sunfish *Lepomis cyanellus* from Neopterygii subclass (*Teleosti infraclass*). A cladogram of transverse hemisections through the right telencephalon revealed the bichir branching on top, the sturgeon branching second from top, the gar branching third from top and the sunfish branching at the bottom (Butler, 2011a). *Epinephalus Coioides* like sun fish belongs to the order of Perciformes and its position in the cladogram of this study will be at the bottom compared to other subclasses of ray-finned fishes.

In some ray-finned fishes, such as the bichir during the eversion process, there is very little migration of the neuronal cell bodies away from the proliferative zone, along the ventricular surface. In others, there was more proliferation and migration such as the sturgeon. In gar and sunfish, the proliferation and migration is significantly greater than the other mentioned fishes (Butler, 2011b). Since, orange spotted grouper belongs to the same order as sunfish and displays similar telencephalic morphology, it must have had a higher rate of proliferation and migration during the eversion process compared to other fishes.

The Perciformes have a lobulated telencephalon rather than a single lobe telencephalon, which is seen in other subclasses. Furthermore the rate of proliferation and migration of neuron cell bodies away from the proliferative zone along the ventricular surface in the eversion process is much more in these fishes as compared to the others. These features together make it less developed and rank it at the lower branch of the cladogram.

The diencephalon of the orange spotted grouper consists of the epithalamus, thalamus and hypothalamus. The epithalamus in teleost fishes comprises the pineal organ and the habenular ganglion. The pineal organ in orange spotted grouper is projected from epithalamus and is situated on top of

the tegmentum in the tectal ventricle space. In contrast, some fishes such as Salmons contain an external epiphysis positioned on top of the optic tectum (Sattari et al, 2002). The hypothalamus consists of the infundibulum and inferior lobes, which act to regulate the pituitary gland. The diencephalic ventricle is obvious in the parasagittal sections between the thalamus and hypothalamus (Genten et al, 2009; Butler, 2011a and Nagarajan et al, 2013).

Saccus vasculosis is a highly vascularized tissue which produces cerebrospinal fluid for the tectal and diencephalic ventricle lumens. This part is located behind the pituitary gland between the two inferior lobes of hypothalamus. This organ is composed of ribbons of cuboidal/columnar epithelium supported by fibrovascular stroma with prominent capillaries and it resembles choroid plexus in mammals. Another hypothesis is that it can also detect water pressure. In Dogfish (*Scyliorhinus canicula*) belonging to the catshark family (Scyliorhinidae), the same pattern for Saccus vasculosis is seen as in orange spotted grouper (Genten et al, 2009; Turkmen et al, 2007 and Butler, 2011a).

In some teleost fishes the mesencephalic structure, tegmentum, does not exist but in *Epinephelus coioides* the tegmentum existed and the tectum area was pretty obvious. The torus semicircularis (TS), which is situated on top of the tegmentum was the principle target of the octavolateral hindbrain. The octavolateral system consisted of 3 mechanosensory systems: the auditory, equilibrium and lateral line systems, which have similar receptor cells called the hair cells. This part was connected to many other areas of the brain and was involved in auditory and lateral line processing. The development of TS reflected the degree of importance of visual sense in different species. The optic tectum was divided into two optic lobes and had a laminar histological structure that consisted of five principal layers. These layers were distinguished by their relative content of nonmyelinated versus myelinated axons and presence

or absence of neurons (Genten et al, 2009 and Mishra and Devi, 2014). In mammals, the optic tectum consists of six layers (Sattari et al, 2002). In goldfish (Cyprinidae family) the optic lobes are the largest lobes of the brain, whereas in orange spotted grouper the most significant part was the cerebellum.

The dorsal part of mesencephalon is cerebellum which in *E. coioides* was the most prominent part of the brain. It was composed of corpus cerebelli and valvula cerebelli. Corpus cerebelli was homologous to this part in mammals, but valvula cerebelli was only present in ray-finned fishes. The corpus cerebelli laid on top of the rostral of rhombencephalon, as in all vertebrates, but the valvula projected forward under the tectum in the tectal ventricle. The cerebellar cortex contains three significant layers: an outer molecular and inner granular with an intermediate ganglionic layer. The outer molecular layer contained parallel fibers, PURKINJE cell dendrites, eurydendroid cell (efferent neuron) dendrites and satellite cells (small neurons). The somata of these elements measured 6 by 8 μm (Genten et al, 2009; Gartner and Hiatt, 2006; Junqueira et al, 1986; Ross and Pawlina, 2005; Ferguson 2006; Mills, 2007 and Bloom and Fawcett, 1968).

The ganglionic layer was under the molecular layer and in teleosts, it contained PURKINJE cells and eurydendroid cells but, in mammals it lacks the eurydendroid cells. Therefore, in teleosts this layer is referred to as the ganglionic layer, whereas in mammals it is called the PURKINJE cell layer. The somata of PURKINJE cells were rounded or pear-shaped, while in eurydendroid cells they were triangular or rhomboid shaped. Eurydendroid cells were larger than the PURKINJE cells and their nuclei were smaller, compared to the latter cells. The granular layer was beneath the ganglionic layer, which contained granule cells that were densely packed small multipolar neurons in appearance. The somata of these cells had a diameter of 3-4 μm (Genten et al, 2009; Gartner and Hiatt, 2006; Junqueira et al, 1986;

Ross and Pawlina, 2005; Mills, 2007 and Bloom and Fawcett, 1968).

The cerebellum in coelacanth and lungfishes (lobe-finned fishes) was comparable to this part in bony fishes (Gonzalez and Northcutt, 2011). But, compared to higher orders of taxonomy, it is not as advanced. The cerebellum of Elephantnose fish (*Gnathonemus petersii*) of the Mormyridae family of Osteoglossiformes order is well developed and consisted of the valvula cerebelli, the corpus cerebelli and the caudal lobes. The cerebellum crest and transitory lobes also existed in this fish, where these additional structures were not seen in orange spotted grouper. This fish contained all 3 layers of cerebellum. Large lobes of valvula cerebelli in this fish had a better developed lateral line system and had an enormous capacity for processing electroreceptive information. This feature was not seen in *E. coioides* (Han et al, 2006; Mcnamara et al, 2005 and Zhang and Han, 2007).

The hindbrain contained the medulla oblongata and it continued to form the spinal cord. The grey substance of teleost fish showed differences in arrangement than that of vertebrates, meaning that the dorsal horns laid so close together, that there was hardly any white substance between them (Genten et al, 2009). In the parasagittal sectionings this feature was not obvious, but it was distinguished in the transverse sections.

In orange spotted grouper cellular elements of the spinal cord contained neurons and neuroglia which were similar to mammals. The neurons contain NISSL substance with rough endoplasmic reticulum in their cytoplasm, Golgi apparatus and a large nucleus. The neurons possessed dendrites and nonmyelinated or myelinated axons. In our study, we were able to identify the neurons, Nissl bodies and neuroglia with the haematoxylin-eosin staining method. The different types of neuroglial cells are hard to distinguish in routine tissue sections and further Immunohistochemical studies, using specific

antibodies against proteins and peptides are required to specify the differences among them. According to previous studies the central neuroglial cells included astrocytes, oligodendroglia and ependymocytes.

Teleost ependymal cells are different in appearance perhaps due to their specialized metabolic activity, since they possess cilia, large amounts of glycogen and many huge mitochondria (Peckham, 2011; Mills, 2007; Junqueira et al, 1986 and Genten et al, 2009).

The brain ventricles consisted of olfactory ventricles, cerebral ventricles, tectal ventricles, diencephalic ventricle and rhombencephalic ventricle (Nagarajan et al, 2012 ; Butlera&b, 2011and Genten et al 2009). In the parasagittal sections, we were able to see the tectal, diencephalic and rhombencephalic ventricles, for the rest, transverse sectioning was required. The Choroid plexus roofed these ventricles and functioned to secrete and absorb the cerebrospinal fluid. In *Oncorhynchus mykiss* (rainbow trout) from the Salmonidae family, the choroid plexus was denser than that seen in *Epinephelus*, perhaps due to more secretion of cerebrospinal fluid required in this fish (Genten et al, 2009; Mills, 2007; Ross and Pawlina, 2005; Junqueira et al, 1986; Bloom and Fawcett, 1968).

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