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Anatomy and Histology of Brain of Zebra Fish

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Abstract: ***The anatomical and histological observations of the brain zebra fishes differ among species, but they resemble each other's in the number of brain compartments, and it is necessary to characterize well the anatomical and histological observations in the brain. Five brain divisions usually observed which are from cranial to caudal; telencephalon or forebrain (contain 2 olfactory lobes and cerebrum), diencephalon (contain epithalamus, thalamus and hypothalamus), mesencephalon or midbrain (contain 2 optic lobes which are connected internally with torus longitudinally and medially with the torus semi - circularis, and optic tegmentum), metencephalon or hindbrain (cerebellum) and myelencephalon or brain stem (medulla oblongata). The ventricular organization composed of the olfactory, lateral, the third, the tectal and the fourth ventricles.***

Keyword: Zebra fish, brain, histology, anatomy

1. Introduction

The zebrafish (Danio rerio) is a tropical freshwater fish with natural inhabitant of rivers (Ganges mainly) of Himalayan region of South Asia especially northern India, as well as northern Pakistan, Bhutan, and Nepal. It belongs to the family of the cyprinids (Cyprinidae) in the class of ray - finned fishes (Actinopterygii) and within this class to the bony fishes (teleost's or Teleostei).

Zebrafish (Danio rerio) are small, almost 3 - 5 cm long. They are native to streams in India and are commonly kept as pets. The males are slender and torpedo - shaped, with black longitudinal stripes and usually a gold colouration on the belly and fins. Females are fat when laden with eggs and have little, if any, gold on their undersides.

Zebrafish was first used as a biological model by George Streisinger (University of Oregon) in the 1970s because it was simpler over mouse and easy to manipulate genetically. Streisinger's colleagues especially Chuck Kimmel in his university got much impressed by the idea of using zebrafish embryo more attractive to study the development of nervous system.

Since it was first described by Francis Hamilton from the Ganges Delta in 1822, the small striped fish commonly known as the 'zebrafish' or 'zebra danio' (Figure 1.1) has become variously a popular aquarium fish (Talwar & Jhingran, 1992) an important bioassay (Von Hertell et al., 1990) and in the last few years a major model in vertebrate physiology and developmental genetics (Vascotto et al., 1997). Several attributes of the species explain its popularity in all these contexts: it reaches a length of only 30mm, is relatively easy to rear and breed in captivity, and produces large numbers of offspring with a generation time as short as four months.

Zebrafish has a lot of physiological and genetic similarities with humans, including the brain, digestive tract, musculature, vasculature, and innate immune system. Also 70% of human disease genes have functional similarities with those of zebrafish.

Zebrafish are easy to raise, with a short generation time of 3 months, and the females can lay hundreds of eggs at weekly intervals. Fertilization is external, allowing easy access to embryos for observation and manipulation. Developing embryos are easily studied under a dissecting microscope since they are transparent.

Zebrafish embryos develop rapidly (in 2±4 days), with a beating heart and visible erythrocytes by 24 h. Another advantage of studying the zebrafish is that, in contrast to other fish, which can be triploid or tetraploid (which makes genetic analysis difficult), it maintains the diploid state.

The zebrafish has been shown to be a useful model for the development of several complex tissues such as the kidney (Drummond, 2000), the olfactory system (Ardouin et al., 2000) and the visual system (Saszik et al., 2000). In the study of haematopoiesis, zebrafish mutants have been observed to demonstrate strikingly similar pheno - types to those in human diseases (Amatruda and Zon, 1999).

Figure 1: Adult zebrafish

Salient features of zebrafish as a model organism

D. rerio is preferred by scientists because of its variety of features that make it useful as a model organism. The embryo develops rapidly outside mother and optically clear and thus, easily accessible for experimentation and observation. The embryo develops very fast, and the blastula stage lasts only for 3 h, while gastrulation gets completed in 5 h; in an embryo that is about 18 h old, very well - developed ears, eyes, segmenting muscles, and brain can be viewed as the embryo is transparent. By 24 h, segmentation gets completed, and most primary organ systems are formed. By 72 h, the embryo hatches out from the eggshell and within the next 2 days starts hunting for food. In a period of just 4 days, the embryo converts rapidly into a small version of adult. The rapid development simplifies development and genetic studies.

The adult zebrafish attains sexual maturity very quickly, having generation time of about 10 weeks, and also this tiny fish has good fecundity rate. When kept under (laboratory conditions) optimal conditions, the zebrafish can lay about 200 eggs per week. The zebrafish is a very hard fish and is very easy to raise.

Use of zebrafish in developmental biology

Much of the pioneer works that established zebrafish as a model organism were done by George Streisinger, Charles Kimmel, and their colleagues. The team of these researchers studied the embryonic axis, cell lineage analysis, embryonic formation, development of central and peripheral nervous systems, muscle development, differential regulation of gene expression, etc.

Many of the critical pathways that control development in vertebrates are highly conserved between human and zebrafish. The zebrafish genome shares a lot of similarities with human genome. About 70% of genes associated with disease in humans have functional homologs in zebrafish.

Angiogenesis

Zebrafish model also has been used in the study of angiogenesis and regeneration. Angiogenesis is the process through which new blood vessels originate from pre - existing vascular structures which play essential role in healthy physiological and pathological conditions.

Being a transparent vertebrate, the zebrafish has emerged as a convenient alternative to study the early development of the cardiovascular system and observe the flow of blood. In zebrafish larvae the vessels and blood flow can easily be visualized by using simple dissecting microscope and also by using fluorescent proteins; the development of the blood vascular system could be examined in great details

Regeneration

The zebrafish exhibits remarkable capacity of regeneration even in adult stages. The caudal fin especially provides an ideal tissue for vascular regeneration studies due to its simple and fine architecture and relative transparency.

Zebrafish as a cancer model system

Being a vertebrate, the zebrafish is an ideal model to study cancer, though humans and fishes are separated from their common ancestry but biology of the cancer in both groups of organisms is the same.

Toxicology and drug discovery

Zebrafish embryos are used as predictive model to assess the toxicity in mammals. The effects of drugs on specific organs have also been studied, and it has been found that organ toxicity is similar in both zebrafish and mammals. The drugs that were used to evaluate the organ toxicity were gentamicin, cisplatin, vinblastine, quinine, neomycin, doxorubicin, dexamethasone, cyclosporin A, caffeine, camptothecin, MPA, fluorouracil, etc.

Human disease and zebrafish

Most of the tissues and organs found in humans and zebrafish are the same except lungs and prostrate and mammary glands. The cloning of mutated genes screened for specific phenotypes in zebrafish has similarities in humans and thus serves as model for human disease and to study underlying mechanisms. The first human disease identified using zebrafish was a blood disorder involving specific defect in haemoglobin production through ALAS2 mutated gene.

Many other mutants which show phenotypic similarities to human disease have been screened and identified. These include neurological disorders, haematological disorder, cardiovascular diseases, muscle disease and cancers,

Parkinson's disease, anxiety, and posttraumatic stress disorder.

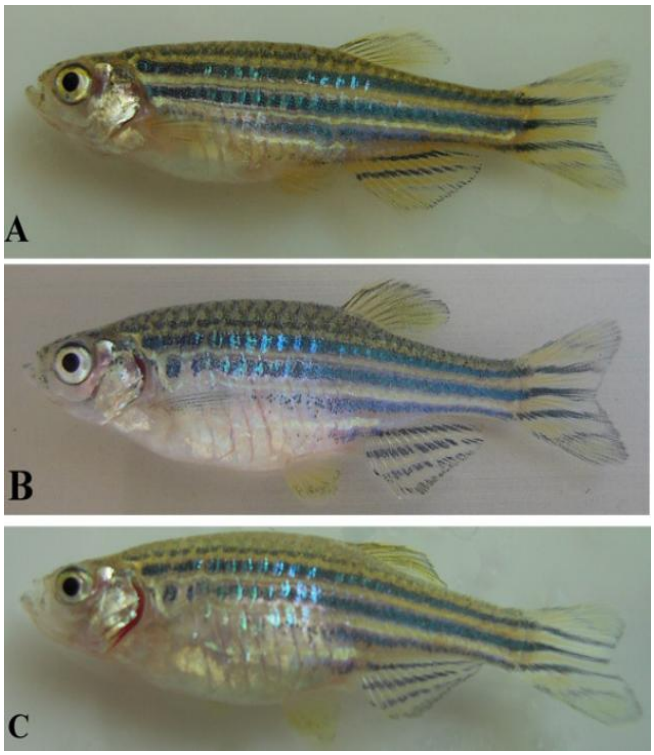


Figure: An illustration of a male zebrafish (A) and female zebrafish (B, C).

2. Review of Literature

Since it was first described by Francis Hamilton from the Ganges Delta in 1822, the small striped fish commonly known as the 'zebrafish' or 'zebra danio' has become variously a popular aquarium fish (Talwar & Jhingran, 1992) an important bioassay (Von Hertell et al., 1990) and in the last few years a major model in vertebrate physiology and developmental genetics (Vascotto et al., 1997). Several attributes of the species explain its popularity in all these contexts: it reaches a length of only 30mm, is relatively easy to rear and breed in captivity, and produces large numbers of offspring with a generation time as short as four months.

The following account outlines the major CNS divisions in the zebra fish and the organization of these divisions into nuclei or laminae, including a description of major tracts and commissures. CNS divisions will be dealt with according to the classical anatomical sequence: telencephalon, diencephalon, mesencephalon, metencephalon (including cerebellum), myelencephalon, and medulla spinalis. Since many tracts and commissures caudal to the diencephalon extend into several brain parts and even into the spinal cord, they are treated in a final separate section. When appropriate, discrepancies between the neuromeric model of Puelles and Rubenstein (1993) outlined above (see: Introduction) and classical brain divisions will be discussed.

The principal terminology applied to nuclei and larger CNS divisions is indicated at the beginning of each major section. For general review articles on fish neuroanatomy, the reader is referred to Nieuwenhuys (1963), Northcutt and Braford (1980), Northcutt and Davis (1983), Nieuwenhuys and Pouwels (1983), and Nieuwenhuys and Meek (1990).

The brain and the spinal cord formed by process named neurulation in which the neural tube in the embryo their walls thickened and for the central nervous system and the hollow tube formed during this process form the ventricular system. In ray - finned (Actinopterygian) fishes some parts of the brain don't reach complete maturation like other fishes (González and Northcutt, 2011).

Actinopterygian fish are classified into several kinds in accordance to developmental changes and structural differences in their brain compartments. However, some brain compartment remains similar between these fishes and other kinds of vertebrates (Butler, 2011).

The terminology of Nieuwenhuys (1963) as modified by Northcutt and Davis (1983) is applied except where noted. In teleosts, the topology of the telencephalon (Tel) is highly distorted (Nieuwenhuys and Meek, 1990).

The telencephalon in actinopterygian fishes protrusion from the pallium except for the cladistians, in which thickening in the walls usually occurs with this protrusion development (Nieuwenhuys, 2011).

The terminology of Braford and Northcutt (1983), Northcutt and Wullimann (1988), and Wullimann and Meyer (1990) is applied except where noted. The diencephalon proper has five major divisions which, in the adult brain, appear in a dorsoventral arrangement. They are the epithalamus, dorsal thalamus, ventral thalamus, posterior tuberculum and hypothalamus. The area praeoptica, although often considered part of the hypothalamus, is treated here in its own right because it constitutes an intermediate region between telencephalon and diencephalon.

The terminology of Nieuwenhuys and Pouwels (1983) is applied except where noted. The mesencephalon includes, dorsally, the (multisensory) optic tectum and, ventrally, the torus semicircularis and the tegmentum.

The sensory torus semicircularis (TS) is the mesencephalic target of ascending octavolateralis systems and lies on top of the lateral tegmentum from where it bulges out into the tectal ventricle. In cyprinids, the central nucleus (TSc) is related to audition and the ventrolateral nucleus (TSvl) is related to mechanoreception (Echteler, 1984; McCormick and Hernandez, 1996).

In Acipenser fish, this process usually detected at the fourth day of old. The epiphysis begins development during the first days of larvae, while other hypothalamic parts developed later. The mesencephalon usually keeps its embryonic shape which is two compartments tectal and tegmental portions; the tegmental poretion usually begins in development before tectal portion. In zebrafish embryos at 26 hpf, transverse sections showed that, the torus semicircularis (TS) develops internally and medially over the tegmentum. Afterward, proliferation becomes restricted to the intermediate portion between the optic tectum (OT) and the TS. It gradually extends during the development and constitutes the peripheral midbrain layer (PML) between the OT and TS (Recher et al., 2013b).

The terminology of Nieuwenhuys and Pouwels (1983) is applied except where noted. The rhombencephalon (hindbrain) is often divided into a rostral metencephalon and a caudal myelencephalon. With the exception of the cerebellum, the ventral (medullary) remainder of the metencephalon can be separated only arbitrarily from the more caudal myelencephalic portion of the medulla oblongata. Thus, we treat cerebellum and medulla oblongata as entities here. Medulla oblongata and tegmentum are collectively referred to as brain stem. The terms metencephalon and myelencephalon are only meaningful in mammals and birds. In those derived vertebrates, the metencephalon appears to be clearly separable from the myelencephalon as it exhibits a large dorsal cerebellum and ventral pons, which consists of relay neurons for cortical fibers to the cerebellum.

Two laterally projected compartments form the cerebellum and later they fuse together and form the ventricular cavity. At first the corpus cerebelli begins in development then followed directly by the valvula cerebella. The torus longiudinalis deign growth with granular eminences and before optic tectum and cerebellar crest development (Candal et al., 2005) in trout and medaka fishes, (Bäuerle and Rahmann, 1993) in Oreochromis mossambicus, (Toyoda and Uematsu, 1994) in Pagrus major (red seabream) and (Sprague et al., 2001; Wullimann and Knipp, 2000) in zebrafish.

The hindbrain in fish composed of the pons, cerebellum, and myelencephalon. In the embryos a dorsal protrusion from the neural tube wall and the alar plates, forms the medulla oblongata. A choroidal plexus which is lining the ventricular systems is responsible for cerebro - spinal fluid formation in fish (Redzic et al., 2005).

Laterally the ventricular system develops which later form all kinds of glial and neuronal cells for formation of various brain regions (Butler and Hodos, 2005).

In most vertebrates, the ventricular system extends laterally within the telencephalic hemispheres, forming the lateral ventricle. This ventricle connected with the tectal ventricle which in turn connects it to the third ventricle. The cerebral aqueduct is a thin canal connecting the third ventricle to the fourth ventricle in the hindbrain (Butler and Hodos, 2005).

The rhombencephalic reticular formation can be divided into midline, medial, and lateral columns (Nieuwenhuys and Pouwels, 1983). The inferior olive (10) is a large nucleus at the ventral periphery of the caudal brain stem. It is the source of climbing fibers reaching the cerebellum in teleosts (Finger, 1983; Wullimann and Northcutt, 1988; 1989)

The fourth ventricle extends backward toward the central canal of the spinal cord. The brain of Dolloidraco fish has many unusual characters in their ventricular system, like presence of some sub - ependymal protrusions, well developed circumventricular organs, a ventricle in the corpus cerebellum and subarachnoid cisterns (Eastman and Lannoo, 2003).

In larvae at the age of 15 - days, there was a connection between lateral ventricle and the preoptic recess, which is connected to the third ventricle in the diencephalon via the interventricular foramen. The cerebral aqueduct lies between the cerebellar ventricle and the tectal ventricle. The fourth ventricle was visualized in the center of the medulla oblongata and continued as the central canal into the spinal cord (Tavighi et al., 2015).

The terminology of Nieuwenhuys and Pouwels (J 983) is applied except where noted. Only the rostral spinal cord at the level of the entrance of the second dorsal root (DR) is characterized here. The second dorsal root is treated here, because the first dorsal root is minute. (Note, however. that the corresponding first ventral root is huge and likely innervates hypaxial (somatic) musculature in the lower jaw.) First dorsal and ventral spinal roots are located approximately 100~150micrometre caudal to the commissure infima of Haller. The second dorsal root, shown in this atlas, lies about 500~800 micrometre caudal to that commissure.

While in action - pterygian fishes, due to the eversion of the telencephalon during embryonic growth, the olfactory ventricle and lateral ventricle together formed a T - shaped telencephalic ventricle (Northcutt and Jr, 1980; Nieuwenhuys, 1998).

3. Methodology

Study Material

Zebrafish were housed in 25 - 30 litre tanks, which were arranged on shelves in a self - contained stock room within the School of Biology aquarium unit. The room was heated to a temperature of 23–25 °C using an ‘Auto heat’ fan heater in combination with a built - in room temperature control. Lighting was provided by fluorescent ceiling lights and was set to a 14h light: 10h dark cycle. Compressed air was supplied to the room from a central source in the aquarium. Fish in the tanks were provided with a gravel substrate and imitation plastic plants made out of green mesh. Filtration was supplied by one Algard ‘Bio foam 45’ air - driven biological filter unit per tank. Maximum density of fish was 35 adults per tank. Tank water was topped up where necessary, with a 30% water change done every two to three days. All tap water was aged in buckets for a minimum of two days before introduction to tanks, Fish were monitored daily for signs of distress, with extra water changes being performed when required.

System Maintenance Protocol

Table: Food and Habitat Features of Danio rerio in laboratory

No.	Temp.	pH	Nature of water	Substrate types	Date and time
1	18.6	6.5	clear water, bottom visible	pebbles, gravels and sand	11.10.2022 and 10:45 AM
2	17.7	6.35	clear water, bottom visible	pebbles, gravels and sand	12.10.2022 and 10:25 AM
3	18.3	6.75	clear water, bottom visible	pebbles, gravels and sand	13.10.2022 and 10:55 AM
4	17.5	6.25	clear water, bottom visible	pebbles, gravels and sand	14.10.2022 and 10:23 AM
5	16	6.65	clear water, bottom visible	pebbles, gravels and sand	15.10.2022 and 10:39 AM
6	16.8	6.45	clear water, bottom visible	pebbles, gravels and sand	16.10.2022 and 11:15 AM
7	17.3	6.7	clear water, bottom visible	pebbles, gravels and sand	17.10.2022 and 10.32 AM

This protocol describes regular care and maintenance of a zebrafish laboratory. Zebrafish are now gaining popularity in genetics, pharmacological and behavioural research. As a vertebrate, zebrafish share considerable genetic sequence similarity with humans and are being used as an animal model for various human disease conditions. The advantages of zebrafish in comparison to other common vertebrate models include high fecundity, low maintenance cost, transparent embryos, and rapid development.

1) Zebrafish are kept in a circulating system that continuously filters and aerates the system water to maintain the water quality required for a healthy aquatic environment. The circulating system also helps to filter excess food and fish excreta. Different companies provide zebrafish systems. The room temperature or the tank temperature is generally maintained between 26 - 28.5 °C and the lighting conditions are 14: 10 hr (light: dark). Multiple lines of fish (e. g., transgenic, mutant, wild type) can also be housed on the same system.
2) A set of different kinds of filters are used in the system. In our system, water from all the tanks passes through a 120 - micron filter pad, 50 - micron canister filter, biological filter, active carbon absorption filter and UV disinfection filter before being circulated back into the tank. Dechlorinated/aged water is used in the zebrafish system. Water can be de - chlorinated by ageing for at least 48 hr, under ideal condition.
3) The pH of the system water should be checked daily and maintained between 6.8 and 7.5. When necessary, sodium bicarbonate should be used to increase the pH.
4) Fish tanks should be cleaned regularly. To clean a fish tank, close the water flow to this tank, drain excess water by tilting the tank backwards and remove the tank carefully from the system. Dirt and algae growth will be apparent on the bottom and sides of the tank.
5) Carefully transfer the fish into this tank with a fish net. Close the lid and transfer the name tag of the tank. Carefully place the clean tank into the system and switch on the water supply. To decontaminate the fish net, spray with 70% ethanol, rinse in water, and let it dry before re - using. Remove the baffle from the dirty tank and spray both parts with 70% ethanol. Rinse thoroughly with tap water and allow the tank and baffle to dry fully before re - using.
6) The circulating system filters have to be checked and changed regularly to ensure their proper function. These filters should be changed regularly to ensure proper and clean water supply to all the fish tanks.
7) The 120 - micron filter pad is usually repositioned or replaced daily, The Canister filter should be changed weekly. To change the canister filter, remove the filter unit by twisting anticlockwise with a wrench or hands. The carbon filter should be changed fortnightly (every two weeks). To change the carbon filter, remove the carbon filter unit carefully with a wrench. Discard the used activated carbon and replace it with new activated

carbon. Re - fit the carbon holder and place it back into the filter unit.

8) **Feeding:** Zebrafish can be fed with dry food (food size from 100 microns for larvae to 300/400 microns for adult fish).

Zebrafish should never be overfed as this may increase the nitrate level in the water, possibly affecting their breeding, or viability, as some fish may die due to overeating. We recommend providing no more food during any one feeding than a tank of fish can finish within 10 min.

When feeding on the Aquatic Habitats systems we usually turn off the water pump and air pump to allow the fish to eat the food for 10 min. This decreases the amount of food that is washed into the filters. However, users must be careful to remember to turn on these pumps again afterwards.

4. Material and Method

Histology

Histology has been used for more than a century to visualize cellular composition and tissue architecture in nanometer - to millimeter - to centimeter - scale tissues from diverse multicellular organisms. Its diagnostic power is dependent on the detection and description of changes in cell and tissue architecture. Normal and abnormal cytological features indicative of physical, inflammatory, and neoplastic causes of disease are readily distinguished using histology. Fifty adult specimens of *Dania rerio* were processed in the course of this study.

The fish were then decapitated, and the skulls were opened dorsally to expose the brain. Fresh tissue specimens will come from aquarium tank. It should be noted that they can very easily be damaged during removal of brain from the zebra fish. It is important that they are handled carefully and appropriately fixed as soon as possible after dissection.

Step 1: Fixation

After the brains were removed from the skulls, The fish brain is placed in a liquid fixing agent (fixative) such as 4% buffer formalin solution (formalin) fix for one day. This will slowly penetrate the tissue causing chemical and physical changes that will harden and preserve the tissue and protect it against subsequent processing steps (so that stop post - mortal changes).

Step 2: Washing

Washing of brain of distilled water three times each for 30 minutes.

Step 3: Dehydration

Because melted paraffin wax is hydrophobic (immiscible with water), most of the water in a specimen must be removed before it can be infiltrated with wax. Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. A series of increasing concentrations is used to avoid excessive distortion of the tissue.

1) 30% ethanol 30 min
2) 50% ethanol 30 min
3) 70% ethanol 30 min (continue with alcohol series or we can leave for one or two days)
4) 90% ethanol 30 min
5) 100% ethanol 30 min
6) 100% ethanol 30 min

Step 4: Clearing

Unfortunately, although the tissue is now essentially water - free, we still cannot infiltrate it with wax because wax and ethanol are largely immiscible. We, therefore, have to use an intermediate solvent that is fully miscible with both ethanol and paraffin wax. This solvent will displace the ethanol in the tissue, then this, in turn, will be displaced by molten paraffin wax. This stage in the process is called “clearing” and the reagent used is called a “clearing agent”.

A popular clearing agent is xylene, and multiple changes are required to completely displace ethanol.

1) xylene 15 min
2) xylene 10 min

Step 5: Infiltration

The tissue can now be infiltrated with a suitable histological wax. Although many different reagents have been evaluated and used for this purpose over many years, the paraffin wax - based histological waxes are the most popular. A typical wax is liquid at 60°C and can be infiltrated into tissue at this temperature then allowed to cool to 20°C, where it solidifies to a consistency that allows sections to be consistently cut. These waxes are mixtures of purified paraffin wax and various additives that may include resins such as styrene or polyethylene. Which allow tissues infiltrated with the wax to be sectioned at a thickness down to at least 2 µm, to form ribbons as the sections are cut on the microtome, and to retain sufficient elasticity to flatten fully during flotation on a warm water bath.

1) wax 60 min
2) wax 60 min

Step 5: Embedding or Blocking Out

Now that the specimen is thoroughly infiltrated with wax, it must be formed into a “block” with the help of “L - pieces”, which can be clamped into a microtome for section cutting. This step is performed using an “embedding centre” where L - pieces are filled with molten wax and the sample (brain) is placed in it. Keep the prepared block in refrigerator in the 4’C.

Step 6: Trimming

Trim the block with sharp knife properly then fix in its cassettes leave it as for one to two hours. When this is completed, the block with its attached cassette can be removed from the mold and is ready for microtomy. After two hours, tissue sections are cut with the help of microtome and spreading of ribbons in hot water bath and takeout the tissue section on the glass slide.

Even with expert proficiency using histological tools and equipment, slice thickness is limited to ~10 µm of cutting paraffin blocks. Moreover, it is manageable to generate complete sets of sections for large numbers of whole brain analysis of organisms.

The brain of the zebrafish Danio rerio: an overview

CNS divisions of zebra fish will be dealt with according to the classical anatomical sequence: telencephalon, diencephalon, mesencephalon, metencephalon (including cerebellum), myelencephalon, and medulla spinalis.

1.1. Adult brain anatomy

1.1.1. Telencephalon

The olfactory bulb of zebra fishes was a spherical or oval and joined to the telencephalon by a very long olfactory tract, the olfactory bulbs were large and about half the volume of the telencephalic lobes (Eastman and Lannoo, 2008). The most rostral telencephalic divisions are the paired olfactory bulbs. The primary olfactory fibers (nervus 01 - factorius, I) entering the olfactory bulbs are the axons of the olfactory receptors, which are of placodal origin and, by definition, not part of the CNS. The rest of the telencephalon comprises two subdivisions, area dorsalis and area ventralistelencephali.

1.1.2. Diencephalon

The diencephalon formed from the epithalamus, thalamus (posterior tuberculum, ventral thalamus, and dorsal thalamus), preoptic area, hypothalamus, synencephalon, some optic nuclei and the pretectum (Butler and Northcutt, 1993).

The area praeoptica, although often considered part of the hypothalamus, is treated here in its own right because it constitutes an intermediate region between telencephalon and diencephalon.

The thalamus of zebra fish composed from the thalamus proper, the habenula, and the prethalamus (Mueller and Wullimann, 2016). The thalamus is located dorsolateral to the hypothalamus, and rostral to the tegmentum of the mesencephalon. The epi - thalamus is composed of the habenula and its commissure. The synencephalon is the region in between the optic tegmentum and the thalamus (Puelles and Rubenstein, 1993). According to Braford and Northcutt (1983), the synencephalon designates a series of structures which are intermediate between the dorsal diencephalon and mesencephalon.

1.1.3. Mesencephalon

The mesencephalon contains optic tectum dorsally and optic tegmentum ventrally. The dorsal tectum evaginates into two bilateral lobes attached dorsally with the tectal commissure and is composed of the optic tectum. The optic tectum (TeO) is the most complex layered structure in the zebrafish brain. It consists of four zones (periventricular grey zone, deep white zone, central zone, and superficial grey and white zone), which can be further subdivided into 15 layers (Northcutt, 1983). Different from all other vertebrates.

The tegmentum is bordered rostrally by the synencephalon, the dorsal thalamus, and the posterior tuberculum; the tegmentum and is related dorsally to the valvula cerebelli, related laterally to the optic tectum and ventrally is related to the inferior lobe of the hypothalamus (Eastman and Lannoo, 2001).

1.1.4. Rhombencephalon or Hindbrain (metencephalon and myelencephalon)

The rhombencephalon (hindbrain) is often divided into a rostral metencephalon and a caudal myelencephalon. With the exception of the cerebellum, the ventral (medullary) remainder of the metencephalon can be separated only arbitrarily from the more caudal myelencephalic portion of the medulla oblongata. Thus, we treat cerebellum and medulla oblongata as entities here. Medulla oblongata and tegmentum are collectively referred to as brain stem.

The cerebellum is lower in size. The cerebellum (Ce) of the zebrafish has three parts: the vestibulolateralis lobe, the corpus cerebelli (CCe), and the valvula cerebelli.

The medulla oblongata contains anterior and posterior parts both are ovoid in shape but the anterior one is slightly larger in size than the posterior part. The vagal lobe formed from two lobes cylindrical in shape. It located posterior to the facial lobe and dorsal to the medulla oblongata (Abrahão et al., 2015).

The medulla oblongata (MO) contains the sensory and motor nuclei of the trigeminal (nervus trigeminus, V), abducens (nervus abducens, VI), facial (nervus facialis, VII), octaval (nervus octavus, VIII), glossopharyngeal (nervus glossopharyngeus, IX) and vagal (nervus vagus, X) nerves.

Medulla spinalis - Only the rostral spinal cord at the level of the entrance of the second dorsal root (DR) is characterized here. The second dorsal root is treated here, because the first dorsal root is minute. They lie in the peripherally located white matter. The white matter of the spinal cord can be subdivided here into dorsal, lateral (which consists of a dorsal and a ventral part).

5. Results and Observation

5.1 Histology

General Brain Histology

5.1.1. Telencephalon

The olfactory bulb formed from four layers which from the outside inwards were: the olfactory nerve fiber layer, the glomerular layer, the plexiform layer and the granule cell layer (Alonso et al., 1989). The cerebrum consisted of a single layer in all teleosts (Ito and Yamamoto, 2009; Sharareh etal., 2013) but was lobulated in zebra fish (Sharareh et al., 2013).

5.1.2. Diencephalon

The diencephalon formed from, dorsal epithalamus which located under the optic tectum, and contains the pineal gland, the habenular ganglion and saccus dorsalis, or telachoroidia, which located dorsorostraly to the diencephalon. The thalamus, it was in the middle, situated under the third ventricle between the tegmentum and the hypothalamus. (Eastman and Lannoo, 2004).

The most posterior region of the diencephalon is the hypothalamus, which considered the main structural complex and formed from the inferior lobes and the infundibular region. As well as, specialized structures,

including the pituitary gland and the saccusvasculosus (FRANCK, 2009; Groman, 1982). The saccusvasculosus located between the two caudal parts of the inferior lobes of the hypothalamus, beneath the pituitary gland (Sharareh et al., 2013).

5.1.3. Mesencephalon

The most obvious part of the midbrain is the optic lobe; it has six histologically distinct layers; the stratum marginale, stratum opticum, stratum fibroetgricialem, stratum album central, stratum griseum central, and stratum periventriculae (Eastman and Lannoo, 2001, 2004).

5.1.4. Hindbrain

The corpus cerebelli is the largest part of the brain in zebra fish (Abrahão et al., 2015). The cerebellum function in receiving the sensory inputs and transferring it toward the motor nuclei. The cerebellum (metencephalon) was composed of the corpus and the valvula cerebella (Lee and Bullock, 1984). The histological structure of the cerebellum gray matter was consisting of; outer molecular, Purkinje cell, and inner granular layers (Eastman and Lannoo, 2008).

5.1.5 Myelencephalon

The myelencephalon principally composed of the medulla oblongata, as the stem of the brain, and the paired vagal lobes (Lagler et al., 1977). The medulla oblongata structure affected by feeding habits of the fish especially the facial, vagal and somatic sensory lobes (Sreekala et al., 2011). Thevagal lobe composed of sensory layer, a fiber layer and a motor layer (Morita et al., 1983).

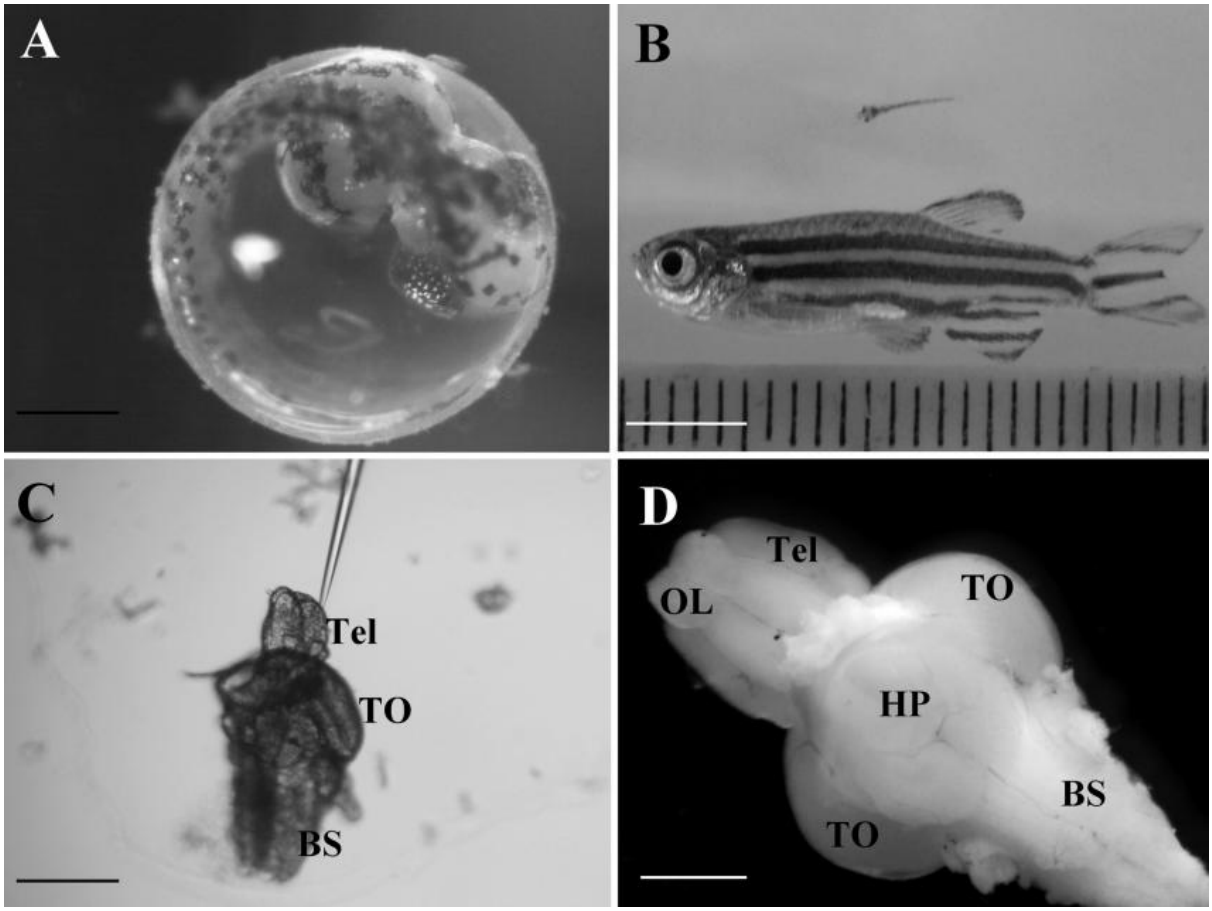


Figure: Zebrafish model

A: photograph showing a zebrafish embryo at 3 days postfertilization.
B: a 5 - days postfertilization larva (top) and 5 - month - old adult zebrafish (bottom).
C: whole brain of a 5 - days postfertilization larva with a recording electrode over the telencephalon (Tel). Tectum opticum (TO) Brain stem (BS).
D: brain of a 5 - month - old adult zebrafish (ventral side). Olfactory lobe (OL), hypothalamus (HP).

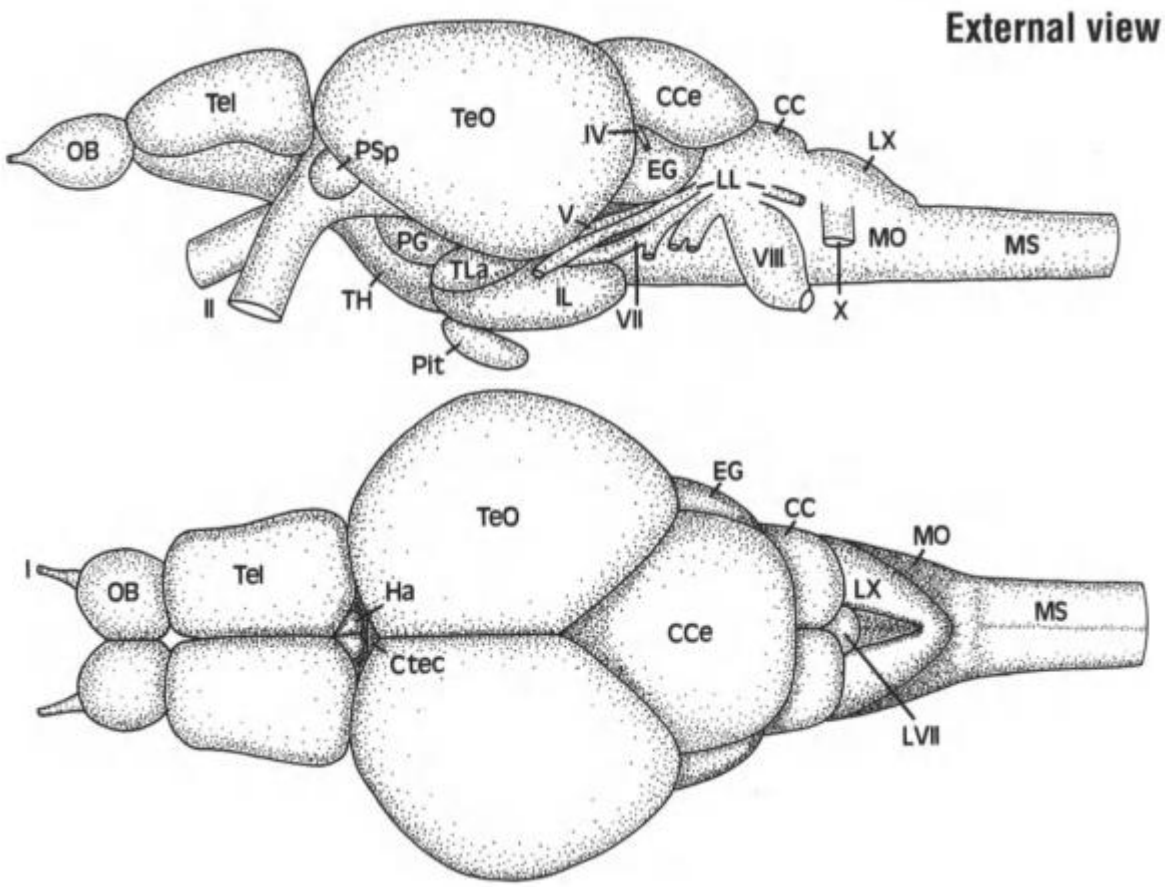
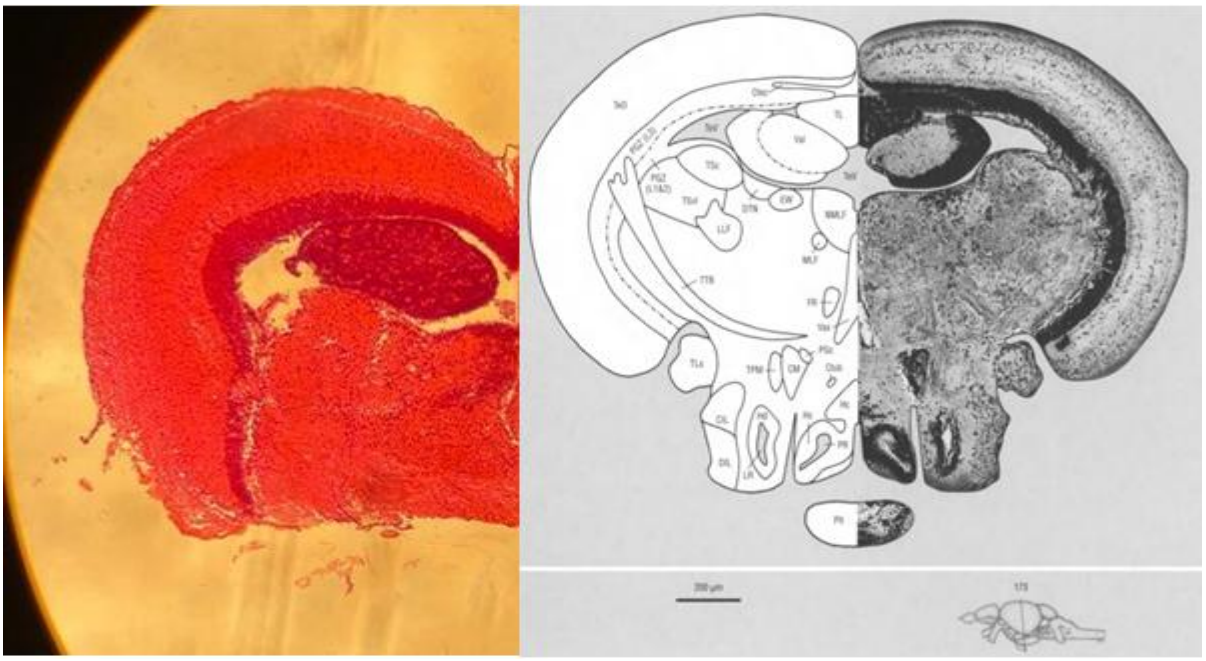


Figure: Lateral (a) and dorsal (b) views of the adult zebrafish brain. Thetelencephalon comprises a dorsal and a ventral telencephalic area as well as an olfactory bulb, which is entered rostrally by the olfactory nerve. The diencephalon is located rostroventral to the midbrain and is largely covered by the optic tectum. Externally visible portions of the diencephalon include the optic nerve, the preglomerular area, the torus lateral is, the habenula, and the hypothalamus. The hypothalamus can be separated into a rostromedialtuberal hypothalamus and paired lateral lobes. Saccus dorsalis and epiphysis were removed during preparation and are not shown. The brainstem, which harbors mostcranial nerve roots except for the optic and olfactory nerves, is covered rostrally by the cerebellum (i. e. the corpus cerebelli and the granular eminence), and includes more caudally the crista cerebellaris, the prominent paired vagal lobes, and the unpaired facial lobe. Finally, the medulla oblongata grades into the spinal cord (medulla spinalis).



6. Conclusion

The anatomical and histological observations of the zebrafishes brain differ among species, but they resemble each other's in the number of brain compartments, and it is necessary to characterize well the anatomical and histological observations in the brain of each particular kind of fishes for doing further subjects in the brain. Five brain divisions usually observed which are from cranial to caudal; telencephalon or forebrain (contain 2 olfactory lobes and cerebrum), diencephalon (contain epithalamus, thalamus and hypothalamus), mesencephalon or midbrain (contain 2 optic lobes which are connected internally with torus longitudinally and medially with the torus semi - circularis, and optic tegmentum), metencephalon or hindbrain (cerebellum) and myelencephalon or brain stem (medulla oblongata). The ventricular organization composed of the olfactory, lateral, the third, the tectal and the fourth ventricles. The adult neurogenesis process is usually observed in the fish brain, unlike mammal's brain. The adult neurogenesis usually detected in cerebellum, optic lobe, and telencephalon.

The embryonic neural tube forms the several compartments of the brain and the spinal cord by the division of the neural stem cells. The hollow neural tube contains cerebrospinal fluid which secreted from ependymal cells lining it. This tube differentiates to form the ventricular system which formed from the olfactory ventricle and lateral ventricle present in the telencephalic hemispheres. The olfactory lobe is spherical or oval or pyriform in shape, its shape and size differ according to kind of fish, and it connected via olfactory tract to the olfactory mucosa and joined in the other side by olfactory tract to the cerebrum. The cerebrum histologically formed from a single layer in all teleosts and danio specieses and anatomically can be divided into dorsal and ventral parts. The epithalamus formed from the pineal gland, habenular ganglion and saccus dorsalis. The nucleus glomerulosus is the most visible part of the thalamus in histological slides of most kinds of fishes. The hypothalamus consists of the inferior lobes, infundibular region, saccusvasculosus and pituitary gland. In some types of fish which depend mainly on vision, the optic tectum is the most significant part of their brains.

The cerebellum is the most prominent structure it is formed from corpus cerebella and valvula cerebella. The medulla oblongata is also large in size; it composed from vagal and facial lobe which responsible for the taste sensation. It can be concluded that there are many fishes their brains are still not fully described like loaches, gray mullet and catfishes and many others. Also, the mechanism of adult neurogenesis is not well understood in spite a lot of many recent research papers give significant results but still need further studies in it. We still need to do more comparison between fish and mammalian brain in relation to adult neurogenesis.

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