PARTICULARITIES OF OLFACTORY SYSTEM IN ZEBRA FISH (*DANIO RERIO*)

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Abstract
Histological study of olfactory bulbs and peripheric olfactory organs were made on 10 adult zebra fiskes (Danio rerio), both sexes, using paraphin embeding method and HEA and PAS stain. Zebra fish is a very good model for studying the mecanisms for odour detection, olfactory changes of the environement and behavioural effects of them. Olfactory system in zebra fish is formed by a pair of olfactory peripherical organs or rosets, situated in nasal cavity, conected at the olfactory bulbs. Sensorial region of the rosete have an characteristic pseudostratified columnar epithelium consisting mainly from sensitive olfactory neurons. Olfactory bulb is a cerebral structure situated in the most rostral region of the anterior brain, conected to olfactory bulb by a short olfactory nerf. It is the central receptor when the olfactory informations are prepared and sent to telencephalic regions.
Key words: *zebrafish, olfactory system, histology*

Introduction

Danio rerio or zebrafish belongs to the family Cyprinidae, genus Danio, comes from India and has become an important tool in neuroscience research due to its genetic traceability, molecular and physiological preservation, small body size, easy of *in vivo* experimental manipulations and rich behavioral repertoire. Zebrafish models and tests are particularly useful in genetic research, in neurophenotyping, in the screening of CNS drugs, toxicology (Petrovici et all., 2020), as well as in the modeling of complex neurological and psychiatric disorders.

In the last decade, technological evolution has opened up new opportunities to study neural circuits. These include molecular approaches for identifying, labeling, and manipulating certain types of neurons in the brain, studying behavior, advances in extracellular recording techniques to measure the triggering potentials of multiple neurons in animals, and intracellular recording methods. In addition, compared to mammals, the brains of adult zebrafish have a high level and a number of proliferative areas of regeneration. The general organization of the central nervous system of zebrafish is indeed very similar to that of other vertebrates and approaching the nerve segments in an antero-posterior order was observed that it is structured in four areas: forebrain, midbrain or middle brain, rhombencephalon or posterior brain and spinal cord.

The olfactory system, composed from the olfactory organs and the olfactory bulb, allows organisms to interact with their environment by detecting odor signals. Smell mediates behaviors that are essential for survival, such as feeding, mating, social behavior, and hazard assessment. The olfactory bulb (OB) is an associated brain structure located in the most rostral region of the anterior brain, connected to the olfactory organ by a short olfactory nerve; is the central relay of the olfactory system in which olfactory information is processed and transmitted to the telencephalic areas.

Materials and methods

The study was conducted on 10 zebrafish, 5 males and 5 females. The samples taken were the fish entirely, initially placed for 4 hours in 4% formaldehyde. After this prefixation, they were sectioned mid-sagittally and transferred to the Bouin fixator for 48 hours. The pieces were dehydrated, clarified, embedded in paraffin, sectioned at 5µm and stained HEA, PAS, PAS- Alcian Blue.

Results

The olfactory system, composed of the olfactory organs and the olfactory bulb (fig. 1), allows to organisms to interact with their environment by detecting odor signals. The telencephalon of the zebrafish consists of olfactory bulbs, the dorsal telencephalon (TD) or pallium and the ventral telencephalon (TV) or subpallium. The telencephalon consists of solid telencephalic lobes, separated by a T-shaped ventricle. The pallium and subpallium can be divided in two domains (TDM-median dorsal telencephalon, TDP-posterior dorsal telencephalon, TVD-ventral telencephalon dorsal part and TVV-telencephalon ventral part). One of the most distinctive features is that TVV is the only telencephalic region in which cholinergic neurons have been detected. Numerous neuroglia (nvg) were also observed in the structure of the zebrafish telencephalon. The axons in the olfactory bulb form the olfactory tracts in the telencephalon.

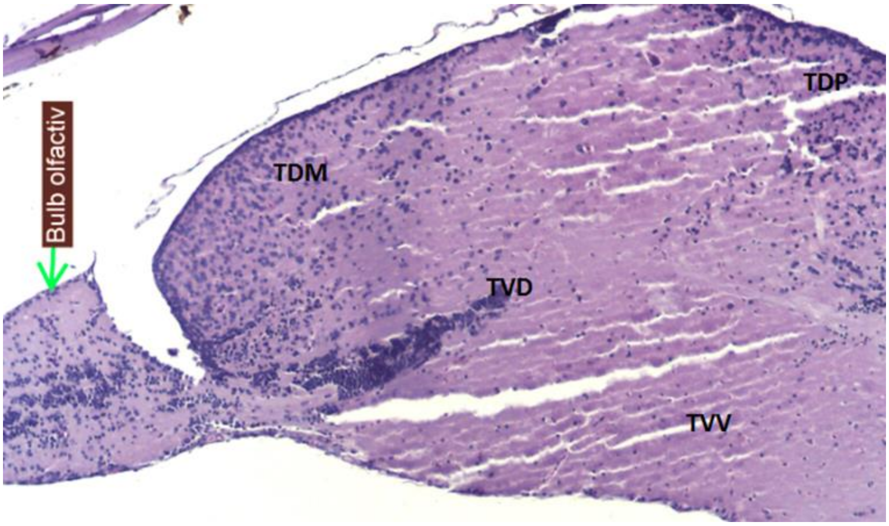

Fig. 1- The telecephalous parenchyma consists of neurons protected by glial cells (astrocytes and oligodendrocytes), as well as microglia and blood vessels. PAS x 40

The general architecture and functional organization of the olfactory system of zebrafish is analogous to that of other vertebrates. It consists of two main structures: a pair of peripheral olfactory organs, or rosettes, located in the nasal cavity connected to the olfactory bulbs (Fig. 2), which is the most frontal region of the rostrum. The olfactory organ of zebrafish contains an epithelium arranged in several lamellae that converge in a non-sensory central area, forming a bilateral, cup-shaped structure known as a rosette.

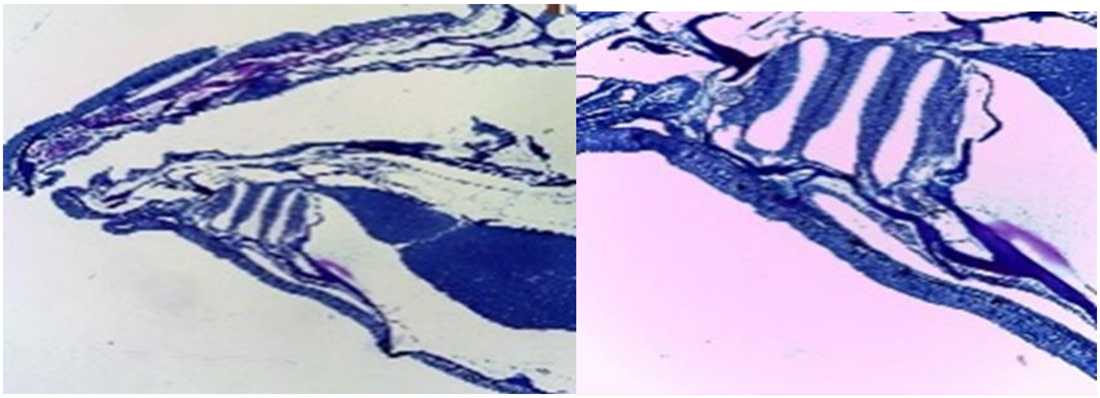

Fig. 2- Structure of the olfactory system of zebrafish: peripheral olfactory organs, or rosettes, located in the nasal cavity connected to the olfactory bulbs. Alcian Blue x100.

The rosette lamellae are composed of a continuous sensory area, located in the central and medial region, as well as a surrounding non-sensory epithelium located dorsally (Fig. 3).

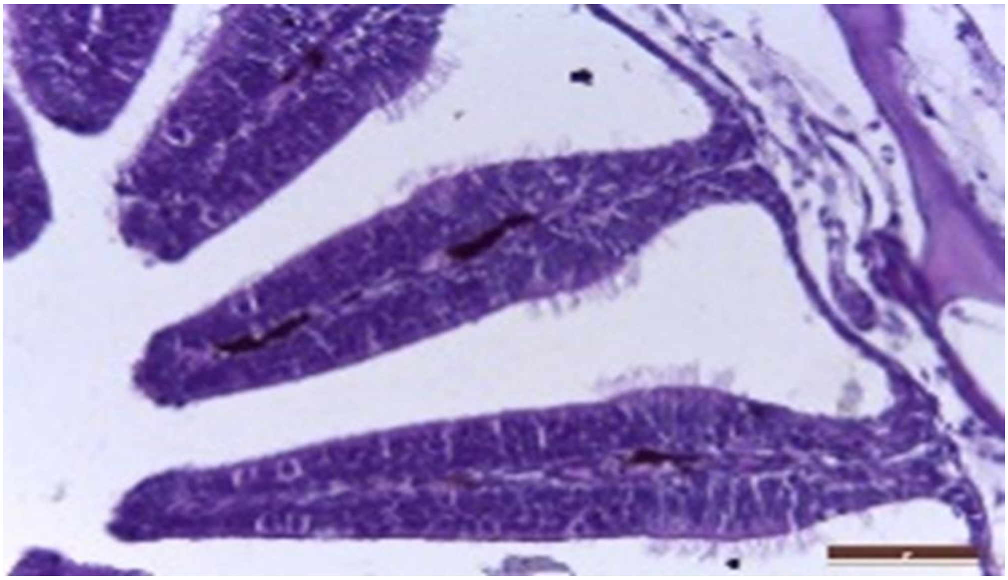

Fig. 3 Peripheral olfactory organs or rosettes, located in the nasal cavity. PAS- Alcian Blue x100

The sensory region of the rosette has a characteristic pseudostratified columnar epithelium consisting mainly of olfactory sensory neurons (OSNs, sensitized by odorants), basal cells and the support cells. There are five types of OSN described in zebrafish (Fig. 4): cills (cl); with microvilli (mv); crypt (cr); Kappe (kp) and pear (pr) neurons described recently. These OSNs have different morphologies, molecular markers and profiles and are differentially located on the entire epithelial layer. Ciliated OSNs are found at the base and have an elongated morphology with a long apical dendrite that continues with cilia. The microvilli OSNs are of intermediate size with apical microvilli that come from a thick dendrite. Crypt neurons are found apically and have a spherical body, appearing as a smaller cell. Kappe OSNs are located on the apical side of the olfactory epithelium (OE) and have a short, globose shape characterized by an apical cap with microvilli.

Pear OSNs, which are also located apically, have a pear-shaped morphology as well as very short apical dendrites (Fig. 5).

Fig.4- Olfactory epithelium composed of ciliated columnar epithelium with olfactory sensory neurons (OSN): contains microvilli (mv); ciliated cells (cl), cryptic cells (cr); kappe (kp) and piriformis cells (pr). PAS Blue Alcian x1000

Fig. 5- The layers of olfactory epithelium (OE) with peripheral basal cells with cills and microvills followed by a layer of nuclei with supporting cells and a layer with nuclei with olfactory cells and further in the center is located the lamina propria. HEA x1000

Discussions

OSNs extend the axonal projections to the olfactory bulbs, forming a fasciculate connecting structure known as the olfactory nerve. OSN axons that reach the olfactory bulbs form discrete structures known as glomeruli, where they form synapses with bulbous mitral cells. Glomerular activity patterns are processed by a distributed network of major neurons, mitral cells, and various types of local interneurons, including granular cells, periglomerular cells, and short-axon cells. These neurons extend the axons to the anterior brain in bundles known as olfactory tracts, where they transmit signals to the respective telencephalic olfactory processing areas to the posterior area of the dorsal telencephalon; the ventral nucleus of the central telencephalon; posterior tubercle and right habenula. Olfactory information is processed and decoded in these telencephalon centers to cause odor-mediated behaviors. In addition to having a well-characterized morphology and neural circuits, the olfactory system of zebrafish has remarkable mechanisms of regeneration, repair and reorganization in the basal states in response to injury. Both the olfactory organ and the olfactory bulb show continuous neurogenesis and neuronal fluctuation throughout the body's life. These characteristics make the olfactory system of zebrafish an ideal model to study the mechanisms of olfactory processing, olfactory dysfunction and regeneration after deterioration.

While ciliated and microvilli cells are also present in higher vertebrates, cryptic cells have been found only in fish. In zebrafish, homologous circuits consist of usually from far fewer neurons than in mice. The olfactory bulb, for example, contains approximately 500 neurons in zebrafish larvae and 20,000 to 30,000 neurons in adults (Mack-Bucher et al., 2007; Wiechert et al., 2010), compared to ~ 10^6 - 10^7 neurons in grown up mices. Therefore, zebrafish allow the sampling of neural activity on a large fraction of neurons in many areas of the brain. Limited sampling is sufficient when the responses are dense and when a calculation can be explained by simple statistical properties of neural activity patterns. For example, the responses of individual neurons in the sensory brain areas are often reduced depending on the average input through an operation called “normalization”. Dense sampling may be required, for example, to define the state of a network, especially when these states are not triggered by an external event, but occur spontaneously. In general, dense sampling becomes important when neuronal activity itself is reduced and when information processing depends on specific subgroups of neurons. Dense measurements and detailed neuron-by-neuron analyzes of activity patterns may therefore be necessary for a rigorous perspective on important neural calculations. Circuits whose function depends on poor activity and the specific structure of activity patterns are likely to be common in vertebrates, for example, in the cortex and cerebellum.

Zebrafish provide a favorable model for studying both the mechanisms underlying the detection of odor and olfactory changes, as well as their behavioral effects.

Conclusions

1. The olfactory system is composed of the olfactory organs and the olfactory bulb and allows organisms to interact with the environment.

2. The olfactory organ consists primarily of a sensory epithelium represented by olfactory sensory neurons (OSNs) that respond to odor or odorant molecules.

3. OSNs are bipolar sensory neurons that extend from the basal lamina to the apical region of the epithelium, where they detect odors in water and are of five types of cells: with microvilli, ciliated, cryptic kappe (kp); and piriform.

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