NEURONAL POPULATION OF THE MESENCEPHALON, RHOMBENCEPHALON AND SPINAL CORD IN ZEBRAFISH *(DANIO RERIO)*

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Abstract
Anatomically central nervous system in zebra fish is represented by telencephalon, diencephalon, mesencephalon, mielencephalon and metencephalon. Histological study of neuronal population of mesencephalon, rombencephalon and spinal cord was made on 10 adult zebra fish, both sexes. Mesencephalon is the median vesicle of the brain having 3 main components: cerebral pedunculs, tegmentum and optic tectum. Tectum has seven layers of fibers and different celular composition. Rombencephalon comprise: cerebelum, ponce and medula oblongata and controls the autonomic functions and equilibrium. Cerebelum is composed by corpus cerebeli and valvula cerebeli, and together with ponce forms the metencephalon.Cerebelum have three layers of neurons: molecular, Purkinje cells and granular. Mielencephalon is represented by medulla oblongata, which is continuing caudally with the spinal cord, and is closing the 4th ventricle. Histologically, medula oblongata have small ovalar cells and small glial cells. In ventral spinal cord four domains are identified: p3, pMN, p1 and 0, composed from neurons having common features.
Key words: zebrafish *(Danio rerio), nervous system, histology*

Introduction

Danio rerio or zebrafish belongs to the family Cyprinidae, genus Danio, comes from India. The information provided by the development, genetics and sequencing research of the zebrafish genome allows their use as a model in various studies (Lieschke and Currie 2007). Genome sequencing, genetic changes that can be induced have led to the creation of numerous models of zebrafish for the study of human tumors, cardiovascular disease, Alzheimer's, Parkinson's, muscular dystrophies (Best and Alderton 2008; Guyon et al. 2007; Lieschke and Currie 2007; Zon and Peterson 2005). In addition, zebrafish has recently been used to assess drug toxicity (Eimon and Rubinstein 2009; McGrath and Li 2008, Strungaru et all., 2018).

The midbrain is the middle vesicle of the brain in developing vertebrates. It comprises three main components, as in other vertebrates, namely the cerebral peduncles, the tegmentum and the optic tectum. The optic tectum (OT) is a key center for processing sensory information that receives most of its afferents from the retina and builds an image of the physical environment. Through connections to multiple regions of the brain, it integrates visually acquired information with motor inputs and outputs to initiate appropriate behavioral responses. Tectum plays major roles in controlling eye movement and sensory-motor actions and has seven tangential layers. These layers have different fibers and cellular compositions.

The posterior brain or **rhombencephalon** includes the cerebellum, bridge and medulla oblongata and controls autonomic functions and equilibrium. The cerebellum consists of the corpus cerebelli and the cerebelli valve and together with the bridge forms the metencephalon. The myelencephalon is represented by the medulla oblongata, the caudal portion that continues with the spinal cord, closing the fourth ventricle and having the role of controlling involuntary vital functions. The terms metencephalon and myelencephalon are more significant in mammals and birds because in these vertebrates the metencephalon seems to be clearly separated from the myelencephalon, which is not the case in zebrafish. The spinal cord is formed from the rest of the neural tube.

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 H.E, PAS, PAS- Alcian Blue.

Results

Optical tectum (TO) is a key center for processing sensory information and contains between 11 and 15 distinct morphological cell types, most (over 90%) being piriform neurons. Ventral to it, below the tectal commissure, in the tectal ventricle (V3), is found torus longitudinalis (TL). Surrounding the posterior tip of the tectum, a thin sheet of cells seals the ventricle that extends between the caudal tip of the tectum and the cerebellum (Fig. 1).

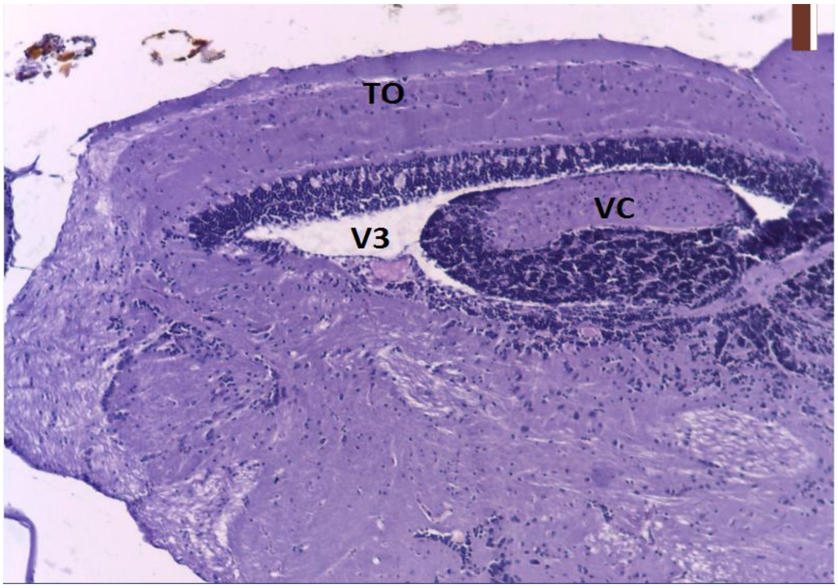

Fig 1. Optical tectum (TO). Ventral, below the tectal commissure, in the tectal ventricle (V3), is found torus longitudinalis (TL) .Col. HE x 40

The areas of the zebrafish tectum as in the case of other teleosts are represented by seven layers that are from the *pia mater* to the ventricle 3, the following: 1-meninges; 2-stratum marginal fibrosum (MS) with non-myelinated axons; 3-layer opticum (SO); 4-stratum fibrosum and superficial griseum (SFGS); 5-stratum griseum centrale (SGC); 6-layer central album (SAC); 7-periventricular stratum (SPV) composed of piriform neurons (Fig. 2).

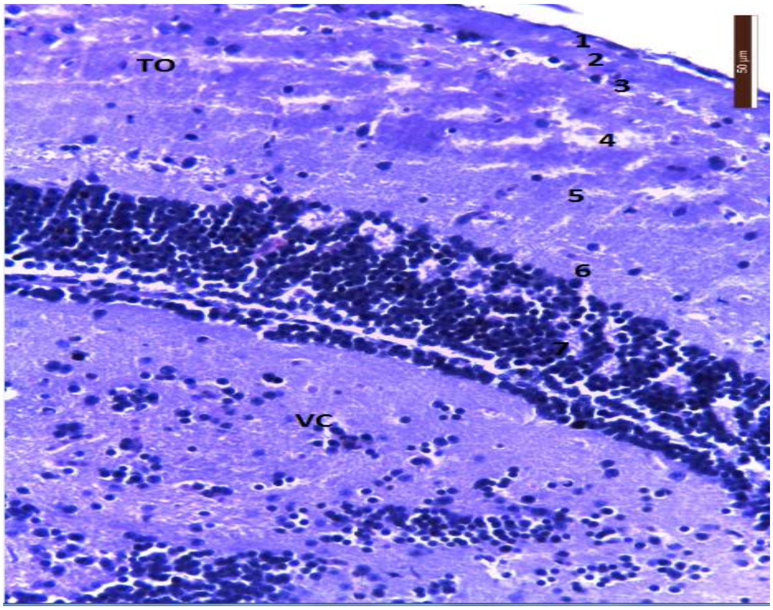

Fig. 2- Optical tectum stratification (TO): 1-meninges; 2-stratum fibrosum marginale; 3-stratum opticum; 4- stratum fibrosum and griseum superficiale; 5-stratum griseum centrale; 6-stratum album centrale; 7-stratum periventriculare, composed from piriform neurons.
Col. Blue Alcian x400

The inputs related to the tectum come from different sensory systems and nuclei. The non-myelinated axons of MS come from the longitudinal torus. SO and SFGS receive most of the retinal terminals. Mechanoreceptive information reaches the SGC and SAC layers of the tectum from the semicircular torus. The telencephalon projects to the GSC (ipsilateral and possibly contralateral). From the diencephalon, numerous pretectal, thalamic and hypothalamic nuclei contribute to the tectum.

Analyzing the caudal division of the three primary divisions of the brain of developing vertebrates, the rhombencephalon, the structure of particular importance is the cerebellum which has a simple laminated architecture consisting of three layers, namely. a molecular layer, a Purkinje cell layer and a granular cell layer (Fig. 3).

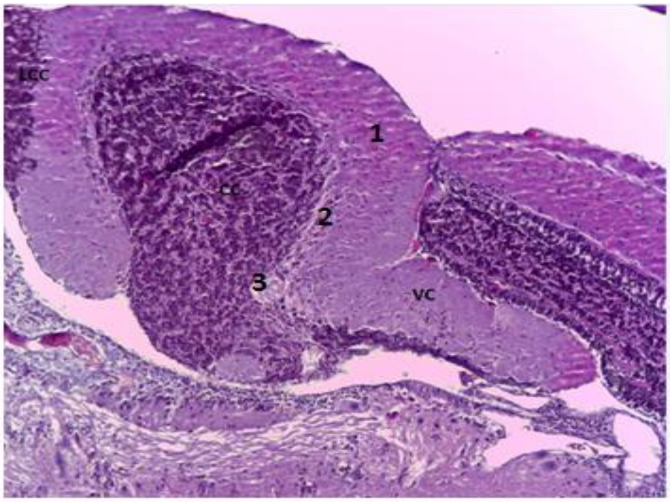

Fig 3- Cerebellum with the 3 structures - corpus cerebelli (CC), cerebelli valve (VC) and lateral vestibular lobe (LCC). The layers of neurons are: 1. molecular layer; 2. Layer of Purkinje neurons; 3. Granular layer. Col. PAS x 40

It has been observed that the granular cell layer consists of excitatory granular cells and inhibitory Golgi neurons and the layer of Purkinje neurons contains Bergmann glial cells and excitatory euridendroid cells. Granular neurons are small and very numerous making this dense. Proliferation of cells are found in all subdivisions of the cerebellum. Although the cerebelli valve extends into the tectal ventricle, it is histologically formed of a granular and molecular layer, to which are added aggregates of large Purkinje cells and euridendroids.

The molecular layer found towards the periphery consists of two types of cells, basket and stellate, which are interneurons. Purkinje cells are among the most complex neurons and are located at the boundary between the two layers (Fig. 4, 5).

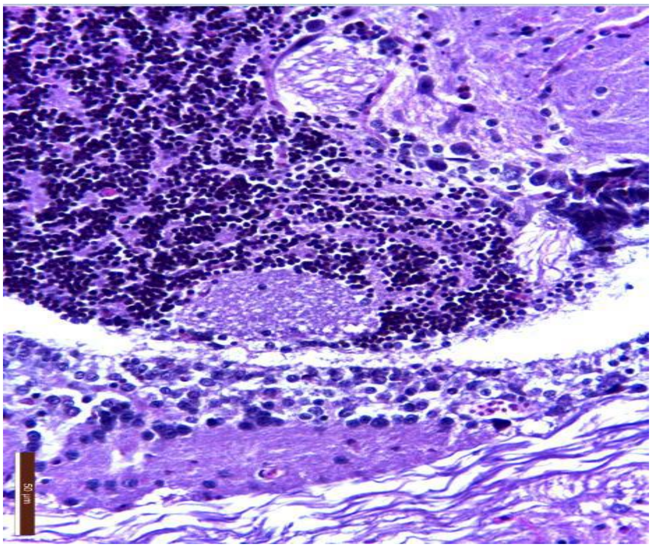

Fig 4- Small, dense granular neurons from the corpus cerebelli.
The central canal is represented by the fourth ventricle - Col. PAS x400

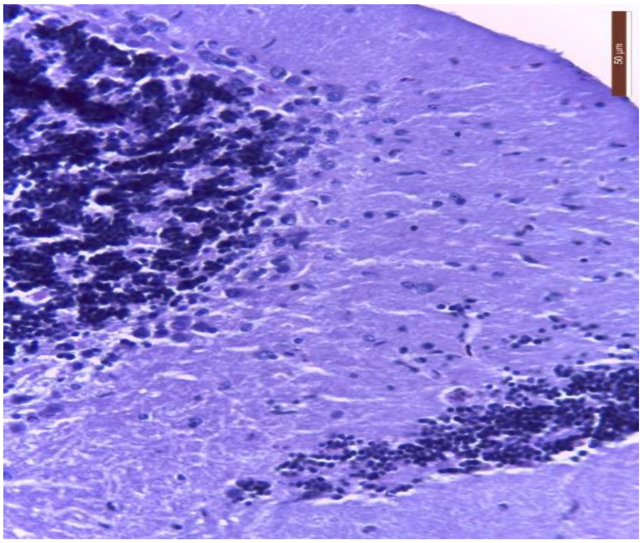

Fig. 5- Cerebellar details- corpus cerebelli. Purkinje neurons and layer of dense, granular neurons.- Col. Blue Alcian x100

In the rostral region, the **myelencephalon** consists mainly of the *medulla oblongata*, as a strain of the brain and the vagus and facial lobes, all of which are connected to the cerebellum at the diencephalon. The central canal as well as the fourth ventricle were found in the middle of the *medulla oblongata*. This region is covered by ependymal cells. The histological details of the *medulla oblongata* highlight oval-shaped neurons and eosinophilic cytoplasm, small glial cells with Nissl corpuscles as well as large circular cells (Fig. 5).

In the spinal cord, the first step toward achieving the diversity observed in adults occurs early in development, with the division of neuronal progenitor cells into distinct domains along the dorsoventral axis in response to signals from local organizing centers. The ventral spinal cord forms four distinct domains, p3, pMN, p1, and p0 (Fig. 7) (Goulding and Lamar, 2000; Jessell, 2000). It is believed that each domain produces neurons that share common features (e.g., axonal trajectories). The next level of complexity appears in each specific area. For example, the p1 domain in mammals produces ipsilateral inhibitory neurons that project ipsilateral with distinct, well-identified functions, such as Ia and Renshaw cells, as well as other ipsilateral inhibitory neurons whose function has not yet been determined (Sapir et al., 2004; Alvarez et al., 2005). Compared to the previous stage of domain formation, the development mechanisms responsible for the complexity resulting from within a domain are less understood.

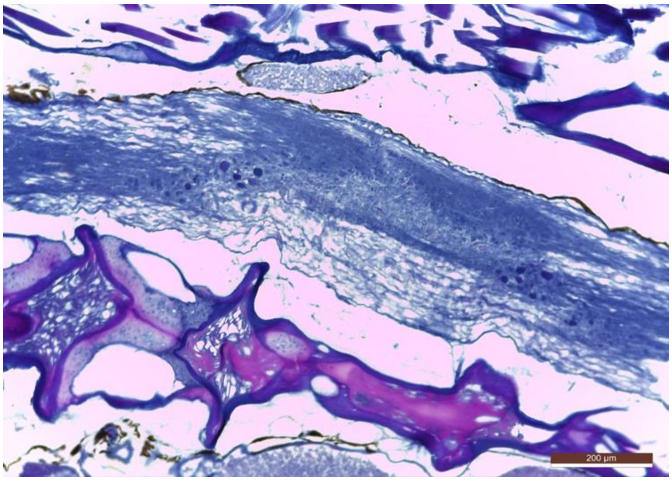

Fig. 6- The medulla oblongata with oval neurons and eosinophilic cytoplasm, small glial cells with Nissl corpuscles as well as large spherical cells. Col PAS-Blue Alcian x100

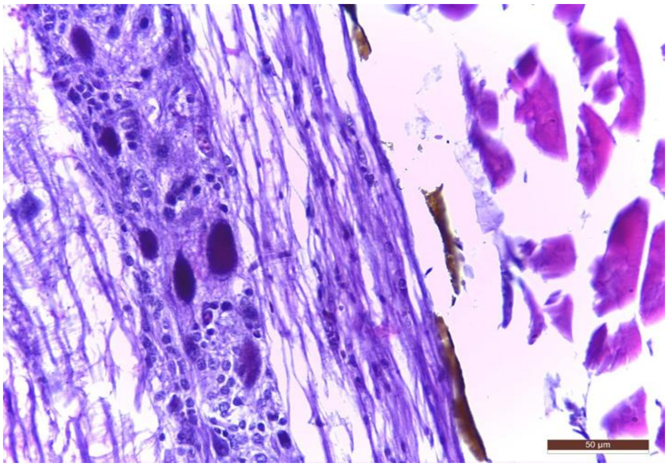

Fig. 7 - The gray matter of the spinal cord is surrounded by the white matter, which contains myelinated nerve fibers. The lumen of the ventricle in the center of the gray matter is lined with ependymal cells. Col. HEA x400

Discussions

Zebrafish is becoming a popular model for studying the structure and function of neural circuits, as it has a variety of advantages over other animal models. Some of these advantages are useful, although not essential, while others allow experiments that are difficult or impossible to perform in other genetic organisms.

Unlike mammals, amphibians and fish do not have a visual cortex (Lázár, 1973; Streidter and Northcutt, 1989). Instead, they have a proportionately larger tectum, which is thought to perform some of the visual processing that the cortex performs in mammals (Nevin et al., 2010; Orger, 2016). The results of this study are in line with the results obtained by Vanegas and col. (1974); Sas and Maler (1986); Meek and Nieuwenhuys (1998) who state that in teleost fish, tectal afferents reach the tectal neuropil, which comprises 7 layers (from outside to inside): the marginal fibrosum layer (MS), which does not receive direct retinal inputs, the opticum layer (SO), fibrosum and superficial griseum layer (SFGS), central stratum griseum (SGC) and central album stratum and periventricular stratum griseum (SAC / SPV). As stated by Miyamura and Nakayasu (2001), and Wullimann (1996), it has been observed that the cerebellum in teleosts is composed of three major parts: the cerebelli valve, the corpus cerebelli and the vestibulolateral lobe with a three-layer structure, and namely a molecular layer, a layer of Purkinje cells and a layer of granular cells with dense neurons, Purkinje neurons being among the most complex neurons. Goulding and Lamar (2000) that in the ventral spinal cord four distinct domains are formed, p3, pMN, p1 and p0 and the histological details of the oblong medulla consist of oval-shaped neurons and eosinophilic cytoplasm, small glial cells with several nuclei, Nissl bodies as well as large spherical cells.

Conclusions

1. The midbrain is the median vesicle of the brain with 3 main components: the cerebral peduncles, the roof and the optic roof. The tectum has seven layers of fibers with different cell composition.

2. The rhombencephalon controls functions and autonomic balance. Cerebelum, a part of the rhombencephalon has three layers of neurons: molecular, Purkinje and granular cells.

3. Histologically, the medulla oblongata is composed of small oval and glial cells.

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