A SIMPLE BRAIN ATLAS OF THE ADULT ZEBRAFISH (DANIO RERIO)

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Abstract
The zebrafish and its brain popularity in scientific research created the necessity of a quick and simple atlas for the examination of the entire central nervous system structure. Sagittal sections of 5 µm were obtained from 10 adult both male and female zebrafish. Luxol Fast Blue-Cresyl Violet staining was performed in serial brain sections from whole adult zebrafish brain for examination of myelin and neuron structure and localization. Neurons can be well established with distinct cell bodies and nuclei in examined cerebral structures from both hemispheres. Cresyl violet is used to stain the neuronal cell bodies and processes which appear in a pink to violet color. Luxol fast blue stain is used to identify myelin in nervous tissue which gets stained in bright blue. The serial brain sections staining with Luxol fast blue-Cresyl Violet gives a quick and complete view of the central nervous system morphology and could be a useful tool in toxicology studies or in the research of neurological and neurodegenerative diseases.
***Key words**: zebrafish, brain, atlas, Luxol Fast Blue-Cresyl Violet*

Introduction

The zebrafish model has proven its advantages for research not only by his shorter body size and lifespan compared to other vertebrate models, but most important by his genomic resemblance to human genome. Thus, many genes involved in human neurological disorders have very well-preserved orthologues in the zebrafish genome, suggesting that the biochemical events underlying the pathogenic mechanisms could be validated in the zebrafish nervous system. In addition, zebrafish show the organization of the central nervous system (CNS) like other vertebrates. Many neural circuits and cell types relevant to the study of human disorders and diseases are preserved in the brain of the zebrafish (Burton, E.A., 2015; Bradford Y. et al., 2017). Also, close correlations have recently been demonstrated between the levels of neurotrophins in various segments of the nervous system and the occurrence of neurodegenerative or neurological diseases in humans (Panula P. et al., 2010; Fontana et al., 2018).

The evolution of research on the CNS in zebrafish has created the need for a more detailed and explicit atlas of the brain of this fish. It was desired to realise a map of the main topographic regions of the brain based on which to easily identify the physiological location of various molecules. This atlas aims to highlight in color the neural populations and nerve pathways in the brain of the adult zebrafish, the most important nuclei and nervous paths of the CNS to facilitate the work of screening for various morphological changes at cellular level, or transcripts of genes or proteins derived from them at molecular level.

Materials and methods

10 healthy adult zebrafish were euthanized by immersion in cold water (4° C). The fixation was performed with 4% paraformaldehyde. Serial cross sections through the fish brain were performed. Samples dehydration was performed by usual method with alcohol series and cleared with xylene. Paraffin cubes were prepared and cut in slices of 5 µm by microtome. Serial sections of the brain (approx. 100 slides/fish) were stained with Luxol Fast Blue-Cresyl Violet and examined in light microscopy. Cresyl violet stained the neuronal cell bodies and processes which appear in a pink to violet color. Luxol fast blue highlights myelin in nervous tissue which gets

stained in bright blue. All areas of the brain were photographed and most significant parts were illustrated. The processing of the images was performed using the Adobe Photoshop program.

Results and discussions

The telencephalon initiates with the paired protuberances called olfactory bulbs that receive the information from the olfactory mucosa thru the olfactory nerve (I) and reach the glomerular layer of the bulbs (fig.1).

The secondary olfactory structures are the lateral and medial olfactory tracts (LOT/MOT) that originate in the mitral cells and connect the olfactory bulbs with the rest of the telencephalon, having implications in sexual (MOT) (fig.2) and feeding behavior (LOT) (fig.3) (Hara T.J., 1992; Kasumyan A. O., 2004).

Each of the olfactory bulb is composed of 4 concentric layers: primary olfactory fiber layer (POF), the most peripheral and rostroventral; glomerular layer (GL); external cellular layer (ECL), containing the large mitral cells; and the internal cellular layer (ICL) (fig.1).

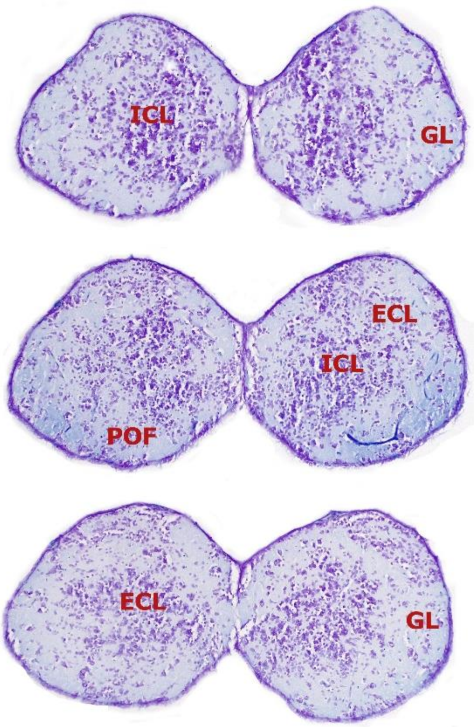

Fig. 1. Serial cross sections of the most rostral telencephalic regions-olfactory bulbs- in adult zebrafish. ECL- external cellular layer of olfactory bulb including mitral cells; ICl- internal cellular layer of olfactory bulb; GL- glomerular layer of olfactory bulb; POF- primary olfactory fiber layer; Luxol Fast Blue-Cresyl Violet X 200.

The main telencephalic body is divided into a dorsal (D) and a ventral (V) telencephalic area, each of them including a dorsal (Dd/Vd), central (Dc/Vc), lateral (Dl/Vl), medial (Dm/Vm) and posterior (Dp/Vp) nucleus (for V) or cell mass (for D) (fig. 3).

Ventral to Dp is located the nucleus taeniae (NT) that, together with Dp, receives part of the secondary olfactory projections. Beside the Vd, other three nuclei line the periventricular area of the V: ventral (Vv), supracommissural (Vs) and postcommissural (Vp) nuclei (fig. 3).

A group of nuclei from the V migrated from the ependymal area of the ventricle and are located mostly periferally. These nuclei are the central/commissural nucleus (Vc) and the lateral nucleus (Vl) (fig.3).

Diencephalon is divided into five main regions located dorsoventrally: epithalamus, dorsal thalamus, ventral thalamus, posterior tuberculum, hyphothalamus and an intermediate region between the telencephalon and diencephalon, the so called area preoptica. The last one includes dorsal and ventral nuclei, most rostral being the anterior parvocellular preoptic nucleus (PPa) continued by the posterior parvocellular preoptic nucleus (PPp) and the suprachiasmatic nucleus (SC), which are located ventrally (fig.4).

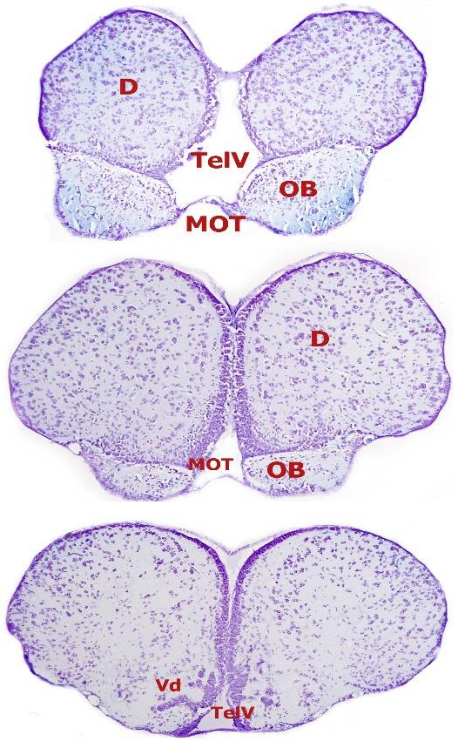

Fig. 2. Serial cross sections of rostral telencephalon in adult zebrafish. D- dorsal telencephalic area; MOT- medial olfactory tract; OB- olfactory bulb; TelV- telencephalic ventricles; Vd- dorsal nucleus of ventral telencephalic area; Luxol Fast Blue-Cresyl Violet X 200.

Dorsally are found the magnocellular preoptic nucleus (PM) and the gigantocellular part of magnocellular preoptic cells (PMg). The preoptic area also contains CRF-expressing neurons that are part of the stress axis and which project onto the anterior pituitary gland (Bally-Cuif, L., & Vernier, P., 2010). Epithalamus is formed by nuclei (dorsal (Had) and ventral (Hav) nuclei of habenula) and two protuberances. The most important structure is the epiphysis, also called the pineal gland, with main roles in the regulation of the circadian cycle and melatonin hormone synthesis.

The preoptic area and the thalamus are some of the main regions of the brain, together with optic tectum, pretectum and accessory optic system, that receive information from the retina (fig. 5).

The largest and most ventrally diencephalic region, hypothalamus, consists of a dorsal, ventral and a caudal zone and also a hypothalamic ventricle, an extension of the diencephalic ventricle (DiV). The ventral and caudal zones form most of the tuberal hypothalamus (TH), while the dorsal region consists mainly of the paired inferior lobes of hypothalamus (IL), into which the DiV prolongs (fig.5; fig.6).

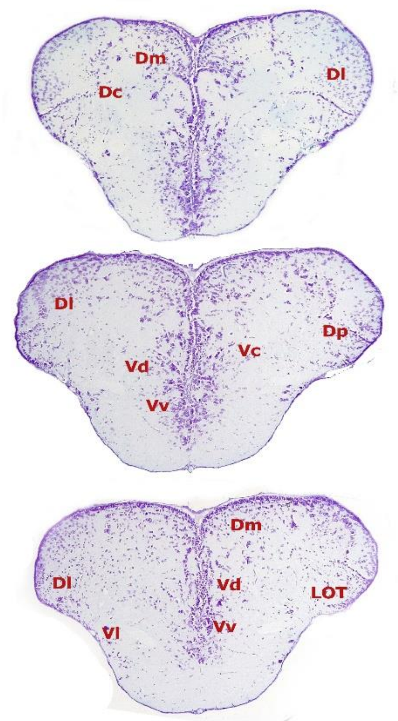

Fig. 3. Serial cross sections of caudal telencephalon in adult zebrafish. Dc- central zone of dorsal telencephalic area; Dm- medial zone of dorsal telencephalic area; Dl- lateral zone of dorsal telencephalic area; Dp- posterior zone of dorsal telencephalic area; LOT- lateral olfactory tract; Vc- central nucleus of ventral telencephalic area; Vd- dorsal nucleus of ventral telencephalic area; Vl- lateral nucleus of ventral telencephalic area; Vv- ventral nucleus of ventral telencephalic area; Luxol Fast Blue-Cresyl Violet X 200.

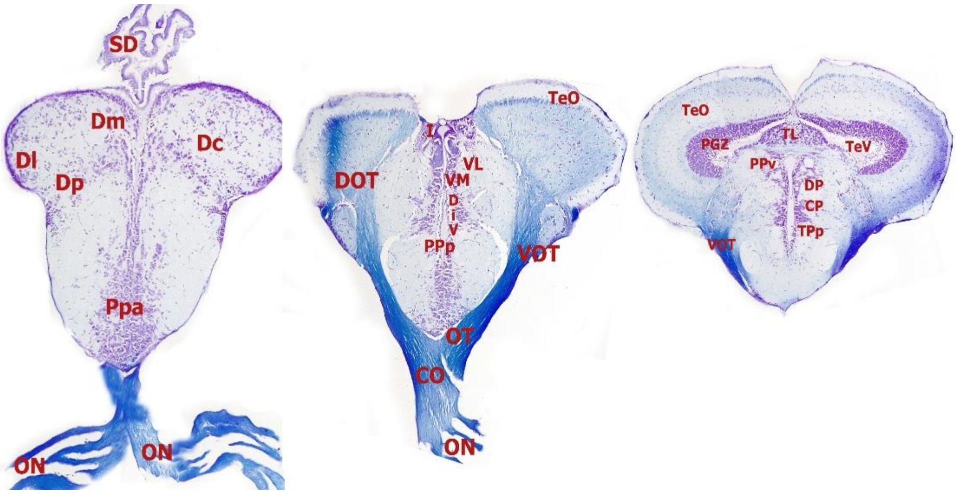

Fig. 4. Serial cross sections of caudal telencephalon, diencephalon and rostral mesencephalon in adult zebrafish. CP- central posterior thalamic nucleus; Dc- central zone of dorsal telencephalic area; DiV- diencephalic ventricle; Dl- lateral zone of dorsal telencephalic area; Dm- medial zone of dorsal telencephalic area; DOT- dorsomedial optic tract; Dp- posterior zone of dorsal telencephalic area; DP- dorsal posterior thalamic nucleus; ON- olfactory nerve; OT- olfactory tract; PGZ-periventricular grey zone of optic tectum; PPa- parvocellular preoptic nucleus, anterior part; PPp- parvocellular preoptic nucleus, posterior part; PPv- periventricular pretectal nucleus, ventral part ; SD- dorsal sac; TeO- tectum opticum; TeV- tectal ventricle; TL- torus longitudinalis; TPp- periventricular nucleus of posterior tuberculum; Val- lateral division of valvula cerebelli; VL- lateral thalamus; VOT- ventrolateral optic tract; VT- ventral thalamus. Luxol Fast Blue-Cresyl Violet X 400.

The hypothalamus projects to the area preoptica, hypophysis and telencephalon and play a role in feeding behavior, reproduction, food intake regulation and aggressive behaviors. Also it was found that it is implicated in the regulation of sleep, heart rate and blood pressure, and the control of body temperature (Bally-Cuif, L., & Vernier, P., 2010).

The mesencephalon has a very large and complex dorsal structure called optic tectum (TeO) and two more ventrally and reduced ones: torus semicircularis (TS) and tegmentum (fig. 5; fig.6). Torus semicircularis receives projections from the telencephalon and consists of a central (TSc) and a ventrolateral nucleus (TSvl), with role in hearing and mechanoreception (fig.5).

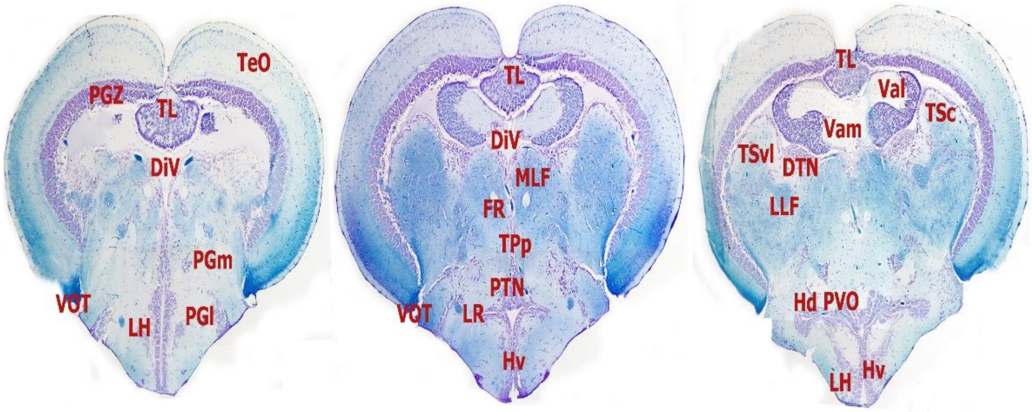

Fig. 5. Serial cross sections of mesencephalon and rostral metencephalon in adult zebrafish. DiV- diencephalic ventricle; DOT- dorsomedial optic tract; DTN- dorsal tegmental nucleus; Hd- dorsal zone of periventricular hypothalamus; FR- fasciculus retroflexus; Hv- ventral zone of periventricular hypothalamus; LH- lateral hypothalamic nucleus; LLF- lateral longitudinal fascicle; LR- lateral recess of diencephalic ventricle; MLF- medial longitudinal fascicle; PGl- lateral preglomerular nucleus; PGm- medial preglomerular nucleus; PGZ-periventricular grey zone of optic tectum; PTN- posterior tuberal nucleus; PVO- paraventricular organ; TeO- optic tectum; TeV- tectal ventricle; TL- torus longitudinalis; TPp- periventricular nucleus of posterior tuberculum; TSc- central nucleus of torus semicircularis; TSvl- ventrolateral nucleus of torus semicircularis; Val- lateral division of valvula cerebelli; Vam- medial division of valvula cerebelli ; VL- lateral thalamus; VOT- ventrolateral optic tract; VT- ventral thalamus. Luxol Fast Blue-Cresyl Violet X 400.

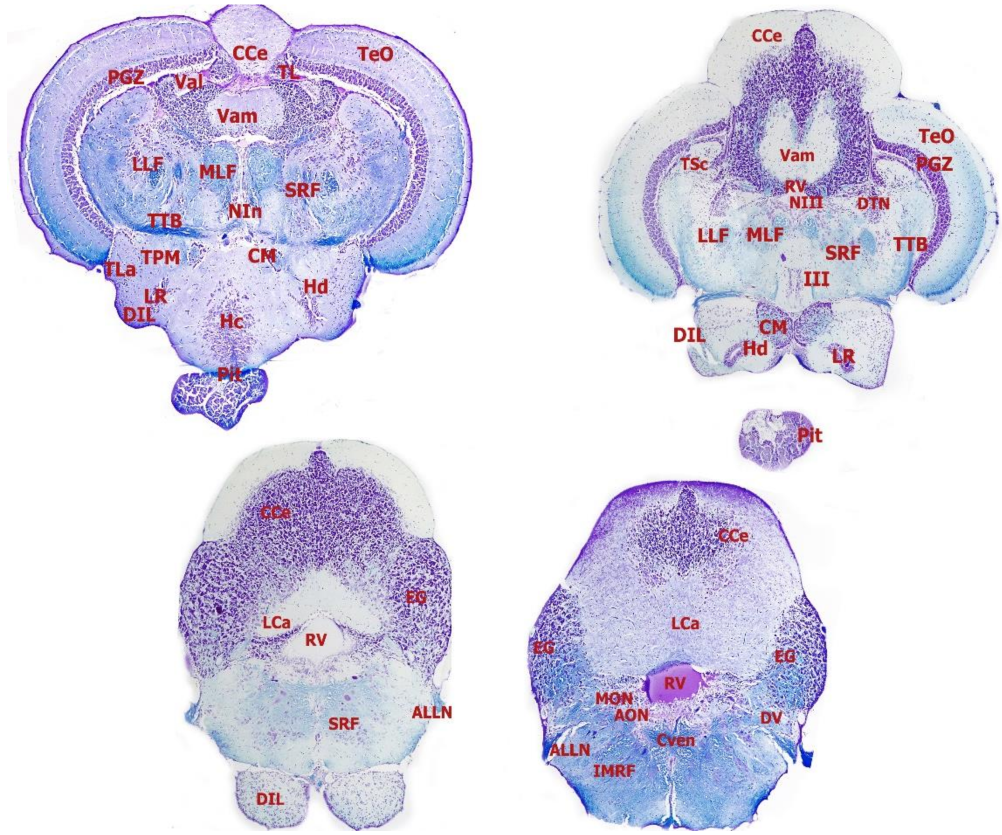

Fig. 6. Serial cross sections of mesencephalon and rhombencephalon in adult zebrafish. ALLN- anterior lateral line nerves; AON- anterior octaval nucleus; CCe- corpus cerebelli; CM- mammillary body; Cven- commissura ventralis rhombencephali; DIL- diffuse nucleus of the inferior lobe; DV- descending trigeminal root; Hc- central zone of periventricular hypothalamus; GC- griseum centrale; Hc- caudal zone of periventricular hypothalamus; Hd- ventral zone of periventricular hypothalamus; IMRF- intermediate reticular formation; LLF- lateral longitudinal fascicle; LCa- lobus caudalis cerebelliLR- lateral recess of diencephalic ventricle; MLF- medial longitudinal fascicle; MON- medial octavolateralis nucleus; NIII- oculomotor nucleus; Nln- nucleus interpeduncularis; ; NLV- nucleus lateralis valvulae; Pit- pituitary; PGZ-periventricular grey zone of optic tectum; RV- rhombencephalic ventricle; SRF- superior reticular formation; TeO- optic tectum; TL- torus longitudinalis; TLa- torus lateralis; TPM- pretecto-mammillary tract; TSc- central nucleus of torus semicircularis; TTB- tecto-bulbar tract; Val- lateral division of valvula cerebelli; Vam- medial division of valvula cerebelli; III- oculomotor nerve. Luxol Fast Blue-Cresyl Violet X 400.

The tegmentum is the most ventral mesencephalic structure and includes cranial nerve nuclei with major role in motor functions (oculomotor-NIII; trochlear-NIV) and parts of the reticular formation (fig.6). It continues with medulla oblongata without a clear demarcation (Wullimann M.F. et al., 1996).

Optic tectum, the most complex layered structure in the zebrafish brain, consisting of neuronal bodies and axons and receives visual information concerning the shape, movement and colour from the retina, thalamus and pretectum. It also integrates inputs from telencephalon, torus semicircularis and reticular formation.

The optic tectum contains three main areas: the superficial and central zones, where the tectal afferents end; the periventricular zone (SPV), where the majority of the tectal cell bodies reside. The outermost layers are stratum marginale (SM) and stratum opticum (SO) that form the superficial zone, followed by stratum fibrosum et griseum superficiale (SFGS), stratum griseum centrale (SGC) and stratum album centrale (SAC) forming the central zone.

The innermost layer, periventricular grey zone of optic tectum (PGZ), is predominantly composed of pyriform neurons, while in the stratum marginale are predominantly found unmyelinated axons. The other layers contain cells scattered among neuronal processes from different sources (Bally-Cuif, L., & Vernier, P., 2010).

The rhombencephalon in zebrafish comprises a rostral division, metencephalon, and a caudal division, myelencephalon, separated only arbitrarily. The main segments of the rhombencephalon are the cerebellum and medulla oblongata (MO).

Medulla oblongata associates with the majority of cranial nerves and their primary motor and sensory nuclei: the trochlear (IV), trigeminal (V), abducens (VI), facial (VII), octaval (VIII), glossopharyngeal (IX) and vagal (X) nerves, as well as the lateral line nerves which includes a component for mechanoreception and electroreception. It also comprises the reticular formation, the raphe nuclei and many ascending or descending fiber tracts (Bally-Cuif, L., & Vernier, P., 2010).

The cerebellum is large and composed of three parts in zebrafish: the vestibulolateralis lobe which includes the medial caudal lobe (LCa) and the paired lateral eminentiae granulares (EO); the corpus cerebelli (CCe), and the valvula cerebelli, which has a medial (Vam) and a lateral subdivision (Val) (Wullimann M.F. et al., 1996).

The cerebellum conserves the classic architecture comprised of a three-layered cortex: the innermost layer is the granular layer, densely packed with granule cells, along with interneurons; Purkinje layer is the middle one and contains the Purkinje cell bodies; the outermost is the molecular layer, which contains the flattened dendritic trees of Purkinje cells (fig.6; fig.7). The main function of the cerebellum is in learning and was found to participate in both spatial and emotional cognition in delay conditioning assays (Yoshida et al., 2004; Rodriguez et al., 2005).

The reticular formation of the rhombencephalic segment is divided into a midline, a medial and a lateral column. The medial column includes the superior, intermediate, and inferior nuclei of the reticular formation, also called superior, intermediate, and inferior reticular formation (SRF/IMRF/IRF) (fig.6; fig.7) (Wullimann M.F. et al., 1996).

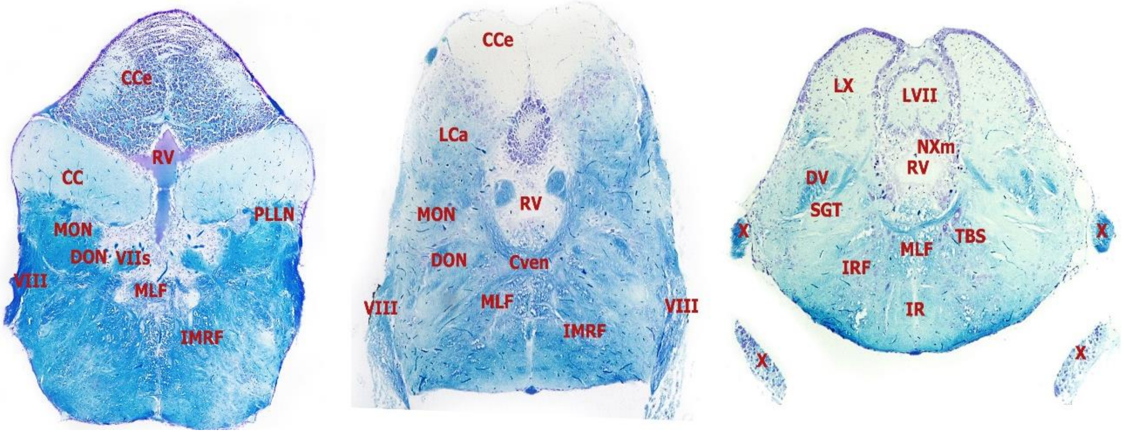

Fig. 7. Serial cross sections of rhombencephalon in adult zebrafish. CC- crista cerebellaris; CCe- corpus cerebelli; Cven- commissura ventralis rhombencephali; DON- descending octaval nucleus; DV- descending trigeminal root; IMRF- intermediate reticular formation; IR- inferior raphe; IRF- inferior reticular formation; LCa- lobus caudalis cerebelli; LVII- lobus facialis; LX- vagal lobe; MLF- medial longitudinal fascicle; MON- medial octavolateralis nucleus; NXm- vagal motor nucleus; PLLN- posterior lateral line nerve; RV- rhombencephalic ventricle; SGT- secondary gustatory tract; TBS- tractus bulbospinalis; III- oculomotor nerve; VIIs- sensory root of the facial nerve; VIII- octaval nerve. Luxol Fast Blue-Cresyl Violet X 400.

The most caudal region of the central nervous system, medulla spinalis or spinal cord, is constituted of the dorsal horn (DH) and of the dorsal root ganglia which extends peripheral axons under the skin to detect a range of mechanical, thermal and chemical stimuli and also of a ventral horn (VH) (fig.8).

The descending spinal projections originate in all divisions of the reticular formation, in the inferior raphe region (but not in the superior raphe), in vestibular and sensory trigeminal nuclei (fig. 7). Furthermore, the nucleus of the medial longitudinal fascicle is an ancestral craniate premotor system descending to medullary and spinal levels (fig. 7; fig.8) (Bally-Cuif, L., & Vernier, P., 2010).

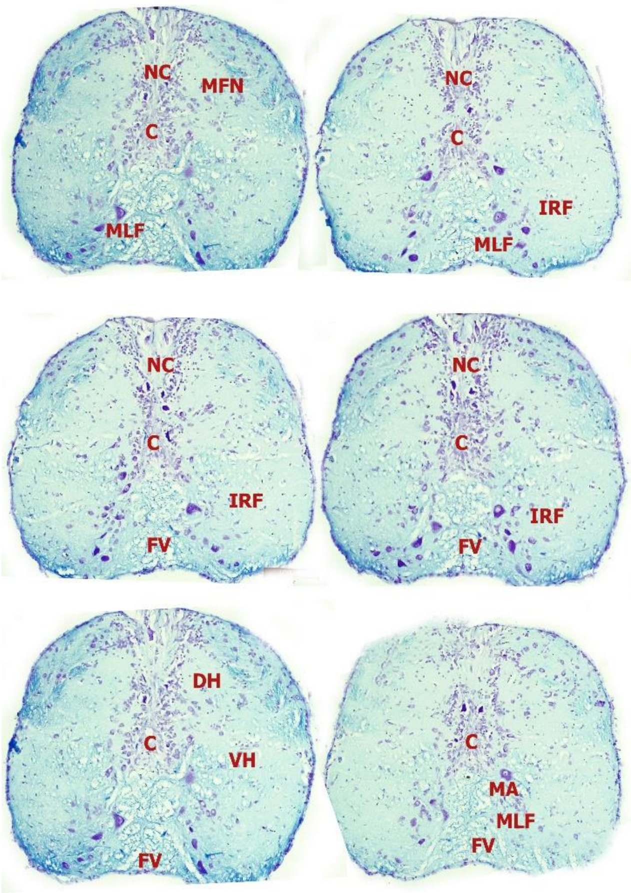

Fig. 8. Serial cross sections of medulla spinalis in adult zebrafish. C- central canal; DH- dorsal horn; FV- funiculus ventralis; IRF- inferior reticular formation; MA- Mauthner axon; MFN- medial funicular nucleus; MLF- medial longitudinal fascicle; NC- commissural nucleus of Cajal; VH- ventral horn. Luxol Fast Blue-Cresyl Violet X 400

Conclusions

Zebrafish shows the basic organization of the nervous system like other vertebrates. Many neural circuits and cell types relevant to the study of human disease are preserved in the central nervous system of this fish (Stewart A. et al., 2014).

The complexity of the anatomical and histological structure of the zebrafish brain is requiring complex research and understanding for the proper and useful manipulation of this fish as a reference model organism for neurogenesis, neuroplasticity, but also neurodegenerative disorders or toxicological mechanisms in other vertebrates, most inquired, in human.

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