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Interaction of Atoms with Grain Surfaces in Steel: Periodic Dependence of Binding Energy on Atomic Number and Influence on Wear Resistance*

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ABSTRACT

The data of our investigations contribute to understanding of cellular mechanisms of the teleost fishes CNS forming in postembryonic development. The revealed peculiarities of structural and neurochemical organization and description of basic histogenetic processes (proliferation, migration and neuronal cell differentiation) during the brain forming in fish, which have signs of fetal organization, widen the existing knowledge about histogenesis of these structures in postembryonic development. It seems conceivable, that during postembrional development in teleost fishes some neurotransmitters and gaseous mediators (NO and H_2S) act as factors, which initiate and regulate the cellular and the tissues processes of genetic program during the brain development. Materials of this investigation define a new experimental model for studying of postembrional neurogenesis processes.

Keywords: Teleostei; Postnatal Neurogenesis; Proliferative Cell Nuclear Antigen (PCNA); Neurotransmitter Signaling; Migration; Tyrosine Hydroxylase; GABA; Development; Pax6; NADPH-diaphorase; Nitric Oxide; Hydrogen Sulfide; Proliferation.

1. Introduction

The fishes brain have unique peculiarity among the vertebrates. It grows with organism during all their life. Thereby, the fishes are an attractive animal model for investigation of the embryonal and postembryonal central nervous system (CNS) development and different impacts on these processes. It has been shown, that in the adult vertebrate brain the system of cambial elements is preserved. Their activity allows to increase the neurons and glia population during all postnatal period [1]. Currently, the mechanisms of pre- and postnatal neurogenesis in fishes, which have long standing fetal state, are unknown. In recent years, considerable attention of different neuroscientists was attracted to the gaseous mediators (NO and H2S) participation in the brain work. Their presence was revealed in the brain of the different vertebrates groups - from cyclostomes to mammals. These researches acquire special meaning in connection with a new data about morphogenetic role of classical and gaseous mediators in the vertebrates CNS development [2].

Pacific salmons were the main objects of our investigation. They present an ancient group of vertebrates and the oldest branch of actinopterygian fishes. Today, the available literature data, concerning information about salmon brain development, interrelations between embryonic and definitive parts in their structure, pre- and postembryonic neurogenesis, organization and establishment of neurotransmitter and modulated brain system are very limited.

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2. Participation of Classical Neurotransmiters in Postembrionic Neurogenesis in the Salmon Brain

The present study allows to suggest, that in the pacific salmon brain exist two forms of intercellular communication throughout different age periods of postembryonic development. The first form occurs on the early stages of postembryonic development and present intercellular interaction, which is realized through paracrine mechanism, during which the cells do not have full-fledged outgrowths (dendrites and axon) and synaptic structure yet. However, such a low differentiated cells by this time are capable to express a specific synthesises: some neurotransmitters and enzymes, synthesizing them, gasotransmitters, transcriptional factors etc (**Figure 1A, C-D**). We believe, that most of signals, which are synthesized during this period, participate in regulation of neuron-targets differentiation and specific phenotype expression, as morphogenetic factors, what corresponds to Ugrumov′s conception [2], concerning the mammalian brain development during embryonic ontogenesis. It is known, that a neuron begins to release typical signal molecules shortly after their formation from cells–progenitors and long before the formation of interneuronal connections occurs. A large proportion of all signaling molecules are involved in autocrine and paracrine regulation of differentiation of neurons–targets and they function as morphogenetic and transcription factors. In mammals the duration of the signal molecules action is limited to certain periods of ontogeny, when processes of differentiation of neurons–targets and the expression of specific phenotype are modulated by a long-term morphogenetic influence. In adult fishes a postnatal neuro- and gliogenesis still occurs in a periventricular area. Already on early postembryonic morphogenetic stages, two systems of neurochemical signaling (dopaminergic and GABA-ergic) exist simultaneously in the *Oncorhynchus masou* brain. These systems exert paracrine and possibly autocrine impacts towards the cell-targets before synaptic contacts shaping occurs and the neurotransmission of specific interneuronal connections begins. The maximal concentration of D_1 dopamine receptors in the eel brain [3] was revealed in the periventricular brain areas (morphogenetic fields), where neurogenesis is preserved during the entire life of the animal. Therefore, the cells, which are located in proliferating brain regions, constitute the targets to dopamine regulation. The zones, which synthesize dopamine and GABA in these brain regions, are localized in a territory of major vascular plexuses (in the forebrain and medulla oblongata). The neurotransmitters (dopamine and GABA) may be released into the portal system blood flow and further into the general circulation, impacting endocrine influence on peripheral organs [4]. Our most recent findings suggest, that dopamine and GABA, in undifferentiated cells of periventricular and subventricular hypophysotropic areas of different age groups of *O. masou*, constitute morphogenetic factors (inductors of the development) themselves.

Along with the paracrine signaling form mentioned, in the salmons during ontogenesis the specific activation systems of forebrain and system of distant (synaptic) intercellular signaling are developed. The nuclei of preglomerular complex constitute the source of these directed connections. The preglomerular complex in fishes is considered a polymodal sensory center of diencephalon, realizing transmission of visual, mechanosensory, octavo-lateral, and acoustic information to the dorsal and ventral regions of the telencephalon [5]. Information on origin, pathways of migration, and phenotype of cells, their lifespan, and functional integration in the course of postembryonic neurogenesis remains at present rather limited. In the brain of a few nonmammalian vertebrates the volume of the sensory projective zones is assumed to increase during the entire life of the animal. This is provided at the expense of proliferation of neural stem cells, located in specific regions, neurogenic niches [6]. This is related with necessity of adaptation of the CNS of such animals to increase in the body dimensions and, respectively, increase in the volume of primary sensory signaling. In agreement with this assumption, we suggest that dopamine, GABA- and NO-ergic systems participate in regulation of basic histogenetic processes: cells migration and differentiation of neuro- and gliospecific lines, because preglomerular nuclei contain morphologically and neurochemically heterogeneous cell populations [7], which represent different ontogenetic stages of main cellular types. The cells formed in proliferative (PCNA-contained) diencephalic zones migrate to preglomerular area, where their further differentiation and growth take place. The presence of D_1 and D_2 dopamine receptors [6, 8] and benzodiazepine receptors B type [9] in these nuclei in the teleost fishes brain confirms these idea. The period of the blood-brain barrier shaping, during the first year of life [10] in the salmon brain may be considered as a critical stage of the paracrine interrelations predominance in the salmon brain. The specific connections shaping, the neuronal processes development and synaptogenesis are occurring in the next ontogenesis period.

We consider, that cells maturing in different parts of the salmon brain occur heterochronycally in many respects. In the caudal brain parts the reticulospinal cells, *raphe nucleus*, V, VII, IX and X nuclei of craniocerebral nerves cells acquire features of phenotypical specialization earlier than in the forebrain structures. In the medullar and spinal cord neurons of one- and two-year-old young cherry salmon *O. masou* full-fledged dendrites and axons are revealed, but their processes have «growth cones», what present the sign of the continued growth and development of these structures in postembryonic period and of their further differentiation. In a three-year-old salmon *O. masou* large differentiated cells, which have expressed TH, GABA and parvalbumin in a spinal cord column motoneurons, nuclei of craniocerebellar nerves, reticulospinal cells and diencephalic nuclei were revealed [11, 12].

Recently the participation of radial glia in postembryonic neurogenesis in a kind of asymmetric mitosis has been shown. One cell remain in periventricular area and have rounded shape, another have a long process, which later is pulled in using somal translocation [13]. The presence of TH- and GABA-ir cells in the territory of the PCNA-ir proliferative zones in one- and two-year-old young cherry salmon *O. masou* and neuromeric structure of diencephalic and medullar brain parts marking, undoubtedly show, that dopamine- and GABA-ergic signalling participate in postembryonic neurogenesis of the *O. masou* brain.

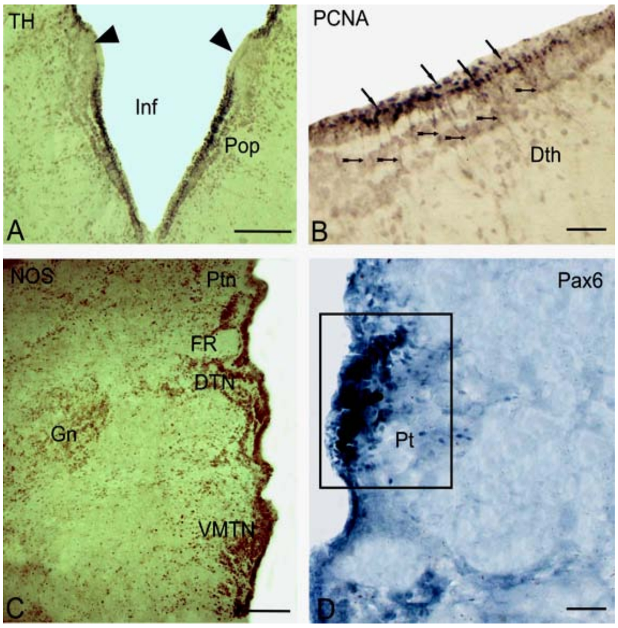

Figure 1. A - immunolocalisation of tyrosine hydroxilase (TH) in parvocellular preoptic nucleus (Pop), B - proliferative nuclear antigen (PCNA) in dorsal thalamus (DTh), C - neuronal nitric oxide synthase (NOS) in pretectal (Ptn), dorsal (DTN), ventro-medial (VMTN) thalamic nuclei, D - transcriptional factor Pax6 in periventricular diencephalon 6-month-old *Oncorhynchus masou*. Immunonegative border of dorsal neuromers on A, delineated by a triangle, the cluster of immunopositive cells on D, delineated by rectangle. Inf – infundibulum, FR – fasciculus retroflexus, Pt – pretectum. Scale: A, C – 100 μm, B, D – 50 μm.

Along with classical neuromediators systems, immunolocalisation of transcriptional factor Pax6 were studied by us. Pax6 is a marker for progenitor cells; the labeling of Pax6 adequately reflects the neuromeric structure of the salmon brain in the different age groups. The results of Pax6 labeling of the salmon brain have shown that this marker is expressed in early youngsters, in the age of 3 and 6 months, as well as in one-year old and adult animals. In one-year-old salmon, we have observed a specific labeling of periventricularly localized cells, creating clusters and domens (Fig. 1D). The investigation of the late-age stages of the salmon has revealed specific accumulations of cells, owing long radially oriented outgrowths, the cells bodies, localized near the brain ventricle lumen or along immunopositive fibres [12].

The PCNA labeling in 1-year-old and salmon adults have shown the presence of a vast population of proliferating cells in periventricular areas of the diencephalon and central grey layer of the medulla (**Figure 1B**). Moreover, on the level of the forebrain distribution of TH-, GABA-, and Pax6-ir cells have labeled a neuromeric construction of the brain; this is confirmed by PCNA labeling of proliferative zones. On the border between the dorsal prosomers P2-P3, we have not detected the immunopositive labeling with TH, GABA, Pax6 and PCNA (**Figure 1A**) In the diencephalon localization of proliferating PCNA-immunogenic zones have corresponded to the prosomeric construction of the forebrain. Thus, we have revealed several active PCNA-ir zones of proliferation, including TH, GABA and NADPH-d (**Figure 2A, B**) expressing cells.

The Pax6 expression was revealed in the glomerular and preglomerular nuclei, what indicate the morphogenetic processes course on a territory of this major sensory centre during the salmon postembryonal development. In glomerular nucleus the Pax6 immunolocalization was revealed in the defined cells populations, which, by our opinion; corresponds to neuroanatomical zones, where differentiation of neurons, passing different types of sensory signaling, take place. We suggest that Pax6 participate in the brain structure regionalization in postembryonic period too. The Pax6 expression in the brain of different salmon age groups indicate, that neurodetermination and migration of cells, occurred in proliferative zones in these ages periods, are regulated by transcriptional factor itself [12]. We hypothesize, that the anterior and medial preglomerular and glomerular nuclei of the salmon constitute the zones of postembryonic morphogenesis, in which postmitotic neuroblasts are participating in formation of a definitive structure of this main sensory center and migrate from cerebral zones, owing primary proliferation.

3. Participation of Gasotransmitters in Postembrionic Neurogenesis in the Fishes Brain

In contrast to mammals, the fishes brain has high neuronal plasticity and is capable to produce new cells during the entire life of the animal [1]. The results of our investigation indicate the presence of nNOS and NADPH-d activity both in neurons and glial cells in the *O. masou* brain. It is probable, that NO in these cells participate in the paracrine control of postembryonic neurogenesis and is functioning as morphogenetic factors (inductors of the development); the similar situation was demonstrated in the mammalian brain [14]. The involvement of NO in postnatal neurogenesis was found in different vertebrates [15]. Two main neurogenesis sites have been identified in the adult mammalian brain. They include the subventricular zone (SVZ), and the subgranular zone (SGZ) of dentate gyrus (DG). The process of neurogenesis is composed of three main steps, which include precursor proliferation, migration; differentiation, integration and survival. It has been demonstrated, that SVZ of mammals is surrounded by nNOS positive neurons [16], and cells expressing nNOS also have been identified in neuronal precursors in DG [17]. These findings suggest nNOS might take part in neurogenesis regulation.

The results of our study allows to suggest, that NO in the *O. masou* salmon medulla periventricular area, which contains PCNA-ir proliferating cells too, can act in different age periods as a regulator of adult neurogenesis, what confirms data on mammals.

Endogenously H_2S is synthesized from L-cysteine by pyridoxal-5'-phosphate-dependent enzymes, cystathionine β-synthase (CBS), and cystathionine γ-lyase (CSE), which are expressed in many tissues. The analysis of localization of CBS, which is an immunohistochemical marker of H_2S in the brain of bony fishes, has been never made before. We found that, in the salmon *O. masou*, CBS labels neurons of the reticular formation, vessels, neurons of the ventral spinal column, and climbing fibers in the cerebellum [18].

In carp *Cyprinus carpio*, the periventricular area of the medulla oblongata and ventral and lateral areas of the cerebellum have contained strongly CBS-stained cells without any outgrowths (**Figure 2C, D**). These CBS-positive cells were found in periventricular zone, corresponding to the area of primary proliferation [18]. Hence, it is logical to hypothesize that H_2S may also work as a regulator of postnatal neurogenesis in the carp brain.

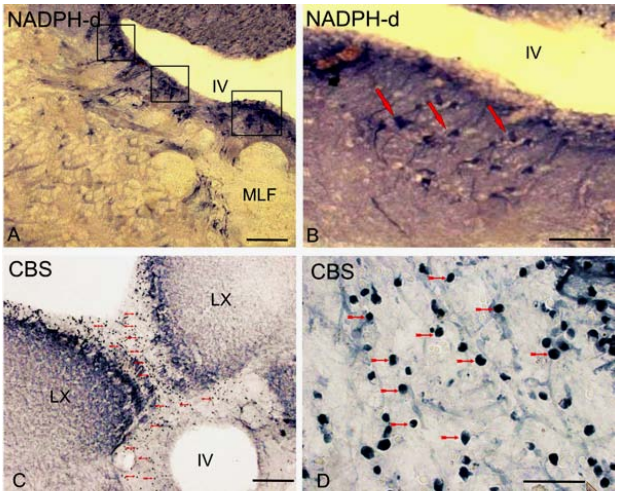

Figure 2. A – clusters of NADPH-d-producing cells (delineated by rectangles) in periventricular area of medulla oblongata of *Oncorhynchus masou;* on B in a large magnification. C - cystathionine β-synthase (CBS) producing cells (red arrows) in periventricular area of *Cyprinus carpio* brain, on D in a large magnification. LX – lobus of vagal nerve, IV – forth ventricle, MLF – medial longitudinal fascicle. Scale: A, C – 200 μm, B, D – 50 μm.

In cyprinoids a periventricular area is free of NADPH/nNOS activity. It seems, that H_2S may function as a signal molecule in a periventricular area of carp. The results of a study performed allows to suggest, that NO in the salmon medulla periventricular area can act as a regulator of adult neurogenesis, while in a periventricular area of medulla oblongata and ventral and lateral zones of cerebellum of capr we have found cells, owing strong CBS immunolabeling (**Figure 2D**). It seems, that NO and H_2S may function as a signal molecules in periventricular area and they can act as a regulators of the adult neurogenesis.

4. Conclusion

The data provided by this study add to our general understanding, that peculiarities of distribution of classical neuromediators (GABA, catecholamines) and gasotransmitters (NO and H_2S) are directly connected with ability of the fishes brain to grow during the animal entire life. We suggest, that some classical neuromediators (GABA, catecholamenes) and gasotransmitters (NO and H_2S) not only regulate functional activity of neurons and modulate synaptic transmission in mature neural networks, but also are regarded as inductors of the fishes brain development (morphogenetic factors) in postembryonic ontogenesis. This confirmation is proved by finding of the phenotypically immature elements, expressing the above mentioned molecules in proliferating brain areas, in the three-year-old salmon brain, and of elements, which owe morphology of radial glia. The presence of enzymes, synthesizing gasotransmitters in the brain areas, which are expressing PCNA, have proved their participation in regulation of postembryonic neurogenesis.

In the fishes, which preserve fetal state during long time (salmon and carp), such markers as NO and H_2S in periventricular proliferative areas may present in different ratios. This is consistent with the hypothesis that in functionally similar complexes in animals the different signal transduction systems may be involved. In contrast to widespread neurogenetic model *D. rerio*, the development of the salmon nervous system occur during long time. As it follows from our data, the development of different CNS structures in the *O. masou* brain is characterized by evident heterochrony, so the cells of caudal brain regions gain features of phenotypical specialization earlier than in the forebrain structures. We suggest that the brain of these animals during a long time preserves the signs of fetal organization and low differentiated cells presence confirms this hypothesis.

The data presented in this study open a new trend in investigation of cellular mechanisms of shaping in structural organization in the postembryonic fishes brain and in examination of morpho-functional manifestations concerning histogenetic processes in different periods of postembrionic ontogenesis. The new priority data received are connected with development of nervous tissue in the pacific salmon brain and with dynamic of the brain shaping and distribution of classical neurotransmitters and gaseous mediators in a context of incessant postembryonic neurogenesis.

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