ECOLOGY

Morphocytochemical Characteristics of Cerebellar Neuronal Populations in Fishes with Various Ambulatory Activities

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The cytoplasmic distribution of basophilic substance and the number of chromatin granules in nuclei of cerebellar neurons were studied. Neuronal proteins were assayed in the molecular, ganglionic, and granular cerebellar layers in fishes of various ecological and morphological groups. A quantitative analysis of Nissl bodies and chromatin granules revealed a polymorphism of neuronal populations. Protein concentrations per body volume of stellate and Purkinje cells in pelagic fishes were higher than in benthophages by 7.9% and 12.3%, respectively. However, the content of protein substances in granular cells of pelagic fishes was 8.9% lower than in those cells of benthophages. Tinctorial heterogeneity of cell populations and peculiarities of the distribution of protein substances in various neurons reflect specific features of structural and functional organization of the cerebellum in fishes with different ambulatory activities.

Key Words: *fish cerebellum; neuronal populations; chromophilia; proteins*

Ecological morphology of the brain, a new branch of evolutional neuromorphology, evaluates the role of exogenous factors, environment, and lifestyle in the formation of specific features of sense organs and related brain structures [1,11,13].

The degree and diversity of ambulatory activity result in enlargement and more complex organization of the central nervous system [7,14]. The cerebellum playing an essential role in the development of the coordination system is responsible for adequate adaptation to environmental conditions. However, histo- and neurochemical peculiarities of cerebellum of aquatic vertebrates (especially lower animals) received little attention. Only the brain stem, cortex, and subcortical ganglia of mammalian brain were studied in this aspect [5,9].

A comparison of morphocytochemical peculiarities of cerebellar neuronal populations in fishes of various ecological and morphological groups would allow us to study adaptation of these animals to environmental conditions and, therefore, to evaluate their "standard". Estimation of this standard at all levels of organization of living matter is important due to biotic and abiotic effects on the body and the influence of anthropogenic factors on the environment.

Here we studied the distribution of basophilic substance, chromatin granules, and proteins in cerebellar neuronal populations and determined the functional activity of neuronal cells. Specific features of these brain neurons associated with particular environmental conditions and various ambulatory activities of fishes were analyzed.

MATERIALS AND METHODS

Two bony fish strains (16 animals weighing 350-450 g) from various ecological and morphological groups were studied: pikes (*Esox lucius*), pelagic animals

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characterized by high ambulatory activity and living in natural freshwater ponds and carps (*Cyprinus carpio*), benthophage animals characterized by low ambulatory activity and living in artificial lakes.

The cerebellum was fixed in Carnoy fluid and embedded in paraffin. Frontal slices (5-7 μ thick) were stained with thionin by the Nissl method. At the level of neuronal populations, a quantitative analysis of neurons was performed by the degree of cytoplasmic chromophilia [9]. The numbers of normo-, hypo-, and hyperchromatic neurons with normal structures and totally hyperchromatic, wrinkled, and neurons with marked chromatolysis were estimated. The first 3 types of neurons were considered as normal neurons maintaining relative stability of biological processes in cell populations. Due to structural peculiarities of granular layer neurons, they were analyzed by the distribution, number, and size of chromatin granules in the nuclei [2].

Dry weights and concentrations of dense substances presented by water-insoluble proteins in the neuron cytoplasm and nuclei were estimated by interferential microscopy on 5-7-μ unstained preparations using a BINAM-L-212 microscope at 535 nm. The minimum and maximum axes of ellipses fitting into the outlines of cells and nuclei were measured using a MOV-1×15 screw micrometer. The areas of profile fields were calculated. Only neurons with preserved structure and nucleolus within the cross-section area were examined. We studied 150 cells of the same population. Protein concentrations in the cytoplasm and nucleus, phase shift values, and cross-section areas of cell structures were entered into corresponding formulas to calculate dry weight of dense substances [4,10]. Results were analyzed by Student's *t* test.

RESULTS

In pikes and carps, normochromatic neurons were prevalent among stellate (SC) and Purkinje cells (PC) of the molecular and ganglionic layers, respectively. Hyperchromatic (dark) and hypochromatic (light) neurons were less abundant. The percentage of hyperchromatic cells in the cerebellar molecular layer in pikes was higher than in carps by 55.8%. However, the percentage of hypochromatic neurons in the population of SC in carps was higher by 82.7%. PC population of the ganglionic layer was characterized by a similar ratio of neurons. Normochromatic neurons were prevalent, and dark cells were more typical than light cells. The presence of solitary totally hyperchromatic neurons was characteristic of the cerebellar ganglionic layer in carps (Table 1).

Tinctorial heterogeneity of cell populations reflects morphological manifestations of neuronal activity and correlates with their metabolism [6,8,11,13]. The presence of hyperchromatic neurons is normally associated with reserve capacities of neuronal populations. Hypochromatism is related to metabolic activity of the population and indicates intense synthesis and transport of various products from the perikarya. Specific features of tinctorial neuronal heterogeneity in molecular and ganglionic layers can be attributed to different activities of cerebellar neurons in pelagic and benthophage fishes living under different conditions. Our data on the ratio of cells with various degrees of cytoplasmic chromophilia can be considered as the "standard" for these populations of cerebellar molecular and ganglionic layers in fishes differing in their environmental conditions, nutrition, and ambulatory activities.

In granular cell populations, neurons containing 4-6 chromatin lumps were prevalent (77.8% and 74.2% in pikes and carps, respectively). However, cells with 2 chromatin lumps in the nuclei prevailed in the cerebellar ganglionic layer in pikes. This was probably due to specific features of the granular cell populations in these animals [2].

The size of cells and the contents of proteins in the cytoplasm and nuclei of cerebellar neurons in pikes were lower than in carps (Fig. 1, *a*). More considerable differences in the contents of protein substances were found in the cytoplasm of granular (by 39.4%) and molecular (by 14.8%) layer cells. However, the concentration of water-insoluble proteins in the cyto-

TABLE 1. Cell Distribution by Cytoplasmic Chromophilia in Neuronal Populations of Cerebellar Molecular and Ganglionic Layers (*M*±*m*)

Cell types	Molecular layer		Ganglionic layer	
	pikes	carps	pikes	carps
Normochromatic	75.1±1.9	65.6±4.0	68.4±4.1	60.9±5.14
Hypochromatic	3.6±0.9	20.8±3.5**	9.7±2.3	12.6±1.4
Hyperchromatic	21.2±1.6	13.6±2.2*	21.9±3.1	24.7±4.1
Totally hyperchromatic	0.1±0.06	—	—	1.8±1.0

Note. *$p<0.05$ and **$p<0.001$ compared with pikes.

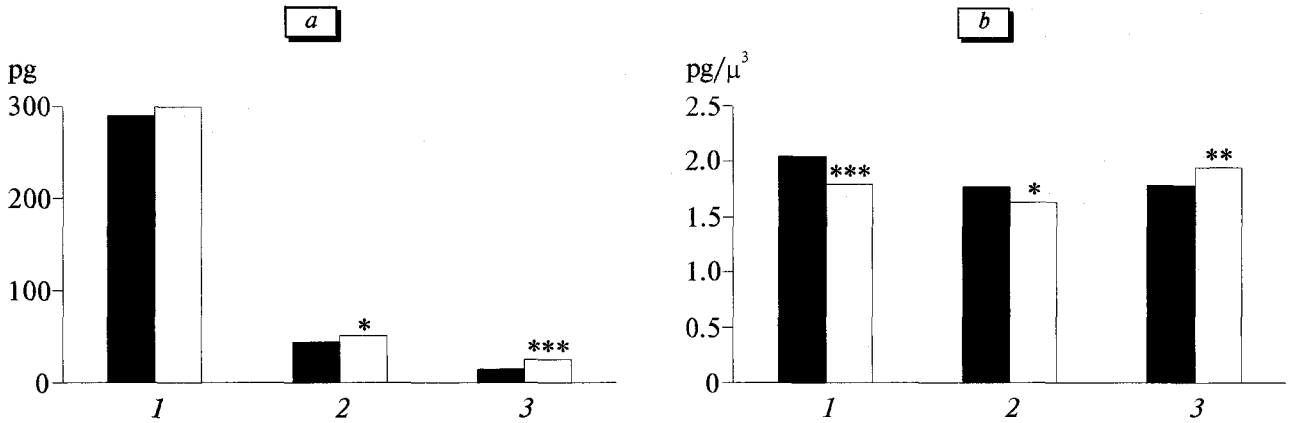

Fig. 1. Content (*a*) and volume concentration (*b*) of proteins in the cytoplasm of cerebellar neurons in bony fishes: pikes (dark bars) and carps (light bars): neurons of ganglionic (*1*), molecular (*2*), and granular layers (*3*). *$p<0.05$, ***$p<0.01$, and **$p<0.001$ compared with pikes.

plasm of molecular and ganglionic layer cells was higher in pikes (by 12.3% and 7.9%, respectively). This concentration in the cytoplasm of granular layer cells was 8.9% higher in carps (Fig. 1, *b*). Protein concentrations in the nucleus were similar in pikes and carps: 0.95 and 0.88 pg/μ³ in SC, 1.07 and 1.04 pg/μ³ in PC, and 1.12 and 1.08 pg/μ³ in the granular layer, respectively. A comparison of proteins in functionally different cerebellar neurons of pikes and carps showed that the content of structured proteins was maximum in PC (the only cerebellar efferent elements characterized by the greatest areas of the cytoplasm and nuclei) and minimum in associative SC of the molecular layer. However, the highest concentrations of dense substances were revealed in the cytoplasm of ganglionic and granular layer cells in pikes and carps, respectively. The content of proteins was higher in nuclei of granular layer cells. The concentration of proteins reflects functional activity of nervous cells [3,5,10]. Similarly directed shifts in the concentrations of dense substances in the cytoplasm and nuclei (in our experiments this was typical of all neuronal populations) indicate stable changes in the protein pool of cerebellar neurons.

Thus, tinctorial heterogeneity of cell populations of the molecular and ganglionic layers, specificity of chromatin organization in the granular layer, and variations of the protein pool in different neuronal populations typical of high-activity pelagic fishes and low-activity benthophage fishes were demonstrated. Therefore, these peculiarities reflect idioadaptation of animals at the population and cellular levels and structural and functional features of the cerebellum in these fishes.

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