Morphocytochemical Assay of Neuronal Populations in Cerebellar Nuclei in Birds and Mammals of Some Ecological and Morphological Groups

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We performed a quantitative morphocytochemical study of neuronal populations in the lateral cerebellar nucleus in birds and mammals of some ecological and morphological groups. Morphological parameters of neuronal density, linear values, and structural nuclear-cytoplasmic index of cells were compared. Specific features of neuronal proteins in cerebellar nuclei were revealed. We revealed objective criteria for adaptive capacities at the level of cell populations in the lateral cerebellar nuclei of animals adapted to various environmental conditions. Our results extend the notion about morphological characteristics of synanthropic birds and rodents that carry infections and invasions hazardous to humans.

Key Words: *birds; mammals; cerebellar nuclei; neuronal populations; density of distribution; protein pool*

Evolution of birds and mammals developed from ancient reptiles of various branches proceeded via different adaptive processes. Some animals adapted to life in air, while others lived on the ground. However, both groups of animals have a well-developed brain and are characterized by complex organization of the forebrain and cerebellum that regulate and coordinate a variety of movements [1,5,11]. Published data show that the lateral cerebellar nucleus in birds corresponds to the dorsal intermediate and ventrolateral lateral nucleus in mammals [1,9-12]. The functional role of the cerebellar nuclei as structures responsible for the regulation and reprogramming of locomotor activity in animals is related to structural and chemical heterogeneity of cell populations [4,8]. A quantitative morphological study of cell populations in subcortical structures should be performed to evaluate the role of cerebellar nuclei in the mechanisms of adaptation of animals living under various environmental conditions and carrying serious infections and invasions.

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Here we evaluated and compared quantitative morphocytochemical characteristics of cell populations in subcortical cerebellar nuclei of birds and mammals of some ecological and morphological groups.

MATERIALS AND METHODS

Experiments were performed on adult animals of 4 species belonging to various ecological and morphological groups. Pigeons (*Columba livia L.*, 275±17 g) inhabit closed landscape areas, are characterized by low locomotor activity and synanthropy, and harbor ornithosis to provide a potential human risk. Sparrows (*Passer domesticus*, 23.0±0.9 g) display high locomotor activity and spent a lot of time in flight. Squirrels (*Sciurus vulgaris*, 406±16 g) are gliding mammals with high ambulatory activity. Gray rats (*Rattus norvegicus*, 242±14 g) inhabit burrows, adapt to various environmental conditions, live close to humans, and carry infectious agents causing about 20 diseases, including pestilence, salmonellosis, pseudotuberculosis, and trichinosis [6].

The animals were decapitated under ether anesthesia. The cerebellum was fixed in Carnoy fluid and

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embedded in paraffin. Sections (6-7 μ thick) were prepared. We examined medium-sized neurons (MSN) in the central part of lateral cerebellar nuclei [4,8].

Quantitative morphometry of neuronal populations was performed on preparations stained with thionin by the Nissl's method. We determined the density of MSN per 1 mm^2, linear size of neurons (area of bodies, cytoplasm, and nucleus), and structural nuclear-cytoplasmic index (ratio between areas of the nucleus and cytoplasm).

MSN proteins were assayed by interferential cytometry [2,3]. Protein concentration and the content of protein substances in the cytoplasm and nucleus of neurons were measured. Each neuronal population included 150 cells.

The results were analyzed using Protein software developed at the Laboratory of Cytochemistry (Institute of Brain, Russian Academy of Medical Sciences).

RESULTS

The lateral nucleus is a phylogenetically young cerebellar structure that transduces signals from the cerebellum to the pons and cerebral hemispheres and coordinates small movements of the limbs [8-11]. In most higher vertebrates neuronal populations of the lateral nucleus primarily include multipolar MSN localized in the central part (Fig. 1).

MSN density decreased in the following order: sparrow>gray rat>pigeon>squirrel. The density of lateral nucleus MSN was maximum in sparrows and surpassed that in gray rats, pigeons, and squirrels by 1.5, 1.8, and 3 times, respectively (Table 1). Sparrows were characterized by a wide variational series for neuronal density per unit area (100-600 MSN). We found that 73.9% cell populations contained 400-500 neurons; 50% of them included 300-400 cells. Variational series were narrow in gray rats and pigeons (100-400 neurons). Maximal densities of MSN (100-300 cells) were observed in 87 and 90% cell populations of these species, respectively. The density of MSN in 57.1 and 33% populations of pigeons was 100-200 and 200-300 cells, respectively. By contrast, the density of MSN in 33 and 54% populations of gray rats was 100-200 and 200-300 cells, respectively. Most cell populations in squirrels (81.9%) were characterized by low density of MSN (up to 200 cells). Our results confirm previous data on the interrelation between cell density in brain structures and body weight of animals (independently on the type of neurons and class of vertebrae) [7].

The average area of MSN bodies decreased in the following order: squirrel>pigeon>gray rat>sparrow. The size of MSN was maximum in mammals with high ambulatory activity and minimum in birds with high locomotor activity (Table 1). The study of neuronal populations in squirrels showed that the area of MSN varied from 200 to 900 μ^2; the area of 64% neurons was 300-600 μ^2. In other animals the area of MSN varied from 100 to 600 μ^2. Neurons with an area of 200-300 μ^2 were prevalent in gray rats (71.3%), sparrows (57.9%), and pigeons (55.9%). The structural nuclear-cytoplasmic index of MSN in highly organized animals was low. The cytoplasm/nucleus ratio in lateral nucleus neurons was 3 (gray rats), 3.4 (sparrows and squirrels), and 4 (pigeons, Table 1).

A negative correlation was revealed between the density and size of MSN in cerebellar nuclei of animals. The higher was the density, the lower was the size of cells, and conversely, the lower was the density, the higher was the size of cells. In the heterogeneous population of birds with high locomotor activity considerable neuronal density was associated with the presence of small cells. In the homogenous population of mammals low neuronal density was associated with the presence of large MSN. Probably, animals of vari-

TABLE 1. Comparative Characteristics of MSN in the Lateral Cerebellar Nucleus of Birds and Mammals Belonging to Various Ecological and Morphological Groups (*M*±*m*)

Parameter	Squirrel	Gray rat	Sparrow	Pigeon
MSN density, 1 mm^2	122.0±14.8	224.0±11.1*	348.0±23.1*	184.0±9.8*
Body area, μ^2	469.3±9.3	266.2±4.0*	250.6±5.2*	295.5±4.6*
Cytoplasm area, μ^2	365.1±7.7	201.2±3.1*	194.1±4.5*	236.7±4.0*
Nucleus area, μ^2	104.2±2.4	65.0±1.5*	56.5±1.2*	58.8±1.1*
Structural nuclear-cytoplasmic index	0.29±0.01	0.33±0.01	0.30±0.01	0.25±0.001
Cytoplasmic protein concentration, pg/μ^3	1.51±0.03	1.58±0.03	2.43±0.05*	1.58±0.04
Nuclear protein concentration, pg/μ^3	0.90±0.02	0.85±0.02	1.44±0.04*	0.95±0.03
Cytoplasmic protein content, pg	555.4±18.8	318.4±8.1*	463.9±13.2**	374.5±11.8*
Nuclear protein content, pg	94.9±3.2	56.0±2.1*	80.3±2.4**	55.9±2.2*

Note. *$p<0.001$ and **$p<0.01$ compared to squirrel.

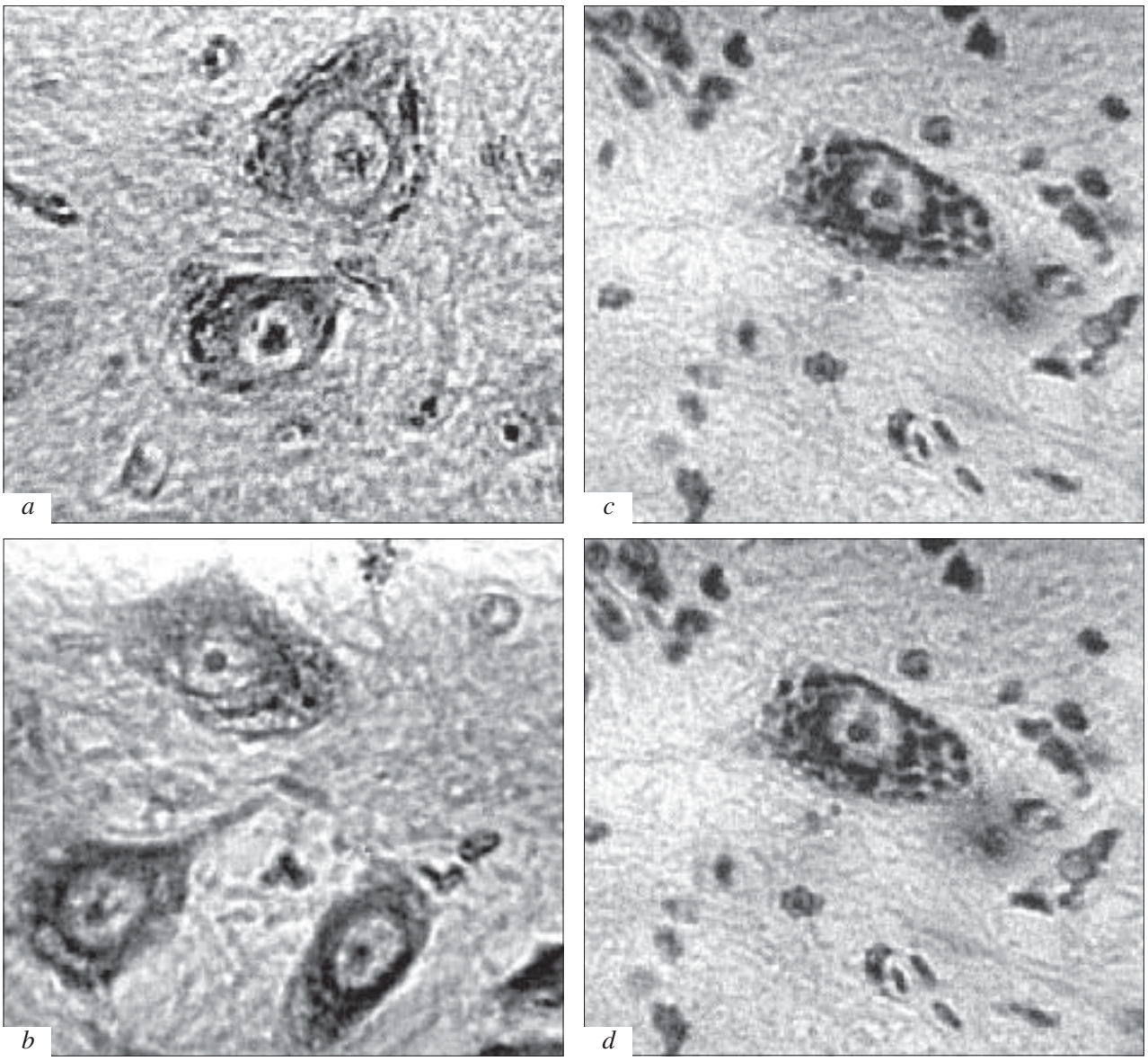

Fig. 1. Medium-sized neurons (MSN) in the lateral cerebellar nucleus of common squirrel (*a*), gray rat (*b*), rock dove (*c*), and domestic sparrow (*d*). Nissl staining, ×400.

ous classes solve the same problems (*e.g.*, rapid changes in synchronization of highly coordinated movements) by different methods. This is realized via the increase in neuronal density in birds and complexity of numerous synaptic connections with cerebellar cortex cells in mammals [4,7,8,11].

The study of protein substances in neuronal populations of lateral cerebellar neurons revealed differences in the distribution and concentration of MSN proteins in birds and mammals. Protein concentration in the nucleus and cytoplasm of MSN was maximum in sparrows. Pigeons and gray rats were characterized by a lower content of proteins in the cytoplasm (by 35%) and nucleus (by 34 and 41%, respectively). Protein concentration in the cytoplasm of MSN was minimum in mammals with high ambulatory activity (Table 1). Cell populations in sparrows, pigeons and gray rats, and squirrels included MSN with cytoplasmic protein concentrations of 0.5-4.0, 0.5-3.5, and up to 3.0 pg/μ^3, respectively. It should be emphasized that in sparrows the heterogeneous cell population contained 73.3% MSN with a protein concentration of 2.0-3.5 pg/μ^3. MSN with a lower content of proteins (1.0-2.0 pg/μ^3) were predominant in the heterogeneous cell population of other animals.

Protein content in structural components of lateral nucleus MSN was high in squirrels. In sparrows protein concentration in the cytoplasm and nucleus of MSN was 14.8 and 14.6% lower than in squirrels, respectively. Protein content in these structures of

pigeons was 31.2 and 40.5% lower than in squirrels, respectively. Protein concentration in the cytoplasm and nucleus of MSN was minimum in gray rats (41.5 and 40.2% lower than in squirrels, respectively). Neuronal populations in squirrels and sparrows were characterized by mosaic patterns. The ratio of cells with a nuclear protein content of 400-600 pg was 40.7 and 42.7%, respectively. Cytoplasmic protein concentration was low (200-400 pg) in 40 and 28% MSN of sparrows and squirrels, respectively. Our results indicate that in phylogenetically different vertebrae with high plasticity of locomotor functions heterogeneity is manifested at the level of protein architectonics in cerebellar nuclei. The content of structural proteins in cells of cerebellar nuclei was high in animals with high ambulatory activity. It should be emphasized that the area of cytoplasm in MSN was maximum in mammals and minimum in birds. These peculiarities can be related to the fact that studied animals similarly increase the safety factor, which gives advantages in their survival and new conditioned reactions to environmental factors [3]. Protein concentration was high in sparrows, but low in squirrels. It was probably related to differences in the metabolism (*e.g.*, protein metabolism) that provides the resolution of similar problems by various methods [2,3]. Optimal values of the protein pool in birds and mammals with different synanthropy (gray rats and pigeons) can be associated with relatively stable living conditions near human dwellings.

Specific quantitative characteristics of neuronal populations in subcortical cerebellar neurons of birds and mammals can be considered as an adaptive normal at the cellular and populational levels of their organization. These data extend the notion of morphological parameters in animals carrying serious infections and invasions.

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