PHYSIOLOGY

Two Types of Projection Neurons in Human Striatum: Peculiarities of Their Somatodendritic Structure in Ventral and Dorsal Striatum

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The somatodendritic structure of projection neurons was morphometrically examined in the nucleus accumbens of human brain. In contrast to reticular neurons, spiny neurons of the nucleus accumbens and dorsal striatum have different somatodendritic structure. In both parts of the striatum, reticular neurons were NADPH-diaphorase-positive.

Key Words: *human brain; neuronal structure; NADPH-diaphorase*

In view of Heimer's concept [10] on the structure of the dorsal and ventral striopallidum, nucleus accumbens can be considered as an integral part of the ventral striatum, which also incorporates the subcommissural striatum except the olfactory tubercle characterized by different structure [4].

In contrast to the dorsal striatum receiving descending afferent projections predominantly from the neocortex, the nucleus accumbens receives fibers primarily from the paleocortex and limbic cortex [2] contacting dendritic spines of spiny neurons, due to which limbic signals directly affect these neurons [12].

The ascending afferent projections of the nucleus accumbens are formed by axons of mesencephalic and thalamic neurons. The mesencephalic projections of the nucleus accumbens terminate on spiny neurons and originate predominantly from the ventral mesencephalic tegmentum, while projections of the dorsal striatum originate predominantly from the substantia nigra [12]. Thalamic afferents of the nucleus accumbens originate from neurons of the midline nuclei, which are considered as the integral elements of the limbic system, while thalamic projections of the dorsal striatum originate from the nuclei connected to the cortex and subcortical structures involved (among other functions) into the work of the motor system [3]. The amygdala afferents of the nucleus accumbens and dorsal striatum originate from common sources: mostly, from the nuclei of the basolateral complex [2]. In dorsal and ventral striatum, projection neurons are presented by spiny neurons and reticular neurons scattered among them [7-9].

We hypothesized that somatodendritic structure of spiny neurons (the major projection cells) would be different in the dorsal and ventral striatum; the structure of reticular neurons would be similar in these subdivisions due to peculiarities of the spatial structure of their dendrites caused by specific mode of information processing [5]. To examine reflec-

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tion of the peculiarities of the afferent connections of the ventral striatum in the arborization pattern of its projection neurons, we carried out a comparative qualitative analysis of the somatodendritic structure of projection neurons (spiny and reticular) in the nucleus accumbens and dorsal striatum.

MATERIALS AND METHODS

Striopallidum of both cerebral hemispheres was isolated from adults males and females (n=5) died of diseases not related to psychic and neurological pathologies.

The specimens were stained by silver impregnation (Golgi technique) according to the protocol accepted in the Department of Neuronal Structure of Brain Research Institute. Histochemical staining for NADPH-diaphorase was also performed. The material taken during autopsy was cut in frontal direction into slices (≤5 mm). For Golgi staining, the specimens were placed into Mueller fluid (3.5% $K_2Cr_2O_7$+1% Na_2SO_4×10 H_2O in water, 1:50 v/v specimen-solution ratio). After 4 days the specimens were washed twice in 3.5% $K_2Cr_2O_7$ water solution, transferred into a mixture of water solutions of 3.5% $K_2Cr_2O_7$ and 1% OsO_4 (4:1), and placed into a thermostat at 37°C for 3 days. Thereafter they were passed in 2 portions of 0.75% $AgNO_3$ water saline and placed into 1% $AgNO_3$ saline for 7 days. The specimens were then dehydrated in ascending alcohols, embedded into celloidin, 120-μ sections were transferred onto slides from absolute alcohol supplied with a few drops of distilled water. The slides with sections were immersed into nonclarified eucalyptus oil for 2 h, passed successively via 3 portions of xylene, and embedded into fir balsam.

Staining for NADPH-diaphorase was performed as described elsewhere [13] with modifications. The sections made on a vibratome (120 μ) were kept in a reaction solution containing of 0.1 M phosphate buffer, 1.2 mM β-NADPH (Sigma), 122.3 μM HCT (Sigma), and 0.3% Triton X-100 (Sigma) for 1.5 h at 37°C. Then the sections were placed on gelatinized slides, dehydrated in a battery of ascending alcohols, and embedded into fir balsam under coverslips.

The preparations of the nucleus accumbens and dorsal striatum stained after Golgi and for NADPH-diaphorase were used to draw the outlines of the

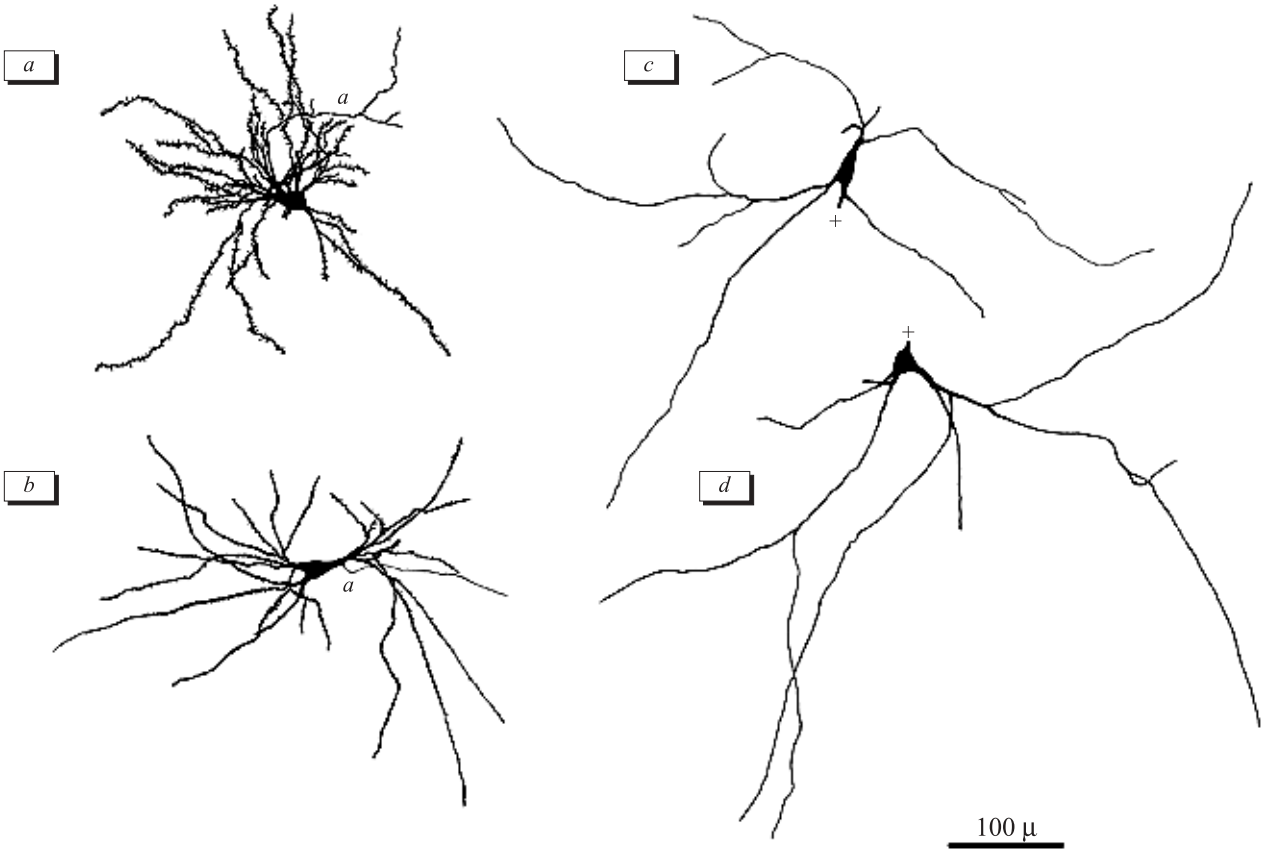

Fig. 1. Outlines of spiny (*a, b*) and reticular (*c*, *d*) neurons in nucleus accumbens (*b*, *d*) and dorsal striatum (*a*, *c*). *a*: axon; +: cut dendrites.

projection neurons and all dendrites using of ORTHOLUX II microscope equipped with a drawing apparatus (Leitz). The neurons were identified by analyzing the outlines of their somatodendritic structure. The morphometric analysis was carried as described earlier [5] with modifications. The following parameters were determined: area of soma, number of dendrites, dendrite branching, maximum radius of the dendrite field, relative radius of this field, mean length of dendrite segment, specific density of dendrites, total length of all dendrites (including cut dendrites). Neuronal area, number of dendrites, and maximum radius of the dendrite field were obtained by direct measurements from the neuron outlines. Other parameters were calculated by the formulas:

$$Ac=Ad\times D$$
$$Ad=Bd\text{-}D_1/D\text{-}D_1$$
$$Er=R/\sqrt{Scl/\pi}$$
$$Qd=Ld/Bd+Gd$$
$$Nds=Ld\times 10{,}000/Sda$$
$$Ldc=Ld/(D\text{-}D_1)\times D,$$

where Scl is area of soma; D is number of dendrites, Ac is total branching of the cell; Ad is dendrite arborization, R is the maximum radius of dendrite field, Er is relative radius of the dendrite field, Qd is the mean length of the dendrite segment, Nds is specific density of dendrites, Lds is total length of all dendrites, D_1 is the number of cut dendrites, Bd is the number of free endings of all dendrites, Gd is the number of all branching points, Ld is the total length of all dendrites on the outline, and Sda is the area of dendrite field.

The data were processed using Mann—Whitney nonparametric *U* test.

RESULTS

Two populations of projection neurons (spiny and reticular) were identified in the nucleus accumbens of human brain on the basis of comparison of their

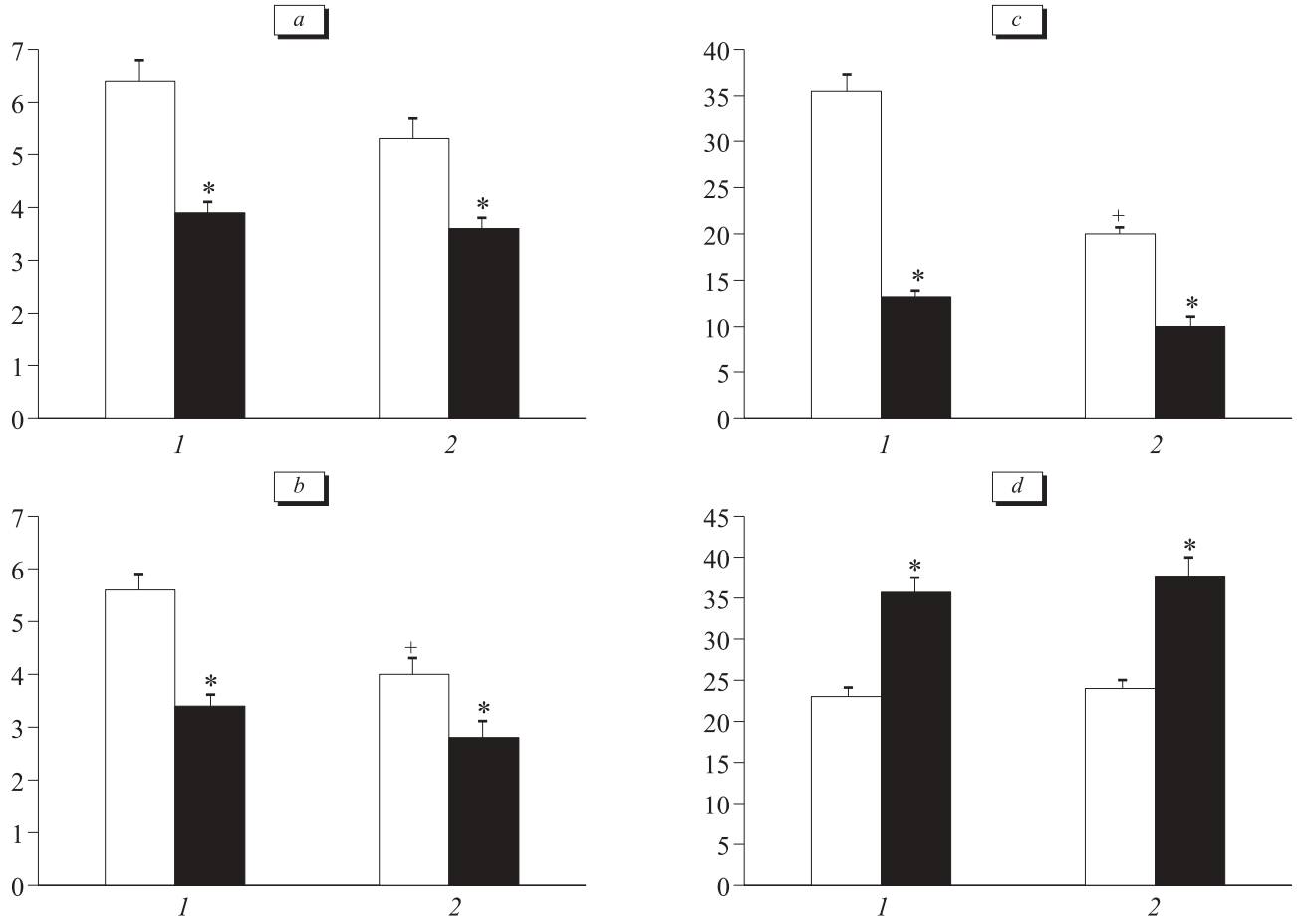

Fig. 2. Somatodendritic structure of spiny and reticular neurons in dorsal striatum (*1*) and nucleus accumbens (*2*). *a*) number of dendrites; *b*) arborization of dendrites; *c*) total arborization of the cell; *d*) relative radius of the dendrite field. Here and in Fig. 3: open and solid bars correspond to spiny and reticular neurons, respectively. $p<0.01$ in comparison with *spiny neurons and +striatal spiny neurons.

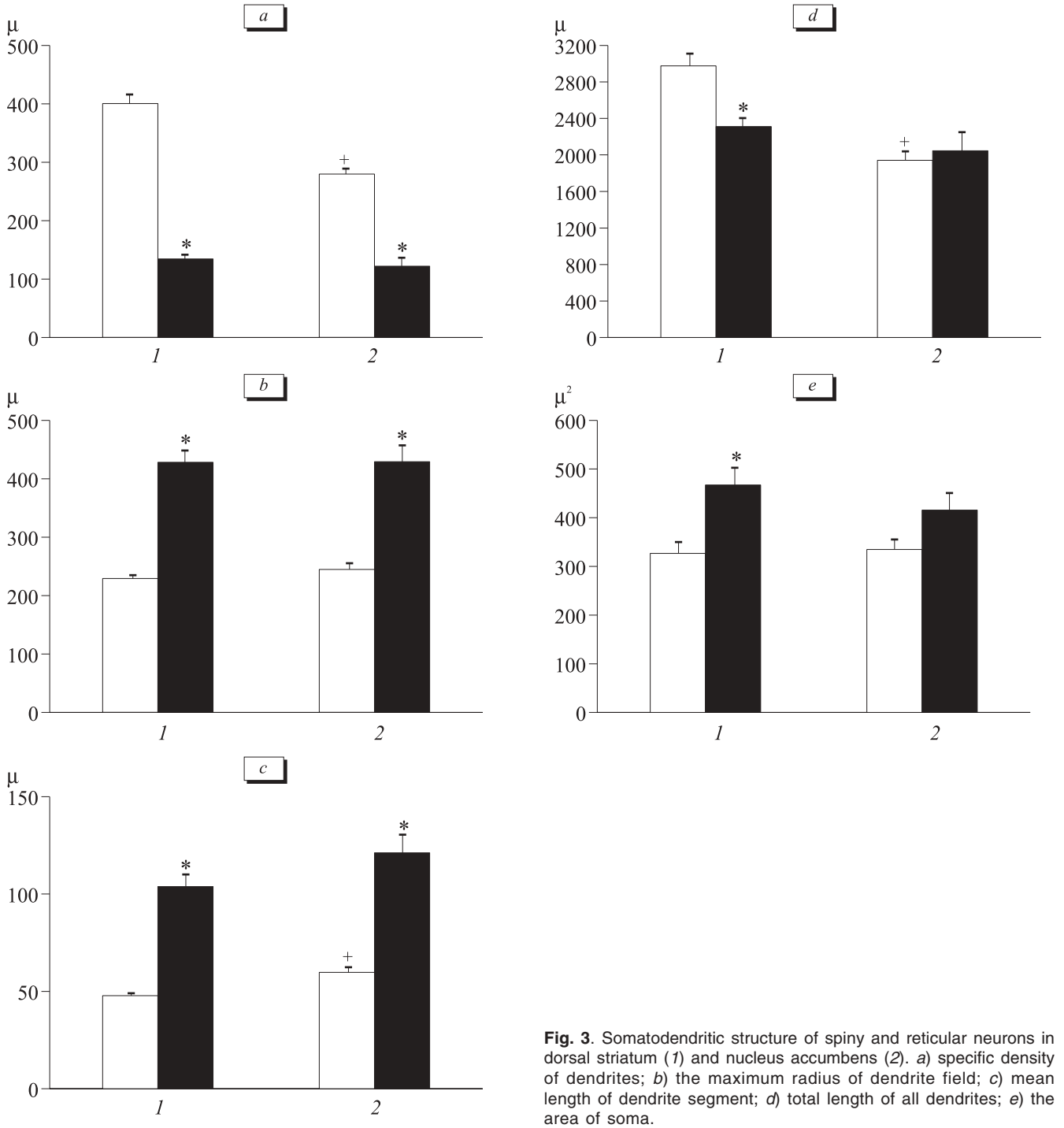

Fig. 3. Somatodendritic structure of spiny and reticular neurons in dorsal striatum (*1*) and nucleus accumbens (*2*). *a*) specific density of dendrites; *b*) the maximum radius of dendrite field; *c*) mean length of dendrite segment; *d*) total length of all dendrites; *e*) the area of soma.

somatodendritic structure and structure of the spiny and reticular neurons in dorsal striatum.

The neurons with small soma and wave-like dendrites covered by spines were found most frequently. The pattern of arborization of these neurons was similar to that of dense-arborizing projection cells of the dorsal aspects of the striatum [12]. These neurons were identified as spiny neurons (Fig. 1, *a*, *b*). As a rule, the dendrites of spiny cells in the nucleus accumbens emerge in fascicles from the opposite poles of the neuron soma (Fig. 1, *b*), while dendrites of similar cells in the dorsal striatum diverge in the radial directions from the neuron soma (Fig. 1, *a*). By Golgi staining, spiny neurons of the nucleus accumbens differed from those of dorsal striatum in 5 of 9 analyzed parameters (Figs. 2, 3).

Despite the fact that densely branching projection neurons of the nucleus accumbens and dor-

sal striatum belong to the same type, they differed by total arborization of the cell, arborization of dendrites, the mean length of dendrite segments, dendrite specific density, and the total length of all dendrites. Therefore, these groups of neurons are characterized by individual peculiarities reflecting specific functional roles of the nucleus accumbens and dorsal striatum correlated with peculiarities of the afferent-efferent connections of these subdivisions of the striatum.

The neurons of other population were rarely found in the preparations. By contrast to spiny neurons, reticular neurons were characterized by other set of morphologic signs: they have large soma and long straight-line rarely branching dendrites (Fig. 1, *c*, *d*). The spines were found only in some parts of dendrites. In the forebrain structures of humans and animals they are described as disseminated elements [7,11], which form ancient integrative and rarely arborizing neuronal system of the brain. This system protrudes continuously along the entire brain axis, reaching the interstitial subdivisions of the spinal cord, reticular formation, hypothalamus, the basal nuclei of the front brain, pallidum, *etc*. [6]. By NADPH-diaphorase staining, reticular neurons of the nucleus accumbens and dorsal striatum did not differ by any parameter (Figs. 2, 3).

In the nucleus accumbens, the spiny and reticular neurons differed by all structural parameters except area of the soma and total length of all dendrites (Fig. 2). In the dorsal striatum, these neurons differed by all parameters (Fig. 2). Thus, spiny and reticular neurons of the nucleus accumbens and dorsal striatum belong to two different populations of the projection cells: the spiny neurons are densely branched cells, while reticular neurons are scarcely-branched ones, indicate their integrity into different neuronal systems of the brain: the dense- and rarely-arborizing ones, respectively.

Our data agree with the hypothesis on availability of poorly branched reticular cells as disseminated elements in the forebrain structures [5].

Using the histochemical reaction on NADPH-diaphorase as a tool in the study of neuronal somatodendritic structure, we found that among two populations of projecting neurons only the rarely-branched reticular cells were NADPH-diaphorase-positive both in the nucleus accumbens and in the dorsal striatum [7]. This selectivity of NADPH-diaphorase activity in the population of rarely-branched projection cells open new vista for the study the functional role of the system of disseminated reticular neurons in the brain, which was described previously by Golgi staining [5].

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