EXPERIMENTAL BIOLOGY

Structural Changes in Spiny Neurons of the Rat Striatum after a Unilateral Injection of 6-Hydroxydopamine

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A decrease in the profile field of bodies and dendrite branching of neurons is revealed. An increase in dendrite lengths and in the area occupied by the bodies of spiny neurons is demonstrated 4 and 12 months postoperation due to the growth of terminal dendritic segments and increased number and length of unbranched dendrites. The positive changes in striatal spiny neurons after injection of 6-hydroxydopamine are indicative of a high level of their plasticity.

Key Words: *spiny neurons; striatum; 6-hydroxydopamine*

The considerable interest paid to the nigrostriatal connections is largely due to their role in the pathogenesis of a number of neurological diseases, including Parkinsons's disease [4]. A model of striatal deafferentation from the substantia nigra with the neurotoxin 6-hydroxydopamine (6-OHDA) [6] is widely used in studies of the nigrostriatal system. It has been demonstrated that the destructive changes in dopamine (DA) endings terminating at the spines of long-axon spiny neurons of the striatum (LSN) are accompanied by a drop of the dopamine level and an increase in the tissue sensitivity to dopamine and its agonists [2]. Injection of apomorphine after the destruction of DA entry in the striatum allows one to indirectly evaluate the degree of damage from the number of contralateral rotations of the animal. Although there have been a good number of papers dealing with this problem, the questions associated with structural changes in striatal LSN so far remain poorly investigated.

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The objective of our study was to investigate the structural changes occurring in LSN of rat striatum, depending on the survival periods after dopamine deafferentation of the striatum with the neurotoxin 6-OHDA.

MATERIALS AND METHODS

Experiments were performed on 60 adult male Wistar rats weighing 300-350 g. The animals were divided into two equal groups (n=30). 6-OHDA (8 μg in 2 μl 0.04% ascorbic acid prepared on normal saline) was injected into the site corresponding to the medial bundle of the forebrain at the midbrain level by the coordinates: A-P +4.3; L +1.4; DV -8.7 [5]. The animals were anesthetized with chloral hydrate (40 mg/kg), and the operation was performed on a stereotaxic apparatus.

The control group included 15 unoperated animals (passive control) and 15 animals after injection of ascorbic acid without the neurotoxin (active control). All operated animals were subdivided into 3 series according to the survival period after the operation: 1 month, 4 months, and 1 year. Each series consisted of 10 experimental rats and

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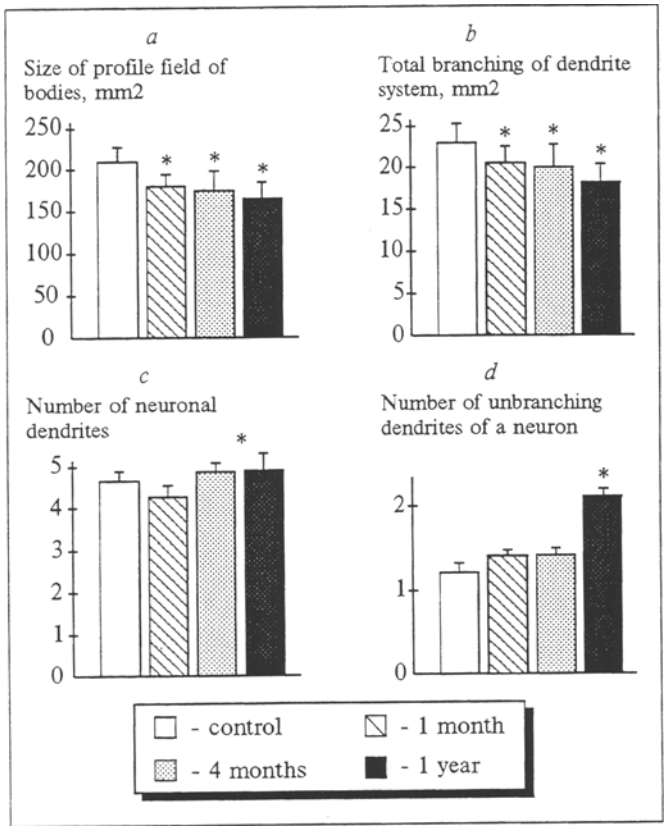

Fig. 1. Changes in structural parameters of spiny neurons of rat striatum at different survival periods after 6-OHDA injection (mean values are shown). Here and in Fig. 2: asterisk indicates significance of differences at $p<0.05$.

5 passive control and 5 active control rats. All experimental rats were tested after 6-OHDA injection by an intraperitoneal injection of apomorphine (0.05 mg/kg), which induced contralateral rotation. The number of rotations was counted every 5 min during a 1-h period. Animals displaying a minimum of 340 rotations per hour, which indicated at least 80% destruction of DA entry in the striatum [3], were used in subsequent studies. After the chosen survival periods the animals were killed and their brains were perfused with 3.5% $K_2Cr_2O_7$ on 10% formaldehyde and treated by the method of Golgi.

Morphological analysis was performed in a series of frontal sections 150-200 μ thick. Precise drawings of striatal LSN magnified 400-fold were subjected to morphometric analysis in a Leits-ASM semiautomatic image analyzer. A total of 320 neurons were analyzed by 28 parameters characterizing soma size and a number of metric and topological characteristics of the dendrite system. The significance of differences between the groups was evaluated using the Kholmogorov-Smirnov and Wilcoxon-Man-Whitney nonparametric tests at $p<0.05$.

RESULTS

There were no statistically significant changes in any of the analyzed parameters between active and passive controls in all 3 series. Quantitative analysis of LSN revealed 8 parameters that proved to be the most significant in the studies of structural changes in these neurons resulting from their DA deafferentation. These parameters were: the area of the profile field of the cell body, total lengths of dendrites; mean length of terminal dendrite segments, mean length of unbranched dendrites; total number of dendrites; number of unbranched dendrites; area of the dendrite field; and neuronal branching.

A decrease in the profile field of LSN bodies (Fig. 1, *a*) and in neuronal branching (Fig. 1, *b*) occurred in all 3 series of experimental rats in comparison with the control. At the same time, there was an increase in a number of parameters in rats surviving 4 months and 1 year after the operation. The most pronounced positive changes in the dendritic system of LSN were associated with the increase of the number of unbranched dendrites in rats which survived for 1 year (Fig. 1, *d*) and with the increase in the total length of

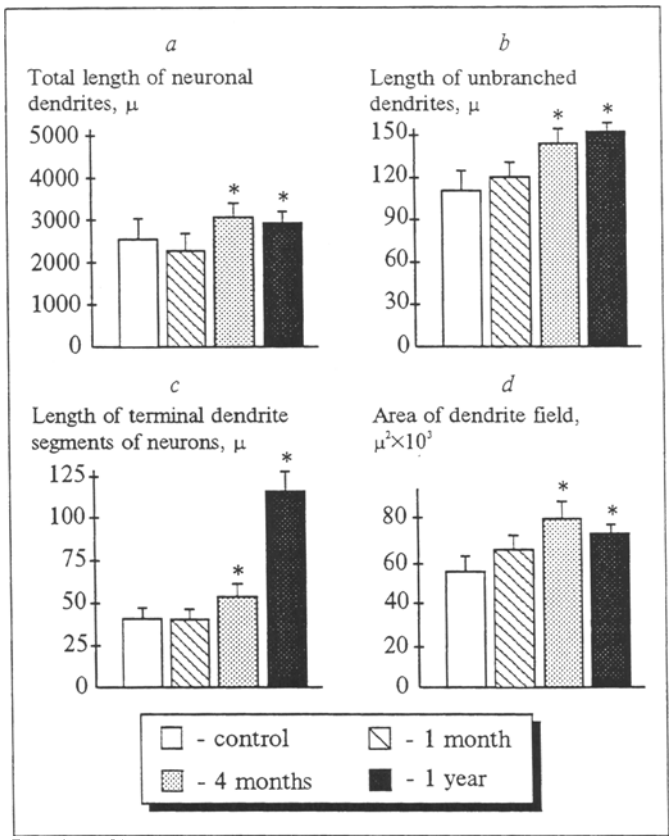

Fig. 2. Changes in structural parameters of spiny neurons of rat striatum in different survival periods after 6-OHDA injection (mean values are shown).

dendrites due to the growth of the terminal segments and the length of unbranched dendrites in rats which survived for 4 months and 1 year after the operation (Fig. 2). Striatal LSN in rats surviving for 1 year were distinguished by a considerable increase in the number of unbranched dendrites (Fig. 1, *d*), which can be regarded as indirect evidence of the search for new afferent sources.

It should be mentioned that the positive changes in striatal LSN were not accompanied by compensation at the behavioral level, judging from the preserved elements of stereotypic behavior and large numbers of rotations after apomorphine probing. It can be assumed that the negative changes in LSN observed 1 month after DA deafferentation of the striatum are associated with transitory destructive changes in striatal neurons in response to the administration of 6-OHDA, in spite of the fact that the dopamine terminals from the substantia nigra constitute only 10-15% of the total input [1]. The positive changes occurring at later survival times in the dendrite system of spiny neurons of the striatum indicate a nonuniform nature of alterations occurring in the dendrite system as a whole and a high level of dendrite plasticity after dopamine deafferentation.

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