MORPHOLOGICAL SIGNS OF RESTITUTION IN THE CAUDATE NUCLEUS AFTER AMPHETAMINE EXCITATION

E. N. Popova and L. B. Verbitskaya

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A light- and electron-microscopic study was made of changes in the structure of neurons and synapses in the caudate nucleus of rats 2 and 3 days after motor excitation induced by amphetamine (10 mg/kg body weight). Electron-microscopic investigations 2 days after amphetamine excitation showed predominance of synthesis over utilization of the intracellular organelles in the neurons of the caudate nucleus; the latter process took place, moreover, in the direction from nucleus to periphery of the cell body. A statistically significant increase in the mean area of cross-section of the nuclei and bodies of the neurons and in the number of impregnated spines per unit length of the dendrites was found. The ultrastructure of the neurons was largely restored to normal after 3 days although the mean area of cross section of the bodies and nuclei of the neurons had not yet reached its initial values. There was virtually no difference in the number of impregnated spines of the dendrites compared with the control, but electron microscopy revealed many synaptic vesicles in the axon terminals.

KEY WORDS: basal ganglia; action of amphetamine; nerve cells.

The object of this combined light- and electron-microscopic investigation was to study the mechanisms lying at the basis of restoration of the structure of neurons and synapses in the caudate nucleus after amphetamine excitation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats. Amphetamine was injected intraperitoneally in a dose of 10 mg/kg body weight, sufficient to cause the development of a motor stereotype that indicated excitation of adrenergic structures, which are widely represented, in particular, in the caudate nucleus. The animals were killed 2 and 3 days after receiving amphetamine. Brain sections were stained by the methods of Nissl and Golgi. The area of cross section of 50 neurons and their nuclei was measured with an ocular micrometer in three experimental and three control rats (magnification 420×). The results were subjected to statistical analysis by the Student–Fischer method. The number of spines in consecutive segments, 50 μ long, along the dendrites of the densely branching neurons of the caudate nucleus also was counted. The spines were counted on a length of 10 μ of the dendrite. The equations used for the evaluation of biological material were employed to analyze the results [6]. Statistical significance was determined by the equation

$$t = \frac{x_1 - x_2}{\sqrt{m_1^1 + m_2^2}},$$

differences being significant for which $t \geq 2$. For the electron-microscopic investigation the brain was perfuses intravitally with a mixture of 1% glutaraldehyde solution and 2% paraformaldehyde solution in phosphate buffer (pH 7.2-7.4) with the addition of 0.01% $CaCl_2$ solution and 7% glucose; postfixation followed in 2% osmium tetroxide solution. Pieces from the dorsal part of the caudate nucleus were embedded in Epon-812 after appropriate treatment. The sections were stained with uranyl acetate and lead citrate.

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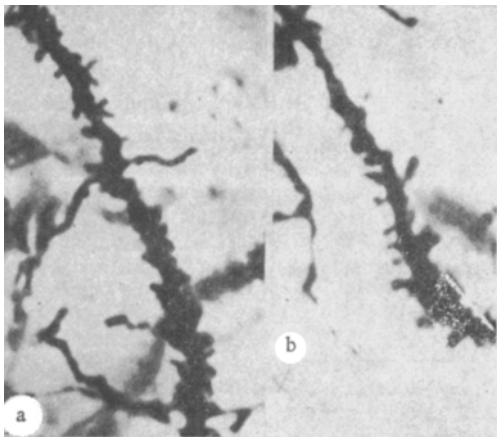

Fig. 1. Structure of dendrite of densely branching neuron in rat caudate nucleus after amphetamine excitation. Golgi, 900×: a) increase in number of impregnated spines 2 days after amphetamine excitation. Axo-dendritic synapses can be seen; b) decrease in number of impregnated spines 3 days after amphetamine excitation.

EXPERIMENTAL RESULTS

The investigations showed that the leading role in the processes of restitution after amphetamine excitation is placed by the nucleus as placenta of intracellular synthesis of RNA and protein. Activation of the nuclear structures could be seen as early as 24 h after administration of amphetamine. Meanwhile, the area of the mean cross section of the nucleus was increased from $60.19 \pm 0.53 \mu^2$ at the height of the stimulant effect of amphetamine to $61.33 \pm 0.69 \mu^2$ (difference statistically significant). Migration of material from the nucleolus into the cytoplasm and the formation of Nissl substance, RNA, and proteins as the result of nucleolar activity were described some time ago after morphological and histochemical investigation [10-13].

Electron-microscopically the perinuclear zone of the cytoplasm was richly supplied with various organelles 2 days after administration of amphetamine. This zone occupied a larger or smaller area around the nucleus and it stood out clearly against the peripheral zone of the cytoplasm, which contained few organelles. Ribosomes and polysomes, lying freely in the cytoplasm, and mitochondria were particularly numerous in the perinuclear zone. Mainly these were small, round mitochondria with a dark matrix and clearly outlined cristae. A few long mitochondria with constrictions, presumably attributable to division of the mitochondria, were seen. Characteristic groups of mitochondria were observed in certain zones of the cytoplasm, which were regarded as functionally more active [1, 7, 8]. The liberation of granules of RNP type from the nucleolus was found

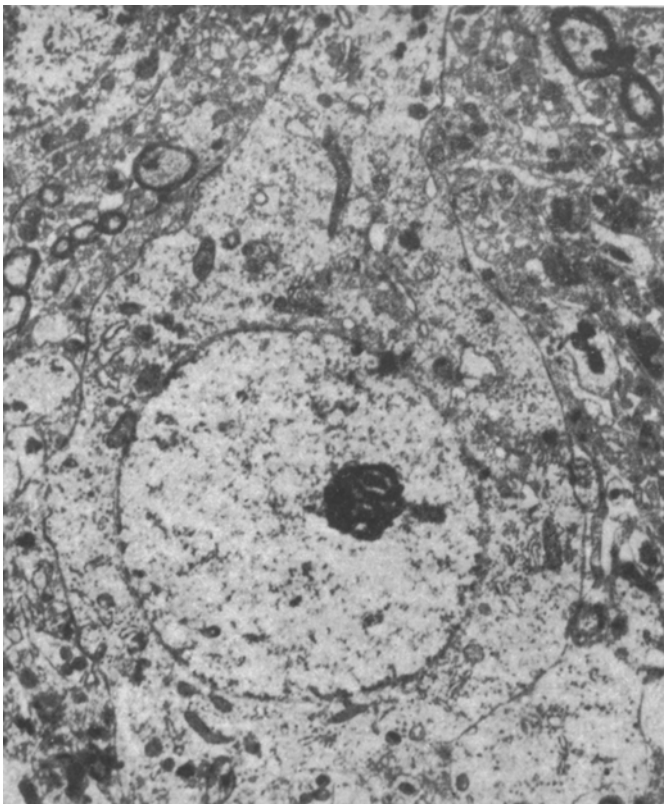

Fig. 2. Ultrastructure of small neuron of caudate nucleus 3 days after administration of amphetamine, 10 mg/kg: 10,000×.

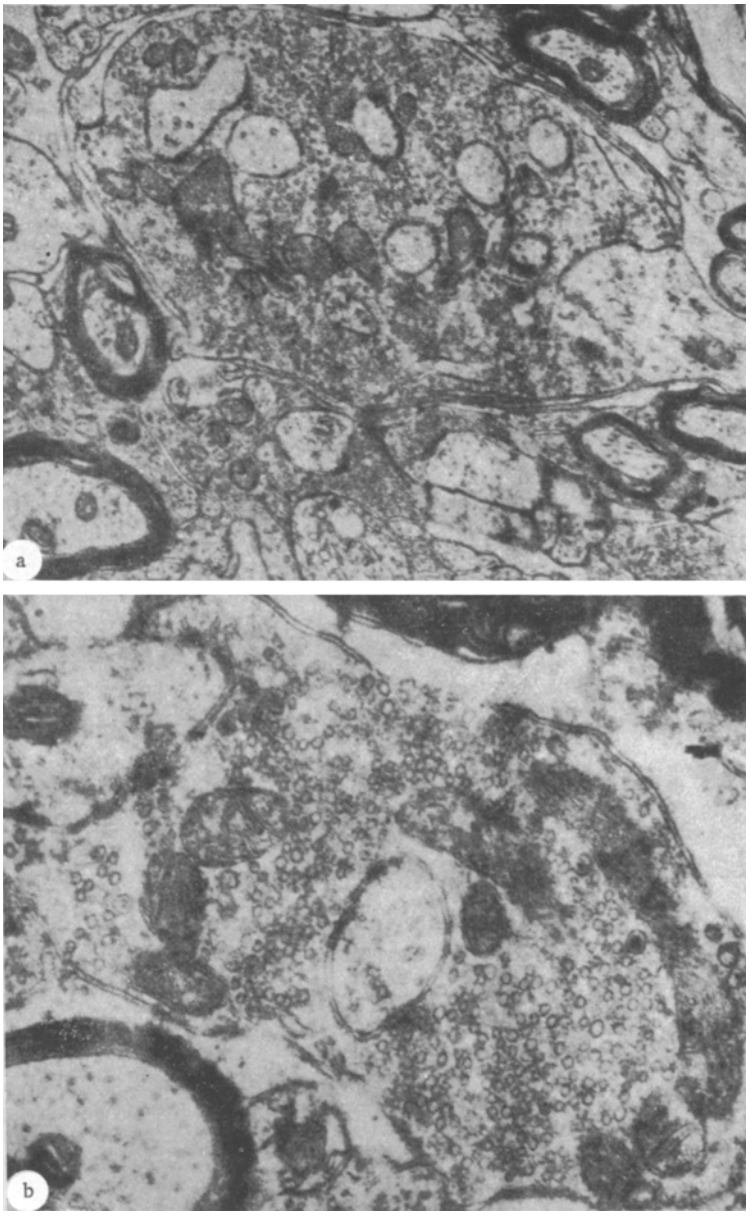

Fig. 3. Synapses invaginated into axon. Axon terminals contain many synaptic vesicles: 3 days after amphetamine administration, 40,000×.

and the outlines of the nuclear membrane were indistinct as the result of the many granules lying on the membrane itself, around the outer nuclear membrane, and in the adjacent layer of cytoplasm. Predominance of synthesis over breakdown of the structural material was observed in conjunction with an increase in the mean area of cross section of the nuclei ($60.30 \pm 0.68\ \mu^2$) and bodies ($86.27 \pm 0.75\ \mu^2$) of the neurons, although compared with the state 24 h after administration of amphetamine these values were a little reduced. It can accordingly be postulated that the period of recovery of the structural organization of the neurons after their hyperfunction is a period of specialized function reflected in the morphological appearance of the cells.

At the light-optical level, increased function of the neuron and the beginning of recovery processes after hyperfunction were reflected morphologically in the same way, as an increase in the size of the cell body and nucleus combined with partial chromatolysis of the Nissl substance. The differences between these states of the neuron were found at the submicroscopic level; the phase of increased function was characterized by a reduction of the number of organelles in the cytoplasm, especially at the periphery of the cell body, whereas the recovery stage was marked by an increase in the number of organelles in the perinuclear zone and a decrease in their number more peripherally.

The intensification of synthesis in neurons of the caudate nucleus 2 days after motor excitation induced by amphetamine correlated with a statistically significant increase in the number of impregnated spines per unit length of the dendrites of the densely-branching neurons: to 8.86 ± 0.183 spines per 10 μ length of dendrite compared with 7.4 ± 0.71 spines 24 h after administration of amphetamine. The number of axodendritic contacts on the spines and trunks of the dendrites was increased (Fig. 1a). Most probably, recovery processes in the cytoplasm took place in response to strengthening of presynaptic afferentation, so that normal relations were restored between processes of synthesis and breakdown of structural materials and the physiological activity of the neuron.

The structure of the neurons and interneuronal synapses was largely restored 3 days after administration of a single dose of amphetamine. However, the mean area of cross section of the neuron bodies (83.59 ± 1.01 μ^2) and their nuclei (57.31 ± 0.90 μ^2) did not regain their original values (79.47 ± 0.41 and 55.02 ± 0.28 μ^2 respectively), although they were less than on the second day after amphetamine excitation, possibly as a result of the slow excretion of the drug from the body, which takes place during the 2-4 days after its injection [5-8].

Electron-microscopic investigations at this time, just as in the control rats, revealed at least two types of neurons to correspond to the pattern of distribution of their intracellular organelles. In the cytoplasm of some cells they were arranged relatively uniformly, but in other neurons their concentration in some places was higher (Fig. 2). The outlines of the nuclear membrane were either indistinct over a certain part of their length, or the nucleus was mainly clearly outlined and the membrane itself sometimes had a number of separate invaginations. Liberation of granules from the nucleolus, localized dilatation of the endoplasmic reticulum, and hypertrophy of individual mitochondria, accompanied by marked swelling of some of them, as is more often found in cells with an irregular distribution of intracellular organelles, were seen. In some cells the cisterns of the endoplasmic reticulum were narrow but were of considerable length and covered by many ribosomes, while at the same time many ribosomes and polysomes were present in the spaces between the cisterns. The number of spines per unit length of the dendrites 3 days after administration of amphetamine was virtually at the control level (7.4 ± 0.1 compared with 7.3 ± 0.1 in the control); axo-dendritic contacts were infrequently seen (Fig. 1B), and under the electron microscope very many synaptic vesicles were found in most axon terminals and contacts were visible on the spines and small branches of the dendrites invaginated into the axon (Fig. 3). The functional significance of ultrastructural changes in the synapses of this sort is unknown. An increase in the number of synaptic vesicles has been found in the ciliary ganglion of birds in the response to orthodromic electrical stimulation [4], in the cerebral cortex of monkeys during general excitation [2], and also in the period of depression of the dendritic excitatory postsynaptic potential in the visual center of the frog in response to electrical stimulation of the optic nerve [3]. If we take into consideration physiological data which show that during recovery of condition-reflex activity of rate 2 days after amphetamine administration the latent periods of the conditioned reflexes are shortened, [9] then the increase in the number of synaptic vesicles in most synapses of the caudate nucleus at that time can be connected with increased functional activity of the synapses.

Microscopic and submicroscopic changes in neurons and interneuronal synapses in the caudate nucleus of rats observed during amphetamine excitation must be classed as functional changes. The functional character of the observed morphological changes is demonstrated by their rapid appearance after the administration of amphetamine and their gradual disappearance after the substances has ceased to act.

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