

process of cytokinesis is disturbed, as is shown by the appearance of large binuclear cells, and some cells after passing through the S period are held up in the G₂ period because of their inability to go into mitosis, they undergo a varied degree of hypertrophy, accumulate in the urinary tubules, and later, are destroyed after different time intervals.

All these disturbances of the cell cycle can be explained by the primary action of mercury on sulfhydryl groups of nuclear and cytoplasmic proteins, or by their secondary action, as a result of disturbance of the blood supply to the kidney tissue and hypoxia, which is accompanied by activation of endogenous phospholipases and lipid peroxidation. The probability likewise cannot be ruled out that the appearance of hypertrophied cells is compensatory and adaptive in character and is connected with intensification of the functions of regenerating cells while in the G₁ period. This is supported by the fact that more hypertrophied cells are found during the period when anuria gives way to diuresis. Evidently a final decision regarding the period in which the hypertrophied cells will be drawn only after experiments with repeated injections of ³H-thymidine.

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QUANTITATIVE EVALUATION OF CYTOARCHITECTONIC REORGANIZATION OF THE LOCUS COERULEUS INDUCED BY 6-HYDROXYDOPAMINE

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The property of the specific neurotoxin 6-hydroxydopamine (6-OHDA) of selectively injuring the catecholaminergic system of the brain is widely used in order to study its structural and functional organization. Experiments with intracisternal injection of 6-OHDA provide the most adequate model of neurological disorders associated with a reduced catecholamine concentration in the brain. When injected in this way 6-OHDA penetrates into the intercellular medium and interacts with catecholamine receptors, so that responses of nerve tissue structures to the initial and gradual damage to the catecholaminergic system can be studied. The main targets for the action of 6-OHDA are dopaminergic structures, but there is evidence that a single injection of 6-OHDA can induce a long-term fall in the noradrenalin level by 70-80% (the main source of the noradrenalin is considered to be the locus coeruleus of the brain stem [2]). We know from postmortem observations that in neurological and mental disorders caused by catecholamine deficiency, the basophilic protein bodies characteristic of neurons of monoamine groups disappear both in the cytoplasm of neurons of the locus coeruleus and in the substantia nigra [7]. Death of neurons of the locus coeruleus has been reported in experiments on rats with the use of 6-OHDA, and in explants of organotypical cultures [3, 5]. Meanwhile other workers found no significant disturbances of the structure of the locus coeruleus under analogous experimental conditions [8]. Thus the data in the literature are scanty and contradictory, yet the obtaining of morphological evidence of structural transformations in the neurons of the locus coeruleus under conditions of injury to the catecholaminergic system is

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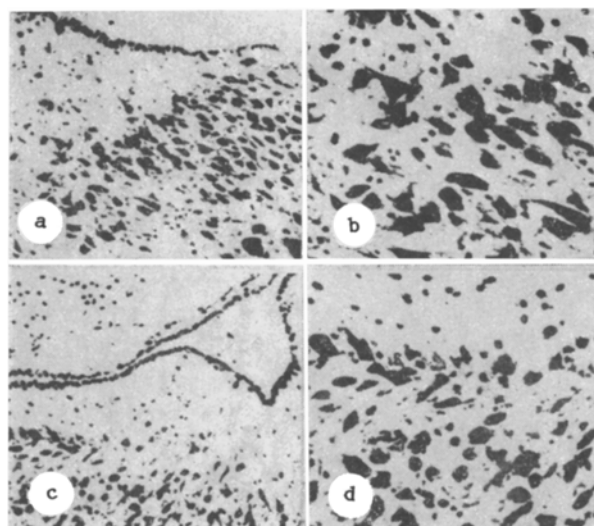


Fig. 1. Locus coeruleus of a rat photographed under magnification of the microscope of 263 (left) and 526 (right). a and b) Normal; c and d) 36 days after injection of 6-OHDA; fixation in 96° alcohol, staining by Nissl's method. On left — objective 20, homal II; on right — objective 40, homal VI.

of great importance for elucidation of the mechanisms of action of 6-OHDA, which may be of endogenous origin [4].

The aim of this investigation was to study the dynamics of the structural transformations of locus coeruleus neurons under the influence of 6-OHDA, using a method of quantitative evaluation of the composition of the neurons under normal and experimental pathological conditions.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats which received a single intracisternal injection of 300 μ g of 6-OHDA, dissolved in 20 μ l of physiological saline. Control animals received an injection of physiological saline alone. The animals were killed 2, 5, 10, and 36 days after injection of 6-OHDA; the brain was removed, fixed in 96° alcohol and, after being thoroughly defatted, it was embedded in celloidin. Sections were cut to a thickness of 12-15 μ and stained with toluidine blue by Nissl's method. A quantitative method was used on histological preparations of the brain of animals killed in the late stages of action of the metabolite (36 days). A "Quantimet-720" system, by means of which the number of cells in the field of vision can be counted and the dimensions of the cross-sections of the neurons calculated automatically, was used for this purpose. The condition of identity of the levels of sections through the locus coeruleus in the experiment and control was strictly observed. Quantitative analysis was carried out on the basis of evaluation of the degree of homogeneity and isotropism of the structures [1, 6], which is based on quantitative comparison of the results of special analysis of images of the locus coeruleus obtained for magnifications K_1 and $K_2 > K_1$ ($K_1 = \times 263$, $K_2 = \times 526$, Fig. 1). The closeness of the studied image to a homogeneous and isotropic structure was assessed by statistical comparison of the "predicted" distribution, constructed for the scale of K_2 , with the distribution of dimensions of cells constructed from the results of measurement of the structural features of the image of scale K_2 . The "predicted" distribution was plotted from data of the original image of scale K_1 , with the a priori assumption of homogeneity and isotropism of the structure. A significant change in the degree of homogeneity and isotropism in the distribution of the neurons served as the criterion of structural changes in the locus coeruleus.

EXPERIMENTAL RESULTS

In the early period of injection of 6-OHDA a typical axonal response of most nerve cells of the locus coeruleus was observed, characterized by dispersion of the chromatophilic substance

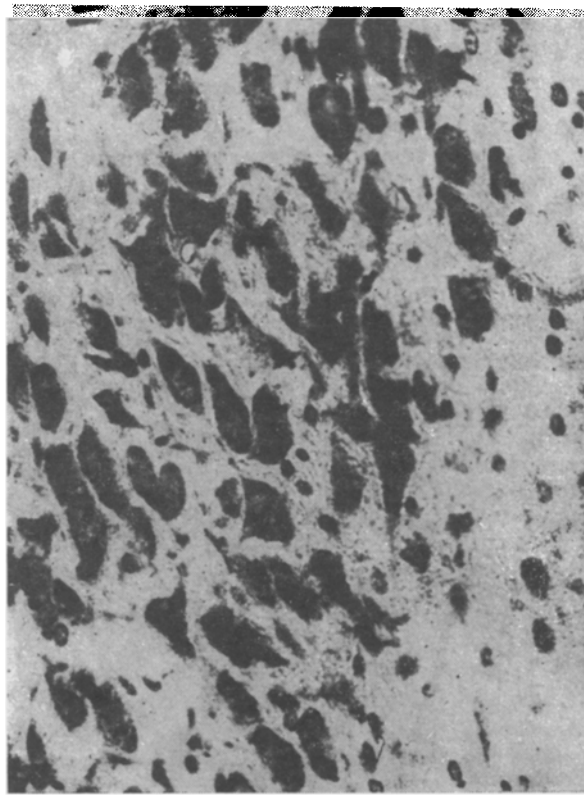


Fig. 2. Central chromatolysis and eccentric location of nucleus of locus coeruleus neurons 2 days after injection of 6-OHDA. Nissl's method, fixation in 96° alcohol. Objective 40, ocular 7.

in the cytoplasm of the neurons, and an eccentric position of the nucleus (Fig. 2). The Nissl substance was palely stained, and a narrow border consisting of separate grains was preserved only along the edge of the cytolemma. On the 5th-10th day of the observations changes in the neurons of the locus coeruleus became stable in character without any increase in the signs of development of progressive pathology of the cytoplasm and nucleus. Most neurons 36 days after injection of 6-OHDA had become hyperchromic, their cytoplasm homogeneous, and the clumps of Nissl substance indistinguishable. In many neurons, as a result of shrinking and changes in the staining properties of the nerve cell, the nucleus could not be identified, although in some of them it was possible to find a relatively pale nucleus with large nucleolus.

The quantitative investigation showed that in both experiment and control the dimensions of the locus coeruleus neurons varied widely from 17.7 to 2268.4 μ^2 , as a result of which the analysis of the degree of homogeneity and isotropism of the structure was carried out within the limits of each class (altogether six classes were distinguished). Histograms of the "predicted" distribution of the number of cells in the image in the control and 36 days after injection of 6-OHDA were approximated to the exponential distribution with the same value of the parameter, which was tested by the chi-square criterion of goodness of fit for a level of significance of 0.05. This meant that the traditional primary statistical analysis of images (counting the number of elements and their size) did not reveal structural changes in the cytoarchitectonic organization of the locus coeruleus as a whole near the influence of 6-OHDA. However, the use of the method of assessing the degree of homogeneity and isotropism as the criterion of structural transformations led to the results given in Table 1, from which it is clear that the distribution of neurons of locus coeruleus measuring from 35.6 to 1139.2 μ^2 is nonhomogeneous and anisotropic. Despite the fact that the distribution of neurons ranging in size from 35.6 to 1139.2 μ^2 was nonhomogeneous and anisotropic both in the control and as it remained 36 days after injection of 6-OHDA, the degree of this anisotropy was significantly altered. Whereas for elements measuring from 35.6 to 284.8 μ^2 the distribution was shifted toward homogeneous and isotropic, for elements measuring from 284.8 to 569.6 μ^2 , on the other hand, it moved away from them. This was evidently due to a change in the numerical composition of the neurons whose cross sections decreased as a result of atrophy, arising under the

TABLE 1. Use of Informational Parameters to Analyze Cytoarchitectonic Structure of the Locus Coeruleus

No. of interval	Boundaries of intervals, μ^2	Number of neurons per image						Character of structure (level of significance $\alpha = 0.05$)	
		real				magnification 526 "predicted"			
		magnification 263		magnification 526					
		control	experiment	control	experiment	control	experiment	control	experiment
1	35,6—71,2	94	46	23	9	23	20	Nonhomogenous	Nonhomogenous
2	71,2—142,4	47	50	15	9	11	10	»	»
3	142,4—284,8	31	79	7	6	17	16	»	»
4	284,8—569,6	42	36	7	11	23	12	»	»
5	596,6—1139,2	22	13	7	6	12	12	»	»
6	1139,2—2278,4	11	3	3	1	8	20		

influence of 6-OHDA. Incidentally, the property of homogeneity and isotropism of structure implies stability of the number of elements of definite size in any distinguishable unit of area of the test field of vision. Thus as a result of the use of the quantitative method, which can reveal coordination of relations between the character of distribution of neurons and their size, it was possible to discover the essential nature of reorganization of the structure of the locus coeruleus and its connection with a change in the quantitative composition of the neurons and the method of their redistribution after administration of 6-OHDA.

It is essential to point out that neurons with an average size of between 569.6 and 2278.4 μ^2 were not involved in the process of general reorganization of the cytoarchitectonic structure of the locus coeruleus. These neurons evidently do not carry dopamine receptors and they continue to function, while maintaining the noradrenalin concentration at a lowered level. Identification reorganization of the structure of the locus coeruleus indicates the compensatory character of changes in the neurons, aimed at making good the catecholamine deficit.

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