ELECTRON-MICROSCOPIC AND CYTOINTERFEROMETRIC STUDY OF CORTICAL NEURONS FROM SECOND-GENERATION OFFSPRING OF PRENEUROSENSITIZED FEMALE RATS

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Previous studies of the effect of preliminary neurosensitization of female rats on the state of the cortical neurons of their offspring revealed changes in ultrastructure of the nucleus and cytoplasm of the nerve cells, a decrease in size of the layer V neurons and in the thickness of the sensomotor cortex, and delay of protein accumulation in the nucleus and cytoplasm compared with the control [2-4].

It was accordingly decided to study to what extent neurosensitization of females is reflected in the CNS of the second-generation offspring.

It is stated in the literature that pathogenic factors acting on the mother can induce a reaction in second-generation animals. This has been shown, for example, in protein deprivation [11] and after removal of the thyroid gland. However, in relation to the nervous system no investigations of this kind have been undertaken.

The discovery of morphological changes in the CNS of the second generation offspring of neurosensitized females may facilitate our understanding of an inborn predisposition to nervous and mental diseases.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats. Females were given an intraperitoneal injection of a 20% saline solution of cortical antigen in a dose of 0.3 mg/200 g body weight three times at intervals of 24 h; the blood antibody titer was determined by the cold version of the complement fixation test. The rats were mated with healthy males 21 days after the first injection. Female offspring born as a result, on reaching sexual maturity (2 months, weight about 200 g) were mated with intact males, and the sensomotor cortex of the offspring of these females was studied at different times after birth — from 2 to 90 days. Intact animals of the same age served as the control.

The ordinary techniques of embedding and staining of the material were used for the electron-microscopic investigation. Preparations were photographed under the Tesla-540 electron microscope. The technique of the cytointerferometric investigation was described in detail previously [4].

EXPERIMENTAL RESULTS

Neurons with a loosely textured nucleus and with numerous vacuoles, both small and large, in it were found quite frequently in the cortex of young rats 2 days old. The mitochondria of the cytoplasm exhibited great variability — from completely intact to swollen, with destroyed cristae. Single ribosomes were often seen against the background of rosettes of polysomes.

The nuclei of some neurons in rats aged 10 days and 2 weeks contained vacuoles surrounded by one or several layers of membranes, often with wavy outlines. Neurons with increased lability of their nuclear membranes were often found (Fig. 1).

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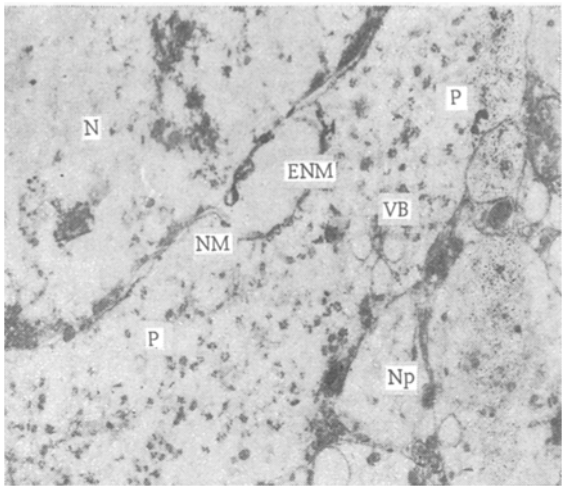

Fig. 1. Neuron of sensomotor cortex of 10-day-old rat, second-generation offspring of neurosensitized females. N) Nucleus, NM) nuclear membrane, ENM) evagination of nuclear membrane, VB) vesicular bodies; P) polysomes; Np) neuropil. 20,000×.

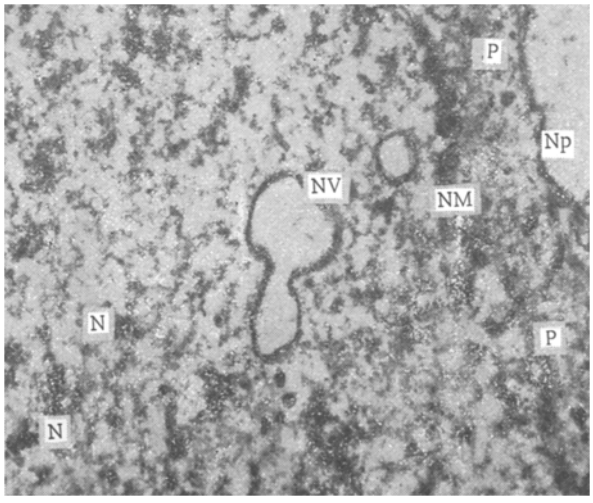

Fig. 2. Sensomotor cortical neuron of rat aged 30 days, second-generation offspring of neurosensitized females. NV) nuclear vacuoles. Magnification 20,000×. Remainder of legend the same as to Fig. 1.

In rats aged 1 month, besides many intact cells there were neurons with varied degrees of vacuolation of the nuclei and lability of the nuclear membranes. The cytoplasm of some neurons contained swollen mitochondria, an increased number of vesicular bodies and free ribosomes, as well as dilated tubules of the endoplasmic reticulum.

The ultrastructure of cortical neurons of rats aged 2 months had the same features as previously (Fig. 2), but the number of intact cells was even greater.

A characteristic feature of the cerebral cortex of the rats of these offspring at all times of the investigation was a large number of neurons with dense dark cytoplasm and nucleus (Fig. 3).

In animals aged 3 months the ultrastructure of most cortical neurons was indistinguishable from the control. Often cells with a loose, vacuolated nucleus and swollen mitochondria were seen.

Cytointerferometric investigation of the cortical cells of rats aged 1 month showed a decrease in the protein content in the nucleus and, in particular, in the cytoplasm compared

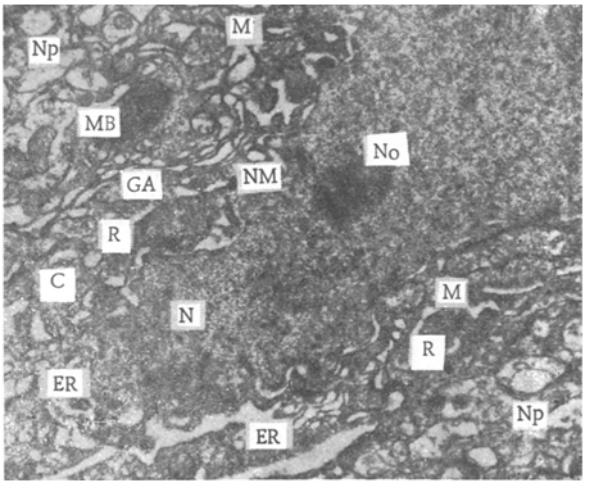

Fig. 3. Sensomotor cortical neuron of rat aged 2 months, second-generation offspring of neurosensitized females. N) Nucleus (dense); No) nucleolus; C) cytoplasm (dark); M) mitochondria; ER) endopolasmic recticulum; R) ribosomes (free); MB) myelin-like body; GA) Golgi apparatus. 15.000×. Remainder of legend as to Fig. 1.

with normal (Table 1): 38 pg in the nucleus and 118 pg in the cytoplasm, whereas normally the corresponding values are 56.6 and 166.6 pg. In rats aged 3 months the protein content in the nucleus and cytoplasm was increased to 68 pg in the nucleus and 178.3 pg in the cytoplasm. However, these values were lower than in the control (77.5 and 202.7 pg, respectively).

The first point to note during analysis of these results is the milder degree of ultrastructural changes in cortical neurons of the second-generation than of the first-generation offspring. One sign of this is the large number of cells with unchanged ultrastructure, another the absence of membranous inclusions of such a diversity of shapes in cortical neurons of the second generation as were found in animals of the first generation [1, 3].

The main distinguishing feature was a tendency for the subcellular organization to be restored in animals of the second generation in the late postnatal period (2 months and, in particular, 3 months), which was not observed in the first-generation offspring of the neurosensitized females.

A characteristic response of neurons of the second generation of rats was the large number of dark cells with a dense nucleus and cytoplasm. It is difficult to explain why this large number of hyperchromic neurons should appear. It has been shown that the level of metabolism in such cells is depressed in both nucleus and cytoplasm [5, 6, 9]. The presence of numerous free ribosomes in their cytoplasm is evidence of lowering of the level of protein synthesis and of neuronal activity [5, 7, 8]. Many hyperchromic cells have been observed in hypoxia, ischemia, and swelling of the brain [1, 10], under the influence of neuroleptics [6], and during procedures inhibiting neuronal function [5]. It is possible that in the present experiment the dark cells, saturated with free ribosomes, may have a lowered level of activity, as reflected in the low protein content in the nucleus and cytoplasm (Table 1).

The mechanisms of many of the phenomena discovered are unknown. For example, membranous inclusions, undergoing structural transformation during growth of the animal, were found in the nuclei of cortical neurons of the first-generation offspring [2]. Virtually no such complex formations were present in the nuclei of neurons of the second generation. Vacuolation of nuclei of some neurons was observed in the second generation at all times of investigation during postnatal development. This phenomenon may be the result of the effect of pathological changes in the nuclear structure of cortical neurons of the parental generation: It is possible that the mothers produced antibodies against nuclear components, and these could have affected the process of formation of nuclear structures of the neurons of the offspring while still in the intrauterine period.

By the age of 3 months the ultrastructure of the neurons was mainly back to normal: The number of dark cells was reduced and the protein index showed a tendency to recover and returned approximately to normal.

TABLE 1. Changes in Content of Solids (in pg) in Nucleus and Cytoplasm of Large Neurons in Layer V of Sensomotor Cortex of Second-Generation Offspring, Aged 30 and 100 Days, of Neurosensitized Female Rats (M ± m)

Time after birth, days	Mean content of solids in nucleus and cytoplasm of cortical neurons			
	control, offspring of intact rats	experiment, second-generation offspring	% of control, taken as 100	
30	56,5±1,6	48,0±1,3	85,0	0,001
	166,4±4,2	138,3±2,0	83,1	0,001
100	77,5±3,9	68,0±2,5	87,7	0,005
	202,7±3,9	178,3±7,6	88,0	0,005

Legend. Numbers above line indicate content of solids in nucleus; below line, in cytoplasm.

Neurosensitization of females before pregnancy thus affects the morphological and functional state of the cortex of offspring not only of the first, but also of the second generation. Changes in the animals of the second generation were less marked than in the first, and by contrast with the first, they exhibit a compensatory trend with age.

LITERATURE CITED

1. N. N. Bogolepov and T. V. Vorob'eva, in: Diagnosis and Surgical Treatment of Cerebrovascular Diseases [in Russian], Leningrad (1974), p. 49.
2. G. R. Dubinskaya, Byull. Éksp. Biol. Med., No. 11, 571 (1980).
3. G. R. Dubinskaya and G. F. Konokotina, Zh. Nevropatol. Psikhiat., No. 7, 875 (1979).
4. P. B. Kazakova and G. F. Konokotina, Byull. Éksp. Biol. Med., No. 1, 11 (1982).
5. Yu. N. Kvitnitskii-Ryzhov and T. Yu. Kvitnitskaya-Ryzhova, Tsitologiya, No. 2, 116 (1981).
6. V. N. Kleshchinov and N. S. Kolomeets, Arkh. Anat., No. 1, 7 (1983).
7. A. V. Nemtsov and G. R. Dubinskaya, Byull. Éksp. Biol. Med., No. 4, 64 (1975).
8. M. Petermann, Physical and Chemical Properties of Ribosomes [in Russian], Moscow (1967), p. 235.
9. Z. Ya. Rubleva, Yu. I. Savulev, and A. S. Pylaev, Zh. Nevropatol. Psikhiat., No. 7, 966 (1977).
10. E. Carrascal, R. Campora, M. Bullen, et al., Arch. Neurobiol., 40, 165 (1977).
11. S. Zamenhof, E. Marthines, et al., J. Nutr. Metab., 14, 262 (1972).