it was possible not only to discover that the cerebellar hemisphere is more vulnerable in systemic circulatory arrest, but also to distinguish its lateral zone as the functional region of the hemisphere which suffers the greatest damage.

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EFFECT OF PRELIMINARY NEUROSENSITIZATION OF FEMALE RATS ON CONTENT OF DENSE SUBSTANCES IN CORTICAL NEURONS OF THE PROGENY

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KEY WORDS: neurosensitization; progeny; neuron; content of dense substances.

The authors showed previously [5, 6] that sensitization of female rats before the beginning of pregnancy with cerebral cortical iosantigen disturbed the course of postnatal neuro-ontogeny in the offspring. These disturbances are expressed as retarded growth of the neurons in size, of the layers of the cortex in width, and of the total and perineuronal glial cells in number. A progressive decrease with age in a quantity of basophilic substances in the cytoplasm of the neurons was found. Since the content of basophilic substances in the cytoplasm of neurons is known to correlate with intracellular protein synthesis and accumulation [8, 10], it was postulated that a disturbance of protein accumulation takes place in the cortical neurons of the progeny of neurosensitized rats.

This paper gives the results of a cyto-interferometric investigation of the content of dense substances (protein) in the large cells of layer V of the sensomotor cortex at different stages of postnatal life of the progeny obtained from neurosensitized rats.

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TABLE 1. Content of Dense Substances in Nucleus and Cytoplasm of Large Neurons in Layer V of Sensomotor Cortex of 30- and 100-Day Old Progeny of Previously Neurosensitized and Intact Female Rats

Age of animals, days	Mean content of substances, pg (M ± m)		Percent of control, taken as 100	P
	control	experiments		
30	56,5±1,6 / 166,4±4,2	35,6±1,4 / 112,3±3,9	63,1 / 67,5	<0,001 / <0,001
100	77,5±3,9 / 202,7±8,7	57,5±1,8 / 112,4±3,9	74,2 / 55,5	<0,001 / <0,001

Legend. Number above the line represents content of dense substances in nucleus, number below the line - in cytoplasm; each mean was calculated on the basis of measurements for 150 neurons.

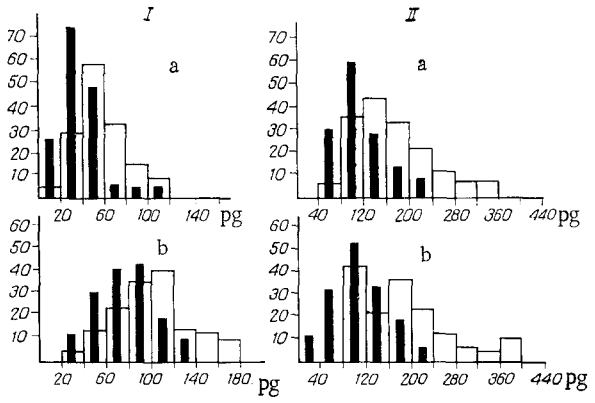

Fig. 1. Histograms of distribution of large neurons in layer V of sensomotor cortex of experimental and control rats based on content of dense substances in nucleus (I) and cytoplasm (II). Animals aged: a) 30 days, b) 100 days. Unshaded column — progeny of intact rats, black column — progeny of neurosensitized rats. Abscissa, content of dense substances in classes.

EXPERIMENTAL METHOD

Noninbred albino rats were used. The method of neurosensitization of the female rats before mating was described previously [6]. The brain of the 30- and 100-day-old progeny of neurosensitized and intact (control) rats was fixed in Carnoy's fluid, dehydrated in alcohols, and embedded in paraffin wax. Unstained sections were glued to protein-free slides and the thickness of the sections was measured with the MIS-11 microscope. After dewaxing the sections were mounted under a coverslip in distilled water and examined in the BINAM L-211 interference microscope. Dense substances (dry weight) of the large cells in layer V of the sensomotor cortex were determined in monochromatic light (wavelength 535 nm) by means of a Senarmon rotary analyzer and compensator (objective 40, ocular 10). The length of the longest (A) and shortest (B) mutually perpendicular axes of an ellpise mentally inscribed within the circumference of the body or nucleus of the neuron was determined with a screw-operated MOV-15x ocular micrometer. The area (S) of the body of the neuron and its nucleus were calculated by the equation $S = \pi ab/4$. The area of the cytoplasm was given by the difference between the areas of the body and nucleus of the cell. The dry weight (m) of the nucleus and cytoplasm was calculated by the equation [2]: $m = \frac{S\delta k}{100\rho}$ (in pg, where δ is the linear value of the phase shift; k a correction for differences in the thickness of the sections, equal to the ratio of the thickness of a 5-μ section to its measured thickness; ρ the coefficient of specific increase in the refractive index, equal to 0.0018). Five pairs of animals were used at each time of investigation (five in the experiment and five in the control); 30 cells were measured in each animal. The content of dense substances was calculated for each neuron. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Mean data on the contents of dense substances in the nucleus and cytoplasm of neurons of the control and experimental animals are given in Table 1. They show that the content of dense substances in the cytoplasm and nucleus of neurons of the 30-day-old experimental animals was significantly lower than that of the control animals of this same age. In neurons from 100-day-old control animals the dry weight was significantly increased in both nucleus and cytoplasm. In the 100-day-old experimental animals an increase in the content of dense substances was observed only in the nucleus; the increase, however, reached only the level found in 30-day-old control animals. Meanwhile the dry weight of cytoplasm of neurons in the 100-day-old progeny remained at its previous level, i.e., that in animals aged 30 days. As a result of this, the difference in the content of dense substances between the experimental and control data was even greater in 100-day-old animals.

Variability in the distribution of dense substances among individual classes of neurons in rats of the different age groups (Fig. 1) reveals a definite dynamics in the control animals. Two dominant classes appeared at the age of 100 days, with maximal dry weight of cytoplasm (80-120 and 160-200 pg) instead of one such class (80-120 pg) at the age of 30 days. In the nucleus, toward the age of 100 days a shift of the dominant class with the maximum of the content of dense substances to the right (80-120 pg) was observed. Meanwhile in the experimental animals the character of distribution of dense substances with age showed no significant change, although in the cytoplasm of neurons of 100-day-old rats the appearance of a class with a lower protein content than the results obtained with 30-day-old animals was observed.

The experimental results show that preliminary neurosensitization of female rats with cerebral cortical isoantigen disturbs the process of accumulation of dense substances (proteins) in the cortical neurons of the brain during the postnatal life of the progeny, expressed as a sharp delay in the increase in dry weight of the neurons. The increase in dry weight of the nuclei of the neurons in 100-day-old experimental animals did not exceed its level in the 30-day-old control rats and was unable to compensate for the deficit of proteins in the nucleus, still less in the cytoplasm, where no increase in their content with age was observed as a general rule.

The progressive deficiency of proteins discovered in the cerebral cortical neurons of the sensitized progeny, in the writers' opinion, reflects a decrease in protein synthesis in the neurons, which has been demonstrated by biochemical studies with the aid of labeled amino acids under similar experimental conditions [9]. This is a logical conclusion if it is recalled that intensification of protein synthesis and accumulation of neurons during ontogeny is closely linked with the differentiation and maturation of nerve cells [1, 11]. If the increase in variability observed by the present writers and other workers [3] in the distribution of dense substances in different classes of cortical neurons indirectly reflects the above-mentioned structural changes in normal animals, the absence of any such dynamics in the experimental animals in the presence of a marked protein deficiency must be evidence of considerable slowing of postnatal neuro-ontogeny. The morphological signs of a disturbance of maturation of cortical neurons and glial cells observed previously [5] in similar experimental material can be explained by the results of the present investigation.

The mechanism of the influence of neuroautoimmune reactions on the developing brain has not yet been fully explained. It can be tentatively suggested that maternal antibrain autoantibodies pass through the placental barrier [7] and may injure the synthetic apparatus of the nerve cells in the antinatal period. This disturbance is manifested to the full and becomes aggravated during the postnatal life of the animal. This hypothesis is perhaps confirmed by the electron-microscopic data obtained previously: the appearance of membranous inclusions and age-progressive vacuolation of the nucleus in cortical neurons of a similar progeny [4].

Considering data in the literature on correlation between protein synthesis and the increase in dry weight of neurons, on the one hand, and the increase in variability of their size during functional maturation of nervous structures, on the other hand, it can be concluded from the results of these investigations not only that development of neurons is retarded, but also that the cerebral cortex as a whole is functionally immature in the progeny of neurosensitized rats. This may perhaps be one cause of the depression of learning processes and the absence of its dynamics observed in 2-month-old animals [5].

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ASYMMETRICAL CHANGES IN HIND LIMB MUSCLE TONE IN RATS AFTER INJECTION OF EXTRACTS FROM THE LEFT AND RIGHT HALVES OF THE BRAIN

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KEY WORDS: peptides; opiate; lateralization of the brain; postural asymmetry.

Brain extracts from rats with unilateral injuries [2-4] or with stimulation [7] of symmetrical structures, if injected into healthy animals, give rise to postural changes in the latter which depends on the character and side of the intervention of the donors. For instance, intracranial injection of brain extracts from animals with experimental vestibulopathy due to hyperactivation or destruction of the vestibular nuclei of Deiters [8] is accompanied by changes of muscle tone in the recipients, which may be in various directions [7]. The further study of this phenomenon was dictated by the need to explain the more general problem associated with chemical lateralization of the brain [12], namely to study the effects of extracts from the left and right halves of the normal brain.†

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. The animals were decapitated, the brain with the cerebellum and medulla was removed, and the brain was divided along the midline into right and left halves, frozen in liquid nitrogen, and kept at −20°C. The frozen tissue was placed in a homogenizer containing 1M acetic acid warmed to 90°C, the homogenizer was kept for 5 min in a boiling water bath, after which the tissue was dispersed for 10 min with a glass pestle [9]. In some experiments extraction with a mixture of chloroform and methanol was used [11]. The suspension was cooled and centrifuged at 10,000 rpm for 15 min (K-24, East Germany). The supernatant was collected and its pH adjusted to 7.0 with concentrated ammonia solution. The residue was separated by filtration and the resulting solution lyophilized. To study the sensitivity of the extracts to proteolysis, a preparation of pronase was used (specific activity 45,000 units/g, from Calbiochem, USA). Samples 1 ml in volume, containing 1.2-1.6 mg "protein" [13] of the extracts, 100 μg pronase, 5×10^{-4}M $CaCl_2$ in 0.05 M borate buffer, were incubated at 37°C for 2 h. The reaction was stopped by immersing the samples in a boiling water bath and heating them for the next 10 min. In some experiments

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†The preliminary data were published previously [6].

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