NUCLEAR AND CYTOPLASMIC RNA IN VISUAL CORTICAL NEURONS OF ADULT RATS DURING VISUAL DEPRIVATION AND PHOTIC STIMULATION

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KEY WORDS: neuron; glia; RNA; visual and motor cortex; visual deprivation; photic stimulation.

Previous investigations [5, 8] showed that functional visual deafferentation in adult animals is accompanied by accumulation of RNA in neurons and perineuronal glial cells in the surface layers of the visual cortex. The main part of the body of cells of this type consists of nucleus, so that it can be predicted that the marked shift in total RNA content in the cell will be due to corresponding changes in nuclear RNA. It was decided to compare the effect of analogous functional deafferentation (due to keeping the animals for a long period in complete darkness) separately on the content of nuclear and cytoplasmic RNA in cells characterized by the opposite relations between nucleus and cytoplasm, namely by predominance of weight of cytoplasm over weight of nucleus.

The object of the present investigation was the cytospectrophotometric determination of quantitative changes in nucleic acids in the nucleus and cytoplasm of large neurons in layer V of the visual cortex of adult rats kept in darkness. For comparison the content of nuclear and cytoplasmic RNA was determined in these same neurons during stimulation by flashes and continuous light, and also (to verify the specificity of the changes observed) in neurons and glia of layer V of the motor cortex.

EXPERIMENTAL METHOD

Adult male rats were kept for 30 days in complete darkness. At the end of this period of visual deprivation some of the animals were stimulated by flashes (frequency 2 Hz) or by a constant light with an intensity of 40 lx for 2 h. For comparison, a group of intact rats also was stimulated by similar flashes or continuous light for 2 h. Control rats were kept for 30 days under standard animal house conditions, in weak, scattered light, and with the ordinary daily rhythm (12 h daylight and 12 h darkness).

All the animals were decapitated without anesthesia, the visual and motor areas of the cortex were removed, fixed by Carnoy's method, and embedded in paraffin wax. In sections stained with gallocyanin and chrome alum the concentration of nucleic acid was determined in the nucleus and cytoplasm of the neurons and also their total concentration for the whole cell body of the perineuronal neuroglia of layer V, by two-wave cytospectrophotometry at 550 and 465 nm. Allowing for the volumes of the cells, nuclei, and cytoplasm, the total content of nuclear and cytoplasmic nucleic acids was calculated (per cell) in relative units. Details of the measurements and calculations, a description of the experiments, and the scheme of the two-wave cytospectrophotometer were given previously [5, 6].

Each mean value for the nucleic acid content was calculated by photometry of 360-600 individual cells, taken from six animals. All calculations and statistical analysis of the numerical results by Student's t-test were carried out on the Minsk-22 computer.

EXPERIMENTAL RESULTS

Although the method of staining used (gallocyanin and chrome alum) revealed total intracellular RNA + DNA, since the DNA content in cells of the mature nervous system is constant,

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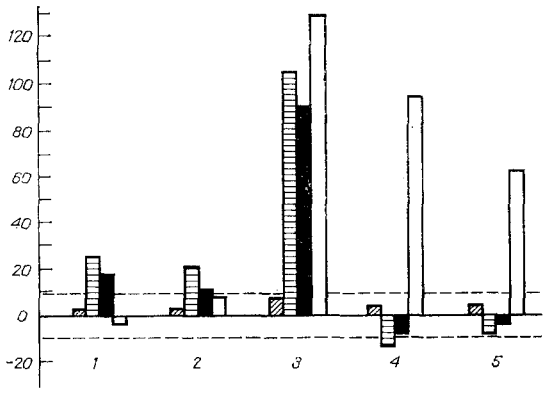

Fig. 1

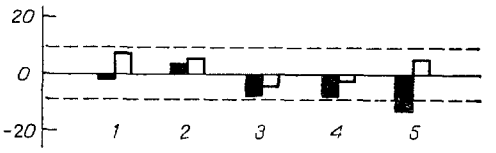

Fig. 2

Fig. 1. Changes in RNA content in neurons and perineuronal neuroglia of layer V of visual cortex of rats exposed to visual deprivation and photic stimulation. Ordinate — relative changes in RNA content compared with control (100%). Oblique shading — nucleus of neurons, horizontal shading — cytoplasm of neurons, black columns — whole body of neurons (nucleus + cytoplasm), unshaded columns — whole cell body of perineuronal neuroglia. Broken lines denote confidence interval for $P < 0.1$. 1) Flashes, 2) continuous light, 3) deprivation, 4) deprivation + flashes, 5) deprivation + continuous light.

Fig. 2. Changes in RNA content in neurons and perineuronal neuroglia of layer V of motor cortex of rats exposed to visual deprivation and photic stimulation. Legend as in Fig. 1.

all changes detected in the total nucleic acid content can be ascribed entirely to RNA.

As Fig. 1 shows, both types of photic stimulation (flashes and continuous light) led to marked RNA accumulation in neurons of layer V of the visual cortex in intact rats. An even greater increase in RNA content was observed in the animals after visual deprivation. In all cases this increase in RNA was localized to the cytoplasm of the neurons: no statistically significant changes could be found in the nucleus. Similar accumulation of cytoplasmic RNA was found in spinal motoneurons [1] after functional motor deafferentation in rats kept for a long time under conditions of hypokinesia.

In an extensive series of investigations [2-4, 7, 8] prolonged visual deprivation led to a decrease in the protein content in the cytoplasm of layer V neurons of the visual cortex. However, those experiments were conducted on newborn animals, in which this functional deafferentation acted on the maturing nervous system. In the present experiments, on the other hand, adult animals were used, in which all connections in the visual system were completely formed. It was this which was evidently responsible for the opposite changes in nucleic acid content in the visual cortical neurons under the influence of early visual deprivation and visual deprivation in adult animals, respectively.

RNA accumulation may be due to stimulation of RNA biosynthesis or a reduction in its breakdown. In the writer's opinion, the second explanation is more likely to be true in these experiments. In models of early visual deprivation it has repeatedly been shown that incorporation of labeled precursors into RNA and proteins of the visual cortex is not stimulated by visual hypofunction, but inhibited [9, 11, 12]. Photic stimulation, on the other hand, leads to activation of incorporation of precursors into RNA of visual nervous structures [10, 12]. Nevertheless, as Fig. 1 shows, photic stimulation of visually deprived animals not only did not give an additive effect but, on the contrary, it led to complete normalization of the cytoplasmic RNA content in the neurons. Photic stimulation did not cause changes in RNA content in the glial cells of intact animals, yet in deprived animals it was still possible to detect a definite normalizing effect of stimulation, although it was quantitatively very weak, especially when flashes were used (Fig. 1).

Investigations by Gershtein [2-4] have shown that keeping animals for a long time from birth in darkness causes a decrease in the protein content in the visual cortical neurons but an increase in protein content in motor cortical neurons. I also determined the RNA content in motor cortical neurons under conditions of visual deprivation. However, this type

of deprivation had no effect on the RNA content either in neurons or in neuroglia in layer V of the motor cortex (Fig. 2).

The results of cytospectrophotometric determination in the cells of the visual cortex indicate definite compartmentalization of RNA metabolism within the neuron–neuroglia system. The nucleus of neurons is metabolically the most stable part; Neither the DNA content nor the RNA content in it (Fig. 1) was changed under the influence of hyper- or hypofunction of the neurons. The cytoplasm of these neurons, and also their glial satellite cells were characterized by greater functional-metabolic lability; meanwhile functionally determined changes in their RNA content were not identical (Fig. 1). It can be tentatively suggested that the presence of at least three compartments in the neuron–neuroglia system, with different levels of sensitivity of metabolism of their macromolecules to functional loads, makes the whole system more flexible and more adaptable to constantly changing conditions of function.

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STRUCTURAL ORGANIZATION OF SYNAPTIC JUNCTIONS OF DEVELOPING ANTERIOR HORN NEURONS OF THE EARLY HUMAN FETAL SPINAL CORD

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KEY WORDS: prenatal human ontogeny; spinal cord; synaptogenesis; synaptic complexes.

The structural organization of developing synapses, especially in early prenatal ontogeny, continues to attract the attention of neurobiologists of widely different specialties. However, despite the steady increase in the number of investigations in this field, synaptogenesis still largely remains unexplained, more especially in man [1-5].

For these reasons a systematic study of the formation of interneuronal connections during human prenatal ontogeny has been started [4, 5]. In the present investigation an attempt was made to establish the basic principles governing the structural organization of synapses in anterior horns of the human spinal cord in the early fetal period of antenatal development (11th-12th weeks of pregnancy).

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