HYSTOCHEMICAL INVESTIGATION OF CERTAIN PROTEINS IN THE CELL STRUCTURES OF THE OPTIC ANALYZER OF ANIMALS BORN IN A STATE OF PREMATURITY (RABBITS) AND MATURITY (GUINEA PIGS) DURING ONTOGENESIS

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Reports in the literature describing biochemical and histochemical investigations point to the participation of proteins in the specific activity of nervous tissue. Differences have been found in the composition and distribution of proteins in functionally differing structures of the brain [7, 8, 16, 19, 20, 26]. It has also been found that the concentration, composition, and distribution of proteins in nervous tissue undergo changes during ontogenesis [1, 14], corresponding to their morphological and functional differentiation [1, 15].

A correlation has been demonstrated between the changes in the concentration and distribution of nucleic acids in the neurons and the establishment of the functions of individual structures of the nervous system [1]. Changes in the RNA content during functional activity of the nervous system have been described [2, 27].

We have made a comparative study of the concentration and distribution of proteins and nucleic acids in the neurons of individual cell structures of the optic analyzer in animals born in states of prematurity (the rabbit) and maturity (the guinea pig) in the course of postnatal ontogenesis. It was assumed that such an investigation would supply additional facts bearing on the association between the functional, morphological, and chemical differentiation of the structures of various elements of the analyzer, as demonstrated histochemically, in the course of ontogenesis.

EXPERIMENTAL METHOD

Investigations were made of the anterior colliculi, the lateral geniculate bodies, and the cortical end of the optic analyzer of 104 rabbits and 68 guinea pigs at different stages of postnatal ontogenesis (at birth, at the age of 1, 3, 5, 8, 11, and 15 days and 1 and 3 months, and after sexual maturity). The morphological data of Rose, Winkler, and Potter were used when taking the material [31, 32, 35].

The concentration and distribution of protein SH- and SS-groups [6, 22], NH_2-groups [7, 34], and COOH-groups [6, 23] were studied in fixed material.

The reaction for the NH_2-group, as described by Pearse [30], gives an indication of the presence of terminal NH_2-groups of protein and of NH_2-groups of the amino acid lysine. By means of the reaction for COOH-groups it was possible to judge the distribution and concentration of proteins containing glutamic acid in their composition. The reaction for thiol groups demonstrated the presence of proteins containing the amino acids cystein and cystine in their composition. It is known that glutamic acid and the sulfur-containing amino acids play an important role in the maintenance of the specific function of the nervous system [12, 33]. The concentration and distribution of nucleic acids (RNA and DNA) were also determined by the reactions of Brachet and Feulgen. The relative concentration of the functional groups of the proteins in the layers of the optic cortex was assessed by means of a type MF-4 microphotometer.

The morphological maturity of the individual layers of the cortical end of the optic analyzer in the various stages of ontogenesis was also judged from their width, which was measured by the usual method under the microscope. As a rule the measurements were made at a distance of 5-7 mm from the sagittal sulcus.

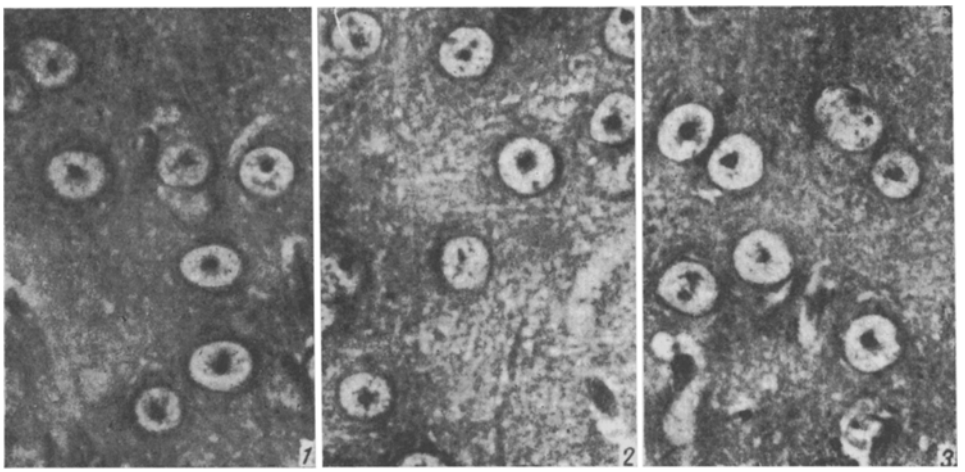

Fig. 1. Distribution of protein COOH-groups in layer IV of the optic cortex of a guinea pig. 1) Newborn; 2) 8 days old; 3) sexually mature. Method of Barrnett and Seligman. Objective 60×, ocular 6×.

EXPERIMENTAL RESULTS

Measurements of the width of the individual layers of the cortex showed that in the animals born in a mature state (guinea pigs) formation of the cortex is largely complete at birth, and that subsequently it grows only very slightly. In the animals born in an immature state (rabbits) formation of the cortex ends a considerable time after birth (about 3 months). From birth until the stage of sexual maturity the total width of the optic cortex in the rabbit increases 2.4 times, and the greatest increase (threefold) occurs in layers II, III, and IV, associated with the performance of complex associative and integrative functions.

It was shown by Brachet's reaction that no significant changes in the concentration and distribution of RNA and DNA took place in the neurons of all structures investigated in guinea pigs during postnatal ontogenesis.

In the newborn rabbit most neurons in the lateral geniculate bodies, the anterior colliculi, and layers V, VI, and VII of the cortex have only a small amount of cytoplasm containing little RNA; a larger amount of RNA is found in the cytoplasm of some nerve cells. The nuclei contain a relatively high concentration of DNA. By the 5th-8th day the amount of cytoplasm has risen sharply, and its content of RNA also shows a considerable increase. Because of growth of the nucleus, the concentration of DNA falls. On the 11th-15th day, i.e., at the time of beginning to see, the cytoplasm becomes rich in RNA and the DNA concentration falls still further on account of a continuing increase in the volume of the nucleus. At this time the concentration and distribution of the nucleic acids in the neurons are similar to those in sexually mature animals. The resemblance becomes complete at the age of about 1 month.

In the very densely packed nerve cells of layers II, III, and IV of the cortical end of the analyzer of the newborn rabbit a high concentration of DNA is seen in the small nuclei; no RNA can be observed in the cytoplasm. On the 5th-8th day, on account of growth of the nerve cells, the DNA concentration in their nuclei has fallen and their cytoplasm contains traces of RNA. On the 11th day the presence of RNA can be clearly distinguished in the cytoplasm of the nerve cells. At the end of the 1st month, when the cytological differentiation of the neurons has attained a high level, the nuclei contain very little DNA but their cytoplasm is rich in RNA. At the end of 3 months more or less the same concentration and distribution of nucleic acids have been established as in the neurons of the same name in sexually mature rabbits. The gradual maturation of the neurons of individual brain structures has also been observed by other investigators [1, 3, 13, 14].

In sexually mature rabbits and guinea pigs the relative concentration of proteins in the upper layers of the cortex (layers II and IV) is equal in the majority of structures. The cytoplasm of the bodies of the overwhelming majority of nerve cells and their processes, the neuroglial cells, and the endothelial cells of the blood vessels contain equal concentrations of all the proteins studied. In other words, in the cytoplasm of the bodies of the neurons and of the remaining tissue elements of these layers of the cortex or, as will be shown later, in the structures situated

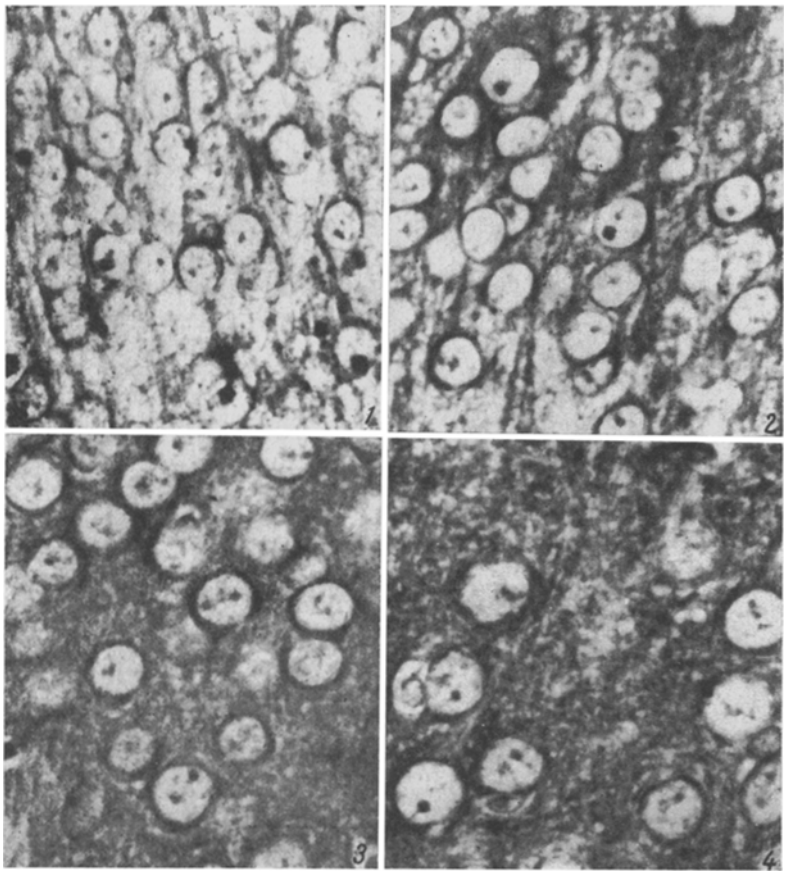

Fig. 2. Distribution of protein COOH-groups in layer IV of the optic cortex of of a rabbit. 1) Newborn; 2) 8 days old; 3) 11 days old; 4) sexually mature. Method of Barrnett and Seligman. Objective 60×, ocular 6×.

between the bodies of the neurons, equal amounts of proteins are present in unit area. In layers III and V the apical portions of the bodies of the neurons, including the apical dendrite, contain less proteins, and the basal portions slightly more, than the surrounding structures. In layers VI and VII the cytoplasm of the bodies of the neurons contains proteins in the same, or a slightly higher concentration, compared with the structures situated between the bodies of the neurons. In the anterior colliculi and lateral geniculate bodies many neurons may be seen in which the protein concentration is higher than in the other structures.

In the neurons of all the structures of the analyzer protein COOH-, SH-, and SS-groups are found in high concentration in the microstructures of the nucleus: the membrane, nucleolus, and chromatin granules; relatively few are found in the karyoplasm. NH_2-groups are also present in fairly high concentration in the karyoplasm.

In the newborn guinea pig the concentration and distribution of functional protein SH-, SS-, COOH-, and NH_2-groups, according to the results of microscopic examination and photometric measurements of the layers of the cortical end of the analyzer, are mainly identical in the structures of all its links with those found in the sexually mature animals (Fig. 1). This agrees with reports of investigations showing that the brain of the guinea pig at birth has attained almost complete maturity as regards its cytoarchitectonic structure, the biochemical processes of its electrical activity, and its ability to form conditioned reflexes [4, 18, 21, 24, 25, 28, 29].

In rabbits, in contrast to guinea pigs, during postnatal ontogenesis important changes are observed in the concentration and distribution of proteins in the cell structures of the various links of the analyzer, appearing in correlation with morphological and functional changes. In the lateral geniculate bodies, the anterior colliculi, and layers V, VI, and VII of the optic cortex of the newborn rabbit, the bodies of the neurons contain more proteins than the structures situated between them. In layers II, III, and IV of the optic cortex, cells of neuroblastic character are

found, giving intensive reactions for NH_2-, SH-, and SS-groups of proteins in their nuclei. The structures surrounding these nerve cells contain lower concentrations of proteins. The cytoplasm of the nerve cells does not differ in the intensity of its reaction from the surrounding structures. In the nuclei of the nerve cells the concentration of COOH-groups is the same as or slightly lower than in the other structures.

During the first 1-3 months of postnatal ontogenesis, in all the cell structures of the analyzer, the protein concentration rises in the cytoplasm of the bodies of the neurons and, in particular, in the intervening structures. The largest changes in the protein concentration take place just before the animal acquires sight (1st-11th day). At the age of 2 weeks the distribution of protein COOH-, SH-, and SS-groups in the neurons of all the investigated structures of the optic analyzer is similar to that in the sexually mature animals; in the subcortical formations and in layers V, VI, and VII of the cortical end of the analyzer this become visible 2-3 days earlier (by the 8th-12th day) than in layers II, III, and IV of the cortical end (by the 11th-15th day after birth). The protein concentration after the rabbit has become able to see continues to increase very slightly.

The characteristic distribution of protein NH_2-groups found in sexually mature rabbits is established later in ontogenesis than that of the SH-, SS-, and COOH-groups: in the lateral geniculate bodies, the anterior colliculi, and in layers V, VI, and VII of the cortical end of the analyzer by the 15th day, and in layers II, III, and IV at the end of the 1st month of postnatal life. However, the concentration of protein NH_2-groups has not reached at this time the characteristic level observed in sexually mature animals (Fig. 2).

This is also demonstrated by oytophotometry of the layers of the cortical end of the analyzer in the rabbit. In the newborn rabbit and at the age of 1 day the maximal relative concentration of SH- and NH_2-groups is found in layers II and IV. Layer III and, in particular, layers V, VI, and VII are characterized by a lower protein concentration. Protein COOH-groups are more numerous in layers II and III. By the 8th day the concentration of protein SH-groups becomes maximal in layers IV and V, as also in the sexually mature rabbits. The concentration of protein COOH-groups characteristic of the sexually mature animal is established on the 3rd-5th day after birth, and the concentration of protein NH_2-groups by the 30th day.

Our findings concur with morphological [10, 11], electrophysiological [5, 17], and physiological [4, 9] data regarding the gradual maturation of the structure and establishment of the function of the various parts of the rabbit's brain during ontogenesis; the most important changes are observed during the first 2 weeks of life. Hence, the cytochemical differentiation (in accordance with the tests used) of the individual formations of the optic analyzer in animals born in a state of maturity (guinea pigs) is largely complete at birth, but in animals born in a state of immaturity (rabbits) it continues until later stages of postnatal ontogenesis, the most marked changes taking place in the period preceding the acquisition of sight.

SUMMARY

A histochemical study was made of the content and distribution of DNA, RNA, and SH-, SS-, COOH-, NH_2-groups of proteins in the laterne geniculate bodies, anterior colliculi and in the visual cortex of rabbit and guinea pigs during postnatal ontogenesis.

At birth the above-mentioned structures of the visual analyzer of the guinea pig exhibited a high degree of cytochemical differentiation and did not differ much from those of the sexually mature animals. In the prematurely born animals (rabbit) cytochemical differentiations completed at a later stage of ontogenesis. The most pronounced changes in the content and distribution of the substances studied were observed during the period preceding the first opening of the eyes.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
