DEVELOPMENT OF THE NEURONS OF THE TROCHLEAR NERVE NUCLEUS IN DOGS DURING ONTOGENESIS

(UDC 611.831.4-018.822-013-019)

R. A. Zumerov

Department of Histology (Head, Professor M. B. Novikov), Astrakhan Medical Institute
Scientific supervisor of work, Professor N. G. Fel'dman
(Presented by Active Member AMN SSSR, V. N. Ternovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 12,
pp. 102-105, December, 1965
Original article submitted June 4, 1964

Very few works [1,2] are devoted to a neurohistological study of the oculomotor apparatus. The neurohistological and histochemical structure of the neurons of the trochlear nucleus during ontogenesis and in connection with the start of functioning of the visual analyzer are especially inadequately investigated.

In the present work, the neurons of the trochlear nucleus during ontogenesis are investigated.

METHOD

The work was carried out on dogs of different ages, beginning with 30-day fetuses and ending with mature animals. The brain was fixed in 12% neutral formalin, 96° alcohol, absolute alcohol, and Muller's, Carnoy's, and Schaeffer's mixtures. The sections were impregnated by the methods of Campos, Buck-Bielschowsky, Bielschowsky-Gros as modified by B. I. Lavrent'ev, and Cajal-Favorskii; staining was by Nissl, for RNA (after Brachet) and DNA (after Feulgen). We determined the basic and total proteins by staining with fast green and by bromophenol blue. To elicit the strength of the RNA bond with the proteins of the nerve cell, the sections were subjected to the action of solutions of crystalline enzymes, pepsin and trypsin, at 37° for from 15 min to 3 h. The control sections were incubated under the same conditions, but without the addition of enzymes. After incubation, we determined the RNA in the sections [3]. We studied the areas of the cell nuclei of the trochlear nucleus by measuring two diameters, the largest and the smallest. The product of these values was taken as a quantitative expression of the area of the nuclei. The obtained data were analyzed statistically.

RESULTS

The trochlear nucleus in dogs of all the investigated ages, beginning from 39-day fetuses, was situated at the level of the inferior colliculi under the gray central substance of the midbrain. On the lower medial side, the trochlear nerve is bounded by the posterior longitudinal fasciculus. The cells of the reticular formation of the midbrain are situated to the side of the trochlear nucleus. In cross-sectional outline, the trochlear nucleus represents a circle whose area increases with age (in the 30-day fetus it is 240 μ^2 and in a mature dog 722 μ^2). The number of cells per unit area of the trochlear nucleus decreases with development. The increase in the area of the trochlear nucleus is due to the development of numerous nerve fibers and glial elements between the neurons and also by an increase in the size of the neurons, themselves, which compose this nucleus.

The shape of the cells of the trochlear nucleus changes during ontogenesis. The neurons are polygonal in 50-day fetuses; for newborn puppies, we could distinguish differently shaped neurons: polygonal, ellipsoidal, and round. For the 18-day-old puppies, there were individual neurons of oval or piriform shape resembling the cells of the mesencephalic nucleus. The development of the neurofibrils in the cells of the trochlear nucleus undergoes a number of changes, their formation occurring earlier in large neurons than in small ones. Single neurofibrils appear in the cell cytoplasm of the 50-day fetus (Fig. 1); in the newborn puppies, they are thickened, concentrated around the nucleus, and are intensely impregnated. In puppies aged 6 days, coarse neurofibrils appear in the cell processes; by the

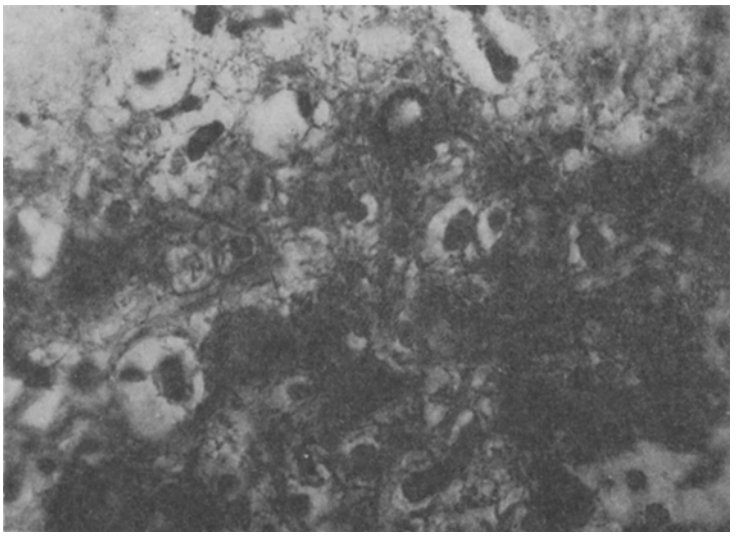

Fig. 1. Trochlear nucleus of 50-day-old dog fetus. Neurons of a multipolar form with short processes; neurofibrils are poorly evidenced. Impregnation by the Buck-Bielschowsky method. Objective 100 ×, ocular 8 ×.

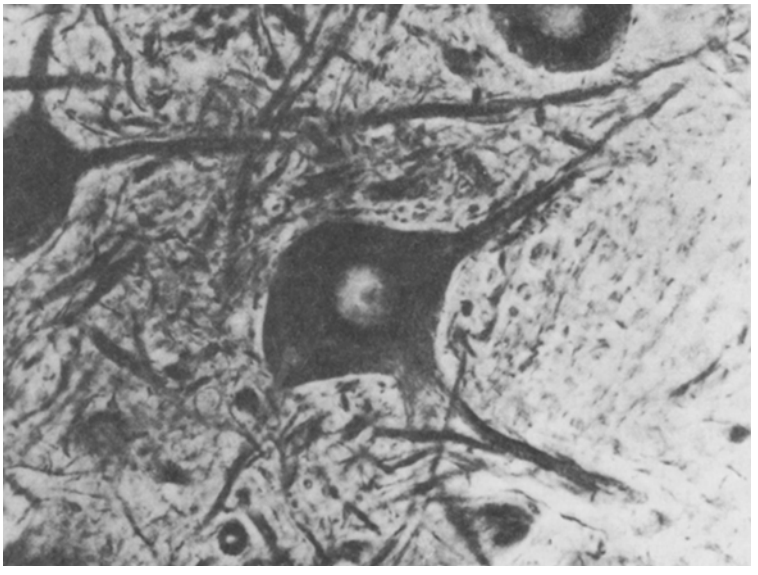

Fig. 2. Trochlear nucleus of month-old puppy. Large multipolar cell with long branching processes; neurofibrils clearly evidenced. Impregnation by the Bielschowsky-Gros method as modified by B. I. Lavren'ev. Objective 100 ×, ocular 8 ×.

18th day, the number of neurofibrils increases and they become more delicate; in month-old puppies, the neurofibrils form a delicate network filling the entire cytoplasm of the neuron (Fig. 2). The chromophil substance in the cells of the trochlear nucleus during development gradually changes from a dustlike substance to small granules, which, on merging, form large granules (Fig. 3) and by a month acquire the structure characteristic for neurons of the stichochrome group. The chromophil substance is formed earlier in large cells than in small ones.

During the maturation of the nerve cells of the trochlear nucleus, the character of the distribution of nucleic acids and nucleoprotides in them changes. The content of DNA in the cell nuclei changes gradually without marked variations. At early stages of development of the nerve cells, the DNA level in the nuclei is higher than in mature cells. An interesting regularity was noted: cells with a large nucleus contain less DNA than cells with a small nucleus.

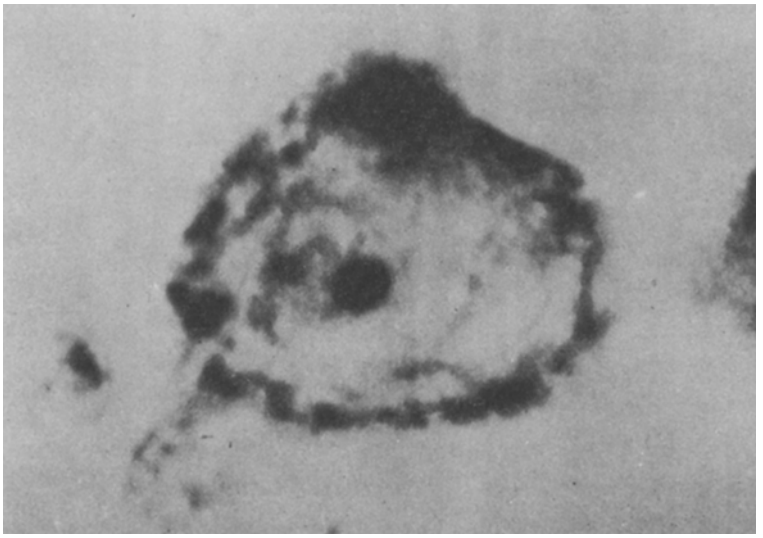

Fig. 3. Trochlear nucleus of 50-day fetus. Small granules of chromophil substance concentrated along the periphery of the neuron cytoplasm. Staining by Nissl. Objective 90 ×, ocular 15 ×.

Actually, during ontogenesis the size of the nuclei increases, whereas the DNA concentration decreases. The nucleus of the glial cells is many times smaller than the nucleus of nerve cells, whereas the Feulgen reaction in the nuclei of the glial elements is appreciably stronger than in the nuclei of the neurons.

The RNA in the cells of the trochlear nucleus of the 30-day fetus was spread out, forming small granules here and there. With further development, the RNA in the nerve cells of the trochlear nucleus at first forms single friable granules situated along the periphery of the cytoplasm, then the granules enlarge and thicken, filling the entire cytoplasm. By one month of intrauterine life, the RNA granules acquire a form typical for mature motor neurons.

At all ages, the nucleoli are pyroninphilic. The larger the neuron, the stronger the reaction to RNA in it. The quantity of RNA especially increases in the studied neurons after 12 days when maturation begins and the eye muscles begin to function.

Beginning with the 30-day fetus up to one month of postnatal life, acid proteins predominate in the cells of the trochlear nucleus, and at a later age the basic proteins predominate. Our data indicate that the formation of the neurofibril apparatus in the neurons of the trochlear nucleus begins at the period when the acid proteins predominate in them. To elicit the relation between RNA and the protein components of the nucleus and cytoplasm, the preparations were treated with trypsin and pepsin with subsequent Brachet staining. The results obtained permit the assumption that the RNA in cells of the trochlear nucleus is strongly bound with the basic proteins. This is confirmed by tryptic hydrolysis in which the basic proteins are split off and the RNA is carried away from the structures along with them. For animals of different ages, a different incubation time with trypsin is needed for RNA staining in the tigroid and nucleolus to disappear completely (30 min for the 30-day fetus and 75 min for a mature dog). The effect of pepsin involves only the disappearance of RNA between granules; it does not affect its content in the tigroid substance. Consequently, we can assume that the diffuse RNA is bound with acid proteins and the RNA of the tigroid does not have such a strong bond with them.

The area of the nuclei of the cells of the trochlear nucleus (see table) markedly increases in puppies up to 12-days old; then the increase in the size and nuclei of these cells somewhat slows down, reaching a maximum by 6 months. In older dogs, we noted a tendency toward a decrease in the size of the nuclei. A certain slowdown in growth of the nuclei of the cells of the trochlear nucleus in puppies after 12 days corresponds to the time of maturation of the animal and the start of functioning of the eye muscles, in particular the superior oblique muscle. In connection with this, we assume that the slowdown in the growth of the cell nuclei at this period is a consequence of

Area of the Nuclei of Neurons of the Trochlear Nucleus in Dogs of Different Ages

Ages of dog	Area of nuclei (M ± m)
Newborn puppy	21.4 ± 0.63
Five-day puppy	36.9 ± 1.18
12-day "	63.3 ± 1.98
Month-old "	70.5 ± 1.92
Six-month "	103.1 ± 2.11
Mature dog	77.9 ± 1.85

Comment: The area is expressed in arbitrary square units of the micrometric ruler. One arbitrary unit is equal to 1.4 (objective 90 ×, ocular 15 ×).

the start of functioning of the superior oblique muscle of the eye. The decrease in the size of the cell nuclei in dogs older than 6 months shows that there exists a limit to their size, indicating the achievement of functional maturity of the neurons.

LITERATURE CITED

1. V. M. Bekhterev, Selected Works [in Russian], Moscow (1954).
2. I. S. Zhurid, Nevropatol. i psikhiatr., 6, 3, 99 (1937).
3. K. A. Kunakov, in the book: Ontogenesis of the Brain [in Russian], Leningrad (1949), p. 166.
4. Chao-te Li, Transactions of the 6th All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Khar'kov (1961), p. 326.
