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NUCLEAR MEMBRANE INCLUSIONS IN CORTICAL NEURONS OF THE PROGENY OF RATS SENSITIZED WITH BRAIN ANTIGEN

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Considerable attention has recently been paid to the role of neuroimmune factors in the pathogenesis of nervous and mental diseases. Modelling of neuroimmune processes may provide the key to the understanding of the fine mechanisms of disturbances of the structure and function of the CNS.

Hardly any references can be found in the literature to the study of the effect of preliminary sensitization of female animals with brain antigens on the development of the brain structures of their progeny. The writer's previous investigations of the CNS of 30-day-old rats whose mothers had been treated in this way [2] revealed a decrease in size of the pyramidal cells in layer V of the sensomotor cortex and a reduced tigroid content of their cytoplasm compared with the control. Electron-microscopic investigation revealed membrane inclusions in the nuclei of the neurons and slight changes in the cytoplasm.

The aim of the present investigation was to study subcellular structural manifestations of the pathology of postnatal development of cortical neurons of an animal whose intrauterine development took place from the beginning under conditions of neuroimmune conflict. An attempt was made to study the dynamics of the ultrastructural changes discovered, by investigating the brain at different stages of postnatal life — from 2 to 60 days.

EXPERIMENTAL METHOD

A 20% solution of cerebral cortical isoantigen was injected intraperitoneally into sexually mature noninbred rats in a dose of 0.3 mg/200 g body weight three times on alternate days. On the 21st day after the first injection the rats were mated with healthy males. The presence of antibodies in the blood was established by the complement fixation test in the cold. The young were born on the 21st-22nd days after mating. The progeny of intact females served as the control. Altogether there were three series of experiments in which 120 experimental and 50 control young rats, obtained from 17 sensitized and nine control mothers respectively, were used. Pieces of sensomotor cortex taken for electron-microscopic investigation were fixed with glutaraldehyde and osmium tetroxide in phosphate

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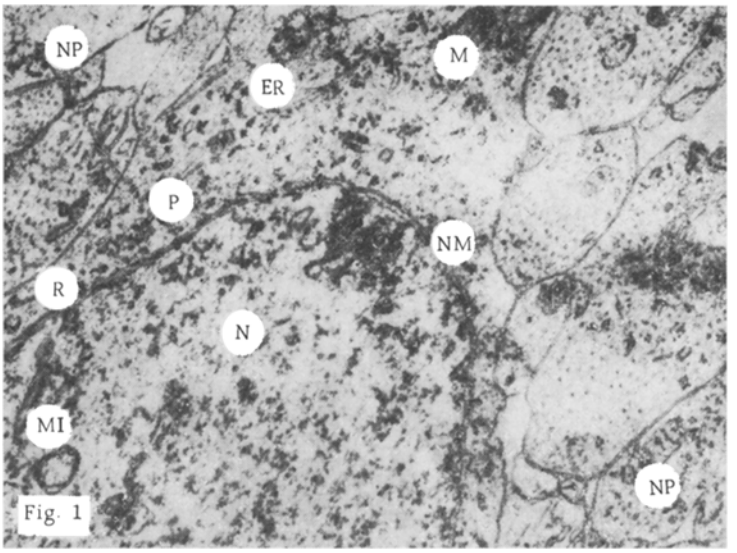

Fig. 1. Sensomotor cortical neurons of 2-day-old rat whose mother had been sensitized with brain antigen. N) Nucleus; NM) nuclear membrane; MI) membrane inclusions; M) mitochondrion; ER) endoplasmic reticulum; P) polysomes; R) ribosomes; NP) neuropil. 16,000×.

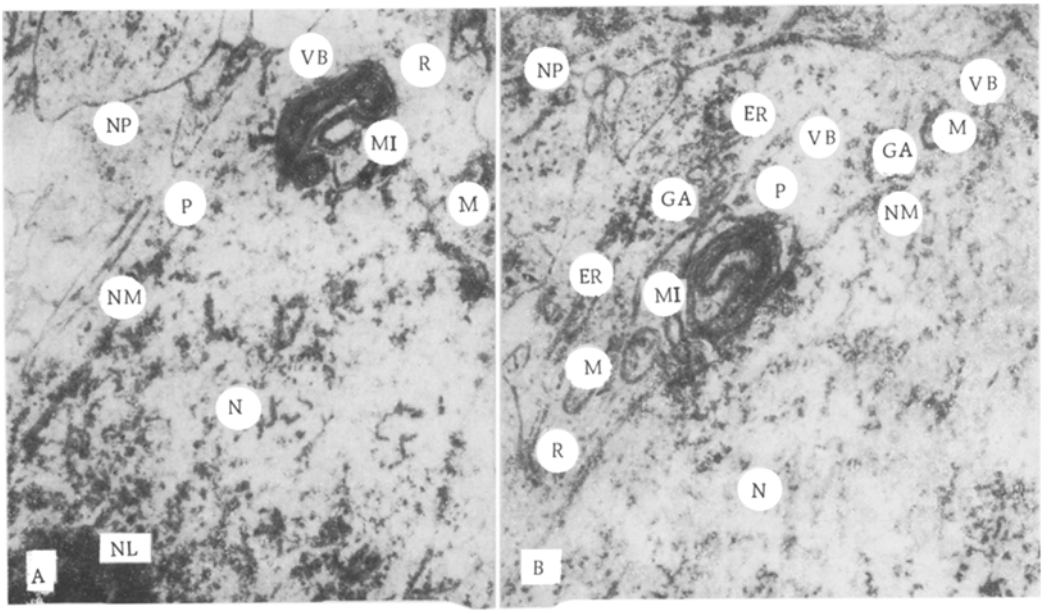

Fig. 2. Sensomotor cortical neurons of 7-day-old rats whose mothers had been sensitized with brain antigen. A: NL) nucleolus; VB) vesicular body; remainder of legend as to Fig. 1. 16,000×. B: GA) Golgi apparatus; remainder of legend as to Figs. 1 and 2A. 16,000×.

buffer (pH 7.2). After dehydration the tissue was embedded in Araldite. Sections obtained on the LKB ultrotome (Sweden) were strained by Reynolds' method and with a solution of uranyl acetate in methyl alcohol and were examined in the TESLA-540 electron microscope.

EXPERIMENTAL RESULTS

The most significant ultrastructural change in the neurons of the experimental young rats was the presence of nuclear membrane inclusions which were not found in the control.

Concentric membrane bodies, small in size and varied in shape (Fig. 1), were found in the nuclei of neurons of 2-day-old rats. The cell cytoplasm was still poorly developed

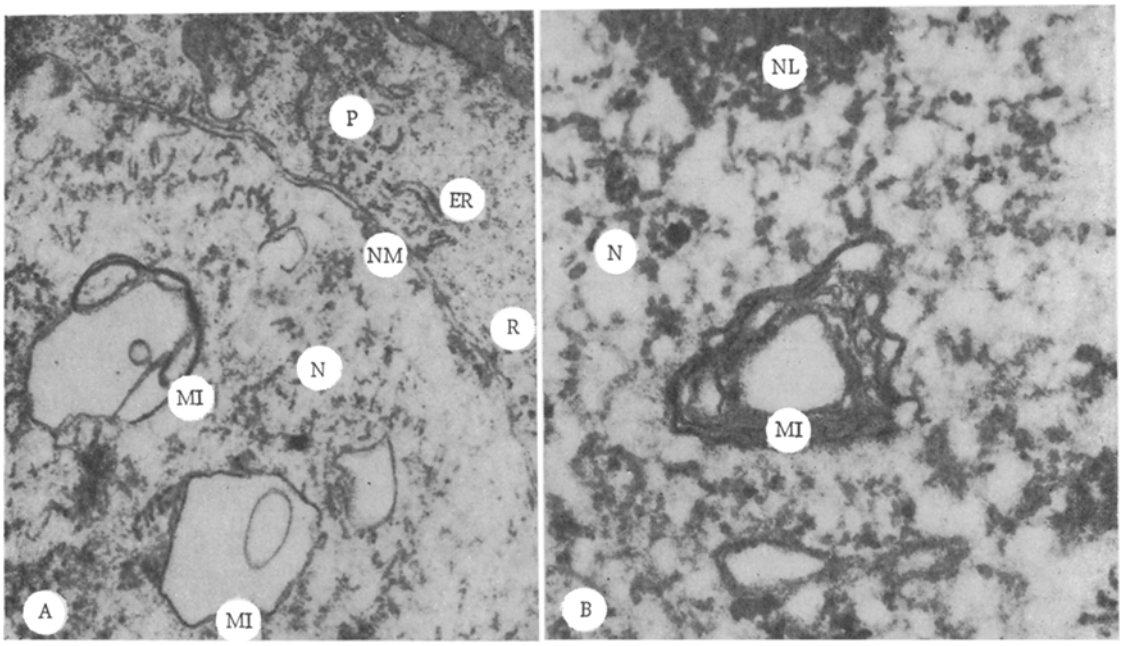

Fig. 3. Sensomotor cortical neurons of 14-day-old (A) and 30-day-old (B) rats whose mothers had been sensitized with brain antigen. Legend as to Figs. 1 and 2. A) 16,000×, B) 22,000×.

and contained few organelles. The cells were arranged close together and had fairly dense nuclei.

Membrane inclusions were larger in the nuclei of rats aged 7 days than in those aged 2 days. Sometimes they had acquired the appearance of concentric stratified bodies whose membranes were studied with ribosome-like particles. Some bodies were located near the nuclear membrane and in close contact with it (Fig. 2). Some bodies consisted of cavities surrounded by a single membrane. The cytoplasm of the cells contained few organelles. In some neurons the channels of the endoplasmic reticulum were dilated and the mitochondria swollen.

In cortical neurons of 14-day-old animals the nuclear inclusions as a rule consisted of cavities surrounded by one or a few layers of membranes (Fig. 3A). The number of these inclusions in the nuclei was greater than at the earlier stages of development. The cytoplasm contained many lysosomes and also vesicular bodies. Swollen mitochondria with homogeneous contents were found.

On the 30th day the structure of the nuclear inclusions was marked by great variability: from cavities bounded by one membrane to stratified membranous formations of different sizes (Fig. 3B). Many vesicular bodies were observed in the cytoplasm of many neurons, just as at the previous time, and bodies with myelin-like or homogeneous contents also appeared. "Giant" mitochondria and mitochondria with a dense matrix and indistinct cristae were seen. In some cells the polysomes were undergoing disaggregation.

In 60-day-old rats the nuclei of the cortical neurons were packed with large cavities bounded by membranes. The state of the organelles differed only a little from that described on the 30th day.

The principal ultrastructural feature distinguishing the cortical neurons of rats whose mothers had been previously sensitized with brain antigen is thus the presence of nuclear membrane inclusions, not found in the control.

Numerous nuclear inclusions (nuclear bodies) in nerve and glial cells have been described in the literature in cases of subacute sclerosing panencephalitis [11, 12], in Alzheimer's and Jakob–Creutzfeldt diseases [10], schizophrenia [8], and other pathological states. In 1967 a classification of these bodies was suggested, distinguishing five types depending on their external appearance [7]. Later, nuclear bodies of the first and second types were qualified as simple, the rest as complex, for they consisted of several components.

Nuclear bodies are found in man and animals under normal conditions also [9, 12, 15]. It has been suggested that they may be intracellular organelles. Their extremely rare discovery under normal conditions is explained by their very small size, so that they do not occur in more than 15-17% of sections [8, 12]. Under pathological conditions, however, the number of these bodies rises sharply, so that they can be observed frequently. Some of the workers cited have emphasized that the structure of the nuclear bodies is identical under normal and pathological conditions [8, 9].

Many workers associate the appearance of nuclear bodies with intensification of cellular activity due to various causes: poisoning by drugs, hormonal hyperactivity, neoplastic and proliferative processes, and immunization [7, 9]. Intranuclear inclusions, in the form of "packets of membranes," have been found in regenerating skeletal muscles [6]. The workers concerned consider that they may be of viral origin. In some publications tubular and lamellar inclusions have been described in nuclei of placental trophoblasts [3]. The nature and function of these inclusions were not clear. Since in many experimental and clinical studies the appearance of nuclear bodies has been due to virus infection [11, 14], some workers have suggested that their presence may be regarded as evidence of the viral character of diseases such as schizophrenia [8] or Alzheimer's disease [10]. However, the authors cited do not rule out the possibility of cellular activation by a virus (slowly acting), leading to the appearance of numerous nuclear bodies.

The chemical nature of nuclear bodies has received very little study. It has been shown that different components of "complex" nuclear bodies differ in their chemical composition [9]. Proteins and ribonucleoproteins have been found in them. "Fibrillary" nuclear bodies did not react with RNase or DNase but were sensitive to trypsin [12]. Many workers consider that nuclear bodies participate directly in protein synthesis [6, 14, 15].

The origin of nuclear bodies likewise is unknown. It has been suggested that some of them may be formed by invagination of the nuclear membrane [3]. "Complex" nuclear bodies are considered to form from "simple" bodies in the course of time [12] and certain nuclear bodies in lymphocytes may perhaps be composed of antigenic material [13].

On the whole, the nature and function of nuclear bodies are still unexplained.

In the writer's view it is interesting that nuclear bodies of identical structure are observed in different pathological processes and functional states of different organs (neurons and glial cells of the CNS, ganglion cells, lymphocytes, skin cells). However, nuclear membrane bodies found in the present experiments were completely different from all types of nuclear bodies described in the literature.

The exact reproduction of membrane bodies in different series of experiments with different batches of animals, but their absence in the control, rules out any possibility of an artefact. Furthermore, changes in the structure of membrane bodies at different stages of postnatal life (from 2 to 60 days) were observed. Initially there were tiny vacuoles bounded by one or more layers of membranes (2 days), next followed larger vacuoles of the same type (7 and 14 days), later stratified membrane bodies appeared (30 days) and, finally, numerous large vacuoles (60 days).

Since the nature of membrane bodies is unknown and it was impossible to compare our data with those given in the literature, it is difficult to judge the functional role of these bodies in the present experiments. The state of the ultrastructure of the cytoplasm of the neuron likewise does not permit any convincing conclusions to be drawn regarding the functions of the membrane bodies. At the present stage of the investigation all that can be said is that membrane bodies essentially appear in nuclei of cortical neurons of rats whose mothers have previously been sensitized by brain antigen. Since membrane bodies can be found as early as on the 2nd day of postnatal life, this suggests that they are formed during the prenatal period. Some electron micrographs suggest that membrane bodies either may be formed in the cytoplasm and are subsequently "taken up" by the nucleus, or are formed by invagination and separation of parts of the nuclear membrane (Fig. 2). Preliminary sensitization of the mothers with brain antigen may perhaps lead to an increase in the lability of the neuron membranes in the progeny, especially the nuclear membrane, and as a result, accumulations of membranous structures appear in the nuclei.

In the course of the animal's life the membrane bodies do not disappear but undergo structural changes, and by the 60th day they occupy a considerable volume of the nucleus. The appearance of such structures in the nuclei must, of course, affect the functioning of

the neurons. For example, the large vacuoles found in the nuclei of neurons of chick embryos after large doses of x-rays have been interpreted as destructive changes in the cells [5]. However, nothing can yet be said about the role of membrane bodies under the conditions of the present experiments. They may perhaps be the product of pathology of development of the CNS as a result of intraplacental action of antibodies on the maturing fetal brain (it has been shown that antibodies pass through the placenta [4]). In all probability this pathology not only does not disappear during life but, on the contrary, becomes consolidated and progressive. This may ultimately lead to considerable disturbances of the brain function of the progeny of mothers sensitized with brain antigen.

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