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MODULAR ORGANIZATION OF THE RAT HYPOTHALAMUS

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Some ideas on the modular organization of the nervous system have now been formulated. This is shown by the rapidly increasing volume of information devoted to functional and morphological aspects of this problem. An extensive survey of the literature, contained in a monograph by a group of authors [1], indicates the penetrating nature of morphological investigations of neuronal assemblies (modules) in the cerebral cortex both of animals and of man. However, a matter of special interest in the complex hierarchy (cortex – subcortex – brain stem) of brain structures is the diencephalic region, which plays a leading role in the development of psychoemotional stress, which is a generator of many pathological states. No data on modules of the hypothalamus could be found in the literature. Accordingly, it was decided to undertake a comprehensive neuromorphological study of the rat hypothalamus, with the aim of detecting modular systems and their role in the development of the stress reaction.

EXPERIMENTAL METHOD

The experimental study was carried out on noninbred male albino rats (24 animals) weighing initially 180-200 g. Emotional stress was produced by immobilizing the rats once or repeatedly by their limbs in the supine position for 3-4 h. In the case of repeated immobilization, the animals were fixed after 1-2 days. The mice were anesthetized with ether and killed after 1, 7, 20, 30, 45, and 60 days of the experiment. Intact rats (six) constituted the control group. The hypothalamic region was fixed in Bouin's fluid, 10% neutral formalin solution, and a 4% solution of paraform ("Fluka," Switzerland). made up in 0.1 M cacodylate buffer. Ultrathin sections were cut on an ultramicrotome, stained, and examined in ÉMV-100 LM and PÉM-100 electron microscopes.

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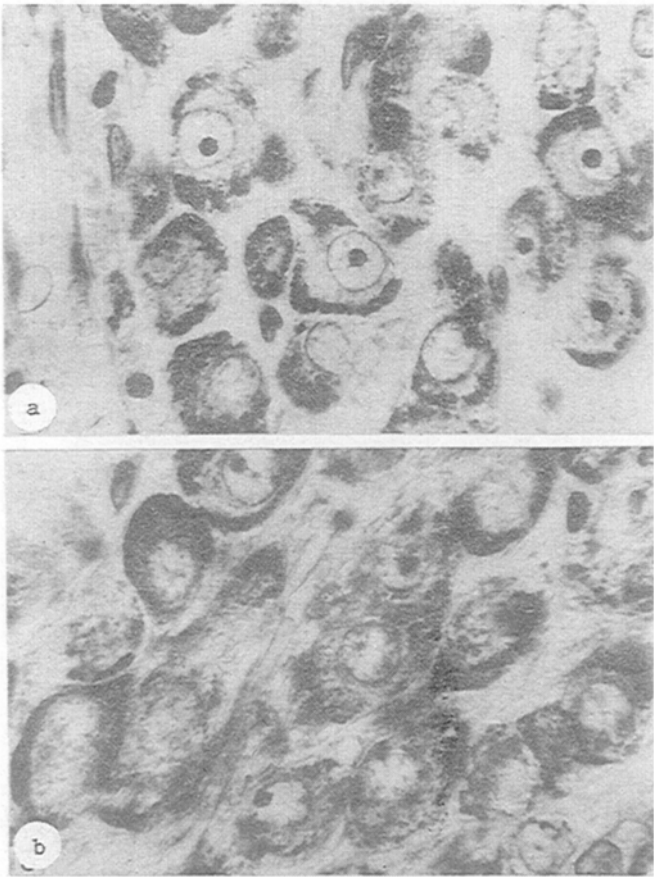

Fig. 1. Structure of supraoptic nucleus. Nissl's stain. 280×: a) control group; b) hypertrophy of neurons, partial chromatolysis, immobilization for 7 days.

The great diversity of cells in the hypothalamus reflects their basic functional specificity – a secretory process, which is accessible for study both at the subcellular level (elementary granules, mitochondria, synapses, etc.), and at the cellular level (changes in shape and size of the cell, nucleus, and nucleolus, Nissl's substance). Accordingly a wide range of techniques was used: preparations were stained by Nissl's method, with Gomori's aldehyde-fuchsine, for DNA and RNA by gallocyanin, impregnation by Bielschowsky's method, electron-microscopic investigation, stereometric investigation – determination of the area of cross-section of the neurons, bulk density of the neurons, specific surface area of the neurons, number of neurons per unit volume, and internuclear distance. Neuronal modules also were studied in a "Microvideomat" microscope ("Opton," Germany), using a microcomputer and the "Stereo-1" program. The numerical results were subjected to statistical analysis by computer. The hypothalamic region of rats was investigated in accordance with the classification suggested by Szentagothai and co-workers [3].

EXPERIMENTAL RESULTS

In the first stages of the experiment (1-7 days) increased functional activity of the neurons was observed in nearly all nuclear formations and areas of the hypothalamic region, expressed in the form of hypertrophy of the neurons (Fig. 1a, b), an increase in the specific surface area of the nerve cells, enlargement of the alimentary granules of secretion, hypertrophy of mitochondria and of the nucleolus of the neurons and of the glial elements (Fig. 2a, b). The greatest functional activity of the neurons was established in the premammillary hypothalamic nuclei.

On the 20th-30th days of the experiment dystrophic changes in the neurons were observed in several hypothalamic nuclear formations, in the form of acute swelling and vacuolation of the cytoplasm, ectopia of the nucleus and nucleolus (Fig. 3a, b), swelling of dendrites, and pericellular and perivascular edema. The most marked dystrophic changes were observed in the paraventricular nucleus, less marked in the supraoptic and dorsomedial nuclei. In

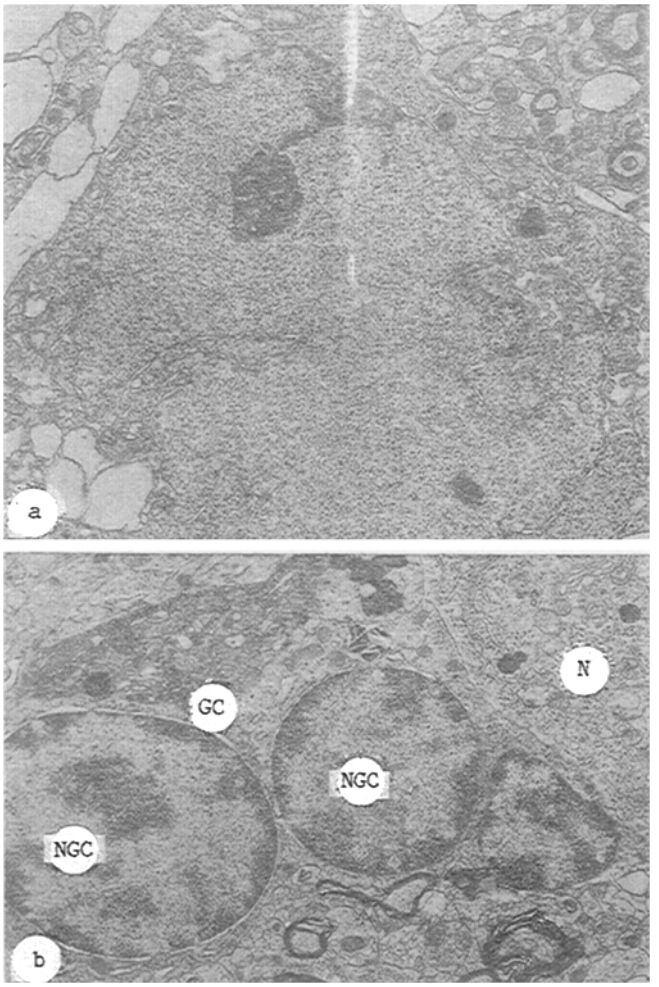

Fig. 2. Ultrastructural organization of neurons, immobilization for 7 days. 6000×; a) arcuate nucleus. Hypertrophy of nucleolus of neuron; b) ventromedial nucleus. Neuron (N) in close contact with glial cells (GC). Nuclei of glial cells (NGC) show marked hypertrophy.

the premammilary and mammillary nuclei neuronal modules exhibited marked hypertrophy, especially in the ventral premammilary nucleus.

With an increase in severity of the stress-inducing factor (45 days of periodically repeated immobilization) the dystrophic processes became more marked, especially in the paraventricular nucleus, in which most neurons had an intensively vacuolated cytoplasm and deformed nucleus. Similar changes were found in the neuronal modules of the anterior hypothalamic area, the lateral hypothalamic region, and dorsomedial nucleus; very slight damage could be seen to the neuronal modules in the supraoptic and suprachiasmatic nuclei, and the retrochiasmal region. Most neuronal modules of the premammilliary and mammillary nuclei and the supraoptic and arcuate nuclei were characterized by hypertophy.

In the final stage of the experiment (60 days) the degree of damage to the neurons in the paraventricular nucleus was very high. In the dorsomedial nucleus signs of hyperchromatosis of the neurons were predominant (Fig. 4a), whereas in the supraoptic nucleus the bulk density of the neurons was reduced, and this was accompanied by hypertrophy of the remaining nerve cells and solitary foci of proliferation of glial cells (Fig. 4b). A very slight degree of damage to the neurons was found in the arcuate nucleus and mammillary nuclei; solitary neurons with signs of dystrophy were found in the ventral premammillary nucleus.

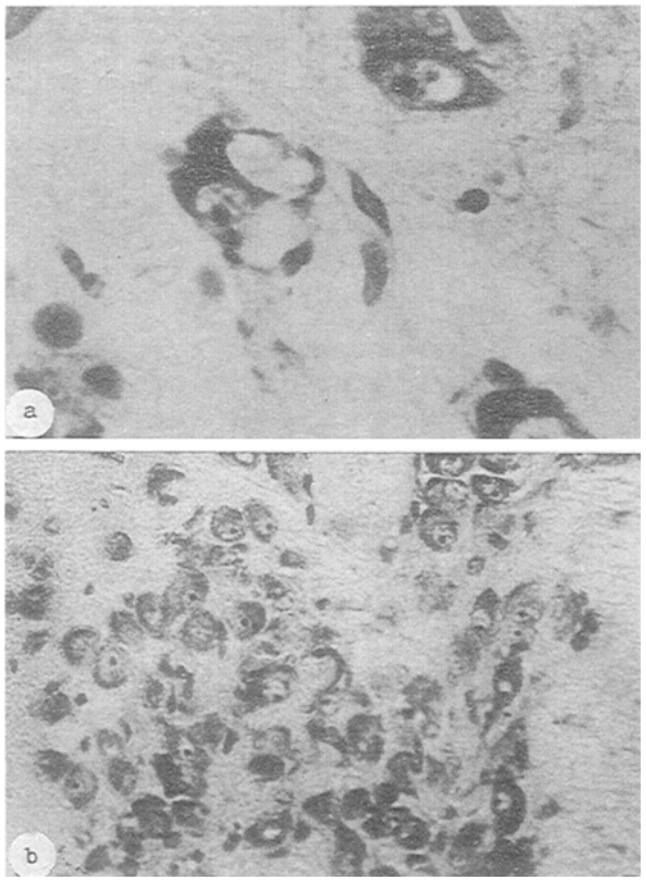

Fig. 3. Structure of cells of paraventricular and supraoptic nuclei after immobilization for 30 days. Nissl's stain. 320×; a) paraventricular nucleus, vacuolation of neurosecretory cells, hyperchromatosis. b) Supraoptic nucleus, hyperchromatosis of single neurosecretory cells. 140×.

The increase in functional activity of neurons in the hypothalamic region during the first days of the experiment is compatible with the generally accepted adaptation syndrome. However, neuronal formations of the paraventricular and dorsomedial nuclei, and some neuronal modules of the supraoptic and suprachiasmatic nuclei were characterized only by a sharp reduction in the density of the neurosecretory granules and by total chromatolysis. Morphometric parameters of these nuclear formations (enlargement of the area of cross section of the neuron, an increase in volume of the nuclei of the neurons, the internuclear distance, etc.) differed from the control group only after 20-30 days of the experiment. This is a fact which deserves special examination. We know that any system consists of excitatory and inhibitory factors (in the hypothalamus these are LHRH and the statins). Evidently even in the initial stage of stress, inhibitory effects predominate at the level of the hypothalamic region, as follows: blocking of nervous formations or neuronal modules, producing stress "mediators" (paraventricular and supraoptic nuclei, secreting CRF and vasopressin) and participating in the pain syndrome (dorsomedial nucleus).

Experimental investigations [2] have shown that during long-term, and especially, repeated exposure to stress factors, activation of the adrenergic and pituitary-adrenal systems becomes steadily weaker with each repetition. This abolition of the stress reaction is independent of exhaustion of the adrenals, for injection of ACTH into pain-adapted animals induces a larger increase in their blood corticosterone concentration than in control animals. These data may be evidence that one factor in adaptation to unavoidable stress situations is activation of regulatory mechanisms which inhibit the output of releasing factors, ACTH and, as a result, release of corticosteroids and catecholamines.

Analysis of data obtained on the immunohistochemistry of the diencephalic region in recent years suggests that modular systems, activated simultaneously with the stress reaction, are localized at hypothalamic level mainly in the premammillary zone. These may include neuronal modules of the lateral mammillary nucleus, the supramammillary and, in particular, the ventral premammilary nuclei. The main mass of neurons secreting antistress neuropep-

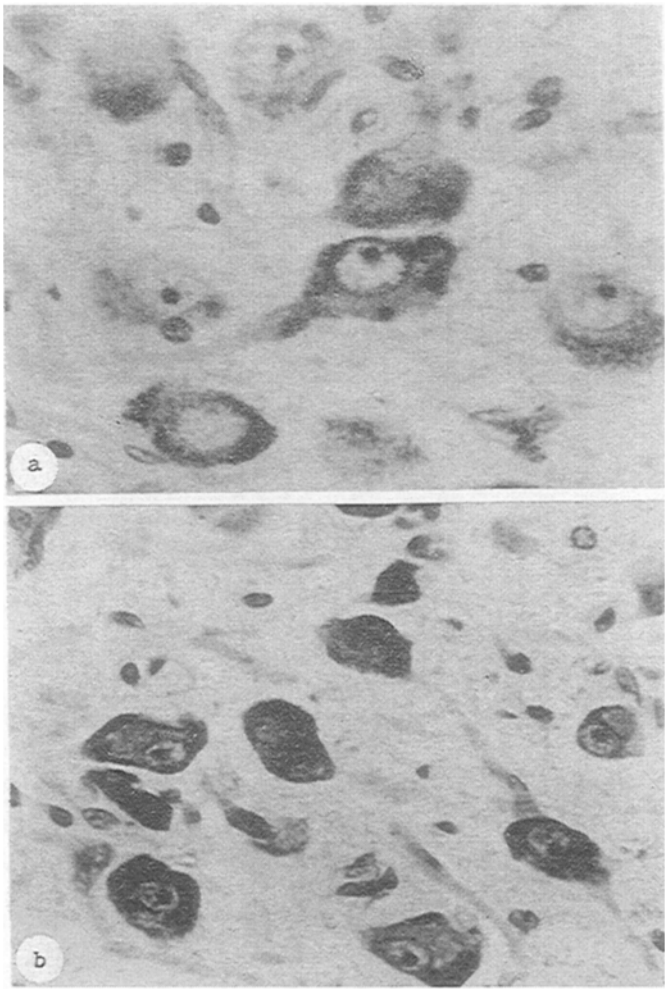

Fig. 4. Structure of hypothalamic neurons after immobilization for 45 days. Nissl's stain. 280×; a) supraoptic nucleus, hypertrophy of neurosecretory cells, reduction of bulk density of neurons. Proliferating glial tissues at site of absent neurons (a). b) Dorsomedial nucleus. Hyperchromatosis of neurons.

tides – substance P, enkephalins, and also GABA-containing neurons, playing the role of a nonspecific inhibitory mechanism, is concentrated [4-8].

Thus two modular systems responsible for development of the stress reaction can be distinguished in the hypothalamic region. The first is involved in activation of the sympathicoadrenal system: paraventricular nucleus, neuronal modules of the dorsomedial nucleus, neuronal modules of the anterior, posterior, and lateral hypothalamic area, and also the supraoptic nucleus. The second exerts an inhibitory effect: ventral mammillary nucleus, supramammillary and lateral premammillary nuclei, and also many neuronal modules of the arcuate nucleus and retrochiasmal region.

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MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF GENETIC ANALYSIS OF OSTEOGENESIS IMPERFECTA IN CULTURED DERMAL FIBROBLASTS

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The modern approach to the study of hereditary diseases presupposes analysis and comparison of all stages of their pathogenesis – from the action of mutant genes to the appearance of the phenotypic picture of the disease at the cell, organ, and whole body levels, as ways of realization of genetic information. The cell is a special apparatus, which constitutes the structural-topographic basis of interaction between genes. Many pathological processes, which are undoubtedly based on molecular defects, are realized through a genetically determined disturbance of cell functions and of the behavior of cellular communities. Under these circumstances, the use of somatic cells cultured in vitro is a unique contribution to the analysis of the pathogenesis of hereditary diseases, for such cells preserve the genetic information of the original organism, while at the same time, they are descended from it. Osteogenesis imperfecta (OI) is a generalized disease of connective tissue, the chief manifestation of which is pathological fragility of the bones. As a result of the clinical variability of OI, four clinical types of it are distinguished [9]. The basic defect in all types of OI is now associated with a disturbance of synthesis and/or metabolism of type I collagen, and for this reason the corresponding changes may be exhibited by cultures of dermal fibroblasts (CDF), which produce this type of collagen sufficiently constantly [8]. Considering the role of the cell in the realization of genetic information and the correlations found between the molecular defects and clinical phenotype of OI, we attempted to discover the cellular mechanisms of the disturbed process of morphogenesis in the space between the molecular defect and the clinical phenotype of OI.

EXPERIMENTAL METHOD

Skin biopsy specimens were obtained from the medial surface of the forearm of 10 patients, eight of whom had type I OI and two had type III OI [9], and also from three clinically healthy volunteer donors. Diploid strains of dermal fibroblasts were obtained by the method developed by Kukharenko and co-workers [2]. A dynamic analysis of growth of CDF was undertaken by a modified method in [6]. Cell strains were compared with respect to the following parameters: 1) days needed by the CDF to reach the stationary phase of growth; 2) the cell density of CDF in the stationary phase of growth; 3) the length and width of the cells with calculation of the ratio between them. The index

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