Regularities in the Formation of Structural and Functional Changes in the Brain in Technogenic Stress

E. V. Kudinova

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Study of structural and functional changes in the brain under the influence of anthropogenic factors showed that limbic brain structures and especially the hippocampus undergo most pronounced reorganization.

Key Words: *limbic brain structures; the hippocampus; mitochondria*

Under conditions of ecological crisis, the study of the biomedical regularities of the action of technogenic stress factors is of increasing importance. Dominant focus of excitation in the central nervous system (CNS) is the basis for specific adaptation aimed at achieving beneficial results. Dominant focus plays an important role in coordination of CNS activities and information storage and processing. Under experimental conditions, the dominant focus can be induced by sending repeated afferent impulses to a specific center. Under conditions of natural behavior, the dominant state of the nerve centers can be caused by metabolic shifts [11]. The study of the reorganization of brain structures in dominant foci is an important problem of neuromorphology and biology [12].

The purpose of the work was to identify brain structures that form the dominant foci of excitation in response to external environmental factors and to determine the characteristics of destructive, compensatory, and reparative changes of neurons in the primary dominant focus of generalized excitation triggering the development of stress-syndrome.

MATERIALS AND METHODS

The animals with high and low threshold of convulsive were kept under standard vivarium conditions in CSRL of Omsk State Medical Academy. The study was performed on 127 sexually mature albino male Wistar rats weighing 170-210 g. The stress syndrome was reproduced using a model of reflex epilepsy: exposure to acoustic stimulation of 86 dB and 102 dB intensity [6] in the kindling mode [5] with an interval between the acoustic stimuli of 48 hours. All procedures of animal keeping and handling were performed in compliance with the guidance of the Ministry of Health Care and Social Development of the Russian Federation (order No. 755, December 8, 1977).

Material for morphological analysis was obtained from animals that were anesthetized with ether after 14, 21, 30, 45, 60 and 90 days and after 4, 7, 10, 15, 20 and 30-fold stimulations, respectively. Verification of brain structures and sectors of the hippocampus was performed using the stereotaxic atlas of adult rat brain [15]. For light microscopy, thin (10 μ) serial frontal sections of the brain placed on slides and stained with 0.1% toluidine blue after Nissl were used. For electron microscopy, hippocampus was isolated. Pyramid fragments of sectors CA1, CA2, CA3, CA4 (5 fragments per case) were incubated with 1% osmium tetroxide for 2 h, washed, and embedded in a mixture of epon and araldite (1:1). The brain from animals demonstrating zero response score (no visual motor excitation) after 3-fold (48 h) acoustic stimulation (86 dB) served as the control.

Electron microscopy was conducted by Prof. E. I. Ryabchikova, (Vector, Novosibirsk). Ultrathin sections

Department of Animals Physiology and Biochemistry, Novosibirsk State Agrarian University, Russia. ***Address for correspondence:*** kudinova_kleo@mail.ru. E. V. Kudinova

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were examined and photographed under a "Hitachi-600H" electron microscope.

The data were processed by methods generally accepted in the biomedical research system using Excel and Statistica 5 [9], according to modern requirements for the analysis of medical data [4].

An integrated methodological approach (light and electron microscopy, morphometric analysis) allowed us to obtain quantitative and qualitative characteristics of neurons and interneuronal synapses in the studied limbic brain structures and sectors of the hippocampus.

RESULTS

Morphometric analysis of different structures of rat brain revealed the general patterns and features of the reorganization of various departments of the neocortex, limbic structures, and brain stem structures. For all investigated brain structures, the typical response to an acoustic stimulation was increased numerical density of destructive changes in neurons and reduced total numerical density of neurons. Systematization of nerve cells in brain structures of albino rats was carried out by two parameters, namely modularity (neuronal ensembles) and size of neurons.

Four main mechanisms of the reorganization of neurogliovascular relationships in response to the acoustic stimulus were identified: 1) prevalence of dysfunction and damage to the vascular system, 2) prevalence of edema, swelling and loss of neurons, 3) predominance of activation of compensatory and reparative processes, 4) adaptation to a new level of neurogliovascular interrelations in the cerebral cortex

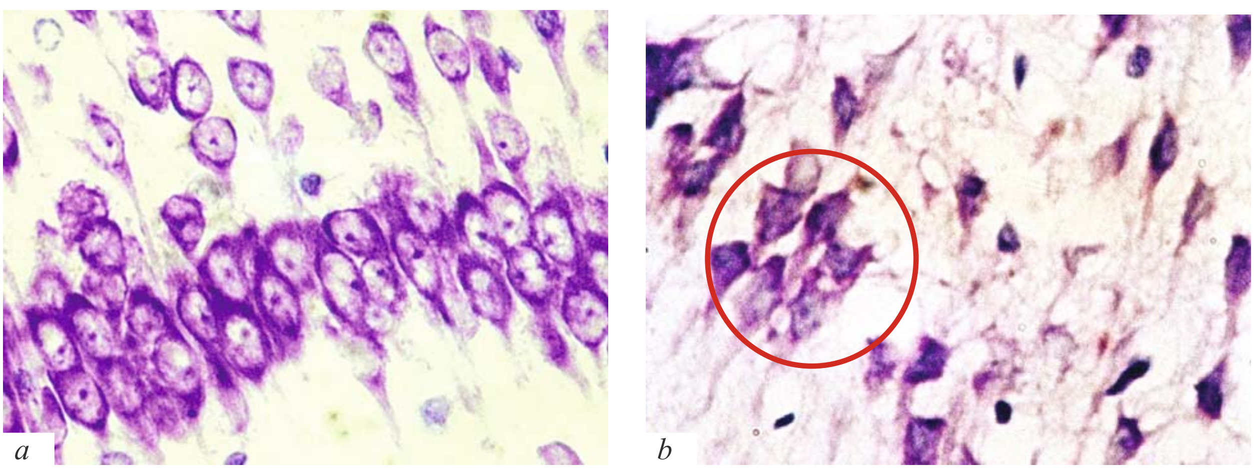

Fig. 1. Hippocampal CA3 region in albino rats (thionine staining after Nissl; ×100). *a*) control: round normochromic neurons with clearly seen light nuclei, nucleoli, and basophilic structured cytoplasm predominate; neuropil is homogeneous without signs of edema/swelling; *b*) hypochromatic and hydropic changes in neurons expressed by the signs of edema/swelling of neuropil and loss of neurons on experimental day 60 (encircled).

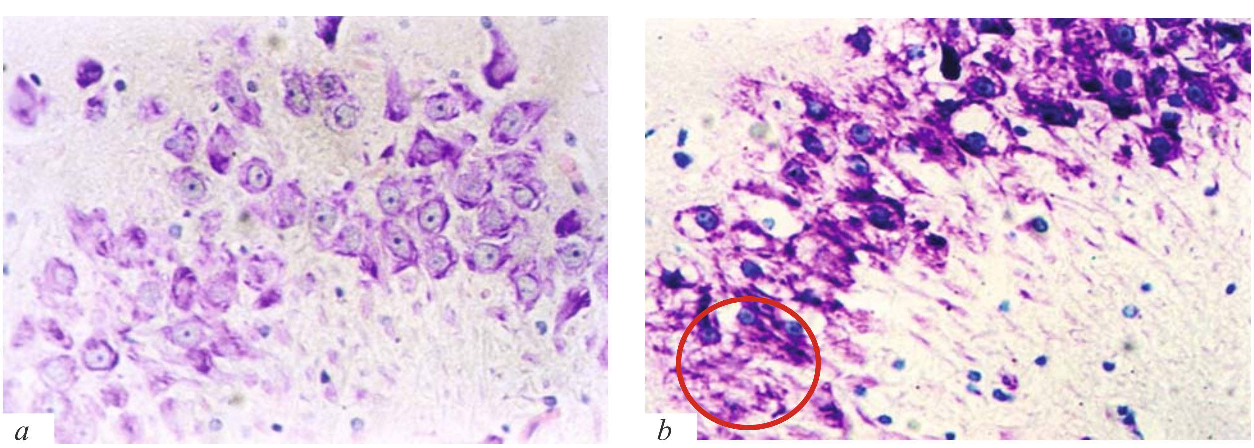

Fig. 2. Hippocampal CA3 region in albino rats at different terms (thionine staining after Nissl; ×400). *a*) control: round normochromic neurons with clearly seen light nuclei, nucleoli, and basophilic structured cytoplasm predominate; neuropil is homogeneous without signs of edema/swelling; *b*) shrunken hyperchromatic neurons, mild symptoms of edema/swelling of neuropil (porosity, coating); days 7-21; *c*) non-shrunken hyperchromatic neurons, signs of edema/swelling of neuropil; days 30-45; *d*) hypochromatic and hydropic changes in neurons, pronounced signs of edema/swelling in the neuropil; days 60-90.

with the risk of formation of pathological systems in the brain. These findings indicate profound restructuring of the neuronal networks, most pronounced (both quantitatively and qualitatively) in the limbic structures modifying the mental and neurological status of the experimental animals. These changes had a temporal and quantitative relationship to acoustic stimulation.

During stress, the hippocampus is one of the first structures undergoing structural and functional restructuring. However, reactive, degenerative, and necrobiotic changes in this compartment of the limbic system quantitatively differ from changes in other brain structures. All the sectors of the hippocampus were involved into structural and functional reorganization; changes in neurons were typically diffuse or focal.

Selective destruction of hippocampal sectors in audiogenic stress with focal loss of neurons and maximum numerical density of irreversibly changed, hyperchromatic shrunken and hyperhydrated neurons in CA3 is shown in Figs. 1 and 2, with the reorganization of the sector field CA1 in all experimental animals (Fig. 3).

Ultrastructural changes in neurons and their processes in different sectors of the hippocampus in audiogenic stress can be divided into reversible and irreversible, destructive, and compensatory-and-reparative. Reversible changes were presented by focal chromatolysis, edema/swelling, and initial stages of cytoplasm dehydration without significant changes in neuronal nuclei (Fig. 4, *a*; Fig. 5, *a*). Pronounced edema and swelling were associated with high number of hypochromatic neurons and irreversible changed shrunken hyperchromatic dehydrated neurons in nuclear structures, especially in hippocampal CA3 (Fig. 4, *b*, Fig. 5, *b*) and in amygdaloid complex. Audiogenic stress stimuli induced reorganization of neuronal populations in limbic structures, especially the amygdaloid complex, thalamic nuclei, and hippocampal CA3 region. Light microscopicy showed that these neurons were presented by ghost cells and hyperchromatic shrunken neurons (Fig. 1, *b*).

Using analysis of variance (ANOVA Kruskal–Wallis test), statistically significant changes in all parameters studied were detected in all regions of rat hippocampus. Reduction of the total numerical density of neurons was observed in field CA1 on day 14 (36.4%); in CA2 on day 21 (52.2%); in CA3 on day 45 (58.8%), and in CA4 on experimental day 21 (50%). The con-

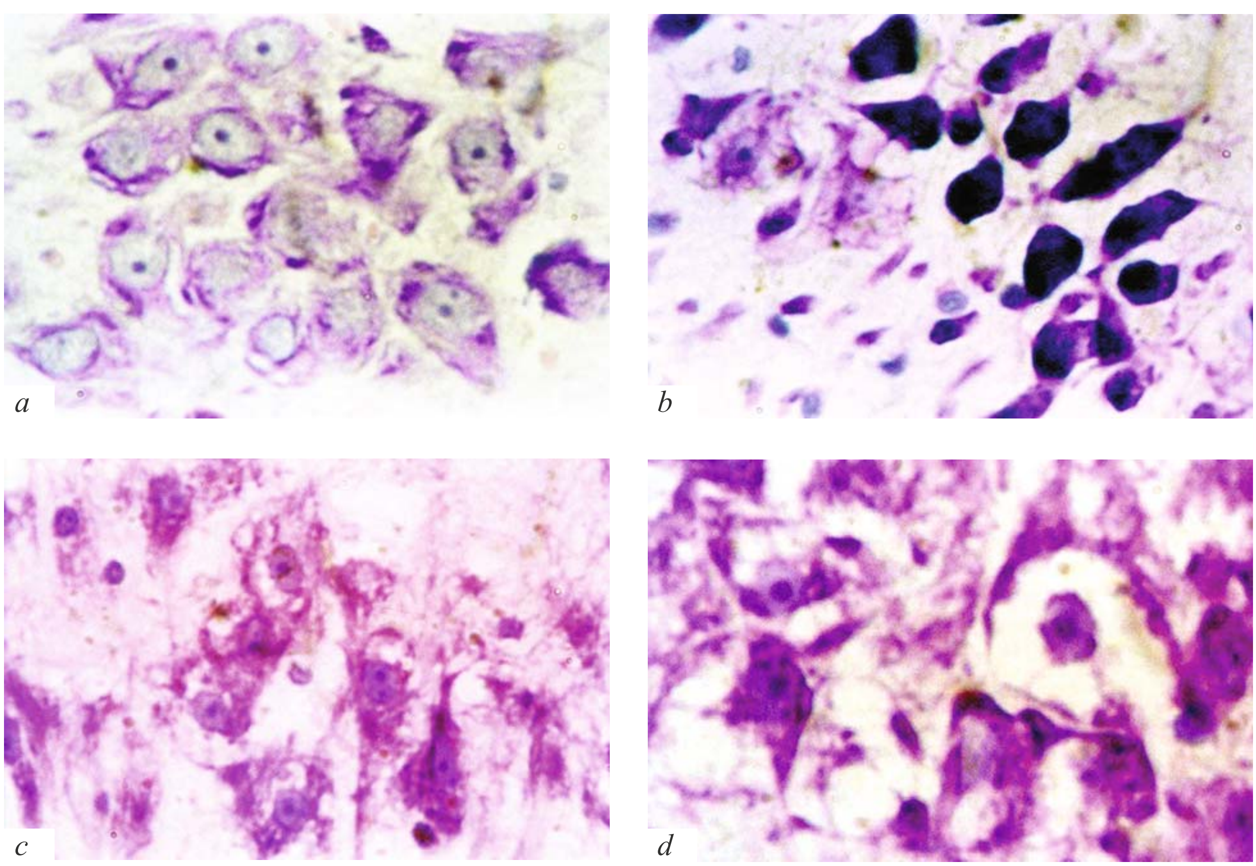

Fig. 3. Hippocampal CA3 region of albino rats ((thionine staining after Nissl; ×200). *a*) control: normochromic neurons with clearly seen light nuclei, nucleoli, and basophilic structured cytoplasm predominate; *b*) non-shrunken hyperchromatic neurons; minor signs of edema/swelling of neuropil (porosity, clarification), appearance of neuronal ensembles (encircled); experimental day 60.

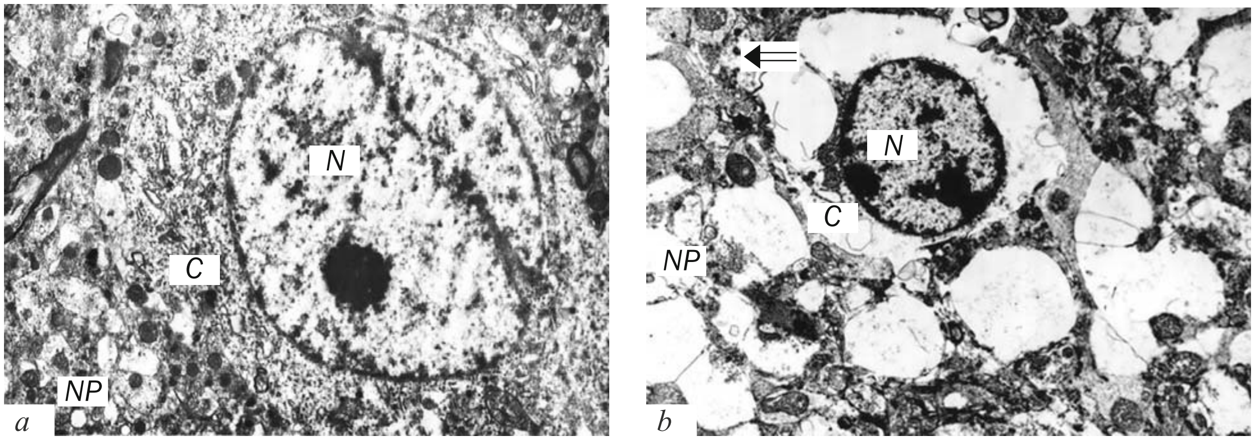

Fig. 4. Pyramidal neurons in CA1 and CA3 regions of albino rat hippocampus 60 days after acoustic stimulation (×17,500). *a*) moderate widening of endoplasmic reticulum, preserved ultrastructure of nuclei and cytoplasmic organelles in CA1; *b*) pronounced manifestations of edema/swelling (arrow) in CA3. N — nuclea, C — cytoplasm, NP — neuronal populations.

tent of irreversibly altered neurons in this group varied significantly, depending on the term. On day 14, shrunken hyperchromatic neurons in CA1, CA2, CA3, and CA4 constituted 27.3, 20.0, 56.5, and 27.8%, respectively. The maximum number of non-shrunken hyperchromatic neurons in CA1 was 60.0% (day 21), in CA2 53.8% (day 45) in CA3 55.6% (day 60), and in CA4 38.9% on day 30.

Multivariate analysis of variance revealed a general pattern of ultrastructural changes in hippocampal neurons. By days 7-14 of the experiment, the decrease was noted in the total numerical density of neurons (45.96%; $p<0.001$). The numerical density of neurons partially recovered on days 21-45 (31.32%; $p<0.05$) probably due to compensatory mechanism for developing adaptation syndrome. On days 45-60, numerical density of neurons sharply decreased again (44.45%; $p<0.05$). Days 60-90 corresponded to the stage of decompensation of the adaptation syndrome and transformation of acute stress to chronic, which was accompanied by reduction of the total numerical density of neurons (55.56%; $p<0.05$).

Of particular interest were intact or reversibly damaged neurons with structural features of high functional activity (hypochromatic CA1 neurons without ultrastructural abnormalities, Fig. 3, *b*, Fig. 4, *a*) and intracellular reparative regeneration due to hyperplasia of the structural components of the cytoplasm and nucleus. These cells were characterized by very high density of ribosomes and mitochondria; secondary mitochondria and residual bodies were also seen (Fig. 6, *b*).

Preserved ultrastructure of hippocampal CA1 neurons with high numerical density of mitochondria and synapses (Fig. 5, *a*; Fig. 6, *a, b*) and reorganization of CA1 region with the appearance of neuron groups can contribute to the formation of the dominant pacemaker area with high information content of neurons and abnormal reverberation of excitation.

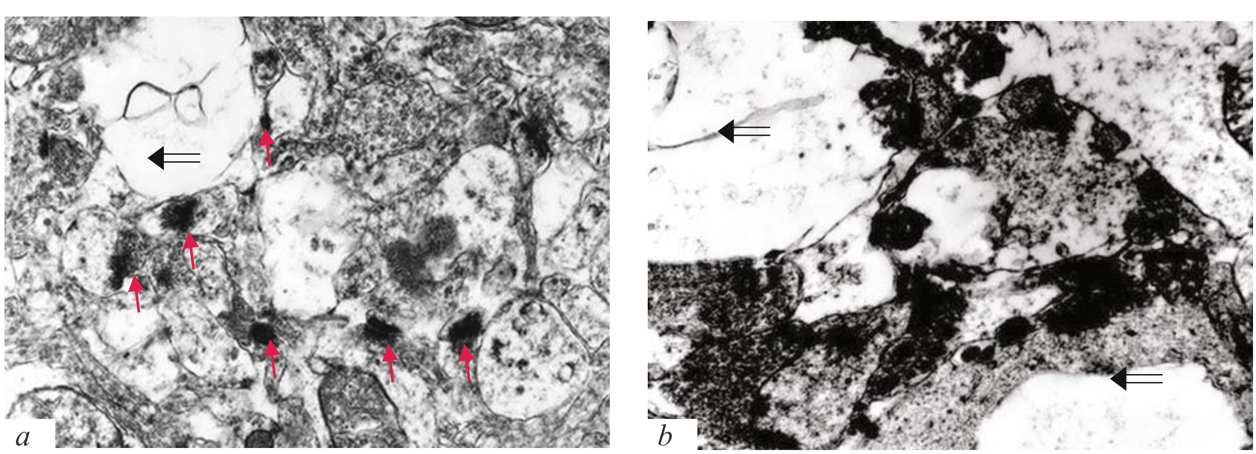

Fig. 5. Neuropil in hippocampal CA3 region of albino rats (×17,500). *a*) high density of small synapses (arrow)against the background of focal edema/swelling of neuropil (double arrow); day 21; *b*, edema/swelling of the neuropil (double arrow), destruction of the terminals (light type), day 60.

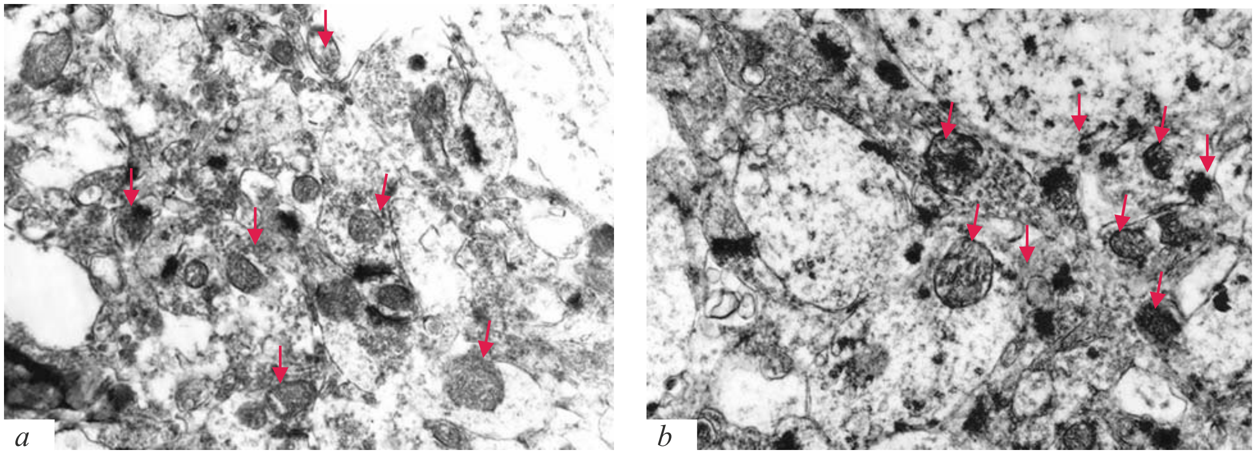

Fig. 6. Neuropil in hippocampal CA1 region of albino rats 60 days after acoustic stimulation. *a*) moderate manifestations of degradation of mitochondria, edema/swelling of the neuropil, mitochondria (arrow, ×15,600); *b*) mild edema/swelling of the neuropil, destruction of cristae in some mitochondria, mitochondria (arrows); high density of small synapses (×17,500).

This restructuring is usually accompanied by marked changes in the integrative and trigger activity of any tuned functional system of the brain or even the formation of its pathological systems. Structurally and functionally preserved synapses enable recovery of the damaged afferent input to neurons through their reparative reorganization. In this case, the mechanisms of hyperplasia, hypertrophy of remaining functionally active synaptic contacts, their cleavage, recombination, and the spatial reorganization are triggered [7].

According to electrophysiological data, "Sommer sector" CA1 is opposed to "Bratz sector" CA3, "resistant"sector has completely degenerated. Sommer sector remained intact with a strong ability to slow the accumulation and storage preservation of information [2], which provokes the appearance of sustained foci of excitation, by activation of additional neurons in volleys of activity in the hippocampus [1], combining hyperactive neurons in generating the strong excitation [10] by fixing all kinds of sensory input [3]. Diffuse reorganization of hippocampal neural circuits alters intercentral relationships in brain structures. These changes manifest in loss of functions (Cannon–Rosenblueth law of denervation) and by the type of qualitative transformation of existing brain neural networks accompanied by the formation of pathological systems. Therefore, congenital or acquired deficiency of natural resistance of the organ under stress conditions can make it a target for pathology, which originally did not affect selectively this organ [8].

Thus, after analyzing published data and results of our experiments, we can conclude that limbic system is the central structure involved in the formation of the stress-syndrome in response to exogenous environmental factors. Hippocampal reorganization of the sector into neuronal ensembles with high information content of neurons and the possibility of formation of the dominant pacemaker area in it promotes the development of stress-syndrome with changes in the integrative and trigger activity of brain structures.

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