PHYSIOLOGY

Structural and Functional Characteristics of Neurons in the Sensorimotor Cortex of Rats with Different Resistance to Emotional Stress

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 8, pp. 124-128, August, 2001
Original article submitted May 24, 2001

Wistar rats behaviorally active in the open field test (resistant to emotional stress) are characterized by polymorphism of neurons in layer V of the sensorimotor cortex and the presence of hyperchromatic cells, which probably determines resistance to emotional stress in these rats. Atrophy of hyperchromatic neurons reflecting transient inhibition of cell activity was noted in Wistar rats subjected to stress. In the sensorimotor cortex of behaviorally passive animals (predisposed to emotional stress) groups of densely packed hyperchromatic cells and pronounced pericellular edema were revealed. In these rats stress caused irreversible changes in cortical neurons and death of some cells. The presence of ischemic cortical neurons in rats subjected to emotional stress suggests that cerebral hypoxia plays a role in structural and functional disorganization of the sensorimotor cortex during emotional stress.

Key Words: *emotional stress; Wistar rats; behavior; sensorimotor cortex; neurons*

Our previous studies showed that the animals can be divided into resistant and predisposed to emotional stress (ES) according to their survival rates in similar conflict situations [4,7,8]. The animals are characterized by different genetically determined and individual resistance to ES. Studies of the behavioral response to a novel situation, parameters of vegetative (orthostatic test), and analgesic reactions allowed us to determine prognostic criteria for evaluation of rat resistance to ES [8]. Rats with different resistance to ES are characterized by different content of catecholamines in brain structures and adrenals [6] and different concentrations of neuropeptides (δ-sleep-inducing peptide, β-endorphin, substance P, and angiotensin II) in the brain and blood [4]. Moreover, expression of *c-fos* in brain structures differs between various animals [7].

We revealed nervous and humoral mechanisms underlying individual and genetically determined resistance of rats to cerebrovascular disorders during ES [6,7]. It was shown that protein contents in neurons of the cerebral cortex considerably varied in different rat strains [2].

Here we studied structural and functional characteristics of neurons of the sensorimotor cortex (SMC) in rats with different resistance to ES and changes in these parameters during experimental ES.

MATERIALS AND METHODS

Experiments were performed on 24 male Wistar rats weighing 200-250 g. Before the experiment, these ani-

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0007-4888/01/1322-0715$25.00

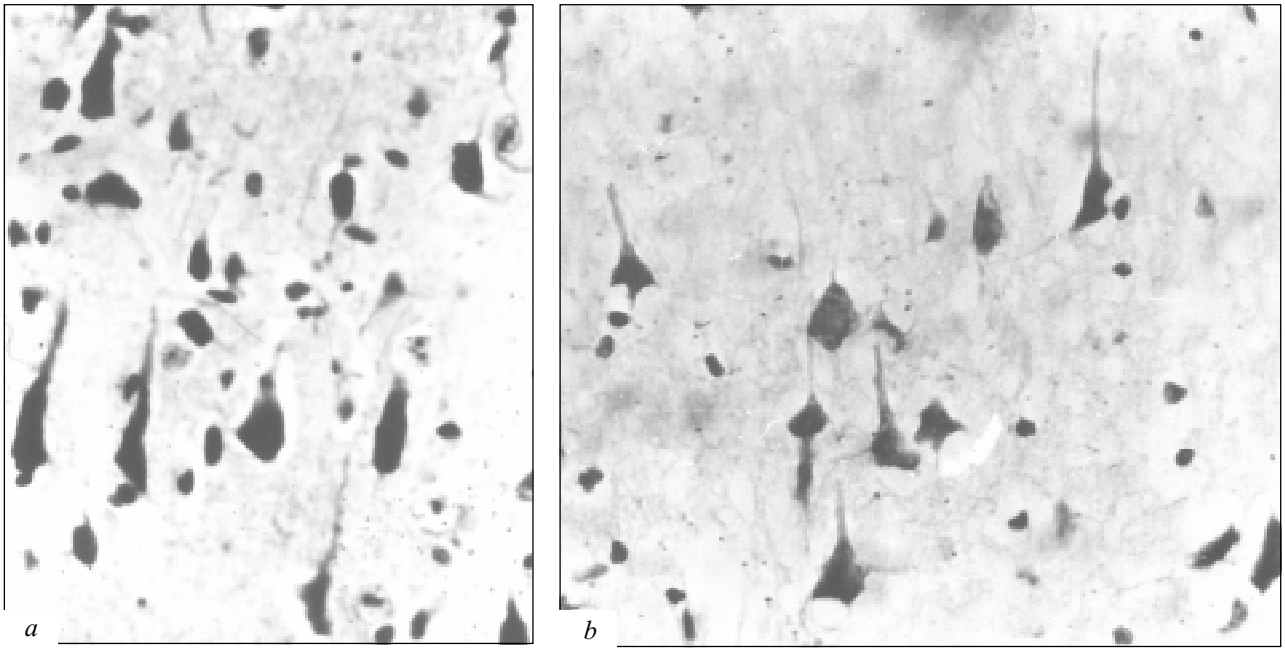

Fig. 1. Neurons of the sensorimotor cortex in control rats resistant (*a*) and predisposed to emotional stress (*b*): sharply hyperchromatic neurons and individual pericellular edema (*a*); hyperchromia, thinning of dendrites, and pericellular edema (*b*). Here and in Fig. 2: Nissl staining, ×300.

mals were kept in cages (10 rats per cage) for 10 days and had free access to food and water.

Individual characteristics and prognostic resistance of rats to ES were evaluated in the open field (OF) test using special software. The following parameters were evaluated: latency of the first motion, latency of entrance into the center of OF, horizontal activity (number of crossed peripheral and central squares), vertical activity (number of peripheral and central rearing postures), exploratory activity (number of explored holes), and vegetative parameter (rate of defecation). Each rat was tested for 3 min.

OF was a round area (diameter 90 cm, wall height 40 cm) illuminated with a 100 W lamp. The floor of this chamber was divided into 37 squares.

Three days after the behavioral test, the rats were immobilized in individual plastic boxes. Electrocutaneous stochastic stimulation of the tail was performed with threshold alternating current (4-6 V, 50 Hz, 1 msec) for 30 sec or 1 min.

After autopsy the adrenals and thymus were weighed.

All rats were divided into 4 groups: resistant (control and ES) and predisposed to ES (control and ES).

Three rats from each group were decapitated 1 h after the start of the experiments. The brain was fixed in neutral formalin, dehydrated in increasing alcohol concentrations, and embedded in paraffin. Brain sections were stained by the Nissl method widely used for evaluation of functional changes in neurons. SMC of

TABLE 1. Count of Different Neurons in 10 Visual Fields of Sensorimotor Cortex Layer V in Rats Resistant and Predisposed to ES (*M*±*m*)

Neurons	Control		Stress	
	resistant	predisposed	resistant	predisposed
Normochromatic	2.4±1.3	0.7±0.5^{++}	—	—
Moderately hypochromatic	3.5±2.0	1.7±0.5^{+}	2.8±1.1	0.15±0.07^{*+}
Moderately hyperchromatic	3.3±1.8	3.0±1.0	1.2±1.0	0.5±0.3*
Sharply hypochromatic	2.4±0.8	1.7±1.1	3.5±1.6	5.0±0.7*
Sharply hyperchromatic	8.8±2.7	7.8±2.1	3.1±1.7**	1.3±0.2*
Ghost cells	2.0±0.3	2.7±1.6	2.5±1.2	4.0±0.7
Ischemic cells	—	—	3.0±1.0*	6.2±1.8^{*++}

Note. $^{*}p<0.01$ and $^{**}p<0.05$ compared to the control; $^{+}p<0.01$ and $^{++}p<0.05$ compared to emotional stress-resistant rats.

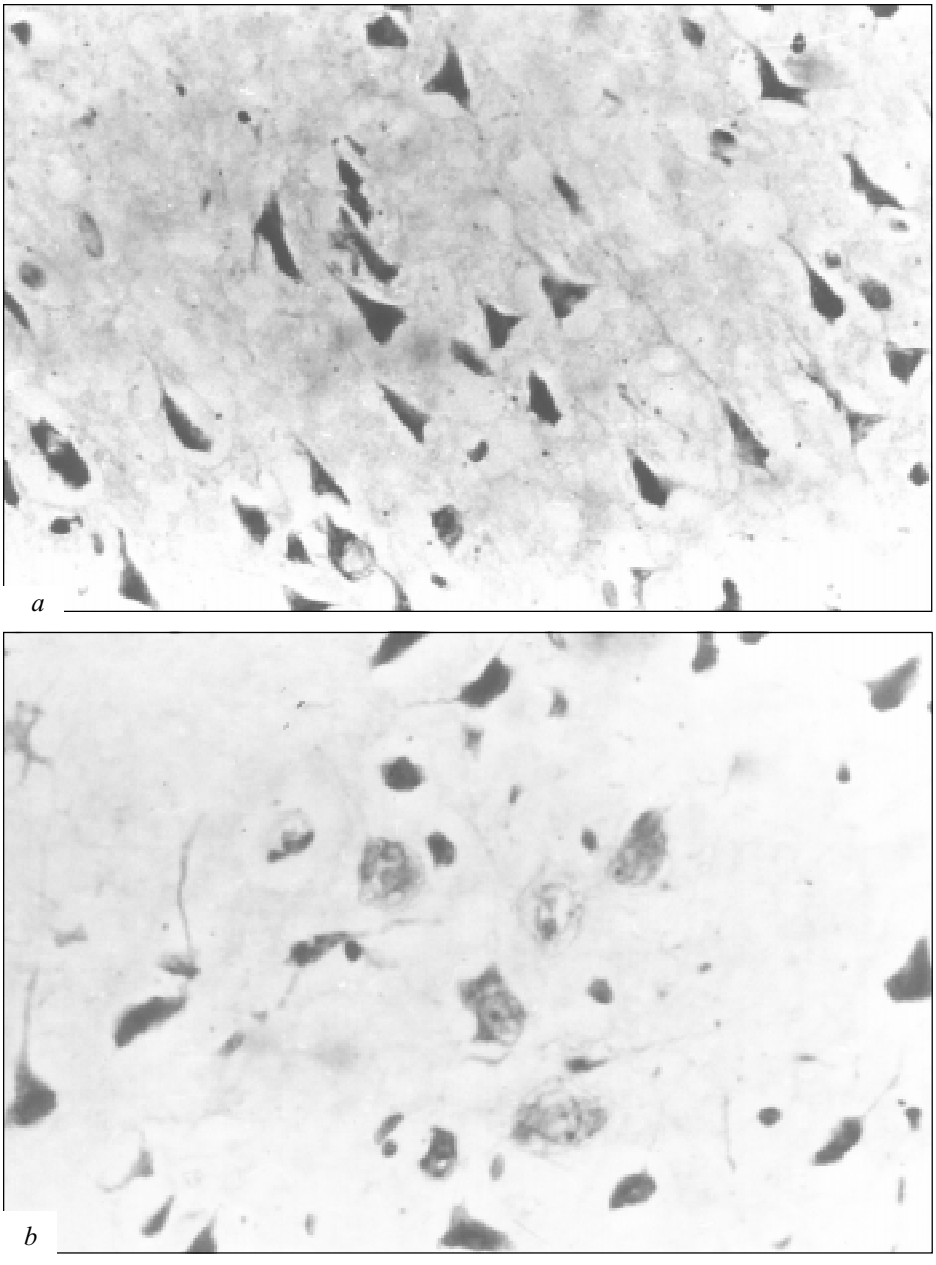

Fig. 2. Neurons of the sensorimotor cortex in stressed rats prognostically resistant (*a*) and predisposed to emotional stress (*b*): atrophy of sharply hyperchromatic neurons (*a*); ischemic neurons, total chromatolysis, edematous atrophy, pericellular edemas, and fragments of neurons (*b*).

the cerebral hemispheres was examined. Apart from qualitative evaluation, structurally different neurons in SMC layer V were counted in 10 visual fields (×200). The results were analyzed by Student's *t* test [1].

RESULTS

Depending on behavioral parameters in the OF test, all rats were divided into active (n=10) and passive groups (n=8). Ambivalent animals (n=6) intermediate by their behavioral pattern were excluded from further experiments.

Our previous studies showed that rats behaviorally active in the OF test are resistant to ES [4,7]. By contrast, animals displaying low locomotor activity are predisposed to ES.

Morphological examination of SMC layer V revealed normochromatic and moderately or sharply hypo- and hyperchromatic neurons in control ES-resistant rats (Table 1). Peripheral and total chromatolysis was typical of moderately and sharply hypochromatic neurons, respectively. The cytoplasm of moderately hyperchromatic neurons contained several small vacuoles. We revealed the presence of individual ghost

cells and signs of satellite penetration into the cell body. Sharply hyperchromatic cells with apical dendrites and pericellular edema were clearly seen (Fig. 1, *a*).

In control ES-predisposed rats the structure of SMC neurons was similar to that in control ES-resistant animals. In rats predisposed to ES the number of cortical neurons (particularly, normo- and moderately hypochromatic cells) was lower, while the count of ghost cells was higher than in ES-resistant animals (Table 1). Sharply hyperchromatic neurons had small bodies, and their thinned dendrites were seen at a long distance. Pericellular edema was found not only in neurons, but also in their satellites and free glial cells (Fig. 1, *b*).

After a 1-h stress exposure normochromatic cells were practically not detected in SMC of ES-resistant rats. The number of sharply hypochromatic cells increased, while the count of moderately hypo- and hyperchromatic neurons decreased (Table 1). Atrophic hyperchromatic neurons with pericellular edema were also seen (Fig. 2, *a*). Pronounced hypochromicity of neurons, their transformation into ghost cells, and vacuolization of the cytoplasm indicated overexcitation of neurons probably accompanied by their exhaustion and death. In these rats we found ischemic neurons with dark nuclei, light homogenous cytoplasm and, more rarely, pericellular edema.

After a 1-h stress exposure normochromatic cells were not found in SMC of ES-predisposed rats. In these animals the count of moderately hypo- and hyperchromatic and sharply hyperchromatic neurons was much lower, while the number of sharply hypochromatic, ghost, and ischemic cells was higher than in ES-resistant rats (Table 1). Individual ischemic cells contained a rod-like nucleus lying perpendicular to the cell body axis. Hypochromatic neurons underwent total chromatolysis with coarse vacuolation of the cytoplasm. Fragments of dark neurons and glial nodules substituting dead neurons were also found (Fig. 2, *b*).

Our experiments demonstrated structural peculiarities of SMC neurons in rats with different individual resistance to ES. In rats prognostically resistant to ES the count of normochromatic (relatively resting) and moderately hypo- and hyperchromatic SMC neurons with high functional activity surpasses that in ES-predisposed animals [5].

Stress leads to atrophic changes in bodies of sharply hyperchromatic SMC neurons in rats prognostically resistant to ES. These reactions are associated with the inhibition of afferent impulses or blockade of generation of efferent impulses in neurons. Formation of pericellular edemas in ES-predisposed rats indicates disturbances in the water-salt metabolism. ES causes partial vacuolar atrophy of neurons in these animals, which reflects severe impairment of the lipoprotein backbone of neurons and can lead to irreversible changes [1]. Accumulation of sharply hypochromatic neurons and ghost cells and substitution of dead neurons with glial elements indicate exhaustion of reserve capacities of SMC neurons in ES-predisposed rats. After a 1-h exposure to stress the count of ischemic neurons in these animals far surpassed the corresponding parameter in ES-resistant rats, which probably reflects the development of severe cerebral hypoxia.

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