Role of Brain Stem in the Mechanisms of Individual Resistance of Rats to Emotional Stress

E. V. Koplik

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It is shown in rats that the structures of brain stem (medial and lateral nuclei) are involved in the mechanisms of individual resistance to emotional stress (immobilization and cutaneous electrostimulation). Bilateral destruction of brain stem structures reduces this resistance, which manifests itself as behavioral changes in the open field test (the behavior of rats resistant to stress resembles that of rats prone to it), increased adrenal hypertrophy, thymic involution, and high mortality under conditions of emotional stress.

Key Words: *behavior; emotional stress; resistance to stress, brain stem*

The resistance of animals to emotional stress (ES) is determined both by genetically determined and acquired features of the organism [4-6]. It was demonstrated that various strains of experimental animals differ in the resistance and the ability to adapt to acute and chronic ES [3,7,8].

Considerable attention has been recently focused on the correlations between various behavioral parameters, for example, in the open field test and emotional reactivity under conditions of stress [7,8,13].

Previously, we showed that rats with higher motor activity in the open field exhibit higher resistance to ES [2]. By contrast, rats with longer latency and lower motor activity are prone to acute ES [2].

Cerebrovisceral disturbances developed in ES are determined predominantly by "stagnant excitation," which is formed primarily in the limbic and reticular structures with obligatory involvement of monoaminergic and peptide systems of the brain [1].

It was demonstrated that functional disconnection of individual limbico-reticular structures changes the response to stress [9,10]. However, central mechanisms underlying formation of the resistance to ES are poorly investigated.

Laboratory of Physiology of Emotions, P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow

In the present study we evaluated the role of brain stem in the resistance to ES.

MATERIALS AND METHODS

Experiments were performed during the spring—summer season on 200 male Wistar rats weighing 200-250 g. The rats were maintained in cages (five in each) and had free access to food and water. Before experiment they were tested in the open filed.

Open field was 90 cm in diameter and had 40 cm walls in height. The box was divided into 37 sectors and illuminated with a 100-W bulb.

The following parameters were evaluated: latency of the first movement, latency of reaching the center, horizontal ambulations (the number of crossed peripheral and central sectors), vertical activity (number of peripheral and central rearings), exploring activity (exploring of "holes"), and number of defecations (the parameter of vegetative balance). All rats were tested for 5 min.

Emotional stress was produced by immobilization with a simultaneous electrocutaneous stimulation. The rats were placed in individual Plexiglass boxes 3 days after the open field test. Electrical stimuli (4-6 V, 500 Hz, 1 msec) were applied stochastically to the tail skin. Each stimulation lasted 30 sec or 1 min.

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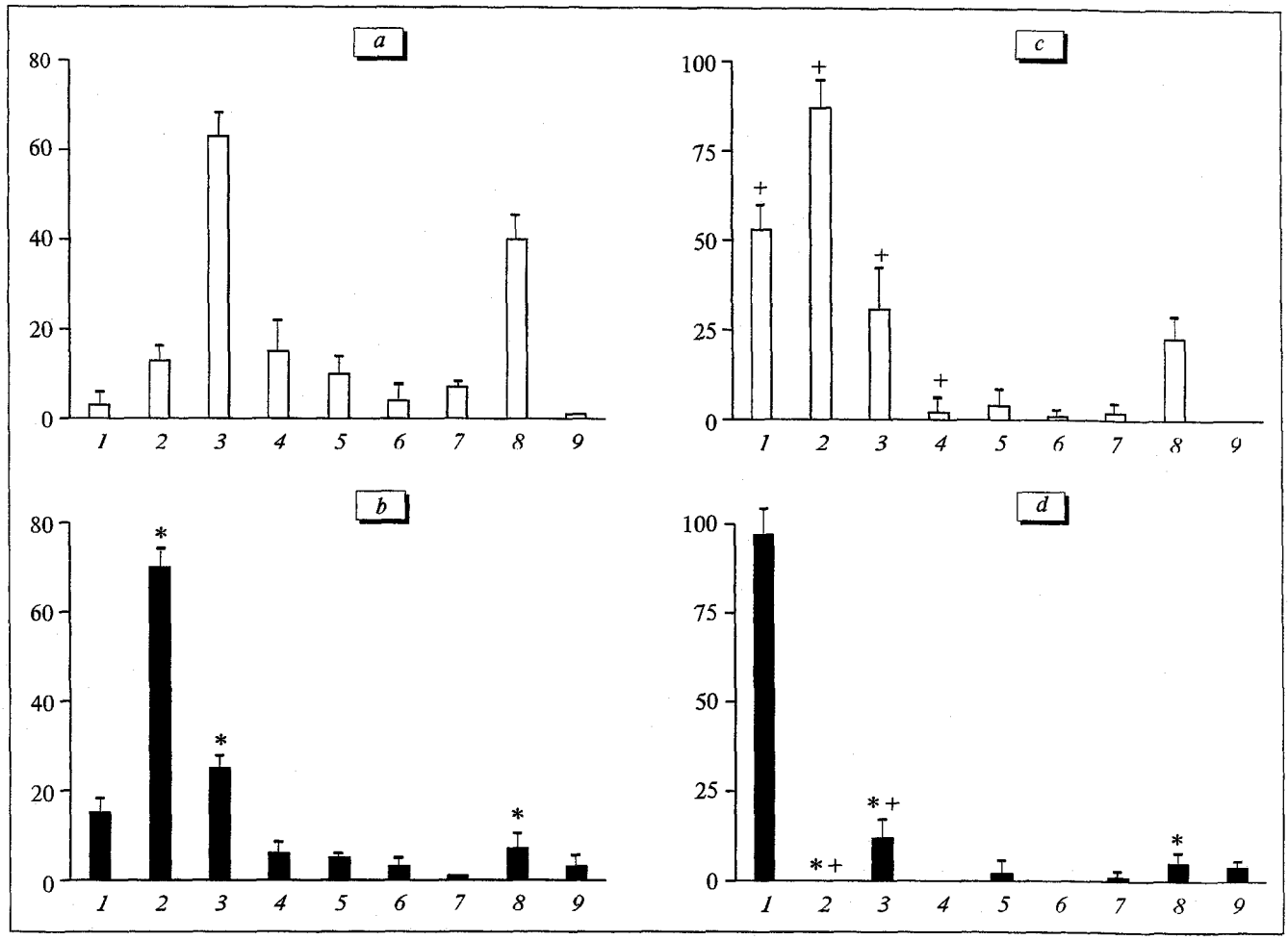

Fig. 1. Behavioral changes in the open field test after brain stem destruction. *1*) latency of the first movement, sec; *2*) latency of reaching the center, sec; *3*) number of crossed peripheral sections; *4*) number of crossed central sectors; *5*) number of peripheral rearings; *6*) number of central rearings; *7*) number of hole explorations; *8*) grooming, sec; *9*) number of boluses. Rats resistant (*a*, n=83) and prone (*b*, n=64) to stress. Rats resistant (*c*, n=40) and prone (*d*, n=38) to stress after destruction of brain stem. $p<0.05$: *compared with the control; *compared with stress-resistant rats.

The following parameters were used as criteria of resistance to ES: 1) behavior in the open field test, 2) changes in the adrenals (hypertrophy) and thymus (involution), and 3) survival under conditions of ES.

Bilateral destruction of the brain stem was performed by anodic polarization (50 μA, 30 sec). The indifferent electrode was placed on the back. Electrodes in the brain stem were inserted according to the coordinates of the stereotaxis atlas of rat brain [11] (AP 7.5 mm, L 0.6 mm, H 4.0-4.5 mm from the skull). Similar operation was performed in control rats (sham-operated) without anodic polarization. After the operation, the rats were treated with penicillin (daily dose 150,000 U per rat) for 3 days. Experiments were performed on day 5 after destruction of the stem. Experimental and control rats were decapitated simultaneously. The destruction was assessed histologically using serial frontal sections stained by the method of Nissl.

Statistical analysis was performed with ANOVA software.

RESULTS

According to the results of the open field test, the rats were divided into three groups. Group 1 consisted of 83 rats with short latencies of the first movement (<3 sec) and of reaching the center (<15 sec), high horizontal motor activity (>80 crossed peripheral and central sectors), high vertical activity (>10 rearings at the periphery and in the center), grooming (>10 sec) and exploring activity (>5 explorations of holes) and a low parameter of vegetative balance (0-1 bolus for 5 min) (Fig. 1).

Group 2 included 64 rats with the latency of the first movement >10 sec and latency of the reaching the center >70 sec, low peripheral and central horizontal activities (up to 40 crossed sectors), low ver-

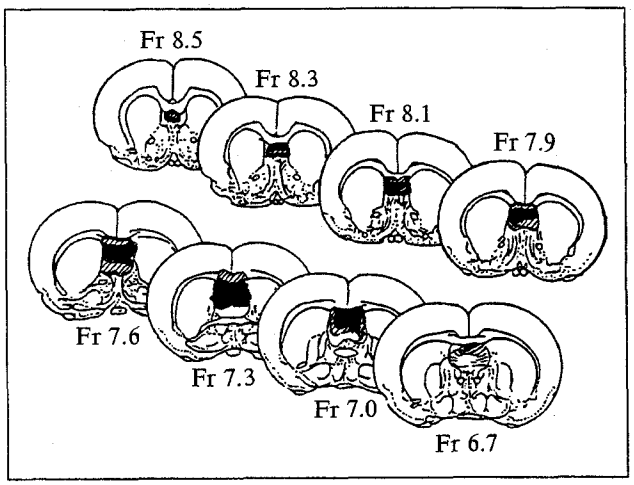

Fig. 2. Destruction of the brain stem. Serial frontal sections (Fr).

tical activity (up to 8 rearings at the periphery and no central rearings), low exploring activity (0-2 explorations of holes) and grooming (up to 10 sec), and higher number of defecations (2-4 boluses for 5 min).

According to our previous findings [2], group 1 rats were regarded as resistant and group 2 rats as prone to ES.

Ambivalent rats (n=53) with parameters intermediate between those of group 1 and group 2 rats were excluded from the experiment.

In 40 rats from the first group and 38 rats from the second group the brain stem was destroyed by anodic polarization after the open field test. The destroyed area (Fig. 2) spread over medial and lateral nuclei, fornix, precommissural fibers, cortico- and septochabenular fascicles, and the fascicle of the streak of Broca. The brain of sham-operated rats was analyzed for the absence of damage to the stem. The rats were tested in the "open field" on day 5 after the operation.

After destruction of brain stem, in group 1 rats the latency of the first movement increased 13-fold compared with the control and the latency of reaching the center increased 5.2-fold. Horizontal motor activity decreased considerably (crossing of peripheral sectors by 60.5% and crossing of central sectors by 90%), vertical activity decreased by 90%, grooming by 56%, exploring activity by 75%, and the parameter of vegetative balance did not differ significantly from the control. In group 2 rats, the latency of the first movement increased 4.7-fold, and the rats never appeared in the center. Horizontal motor activity decreased considerably (crossing of peripheral sectors decreased by 62.5%), vertical motor activity decreased by 90% compared with the control, while grooming, exploring activity, and the vegetative balance parameter did not differ significantly from the control (Fig. 1).

After the open field test, 40 control rats and 40 rats with destroyed brain stem were subjected to acute ES: 1 h in small Plexiglass boxes with aperiodical electrocutaneous stimulation with alternate current.

All sham-operated rats with high motor activity and low parameter of vegetative balance survived under conditions of acute ES.

Four out of sixteen rats with long latency of the first movement, low motor and exploring activities, and high parameter of vegetative balance died during the acute ES experiments.

We analyzed stress-induced changes in the weight of adrenal and thymus and calculated the weight index in control and stressed rats as weight of the organ (mg) per 100 g body weight.

In 20 control rats with high motor activity, this index was 10±0.8 mg/100 g for the adrenals and

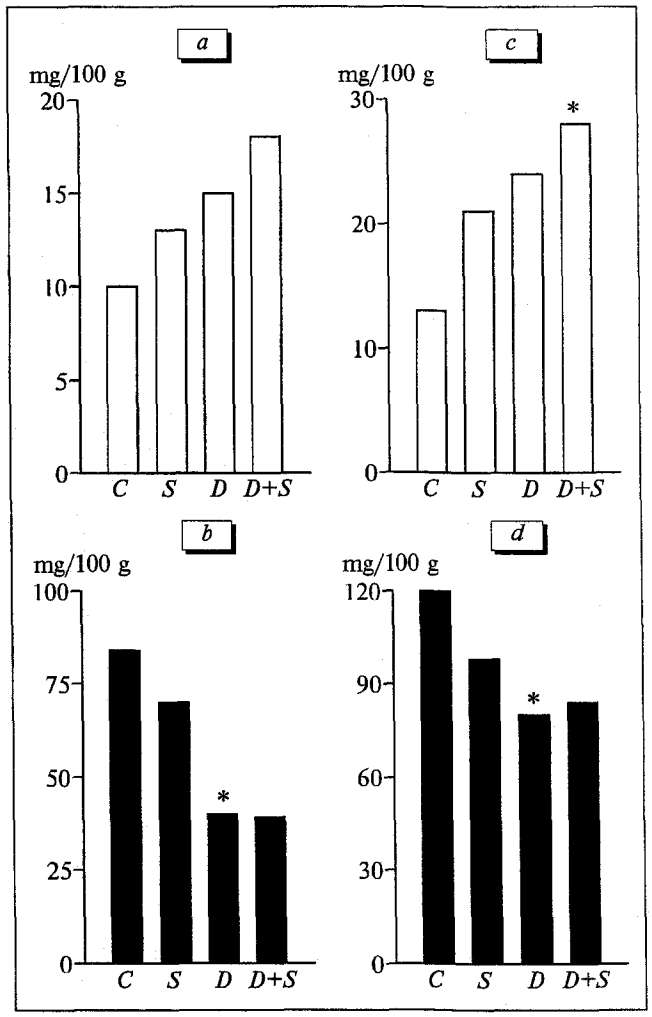

Fig. 3. Changes in the relative mass of adrenals (*a*, *c*) and thymus (*b*, *d*) caused in rats with destroyed brain stem. Animals resistant (*a*, *b*) and prone (*c*, *d*) to stress. *C*) control; *S*) stress; *D*) destruction of brain stem; *D+S*) stress against the background of brain stem destruction. *p<0.05 compared with the control.

100±13 mg/100 g for the thymus. In rats with low motor activity (n=10), this index was 13±0.63 mg/100 g for the adrenals and 86±0.7 mg/100 g for the thymus.

After acute ES, adrenal index increased by 2.2% in rats with high motor activity (n=20) and by 15-20% in rats with low motor activity (n=18) (Fig. 3).

In both groups, stress induced 68-70% involution of the thymus (Fig. 3).

After bilateral destruction of the brain stem, 2 out of 20 rats with high motor activity and low parameter of vegetative balance died under conditions of acute ES. Eleven out of twenty rats with long latency of the first movement, low motor and exploring activities, and high parameter of vegetative balance died under these conditions.

After stem destruction, in 20 rats with high motor activity the adrenal index was 15.4±1.3 mg/100 g and the thymic index was 69.4±17 mg/100 g. In rats with low motor activity (n=20), the adrenal index was 17.2±0.58 mg/100 g and the thymic index was 62±0.5 mg/100 g.

The adrenal index increased by 44.8% in rats with high motor activity (n=20) and by 56% in rats with low motor activity (n=20) (Fig. 3).

Against the background of destroyed brain stem, acute ES induced a 56% thymic involution in rats with low motor activity, while in rats with high motor activity thymic index did differ significantly from the control.

Our results show that medial and lateral nuclei of brain stem are involved in the mechanisms responsible for stress resistance in rats. Bilateral destruction of these structures reduces the resistance to acute ES. This manifests itself as behavioral changes in the open field test (the behavior of stress-resistant rats with destroyed stem resembles that stress-prone rats), increased adrenal hypertrophy, thymic involution, and mortality under conditions of stress.

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