Effects of Different Stress Techniques and Adaptation on Behavioral and Somatic Indices in Rats

O. N. Bondarenko, N. A. Bondarenko, and E. B. Manukhina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 8, pp. 157-160, August, 1999
Original article submitted November 23, 1998

We developed a model of stress (free swimming in a cage) which allows to assess the immediate and long-term effects of emotional stress. This stress induced typical changes in the open field test and ulceration of gastric mucosa. Unlike standard immobilization stress the proposed technique excludes a traumatic factor, it is well reproducible and simple.

Key Words: *stress; adaptation; behavioral and somatic indices, rats*

Currently efforts are underway to understand the mechanisms of stress and adaptation and to find ways and methods of preventing the so-called stress-related diseases. The techniques applied to study the mechanisms of stress in animal experiments usually include a traumatic factor. An alternative approach was proposed by P. V. Simonov: one group of animals is subjected to inescapable electroshock, the animals of the other group hear their vocalizations, which act as a stress factor. A distinct group of psychosocial stress techniques can be divided into 2 categories: 1) generation of laboratory overpopulation, where the number of individuals is regulated by stress; 2) complete social isolation of individuals for a long time [5]. However, these techniques are very complex and laborious and do not meet the international regulations for humane treatment of experimental animals.

The study was aimed at developing of an appropriate and nontraumatic technique which enables to assess both the immediate and long-term effects of emotional stress in animals.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 220-250 g. They were performed in the daytime from 12.00 to 16.00.

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

The proposed stress technique is further referred to as free swimming in a cage (FSC). Experimental chamber for FSC was a standard plastic cage (50×30×20) filled with water (22°C) to a level of 15 cm. Five rats were placed into the cage which was covered with a wire net. The distance from the cover to water was 5 cm. The animals could rear up grasping the net and hang on to it or swim without interfering with each other. The exposure lasted 30 min.

Water immobilization [6] was used as a reference technique. Rats were placed into individual plastic boxes and fixed by the neck with a special clip. The boxes were vertically immersed in water (22°C). The head and neck were above the water level.

After exposure the rats were dried, left for 1 h in a warm place, and then tested in an open field. The open field apparatus was a white round box 120 cm in diameter with 45 cm high walls. The floor was marked in 32 squares 20×20 cm with a hole 2.5 cm in diameter at the corner of each square. The white noise intensity was 62 dB. The rat was placed near the wall and its horizontal activity (HA; peripheral, central, and total) was counted for 4 min.

Vertical activity (VA) in the open field was represented by rearings with and without support which were counted separately. The total VA was calculated after the test.

Adaptation to water immobilization was performed according to a previously described schedule [4]: the animals were immobilized for 5, 10 and 15 min

0007-4888/99/0008-0794$22.00

TABLE 1. Changes in Internal Organs Depending on Stress Model and Functional State of Experimental Animals

Experimental conditions	*n*	Weight, mg		Hemorrhages in stomach, mm²	Animals with hemorrhages, %
		thymus	adrenal glands		
Sated					
control	10	490	38	0	0
water immobilization	10	464	40	0	0
FSC	7	483	56*	1.3*	57*
Hungry					
control	10	485	39⁺	0⁺	0⁺
water immobilization	9	449	50*	4.8*	100*
FSC	10	410*	48*	5.4*⁺	80*

Note. Here and in Table 2: $p<0.05$: *in comparison with intact animals, ⁺in comparison with water immobilization.

on days 1, 2, and 3, respectively. After 2 days, the procedure was repeated. Twenty-four hours after the last session the animals were tested in the open field or subjected to 3-h immobilization and tested 1 h afterwards.

The adaptation to FSC, acute stress, and behavioral testing were performed using a similar schedule. However, considering that the period of FSC was 6 times shorter than immobilization, the time of adaptation was reduced to 1, 3, and 5 min on days 1, 2, and 3, respectively.

Our preliminary experiments showed that FSC and water immobilization exert different effects on sated (free access to food) and hungry (24-h food deprivation) animals.

After the experiments the rats were decapitated. The thymus and adrenal glands were weighed and the area of hemorrhage in the gastric mucosa was measured.

The data were analyzed statistically using the Wilcoxon—Mann—Whitney test.

RESULTS

In sate drats, 3-h water immobilization caused no significant changes in the weight of the thymus and adrenal glands and induced no gastric lesions (Table 1), while FSC significantly increased the weight of the adrenal glands.

Hungry animals after water immobilization or FSC exhibited more pronounced pathology: their adrenal glands significantly increased, while the thymus decreased, hemorrhages appeared in the stomach. Therefore, in further experiments we used only hungry animals.

The total HA of rats subjected to FSC did not differ from the control. However, in the open field they spent all the time at the periphery not entering the central areas (Table 2). VA of these rats consisted of rearings with wall support. The same tendency to move along the walls in the open field test was reported for rats subjected to chronic emotional stress. These rats showed pronounced gastric ulcers and other stress-induced changes in internal organs [2].

After water immobilization the rats displayed significantly lower HA (total, peripheral, and central) in the open field in comparison with the control. However, the structure of their behavior was preserved. The total VA was markedly reduced with preserved proportion between rearings with and without support.

Thus, FSC-induced changes in the structure of the open field behavior point to primarily emotional aspect of this stress condition. The changes in the open field behavior after immobilization stress can be interpreted as a decrease in general locomotor activity.

TABLE 2. Effects of Different Stress Models on Rat Behavior in Open Field Test (n=10)

Experimental conditions	Horizontal activity			Vertical activity		
	in the center	at the periphery	total	rearings without support	rearings with support	total
Control	3.4	44.2	47.6	2.0	10.4	12.4
FSC	0.0*	41.0	41.0	0.0*	6.8*	6.8*
Water immobilization	1.4*	21.1*	22.5*	1.3*	4.9*	6.2*

TABLE 3. Effects of Adaptation to FSC on Behavioral and Somatic Indices in Rats (n=10)

Experimental conditions	Horizontal activity			Vertical activity			Area of gastric hemorrhage, mm²	Animals with hemorrhages, %
	in the center	at the periphery	total	rearings without support	rearings with support	total		
Control (intact)	3.4	44.2	47.6	2.0	10.4	12.4	0	0
Acute stress	0.0*	41.0	41.0	0.0*	6.8*	6.8*	5.4*	80*
6-day adaptation	2.8	34.5	37.3	1.3⁺	11.5⁺	12.8⁺	0⁺	0⁺
6-day of adaptation+acute stress	0.5*	50.2	50.7	0.5*	11.8⁺	12.3⁺	0.3⁺	9⁺

Note. p<0.05: *in comparison with the control (intact animals); ⁺in comparison with nonadapted animals subjected to acute stress.

This decrease can be a consequence of immobilization with compression of neck musculature (traumatic stress).

In further experiments, the open field behavior was studied in rats adapted to FSC (the data on rats adapted to water immobilization have been reported elsewhere [4]). The adapted and control (intact) rats showed similar HA and VA (Table 3) and similar changes in HA after exposure to FSC. At the same time, adaptation prevented the stress-induced changes in VA (rearings with support and total VA). Therefore, at the behavioral level the protective effect of adaptation was manifested only in VA.

In rats, VA is directly proportional to activity of the dopaminergic brain systems [1]. Hemorrhages in the gastric mucosa are known to result from vasoconstriction caused by elevation of blood catecholamines. Our data showed that these two indices changed in parallel: hemorrhages in the gastric mucosa were most pronounced in rats with low VA (due to depletion of brain dopamine). Adaptation to stress equally prevented both the behavioral and autonomic nervous pathology (Table 3).

When planning the experiments it is important to determine the minimum number of animals (n) necessary to obtain statistically significant results. Using the methods of mathematical statistics [3] we found the n values for both stressogenic techniques. It turned out that water immobilization technique requires nearly twice as many animals as FSC (n=15.5 and n=6.8, respectively).

In general, our data showed that the proposed technique has a number of advantages over the standard water immobilization procedure: it is simple and reliable, and stress-induced pathology is primarily of the "emotiogenic" nature.

The work was supported by the Russian Foundation for Basic Research (grant No. 97-04-48370), by Leading Scientific Schools Grant of President of Russia (project No. 96-15-97030), and by the Foundation for Development of Emotional Stress Pharmacology.

REFERENCES

1. N. A. Bondarenko, A. V. Val'dman, and V. A. Kamysheva, *Byull. Eksp. Biol. Med.*, **92**, No. 7, 35-38 (1981).
2. A. V. Val'dman, N. A. Bondarenko, and L. A. Malikova, *Mechanisms of Stress*, Kishinev, (1987), pp. 79-99.
3. Yu. S. Kabatov and M. B. Slavin, *Statistical Methods in Medical Investigations and Reliability of Medical Equipment* [in Russian], Moscow (1976).
4. E. B. Malenyuk, N. P. Aimasheva, E. B. Manukhina, *et al.*, *Byull. Eksp. Biol. Med.*, **126**, No. 9, 274-277 (1998).
5. V. P. Poshivalov, *Neuropharmacological Aspects of Emotional Stress and Drug Addiction* [in Russian], Leningrad (1978), pp. 88-101.
6. K. Kuriyama, K. Kanmori, and Y. Yaneda, *Neuropharmacology*, **23**, No. 6, 649-654 (1984).