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Assessment of Susceptibility of Outbred Albino Rats to the Formation of Depression-Like State of Learned Helplessness

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Learned helplessness (a model of depression-like state) was developed in rats by exposure to repeated inescapable electric stimulation and evaluated by the absence of attempts to escape when it could be performed. In randomly grouped outbred white rats, 37.5% animals after the above procedure meet the criterion of learned helplessness. On experimental day 14, the latent period and the number of applied electric shocks prior to the first escape to the safe compartment in rats with learned helplessness were significantly higher than in the control, but no significant differences in these parameters were observed on day 21. The Porsolt forced swimming test performed on days 14 and 21 revealed no differences from the control group. After the rats were divided into low- and high-active subgroups according to their open field behavior, 35% rats with learned helplessness were in the low-active subgroup group and 30% rats with learned helplessness were in the high-active subgroup. On day 14, the parameters of learned helplessness significantly surpassed the control levels only in the low-active subgroup. Only in rats with learned helplessness and low activity in the open field, the immobility time in the Porsolt test was longer than in control low-active rats. These findings attest to advisability of preliminary splitting of outbred animals by their open-field behavior into low- and high-active subgroups and the use of only animals for modeling learned helplessness.

Key Words: *depression models; learned helplessness; Porsolt test; open-field test; rats*

High prevalence of depression and limited possibilities of modern pharmacotherapy of the disease explain the urgent need to search for novel and highly effective antidepressants, which logically should be based on adequate experimental models. Such models should reproduce the behavioral and neurochemical abnormalities in patients with depressive disorders. However, the diversity of etiological features, pathogenesis, and symptomatology prevents the development of consistent and comprehensive experimental translational model. At this, most researchers believe that objective description of pharmacological features and the mechanism of action of potential antidepressants can be achieved only by combination of various experimental models, in which learned helplessness (LH) plays an important role [9,11].

In rats, LH is formed after repeated exposure to inescapable electric shocks. This behavior is characterized by the absence of attempts to avoid or escape an aversive stimulus under conditions where it is possible to do. According to available data, LH is developed in 50-70% animals depending on experimental conditions and animal strains [5,10]. Some studies reported that the behavioral phenotype under conditions of novelty has a predictive value for LH formation [8].

This work was designed to examine susceptibility to LH formation in random sample of outbred male albino rats and in rats, which had been preliminary subdivided by their activity in the open-field test.

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MATERIALS AND METHODS

The study was carried out on outbred male albino rats (n=196) weighing 180-200 g at the beginning of the experiments, which were obtained from Stolbovaya Animal Breeding Department (Research Center of Biomedical Technologies, Federal Medical-Biological Agency of Russia). The animals were maintained under standard vivarium conditions at natural illumination with *ad libitum* water and food supply. All procedures with animals were carried out in strict adherence to Order No. 199n of Ministry of Health of the Russian Federation (On Establishing the Rules of Good Laboratory Practice; April 1, 2016), Decision No. 81 of Council of the Eurasian Economic Commission (Rules of Good Laboratory Practice of the Eurasian Economic Union in the Field of Drugs; November 3, 2016) and the GOST R-33044-2014 Principles of Good Laboratory Practice. The experimental protocols were approved by the Bioethics Committee of the V. V. Zakusov Research Institute of Pharmacology.

A Shelter system (Neurobotics) operated without the audio signal was used for LH modeling. On experimental days 1 and 2, the rats were placed into the chambers with closed partition and subjected to 30 inescapable electric shocks (0.65 mA, 30 sec pulse duration) through electrified floor. The intervals between the shocks randomly varied (mean 30 sec). The control group consisted of intact animals. LH was assessed on experimental day 3. To this end, the rats were placed into the same chambers, but the door was opened allowing transition to the safe compartment. The animals were subjected to 30 shocks (0.65 mA, 6 sec duration) at variable intervals (mean 30 sec). The criterion for the development of LH was at least 25 escape failures in 30 trials [9]. LH was assessed on days 14 and 21 by the latency and the number of shocks until the first run to the safe compartment and by the behavior in the Porsolt forced swimming test performed in clear Plexiglas cylinder 45 cm in high and 20 cm in diameter (Open Science) filled by $^2/_3$ with water at 25±1°C. One day prior to testing, each rat was adapted by placing into this water-filled cylinder for 15 min. On experimental day, the depressive-like behavior was assessed by the total immobility time measured for each rat during the observation period (6 min).

Novelty-evoked activity was assessed in the open-field test (brightly illuminated white open arena 2 m in diameter divided by radii and 3 concentric circles into 16 peripheral and 8 central segments). The arena was illuminated with 4 shadowless lamps of 100 W each elevated by 1 m above the arena. Prior to experiment, the rat was placed into a dark compartment for 1 min, and then it was transferred to one of peripheral segments of the arena. The number of crossed peripheral segments (peripheral activity), the number of crossed central segments (central activity), the number of visits into the center, the number of rearings (vertical activity), and the number of defecations were recorded over 5 min. Total motor activity was the sum of peripheral, central, and vertical activities.

The results were statistically processed using StatPlus Pro 6.2 software. The normality of distribution was assessed with Shapiro—Wilk test. The intergroup difference between independence samples was analyzed with parametric Student's t test if the data were normally distributed, and dispersion was homogeneous according to Levene's test; otherwise non-parametric Mann—Whitney U test as applied. The differences were considered statistically significant at $p<0.05$.

RESULTS

On the first series of experiments, LH was induced in animals randomly divided into 4 groups (24 rats per group). Testing on experimental day 3 showed that 36 rats demonstrated LH (37.5%). Only these rats and intact controls were used in the following experiments. Retention of depressive-like state was assessed on days 14 and 21 in the Porsolt test (groups 1 and 2) and in Shelter system followed by the Porsolt test in 48 h (groups 3 and 4).

On days 14 and 21, Student's t test revealed no significant differences between the control and LH-rats by total immobility time, the basic index of depressive-like state assessed in the Porsolt test. On day 14, this index was 205±11 sec in LH group and 208±18 sec in control group; on day 21, the corresponding values were 205±11 sec and 193±13 sec.

On experimental day 14, latency of the first transition to the safe compartment and the number of elec-

TABLE 1. Behavior of Rats with LH on Days 14 and 21 after Training (Me (Q1-Q3)

Group	Latency to the first transition to the safe compartment, sec	Number of electric shocks prior to the first escape to the safe compartment
Day 14 after training		
Control (n=10)	54 (37-113)	1.5 (1-2)
Rats with LH (n=7)	570 (407-1543)*	10 (7-26)*
Day 21 after training		
Control (n=10)	53 (31-84)	1 (1-2)
Rats with LH (n=5)	376 (47-1129)	7 (1-19)

Note. Here and in Table 2: *$p<0.01$ in comparison with the control (Mann—Whitney U test)

TABLE 2. Behavior of LA and HA Rats with LH on Day 14 after Training (Me (Q1-Q3)

Group	Latency to the first transition to the safe compartment, sec	Number of electric shocks prior to the first escape to the safe compartment
La rats with LH (*n*=7)	695 (34-1800)*	12 (1-30)*
Control (*n*=8)	23.5 (17.0-63.5)	1 (1.0-1.5)
Ha rats with LH (*n*=3)	1800 (27-1800)	30 (1-30)
Control (*n*=8)	62 (35.0-137.5)	2 (1.0-2.5)

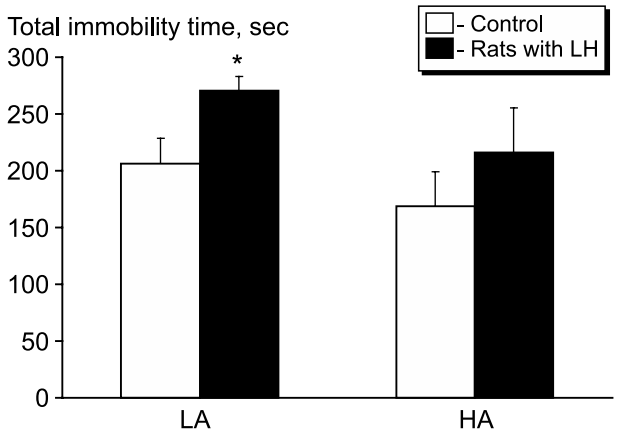

Fig. 1. Total immobility time in the Porsolt test in LA and HA rats with LH on day 16 after LH modeling. *p<0.05 in comparison with the conrtrol (Student's t test).

tric shocks delivered before this transition in LH-rats significantly surpassed the corresponding control values (Table 1). On day 21, these parameters were also higher, although the differences were insignificant (Table 1). Porsolt test also did not reveal the significance differences between total immobility times in LH and control groups. On day 14, these values were 240±20 sec and 188±19 sec in LH-rats and control animals, respectively (Student's t test), whereas on day 21, the corresponding indices were 265±4 sec and 220±30 sec (Mann—Whitney U test).

Thus, significantly longer latency of transition to the safe compartment and a higher number of electric shocks delivered before transition in LH-rats on experimental day 14 suggest that the use of modified procedure of LH modeling ensured retention of LH for up to 14 days. According to published reports, original procedure ensured LH formation in 55-70% animals [5,9,10].

Previous data attest to a relationship between individual typological personality traits and predisposition to the development of depression [12]. To simulate these differences, outbred animals in the second series of experiments were divided according to their open-field behavior, it is known that the depressive-like state is differently formed in animals with different reactions to stressful stimulation [4,7,8].

Based on previous studies [1,2], we formed the groups of rats with high (HA; total motor activity >80) and low (LA; total motor activity ≤35) activities. Of 100 rats, 28 LA and 18 HA animals were selected. Then, each group was randomly subdivided into the control rats and the rats subjected to LH modeling. To develop LH, the rats were trained on the Shelter system as described above. The number of LH-rats did not increase in comparison with previous experiment: we obtained 35% LH-rats in LA group and 30% LH-rats in HA group.

On day 14, the rats were tested in Shelter system and then subjected to Porsolt test in 48 h. Here, we revealed a dependence of the examined parameters on the type of activity in the open-field test: only LA rats with LH demonstrated significantly longer latency and higher number of electric shocks before the first transition to the safe compartment in comparison with control animals. In the HA subgroup, similar elevation of these parameters was observed, but they did not attain the significant level, which can be explained in part by a small number of animals in this subgroup (Table 2). Retention of depressive-like state examined in the Porsolt test showed that total immobility time in LA rats with LH was significantly longer than that in control LA rats, whereas HA rats with LH demonstrated merely a trend to an increase in the total immobility time in comparison with control HA rats (Fig. 1).

Published data on relationship between typological characteristics of rats and predisposition to LH are ambiguous. Some studies showed that congenitaly helpless rats are characterized by the increased exploratory activity in spatial novelty [3,4,8]. At the same time, there are data on susceptibility of LA animals to the development of depressive-like state as revealed by testing in the open field [6], which agrees with our findings.

Thus, retention of depressive-like state, which was observed in this study only in LA rats for 14-16 days after the development of LH indicates advisability to preliminary select LA animals from a random-bred population planned for LH training in order to assess the effects of potential antidepressants in the selected group.

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