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PHYSIOLOGY

Influence of "Enriched Environment" on Behavior and Neurogenesis in Mice Selected by Cognitive Trait

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Mice selected for high score in the extrapolation test (EX line) and kept under conditions of "enriched environment" for 3 months demonstrated changes in locomotor and exploratory activity and enhanced reaction to novelty. The relative brain weight was higher and neurogenesis in the hippocampal fascia dentate was more intensive in this group. In non-selected mice, the changes were similar, but insignificant in many cases.

Key Words: *enriched environment; neophagophobia; brain weight; neurogenesis; selection*

Maintenance of mice and rats in "enriched environment" (EE) [3] induced changes in their behavior, in particular, in the level of anxiety [3,5,6,8]. In mice, living in EE also stimulates neurogenesis of the adult brain [3,5,7,11]. The modifying effect of EE can also depend on the genotype [3,9,10] and can improve genetically reduced cognitive abilities [9,12]. At the same time, there is practically no data on the effect of EE on animals with different cognitive abilities.

We studied the effect of EE on behavior, relative brain weight, and neurogenesis in EX mice (selection for high score in the test for extrapolation of the direction of food stimulus movement) and the unselected population CoEX [2,3] and differences between the animals living in EE and animals maintained in standard cages. In the late generations of this selection (starting from F9), EX mice did not show stably higher scores in the extrapolation test, but they were significantly more successful in solving another cognitive test, the search for entrance into a shelters [2,8] and demonstrated more active reaction to novelty (presentation of new food in a new environment, a test for neophagophobia) [1]. The data for extrapolation and puzzle-box tests performed by these animals are not included in this paper.

MATERIALS AND METHODS

The experiments were conducted on female mice (n=43). The choice of females for the experiment was dictated by the need to exclude possible manifestations of intermale aggression that can also change behavior [3]. EX and CoEX animals from selection F16 were used. At the age of 30 days, the mice were placed for 3 months in EE. Two plastic cages (59×37×20 cm) connected by "tunnels", equipped with feeding racks, watering bowls, various "shelters", and "running wheels" were used to create EE. Control ECS and CoEX animals maintained in standard plastic cages (42.5×26.6×15 cm) served as the control. The number of animals in the groups: 10 animals of EX and CoEX in EE and 9 and 14 animals of EX and CoEX under standard conditions. The studies were conducted in accordance with the requirements of the EU Directive 2010/63/EU.

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The elevated plus-maze (EPM) consisted of 2 closed and 2 open arms and was positioned at a height of 28 cm above the horizontal plane. For 3 minutes of the test was recorded (manually). The time spent in open arms of the EPM, the number of entries into open arms, the number of transitions between closed arms, peeping, the number of vertical postures and grooming and defecation acts were recorded.

In the test for hyponeophagy, the mouse previously deprived of food (water *ad libitum*) for 14-16 h was placed in a small cylindrical chamber (diameter 40 cm, height 25 cm), in the center of which was a small plastic cup with a hinge of new food for the animal (cheese in form of small "cubes") was placed [1]. Within 10 minutes of the test, the time taken by the meal and the weight of eaten food were marked manually.

At the end of the experiment, the mice were euthanized by ether narcosis. The brain weight and body weight were determined and the relative weight of the brain was determined: brain weight (mg)/body weight (g).

The level of neurogenesis was evaluated in the hippocampal dentate gyrus on brain preparations of 8 mice (2 females of each line, from groups kept in EE and under standard conditions) by the immunohistochemical method to identify new cells expressing Ki-67 marker [4]. Cell counts were calculated visually under an Olympus CX-41 microscope with a fluorescent attachment by determination of the average number of cells in the cut.

Statistical processing of the results was conducted with a two-factor ANOVA (program Statistica 6.0) with a built-in post-hoc function by the method of least squares (LSD test).

RESULTS

The effect of EE on mouse behavior in the EPM test was detected in animals of both genetic groups. The line factor (ANOVA F1-31=4.25, $p<0.05$) with a longer time in both control groups significantly influenced the time spent in the center, *i.e.* the indicator "fear of open illuminated space". In other words, mice of both genotypes from EE more actively avoided the open part of EPM. However, the number of peeping in the illuminated part of the EPM in EX mice in EE was greater than in EX mice kept under standard conditions ($p<0.01$), whereas in both CoEX groups, the ratio was inverse, but insignificant. ANOVA also revealed the influence of the "medium" factor on the number of crossing between the closed arms of the EPM ($F_{1-31}=7.16$, $p<0.05$). In EX and CoEX mice kept in EE, there the number of crossings was higher than in mice kept under standard conditions, and the differences were significant in EX (but not in CoEX) (4.13±1.00 and 0.57±1.00, $p<0.01$). This parameter is considered usually as the index of the general level of locomotion. This is correlated with higher number of acts of exploratory behavior (vertical postures) revealed in the EPM test in EX and CoEX mice kept in EE in comparison with the same mice kept under standard conditions (in case of EX from EE group, the number of acts was significantly higher: 13.0±1.8 and 9.71±1.93; $p<0.01$). The differences between mice kept under "enriched" and standard conditions by the number of entries into open arms of the maze, "hanging", and grooming episodes had similar sign: these parameters were higher in mice from EE group, but the differences were insignificant. Thus, environmental enrichment led to an increase in overall activity of

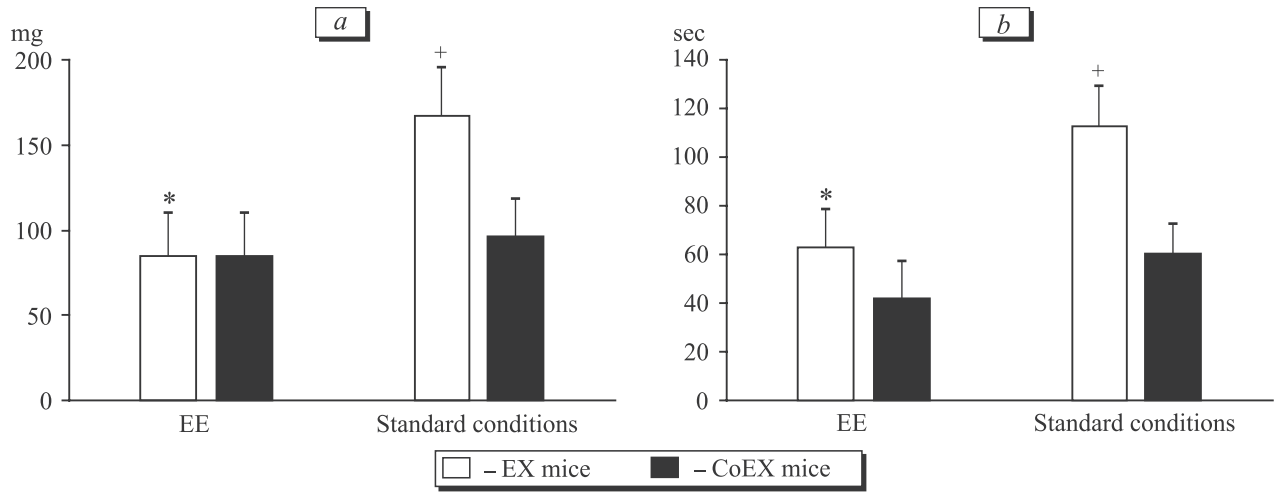

Fig. 1. Test for hyponeophagy. *a*) Weight of food (new), eaten in 10 min of the test; *b*) time spent for food consumption. Here and in Fig. 2: $p<0.05$ in comparison *with the corresponding group under standard conditions, +with the CoEX group.

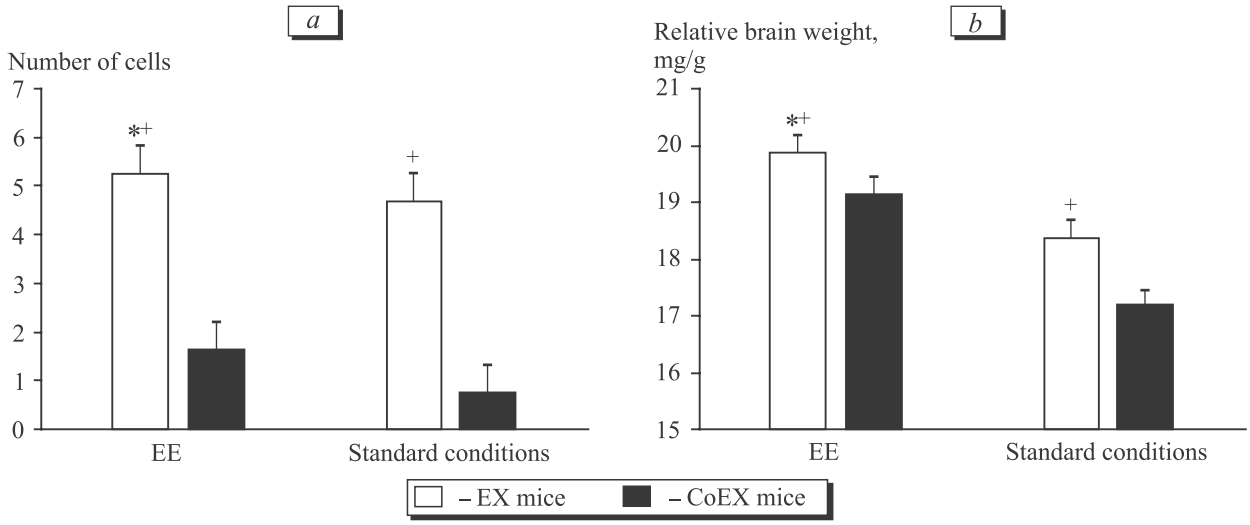

Fig. 2. Morphological parameters of the brain in mice. *a*) Number of new cells immunopositive for Ki-67 (mean values per 1 slice, 8 slices in each group). *b*) Index of relative brain weight: brain weight (mg)/body weight (g).

mice in both groups, but these differences were more pronounced in the mice, for which the higher parameters of research behavior were characteristic.

The comparison of mice in the test for hyponeophagy (Fig. 1) showed that EX mice from EE group demonstrated more pronounced fear of new food in the new environment: they ate significantly less food during the test and spent less time eating than EX mice kept under standard conditions. The differences for eaten food and in time of food intake between CoEX mice kept in EE and under standard conditions were insignificant. At the same time, the same parameters of "standard" groups also differed significantly, but EX mice were more active in this test, and hyponeophagy was significantly less pronounced in them than in CoEX mice, which agree with the previously described differences in this test for mice of these groups [1]. Thus, in this test, as well as in EPM, it was possible to observe higher anxiety of the mice from EE.

Thus, the behavior of mice of the two genetic groups kept in different conditions varied: EX mice kept in EE were more active and performed more acts of exploratory behavior (rearing postures).

ANOVA revealed a significant effect of the "line" factor (F_{1-31}=40.5, p<0.05) on the number of new cell elements (neurogenesis). Post-hoc analysis showed that EX mice from EE group significantly differed from the other three groups by the number of cells stained with antibodies to Ki-67 protein (marker of new cells). In other words, these animals had higher level of neurogenesis (Fig. 2, *a*). The differences between groups of CoEX mice kept in EE and under standard conditions were similar, but less expressed. The relative weight of the brain was also higher in EX and CoEX mice kept in EE than in the corresponding "standard" groups kept under standard conditions (Fig. 2, *b*).

Thus, the study revealed differences in the response of mice with higher cognitive performance to EE in comparison with control animals. Maintenance in EE was accompanied in EX mice by more pronounced fear of illuminated areas of EPM and fear of novelty (neophagophobia) in comparison with mice kept under standard conditions. Increased anxiety in animals from EE was reported by other authors on other models [4,6]. The stimulating effect of EE, which is more pronounced in EX mice, suggests that selection for cognitive signs caused changes in the level of proliferative processes in the forebrain.

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