METHODS

Selection of Anxious Mice by a Three-Stage Test in an Elevated T-Maze

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Daily testing of SHR and C57Bl/6 mice during 3 days shows an increase of the number of mice with a high level of anxiety, which was determined according to the ratio of the number of entries into the light arms to the total sum of entries into the light and dark arms of an elevated T-maze. Such results were obtained after 3 testings every 3 days in C57Bl/6 but not in SHR mice. This procedure is proposed for the selection of anxious individuals as objects for an anxiety model.

Key Words: *anxiety; mice; selection; elevated T-maze*

In experiments with putative endogenous anxiogens [3] and anxiolytics [4] in an elevated T-maze it was previously noted that in the control under conditions of repeated tests the number of mice with a high level of anxiety was increased according to the test indexes. We decided to verify this observation with the aim of determining whether repeated tests can be used for the selection of anxious mice for experiments with anxiolytics and anxiogens.

MATERIALS AND METHODS

Outbred (SHR) and inbred (C57Bl/6) male mice aged 6 weeks weighing 19-20 g (Rappolovo nursery) were kept in groups of 8 animals in 20×15×10-cm metal boxes prior to the first test in the maze. According to the results of the first test the animals of both groups were divided into three subgroups as follows: high-anxiety (HA), low-anxiety (LA), and moderate-anxiety (MA). The criterion for such a classification was the ratio (RAT) of the number of entries into the light arms to the sum of entries into the light and dark arms. This index is a standard one for estimating levels of anxiety in mice and rats [1,2]. The lower the index, the higher the level of anxiety, and vice versa. Another index, the amount of time spent in the light arms, which is routinely used in tests involving a maze, including our experiments [3,4], was not used in the present study due to the marked variations of data in the control. Initially the two groups of mice differed in the RAT value: outbred - 0.43 (the mean value for 60 mice) and inbred - 0.34 (the mean value for 56 mice), that is, the inbred mice were on the whole more anxious. Ratios of different magnitudes were accordingly chosen for the separation of individuals within a group. Outbred HA rats had a the RAT lower than or equal to 0.26, LA a value greater than or equal to 0.40, and MA a value between 0.26 and 0.40. For the inbred animals RAT was 0.20, 0.33, and between 0.20 and 0.33, respectively. Then the subgroups were compared to one another within one group but not between groups. Each animal kept its individual tag throughout the tests, enabling it to be identified and its "transition" to be followed from one subgroup to another during the second and third tests. For the one or two days between tests all 28

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TABLE 1. Distribution of the Number of Anxious Animals among Outbred and C57Bl/6 Mice after Repeated Testing in an Elevated T-maze

Group (total number of animals)	Number of animals on test day:		
	1	2	3
Outbred (28):			
HA	0	9	14
LA	19	7	9
MA	9	12	5
C57Bl/6 (28):			
HA	6	13	21
LA	13	10	4
MA	9	5	3

mice were kept together in the vivarium in 55×25×20-cm cages. In the laboratory before the experiment the mice were divided into subgroups according to the results of the previous test and kept in groups of 5-8 in boxes 20×15×10 cm in size.

The maze is made of plywood (crossed school rulers) and pasteboard (walls of the closed arms). The open arms measure 5×30 cm, the closed arms 5×30×15 cm. The closed arms have no roof. Lighting above the open arms is 80-90 luxes in the middle of the closed arms 15-17 luxes. The maze is fixed at a height of 50 cm. The duration of testing is 3 min. The mouse was placed at the crosspoint its head facing the farther open arm. The experiments were carried out according to two protocols: 1) the mice were tested on three days with 24-hour intervals; 2) the mice were tested three times with a 2-day interval at the third test. Locomotion was measured separately in 20×15×10-cm boxes during 3 min according to sectors crossed.

The reliability of differences between the size of subgroups of one group in the first test and between the 1st and 3rd tests was determined according to the χ^2 test, and that between the indexes of locomotion according to the Mann-Whitney *U* test.

RESULTS

Protocol No. 1. After the first testing the HA subgroup was the smallest in both groups, while LA was the largest subgroup (Table 1). The number of HA mice increased in both groups in the course of testing, whereas the number of LA and MA animals decreased. The HA subgroups increased in size because mice from the LA and MA groups "transitioned" into them. The total number of entries into the open and closed arms (or locomotion in the maze) in the 1st test was lower in the inbred mice than in the outbred mice 10.3±0.9 and 17.4±1.8, respectively. Locomotion in the boxes on the 1st day of testing was reliably lower in HA than in LA in both groups. On the 3rd day of testing locomotion in the boxes was reliably lower in intensity than on the 1st day, probably due to extinction of the orienting reflex. The difference between HA and LA was leveled.

Protocol No. 2. Among the inbred mice the number of HA animals rose by the 3rd test from 8 to 20, while among the outbred mice it was practically unchanged. The LA subgroup was diminished in inbred animals from 14 to 2, while in the outbred mice it showed a rise from 8 to 15 individuals. Differences between the two groups taken from the same nursery were previously noted by us. In the same maze, however, the anxiogenic effect of phenylethylamine and phenamine was observed only in the outbred mice. Among the outbred mice from that nursery the "anxious" individuals (according to assessment in the maze) differed from the "nonanxious" in a smaller number of the binding sites for ^{3}H-flunitrazepam and ^{3}H-muscimol in the brain cortex [5].

Selection of HA, LA, and MA mice with the aid of such a simple and quick procedure as three-stage tests in a maze may be a promising technique in the study of anxiolytic and anxiogenic drugs. Animal groups without selection are shown to be heterogeneous and the variations in drug effects in HA, LA, and MA mice may be masked by averaging the data. From the practical and theoretical points of view it is of interest to know whether the anxiety of HA mice selected in the maze will manifest itself in other models of anxiety, such as in a dark-light chamber or in the test of social interaction. The answer to this question will help us better understand the degree to which mouse anxiety is universal and how specific this particular anxiety model is.

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