PHYSIOLOGY

Orientation and Exploratory Behavior and Anxiety of CBA Mice with Anosmia Induced by N-Trimethylindole (Skatole)

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The behavior of CBA mice in the hole-board test and elevated plus maze was studied after intraperitoneal injection of skatole leading to destruction of the epithelium in the main olfactory system. Locomotor and exploratory activity and degree of anxieties were low in intact mice. Anosmia was accompanied by an increase in orientation and exploratory activities and degree of anxiety.

Key Words: *skatole; anosmia; orientation and exploratory activities; anxiety*

The olfactory system provides perception of biologically important signals. Olfactory signals are perceived by receptor cells in the nasal mucosa, transduced to mitral cells in the olfactory bulb glomerular layer, and conducted via centripetal fibers to brain structures. Various structures of the limbic system, including the anterior olfactory nucleus, piriform cortex, amygdaloid nuclei, and anterior hippocampus, receive direct projections from the olfactory bulb [4,5]. Neuroanatomical relations of the olfactory analyzer with the limbic system and other emotiogenic structures of the brain suggest the interrelation between olfaction and emotional behavior [1,2]. However, it remains unclear whether the olfactory system plays a role in the orientation and exploratory behavior and its emotional component.

Here we studied orientation and exploratory behavior, anxiety, emotionality, and risk behavior in CBA mice with anosmia induced by intraperitoneal injection of N-trimethylindole (skatole).

Intraperitoneal injection of N-trimethylindole is followed by damage to the olfactory epithelium and degeneration of receptor neurons in the major olfactory system [7,9], but has no effect on the accessory olfactory system [6]. Immunohistochemical studies showed that skatole induces ultrastructural changes in the olfactory bulb leading to impairment of olfactory signal transduction from the olfactory epithelium to the olfactory bulb. Hence, the animals lose the ability to detect these signals. The olfactory epithelium recovers not earlier than 3 weeks after skatole treatment [9].

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MATERIALS AND METHODS

Experiments were performed on 2-2.5-month-old male CBA mice (n=40). The animals were maintained in a vivarium under standard conditions and 12:12-h light/dark cycle and had free access to water and food.

Animal behavior was studied in the hole-board test. The chamber (50×50×20 cm) was divided into 16 squares and had 16 round holes in the floor. The animal was placed in a corner of this chamber. The latency of crossing the first square, horizontal locomotor activity (ambulation, *i.e.*, number of crossed squares), number of explored holes, vertical locomotor activity (rearing postures, *i.e.*, number of hindlimb rearing), and grooming behavior were recorded for 5 min. The behavioral test was performed under red-light and white noise conditions.

For more comprehensive evaluation of orientation and exploratory activities, degree of anxiety, and/or risk behavior of animals we used the elevated plus maze (EPM) test. EPM consisted of four 25-cm arms: two opposite dark arms had side and end walls (10 cm), two other light arms were open. The animal was placed in an illuminated central area of the maze. The latency of transition to the dark compartment, time spent in illuminated compartments (except for the latency), number of entries into the light compartments, number of overhanging from light arms of the maze, horizontal locomotor activity (transition between dark compartments, movement in light arms), vertical locomotor activity (rearing postures in dark and light), looking out from the dark compartment, and grooming episodes were recorded for 3 min.

Dysfunction of the main olfactory system in animals of treatment groups was induced by intraperitoneal injection of 200-220 mg/kg skatole oil solution [6,7]. Control mice received an equivalent volume of the solvent. The degree of anosmia in animals of both groups was estimated by the presence or absence of avoidance behavior in response to a strong unpleasant odor of ammonia.

Statistical treatment of data involved ANOVA with LSD test. We calculated the mean value, error of the mean, dispersion, and standard deviation.

RESULTS

The total horizontal locomotor activity (ambulation) in anosmic mice was much higher than in control animals (106.1±6.44 and 44.5±4.77, $p<0.001$). The control mice crossed 10 squares over the 1st minute. The number of squares crossed by these animals decreased during the 2nd and 3rd minutes, but increased during the 4th and 5th minutes (Fig. 1, *a*). In treated mice this parameter progressively decreased from the 1st to the 5th minute. Intergroup differences in locomotor activity were significant over the first 4 min of the study.

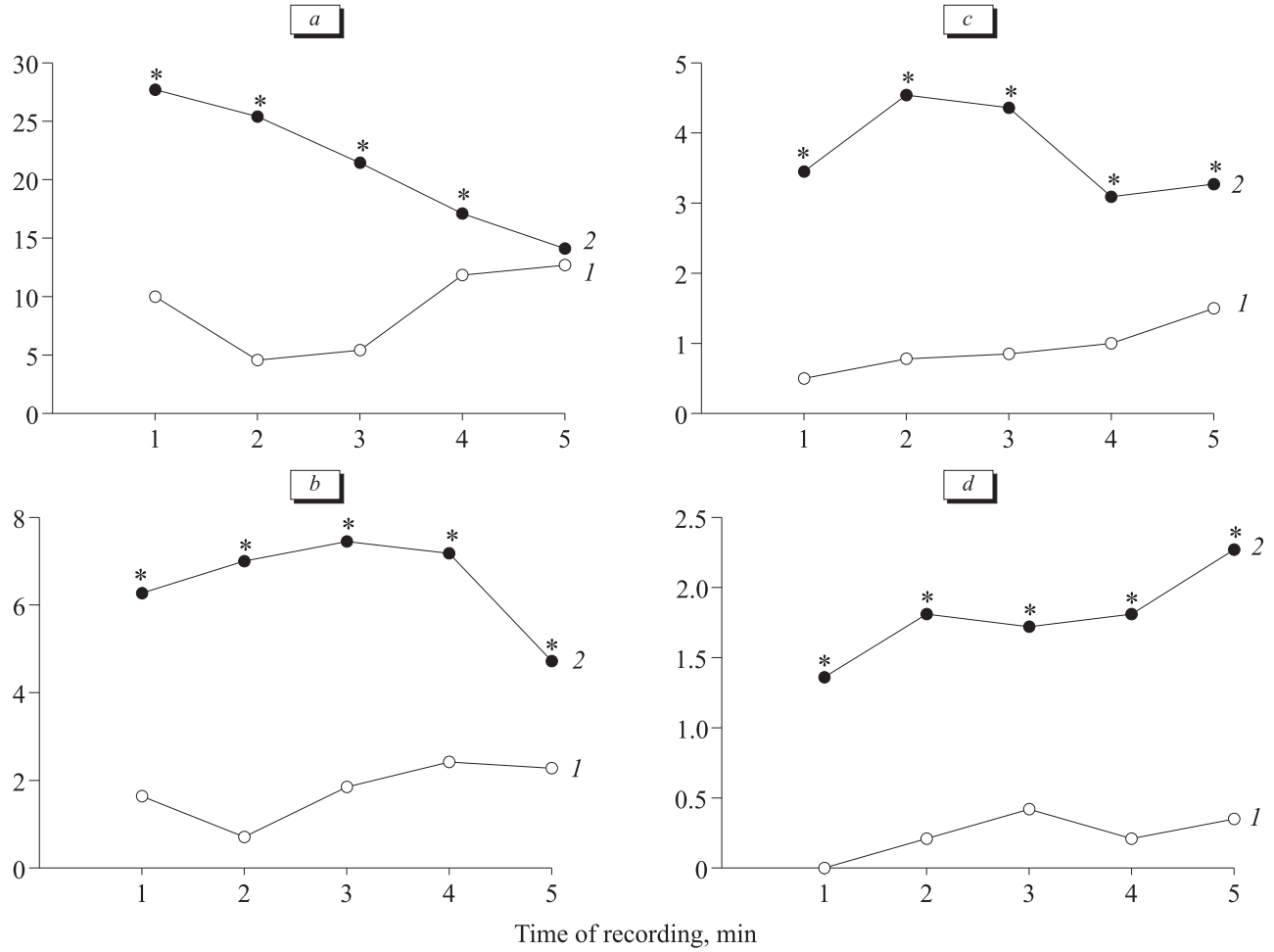

Fig. 1. Behavior of CBA mice in the hole-board test. Horizontal locomotor activity (ambulation, *a*), vertical locomotor activity (rearing postures, *b*), hole reactions (exploration of holes, *c*), and grooming (*d*). *$p<0.0001$ compared to the control. *1*) control group, *2*) treatment group.

The total number of holes explored by control and treated mice was 8.9 and 32.6, respectively ($p<0.00001$). Exploratory activity of control mice remained low in various periods of the study. The number of explored holes was lowest during the 2nd minute, but increased by the 5th minute. Exploratory activity of treated animals remained practically unchanged over the first 4 min (Fig. 1, *b*). Exploratory activity of treated mice decreased in the 5th minute, but exceeded that of control animals.

The total number of rearing postures in control and anosmic mice was 4.6 and 20, respectively ($p<0.0001$). The number of rearing postures in control animals was low in the initial stage of study, but increased by the 5th minute. The basal level of vertical activity in treated mice exceeded that in control animals and remained practically unchanged by the end of the study (Fig. 1, *c*).

The total number of grooming episodes was low in control mice, but high in anosmic animals ($p<0.0001$). This parameter underwent similar changes in mice of both groups (Fig. 1, *d*). Grooming episodes in control animals were not detected during the 1st minute, but this parameter slightly increased in the follow-up period. Grooming activity of treated mice remained high by the end of the study.

The latency of crossing the first square in control mice was longer than in anosmic animals (12.3 and 3.5 sec, respectively, $p<0.0006$).

Therefore, control mice are characterized by low orientation and exploratory activities (small run, low number of rearing postures and explored holes), which is confirmed by long latency of crossing the first square. Control animals exhibited low grooming activity. This finding illustrates reduced emotionality and, probably, low fear of control mice. The development of anosmia in CBA mice was accompanied by a significant increase in the test parameters of orientation and exploratory activities.

TABLE 1. Behavior of CBA Mice in the EPM Test

Parameter	Group	
	control	treatment (skatole)
Number of entries into light compartments	3.00±1.64	4.60±0.30*
Rearing postures in the light compartment	0.46±0.91	5.09±0.83*
Grooming	1.06±1.16	8.81±1.47*
Overhanging	2.013±2.190	9.18±1.00*
Looking out	3.20±2.36	4.72±0.33*

Note. *$p<0.0001$ compared to the control.

Experiments with control mice showed that the latency of entry into the dark compartment of EPM and time spent in illuminated areas are 6.9 and 47.33 sec, respectively. The corresponding parameters in anosmic animals were much higher compared to the control (19.3 and 75.5 sec, respectively, $p<0.001$). In control and treated mice, the time spent in the light compartments significantly exceeded the latency (by 7 and 4 times, respectively). Locomotor activity of control animals in illuminated compartments remained low (0.26±0.59 and 0.06±0.25 sec in the 1st and 2nd light compartments, respectively). This parameter in anosmic mice was much higher than in controls: 3.45±0.36 ($p<0.001$) and 1.36±0.43 sec ($p<0.002$), respectively).

The numbers of overhanging from arms of the maze, rearing postures in illuminated arms, transition in illuminated arms of the maze, looking out episodes, and entries from the dark arm into the light arm in control mice were much higher than in treated animals (Table 1). However, no intergroup differences were revealed in the number of rearing postures in the dark compartment and transition between the dark compartments.

The behavioral act is a result of potential behavioral reaction and its realization, *i.e.* the researcher investigates behavioral reactions that the animal dared or was able to perform. Experiments on mice of both groups showed that the number of looking out episodes coincides with the number of entries into the light compartment (3.0 and 3.2, respectively, in control animals; 4.6 and 4.7, respectively, in treated animals, $p<0.001$). The behavior of mice probably reflects the state of anxiety and/or fear in a novel situation.

Grooming activity was very low in control mice. High grooming activity of anosmic animals reflects increased anxiety and stress in these mice [8].

Our results suggest that skatole-induced anosmia is accompanied by the increase in orientation and exploratory activities and grooming of CBA mice. Published data show that high level of grooming corresponds to low exploratory activity [8]. In our study, the biological significance of grooming act is the preparation (cleaning) of olfactory pathways for new olfactory signals. The absence of anticipated olfactory signals due to sensory deprivation explains peculiar behavior of treated animals. High grooming activity of these mice reflects the state of anxiety and/or conflict between fear motivation and exploratory behavior.

Consequences of destruction of the olfactory epithelium illustrate severe dysfunction of the brain.

The olfactory bulb is an important component of the limbic system, which probably plays a role in orientation and exploratory activities, anxiety, and emotionality of animals [3]. This assumption is supported by the data on the existence of close neuroanatomical relations between the olfactory bulb and various brain structures.

The results of our study suggest that skatole-induced anosmia modulates orientation and exploratory activities and anxiety of CBA mice.

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