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Modulation of Orientation and Exploratory Behavior in Mice during Development of Humoral Immune Response

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We demonstrated opposite changes in the orientation and exploratory behavior of (CBA×C57Bl/6)F_1 mice in the open field test during the formation of primary humoral immune response. These changes depended on initial behavioral activity: the exploratory behavior was suppressed in animals with initially high activity, stimulated in animals with medium reaction, and remained unchanged in mice with initially low activity. The detected changes in the exploratory behavior during the formation of immune response were leveled in the total population (not divided by initial behavioral status).

Key Words: *exploratory behavior; humoral immune response; cytokines*

Afferent organization of the interactions between the immune and nervous systems, mechanisms of brain response to activation of the immune system, and participation of immunogenic factors in the regulation of brain functions are now actively explored [1,6]. Modulation of integral parameters of higher nervous activity, specifically behavior, during the formation of immune response is a very interesting problem. Progress in this field will extend potentialities of immunoregulation (through correction of behavioral functions) and correction of behavior (via modulation of immunity parameters). However, the interrelationships of between animal behavior and immune response are little studied, and published reports are contradictory [3,15].

We investigated the orientation and exploratory activity (OEA) during the formation of the primary humoral immune response in mice with different initial behavioral status.

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MATERIALS AND METHODS

Experiments were carried out on 3-month-old male (CBA×C57Bl/6)F_1 mice (n=92) from the Breeding Center of Institute of Pharmacology (Tomsk). Before the experiment the animals were kept in cages (10 animals per cage) under standard vivarium conditions (standard diet with free access to water and normal day/night regimen) for at least 2 weeks. All experiments were carried out from 10.00 to 14.00. The mice were intraperitoneally immunized with sheep erythrocytes (SE, 5%, 0.5 ml/animal). Controls were injected with the same volume of RPMI-1640 under the same experimental conditions. OEA was evaluated in the open field test [2] at the peak of the immune response (day 5 after injection of the antigen). The study was carried out in a large chamber (100×100 cm, 100 squares) surrounded with 40-cm plastic walls and lighted with a shadowless lamp (100 W) placed at a height of 100 cm. The animal was put in a corner square and its activity was recorded for 5 min (separately for each minute). The number of crossed central and peripheral squares, number of vertical postures (free and with sup-

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port), and total motor activity (MA) were evaluated. Emotional state was evaluated by the number of boluses.

Interleukin-1 receptor (rIL-1) gene expression in the brain was evaluated by RT-PCR. Total RNA was isolated [11] and reverse transcription and amplification reactions were carried out as described previously [8]. Primers for rIL-1 and β-actin (internal standard) were synthesized in accordance with the structure described previously [8]. PCR products were visualized in a densitometer (Pharmacia-LKB), semiquantitative analysis of the results was carried out using Image-Master VDS Software.

The results were statistically processed using Student's *t* test and paired Mann—Whitney test. The differences were considered significant at $p<0.05$.

TABLE 1. Effect of Immunization with SE on OEA of (CBA×C57Bl/6)F_1 Mice in the Open Field Test (*M*±*s*)

MA	Control	SE
Horizontal		
peripheral	93.7±19.8	68.0±40.0
central	10.2±3.6	5.2±11.2
total	104.0±26.9	76.1±46.7
Vertical		
free	1.5±0.4	3.3±1.1*
leaning against the wall	2.7±2.0	3.1±3.7
total	4.1±1.4	3.8±4.8

Note. *$p<0.05$ *vs.* the control.

RESULTS

Only one component of OEA, rearing without support, increased significantly during the development of primary humoral immune response to SE (Table 1). This effect probably resulted from activation of the brain dopaminergic system accompanying the formation of the immune reaction [5].

We previously showed that (CBA×C57Bl/6)F_1 mice can be divided into 3 groups by OEA and these groups demonstrate different reactions to antigens [7, 12]. In the next experimental series we evaluate behavioral changes during the formation of humoral immune response in mice with different behavioral status. The animals were divided into 3 groups with high, medium, and low OEA (by the results of preliminary testing) [12].

We observed opposite changes in parameters of behavioral reaction to immunization (Table 2). In mice with initially high OEA immunization decreased peripheral ($p<0.01$) and central ($p<0.01$) horizontal MA and free vertical activity ($p<0.01$). By contrast, in animals with medium OEA this reaction was enhanced, which manifested in increased peripheral ($p<0.05$) and total ($p<0.05$) horizontal MA and free rearings ($p<0.01$). Open field behavior of mice with initially low OEA after immunization remained unchanged. Emotional status of immunized and control mice was similar.

CNS reactions (including behavioral) to activation of the immune system are mediated by cytokines (IL-1, -2, -10, tumor necrosis factor-α, *etc.*) and neurotransmitter systems of the brain [1,6,10,13,14]. According to published data and our observations, activity of these systems closely correlates with OEA. For example, differences in specific activities of monoamine oxidases A and B, cholinesterase, and choline

TABLE 2. Effect of Immunization with SE on OEA of (CBA×C57Bl/6)F_1 Mice with Initially Different levels of OEA in the Open Field Test (*M*±*s*)

MA	OEA level					
	high		medium		low	
	control	SE	control	SE	control	SE
Horizontal						
peripheral	193.3±11.7	82.0±62.9*	67.4±27.4	104.9±36.8**	20.6±20.3	17.2±19.5
central	27.3±6.1	8.3±10.2*	3.9±3.0	7.0±6.6	0.3±0.7	2.0±6.7
total	220.5±15.6	97.0±76.1*	71.3±29.1	112.0±41.8**	20.9±20.4	19.2±22.3
Vertical						
free	4.5±1.3	0.8±1.7*	0±0	1.1±1.0*	0±0	0.1±0.1
leaning against the wall	5.8±2.9	4.3±3.4	2.3±2.1	4.8±3.3	0±0	0.3±1.0
total	10.0±1.4	5.1±4.3	2.3±2.1	5.9±4.3	0±0	0.4±1.1

Note. *$p<0.01$, **$p<0.05$ *vs.* the control.

acetyltransferase in the sensorimotor cortex [4] and a relationship of dopamine- and serotoninergic systems with behavioral reactions [4-6] were shown for Wistar rats with high and low MA in the open field test. Differences in expression of rIL-1 gene by brain cells were detected in mice with different levels of OEA: 25.82, 13.75, and 39.24 opt. dens. units, respectively, for animals with high, medium, and low levels of OEA (all $p<0.01$). IL-1 is a major cytokine produced during immunogenesis; it is characterized by numerous central effects modifying behavioral reactions [6,9,10]. It is possible that the detected reduction of OEA parameters after immunization of mice with initially high level of this behavioral reaction can be a manifestation of the suppressive effect of IL-1 on this reaction. On the other hand, a relatively low baseline expression of rIL-1 gene in the brain of mice with medium OEA in this situation can explain predominance of the effects of other cytokines, produced in the course of immunogenesis, *e. g.* IL-1, -6, and -10, which stimulate the studied behavioral reaction [13,14].

Hence, the effect of humoral immune response on animal behavior cannot be evaluated unambiguously because of a complex of relatively independent mechanisms realizing the behavioral effects of immune cell activation. On the other hand, our data indicate that changes in OEA of mice during the formation of primary humoral immune response depend on the initial behavioral status of animals.

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