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Immunomorphological Characteristics of Animals with Different Levels of Orientation and Exploratory Behavior

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We revealed some relationships between the level of orientation and exploratory behavior, functional activity of the immune system, and structural and functional organization of the CNS in animals. Significant differences in brain morphology and expression of cytokine IL-1β, type I IL-1 receptor, and erythropoietin receptor genes in brain cells were detected in (CBA×C57Bl/6)F_1 mice with different initial levels of orientation and exploratory behavior. Immunocompetent cells of mice with high and low levels of exploratory behavior differ by spontaneous and mitogen-induced proliferative activity. The formation of humoral and cellular immune response in these animals causes opposite changes in exploratory behavior and the type of these changes depends on the initial level of this behavioral reaction.

Key Words: *exploratory behavior; brain; immune system; cytokines*

The data on phenotypical and functional similarity of the immune and nervous system accumulated during the last 15-20 years permit us to speak about their close cooperation during performance of basic functions. The study of fundamentals, regularities, and mechanisms of relationships between the immune system and higher nervous activity seems to be an important task. Orientation and exploratory behavior as a manifestation of higher nervous activity is one of the most important types of behavior providing information about the environment and an important psychological mechanism of adaptation in higher vertebrates.

We studied the relationships between the level of orientation and exploratory behavior (OEB), brain morphology, and functions of the immune system in animals.

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MATERIALS AND METHODS

The study was carried out on 3-month-old (n=180) male (CBA×C57Bl/6)F_1 mice from the Laboratory Animal Breeding Center of Institute of Pharmacology, Siberian Division of Russian Academy of Medical Sciences (Tomsk). Before the experiment the animals were kept in a vivarium in cages (10 per cage) on standard diets with free access to water under conditions of normal day/night schedule. The experiments were carried out from 10.00 to 15.00. The orientation exploratory behavior was evaluated using the open field test [2] in a 100×100 cm rectangular box divided into 100 squares with 40-cm plastic walls, illuminated with a 100-W shadow less lamp hanging 100 cm above the center of the field. A mouse was placed into the corner of the field and its activity per minute was recorded over 5 min. The number of crossed central and peripheral squares, rearing (free and with wall support), and total motor activity were recorded. The degree of emotional strain was evaluated by the number of fecal boluses.

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For morphological analysis the brain was fixed in neutral formalin, dehydrated in ascending alcohols, embedded in paraffin, and the sections were stained by Niessle's method. The sensorimotor cortex of the cerebral hemispheres was examined. Qualitative analysis was carried out in 25 visual fields.

The proliferative response of splenocytes and thymocytes was evaluated using routine lymphocyte blast-transformation test. The cells were suspended RPMI-1640, 5% inactivated FCS, 2 mM L-glutamine, 10 mM HEPES buffer, 5×10^{-5} M 2-mercaptoethanol (Sigma), and 80 μg/ml gentamicin and transferred to 96-well round-bottom plates (Linbro) in a concentration of 5×10^5 thymocytes and 10^5 splenocytes per well (in 50 μl). Mitogen (suboptimal concentrations of *E. coli* 026:B6 LPS, Sigma) and ConA (Pharmacia) in concentrations of 25 and 1 μg/ml, respectively (50 μl each), and/or culture medium to the final volume of 150 μl/well in complete medium were added to wells. The cells were cultured for 72 h at 37°C and 5% CO_2. ^{3}H-thymidine (1 μCi/well) was added 16 h before the end of incubation. After incubation the cells were transferred to special fiberglass filters (Flow Lab. Inc.) using an automated 12-channel Cell-harvester-530 (Flow Lab. Inc.). Dried filters were put into vials with 10 ml toluene scintillator and radioactivity was measured in a liquid scintillation counter (in cpm).

The level of IL-1β, type I IL-1β receptor (IL-1R), and erythropoietin receptor genes in mouse brain was evaluated by reverse transcription-PCR. Summary RNA was isolated as described previously [12], RT-PCR was carried out as described elsewhere [10]. Primers for IL-1β, IL-1R, erythropoietin receptor, and β-actin (used as internal control for standardization and leveling of the results of analysis of DNA samples) for PCR were synthesized according to the structure described previously [10]. PCR products were visualized in a densitometer (Pharmacia-LKB), semiquantitative evaluation of the results was carried out using Image Master VDS Software. The results were expressed in optical density units.

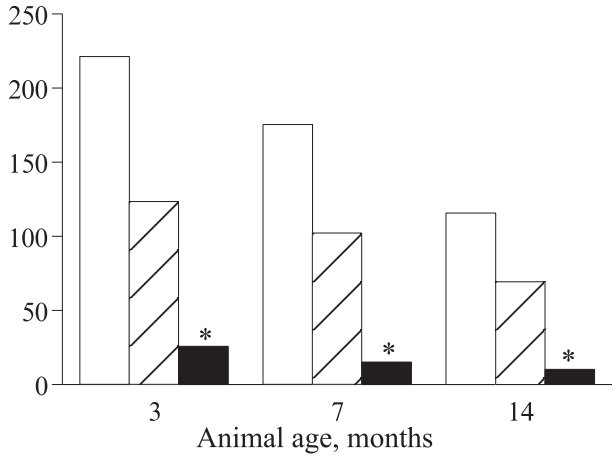

Fig. 1. Changes in total motor activity of (CBA×C57Bl/6)F_1 mice with different levels of orientation and exploratory behavior (OEB) in the open field test during ontogeny. Ordinate: number of crossed squares over 5 min. Light bars: mice with high OEB; cross-hatched bars: mice with medium OEB; and dark bars: mice with low OEB. *$p<0.05$ compared to animals with low OEB.

The data were statistically processed using Student's *t* test and Mann—Whitney's paired test (Jandel Sigma Plot and Statistica software). The results were presented as $M\pm S$. The differences were considered significant at $p<0.05$.

RESULTS

By their behavior in the open field, (CBA×C57Bl/6)F_1 mice were divided into 3 groups (high, medium, and low levels of OEB) [5, 14]. Analysis of animal behavior in the ontogeny (up to the age of 14 months) showed that stability of the detected differences between the groups (Fig. 1) indicates stability of this behavioral characteristic for each animal.

The animals with different levels of OEB were characterized by specific features of the immune status [5]. Spontaneous and mitogen-induced proliferative activity of immunocompetent cells differed significantly in mice with high and low levels of OEB (Table

TABLE 1. *In Vitro* Study of the Proliferative Activity of Thymocytes and Splenocytes of (CBA×C57Bl/6)F_1 Mice with Different Levels of OEB in the Open Field Test ($M\pm S$, cpm)

Parameter			OEB level	
			high	low
Splenocytes	spontaneous activity		1611.8±282.4	609.7±73.9**
	induced	by ConA	71 938±30 854	39 880±10 160*
		by LPS	11 551.3±1403.0	3228.7±396.3**
Thymocytes	spontaneous activity		527.8±86.6	242±77**
	induced	by ConA	12 935.2±3684.8	3109.3±447.1*

Note. *$p<0.05$, **$p<0.01$ compared to animals with high OEB.

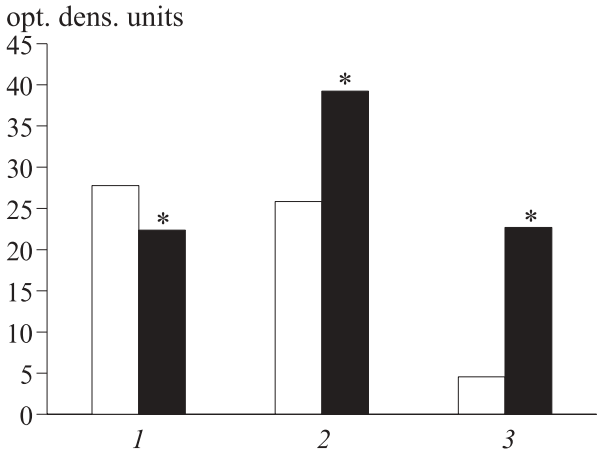

Fig. 2. Gene expression: erythropoietin receptors (*1*), type I IL-1 receptors (*2*), IL-1β (*3*) in the brain of (CBA×C57Bl/6)F_1 mice with high (light bars) and low (dark bars) OEB in the open field test. **p*<0.05 compared to animals with high OEB.

1). The level of animal OEB was directly related to the level of developing delayed hypersensitivity reaction, characterizing the cellular immune response; in addition, these parameters were inversely related to the emotional level. This regularity was revealed for BALB/c, C57BL/6, (CBA×C57Bl/6)F_1 mice and for OXIS and Wistar rats characterized by low and high exploratory activity in the open field test, respectively [7,14]. The formation of cellular and humoral immunity in (CBA× C57Bl/6)F_1 mice with different OEB caused variously directed changes in this behavior. The type of changes depended on the initial behavioral status of animals [6,8]. CNS reactions, including behavioral, to activation of the immune system are largely mediated by cytokines (IL-1, 2, 6, 10, 13, 15, TNFα, *etc.*) and neurotransmitter systems of the brain [4,11, 13,15]. Activity of these systems is linked with animal OEB according to published data and our findings. We detected significant differences in the expression of IL-1β, IL-1R, and erythropoietin receptor genes in brain cells of (CBA×C57Bl/6)F_1 mice with different OEB levels (Fig. 2). Behavioral reactions are related to the dopamine- and serotoninergic systems [3,9]. Specific activities of types A and B monooxidase, cholinesterase, and choline acetyl-transferase in the brain differed significantly in Wistar rats with high and low activity in the open field test. The differences in the neurotransmitter system activities were most pronounced in the sensorimotor cortex [3]. The study of the morphology of the cerebral sensorimotor cortex in (CBA×C57Bl/6)F_1 mice with different levels of OEB showed some specific features in these groups. In mice with low OEB the number of neurons was lower than in mice with high OEB (93.6±16.5 and 133.2±25.5 respectively, $p<0.05$), there were groups of compressed cells and pericellular edema (93.7±23.0 and 69.0±9.4, $p<0.05$), and slightly increased number of ghost cells (15.8±5.8 and 12.9± 5.1, $p>0.05$). In mice with high OEB level the number of neurons increased and these cells were hypertrophic with hyperchromatic nuclei reflecting their hyperfunction. These characteristics of the structural and functional organization of the sensorimotor cortex were also detected in Wistar rats differing by behavioral parameters in the open field test [1]. The results indicate high morphofunctional plasticity of cortical structures in CNS caused by individual behavioral characteristics of animals.

Hence, animals with different OEB are characterized by specific features in the functioning of the immune system and structural and functional organization of CNS. The detected relationships will help to more accurately determine the mechanisms regulating congenital and acquired forms of behavior.

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