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Activity of Cell Immune Response and Open Field Behavior in Wistar and OXYS Rats

E. V. Markova, L. A. Obukhova*, and N. G. Kolosova**

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The state of the thymus, activity of cell-mediated immunity, and open field behavior were compared in Wistar and OXYS rats (premature aging). Early involution of the thymus was revealed in OXYS rats, which by the type of morphological changes corresponded to accidental involution and was associated with low level of delayed hypersensitivity reaction and decreased motor and exploratory behavior.

Key Words: *thymus; delayed hypersensitivity; motor exploratory behavior*

Neurochemical peculiarities of the brain determining individual behavior are essential for immune reactions. Experiments on mice demonstrated close relationships between behavioral characteristics and parameters of cell immunity; exploratory activity can be modified via modulation of the cell immunity [4,10]. We compared some parameters of cell immunity in Wistar and OXYS rats; this latter strain is characterized by changes in the cognitive and emotional spheres typical of aged humans and animals, one of these shifts being essential inhibition of exploratory behavior [3]. We studied induction of delayed hypersensitivity (DH) and the state of the thymus as one of the central organs of the immune system responsible for cell immunity. Individual differences in animal behavior in an open field (OF) were preliminary determined.

MATERIALS AND METHODS

The study was carried out on 60 male Wistar and OXYS rats aged 3 months at the Laboratory of Animal Breeding, Institute of Cytology and Genetics, in which the strain of untimely aging OXYS rats was derived [9]. The animals were kept under standard vivarium conditions on standard rations with free access to water. Experiments were carried out at 10.00-15.00. Individual differences in animal behavior were studied in the OF test. OF was a large (100×100 cm, 100 squares) rectangular box with plastic walls 40 cm high, illuminated with a shadowless lamp (100 W) hanging at a height of 1 m above the center of the field. The animal was placed into the corner of the field and its motor and exploratory behavior was evaluated by counting the number of squares crossed and rearings over 5 min; emotional strain was evaluated by the number of grooming reactions, fecal boluses, and the latency of entry into the center of OF [1].

In some animals the thymus was isolated and examined under a light microscope. The thymuses were weighed for estimation of the weight index (mg/100 g body weight), fixed in Tellesnitskii fluid at 4°C, and embedded in histoplast. Serial sections were stained with hematoxylin and eosin, azur B, and eosin Y. Morphometry of structural components of the thymus was carried out by dot counting followed by calculation of absolute volumes [7].

For evaluation of delayed hypersensitivity reaction, the animals were immunized intraperitoneally

Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences; *Institute of Lymphology, Siberian Division of Russian Academy of Medical Sciences; **Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk. ***Address for correspondence:*** evgeniya_markova@mail.ru. Markova E. V.

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TABLE 1. Behavioral Activity of Wistar and OXYS Rats in the Open Field Test (*M*±*m*)

Animals	Latency, sec	Number of crossed squares	Number of rearings	Grooming	Defecations
Wistar (*n*=20)	261.0±17.9	106.9±15.8	10.9±1.4	3.4±0.6	3.6±0.5
OXYS (*n*=20)	265.0±25.3	42.6±7.4*	4.1±0.7*	1.0±0.2*	5.1±0.6
p	0.59	0.0007	0.0002	0.0007	0.055

Note. *compared to Wistar rats.

with sheep erythrocytes (0.5%, 5 ml). The challenge dose of the antigen (50%, 0.5 ml) was injected under the hind paw aponeurosis after 96 h. The formation of DH was evaluated 24 h after the challenge by the degree of paw swelling (its thickness in comparison with the control hind paw of the same animal after injection of RPMI-1640). The reaction index (RI) was estimated for each animal using the formula: RI=(Rexp-Rc)/Rc and expressed in percent [11].

The results were processed statistically using ANOVA factorial analysis using Statgraphics software. The differences were significant at $p<0.05$.

RESULTS

Studies of animal behavior in OF confirmed the data on appreciable inhibition of motor and exploratory behavior in OXYS rats: horizontal and vertical activity of animals (number of crossed squares and rearings) decreased 2.5-fold compared to those in Wistar rats (Table 1), the number of boluses was 1.4 times higher than in Wistar rats, and the incidence of grooming reactions was 3.4 times lower, which confirms increased anxiety of these animals. The latency of the first excursion into the center was virtually the same in both strains, which, considering chaotic trajectory of movements in OXYS rats, can be attributed to impaired spatial orientation.

Progressive involution of the thymus, inhibition of its function, and gradual loss of autotolerance are typical manifestations of aging. Gravimetric and morphometric studies revealed considerable differences in the state of thymus between OXYS and Wistar rats. In OXYS rats weight index and total volume of the thymus decreased 1.8- and 1.9-fold, respectively, volumes of the cortical and medullar layers decreased 2.1- and 1.9-fold, respectively, and volume of the connective tissue stroma decreased 1.3-fold compared to corresponding parameters in Wistar rats (Table 2). Microscopic examination of the thymus from OXYS rats showed decreased density of lymphoid cells in the cortical matter and medulla, decreased number of blasts in the subcapsular cortical zone. Cells with pyknotic nuclei, macrophages containing pigment inclusions and/or fragments of dead lymphocyte nuclei, plasma cells were more often seen in the cortical matter. In OXYS rats the number of degranulated mast cells in the capsule and interlobular septae of the thymus was higher than in Wistar rats. These results indicate earlier involution of the thymus in OXYS rats, which by the type of morphological changes looks like accidental involution. Accidental involution usually results from stress exposure [5], and presumably, high sensitivity of OXYS rats to oxidative stress serves as the factor triggering accidental involution of the thymus in the absence of external stimuli.

Decreased reactivity of cell immunity is a manifestation of thymic changes in OXYS rats. The development of delayed hypersensitivity reaction in response to sheep erythrocytes in OXYS rats was 2.4 times less pronounced than in Wistar rats (37.1±9.6 *vs.* 90.0±8.7%, $F(1.30)=251.42$; $p<0.05$).

Brain structures modulating the intensity of immune response are the posterior and anterior hypothalamic fields, hippocampus, reticular formation of the midbrain, raphe nuclei, and amygdala. High activity of

TABLE 2. Morphometric Parameters of the Thymus in Wistar and OXYS Rats (*M*±*m*)

Parameters	Wistar (*n*=5)	OXYS (*n*=5)
Total volume of thymus, mm^3	99.54±4.84	51.59±9.86**
Volume of cortical matter, mm^3	71.85±4.69	34.58±6.81**
Volume of medullary matter, mm^3	20.05±1.32	10.56±2.25**
Volume of capsule and lobular septae, mm^3	9.44±0.78	6.44±0.94***
Weight index, mg/100 g	110.03±6.91	62.11±5.95*

Note. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to Wistar rats.

the serotoninergic system of the midbrain raphe nuclei realized through the hypothalamic-pituitary-adrenal axis can lead to suppression of the immune functions [8]. OXYS rats are characterized by considerable changes in activity of the serotoninergic system including increased serotonin content in the midbrain of young animals [6] and appreciable decrease of monoamine oxidase A activity in the brain stem [2]. The detected behavioral peculiarities of OXYS rat can be due to the above-mentioned changes in the neurochemical organization of the brain and function of the immune system in these animals.

Hence, the relationship between activity of the cell immunity and behavioral patterns detected in mice is also characteristic of early aging OXYS rats; these animals, apart from decreased exploratory activity, are characterized by early involution of the thymus, paralleled by inhibition of cell immunity.

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