Emotional State and One-Trial Learning in OXYS Rats with Hereditarily Elevated Production of Oxygen Radicals

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Comparative analysis of unconditioned and conditioned behavior of Wistar and prematurely aging OXYS rats revealed that the latter have significantly reduced locomotor and exploratory activities, increased anxiety in the elevated plus-maze test, spatial disorientation, and abnormal associative learning. OXYS rats can be used as a biological model for studying molecular, neurobiological, and neurochemical mechanisms of brain aging.

Key Words: *aging; OXYS rats; free radicals; anxiety; orienting response; one-trial learning*

Accumulation of oxidized lipids, proteins, and DNA in brain mitochondria and inhibition of bioenergetic processes play an important role in aging and pathogenesis of neurodegenerative diseases [6]. Ameliorating effects of antioxidants on impaired memory, attention, and associative learning [5] support the correlation between age-related changes in human and animal behavior and cognitive functions and oxidative damage to macromolecules. Similar nature of cognitive, neurobiological, and neurochemical changes in aging human and animal brain [7] makes it possible to use biological models for studying the mechanism of physiological aging and relevant mental pathologies. In this context OXYS rats [8] are of considerable interest. This strain was bred at the Institute of Cytology and Genetics of Russian Academy of Science by selection and inbreeding of more than 65 generations of Wistar rats sensitive to the cataractogenic effect of galactose [3]. These rats are characterized by enhanced production of $OH^{\bullet}$ radicals in the liver and myocardium [14], accumulation of lipid and protein oxidation products in tissues, damage to DNA and cell membranes [14,15], and inhibition of mitochondrial oxidative phosphorylation [4]. These processes underlie premature aging and the development of degenerative diseases (cataract, cardiomyopathy, scoliosis, and emphysema) in OXYS rats [14]. There is only one study of brain functions in OXYS rats, which was performed on animals from the 5th and 6th generations and revealed no abnormalities in active avoidance acquisition. The authors noted only reduced retention of the conditioned response [2]. The present study was aimed at the analysis of conditioning under conditions of one-trial learning and evaluation of the attendant behavior.

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MATERIALS AND METHODS

The study was carried out on 4-month-old OXYS and Wistar male rats (n=79) from breeding stock of the Institute of Cytology and Genetics. The rats were trained one-trial passive avoidance (PA) according to a standard protocol [9] in an experimental chamber consisting of illuminated (safe) and dark (dangerous) compartments. The latency of transition from the illuminated to the dark compartment was recorded (time of recording 180 sec). The rats were first familiarized with the chamber and after 24 h exposed to one-trial learning when the entry to the dark compartment was punished with an electroshock (0.75 mA, 2 sec). The tests for PA retention were performed on days 1 and 7 after conditioning.

Animal behavior were studied in the open field (OF) test. OF was an open 1×1 m platform lined into

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100 squares and illuminated with a 100 W bulb. The rat was placed on the corner of the platform and orienting and exploratory activity was evaluated by counting the number of crossed squares and rearings for 5 min. Emotional component was evaluated by the rate of defecation, grooming, and visits to the center. Anxiety was evaluated in an elevated plus-maze (EPM) [12] 48 h after OF testing. The following parameters were measured: time spent in central zone, open and closed arms of the maze, the number of open-arm, center, and closed-arm entries. The ratio of open-arm entries to the total number of transitions was calculated. Rearings, grooming reactions, peepings out, and defecations were additionally counted.

The data were analyzed statistically using Student's t test and one- and two-factor analysis.

RESULTS

Compared to Wistar rats, OXYS rats showed significantly lower horizontal ($p<0.01$) and vertical ($p<0.05$) activities in OF, *i. e.* suppressed locomotor and exploratory behavior (Table 1). The latency of the first visit to the central zone and the frequency of grooming acts were also significantly below the control ($p<0.05$). It did not imply, however, reduced anxiety in these rats as it could be suggested from the latter two indices. These characteristics were associated with slightly disturbed spatial orientation and consequently rather chaotic scanning compared to more organized strategy of exploratory activity in Wistar rats, which visited the central zone only after the exploration of the periphery.

The EPM test revealed high anxiety in OXYS rats: the time spent in open space and the number of entries into open arms were far below the corresponding indices in Wistar rats (Table 2). There was some difference (insignificant) between the OXYS and Wistar rats in the ratio of open-arm entries to total transitions.

TABLE 1. Locomotor and Exploratory Activity in Open Field Test ($M\pm m$)

Index	Wistar	OXYS
Crossed squares	76.3±6.0	44.2±5.5*
Rearings	14.8±2.0	10.2±1.3*
Latency of entry to central zone, sec	136.1±25.6	67.3±14.2*
Grooming	4.0±0.7	1.8±0.3*
Defecation	4.1±0.5	5.0±0.5

Note. *$p<0.05$ compared to Wistar rats.

Similarly to the OF test, the EPM test revealed significantly reduced locomotor (horizontal) and exploratory (vertical) activities in OXYS rats.

During familiarization with the experimental chamber, OXYS rats showed a higher rate of transition to the dark compartment compared to Wistar rats ($F_{1.36}=6.56$; $p<0.01$, Fig. 1). This can also be attributed to weak response to novelty, which can impair learning [11]. During conditioning they showed an increased responsiveness to pain stimulus and delayed escape from the dangerous compartment (3-5 sec delay). All these factors can interfere with acquisition of new information and prevent the formation of memory traces.

Two-factor ANOVA applied for the analysis of transition latencies revealed a significant interaction between the group and day factors ($F_{2.72}=8.7$; $p<0.001$). The results of paired comparison showed that on day 1 after conditioning OXYS rats had a shorter transition latency and a lower defecation rate than Wistar rats ($p<0.05$). The control rats showed spontaneous extinction of the conditioned response on day 7 after learning ($p<0.01$ in comparison with day 1), while OXYS rats demonstrated its enhancement ($p<0.05$ in comparison with day 1). The same tendency was revealed

TABLE 2. Behavioral Indices of Wistar and OXYS Rats in Elevated Plus-Maze ($M\pm m$)

Behavior	Wistar (n=20)	OXYS (n=21)	$F_{1.39}$
Open-arm entries	1.3±0.3	0.5±0.2***	4.41
Center visits	4.0±0.5	2.0±0.3**	8.44
Closed-arm entries	4.8±0.6	3.2±0.4**	6.24
Transition ratio	18.3±4.3	9.3±3.3	2.53
Time spent in open arms, sec	70.4±9.9	30.0±5.9*	12.40
Time spent in closed arms, sec	223.3±10.6	262.3±15.4***	4.28
Peeping out	7.9±1.1	6.6±1.1	0.79
Rearing	8.9±1.2	4.8±0.5**	9.40
Grooming	2.7±0.4	4.7±0.5	7.78
Defecation	4.0±0.8	3.0±0.6	0.13

Note. *$p<0.001$, **$p<0.01$, ***$p<0.01$ in comparison with Wistar rats.

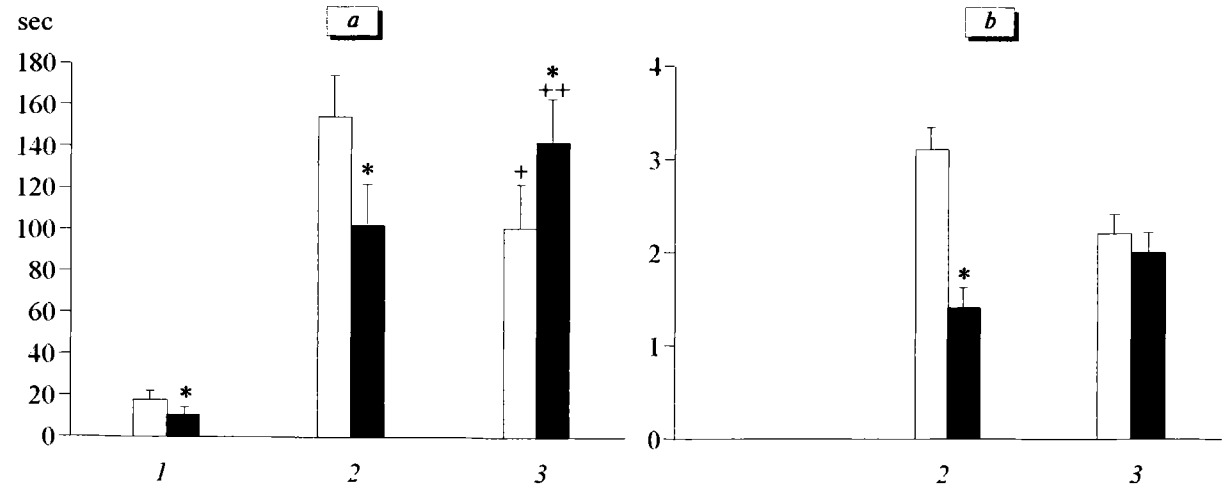

Fig. 1. Retrieval and retention of passive avoidance response in Wistar (open bars) and OXYS (filled bars) rats: latency (*a*) and rate of defecation (*b*). *1*) first familiarization; *2*) 1 day after conditioning; *3*) 7 days after conditioning. *$p<0.05$ compared to Wistar rats, +$p<0.01$ ++$p<0.05$ compared to day 1.

for defecation rate. This enhancement probably indicates impairment of inhibitory processes in OXYS rats.

There is no direct evidence that unconditioned behavior of OXYS rats and impaired learning in one-trial paradigm are due to modulatory effects of oxygen radicals. However, previous studies revealed decreased activity of monoaminoxidase A in the brain stem of OXYS rats [1]. Changes in the monoaminergic system can cause the above-described behavioral peculiarities typical of physiological aging [10,13] appearing in 28-30 month-old rats. Therefore, this model is suitable for studying the molecular and neurochemical mechanisms of both physiological and premature brain aging.

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