AGE CHANGES IN PATTERNS OF EXPLORATORY BEHAVIOR AND PROCESSES OF LIPID PEROXIDATION OF FEMALE RATS DEPENDING ON THE STAGE OF THE REPRODUCTIVE CYCLE

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Abstract. The paper presents the results of a study of patterns of exploratory behavior and processes of lipid peroxidation in liver tissue in young and old female rats, depending on the stage of the estrous cycle. The studies were carried out on females in the estrus and diestrus stages. It was shown that females at the age of 2-3 months were characterized by regular reproductive cycles with an average duration of 4.5 ± 0.11 days, which corresponds to the normal estrous cycle of sexually mature rats, which includes 4 consecutive stages. Animals 24 months old had cycles different from the normal physiological cycles of young animals. The work shows that in young sexually mature females the motor activity in the open field test is higher, and the level of anxiety is lower in the estrus stage compared to the analogous indicators in the diestrus stage. In young females, the baseline MDA level was significantly higher in the diestrus stage relative to that in the estrus stage. Patterns of exploratory behavior and the level of LPO depended on the age of the animal. Old rats had an increased level of anxiety regardless of the stage of the reproductive cycle, while young females were characterized by higher patterns of exploratory activity. In addition, old female rats had a higher level of free radical oxidation in comparison with young animals.

Keywords: exploratory behavior, level of anxiety, free radical oxidation, estrous cycle.

Introduction

According to the WHO concept, the physiological aging of the organism is considered as a biological process that determines the age-related extinction and deterioration of the physiological functions of the organism, increasing the risk of a number of diseases. With age, the biological, chemical and physical functions of neurons change, which leads to memory impairment, changes in behavioral responses, weakening of cognitive functions, increased levels of anxiety and depressive manifestations. Integral signs of aging are also worsening blood circulation, hormonal imbalance, slowing down the excretion of metabolic products, anemia, which creates conditions for the steady development of hypoxia at the tissue level and a decrease in the energy potential of cells. The result of CNS aging is a violation of the ability to analyze the environment, shifts in behavioral and emotional reactions, memory impairment, a decrease in mental and physical performance, motor activity, reproductive function, regulation of homeostasis, and many other processes (Bulgakova *et al.*, 2020; Sidenkova *et al.*, 2021). Every year the number of people with disorders of higher nervous functions associated with age, the negative impact of extreme, social factors is increasing. Aging is one of the main risk factors for common neurodegenerative disorders (Hou *et al.*, 2019).

It has now been proven that free radical processes are enhanced with age (Artemenkov, 2018; Dubinina & Pustygina, 2017; Meshchaninov & Shcherbakov, 2015). Many tissues in old animals have an increased level of oxidative stress compared to similar tissues in younger animals, which contributes to the development of many age-related pathologies and, possibly, to the normal aging process (Daenen *et al.*, 2019; Liguori *et al.*, 2018). Types of reactive oxygen species (ROS) include the superoxide anion produced by the respiratory chain and various oxidases, the hydroxyl radical produced by the reaction of hydrogen peroxide with Cu+ or Fe2+, and NO produced in response to increased intracellular Ca2+ levels. During aging, increased production of ROS and a decrease in antioxidants lead to a redox imbalance, causing age-related disorders (Dubinina & Pustygina, 2017).

The natural fluctuation of sex hormones during the reproductive cycle affects the current psychophysiological status of the organism (Sbisa *et al.*, 2017). With aging, adaptive, motor, and behavioral reactions and their hormonal component, the role of which in different phases of the estrous cycle of females, change unequally, has not been studied enough. Aging is accompanied by a disturbance of the reproductive function, while in women and female animals there is a disorder in the regularity of the sexual cycles. At the same time, in female rats, lengthening, shortening, or loss of the stages of reproductive cycles can occur (Kotelnikov & Kotelnikova, 2005).

The purpose of the work is to supplement the ideas about changes in the patterns of exploratory behavior and the level of lipid peroxidation depending on age and the reproductive cycle stage.

Materials and Methods

The study was performed on 48 white nonlinear young mature (3–4 months) and old (24 months) female rats, which were kept under standard vivarium conditions with free access to food and water. The proportion of animals for each group was 24 females. All experiments were performed in accordance with the National Standard of the Russian Federation GOST R-53434-2009 «Principles of Good Laboratory Practice», Order of the Ministry of Health of the Russian Federation No. 199n dated April 1, 2016 «On Approval of the Rules for Good Laboratory Practice» and the European Convention Directive 2010/63/EU of 22 September 2010. The maintenance of laboratory animals and the performance of all manipulations met the requirements of regulatory documentation and the protocol of the Ethics Committee of Astrakhan State University named after V.N. Tatishchev No. 8, February 27, 2023.

All experiments were carried out in the autumn-summer period. The animals were taken out of the experiment by rapid decapitation under Nembutal anesthesia (40 mg/kg b.w.), which was administered to the animals intraperitoneally.

The level of free radical oxidation was determined by the level of the initial malondialdehyde (MDA), the rate of spontaneous and ascorbate-dependent lipid peroxidation (LPO) in the liver tissue, by the thiobarbituric method (Meshchaninov & Shcherbakov, 2015).

Patterns of exploratory behavior were assessed in the «open field» test (Buresh *et al.*, 1991). The exposure time for each animal was five minutes. The test evaluated eight visually different parameters: the latent period of the first movement from the center of the field (sec.), the number of crossed central squares (number), the number of crossed peripheral squares (number), the number of central leg stand without support on the side (number of in), the number of peripheral leg stand supported on the side (number), the number of peeps into the minks (number), grooming (number) and the number ofdefecation boluses (number).

Before the experiment, the females were handled (tamed by hand) to eliminate the masking effect of fear that arises in animals when picked up by the experimenter (Kondratenko & Lomteva, 2003). In the course of the experiment, in order to determine the rhythm of the functioning of the ovaries, vaginal smears were analyzed daily. Preparation and analysis of smears were performed according to the following procedure.

Smears (vaginal swabs) were taken with a thin eye pipette, placed on a glass slide and examined under a microscope at 300 times magnification in a slightly darkened field (Vladimirskaya *et al.*, 2011).

The estrus cycle of the rat consists of 4 phases: proestrus (pre-estrus), estrus (estrus), metaestrus (post-estrus) and diestrus (interestrus, or resting stage). Each stage of the estrous cycle corresponds to a certain cellular composition of the vaginal smear. Females were taken into the experiment only in the period of diestrus and estrus, since these stages are distinguished by clear behavioral features (Vinogradova *et al.*, 2018; Kondratenko & Lomteva, 2003).

Statistical processing of the results of the study was carried out by calculating the arithmetic mean (M), the error of the arithmetic mean (m), and presented as $M \pm m$. Differences

between groups were assessed using Student's t-test with Bonferoni correction; results were considered significant at $p < 0.05$.

Results

The study of the reproductive cycle showed that female rats aged 2-3 months were characterized by regular sexual cycles lasting 4.5 ± ± 0.11 days, which corresponds to the normal estrous cycle of mature rats. Each estrus cycle included 4 successive stages (proestrus, estrus, metaestrus and diestrus). Animals 24 months of age had cycles different from the normal physiological cycles of young animals. They were characterized by longer ovarian cycles, with a prolonged diestrus stage or prolonged persistent estrus, or the absence of cyclicity.

The results of studying the patterns of exploratory behavior in young females depending on the stage of the sexual cycle were as follows. In the estrus stage, compared with the diestrus stage, the time of the latent period of the first movement was significantly lower ($p < 0.05$), and the number of crossed peripheral squares was significantly less ($p < 0.01$). Vertical activity in the estrus stage was slightly higher compared to the same indicators in the diestrus stage, this was manifested in the number of central and peripheral racks. The number of studied minks changed insignificantly. Emotional reactivity in terms of the number of grooming reactions was somewhat lower in the estrus stage compared to that in the diestrus stage. The indicator of autonomic balance in the form of the number of boluses of defecation in the estrus stage, compared with the diestrus stage, decreased to zero (Table 1).

Old animals also showed differences in patterns of exploratory behavior depending on the stage of the estrous cycle (Table 1). Thus, there was a decrease in the number of crossed segments at the periphery of the field in the estrus stage in comparison with the same indicator in the diestrus stage ($p < 0.01$). Latency time and motor activity in the center of the «open field» did not practically differ between the stages of the cycle. The number of peripheral leg stand was lower in animals in the estrus stage relative to that of females in diestrus ($p < 0.05$), while the number of central leg stand changed less significantly. The number of minks studied in female rats tended to increase in the estrus stage compared to diestrus. Grooming reactions and the number of boluses of defecation in the studied animals did not practically change between the stages of the cycle.

When comparing the patterns of behavioral responses in young and old animals, the following results were obtained (Table 1). The latency time of the first movement was somewhat higher in young animals compared to that in old rats, regardless of the stage of the sexual cycle. Horizontal motor activity in the form of the number of crossed squares on the periphery and in the center of the field was lower in young animals relative to the indicators in old female rats in both stages of the estrous cycle ($p < 0.05$). The number of uprights was also lower in young animals than in old females, but only in the diestrus stage. The number of exploratory visits to burrows in the diestrus stage was significantly higher in young animals than in old rats. Emotional reactivity in the form of grooming reactions and the indicator of autonomic balance in the form of the number of boluses of defecation were significantly lower in young female rats relative to similar indicators characteristic of old females, regardless of the stage of the reproductive cycle.

Thus, the parameters of exploratory behavior changed depending on the age and hormonal status of the animal. Old rats were distinguished by an increased level of anxiety, regardless of the stage of the reproductive cycle. Young females differed from older animals in higher exploratory activity. Preliminary handling of female rats made it possible to reveal the dependence of the exploratory behavior in mature animals on the stage of the estrous cycle: the motor activity in females was higher, and the level of anxiety was lower in the estrus stage compared to similar indicators in the diestrus stage.

The processes of free radical oxidation had their own characteristics. The rate of lipid peroxidation in young rats did not depend on the stage of the reproductive cycle, while the initial level of MDA was significantly higher in the diestrus stage relative to that in estrus (Table 2).

Table 1

Patterns of exploratory behavior of young and old animals depending on the reproductive cycle stage

Indicators of the Open field test	Cycle stage	Young animals	Old animals
Latent period of the first movement from the center of the field, sec	diestrus	4.1 ± 0.80	2.4 ± 0.29
	estrus	2.2 ± 0.36 Δ	2.0 ± 0.23
Number of crossed center squares	diestrus	4.9 ± 0.96	5.2 ± 0.33
	estrus	3.2 ± 0.12 +	5.2 ± 0.89
Number of crossed peripheral squares	diestrus	34.6± 2.30 +	45.8 ± 3.72
	estrus	22.0 ± 2.90 ΔΔ +	30.3 ± 2.49 ΔΔ
Number of central leg stand	diestrus	0	0.6 ± 0.14
	estrus	1.6 ± 0.57	1.2 ± 0.30
Number of peripheral leg stand	diestrus	2.8 ± 0.40 ++	5.8 ± 0.69
	estrus	4.0 ± 0.65	3.6 ± 0.61 Δ
Number of peeps into minks	diestrus	10.1± 1.97 ++	3.8 ± 0.38
	estrus	6.3 ± 1.51	5.1 ± 0.75
Number of grooming reactions	diestrus	5.3 ± 1.80	6.3 ± 1.10
	estrus	3.9 ± 1.03 +	9.5 ± 2.00
Number of defecation boluses	diestrus	0.9 ± 0.32	1.8 ± 0.48
	estrus	0	1.2 ± 0.36

Note: Δ - significance of differences between cycle stages, + - significance of differences between old and young animals Δ (+) – $p < 0.05$; Δ Δ (++) – $p < 0.01$; Δ Δ Δ (+++) – $p < 0.001$.

Table 2

Processes of free radical oxidation of young and old female rats depending on the stage of the estrous cycle

LPO processes	Cycle stage	Young animals	Old animals
The rate of spontaneous LPO	diestrus	17.0 ± 1.32 +++	37.8 ± 5.02
	estrus	20.8 ± 1.79 +	51.5 ± 7.32
Ascorbate-dependent LPO rate	diestrus	53.3 ± 6.30	65.97 ± 6.54
	estrus	43.5 ± 5.81	52.3 ± 6.20
The initial level of MDA	diestrus	5.1 ± 0.56 +++	4.5 ± 0.81
	estrus	1.5 ± 0.22 ΔΔ +	2.85 ± 0.43

Note: Δ - significance of differences between cycle stages, + - significance of differences between old and young animals Δ Δ (+) – $p < 0.05$; Δ Δ (++) – $p < 0.01$; Δ Δ Δ (+++) – $p < 0.001$.

In old animals, the processes of lipid peroxidation did not depend on the stage of the estrous cycle. Young animals were characterized by a lower rate of spontaneous LPO and the initial level of MDA, in contrast to the indicators in old female rats, regardless of the stage of the estrous cycle (Table 2).

Discussion

A higher level of the initial malondialdehyde in female rats at the diestrus stage compared to the estrus stage may be associated with fluctuations in sex hormones during the ovarian cycle, as many authors point to the antioxidant effect of sex hormones. In particular, the antiradical

and antioxidant effects of estrogens have been shown (Mikheev *et al.*, 2010; Sashkov, 2009).

The patterns of exploratory behavior of animals in the estrus stage were characterized by a shorter latency time of the first movement, as well as a decrease in motor activity on the periphery of the open field, this may indicate a lower level of anxiety in the estrus stage, which is also confirmed by a decrease in the number of grooming reactions and the number of defecation boluses at this stage. It has been shown that a low level of anxiety and an increase in sensitivity to anxiolytic drugs are characteristic of the stages of proestrus and estrus (Sashkov, 2009). Anxiety is closely dependent on fluctuations in sex hormones during the reproductive cycle, since it is known that progestins and their metabolites are modulators of the activity of excitatory and inhibitory receptors in the central nervous system. There is evidence of the interaction of progestins with the benzodiazepine system of the brain, which determines emotional arousal, fear and anxiety to a greater extent (Guillen-Ruiza *et al.*, 2021). Most researchers note the anxiolytic properties of exogenous progesterone (Zhukov *et al.*, 2020).

Motor activity in female rats in the estrus stage was higher compared with the diestrus stage. Changes in the motor activity of animals at different stages of the estrous cycle, found in our experiments, may be associated with a change in the activation of the dopaminergic system of the brain. There is evidence of a change in the functional activity of the dopaminergic system depending on the level of sex hormones (Vinogradova & Chaadaeva, 1994). In the stage of late proestrus and estrus, when there is an increase in the secretion of progestins and estrogens, the level of dopamine and its metabolites in the brain tissues increases, and in the diestrus stage it decreases (Vinogradova, 1999).

In older females, there was a violation of ovarian cycles, which manifested itself in a change in the duration of the stages of the reproductive cycle before the establishment of a permanent estrus or diestrus, or the loss of any stage of the cycle, which is consistent with the literature data (Zhukov *et al.*, 2020). According to the indicators of exploratory behavior, old females showed great motor activity on the periphery of the open field, which, combined with a high level of grooming and low research activity, may indicate a high level of anxiety in this age group. It is shown that the elderly are characterized by an increase in the role of negative emotions, a decrease in the variability of emotional states. There is an age-related increase in the excitability of negative emotional zones of the hypothalamus, a decrease in the thresholds of negative emotional reactions. With aging, rats have prerequisites for shifting the emotional balance towards negative manifestations, there is evidence that feelings of anxiety, fear, and confusion become more frequent in old age (Artemenkov, 2018; Sidenkova *et al.*, 2021; Hou *et al.*, 2019; Liguori *et al.*, 2018).

Age-related changes in animals were also manifested in indicators of lipid peroxidation. This was expressed by a higher initial level of malondialdehyde, and to a greater extent in the estrus stage. With age, free radical processes increase, which is reflected in the free radical theory of aging (Dubinina & Pustygina, 2017; Liguori *et al.*, 2018).

Conclusion

Thus, the parameters of exploratory behavior in the «open field» test depend on the age of the animal. Old rats are characterized by increased anxiety regardless of the stage of the reproductive cycle. Young females differ from older ones by higher research activity. Preliminary handling of animals revealed the dependence of exploratory behavior in mature female rats on the stage of the estrous cycle: the motor activity of females is higher, and the level of anxiety is lower in the estrus stage compared with similar indicators in the diestrus stage.

Older female rats have a higher level of free radical oxidation.

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