

Age-Related Peculiarities in Steroid Hormone Secretion and Behavior in Prenatally Stressed Female Rats in Novel Environment

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We studied the effects of immobilization of female rat during days 15-18 of pregnancy on the behavior in novel environment (open field test) and blood level of steroid hormones in their female offspring depending on the cycle phase and age. The rats were tested at the age of 3, 12, and 24 months. Locomotor and exploratory activity and anxiety of control rats depended on the phase of estrous cycle. Age-related changes in the studied parameters were noted: locomotor and exploratory activity decreased and anxiety increased against the background of reduced secretion of the main ovarian hormones with age. In addition, blood estradiol level and behavior in novel environment virtually did not depend on age and estrous cycle phase in prenatally stressed females. Our findings suggest that maternal stress has a modulatory effect on relationship between behavioral type and estrous cycle stage, as well as on age-related pattern of behavioral reactions.

Key Words: *perinatal stress; open field; estradiol; progesterone; rat*

Extensive experimental and clinical data accumulated during recent decades showed that sex hormones play an important role in the regulation of non-sexual forms of behavior in addition to realization of reproductive functions. In light of this, estradiol similarly to progesterone, produces pronounced effects on CNS and affects some important brain functions such as learning, memory, emotions, affective states, motor activity, and anxiety [1,5,6,11-13]. Moreover, both pathology of the pituitary-ovarian system and fluctuations of its activity throughout the ovarian cycle determine the development of various neuropsychic disorders [4,9,10,14]. Age-related changes in female organism associated with loss of reproductive functions are also characterized by significant changes in emotional sphere which can be normalized by replacement therapy [11,15].

We previously showed that exposure of pregnant rats to stress during the last third of gestation nega-

tively affects reproductive function of their female offspring [7] depending on animal age. Moreover, activity of pituitary-adrenocortical system and especially its feedback regulation were affected in prenatally stressed females, and these disturbances increased with age of the animals [3]. In addition, prenatally stressed females were characterized by lower locomotor activity and higher anxiety even in young age [2]. It can be hypothesized that apart from hormonal disturbances, significant behavioral changes can develop with age in females prenatally exposed to stress. In this connection, this study was focused on investigation of behavior of prenatally stressed female rats in novel environment (open field test) depending on the phase of the estrous cycle and age of animals. Obtained results of behavioral test were compared with the corresponding changes in secretion of sex steroid hormones.

MATERIALS AND METHODS

The experiments were performed on female offspring from 10 primigravida female Wistar rats kept under

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standard vivarium conditions with no water and food restriction. Pregnant females ($n=5$) from gestation days 15 through 19 were exposed to daily immobilization stress in narrow plastic cages ($20 \times 7 \times 6$ cm) under conditions of bright illumination. The control group included intact pregnant females ($n=5$). Offspring remained with mothers until the age of 30 days, and thereafter 6 animals per cage.

At the age of 3 and 12 months, the females from the experimental and control groups were divided into groups of estrus and diestrus ($n=7$ in each subgroup; examination of stained vaginal smears); blood samples from the caudal vein were taken to determine progesterone and estrogen levels. Two-year-old females from both groups were characterized by long (more than 10 days) cycle with protracted diestrus and inexplicit other phases; therefore, the blood for hormonal analysis was taken in diestrus. The levels of sex steroid hormones were determined in blood plasma using enzyme immunoassay with standard kits (Chema-Medica).

Rat behavior in new environment was evaluated in open field test according to the method described previously [2], where 5 min registration included locomotor activity, number of rearings and defecations, time of freezing, as well as time in central quadrants, which reflects anxiety level. Considering the fact that female behavior varies according to the phase of the estrus cycle, to test cycling animals (3 and 12 months) 8 rats in estrus and 8 rats in diestrus were selected from each group. Old 24-month-old females ($n=9$) were tested in diestrus. Behavioral tests were performed from 11 a.m. to 1 p.m.

All investigations were performed in accordance with ethic principles stated in European Convention

for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Between-group comparisons were performed using one-way ANOVA with subsequent post-hoc Newman-Keuls test. The differences were considered significant at $p < 0.05$.

RESULTS

Hormonal analyses demonstrated clear-cut dependence between blood estradiol concentration and stage of estrus cycle in 3-month-old females from the control group (Fig. 1, *a*). Similar results were obtained for 1-year-old control females; however, it should be noted that estradiol level in two investigated phases of the cycle were lower than in young animals, although these differences did not attain the level of significance. Prenatal stress decreased blood estradiol concentration in 3- and 12-month-old female rats in estrus, which leveled hormonal differences between the cycle stages (Fig. 1, *a*); moreover, significant decrease in estradiol level in estrus was observed in 12-month-old animals in comparison with 3-month-old animals. Significant reduction in estradiol concentration was detected in the blood of 24-month-old rats (diestrus) from the control group, whereas hormone level in prenatally stressed animals was the same as in 12-month-old rats and higher than in the control.

It was also shown that progesterone level in control cycling animals (3- and 12-month-old) during diestrus was higher than during estrus (Fig. 1, *b*). Prenatal stress did not affect progesterone level fluctuation during estrus cycle; moreover, hormone concentration in 3-month-old animals during diestrus substantially surpassed the control values. At the age of one year,

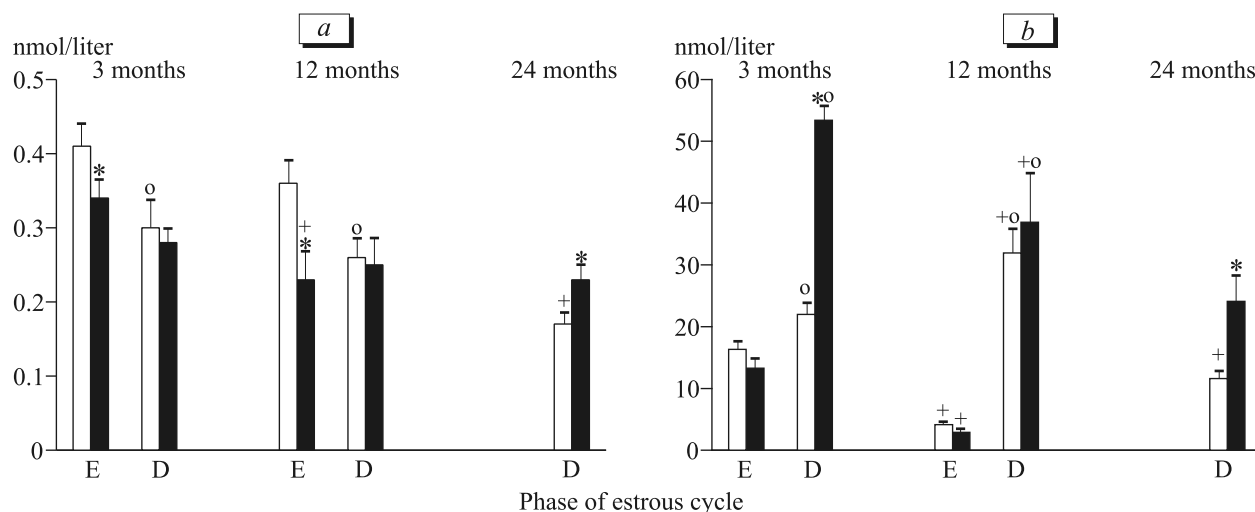


Fig. 1. Effects of prenatal stress on age-related changes in estradiol (*a*) and progesterone (*b*) concentrations in female rats. Here and in Fig. 2: light bars: intact rats, dark bars: prenatally stressed females. E: estrus; D: diestrus. $p < 0.05$ compared to *intact rats, °corresponding phase at the previous term, +estrus.

females from two groups had no differences in progesterone levels, meanwhile a decrease in hormone concentration during both stages of the cycle in comparison with the corresponding parameter in 3-month-old animals was noted in both the control and prenatally stressed group. In 24-month-old control females, progesterone levels were lower than in 3- and 12-month

animals in diestrus, whereas the age-related decrease was less pronounced in animals with prenatal stress.

It should be noted that control females exhibited age-related gradual decrease in the levels of both ovarian hormones, which is consistent the theory of hormonal aging [8], whereas prenatally stressed animals showed age-related changes only for progesterone.

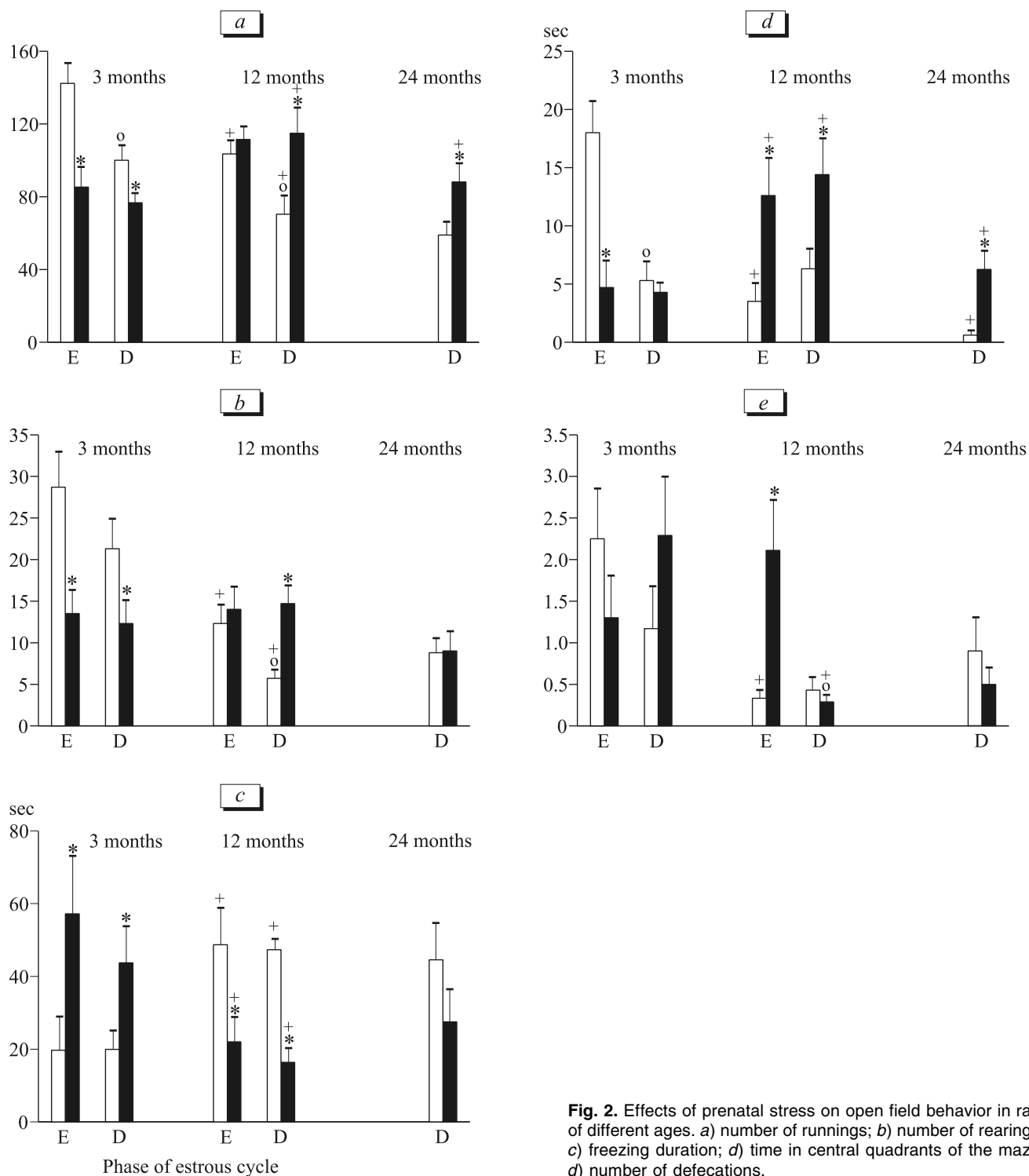


Fig. 2. Effects of prenatal stress on open field behavior in rats of different ages. a) number of runnings; b) number of rearings; c) freezing duration; d) time in central quadrants of the maze; e) number of defecations.

Estradiol concentration in females born from dams exposed to stress at the end of gestation did not depend on the phase of estrous cycle and virtually during the whole life.

Evaluation of exploratory behavior of 3- and 12-month-old females under conditions of novel environment revealed relationships between locomotor activity, anxiety, and phase of estrus cycle (Fig. 2). At the age of 12 months, locomotor activity, number of rearings, and emotionality decreased, while freezing duration increased in both phases of the estrous cycle and anxiety significantly increased during estrus, which was seen from reduced time spent in central quadrants of the field. Exposure of pregnant dams to stress eliminated the dependence between animal behavior and estrus cycle phase in their female offspring. Young females with prenatal stress were characterized by lower number of runs and rearings, longer freezing reaction, and higher anxiety in comparison with counterparts from the control group. Substantial decrease in freezing duration and anxiety was noted in 12-month-old females from experimental group in both phases of the cycle in comparison with 3-month-old animals. As a result, 12-month-old prenatally stressed animals demonstrated higher locomotor and exploratory activity in diestrus, high emotionality in estrus, lower freezing duration and lower anxiety in both phases of the cycle compared to control counterparts. Increased locomotion and reduced anxiety in comparison with the control were observed in 24-month-old prenatally stressed females during diestrus.

Summarizing the results of the experiment one may note that locomotor activity, number of rearings, and emotionality decrease with age, while freezing duration and anxiety increase. Nevertheless, locomotor and exploratory activities in prenatally stressed animals were virtually constant through the life; however, opposite age-related changes in freezing duration and anxiety levels were noted in comparison with the control. In addition, the studied parameters of exploratory behavior in cycling prenatally stressed animals were independent from the phase of the estrus cycle. Basing on the hormonal analyses, one may suggest that the absence of changes in exploratory behavior in prenatally stressed females in different phases of the cycle and in different age is determined by the absence of fluctuations of estradiol, its level remained almost constant through the estrus cycle and through the life of the animals.

The effects of estrogen on non-reproductive behavior in both humans and rodents were extensively

investigated. In this respect, the highest effect was observed for emotions. In humans, decreased estrogen levels result in depression, irritability, anxiety, panic disorders, and cognitive dysfunctions [10,14]. The relationship between non-reproductive behavior (anxiety, fear, learning) and blood estrogen level was also noted in rodents [5,11]. Moreover, replacement therapy improved emotional component of behavior in both ovariectomized females and postmenopausal women [5,11,15]. Although there are contradictory data on the effects of ovariectomy on animal behavior in the open field test [5], we consider, that the observed changes (or their absence) in the behavior of aging control and prenatally stressed females are also determined by estrogen concentration in the blood.

Thus, obtained data indicated that maternal stress modifies the relationship between behavioral pattern and phase of estrus cycle as well as the age-related pattern of behavioral reactions of female rats most likely determined by the absence of significant estradiol fluctuations.

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