ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XLIV, 21 SECTIO D 1989

Katedra Histologii i Embriologii. Akademia Medyczna w Lublinie
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Studies on the Effect of Stress on the Estrus Cycle in Rats

Badania nad wpływem stresu na cykl płciowy u szczurów

Исследования влияния стресса на половой цикл у крыс

Rats were studied for susceptibility to restraint stress in different phases of the estrus cycle. Susceptibility was measured by the duration of disorders of the estrus cycle. It was found that in proestrus and estrus the disorders of the cycle were most prolonged. During lower sexual activity female rats showed considerable resistance to stress, disorders of the estrus cycle being short-lasting.

The rapid advance of civilization and ever-growing pace of life produce conditions where we are subjected to the more and more intense stress situations. It is common knowledge that both men (2, 15, 17) and animals (6, 10, 14) are susceptible to stress. It must be emphasized that organism's reactivity to stressors is not constant but varies depending on the physiological condition of the organism. There are probably many factors that change stress reactivity, causing the organism to be more or less susceptible to stress. In his studies on male rats Ader (1) has found that susceptibility to restraint stress measured by the occurrence of gastric ulcers depends on circadian motor activity of the animals. In female rats circadian activity is additionally modified by increased motility in proestrus and estrus (3, 8). If susceptibility to restraint stress indeed depends on the level of motor activity, it must be expected that it should likewise be subject to the periodic changes in the estrus cycle. The purpose of the present work was to corroborate this thesis.

MATERIAL

The experiments were performed on 44 twelve-week old white female Vistar rats weighing 160—200 g. The animals were kept in single cages in natural light conditions. Phases of the estrus cycle were determined on the basis of vaginal smears taken daily for 8 days. The smears were stained

according to the Shorr method (12). Interpretation of cytohormonal pictures was made on the basis of colouring, number, shape, kind and pattern od arrangement of vaginal epithelial cells, of the condition and size of cell nuclei and the amount of leucocytes and mucus in vaginal swab. The animals were then subjected to four-hour restraint stress in different phases of the estrus cycle at a temperature of $+4^{\circ}C$ (11). After that time the animals were freed immobilization nets and returned to their cages where they remained until the end of the experiment. Daily vaginal swabs were taken from the female rats after stress and examined according to the Shorr method (12). The results were assessed with the medial and the mean semi-interquartile range.

RESULTS

In all studied animals a four-day estrus cycle was found that was characterized by distinct phases: diestrus, proestrus, estrus and matestrus. Susceptibility to stress in particular phases of the estrus cycle was assessed according to duration of disorders of the cycle. The four-hour restraint stress and low temperature produced changes in the cytohormonal picture. The picture of vaginal swab after stress resembled diestrus or metestrus: it was however, non-typical and could not be assigned to any of the phases. In the vaginal smear after stress there were profuse streaks of mucus with intermedial cells and numerous leucocytes. Syndrome of dissociation of the estrus cycle was shown in Fig. 1.

The criterion of return to the normal estrus cycle was the day on which a lagible vaginal smear was found that permitted to determine the phase of the cycle and beginning from which a regular estrus cycle still persisted for at least 8 following days. The results were presented in Fig. 2.

It was found that if restraint stress was elicited in diestrus, disorders of the estrus cycle were the shortest for the medial value of duration of the estrus cycle disorders ranged between 10.0 ± 4.0 days. If, however stress was elicited in proestrus or estrus these disorders lasted the longest, ranging 27.0 ± 3.5 and 25.0 ± 2.5 days respectively. Immobilization of the animals during metestrus produced disturbances of the estrus cycle that lasted 14.0 ± 2.5 days. Most frequently, the first distinct phase after the syndrome of the estrus cycle dissociation stopped was the estrus phase.

DISCUSSION

The results indicate that susceptibility to stress, measured by duration of the estrus cycle disorders, varies with the phases of the estrual rhythm. During increased estrual activity female rats proved to be the most sensitive to stress factors. By far greater resistance to stress was found in females during lower estrual activity in diestrus, which was manifested in short-lasting disorders of the

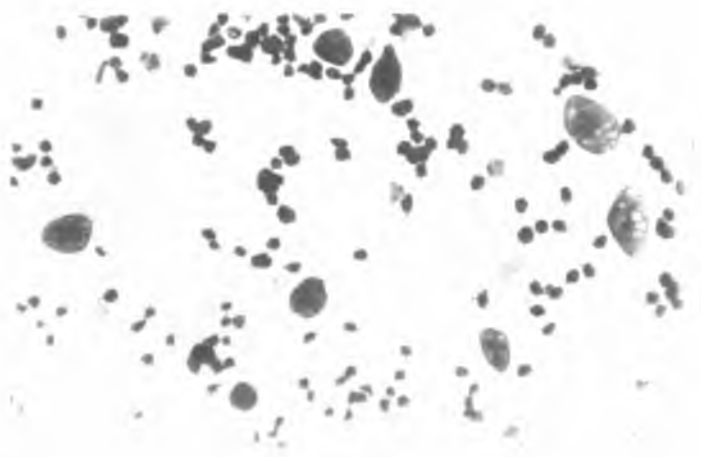


Fig. 1. Syndrome of dissociation of estrus cycle; intermedial cells of vaginal epithelium profuse mucus and leucocytes are visible; magn. ca 200 ×

estrus cycle. The causes of the observed changes in susceptibility to stress during the estrus cycle can be numerous and complex.

There is some analogy between the results of this study and those obtained by Ader (1), who has demonstrated that susceptibility of male rats to restraint stress depends on the circadian activity of the animals. In rats restrained at the beginning of their highest motor activity, which mostly occurred between 18:00 and 03:00 hrs, the most intense gastric ulcerations were observed. However, in rats restrained during their lower motor activity gastric ulcers were not reported.

The effect of motor activity on stress reactivity was studied by Sines (13), who advanced a thesis that the race of rats characterized by high level of motor activity was more susceptible to restraint-induced gastric ulcers.

The increased susceptibility of the organism to stress can be due to a significant increase of the motor activity of female rats during diestrus and estrus. The circadian cycle of motor activity is additionally modified by the rhythm of motility changes corresponding to estrual rhythms. Female rats during proestrus and estrus are far more motile than in the other phases of the cycle (3, 8).

Therefore, just as in Ader's study (1), where rats at the peak of their circadian motor activity were found to be most sensitive to the ulcerogenic factor, which was long-lasting immobilization, likewise in the present work an increase in the motor activity of the animals during proestrus and estrus caused that the identical immobilization proved to be a stronger stress stimulus.

Another cause of the reported disorders of the estrus cycle could be changes in gonadotropin secretion under stress. This is supported by the results obtained by Than Do and Kurcz (16), who found significantly lower fertility in rats subjected to different stress factors. One of the conditions of gonadotropin release is known to be the adequate level of brain serotonin (5). A pharmacological reduction of serotonin level in the central nervous system by reserpine administration inhibited the estrus cycle at the phase of diestrus (4). Studies by Labhsetwar and Zolowick (9) confirm the role of serotonin component in ovulation. Since the present study was also performed on rats and the applied stress was similar in character, the level of brain serotonin should be subject to similar variations. It could thus be inferred that the consequence of serotonin reduction in the central nervous system could be insufficient gonadotropin secretion, which was especially felt by the organism during the greatest requirement for these hormones i.e. during proestrus and estrus. The estrus cycle disorders were then very heavy because long-lasting. During diestrus, however, when the estrual activity of female rats is weaker, the effects of changes in brain serotonin level and gonadotropin deficit were less felt and manifested in short-lasting disorders of the estrus cycle.

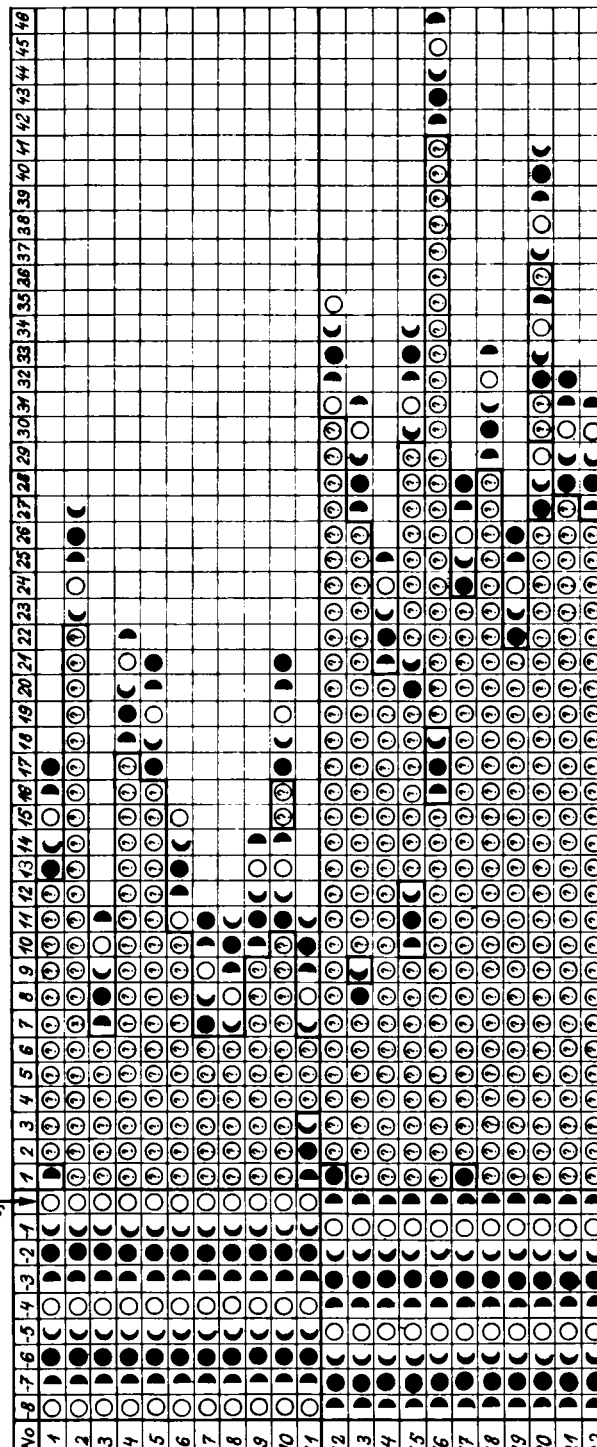
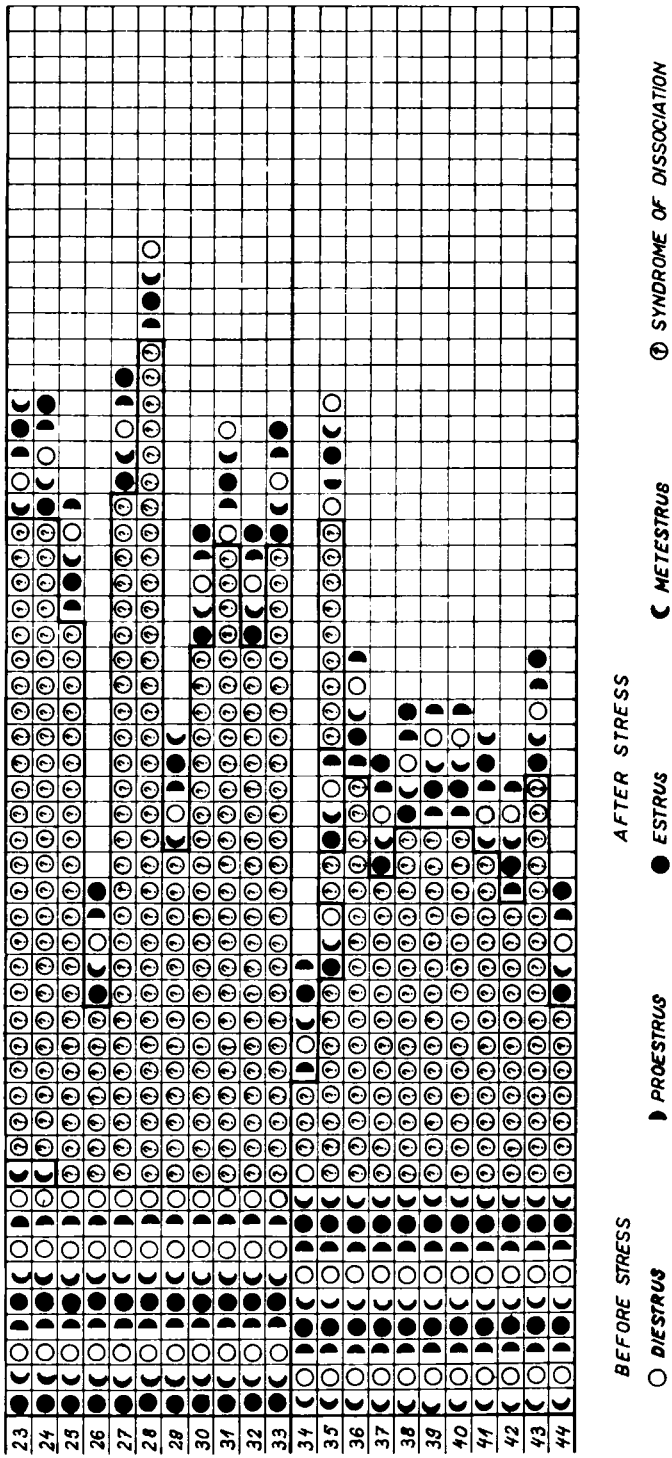


Fig. 2. The effect of restraint stress on the estrus cycle of individual female rats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BEFORE STRESS
AFTER STRESS
○ DIESTRUS
◗ PROESTRUS
● ESTRUS
☾ METESTRUS
Ⓣ SYNDROME OF DISSOCIATION

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Otrzymano 1989.05.18.

STRESZCZENIE

Badano podatność zwierząt na stres immobilizacyjny i niską temperaturę w różnych fazach cyklu płciowego. Miarą podatności na stres był czas trwania zaburzeń cyklu rujowego. Stwierdzono, że w fazach *proestrus* i *estrus* zaburzenia cyklu płciowego utrzymywały się najdłużej. W okresie zmniejszonej aktywności płciowej samice wykazywały znaczną odporność na stres, a zaburzenia cyklu płciowego były wtedy krótkotrwałe.

РЕЗЮМЕ

Исследовано податливость животных на иммобилизационный стресс и низкую температуру в разных периодах полового цикла. Мерой податливости на стресс было время продолжания расстройства полового цикла. Констатировано, что в периодах *proestrus* и *estrus* расстройство полового цикла удерживалось длиннее всего. В периоде сниженной половой активности самки проявляли значительную сопротивляемость на стресс, а расстройство полового цикла было тогда кратковременное.

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Nakład 600 egz. + 25 nadb. aut., ark. wyd. 12,5, ark. druk. 10 + 18 str. wkl kred. Papier druk. sat. kl. III, B1, 70 g. Oddano do składania w październiku 1990 r., podpisano do druku w październiku 1991 r. wydrukowano w grudniu 1991 r. Cena zł 19.000,—

Lubelskie Zakłady Graficzne, Lublin, ul. Unicka 4, zam. 1348/90

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