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RESEARCH ARTICLE

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# A Comparative Study of Maternal Behavior Dynamics in Wistar Rats and Rats Selected for Predisposition to Catatonic Reactions (GC Rats)

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**Abstract**—Maternal behavior is an important factor for the offspring’s development in mammals. Particular traits of maternal behavior in early ontogenesis may have a lasting effect on a number of behavioral and physiological parameters of offspring in their adulthood. In this study, the maternal behavior of GC rats (GC stands for “genetic catatonics”) and control Wistar rats from the first to the 20th day after delivery was examined. Observations were carried out in a home cage during the day using an automated video recording system without the experimenter’s presence. GC female rats were found in the nest and nursed pups more often as compared to the female Wistar rats. Wistar female rats “rested” outside the nest more often during the day, along with less frequent manipulations with nest material and less frequent self-grooming. The change in the frequency of maternal behavior patterns as the offspring matured had similar dynamics in both rat strains. When comparing activity in the light and dark phases of the light/dark cycle, it was found that GC females had a greater activity outside the nest at night. It was assumed that distinguished maternal behavior of catatonic rats might be due to greater anxiety in GC rats as compared to control rats.

**Keywords:** maternal behavior, catalepsy, catatonia, GC rat strain, circadian rhythm, animal model

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## INTRODUCTION

The influence of early environment on the establishment of organism functions is currently doubtless. The mother is one of the most essential factors forming the early environment in mammals. Maternal behavior in the critical periods of ontogenesis specifically alters the gene expression in different brain regions of offspring [1, 2], having a lasting effect on their neuroendocrine, behavioral, and cognitive characteristics [3, 4].

This study was conducted on the GC rat strain (GC stands for “genetic catatonics”), taken as an experimental model of catatonic disorders [5, 6]. Catatonic syndrome comprising both stupor and psychomotor agitation in humans is associated with a number of diseases, including affective disorders and schizophrenia [7]. It is known that maternal behavior of women suffering from schizophrenia or depression has several specific traits, particularly, avoiding contacts with their children and reduced reactions to outgoing signals [8–10]. Psychopathological models provided the information about the changes in maternal behavior of experimental animals. FSL (Flinders Sensitive Line) rat strain offered as a genetic model of depression showed poorer maternal behavior than Sprague–

Dawley and FRL (Flinders Resistant Line) strains [11]. Lack of maternal motivation was demonstrated in the WAG/Rij (Wistar Albino Glaxo from Rijswijk) rat strain with absence epilepsy characterized by depression-like behavior [12, 13]. Maternal behavior of GC rats was previously studied [14]; however, we faced the pathomorphosis of cataleptic reaction in the process of strain selection. If the catatonic genotype appeared phenotypically as increased predisposition to freezing at the early stages of selection, animals demonstrated vocalization and paroxysmal running and jumping at this stage [15]. Therefore, the question occurred whether other patterns of behavior also changed in GC animals.

It is known that patients with affective disorders and psychopathologies have impaired circadian rhythms [16, 17]. This fact aroused interest in the specifics of diurnal activity of GC rats with pathological behavior.

The aim of this research was to investigate the traits of maternal behavior and diurnal activity of female rats with catatonia. In order to do this, the behavior of nursing Wistar female rats was compared with GC rats using a 24-h video recording for the first time.

## MATERIALS AND METHODS

**Experimental animals.** The experiments were performed using white rats (*Rattus norvegicus*) of GC and Wistar strains. The GC strain was bred in the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences (Novosibirsk) selecting rats from Wistar population by exaggerated reaction of passive-defensive freezing [5]. Experimental animals were kept in a conventional vivarium of the ICG SB RAS SB RAS grouped by four animals of the same sex under natural lighting regimen and provided with free access to water and food. Water and food were refreshed daily. At the age of 4 months, males were paired with virgin females virgin females (b.w. 250–300 g). Pregnant females were isolated in standard cages (50 × 33 × 20 cm) equipped with high-resolution video cameras allowing both day- and nighttime recording [18]. The experiment was conducted in autumn (dawn–sunset: 8:30 a.m.–6:00 p.m.). Since the maternal behavior is affected both by the number of pups and artificial changes in this number [19], the sizes of litter were not normalized. Experimental groups consisted of six female rats of each strain with six to ten pups in their litters.

**Investigation of maternal behavior.** The day of delivery was accepted as the zero day and maternal behavior was analyzed in a home cage from the first to the 20<sup>th</sup> days after delivery. Female rat behavior was recorded automatically six times a day (from 2:00 a.m., 9:00 a.m., 2:00 p.m., 5:00 p.m., 8:00 p.m., and 11:00 p.m.). With this purpose, a 10-second video record was made every 3 min within 1 h (20 times per session) and the behavioral patterns of females and staying in or outside the nest were tracked. Overall, 120 observations were made throughout the day for each female. Pup-directed behavioral patterns (nursing, licking, grooming, and carrying) and individual behavioral patterns (eating or drinking, vertical and horizontal motor activity, self-grooming, manipulations with nest material, sitting or lying rest away from pups) were analyzed. Three nursing poses were distinguished: (a) arched-nursing, when a mother leaned over pups, its back arched and paws splayed; (b) blanket-nursing, when a mother almost lays over pups with its back flat; (c) passive-nursing lying on its side or back [20]. Furthermore, the integral parameter of activity outside the nest was assessed, including all the individual patterns of female behavior apart from resting.

The total observation period was divided into four stages of 5 days (1–5, 6–10, 11–15, and 16–20 days after delivery) for further analysis. The end results were presented as overall number of observations of one or another pattern for each period (maximally possible number of pattern observations per period was 600). When assessing the diurnal activity, the results obtained at light (9:00 a.m. and 2:00 p.m.) and dark (2:00 a.m. and 11:00 p.m.) hours were summed

(maximally possible number of pattern observations for each time of the day was 200).

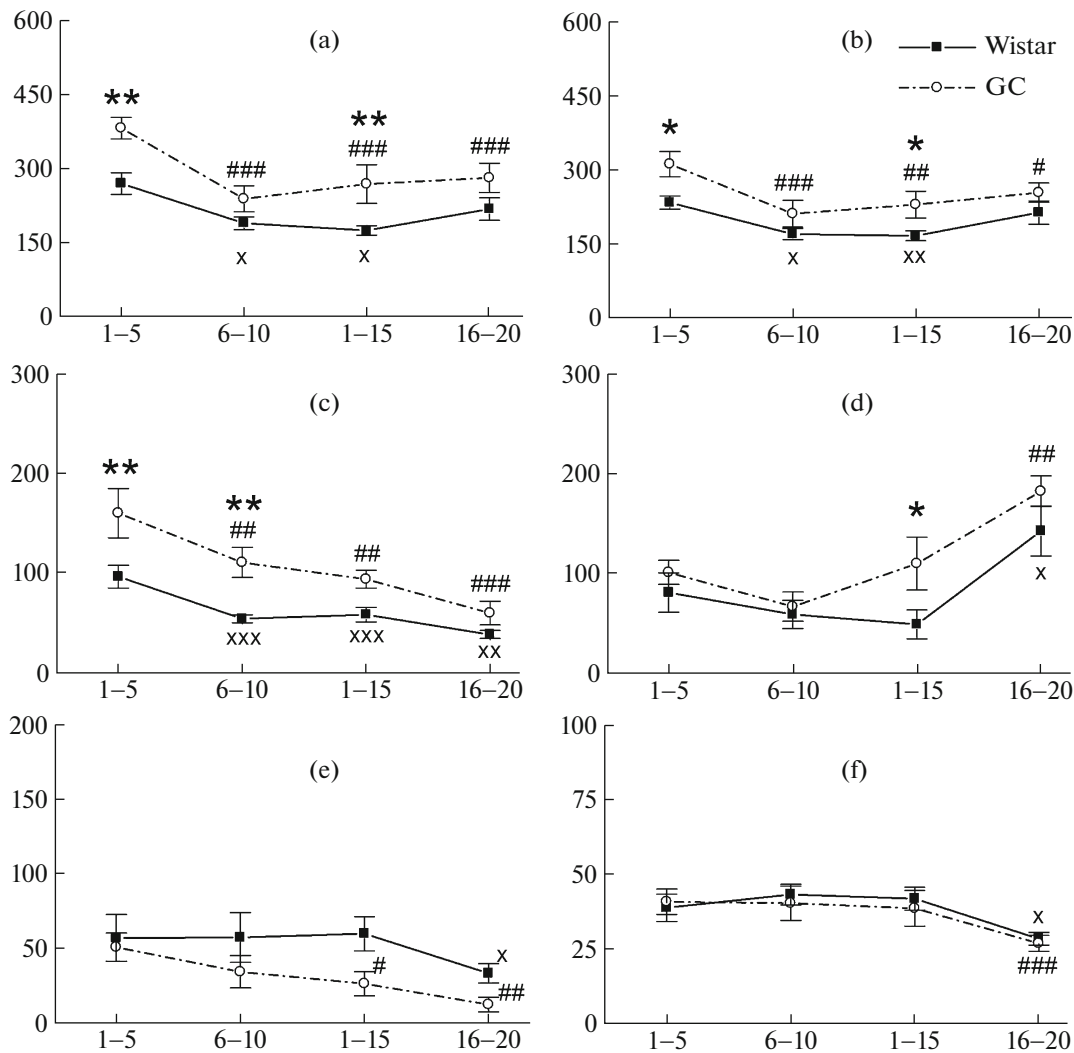
**Statistical analysis.** Distribution normality check was conducted using the Shapiro–Wilk test. One-way repeated measurement ANOVA was used with the genotype factor to detect interstrain differences between normally distributed data. Post hoc Fisher's LSD test was applied to identify the contributions of genotype or observation period factors. The interstrain differences were compared within one period of observation. The effect of the observation period was estimated by comparing the first and further periods. For nonnormal distributions, interstrain comparisons were performed with the Mann–Whitney U–test. Comparison of day and night time activities within one strain was made using the t-test for independent samples. The differences were considered significant at  $p < 0.05$ . Statistical analysis was conducted in Statistica 6.0 (StatSoft, United States) software. Figures present mean and standard errors ( $M \pm SEM$ ).

## RESULTS AND DISCUSSION

The present study revealed the influence of genotype on certain behavioral patterns of females directed to pups (Figs. 1a–1c). GC rats were found in the nest more often ( $F_{1, 10} = 9.49, p < 0.02$ ) and used a higher overall number of nursing positions ( $F_{1, 10} = 7.07, p < 0.03$ ) and amount of blanket-nursing ( $F_{1, 10} = 11.35, p < 0.008$ ). No effect of genotype factor was detected on the amount of passive- and arched-nursing (Figs. 1d, 1e). Moreover, no interstrain differences were discovered in pup licking and grooming (Fig. 1f). At the first observation period (first to fifth days after delivery) Wistar female rats carried pups more often ( $p < 0.03, U = 3.5$ ). This is a relatively uncommon form of behavior registered sporadically during the second and third periods and absent in the fourth period.

The individual behavior differed between strains (Figs. 2a–2c). GC female rats manipulated with nest material more ( $F_{1, 10} = 12.89, p < 0.005$ ) and self-groomed often ( $F_{1, 10} = 16.05, p < 0.002$ ). Wistar female rats spent more time sitting or lying away from pups ( $F_{1, 10} = 17.02, p < 0.002$ ).

GC female rats remained in the nest and nursed pups more than Wistar rats, thus demonstrating a higher level of maternal care (Figs. 1a, 1b). The literature data provide strong evidences of the correlation between increased care after pups and higher anxiety of mother. These data were obtained from nursing Wistar female rats differing in the anxiety level [21], bHR (bred High Responder) and bLR rat strains (bred Low Responder) [22], HAB (High Anxiety-related Behavior) and LAB (Low Anxiety-related Behavior) rat strains [23, 24], and High Anxiety-related Behavior) and LAB (Low Anxiety-related Behavior) mouse strains [25] differing in anxiety levels in an elevated

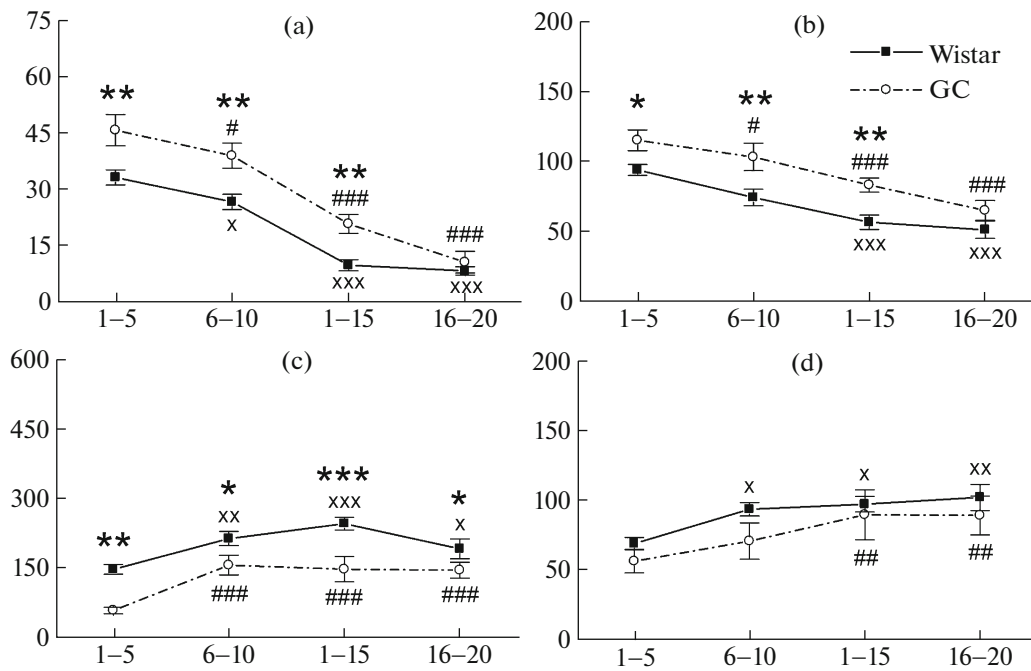


**Fig. 1.** Parameters of pup-directed behavior of GC (dashed line) and Wistar female rats (solid line) from the first to the 20th days after delivery. (a) Staying in nest; (b) overall nursing; (c) blanket-nursing; (d) passive-nursing on the side; (e) arched-nursing; (f) licking and grooming of pups. Data are presented as mean and standard error ( $M \pm SEM$ ).  $N = 6$  for each group.  $y$ -Axis represents the number of observations;  $x$ -axis shows the day after delivery. \*  $p < 0.05$ , \*\*  $p < 0.01$  for GC compared with Wistar strains; #  $p < 0.05$ , ##  $p < 0.01$ , ###  $p < 0.001$  compared to first to 5 days after delivery in GC strain;  $x$   $p < 0.05$ ,  $xx$   $p < 0.01$ ,  $xxx$   $p < 0.001$  compared to first to 5 days after delivery in the Wistar strain.

plus-maze. GC rat strain is characterized by increased anxiety in behavioral tests and higher amplitude of startle reflex, suggesting a more expressed fear response [6]. From this perspective, maternal type observed in the GC rat strain coincides with other experimental findings. On the other hand, we have earlier showed that GC rats contacted with pups less than Wistar females [14]. The source of intensified maternal care of rats with catatonia seen in the present study requires further research. It can be underlain by endogenic mechanisms providing the pathomorphosis of cataleptic reaction observed during the selection of GC rats [15]. Moreover, maternal behavior highly depends upon the external factors and stressful agents [26]. The method of tracking animal behavior can also affect the results: thus, other authors received opposite

results on the nursing frequency of hypertensive mothers compared to control when studying the behavior of SHR (Spontaneously Hypertensive Rats) strain in the presence of an observer and under video observation [27]. In our previous study [14], we applied a visual registration of behavior and performed a home-cage retrieval test, whereas the nursing mothers were not exposed to stress in this experiment.

Grooming, licking of pups, and arched-nursing are the crucial components of maternal behavior contributing to the psychoemotional and neuroendocrine characteristics of the offspring [2]. No interstrain differences were detected in these parameters (Figs. 1d, 1e). Pup carrying—registered more commonly in Wistar rats over the first 5 days of lactation—is considered



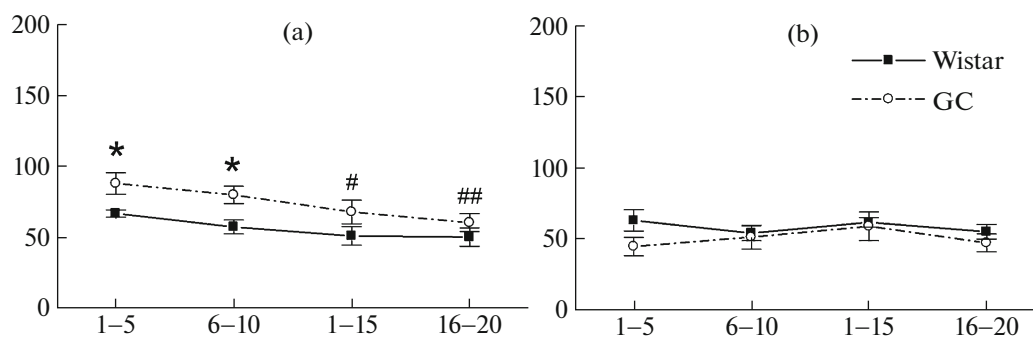
**Fig. 2.** Parameters of individual behavior of GC (dashed line) and Wistar female rats (solid line) from the first to the 20th days after delivery. (a) Manipulation with nest material; (b) self-grooming; (c) rest; (d) eating and drinking. Data are presented as mean and standard error ( $M \pm SEM$ ).  $N = 6$  for each group.  $y$ -Axis represents the number of observations;  $x$ -axis shows the day after delivery. \*  $p < 0.05$ , \*\*  $p < 0.01$  for GC compared with Wistar strains; #  $p < 0.05$ , ##  $p < 0.01$ , ###  $p < 0.001$  compared to the first to 5 days after delivery in GC strain; x  $p < 0.05$ , xx  $p < 0.01$ , xxx  $p < 0.001$  compared to the first to 5 days after delivery in Wistar strain.

as proactive maternal behavior and a parameter of high maternal motivation [24]. However, this form of behavior is mainly discussed in literature in the context of tests with disturbed familiar environment of nursing dams (for instance, pup retrieval test) [11, 28], and we have earlier demonstrated that Wistar females carried pups more successfully [14]. Pup carrying by dams is also initiated by ultrasonic vocalization of pups produced when they feel discomfort [11]; therefore, definitive classification of this pattern as “better” or “poorer” maternal behavior in a home cage seems problematic.

As shown in Figs. 1 and 2, the dynamics of maternal behavior was generally the same in rats of both strains. No interaction of genotype and observation period factors was identified. Reduced frequency of resting inside nest ( $F_{3,30} = 14.47, p < 0.0001$ ) and nursing ( $F_{3,30} = 9.80, p < 0.001$ ) was registered after 6–10 days from delivery. By the third week after delivery, statistical differences between these factors disappeared in Wistar rats compared to the first period of observation. In this regard, it should be emphasized that, while pup nursing was initiated by dams at the early stages of ontogenesis, 3-week-old pups were capable of managing the frequency of feeding on their own [29]. Therefore, detected differences could also be associated with the activity of pups. The reduction in pup grooming rate ( $F_{3,30} = 11.85, p < 0.0001$ ), num-

ber of self-grooming episodes ( $F_{3,30} = 23.27, p < 0.001$ ), and manipulations with nest material ( $F_{3,30} = 84.5, p < 0.0001$ ) was seen along with offspring maturation. In addition, the frequency of dams’ drinking and eating gradually elevated ( $F_{3,30} = 8.35, p < 0.001$ ) as well as the increase of dams’ resting ( $F_{3,30} = 15.71, p < 0.0001$ ) starting from the second period compared to the first 5 days after birth. Over the last period of observation, Wistar rats demonstrated the decreased number of resting episodes compared with the third period ( $p < 0.05$ ), and the difference with the first period remained statistical.

The analysis of frequencies of different nursing postures (Figs. 1c–1e) revealed that only the number of passive-nursing on the side ( $F_{3,30} = 14.16, p < 0.0001$ ) increased in contrast with limited arched-nursing ( $F_{3,30} = 5.69, p < 0.003$ ) and blanket-nursing ( $F_{3,30} = 22.12, p < 0.0001$ ) by the third week of rearing. Similar results were seen in bHR and bLR rat strains. It was shown that the nursing frequency in passive posture was growing together with pup maturation [30]. It is known that the milk letdown is not equally effective in different postures. It is thought that arched-nursing provides the maximal milk letdown. On the contrary, the amount of excreted milk is minimal when lying on the side [31]. The observed proportion of nursing postures probably reflects the reduced



**Fig. 3.** Activity of GC (dashed line) and Wistar female rats (solid line) from the first to the 20th days after delivery at day and night hours. (a) Activity in the dark phase; (b) activity in the light phase. Data are presented as mean and standard error ( $M \pm SEM$ ).  $N = 6$  for each group.  $y$ -Axis represents the number of observations;  $x$ -axis shows the day after delivery. \*  $p < 0.05$ , \*\*  $p < 0.01$  for GC compared with Wistar strains; #  $p < 0.05$ , ##  $p < 0.01$  compared to the first to 5 days after delivery in GC strain.

milk demand of pups in the process of transition to a mixed feeding type.

Comparison of total mother activity outside the nest throughout the day did not identify interstrain differences in any of the experimental periods. Genetic differences were detected when comparing the activity parameters of dams during the day and night hours separately (Fig. 3). A higher nighttime activity was shown in GC female rats compared to Wistar rats ( $F_{1,10} = 10.49$ ,  $p < 0.009$ ) and to their individual activity during the day ( $p < 0.004$  for the first period,  $p < 0.04$  for the second period). In Wistar rats, the activity level at daytime did not differ from the night hours. Moreover, the period of observation ( $F_{3,30} = 5.79$ ,  $p < 0.003$ ) was shown to influence on the activity level of nursing dams at night, specifically, the activity decreased with pup maturation. The correlation between the female activity during the day and pup age was not observed.

Rats are nocturnal animals with their peak activity at dark hours. It is known that laboratory rats are normally less active in the period of lactation and the diurnal activity variations are less prominent [32]. Literature data suggest higher activity of nursing bHR dams at the dark time of the day characterized by greater anxiety compared with low-anxiety bLR strain [30]. Studies of depression-like behavior in female rats after weaning found that mothers with depression-like behavior demonstrated increased activity during the dark phase of the light/dark cycle during lactation [33]. Enhanced activity of nursing GC females at night is similar to the one in rats with high anxiety and depression-like behavior. It is noteworthy that a number of characteristics of the GC rat strain are related to homologous parameters seen in depression [6].

Circadian rhythms of GC female rats have not been previously investigated; however, researches involving males allowed presuming the impairment of circadian rhythms in this rat strain. Thus, there were differences between the diurnal fluctuations of monoamine oxi-

dase activity in the brain [34], enzymatic activity in blood lymphocytes, and diurnal variations of cell count in lymphoid organs in GC and Wistar rat strains [35]. Experimental and epidemiological researches provided strong evidences of the connection between disturbed circadian rhythm and psychoemotional disorders [16, 17, 36]. Discovered differences in diurnal activity of GC females actualize further exploration of circadian rhythms in catatonic animals.

To summarize, the study demonstrated that, when nursing in the absence of interference in the maternal environment, GC female rats spending more time inside the nest and nursed pups more frequently than Wistar female rats. These traits of maternal behavior are probably associated with greater anxiety in rats of the catatonic GC strain than in control animals. Observed differences in the activity of nursing GC dams at light and dark hours suggest the alteration of circadian rhythm in these animals.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of Interest.* The author declares that she has no conflicts of interest.

*Statement on the Welfare of Animals.* International bioethical guidelines (European Union directive 2010/63/EU, September 22, 2010) for the care and experimental use of animals were followed.

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