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Changes in Sociability and Preference for Social Novelty in Female Rats in Prolonged Social Isolation

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Chronic stress due to social isolation (SI) can lead to distress with negative consequences for both humans and animals. Numerous disorders caused by SI include disorders in the emotional-motivational domain and cognitive functions, as well as changes in social behavior. There are currently no data identifying the sequelae of SI when its duration is significantly increased. Although female rats have been shown to be highly sensitive to stress, research on them is lacking. The present study assessed sociability and preference for "social novelty" in a three-chamber social test in female Wistar rats in two series of experiments at different time points during prolonged SI, which began at adolescence and continued to ages 5.5 and 9.5 months. At two months of SI, rats showed an increased preference for a social object over a non-social object (increased sociability) simultaneously with the appearance of signs of a decrease in the preference for a new social object over an already familiar social object (signs of a decrease in the preference for social novelty). In a social interaction test, the rats also displayed increases in the durations of social contacts, including aggressive interactions; they showed a decrease in exploratory risk assessments (head dips from the open arms) in the elevated plus maze test and a decrease in exploratory activity. After SI lasting 8.5 months, the rats showed signs of social deficit and a marked decrease in the preference for social novelty. No signs of increased aggressiveness were found. Thus, the impact of SI on social behavior depended on its duration and, we believe, was accompanied by a change in coping strategies.

Keywords: stress, social isolation, female rats, sociability, preference for social novelty, anxiety, aggression, motor and exploratory activity, coping strategies.

Chronic stress, which is accompanied by overactivation of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis, which support the functioning of the mechanisms of allostasis, leads to allostatic load, which may result in the development of neuroplastic rearrangements in the brain and the formation of the pathophysiological basis for mental and behavioral disorders [McEwen, 2004; McEwen, Gianaros, 2011; Ieraci et al., 2016]. Changes in behavior in response to stress are based on emotional and cognitive assessment of a situation, which allows the subject to develop a coping strategy [Dantzer, 2016]. A special place among chronic stresses of various origins affecting species with social living, including humans, is occupied by psychosocial stress caused by a decrease in the number of social contacts, or social isolation (SI) stress, which can lead to psychological distress with adverse consequences for both humans [Holt-Lunstad et al., 2010] and animals [Mumtaz et al., 2018]. The development of distress in humans in conditions of forced long-term SI has been confirmed by observations during the COVID-19 pandemic [Gorenko et al., 2020; Mann and Walker, 2022; Karpenko, 2020]. In rats, which are social animals [Schweinfurth, 2020], SI leads to the development of numerous disorders, which, in addition to changes in the state of the neuroendocrine system and the neurotrophic factors system, as well as neurochemical, physiological, and anatomical parameters, include disorders of the emotional and motivational domains and cognitive functions [Heidbreder et al., 2000; Fone and Porkness, 2008; Mumtaz et al., 2018; Arakawa, 2018].

Many studies have found sexual dimorphism in the emotional and motivational behavior of intact rats of dif-

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ferent strains. For example, adult female Sprague–Dawley and Wistar rats were less anxious than males in the elevated plus maze (EPM) test and their motor/exploratory activity was higher than that of males [Pavlova et al., 2020; Krupina et al., 2020; Scholl et al., 2019]. The expression of anxiety-like behavior in rats depends on the animal's sex, which is important to take into account in translational studies. Sex differences in stress responses are expected and have been demonstrated in numerous studies, especially in stress in animals in the early developmental period [Bale and Epperson, 2015; Oyola and Handa, 2017; Bangasser et al., 2018; Heck and Handa, 2019].

However, most studies have been performed using males; this bias is particularly notable in neuroscience and biomedical research [Beery and Zucker, 2011]. A biopsychosocial approach [Hirnstein and Hausmann, 2021], focusing on studies using females [Couzin-Frankel, 2014], has been proposed with the aim of filling the gap in studies of sex differences in brain reactivity. This approach should identify the mechanisms determining the different predispositions of individuals of different sexes to developing psychoneurological disorders etiologically associated with stress [Bangasser et al., 2018; Heck and Handa, 2019].

SI stress beginning at an early age and lasting from several weeks to 2–3 months usually, though not always, provokes increases in anxiety levels and motor activity in male rats of different strains (cited in reviews [Fone and Porkness, 2008; Lukkes et al., 2009; Mumtaz et al., 2018; Begni et al., 2020]), and these changes are regarded as part of an adaptive response, suggesting an increase in risk avoidance behavior in a threatening environment [McEwen et al., 2012]. As shown in recent years, SI can also change anxiety levels in females, though the data are contradictory. For example, brief SI in adolescence increased anxiety levels in female Sprague–Dawley rats over that in females kept in groups, to a level no different from that in males [Leussis and Andersen, 2007], while combined stress due to chronic SI in adolescence aggravated by periodic social threat or restriction of freedom did not increase the anxiety level in the EPM test in Wistar females, though it did increase motor and exploratory activity, females differing from males in these responses [Bourke and Neigh, 2011]. Impairments in other behavioral indicators, such as depression-like behavior, pain sensitivity, and learning, under the influence of SI, were more marked in female rodents than males [Bourke and Neigh, 2011; Hong et al., 2012; Beery and Kaufer, 2015]. It was suggested on the basis of overall observations that the manifestation of behavioral disturbances induced by SI in females takes longer than in males [Fone and Porkness, 2008], generating the need to model long-term SI.

Our studies using an SI model in rats, starting at an early age and lasting up to nine months, also revealed more marked cognitive impairments in females than males in terms of spatial memory and associative learning in the late stages of isolation [Krupina et al., 2020]. Despite the fact that the basal serum corticosterone levels in male and female rats at nine months of SI were no different from controls, levels were higher in females than males, while relative adrenal weight was greater in SI conditions than in animals kept in groups, but only in females. This directly indicates that SI lasting up to nine months has a powerful stressor action, the response to which is very pronounced in female rats.

Changes in behavior induced by experimental chronic SI stress also include changes in social behavior [Mumtaz et al., 2018]. Rats develop impairments to sociability and social memory [Arakawa, 2018; Tanaka et al., 2019]. Our studies have shown that SI lasting 2–3 months provokes increases in social contacts in male Wistar rats [Krupina et al., 2015; Khlebnikova et al., 2018]. Ferdman et al. [2007] studied rats of this same strain and found an increase in social contacts in male rats but not in female rats after 10–11 weeks of SI. However, there are as yet few data on the effects of long-term SI on social interaction of rats, especially females, in different models involving direct social contact of animals or assessment of social preference. Social interaction has age-specific characteristics at different stages of ontogeny [Einon and Morgan, 1977; Arakawa, 2003, 2018]; as a result, the effects of SI depend on the stage of development and the duration of isolation of the subject. Changes in social behavior can be used to assess not only the effects of stress as such, but also adaptation to the effects of stress when repeated multiple times [Agrawal et al., 2011].

As far as we know, there are as yet no data on how long-term SI starting in adolescence and lasting continuously into adulthood affects social behavior in female rats at different time points of isolation. The aim of the present study was to assess sociability and preference for "social novelty" in parallel with assessment of levels of anxiety and motor activity in different tests in female rats at different periods of long-term social isolation starting in early postnatal ontogeny and lasting up to 8.5 months.

Methods. Studies used female Wistar rats born and raised in the breeding facility of the Science Research Institute of General Pathology and Pathophysiology (Mercury system, registration number: RU 1487336). Starting from birth and continuing throughout the study, animals were kept in standard animal house conditions with natural light changes and free access to water and food (Laboratorkorm, Russia). All procedures and experiments on animals were carried out in compliance with the *Rules for Good Laboratory Practice*, approved by Order No. 199n of the Ministry of Health of the Russian Federation of April 1, 2016, state standard GOST 33215-2014, 33216-2014 *Guidelines for the Maintenance and Care of Laboratory Animals*, and Directive 2010/63/EU of September 22, 2010, on the protection of animals used for scientific purposes. Studies were carried out under the supervision of the Ethics Committee of the Science Research Institute of General Pathology and Pathophysiology.

Modeling of social isolation. Two series of studies lasting several months were run consecutively for two years

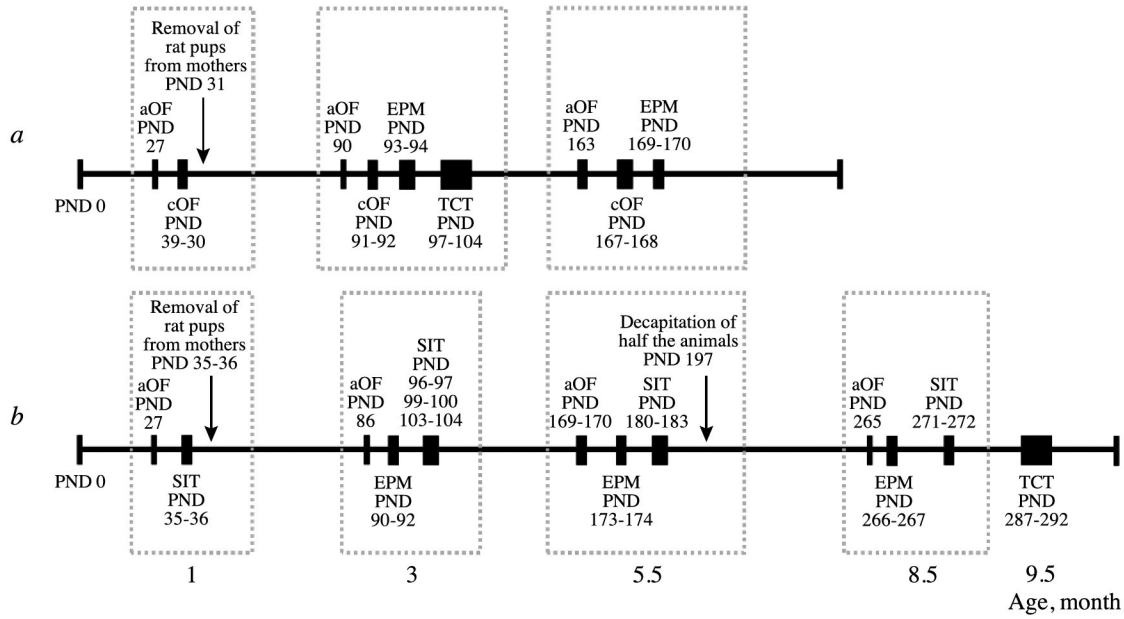

Fig. 1. Experimental design. *a*) Series 1; *b*) series 2. Postnatal day 0 (PND 0) is the day on which the animals were born; aOF – automated open field test; cOF – classical open field test, EPM – elevated plus maze test; TCT – three-chamber social test; SIT – social interaction test. The day the rat pups were weaned from their mothers coincided with the day of the beginning of social isolation in rats of the experimental group.

using a similar scheme. The study design is shown in Fig. 1. Rat pups were born at the same time of the year, i.e., late December–early January; thus, all subsequent assessments of rat behavior at each time point in both series were obtained during the same season. The rats' day of birth was taken as postnatal day (PND) zero. On PND 1, each female was left with 5–7 pups from different litters for feeding in order to reduce the influence of the genetic factor and the level of maternal care on the behavior of the offspring. Rats were weaned from mothers on PND 31 in series 1 and PND 35-36 in series 2, corresponding to adolescence [Lukkes et al., 2009; McCormick and Green, 2013]. The difference in weaning times was due to some differences in the set of behavioral tests in the two series. Rats aged one month underwent primary assessment of motor activity in an automated open field test (the test is described below) and, taking into account the weight of the animals, control and experimental groups of rats were formed in such a way that the groups were no different in terms of this indicator. Groups in series 1 were Control group C1 (n = 30) and social isolation group SI1 (n = 31); groups in series 2 were Control group C2 (n = 31) and social isolation group SI2 (n = 30). Starting from the day of weaning from mothers and continuing throughout the experiment, animals of the control groups were kept in groups of 4–5 individuals in cages made of opaque polypropylene of size 57.0 × 37.0 × 19.0 cm, while animals of the experimental groups were kept singly in cages of size 36.5 × 20.5 × 14.0 cm until the animals reached age 5.5 months in series 1 and 9.5 months in series 2. Rats of the control and experimental groups in both series were kept in the same conditions throughout the experiment, in the same room in the animal house, which also contained cages with males. Rats kept alone were able to perceive olfactory, auditory and, to a lesser extent, visual signals from other rats, but were completely deprived of tactile interaction, i.e., the possibility of direct social contact. In addition, these rats were kept in lower-height cages than the group animals, which could to some extent restrict their motor activity. The SI regime in rats of the experimental groups was disturbed when the cages were cleaned, twice a week, and during planned testing. Rats of all groups were weighed once a week during the test periods.

By the end of the experiments, the durations of SI were 4.5 months in series 1 and 8.5 months in series 2. In series 2, when the animals reached age six months and after the behavioral studies were complete, half the rats of the experimental and control groups were decapitated using a guillotine, and samples of brain structures were taken for subsequent biochemical analysis (the data will be presented in another report). Thus, at the last stage of the behavioral study of rats at age 9.5 months, the C2 group contained 14 animals and the SI2 group contained 16 animals.

Assessment of motor and exploratory activity. *Automated open field test*. An automated open field (aOF) with transparent walls (arena of size 48 × 48 × 21 cm) was used to assess the horizontal (motor, distance traveled, cm) and vertical (exploratory, number of rearings) activity of rats for periods of 10 min under soft room lighting (17 lx); Opto-Varimex system software (Columbus Instruments, USA) was used. After each animal had been tested in this and all other (see below) tests, the experimental chamber was wiped with 70% alcohol and dried with a towel.

In series 1, motor activity in this test was assessed in rats at ages 1, 3, and 5.5 months; in series 2, testing was at ages 1, 3, 5.5, and 8.5 months.

Assessment of anxiety-like behavior. *Classical open field (cOF) test*. Decreases in the preference for the center of the OF are known to characterize increases in anxiety in rodents [Mohammad et al., 2016]. Testing was carried out in the conditions of the classical open field (a round white arena 120 cm in diameter, divided into 20 squares of side 20 cm and surrounded by opaque walls 28 cm high) under bright illumination (500–510 lx at the center, 400–410 lx at the periphery of the OF). The inner central circular zone of diameter 28.3 cm was taken as the center of the field and the outer zone, adjacent to the OF wall, accounting for 20% of the radius of the OF, i.e., 12 cm, was taken as the edge zone. Total duration was 5 min. Along with assessment of motor activity in terms of the number of squares crossed and exploratory activity in terms of the number of vertical rearings, the latent period of entry into the edge zone of the OF (sec) was assessed, along with the time spent in the edge zone (sec) and the time spent in the center of the OF minus the latent period of exit from the center after the rat was placed in the OF (henceforth the time spent in the center). This method was used to assess anxiety levels in rats aged 1, 3, and 5.5 months in series 1 only.

Elevated plus maze test (EPM). A standard approach was used to assess anxiety in the EPM [Pellow et al., 1985] using a modern protocol [Ari et al., 2019] and the equipment and software of the VideoMot2 video system (TSE System, Germany). The dimensions and structure of the maze, which had two open arms (OA) and 2 closed arms (CA), are described in detail in [Krupina et al., 2015]. Illumination over the central zone of the EPM was 24 lx. The rat was placed in the center of the maze with its head towards one of the OA. The durations of the animals' stays in the OA and CA were determined, and the preference for the OA was calculated as the ratio of the duration of the rat's stays in the OA to the total duration of the stay in the EPM arms; the total distance traveled in the EPM (cm) was used to assess the animals' motor activity; mean movement speed (cm/sec) was also evaluated, along with the numbers and durations of vertical rearings and head dips from the OA. Test duration was 5 min. In series 1, anxiety levels were assessed in the EPM in rats at ages 3 and 5.5 months; in series 2, assessments were at ages 3, 5.5, and 8.5 months.

Assessment of social behavior. Two types of test were used to assess the rats' social behavior: (1) a three-chamber social test (TCT), which assessed the rats' sociability, i.e., the choice between an unfamiliar social object (a conspecific in conditions of restricted motor activity) and a non-social object, and preference for social novelty, i.e., the choice between familiar and unfamiliar conspecifics; (2) a social interaction test (SIT), which assessed the social contacts of rats with conspecifics, with both in conditions of free behavior.

Three-chamber social test. Tests were run in an apparatus consisting of a gray plastic box of size 120 × 80 cm, divided by two partitions with doors into three equal chamber parts (40 × 40 × 80 cm). For adaptation, 24 h before testing, rats of the experimental and control groups were placed one at a time in the empty three-chamber box and were allowed to explore it for 5 min. Intact rats of the same sex and age used for testing as social objects and not included in the experimental groups were also adapted to the environment 24 h before testing by placing them in a cylindrical cage (20 cm in diameter and 30 cm high) for 5 min. The walls of the cylinder consisted of metal rods. The next day, during the experiment, the central chamber was left empty, cylinder cages were placed in the two outer chambers, one cylinder containing a rat as a social object (these rats were kept in another animal-house room throughout the study), the second cylinder being left empty. The test rat was placed in the central chamber for 1 min to adapt, and the doors were then opened and the test rat was allowed to explore all three chambers for 10 min (Step 1). After 10 min, the doors were closed again and the experimental rat was returned to the central chamber. At this time, a second rat (a new, unfamiliar social object) was placed in the second, previously empty, cylinder. The doors were again opened and the experimental rat was allowed to explore all three chambers for 10 min (Step 2). At each step, the time spent by the test rat in each chamber with objects and the number of visits to these chambers were evaluated; the times spent by the rat at each of the objects and the numbers of approaches to the objects were recorded. Approaches to the object were taken as approaches of the rat's nose to another rat in the cylinder or to an empty cylinder, i.e., active examination of the object, at a distance of no more than 2 cm from the metal bars of the cylinders.

Sociability at test Stage 1 was assessed in terms of the preference for a social chamber and a social object using the formulas presented by [McKibben et al., 2014]:

(1) [(time spent in chamber with a social object) – (time spent in chamber with an empty cylinder)]/(total time spent in these two chambers) × 100%;

(2) [(time spent near the social object) – (time spent near the empty cylinder)]/(total time spent near the two objects) × 100%.

Similarly, indicators of social novelty were calculated at Stage 2 of testing: in terms of preference for a chamber containing a novel social object (an unfamiliar rat) and the new social object itself over an already familiar social object.

The three-chamber social test in series 1 was performed in rats at age three months (the duration of SI in rats of the experimental group was two months), while in series 2, the test was performed in rats at age 9.5 months (the duration of SI in rats of the experimental group was 8.5 months).

Social interaction test. Social interaction was assessed in a plexiglass cage (37.0 × 57.0 × 19.0 cm) which was unfamiliar to the rats, in an experimental room under red light: illumination above the center of the cage was 7 lx.

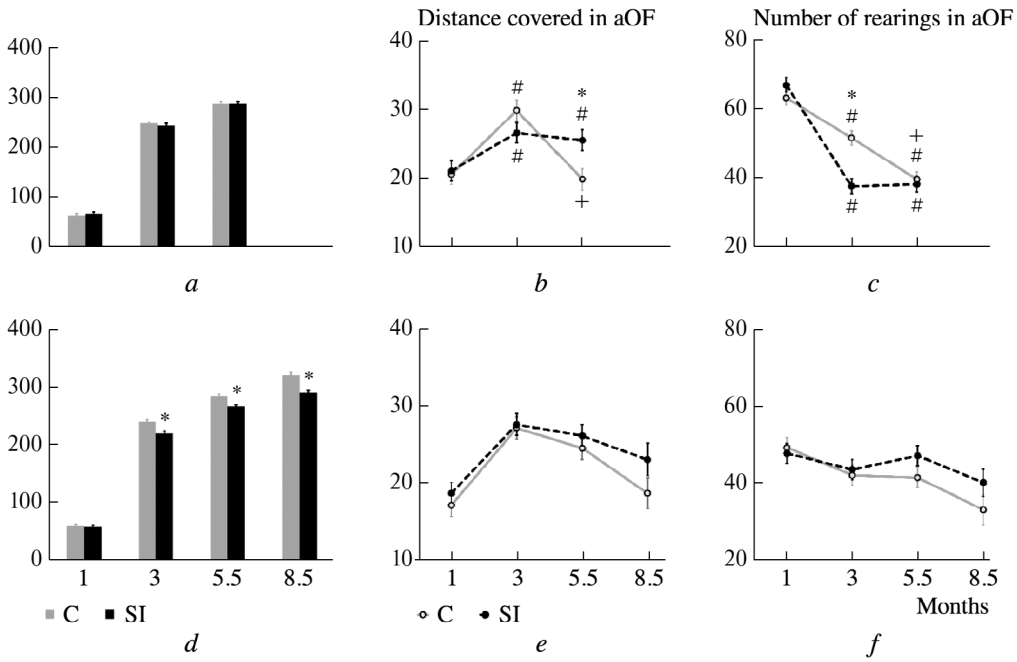

Fig. 2. Weight and motor (horizontal) and exploratory (vertical) activity in rats kept in conditions of social isolation (SI) (dotted line) compared with control rats (C) kept in groups (solid line) in the automated open field test in two series of studies. The vertical axis shows: *a, d*) weight, g, in series 1 and series 2 respectively; *b*, *e*) distance covered in 10 min of observation, cm, in series 1 and series 2 respectively; *c*, *f*) numbers of rearings for 10 min of observation in series 1 and series 2 respectively. The horizontal axis shows rats' age. $^{\#}p < 0.05$ compared with rats of the same group at age one month; $^{+}p < 0.05$ compared with rats of the same group at age three months; $^{*}p < 0.05$ compared with the control group of rats at the same age (two-way analysis with repeat measures, post hoc Newman–Keuls test).

The method of Schneider and Przewłocki [2004] with minor modifications was used to compare the behavior of pairs of rats formed from animals of either the experimental or control group, i.e., the unit for data collection was a pair of rats [Hermes et al., 2011; Campos et al., 2013]. Pairs were selected in such a way that animals from the same group had not met each other earlier in each test period and the difference between their weights was no greater than 15%. Test duration was 15 min. The durations of active non-aggressive social contacts were assessed: sniffing, social grooming (licking), crawling under or climbing onto a partner, following not ending in aggression, and aggressive contacts, i.e., following turning into aggressive interactions, attacks/fights, biting, and aggressive grooming (biting causing the partner to vocalize) during testing.

This method was used to evaluate social interaction in rats aged 1, 3, 6, and nine months in series 2 only.

Statistical data processing was carried out in Statistica for Windows 12.0 after prior testing of the hypothesis that distributions were normal using the Kolmogorov–Smirnov and Lilliefors tests. If this hypothesis was not rejected, parametric methods of analysis were used; if the hypothesis was rejected, nonparametric methods were used. Taking account of the fact that in series 2, the last observation time point (at age 9.5 months) included half as many rats in groups as at other stages, while in series 1, the final observation stage also included, for a number of reasons, a smaller number of rats than at the first stage of observation, parametric two-way ANOVA without repeat measures was used to assess the dynamics of the animals' behavior during the experiment. The effects of the Housing (two levels: isolation and control in series 1 and 2) and Age (three levels: 1, 3, and 5.5 months in the aOF test and two levels: 3 and 5.5 months in the EPM test in series 1; four levels: 1, 3, 5.5, and 8.5 months in the aOF test and three levels: 3, 5.5, and 8.5 months in the EPM test in series 2) factors were evaluated. Post hoc analysis was run using the Newman–Keuls test. Data from two independent groups of rats were compared using the nonparametric two-tailed Mann–Whitney U test for independent variables; within-group comparisons at different time points were made using nonparametric Kruskal–Wallis analysis of variance (ANOVA) followed by multiple comparison of mean ranks. In the social interactions test, the proportion of pairs of rats in which at least one of the animals exhibited aggressive behavior was assessed using Fisher's exact test (FET, two-sided test). The significance level was taken as 5%. Data tested using parametric criteria are presented as $M \pm SEM$; data tested using non-parametric criteria are presented as the median with first and third quartiles.

Results. ***Body weight.*** In series 1, only the Age factor was found to influence the rats' body weight: $F(2,161) = 1350.5$; $p < 0.001$. Post hoc analysis showed that increasing age of rats was associated with increased body weight (increases in weight at each next test period compared with the previous period in the experimental and control groups were statistically significant; $p < 0.002$) (Fig. 2, *a*).

TABLE 1. Indicators of Anxiety-Like Behavior in the Classical Open Field Test in Series 1 in Rats Kept in Social Isolation (SI) Compared with Rats Kept in Groups (C). Group Designations Indicate Series Number

Behavioral indicator	Age 1 month		Age 3 months		Age 5.5 months	
	C1 (n = 31)	SI1 (n = 30)	C1 (n = 29)	SI1 (n = 30)	C1 (n = 27)	SI1 (n = 30)
Time spent in center of OF (sec)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	6.0• (0.0; 11.0)	3.0• (0.0; 7.0)	0.0• (0.0; 9.0)	0.0 (0.0; 4.0)
Time spent in edge zone of OF (sec)	290.5 (287.0; 294.0)	292.0 (290.0; 296.0)	266.0• (247.0; 275.0)	276.0• (259.0; 286.0)	280.0• (261.0; 289.0)	288.0*• (279.0; 295.0)
Latent period of entry into edge zone of OF (sec)	6.0 (4.0; 8.0)	4.0 (3.0; 6.0)	10.0• (6.0; 12.0)	10.0• (6.0; 15.0)	8.0 (5.0; 14.0)	5.0* (4.0; 8.0)

Data are presented as medians with the first and third quartiles. *$p < 0.05$ compared with age-matched group C1 (Mann–Whitney U-test); •$p < 0.05$ compared with rats of the same group at age one month (Kruskal–Wallis ANOVA followed by multiple comparison of mean ranks); n is the number of rats in the group.

In series 2, rats' body weight was influenced by both the Age ($F(1,204) = 38.548$; $p < 0.001$) and Housing ($F(3,204) = 1662.205$; $p < 0.001$) factors, as well as their interaction: $F(3,204) = 4.388$; $p = 0.005$. As in series 1, increases in weight at each next test period compared with the previous period in the experimental and control groups were statistically significant ($p < 0.001$), though starting from age three months, the weight of rats under SI conditions was less than the weight of control rats (Fig. 2, *d*), that is, SI led to a decrease in the weight of animals.

Motor and exploratory activity. *Automated open field test. Series 1.* Two-way ANOVA showed that the Age factor influenced the total locomotor activity of rats over the 10-min test period: $F(2,170) = 13.183$; $p < 0.001$; post hoc analysis using the Newman–Keuls test showed that the motor activity of rats at age three months was higher than at age one month and was greater than in animals at age 5.5 months (in both cases, $p < 0.001$). The Housing factor had no effect, though the interaction of the Age and Housing factors did: $F(2,170) = 4.297$; $p = 0.015$. At age 5.5 months, motor activity of rats kept in isolation was still statistically significantly higher than in this group of rats at age one month and was greater than in rats of the control group. In rats of the C1 group at age 5.5 months, motor activity decreased to baseline at age one month (Fig. 2, *b*).

The Housing and Age factors influenced vertical activity in female rats: $F(1,170) = 5.012$; $p = 0.026$ and $F(2,170) = 83.087$; $p < 0.001$ respectively. Vertical exploratory activity was lower in females subjected to SI compared with animals and decreased with age, regardless of keeping conditions. An Age × Housing interaction was found: $F(2,170) = 9.183$; $p < 0.001$. In rats of the SI1 group at age three months (after two months of isolation), the decrease in the number of rearings compared with that before SI at age one month was greater than that in the C1 group, which led to a statistically significant difference between the SI1 and C1 groups at age three months (Fig. 2, *c*).

Series 2. Two-way ANOVA revealed a statistically significant effect of the Age factor on the level of motor activity in rats: $F(3,204) = 16.914$; $p < 0.001$; post hoc analysis showed that motor activity was greater at ages three and 5.5 months than at age one month and was greater than in rats at age 8.5 months (in all cases, $p < 0.001$) (Fig. 2, *e*). The effect of the Housing factor did not reach the level of statistical significance: $F(1,204) = 3.041$; $p = 0.083$. No interaction between these factors was found.

Two-way ANOVA showed that the Age factor influenced vertical exploratory activity in rats: $F(3,203) = 4.771$; $p = 0.003$. At age 8.5 months, this was lower than at ages one, three, and 5.5 months: $p < 0.001$, $p = 0.042$, $p = 0.030$ respectively (Fig. 2, *f*). The Housing factor and the Age × Housing interaction had no effect.

Thus, the aOF test revealed the effect of age on the levels of motor and exploratory activity in rats in both series. Keeping conditions had no direct effect on the level of motor activity but could alter the levels of motor and exploratory activity of rats depending on age.

Anxiety-like behavior. *Evaluation of anxiety levels in the classic open field. Series 1.* No differences were found between rats of the experimental and control groups in terms of locomotor and exploratory activity at any of the test periods ($p > 0.05$ for all comparisons).

Indicators of anxiety levels in the cOF in rats kept in isolation and in groups are shown in Table 1.

Statistically significant between-group differences in indicators were revealed only at age 5.5 months: rats kept in SI conditions spent more time in the edge zone of the OF and quickly entered the edge zone.

Within-group comparisons revealed similar changes in the experimental and control groups of rats: compared with age one month, the latent period of entry into the edge zone of the OF at age three months was increased [SI1: $[H(2, N = 87) = 22.830, p < 0.001$; C1: $H(2, N = 90) = 8.571; p = 0.014]$, as was the time spent in the center of the OF [SI1: $H(2, N = 87) = 12.393, p = 0.002$; C1: $H(2, N = 90) = 21.759; p = < 0.001]$. The time spent in the edge zone by rats of both groups at age three months, conversely, was less than that at age one month [SI1: $H(2, N = 87) = 33.058, p < 0.001$; C1: $H(2, N = 90) = 28.913, p < 0.001]$.

Thus, one of the main indicators characterizing the anxiety level in this test indicated that the time spent in the central zone of the OF by rats of the SI1 group at age three

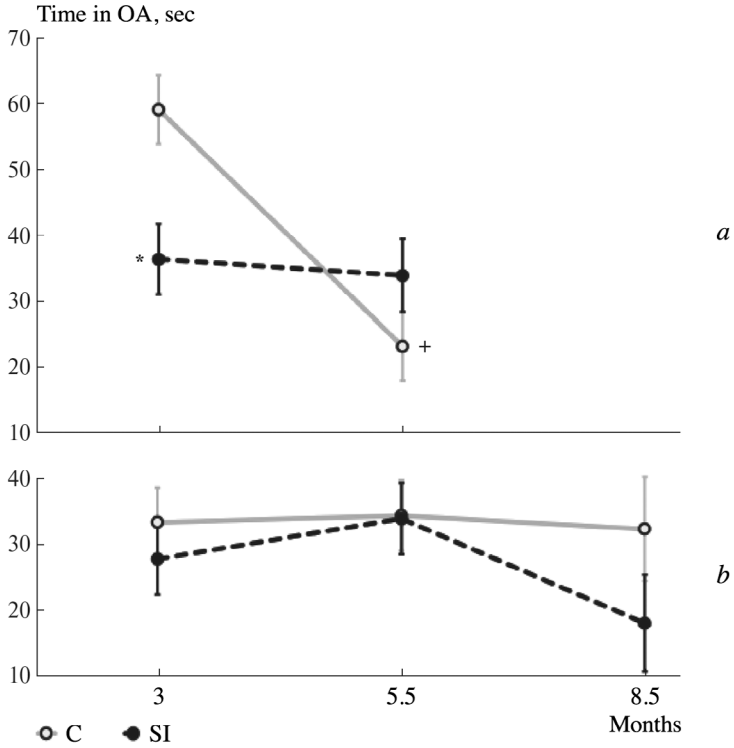

Fig. 3. Durations of stays in the open arms of rats kept in conditions of social isolation (SI) (dotted line) compared with control rats (C) kept in groups (solid line) in the elevated plus maze test in two series of studies. *a*, *b*) Series 1 and series 2 respectively: the vertical axis shows time, sec; the horizontal axis shows rats' age. *$p < 0.05$ compared with the control group of rats aged three months; $^{+}p < 0.05$ compared with rats of the same group at age three months (two-way analysis with repeat measures, post hoc Newman–Keuls test).

months did not differ from that of rats in the C1 group; however, at age 5.5 months, rats kept in SI conditions showed signs of greater avoidance of open space than control rats.

Elevated plus maze test (EPM). Series 1. Two-way ANOVA demonstrated an effect for the Age factor on the length of stays of rats in the OA [$F(1,112) = 12.894$; $p < 0.001$], along with an interaction between the Age and Housing factors [$F(1,112) = 9.797$; $p = 0.002$] (Fig. 3, *a*). As compared with age three months, the duration of stays in the OA in rats aged 5.5 months decreased: reductions occurred only in rats of the control group. At age three months, rats of the SI1 group spent less time in the OA compared with those of the C1 group.

The Age factor influenced the preference for OA in the EPM (two-way ANOVA, $F(1,112) = 9.774$; $p = 0.002$) and there was an Age × Housing interaction ($F(1,112) = 8.574$; $p = 0.004$). A decrease in OA preference with age was found only in rats kept in groups, while OA preference remained at a low level at both test periods in rats kept in SI conditions; at age three months, the preference for OA was lower in rats kept in isolation than that in control rats. These results indicate that in series 1, anxiety indicators were higher in three-month-old rats subjected to two months of SI than in rats in the control group.

In series 1, the Age and Housing factors had no effect on the total distance covered or the average speed of movement of rats in the EPM; these factors did not interact ($p > 0.05$ in all cases).

Between-group analysis using the Mann–Whitney test showed that the number of head dips was smaller and the duration of head-dipsfrom the OA of the EPM was shorter in rats of the SI1 group than those of the C1 group at age three months (Table 2).

Kruskal–Wallis ANOVA showed a decrease with age in the number of head dips from the OA only in the C1 group [$H(1, N = 60) = 24.405$; $p < 0.001$], with a decrease in their duration [$H(1, N = 60) = 18.618$; $p < 0.001$].

Series 2. The Age and Housing factors had no effect on the length of stays of rats in the OA (Fig. 3, *b*) or on the preference for the OA; no interaction of factors was found ($p > 0.05$ in all cases).

These factors also had no influence on the total distance covered or the average speed of movement of rats in the EPM, and no interaction of factors was found ($p > 0.05$ in all cases).

Between-group analysis using the Mann–Whitney test showed that the numbers of rearings and head dips were smaller and the duration of head dips was shorter in the EPM in rats of the SI2 group at age three months than in the C2 group (see Table 2).

Only the C2 group showed a decrease in the number of rearings in the EPM with age [$H(2, N = 75) = 14.488$;

TABLE 2. Indicators of Anxiety-Like Behavior in the Elevated Plus Maze Test in Rats Kept in Social Isolation (SI) Compared with Rats Kept in Groups (C) in Two Series. Group Designations Indicate Series Number

Behavioral indicators	Number of rearings	Duration of rearings, sec	Number of head dips from OA	Duration of head dips from OA
Age 3 months	Series 1			
C1	11.0 (8.0; 14.0)	29.4 (16.0; 36.4)	10.0 (5.0; 14.0)	15.4 (6.8; 23.1)
SI1	11.0 (8.0; 13.0)	24.5 (19.2; 41.1)	6.0* (3.0; 9.0)	7.5* (2.7; 11.5)
Age 5.5 months	Series 1			
C1	10.0 (7.0; 14.0)	31.7 (19.8; 28.7)	1.5•• (0.0; 6.0)	1.5•• (0.0; 8.2)
SI1	8.0 (6.0; 11.0)	29.2 (22.1; 37.6)	3.0 (0.0; 7.0)	4.8 (0.0; 14.9)
Age 3 months	Series 2			
C2	13.0 (10.0; 17.0)	27.5 (20.0; 34.5)	5.0 (2.0; 10.0)	5.6 (1.8; 13.9)
SI2	10.0* (8.0; 14.0)	16.5# (13.6; 33.7)	3.0* (0.0; 6.0)	3.4* (0.0; 6.4)
Age 5.5 months	Series 2			
C2	9.0• (7.0; 13.0)	27.9 (17.8; 40.5)	3.5 (2.0; 6.0)	5.7 (1.8; 8.7)
SI2	9.5 (7.0; 14.0)	27.7 (18.4; 32.9)	3.5 (0.0; 7.0)	5.4 (0.0; 16.9)
Age 8.5 months	Series 2			
C2	8.0• (5.0; 11.0)	30.8 (19.8; 46.7)	3.0 (0.0; 6.0)	11.4 (3.0; 17.8)
SI2	8.0 (5.0; 10.5)	24.8 (15.3; 32.2)	1.5 (0.0; 3.5)	1.6 (0.0; 10.9)

Data are presented as medians with the first and third quartiles. *$p < 0.05$; #$p < 0.08$ compared with age-matched control group (Mann–Whitney U-test); ••$p < 0.001$; •$p < 0.05$ compared with rats of the same group at age three months (Kruskal–Wallis ANOVA followed by multiple comparison of mean ranks). Series 1, age three months: SI1, $n = 29$; C1, $n = 30$; age 5.5 months: SI1, $n = 27$; C1, $n = 30$. Series 2, age three months: SI2, $n = 30$; C2, $n = 31$; age 5.5 months: SI2, $n = 30$; C2, $n = 30$; age 8.5 months: SI2, $n = 16$; C2, $n = 14$, where n is the number of rats in the groups.

$p < 0.001$]: this was reduced in rats aged five and 8.5 months, as compared with rats aged three months.

Thus, indicators of the activity of rats in the OA in the EPM test were not directly affected by keeping conditions in either series, though changes could be produced in control rats depending on age. In both series, the number and duration of exploratory risk assessments (head dips) were reduced in rats after two months of SI. However, SI for up to 7.5 months did not alter the rats' vertical exploratory activity. Neither age nor keeping conditions had any effect on the animals' motor activity.

Social behavior. *Three-chamber social test. Series 1.* In this series, at Stage 1, after two months of SI, the time spent in the chamber with the social object (a rat) and the time spent near the social object were increased in rats compared with controls; the time spent near the empty cylinder was reduced (Fig. 4). The increase in preference for the chamber with a social object to the chamber with a non-social object in rats of the SI1 group compared with rats of the C1 group did not reach the level of statistical significance, while the preference for a social object over a non-social one was statistically significant (Table 3).

At Stage 2, rats in the SI1 group, as compared with control animals, showed an increase in the time spent near the familiar rat, along with an increase in the number of approaches to the unfamiliar rat (see Fig. 4). There was no statistically significant change in preference for the chamber with an unfamiliar rat over the chamber with a familiar rat, but there was a marked trend towards a decrease in preference for the novel social object over an already familiar social object (see Table 3).

Series 2. At Stage 1 in this series, rats that were in social isolation for 8.5 months made a smaller number of visits to the chamber with a social object (a rat) than rats in the control group and the time spent near the rat was shorter (Fig. 5). However, the reductions in preference for the chamber with a social object over the chamber with a non-social object and for a social object over a non-social object were not statistically significant (see Table 3).

At Stage 2, rats of the SI2 group, as compared with rats of the C2 group, spent more time in the chamber with a familiar rat and less time in the chamber with an unfamiliar rat (a novel social object). The number of approaches to a familiar rat in isolated animals was also increased from that in controls (see Fig. 5). Rats of the SI2 group showed a tendency to an increase in the duration of approaches to a familiar rat compared with rats of the control group: 63.0 (16.0; 122.0) sec and 33.0 (9.0; 42.5) sec respectively, $p = 0.069$, Mann–Whitney test. After 8.5 months of SI, the preference for the chamber with an unfamiliar rat over the chamber with

TABLE 3. Measures of Sociability and Social Novelty in the Three-Chamber Social Test in Socially Isolated Rats (SI) Compared with Rats Kept in Groups (C) in Two Series of Studies. Group Designations Indicate Series Number

Indicators	Age 3 monthsSeries 1		Age 9.5 monthsSeries 2	
	C1	SI1	C2	SI2
Stage 1. Preference for chamber containing social object (rat), %	23.0 (–7.0; 38.9) (n = 28)	45.9$^{\#}$ (17.7; 62.8) (n = 26)	56.3 (–0.3; 70.2) (n = 11)	38.0 (–6.1; 62.5) (n = 12)
Stage 1. Preference for social object (rat), %	45.3 (12.5; 67.1) (n = 28)	73.1* (55.1; 80.0) (n = 26)	59.2 (31.3; 68.8) (n = 11)	38.3 (0.9; 60.2) (n = 12)
Stage 2. Preference for chamber containing novel social object (unknown rat), %	28.7 (–15.0; 56.0) (n = 24)	12.0 (–23.7; 61.3) (n = 26)	24.5 (–14.2; 74.1) (n = 8)	–31.0* (–59.0; –18.4) (n = 12)
Stage 2. Preference for novel social object (unknown rat), %	51.5 (–16.7; 82.6) (n = 24)	25.5$^{\#}$ (–17.5; 66.7) (n = 26)	54.9 (–11.7; 80.9) (n = 8)	–18.1* (–37.9; 38.3) (n = 12)

Data are presented as medians with the first and third quartiles. *$p < 0.05$, $^{\#}p < 0.083$ – compared with age-matched control group (Mann–Whitney U-test); n is the number of rats in the group.

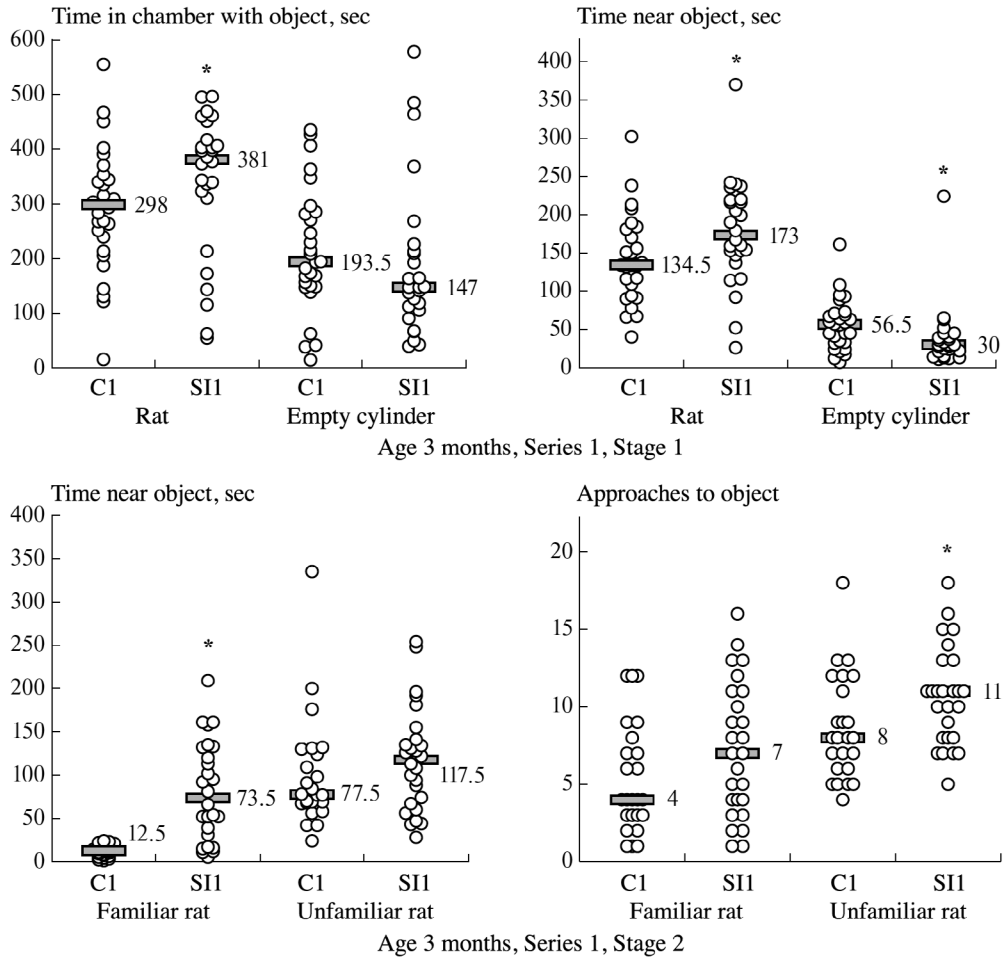

Fig. 4. Behavioral parameters in the three-chamber social test (series 1) in rats kept in conditions of social isolation (SI) for two months compared with control rats kept in groups (C). Group designations indicate series number. Horizontal axes show: "Rat" – chamber containing a social object; "Empty cylinder" – chamber containing a non-social object; "Familiar rat" – a chamber with a rat with which there had been contact at Stage 1; "Unfamiliar rat" – chamber with a new rat. Numbers of animals in groups at Stage 1: C1 group n = 28, SI1 group n = 26; at Stage 2: C1 group n = 24; SI1 group n = 26. Gray rectangles show the median; median values are indicated by numbers alongside. *$p < 0.05$ compared with group C1 for the corresponding parameter in the same chamber (unpaired nonparametric Mann–Whitney test).

a familiar rat and the preference for a novel social object over a familiar social object in rats were reduced compared with animals of the control group (Table 3).

Thus, the results obtained by testing rats in the TCT showed that the sequelae of SI of different durations on sociability and the preference for social novelty in rats were

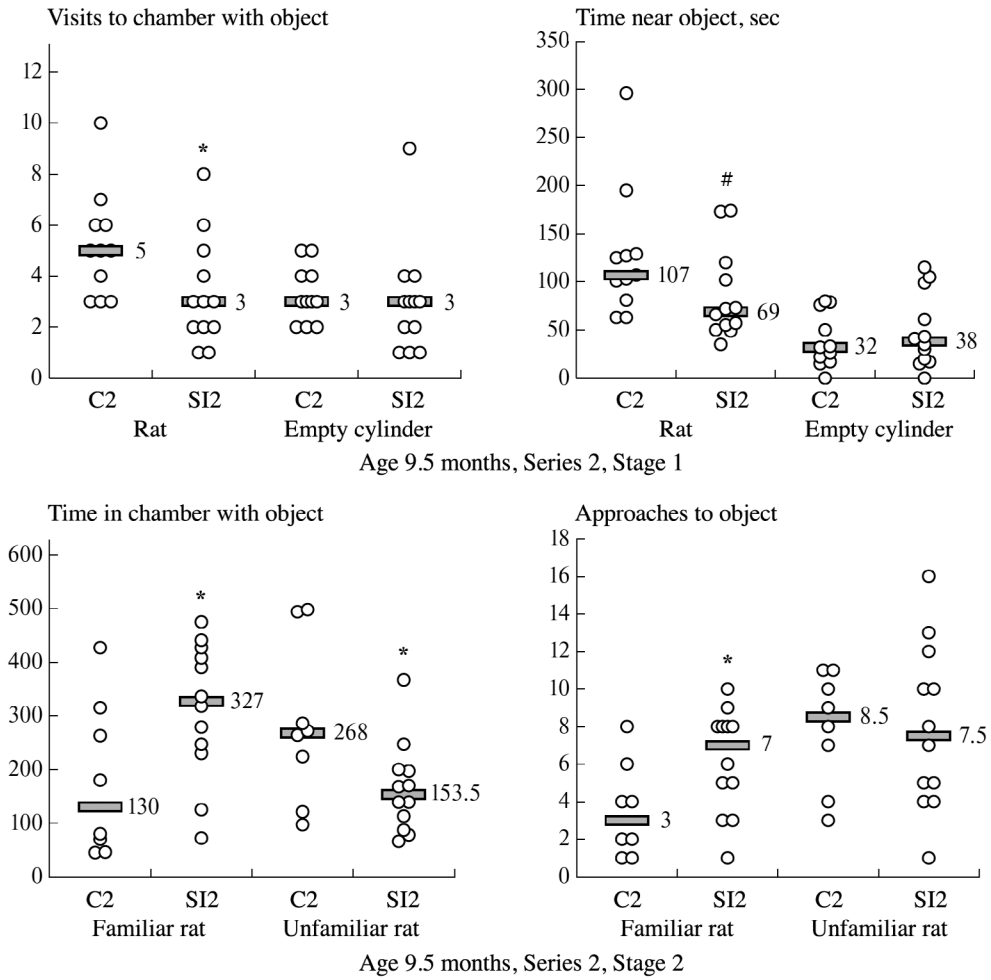

Fig. 5. Behavioral parameters in the three-chamber social test (series 2) in rats kept in conditions of social isolation (SI) for 8.5 months compared with control rats kept in groups (C). Group designations indicate series number. Horizontal axes show: "Rat" – chamber containing a social object; "Empty cylinder" – chamber containing a non-social object; "Familiar rat" – a chamber with a rat with which there had been contact at Stage 1; "Unfamiliar rat" – chamber with a new rat. Numbers of animals in groups at Stage 1: C2 group $n = 11$, SI2 group $n = 12$; at Stage 2: C2 group $n = 8$; SI2 group $n = 12$. Gray rectangles show the median; median values are indicated by numbers alongside. $^{*}p < 0.05$, $^{\#}p = 0.051$ compared with group C2 for the corresponding parameter in the same chamber (unpaired nonparametric Mann–Whitney test).

different. After two months of SI, the preference for a social object over a nonsocial object in rats was significantly higher than the control level (by more than 60%, see Table 3; Stage 1). After 8.5 months of isolation, the sociability of the rats of the experimental group in terms of this indicator was no different from that of animals in the control group, though there was a slight decrease (by about 35%, see Table 3). After two months of SI, rats showed only a tendency to a decrease in the preference for social novelty, while after 8.5 months of isolation, the decrease in the preference for social novelty was very marked.

Social interaction test. Series 2. Between-group comparison did not reveal any differences in the durations of non-aggressive and aggressive interactions in rats of the experimental and control groups at age one month. However, at age three months, the durations of both non-aggressive and aggressive social contacts in rats of the SI2 group were greater than those in rats of the C2 group (Fig. 6), so two months of SI led to increased social interactions of rats, independently of the type of these interactions.

At age six months – after five months of SI – rats retained signs of increased social interaction as compared with control rats. At age nine months, no statistically significant differences were found between rats of the SI2 and C2 groups in terms of the durations of either non-aggressive or aggressive social interactions.

Within-group nonparametric analysis of Kruskal–Wallis ANOVA revealed no changes in the duration of nonaggressive social interactions in the control group of rats over the observation period ($p > 0.05$). Aggressive interactions in adult rats kept in groups decreased to an almost complete absence by age three months as compared with age one month and did not increase further [$H(3, N = 52) = 20.753$; $p < 0.001$; as shown by post hoc analysis results, $p < 0.02$].

Rats in the SI2 group at ages three and six months, as compared with age one month, showed statistically sig-

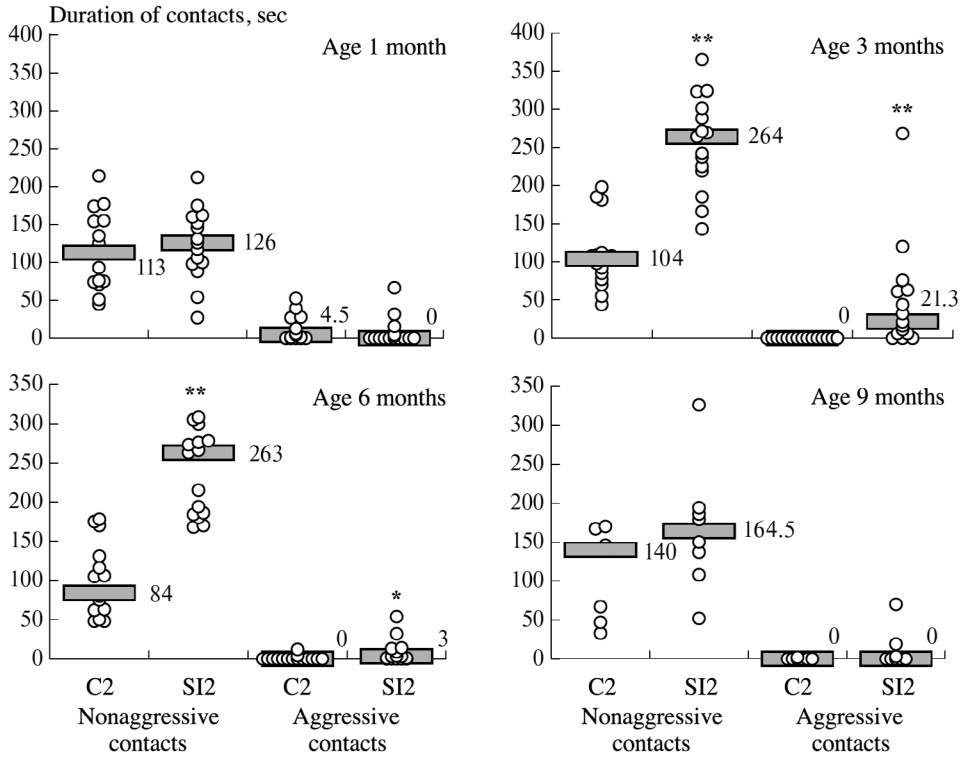

Fig. 6. Duration of social contacts in the social interaction test (series 2) in rats kept in conditions of social isolation (SI) compared with control rats kept in groups (C). Group designations indicate the series number. The vertical axis shows the duration of contacts, sec. At ages one, three, and six months, the numbers of pairs in all groups were $n = 15$; at age nine months the C2 group contained $n = 7$ and the SI2 group contained $n = 8$. Gray rectangles show the median; median values are indicated by numbers alongside. $^{**}p < 0.001$; $^{*}p < 0.05$ compared with the C2 group for the corresponding parameter at the same observation period (unpaired nonparametric Mann–Whitney test).

nificant increases in the duration of non-aggressive social contacts [H (3, $N = 53$) = 27.039, $p < 0.001$; post hoc analysis gave $p < 0.001$ in both cases]. Rats aged nine months – after eight months of SI – displayed a tendency to maintain an increased duration of non-aggressive social contacts as compared with age one month (post hoc analysis gave $p = 0.096$). The duration of aggressive contacts in rats in the SI2 group exceeded the values at age one month only at age three months [H (3, $N = 53$) = 10.703, $p = 0.013$; post hoc analysis gave $p = 0.020$].

The relative number of pairs in which rats demonstrated aggressive behavior was higher in the SI2 group at ages three and six months (FET, $p < 0.001$ and $p = 0.004$ respectively).

Thus, SI lasting up to at least five months was accompanied by increases in social interactions of both non-aggressive and aggressive nature. Social interactions in rats of the experimental and control groups were no different by eight months of SI.

Discussion. The two series in this study yielded experimental evidence showing that the duration of SI affects social behavior in female rats. The fact of an increase in the preference for a social object in female rats in series 1 in the TCT after two months of SI, i.e., the fact of an increase in the sociability of rats at age three months, is in good agreement with the increase in the duration of social contacts of rats in the SIT at this duration of SI in series 2 (See Fig. 6). The absence of any differences in the duration of non-aggressive social contacts in the SIT in series 2 after eight months of SI in rats of the experimental groups as compared with control rats is also in good agreement with the absence of any statistically significant differences in their sociability in the TCT after 8.5 months of SI. Thus, SI lasting two months increased social preference in female rats, while the longer period of SI – 8 months or more – resulted in a decrease in social preference to the control level, and, according to our data, signs of deficient sociability could even appear.

The increases in social interaction and social preference found in rats at age three months – after two months of SI – cannot be explained by an increase in the animals' motor activity, as the motor activity of isolated females was no different from that of females in the control group in both series at this test time point. The vertical exploratory activity of rats after two months of SI was even lower than that of animals in the control group (in the aOF test in series 1 and in the EPM test in series 2).

It should in particular be noted that behavioral indicators in female Wistar rats of the control groups also changed with age: there were decreases in motor and exploratory activity with age in the aOF test (series 1 and 2) and increases in anxiety levels in the EPM test (series 1). Similar age-related changes in motor, exploratory activity, and, to a lesser extent, anxiety, have been seen in male and female

rats of different strains and populations, including Wistar, throughout the lifetimes of the animals [Altun et al., 2007; Rykova et al., 2011]. Despite the fundamental similarity of the dynamics of motor and exploratory activity in rats of the control groups in the two series reported here, some between-series differences are also clearly visible (Figs. 2 and 3). As noted above, the same indicators were measured in these series in the same seasons (and, as a rule, in the same months) of the year for two consecutive years. With this in mind, we believe that the differences found in these studies may be due primarily to variability in the behavior of the outbred Wistar rat populations used in the present study, rather than the influences of other factors – daily/seasonal light rhythms, etc. This suggestion is supported by, for example, data obtained by Stepanichev et al. [2016]. In this study, male rats from a heterogeneous outbred Wistar population, which were initially divided according to the level of orientational-exploratory behavior in the open field test, showed different responses to exposure to chronic combined stress in terms of anxious-depressive behavior.

It is possible that the heterogeneity of the Wistar population led to differences in body weight dynamics in the rats of the experimental groups in our two series of studies: in series 2, rats of the experimental group weighted less than those of the control group, starting from two months of SI and continuing to the end of the observation period. Numerous assessments of the weight of rats in conditions of shorter SI than that used in the present study have shown that changes in behavior are not accompanied by stable changes in body weight, though the data are contradictory (cited in [Fone and Porkess, 2008; Beery and Kauffer, 2015]). Our previous studies – on male Wistar rats – did not find any changes in body weight after two and three months of SI [Krupina et al., 2015; Khlebnikova et al., 2018]. However, after nine months of SI, male rats, but not female rats, showed a decrease in weight as compared with the weight of control animals [Krupina et al., 2020]. Carnevali et al. [2020] showed that adult females of the Wild-type Croningen strain gained weight more slowly during six weeks of SI than females of the control group. In contrast, female Sprague–Dawley rats weighted more than control rats after three weeks of SI starting in adolescence [Jahng et al., 2012] and after two months of SI starting immediately after weaning [Hermes et al., 2011]. One early study of SI in inbred Wistar rats showed weight loss in both males and females after 13 weeks of SI [Hatch et al., 1965]. The effect of SI on the animals' body weight appears to depend on the strain (or population) of rats, the time of onset of SI, its duration, and, in the case of outbred strains, on the heterogeneity of the cohorts used. In the present study, rats of the experimental groups in both series showed increased in sociability at age three months – after two months of SI – regardless of whether or not their body weight differed from that of rats in the control groups. Weight loss associated with loss of muscle mass begins to influence the behavior of elderly rats reaching age two years [Altun et al., 2007].

Might the increases in sociability in the TCT and the duration of social contacts in the SIT be related to the change in the anxiety level in rats after two months of SI? In series 1, rats at two months of SI spent less time in the OA in the EPM test than rats of the control group (see Fig. 3, *a*), which can be regarded as a sign of a higher anxiety level. As anxiety levels in rats aged one month (before the onset of SI) were not assessed in this test, it cannot be ruled out that rats of the experimental and control groups might have different anxiety levels at baseline. However, in the cOF test, the rats of the experimental and control groups showed no differences in baseline anxiety levels at age one month (see Table 1). Results of assessing anxiety levels in rats in different tests could differ for a number of reasons, primarily due to different anxiogenic contexts [O'Leary et al., 2013; Mohammad et al., 2016]. Nevertheless, we believe that the supposition that there might be an initial difference in the anxiety level in the EPM can be disregarded. It is, however, possible either that the anxiety level in rats of the control group decreased at age three months compared with baseline or that the anxiety level in rats of the experimental group increased at this age, after two months of SI. In series 2, there were no signs of increased anxiety in rats of the SI group in the EPM test. Our previous studies on Wistar males subjected to two months of SI also yielded contradictory data: anxiety levels in the EPM test after two months of SI could increase [Khlebnikova et al., 2018] or remain unaltered [Krupina et al., 2015]. These assessments do not contradict those reported by other authors in terms of changes in anxiety levels in rats under SI conditions. After seven weeks SI, Yildirim et al. [2012] found no changes in anxiety levels in either male or female Wistar rats in the EPM test. The absence of any changes in anxiety in this test after 26 days of SI was reported by Joshi et al. [2017], who used male and female Long Evans rats, while Hellemans et al. [2004], conversely, found an increase in anxiety in male rats of the same strain after seven weeks of SI. Taking the whole of these conflicting data, we believe that our earlier suggestion that the change in the anxiety level in the EPM test in male rats under SI conditions cannot be regarded as a stable characteristic of behavioral disorders [Khlebnikova et al., 2018], is a view which apparently can be extended to female Wistar rats. Thus, the increase in sociability in these animals after two months of SI in two tests assessing social behavior occurred without any obvious connection with the anxiety level. We have previously observed a similar picture in males: the duration of social contacts in the SIT after two months of SI was increased from the control level, regardless of whether or not the anxiety level changed [Krupina et al., 2015; Khlebnikova et al., 2018].

However, after 4.5 months of SI, signs of a change in the behavior of the animals in the edge zone of the OF were found. This zone is regarded as the thigmotaxis zone. Benzodiazepine drugs have been shown to facilitate excursions from the thigmotaxis zone to the center of the OF, and

this effect is associated with the decrease in stress-induced inhibition of exploratory activity in a dangerous unfamiliar environment seen after administration of these substances, but not with their anxiolytic effect as such [Prut and Belzung, 2003]. An increase in thigmotaxis in the stressful environment of an unfamiliar OF was found in Long–Evans rats, regardless of gender, after 26 days of SI [Joshi et al., 2017]. These ideas suggest that in the present study, SI, leading to faster entry of rats into the edge zone of the OF and an increase in the time spent in it, i.e., increasing thigmotaxis, actually enhances stress-induced inhibition of exploratory activity in animals in a dangerous environment. It is likely that SI increases sensitivity to stress in females. This suggestion is in good agreement with previous data from our studies on the high vulnerability of female rats to prolonged SI stress, which was confirmed by, inter alia, the greater time spent by socially isolated rats in the edge zone of the Morris water maze, i.e., the thigmotaxis zone [Krupina et al., 2020].

In both series, the EPM test also showed a decrease in the number and total duration of head dips from the OA of the maze in rats after two months of SI compared with rats of the control groups. Interpretation of the decrease in the extent of head-dipping behavior in the EPM is ambiguous [Ennaceur, 2014]. This decrease can be regarded as an indicator of a decrease in exploratory risk assessment [De Jesús-Burgos et al., 2012], in which case we can speak of decreases, in both series, in exploratory activity in rats kept in isolation for two months. A decrease in the number of head dips from the OA can also be seen as evidence of an increase in anxiety levels in rats, as the number of head dips correlates directly with exploration of the OA of the maze [O'Leary, Gunn, Brown, 2013]. The data obtained in the present study support this interpretation: in series 1, rats of the control group displayed a decrease in the preference for the OA (an increase in the anxiety level) at age 3 to 5.5 months and this was accompanied by decreases in the number and duration of head dips; three-month-old rats kept in isolation made shorter stays in the OA compared with controls, and this was combined with a lower extent of head-dipping behavior (see Fig. 3, *a* and Table 2). However, it is of note that no decrease in visit duration in the OA was found in rats in series 2, while the head dips were below the level seen in controls. Increases in excursions to the OA in rodents under the influence of a number of anxiolytics can be accompanied by both an increase and a decrease, or even the absence of a change, in the frequency of head dips (cited by review [Ennaceur, 2014]). Working from the whole of these data, we suggest that head dips from the OA in the EPM may be an additional characteristic of the behavior of rats in experimental conditions producing a conflict of motivations, though it is presently difficult to provide an unequivocal answer to the question of whether changes in these indicators indicate an impairment to exploratory behavior or a change in the level of anxiety.

The desire of female rats to increase social contacts, including aggressive social contacts (in series 2), seen in both series after two months of SI, can be regarded as demonstrating maladaptive and inadequate social interactions due to social deprivation, that is, as a manifestation of distress. But the problem can be approached differently. Social interaction itself plays an important role in coping with the SI stress experienced during adolescence more effectively, with development of an adaptive social phenotype which is useful in situations requiring social belonging for the survival of a social animal species [Rivera-Irizarry et al., 2020]. The strengthening social interaction induced by unfavorable social conditions, including SI stress, contributes to mitigating this stress and overcoming its effects and also to the development of stress resistance, that is, it contributes to so-called "social buffering" [Beery and Kaufer, 2015]. Social buffering in turn helps to reduce the level of stress-induced anxiety [Smith and Wang, 2014]. From these positions, it can be suggested that the increase in sociability in female rats after two months of SI is a strategy for overcoming isolation for this period of time. The data obtained in our studies are not consistent with data reported by Tanaka et al. [2019], who found a decrease in social preference in female Long–Evans rats exposed to SI for about two months immediately after weaning. However, as already noted [Arakawa, 2003], the effect of SI on anxiety-related behavior in rats depends on the developmental period of the animals when isolation was initiated. Tanaka et al. [2019], started SI on postnatal day 21, immediately after weaning rat pups from their mothers, i.e., in the preadolescent period; in the present study, rat pups were placed in SI conditions when they were already in adolescence. We also cannot exclude the possibility that the discrepancy between the data obtained in the present study and those reported by [Tanaka et al., 2019] is associated with the use of different rat strains (Wistar and Long–Evans respectively), as it has been shown that the rat strain influences the effects of stress [Faraday, 2002; Martis et al., 2018].

It should be noted that the decrease in preference for social novelty in rats after 8.5 months of SI, as compared with control animals, was not accompanied by any changes in motor or exploratory activity or changes in anxiety levels in the EPM. Our previous studies also found no differences from controls in female rats aged 8.5 months – after 7.5 months of SI – in terms of motor activity [Krupina et al., 2020] or anxiety [Shirenova et al., 2021]. Thus, the impairment to the preference for social novelty in rats found in the later stages of SI cannot be associated with changes in these indicators.

Evaluation of the preference for social novelty can be attributed to cognitive functions characterizing social recognition, so the impairment to the preference for social novelty suggests development of disorders in the processing of social information and social memory [Seillier, Giuffrida, 2016]. The data obtained in the present study indicate a possible progressive deterioration of social memory in rats as the duration of SI increases.

The results of this study do not contradict an interesting hypothesis discussed in [Matisz et al., 2021], the essence of which is that chronic stress switches the behavior of rats from environmental exploration to the exploitation of known resources to meet current and unknown future needs and avoid many possible threats. Studies on adult male Long–Evans rats showed that after five weeks of chronic mild unpredictable stress, including SI as one of the stressors (twice a week all night, once a week for a day), the animals displayed increased sensitivity to a threatening context (they ran a greater distance at a greater speed in the presence of a predator odor than control rats in the OF test) and decreased exploratory and consummatory/food-procuring behavior (they explored less and licked feeders less in a competitive selection task). The authors took the view that the shift in behavior from exploration to exploitation of known resources gives stressed animals an advantage in adapting to the environment; it can be regarded as adaptive because exploration requires time and energy and is associated with increased risk as compared with using the familiar option. In the present work using a chronic SI stress model, we did not assess the consummatory behavior of female rats as such, but we evaluated exploratory behavior, risk avoidance behavior, and motor activity. A decrease in exploratory activity compared with control values was found in rats in both series after two months of SI. Signs of increased risk avoidance behavior in the classical OF test (rapid departure from the center to the edge zone and more time spent in this zone, see Table 1) were found in series 1 in rats aged 5.5 months – after 4.5 months of isolation – which coincided in time with an increase in the motor activity of animals in the automated OF (see Fig. 2, *b*). An enhanced motor response, according to the authors of the hypothesis [Matisz et al., 2021], may be due to the inability to use passive coping strategies when sensitized to threats or when avoidance behavior is increased, i.e., it may reflect increased arousal. Within the framework of the hypothesis of a shift in behavior under the influence of chronic stress from exploration to the exploitation of known resources, a decrease in preference for a new social object, up to the complete absence of such a preference, can be regarded as a decrease in motivation to explore a new object. Thus, the decrease in the preference for social novelty may reflect not only impairments to social memory, but also a change in the nature of motivational activity.

The changes in sociability indicators found in the present study and the degradation of the preference for social novelty in rats after SI lasting 8.5 months as compared with SI lasting two 2 months is apparently accompanied by a change in coping strategies. In accordance with the model of allostatic states proposed by Kupriyanov and Zhdanov [2014], it can be suggested that the allostatic load is moving to a different, higher level.

Conclusions. 1. Similar age-related changes in the weight of female Wistar rats kept in groups or in conditions of SI starting in adolescence at age one month and continuing uninterrupted to age 5.5 or 9.5 months were found in two series of studies. The weights of rats of the experimental and control groups increased with age. However, in one of the series, the body weight of SI rats from two months of isolation was less than that of control animals kept in groups.

2. In both series, the automated open field test showed similar dynamics of motor activity in rats kept in groups and in SI conditions. Motor activity in adult rats was greater than in adolescents and decreased starting from 5.5 months. Vertical exploratory activity in both series, regardless of keeping conditions, decreased with age. However, in one of the series, changes in parameters in groups depended on age: exploratory activity at age three months was lower and motor activity at age 5.5 months was higher in rats kept under SI conditions than in animals kept in groups.

3. In both series, keeping conditions had no direct effect on anxiety levels of rats in the elevated plus maze test in terms of activity in the open arms. However, in one series, measures of anxiety in rats after two months of SI were higher than those in control rats. In this series, anxiety in control rats at age 5.5 months was greater than at age three months. The extent of exploratory risk assessments (head-dipping behavior) was reduced after two months of SI in rats in both series. Neither age nor keeping conditions had any effect on the motor activity of the animals.

4. In the classical open field test, anxiety levels in adolescent and adult rats kept in SI conditions or in groups at ages one and three months were no different, though at 4.5 months of SI, rats showed signs of avoiding open spaces, spending more time in the periphery of the open field.

5. In the social interaction test, increases in the durations of social contacts of non-aggressive and aggressive nature were found in rats after two and five months of SI. There was no difference in the nature of social interaction between rats from the experimental and control groups after eight months of SI.

6. The duration of SI influenced sociability and the preference for social novelty in rats. Experiments in a three-chamber social test in rats after two months of SI showed that the preference for a social object over a non-social object was higher than in control rats, while the animals showed signs of decreased preference for a new social object over a previously familiar social object. After 8.5 months of SI, the preference of rats for a social object over a nonsocial one did not differ from that in the control group, though the preference for social novelty was reduced.

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