

Social Isolation during Peri-Adolescence or Adulthood: Effects on Sexual Motivation, Testosterone and Corticosterone Response under Conditions of Sexual Arousal in Male Rats

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Abstract

Reproductive functions in adult organism are known to be affected by different factors. Effects of social environment at the postnatal ontogenesis attract particular attention since it has deep impact on the development of physiological and emotional state of an individual. Effects of chronic social isolation at different ages on male sexual motivation, testosterone and corticosterone response under conditions of sexual arousal were studied in Wistar rats. After weaning at 21 day of age, rats of one group were isolated for six weeks and after that they were housed in groups of five per cage for ten weeks (Iso3-9). Rats of the second group were housed in groups of five animals per cage till 13 weeks of age, and then they were isolated for six weeks (Iso13-19). Rats of the control group were housed in groups during the experiment. Adult 19 week-old male rats were tested under conditions of sexual arousal. The expression of sexual motivation was estimated as the behavioral activity of a male at the transparent perforated partition separating a receptive female. Isolation of adult male rats reduced the number of approaches to the partition, while the period of time a male spent at the partition was not changed and testosterone response was enhanced as compared to control rats. Chronic social isolation during peri-adolescence reduced sexual motivation and prevented arousal-induced elevation of testosterone. Plasma corticosterone increases at sexual arousal in the two groups of isolated rats did not differ from that in controls. Our results are evidence that social isolation during the post-maturity stage (Iso13-19) did not diminish the manifestation of sexual motivation and hormonal response to a receptive female, while isolation during peri-adolescence attenuated behavioral and hormonal expression of sexual arousal in adult males.

Key Words: post-weaning or adult social isolation, re-socialization, male sexual arousal, sexual motivation, testosterone, corticosterone

Introduction

Reproductive functions in adult organism are

known to be affected by different factors including social stress (24, 38). In adult male mice, intruder males failed to display copulatory behavior towards

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Received: October 4, 2011; Revised: December 15, 2011; Accepted: March 16, 2012.

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estrous females following repeated defeat in confrontation with an aggressive resident (33). Defeated rats also showed a reduced frequency of mounting behavior (28), elongation of intromission and ejaculation latencies (27). Moreover, plasma testosterone elevation in the presence of a potential sexual partner was not observed in the defeated animals (27, 28). It should be noted that not only social defeat but also social victories experienced in the repeated daily agonistic confrontations in male mice were followed by a significantly lower sexual motivation and hormonal response (18). On the other hand, total absence of social contacts under conditions of social isolation also resulted in a decrease in copulatory efficiency in individually housed rats (7) and in increases in ejaculation latency (37). However, the effects of social isolation on initial stages of sexually motivated behavior, *i.e.* sexual arousal, remain unclear.

Effects of social environment at the postnatal ontogenesis attract particular attention since it has deep impact on the development of physiological and emotional state of an individual. In animals with social organization, upbringing in a group of peers with manifestation of juvenile play behavior and related emotional and physical responses seems to play a crucial role in the development of adequate responses to social stimuli. It was shown that chronic social isolation for various periods during peri-adolescence (from weaning till the young adult age) disturbed brain maturation (10, 19, 31) and altered social behavior at adulthood (34, 35). The data on the effects of post-weaning isolation on male copulatory behavior are mixed. Cooke and colleagues (10) showed that males which had been socially isolated from 21 to 90 days of life had poorer copulatory performance, evaluated as the ratio obtained by the number of intromissions divided by the number of mounts+intromissions (hit rate). Besides, these males achieved significantly fewer non-contact erections and displayed them later than did controls (10). However, there are data indicating that juvenile isolation did not influence the capacity to perform sexual motor acts (the number of mounts, intromissions and ejaculations), but the number of anogenital sniffing bouts was decreased in isolates as compared to non-isolated controls, and the latency until the first anogenital investigation was significantly increased in isolated rats (35). These data let us suggest that social isolation provokes behavioral deficits even at the initial stage of sexual behavior, so-called sexual arousal, which triggers all the following stages of sexual performance. But experimental verification of this hypothesis has not been carried out yet. Moreover, the behavioral and hormonal responses to the environmental challenges in adolescent individuals could be inherently different from that of adults due the neuroadaptive differences

and that issue should also be verified.

It is well established that male rat reproductive behavior, including sexual motivation and performance, is dependent on circulating levels of testosterone (4-6). Sexual arousal is also accompanied with a moderate plasma corticosterone increase (6, 9). The effects of social isolation during post-weaning period or adulthood on the endocrine function of testes and adrenal cortex under conditions of sexual arousal are yet to be investigated.

To answer these questions, we compared the expression of sexual motivation and hormonal response at exposure to a receptive female in male rats after chronic social isolation at different ages. Adult 13 week-old males or juvenile three-week old male rats were socially isolated for six weeks. To identify the effects of chronic isolation during peri-adolescence (from three to nine weeks of age), the rats from the latter group were united in groups after isolation for re-socialization for ten weeks.

Materials and Methods

Subjects

The Wistar rat strain maintained at the Institute of Cytology and Genetics was used in the experiments. The animals were given food and water *ad libitum*. To produce offspring, a male was introduced into a cage with three females. Pregnant females were individually housed. The day of birth was taken as day 1 of life. The litters consisting of both males and females were reduced to eight pups, the number of males was maximized, and at least two females per litter were left. All experimental procedures were carried out in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC).

Experimental Design

The rat litters were weaned on day 21 of age, and males were randomly divided into three groups. The control rats were kept in groups of five (Control Group, $n = 20$) in $53 \times 32 \times 19$ cm opaque plastic cages. The rats of the second group were reared like control group until 13 weeks of age, and they were then housed individually in opaque $28 \times 15 \times 10$ cm plastic cages for six weeks (Iso13-19, $n = 10$). The rats of the third group were housed individually in opaque $28 \times 15 \times 10$ cm plastic cages just after weaning for six weeks (Iso3-9, $n = 20$) and after that they were housed likewise as the control animals, in groups of five per cage, and left undisturbed for ten weeks. It should be noted that the duration of isolation and group-housing were equal in rats of both groups ex-

posed to social isolation. All the animals were tested at the age of 19 weeks.

Test for Sexual Motivation

To evaluate sexual motivation, the partition test was used. This test was validated for measuring expression of sexual motivation and plasma testosterone response in naïve male rats and mice (1). Each male rat was placed in a 52 × 33 × 20-cm experimental cage divided by a holed transparent partition into two compartments for three days for adaptation prior to experimenting and to remove the group effect in control group of group-reared rats and in Iso3-9 rats which were re-socialized after period of isolation. The perforated transparent partitions were designed so that the males could smell, see and hear receptive females but were not able to establish any physical contact associated with mating. Test sessions were run at 20:00-22:00 under red light. ITH-LINE-36-RED LED lamp with CREE LEDs produced by Industrial Technology company (Novosibirsk, Russia) was used for illuminating the testing room. The animals were given 5 min for adaptation to novel lighting conditions, and their spontaneous activity near the partition was assessed during the subsequent 10 min. Then, each male was exposed to a receptive female. The amount of time a tested male spent actively exploring the partition over 10 min and the number of approaches it made to the partition over this interval were recorded. Then a male was kept for a further 10 min at exposure to a receptive female and blood samples were taken for hormonal assays. The total duration of the test session was 35 min. Each female was used to test only one male.

In the females, estrus was induced by β -estradiol (Sigma-Aldrich Co, St. Louis, MO, USA) diluted in peach oil and administered subcutaneously 48 h before testing with a dose of 20 μ g/rat and progesterone (Dal'chipharm, Khabarovsk, Russia) diluted in peach oil and injected subcutaneously 4 h before testing in a dose of 1 mg/rat. The volume of injection was 0.2 ml/rat.

Blood Sampling and Hormone Assays

To estimate testosterone and corticosterone levels at sexual arousal, blood samples were taken after 20 min of exposure to a female by cutting off about 2 mm of the tail tip. Duration of the procedure did not exceed 2 min. To evaluate basal evening plasma levels of corticosterone and testosterone at testing conditions but in the absence of a receptive female, rats were exposed to the same experimental conditions for 35 min one week after the behavioral test. Blood samples were taken as described above.

Plasma testosterone radioimmunoassay was performed using a highly specific antiserum and [3 H] testosterone (Amersham, Bucks, U.K.) as described earlier (1). The percentage of cross-reactions shows specificity of antiserum used for testosterone (100%), dihydrotestosterone (62%), androstenediol, androstenedione, androsterone, progesterone, estradiol, cortisol (0 to < 0.5%); CV% intra-assay = 8; inter-assay = 6. The corticosterone level was measured in 50 μ l of rat plasma by a competitive protein-binding radioassay technique as described in details earlier (23). Steroid-free plasma of female rats was taken as the source of corticosteroid-binding globulin. In the samples, endogenous corticosteroid-binding globulin was inactivated by heating at 75°C for 10 min. Equivalent amounts of steroid-free rat plasma were added to the standard curve tubes before heating. A dextrane-coated charcoal method was used for separation of bound and free steroids. The standard curve range was 1-50 ng of corticosterone. Intra- and inter-assay coefficients of variation were less than 8% and 12%, respectively.

Data Analysis

Since all the parameters studied for each rat were obtained twice, with an empty adjacent compartment and in the presence of a receptive female, the two-way ANOVA for repeated measures (Statistica 7.0) was carried out for data analysis with the following independent variables: rearing conditions (control, Iso3-9, or Iso13-19 group) and test conditions (empty neighbor compartment or female exposure). Data were expressed as means \pm SEM. The group means were compared using Newman-Keuls *post-hoc* test. A difference was considered statistically significant at $P < 0.05$.

Results

The indices measured in the partition test (the number of approaches to the partition and the time spent by a male at the partition) were significantly affected by the presence of a receptive female behind the partition and rearing conditions. Two-way ANOVA for repeated measures revealed significant effects of the presence of a receptive female ($F_{(1,47)} = 169.4, P < 0.001$) and rearing conditions ($F_{(2,47)} = 9.9, P < 0.001$) on the number of approaches to the partition that corresponded to the general arousal and locomotor activity, and the interaction of the factors ($F_{(2,47)} = 16.3, P < 0.001$). No group difference in the number of approaches to the partition was found at empty neighboring compartment (Fig. 1A). At exposure to a receptive female, males of all groups made significantly more approaches to the partition than at

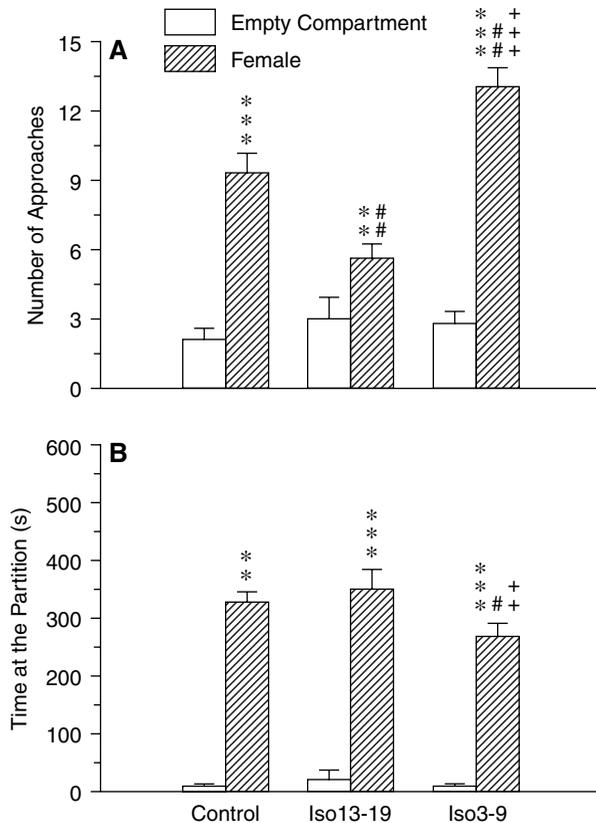


Fig. 1. The effects of chronic isolation for six weeks at different ages (from 13 to 19 weeks of age or from 3 to 9 weeks of neonatal live) on sexual motivational behavior induced by the presence of a receptive female behind the partition in adult male rats. Number of approaches to the partition (A) and time spent at the partition (s) (B) separating empty compartment (during 10-min exposure) are displayed in open bars. The alterations in behavioral activity after a receptive female introduction (during 10-min exposure) are presented in hatched bars. Data are expressed as means \pm SEM. $**P < 0.01$, $***P < 0.001$ vs. behavioral activity towards empty compartment; $\#P < 0.05$, $\#\#\#P < 0.001$ vs. control group; $++P < 0.01$, $+++P < 0.001$ vs. Iso13-19 group.

exposure to an empty compartment. However, rats of the Iso13-19 group made less approaches while males of the Iso3-9 group made more approaches to the partition as compared to control males.

A significant effects of the presence of a receptive female ($F_{(1,47)} = 594.4$, $P < 0.001$) and rearing conditions ($F_{(2,47)} = 3.4$, $P < 0.05$) on the main index of sexual motivation in the test, the time spent by a male at the partition, were also observed. Moreover, significant effect of the interaction between the factors ($F_{(2,47)} = 3.4$, $P < 0.05$) indicated that the behavioral difference between control and isolated males was manifested only at the presence of a receptive female behind the partition. At exposure to empty compartment, the time at the partition did not vary between all

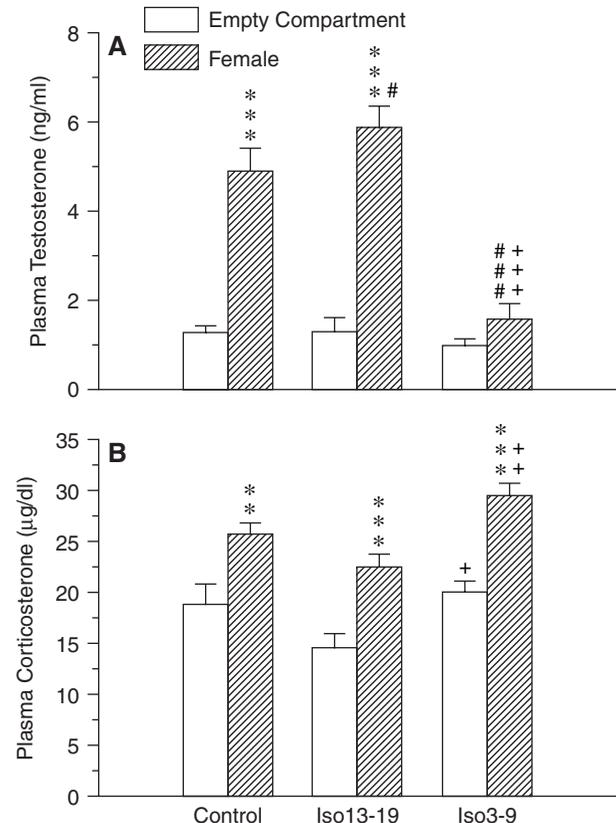


Fig. 2. The effects of chronic isolation for six weeks at different ages (from 13 to 19 weeks of age or from 3 to 9 weeks of neonatal live) on hormonal response induced by the presence of a receptive female behind the partition in adult male rats. Basal testosterone (ng/ml) (A) and corticosterone (μ g/dl) (B) levels are displayed in open bars, the alterations in plasma testosterone and corticosterone concentrations after 20-min exposure to a receptive female are presented in hatched bars. Data are expressed as means \pm SEM. $**P < 0.01$, $***P < 0.001$ vs. hormonal response to an empty compartment; $\#P < 0.05$, $\#\#\#P < 0.001$ vs. relevant control group; $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$ vs. relevant Iso13-19 group.

experimental groups, while introduction of a receptive female caused an augmentation of the parameter depending on the age at which males had been socially isolated (Fig. 1B). Male rats of the Iso13-19 group spent as much time as control males did at the partition while the Iso3-9 males were less interested in a receptive female, and the time they spent at the partition was significantly decreased.

Significant influence of a receptive female ($F_{(1,47)} = 90.7$, $P < 0.001$), rearing conditions ($F_{(2,47)} = 30.6$, $P < 0.001$) and the interaction between the factors ($F_{(2,47)} = 16.3$, $P < 0.001$) on plasma testosterone levels was found. Basal testosterone concentrations did not vary in the plasma of the experimental males (Fig. 2A), while testosterone response to receptive female intro-

duction depended on the rearing conditions. In males isolated at adult age (Iso13-19), the testosterone level at exposure to a receptive female was significantly higher than that in control males, whereas males isolated right after weaning (Iso3-9) despite the subsequent re-socialization did not display significant plasma testosterone elevation in response to a receptive female exposure (Fig. 2A).

Plasma corticosterone levels in males were influenced by rearing conditions ($F_{(2,47)} = 6.9$, $P < 0.01$) and the presence of a receptive female ($F_{(1,47)} = 63.7$, $P < 0.001$). The effect of interaction between these factors on plasma corticosterone levels was not significant ($F_{(2,47)}$, $P < 1$). The main effect of rearing conditions in the overall analysis was due to a slightly lower corticosterone level in Iso13-19 group. At exposure to an empty compartment, the corticosterone level in males of the Iso13-19 group was significantly lower in comparison with that of males of the Iso3-9 group ($P < 0.05$), and there was a tendency to decrease compared with the control males ($P = 0.06$). Under conditions of sexual arousal, corticosterone levels augmented in males of all groups, and there were no significant differences between males of the control group and both groups of socially isolated males. However, corticosterone level at exposure to a receptive female in males of the Iso13-19 group was substantially reduced compared to that in rats of the Iso3-9 group (Fig. 2B).

Discussion

Although social deprivation produces wide-ranging behavioral and neurochemical effects, it appears that these effects are determined by a number of factors, the most critical factor being the age or developmental stage during the period of deprivation (2, 3, 13). During peri-adolescence, pups develop behavioral patterns of adult individuals while playing and communicating with peers and adults, that also influences development of CNS (10, 19).

Behavioral consequences of social isolation during juvenile period differ from the effects of social isolation during adulthood. It has been shown that isolation during the juvenile stage alters behavior in the open-field test in a direction opposite to that of isolation during the post-maturity stage (2). In the present study we have also found opposite effects of social isolation during peri-adolescence and adulthood on the behavior of males under condition of sexual arousal. In males of the Iso3-9 group, the number of approaches to the partition was significantly augmented in comparison with control group-housed rats, whereas they spent significantly less time exploring the partition separating a receptive female than rats of two other experimental groups.

Hence, the males isolated right after weaning expressed the increased locomotor activity at the partition and reduced interest to potential sexual partner since the time at the partition corresponds to the expression of sexual motivation while the number of approaches reflects rather locomotor activity and general arousal as revealed earlier (1, 30). The similar behavioral effects were observed by Toth *et al.* (34) while studying the behavior of isolation reared rats in the resident-intruder test. The isolated rats demonstrated an increased frequency of behavioral bouts with significant decrease of their duration. The authors have called this phenomenon "behavioral fragmentation" and considered it a form of behavioral arousal. In contrast to males of the Iso3-9 group, males of the Iso13-19 group manifested normally expressed sexual motivation and reduced number of approaches.

One may suggest that decreased expression of sexual motivation in males of the Iso3-9 group is related to the phenomenon of disturbed social motivation. It is known that peri-adolescent social isolation of rats can affect social behavior in the same-sex social interaction tests used in the end of isolation period but the data are contradictory. Some data showed a decrease in the parameters of social behavior in isolated rats (32), while other reports indicated an increase of these parameters (12, 15).

Since the rats of the Iso3-9 group underwent re-socialization after the isolation period, we have compared our results with the studies that included re-socialization into experimental design. It was shown that juvenile isolation during four to five weeks followed by one- (35) or three-week (36) re-socialization suppressed the motivational properties of social behavior such as frequency and duration of anogenital sniffing and approaches to the test partner, respectively, in six-week old and adult rats. In another experiment, rats isolated for three weeks after weaning followed with three-week re-socialization also showed increased fear behavior and reduced social contacts in the social interaction test (20). Simultaneously, there are data which indicate that disturbances in social interaction induced by post-weaning six-week long social isolation are fully attenuated after 72 h of re-socialization in male groups (17). Moreover, Meng *et al.* (25), who applied social isolation from the 21st to 48th postnatal day followed by re-socialization from the 49th to 76th postnatal day found that before re-socialization, isolated rats showed markedly more social interaction behavior than socially reared rats, while after re-socialization there was no significant difference between the two groups. Thus, the data on the long-term effects of post-weaning isolation followed by re-socialization on the social behavior of male rats appeared to be contradictory. The incon-

sistency in the results can be explained by the differences in duration of isolation and re-socialization and different testing conditions. In our own recent experiment, we used the same model of post-weaning social isolation with subsequent re-socialization and compared its effects on sexual arousal in two rat strains, Wistar and ISIAH, an inbred rat strain with inherited stress-induced arterial hypertension selected from Wistar population (21, 22). We have found a decrease of sexual motivation in both lines, a more pronounced in hypertensive rats. The reaction of ISIAH rats, whose sexual motivation was more vulnerable to isolation effect, did not differ from group-housed male rats in behavioral response to a male behind the partition (8). Noteworthy, the activity towards a receptive female was reduced in ISIAH males isolated at post-weaning period with subsequent re-socialization down to the level of communicative behavior towards male (8). Thus, we suggest that reduction in duration of exploration of the partition separating the male from a receptive female in males socially isolated during peri-adolescence observed in the present study refers to the decrease in sexual motivation.

This suggestion was further supported by data on the plasma testosterone levels after 20-min exposure to a receptive female. The elevation of testosterone level in plasma of a male at exposure to sexual partner is well-established marker of sexual arousal (6, 29). Earlier, we demonstrated in mice or rats that at analogous testing conditions, an increase in plasma testosterone level occurred in the presence of a receptive female but not a male (1, 8). In the present study, the testosterone levels under conditions of exposure to empty compartment did not vary significantly between all experimental groups. Thus, the observed alterations in sexual motivation did not seem to depend on the basal testosterone levels. After the introduction of a receptive female the hormone levels significantly augmented in control males and males of Iso13-19 group, and in the latter the activated level was even higher than in control rats. On the other hand, testosterone levels after exposure to a receptive female did not differ from the basal testosterone level in males socially isolated during peri-adolescence. Thus, we revealed the disturbances in sexual arousal in rats socially isolated during peri-adolescence which might be triggered by alterations at the initial stage of sexual behavior detected in these males. However, we have not found any damaging effect of social isolation during adulthood on male sexual arousal.

Sexual arousal in males under conditions of direct contact between animals is accompanied with an increase in the plasma corticosterone level (6). The presence of a receptive female behind the perforated partition also induced adrenocortical activation

in rats (8). Here, we found that plasma corticosterone levels at exposure to empty compartment and arousal-induced increase in hormone concentration in both Iso13-19 and Iso3-9 groups did not differ from the hormone levels in control rats. These results are in good agreement with findings of other authors who investigated the adrenocortical function in rodents isolated at adult age and found no signs of chronic stress or enhanced acute stress response (11, 14, 16, 26). As for the adrenocortical function in the Iso3-9 rats, similar results were obtained in our earlier experiments conducted on Wistar and ISIAH rats using the same model of social isolation (8). These data are also concordant with the results from other studies revealing that long post-weaning social isolation with subsequent long re-socialization did not affect both basal and restraint stress-induced corticosterone levels in rats (20). Other investigators indicated that adult rats which had been isolated for two weeks during a post-weaning period with subsequent prolonged re-socialization did not differ from group-housed control in the dynamics of plasma corticosterone levels during 15 min after 5-min social defeat but had slower restoration of the initial hormone level (35). On the other hand, corticosterone levels in males reared in isolation without re-socialization were twice as much as in group-reared controls in response to exposure to socially significant stimulus (attack of a resident male) (34). Thus, the effect of isolation during post-weaning period on adrenocortical function seems to depend on the presence or absence of the re-socialization period.

Despite the absence of significant differences between both groups of isolated males *vs.* control males in adrenocortical response to sexual arousal, it is noteworthy that males isolated during adulthood differed significantly from males isolated during peri-adolescence. The data suggest that HPA axis in rats isolated during peri-adolescent period of life with subsequent re-socialization is more sensitive to sexually meaningful stimulus than that of rats isolated at adulthood.

Thus, the present study has presented evidence that the effects of social isolation in male rats on their sexual motivation and testosterone response induced by the exposure to a receptive female depend on the stage of development when males underwent the social deprivation. Rats reared after weaning in groups and isolated at an adult age demonstrate normal sexual motivation and increased testosterone response induced by exposure to a receptive female. On the other hand, isolation during periadolescence despite the following re-socialization produces a decrease in the expression of sexual motivation and prevents plasma testosterone elevation at exposure to a potential sexual partner in adult male rats.

Acknowledgments

This study was partially supported by the Molecular and Cell Biology Program of the Russian Academy of Sciences (grant no. 6.9) and Russian Foundation for Basic Research (grant no. 12-04-00549-a).

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