Semax Attenuates the Influence of Neonatal Maternal Deprivation on the Behavior of Adolescent White Rats

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Maternal deprivation in the early postnatal period significantly affects the behavior and development of different animals. Here we studied delayed effects of daily maternal deprivation (5 h/day) on physical development and behavior of white rats during postnatal days 1 to 14. Here we studied the possibility of reducing the negative consequences of deprivation by daily intranasal treatment with Semax, an analog of $ACTH_{4-10}$, in a dose of 0.05 mg/kg from postnatal days 15 to 28. It was found that maternal deprivation decelerated the growth of young rats, boosted physical activity and emotional reactivity in novel environment, and increased anxiety in one-month-old animals. Semax weakened the impact of deprivation on animal body weight and normalized the levels of anxiety in rats.

Key Words: *maternal deprivation; Semax; body weight; anxiety; locomotor activity*

Pain and stress experienced by newborns can later result in disturbances in the nervous system development and significant behavioral changes [4]. Impairmant of social and emotional connections between the mother and her child during the first year of life is a stressful factor increasing the risk of various mental illnesses [13]. Several authors reported a correlation between neonatal impacts and behavioral disorders of adults, but this issue remains poorly studied [5]. Animal experiments make it possible to study the dependence of delayed behavioral changes on the duration and nature of the stressful factors and facilitate the search for neonatal stress management techniques.

Maternal deprivation (MD) in the early postnatal period has distinct behavioral and phenotypic consequences in animals of various species [10]. The delayed effects of chronic MD depend on the duration of daily mother-infant separation. Short-term deprivation (15 min/day) favorably affects further development of animals: they demonstrate lower anxiety, increased exploratory activity, and better learning capacities [8]. Long-term chronic MD (3-6 hours per day during the first postnatal weeks) produces long-tern delayed behavioral changes and was regarded as a model of neonatal stress. In most experiments, maternally deprived rats exhibited increased anxiety [11]. In experiments analyzing the effects of MD on locomotor activity and novelty seeking, both enhanced [14] and reduced exploratory activity [11] were observed by different authors. Different authors also reported impaired [7] or, on the contrary, improved spatial learning in animals subjected to long-term MD [12]. Thus, the delayed effects of neonatal stress caused by chronic long-term MD are poorly studied and the results are contradictory.

Heptapeptide Semax (MEHFPGP) is an analog of $ACTH_{4-10}$ fragment characterized by long-term neurotropic activity [2,6,9]. Semax is used as a nootropic and neuroprotective drug [1]. Experiments on animals have shown that chronic neonatal administration of

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Semax improved exploratory behavior and reduced anxiety of rats at later terms [3].

Here we studied the delayed effects of chronic long-term MD and tested the possibility of their correction with Semax.

MATERIALS AND METHODS

Experiments were performed on young outbred white rats of both sexes. The animals were kept under standard vivarium conditions with free access to food and water and 12-h light regimen. A total of 99 animals from 10 litters were used. Semax was synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences.

The pups in each litter were divided into 3 groups: controls (n=34), MD (animals subjected to MD; n=31), and MD+Semax (animals subjected to MD and receiving Semax; n=34). Rat pups in the control group remained with the dams. Animals of groups MD and MD+Semax from postnatal days 1 to 14 were daily placed in individual boxes for 5 hours. During the isolation, the pups were kept in silence at a temperature of 25±2°C and moderate illumination. On postnatal days 15-28, the pups in the MD+Semax group daily received aqueous solution of Semax intranasally in a dose of 0.05 mg/kg. Pups in the control group and MD group received an equivalent volume of solvent at the same time. The animals were daily weighed on days 15-28; at the age of 30-31 days, general locomotor activity, exploratory behavior, and anxiety were evaluated.

General locomotor activity and exploratory behavior of the animals were evaluated using the open field test (OFT). The animals were placed in the center of a round arena 80 cm in diameter and ambulation (number of crossed squares), rearings, and entries into the centre, as well as grooming behavior were recorded over the first 2 min in silence and under red light.

Animal anxiety was evaluated using the elevated plus-maze test (EPM). EPM consists of four arms diverging from the center (length 35 cm, height of the walls 20 cm). Two oppose arms are shaded and have walls at the ends; two others are illuminated and opened. The maze was positioned at a height of 50 cm from the floor. The animal was placed in the center of the maze and the total time spent in illuminated compartments, number of open arm visits, and the number of overhangs from the open arms of the maze were recorded over 3 min.

When processing the results, the values for each litter were standardized to the corresponding control (because of parameter variability in different litters). The means and distributions were compared using one- and two-factor analysis of variance (ANOVA), Mann–Whitney U test and Fisher exact test. The differences were considered significant at p<0.05.

RESULTS

Body weight of pups was measured over 2 weeks after MD. Two-factor ANOVA (factor 1, age; factor 2, sex) demonstrated significant dependence of body weight gain on animal sex ($F_{4,364}$=2.90; p<0.02), therefore changes in this parameter were analyzed separately for males and females. It was found that females in MD and MD+Semax groups at the age of 15 and 18 days had lower body weight than controls (Fig. 1, *a*). Subsequent measurements revealed no significant differences in body weight of females exposed to MD from the control. Semax had no effect on body weight gain in female rats subjected to MD. In males of MD group, body weight was significantly lower than in controls throughout the study (Fig. 1, *b*). In males

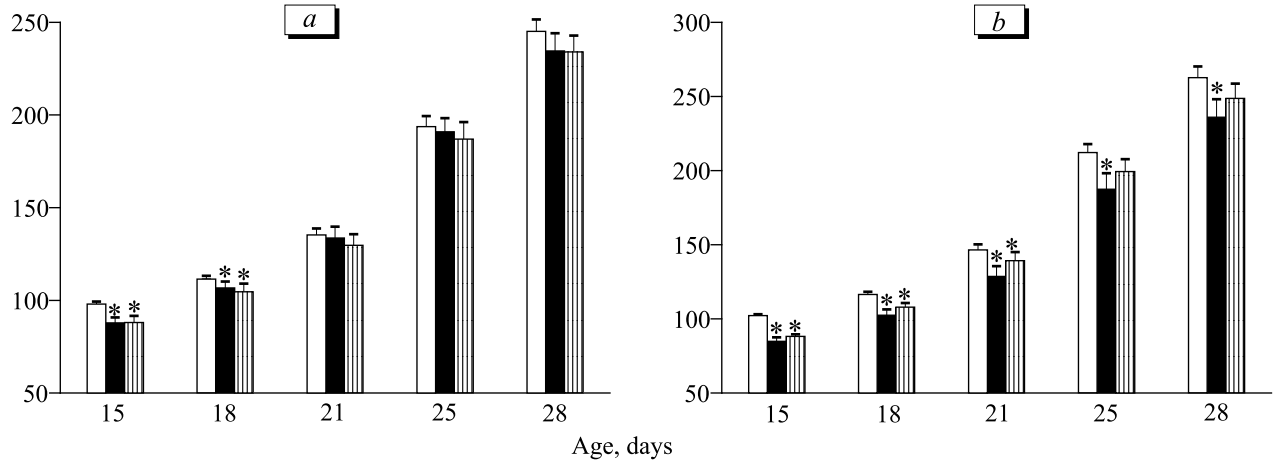

Fig. 1. Body weight gain in female (*a*) and male (*b*) rats. Here and in Figs. 2 and 3: ordinate: % of control at the first weighing; open bars: control; dark bars: MD; hatched bars: MD+Semax. p<0.05 in comparison with: *control; +MD. Data are presented as *M*±*SEM*.

of MD+Semax group body weight was significantly lower than in controls on days 15-21, but subsequent measurements revealed no differences from the control. In addition, body weight of males in MD+Semax group at the age of 21 days was significantly higher than in males of MD group; a tendency to increase this indicator was also found at the age of 25 days ($p<0.1$).

Evaluation of exploratory behavior and anxiety level using two-way ANOVA (factor 1, group; factor 2, sex) revealed no significant interactions between these factors ($F_{2,91}<1.7$; $p>0.20$), therefore the results are presented for the whole groups.

Animals of MD group demonstrated significantly longer run length in OFT, greater numbers of entries into the centre, and higher grooming levels in comparison with controls. Vertical activity (number of rearings) reflecting the level of exploratory activity [14] did not differ from the control (Fig. 2). Similar behavioral changes were observed in MD+Semax group. Analysis of MD-induced changes in OFT behavior attested to an increase in motor activity, but not exploratory behavior, because vertical activity did not differ in the experimental and control groups. Enhanced grooming reflects increased emotional strain in animals subjected to MD. Thus, long-term MD leads to hyperactivity and high emotionality in novel environment in one-month-old rats. Semax did not change these effects of MD.

Testing in EPM revealed a significant decrease in the time spent in open arms and number of visits to open arms in rats of MD group; the number of overhangs ($p<0.10$) tended to decrease in comparison with the control (Fig. 3), which attested to increased anxiety. In MD+Semax group, the number of overhangs significantly increased in comparison with the control group; other parameters did not significantly differ from the control. The number of open arm visits and the number of overhangs in this group were significantly higher than in MD group; a tendency to an increase in the total time spent in open arms in MD+Semax group compared to MD group was also noted ($p<0.1$). Hence, administration of Semax to animals subjected to MD normalizes the level of anxiety at the age of 1 month.

Thus, chronic long-term MD slows down the growth of rats, which agrees with published data [15]. However, sex-dependence of this effect has not been studied. Our studies have shown that the impact of MD on body weight of animals is sex-dependent: the effect is more pronounced in males. Administration of Semax after the completion of MD attenuates the effects of neonatal stress on the rate of somatic growth.

Long-term repeated MD is known to induce long-term delayed changes in animal behavior. However, opposite changes in exploratory behavior and anxiety

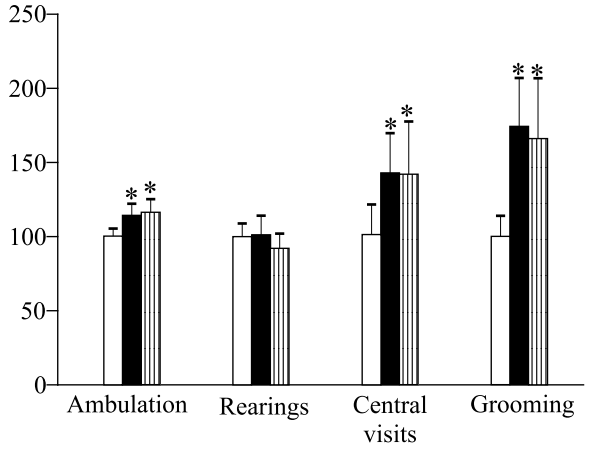

Fig. 2. Parameters of rat behavior in the open-field test.

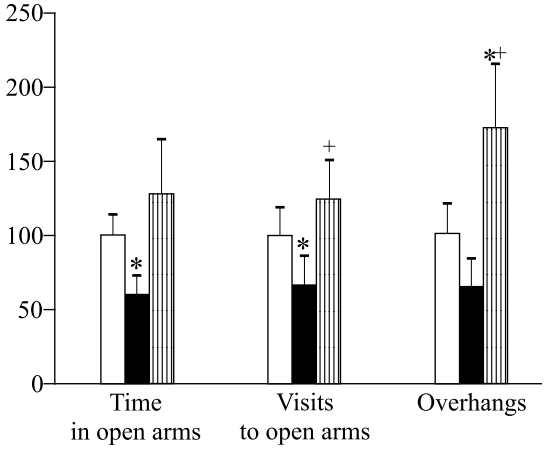

Fig. 3. Parameters of rat behavior in elevated plus-maze.

level in rats subjected to MD were reported, which can be explained by both differences in the experimental protocols (different periods and duration of MD) and different animal age during testing [11,14]. Our experiments show that daily MD leads to hyperactivity of animals in novel environment and increased anxiety in adolescent animals. Chronic administration of Semax after completion of MD course partially compensates for the negative effects of MD on animal behavior.

Our experiments showed that daily MD for 5 h per day for 1-2 weeks affects the development of rats and causes delayed behavioral changes. The animals subjected to MD demonstrated somatic growth delay, hyperactivity, enhanced emotionality, and anxiety at the age of 1 month. Chronic administration of Semax to rats subjected to MD attenuates the negative effects of neonatal stress.

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