Research article

Effects of swimming exercise on morphine-induced reward and behavioral sensitization in maternally-separated rat pups in the conditioned place preference procedure

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HIGHLIGHTS

• Maternal separation increased morphine preference and behavioral sensitization.
• Swimmer maternally separated pups exhibited a decrease in CPP in both sexes.
• Swimming decreased behavioral sensitization in maternally separated male pups.

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ABSTRACT

This study was designed to examine the effects of swimming exercise during adolescence on morphine-induced conditioned place preference (CPP) and behavioral sensitization in maternally separated male and female rat pups. Male Wistar rats were allowed to mate with female virgin Wistar rats. Pups were separated from the dam daily for 180 min during postnatal days 2–14. All pups were weaned on day 21. The exercising pups were allowed to swim (60 min/d, five days per a week, for 30 days) during adolescence. Then, rat pups were tested for behavioral sensitization and the CPP induced by morphine. Maternal separation produced a significant increase in morphine-induced CPP in both sexes, behavioral sensitization in male pups and tolerance to morphine-induced motor activity in female pups. Swimmer pups separated from the dam exhibited a decrease in morphine-induced CPP in both sexes and behavioral sensitization in male pups than those of their control pups. The present results have shown that swimming exercise during adolescence may exert a protective effect against morphine-induced reward and behavioral sensitization in adult male and female rats following maternal separation.

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1. Introduction

Several studies have shown that early maternal separation in infants (15 min to 6 h during the first 2 weeks of life) can permanently alter various aspects of behavior in adulthood [24,40], produce structural disruptions in the brain [2,31,43], dopaminergic alterations [3,28]. Also, it reported that maternal separation increase ethanol preference [7,18], sensitivity to ethanol-induced locomotor activity [9], and lead to a basal hypoisactivity of the enkephalinsergic system and vulnerability to morphine dependence and changes in sensitivity to morphine in adulthood rats [20,21,41]. Thus, the reversal or prevention of the brain alterations induced by maternal separation could be a useful method for the prevention and treatment of drug addiction in adulthood. We have previously shown that the swimming [8] and voluntary exercise [30] diminished the severity of physical and psychological dependence in both morphine-dependent and withdrawn rats and also voluntary consumption of morphine in the rat pups born from morphine-dependent mothers after wheel running [16]. Previous studies have shown exercise reduces the rewarding efficacy of

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methamphetamine [5] and cocaine [38], morphine [26] and self-administration of morphine [17]. Taken together, these findings indicate that exercise has a rewarding effect and elicits neuroplastic changes in the mesolimbic dopaminergic reward pathway [15]. Therefore, the aim of this study was to investigate the effects of swimming exercise during adolescence on morphine-induced conditioned place preference (CPP) and behavioral sensitization in maternally separated male and female rat pups.

2. Materials and methods

Male Wistar rats (250 ± 10 g) were allowed to mate with female virgin Wistar rats (250 ± 10 g) during a 24 h period. After Observation of vaginal plug, each pregnant dam (n = 16) was individually housed in polycarbonate cages with a 12 h light/dark cycle, at temperature 22 ± 4 °C and food and water were available ad libitum [17]. Postnatal 0 (PND 0) was the day of birth and maternal separation was conducted from PND2 to PND14 for 3 h from 10:00 AM to 13:00 PM (see Fig. 1). Each separation consisted of removing dams from the home cage and placing pups with their cages in a different room, such that the pups were kept together in the rearing site while room temperature was maintained at 32 ± 0.5 °C (days 2–5) or 30 ± 0.5 °C (days 6–14) for 3 h [13,44]. Dams and their litters were housed in polycarbonate cages filled with soft wood shavings. Control dams and pups were reared under standard conditions and their cages were cleaned twice-weekly that was similar to that of the pups separated from mothers, but never separated. The pups were weaned at 21 days of age from the dams and housed together in cages with same-sex littersmates. To attenuating litter-size-induced variability in body weight of pups, one or two pups of each sex from each litter randomly assigned for each group. 56 male pups and 56 female pups were assessed in this study. The pups (n = 5–8/sextomeperiment/rearing group) were randomly divided into four groups according to sex into two separate experiences; pups that were not separated from mothers and had no swimming exercise (No MS/No Swim); pups that were not separated from mothers and had performed swimming exercise (No MS/No Swim); pups separated from mothers and had no swimming exercise (MS/No Swim); and pups separated from mothers and had performed swimming exercise (MS/Swim). All the experimental procedures were conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (were approved by University Ethics Committee).

2.1. Exercise protocol

Daily swimming exercise was performed in a blue circular pool (140 cm in diameter and 50 cm high) filled to a 25 cm depth with 32 ± 1 °C water. The pool did not contain any place to rest. Exercise was performed between 9:00–11:00 a.m., using our previously described system [9]. After swimming, rats were towed dry and kept warm by electric heater. To acclimate to the new environment, all rats were adapted to water before beginning the experiment, which took four days. This adaptation started by placing the animals for 5 min into the swimming pool with shallow water in which they could stand. At the second day, rats spent 5 min in head-high water in which they might start swimming. At the third day, the water was deep enough so they had to swim for 5 min. On the fourth day, rats had to swim for 15 min [37]. The training period gradually and progressively began for 30 min on the first day until 60 min continuous on the last day, and it was performed 5 days per week for 4 weeks (Fig. 1).

2.2. Conditioned place preference

The conditioned place preference paradigm was used to study the rewarding effects of morphine that was based on [5,40] models. It was made from wood and was composed of two distinct chambers (A and B) with (30 × 30 × 30 cm) dimensions, separated from each other by a neutral chamber (30 × 15 × 30 cm) with a red background and having guillotine gates. Each chamber of A and B had a black background or white with the different pattern of white or black stripes (vertical or horizontal), respectively. According to the study of Xu, et al. [45], the procedure was performed for 9 days

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**Fig. 1.** Timeline of experiments.
and behavioral activity (time spent and the number of visits to the each chamber with back-and-forth motions during pre- and post-conditioning test sessions, to determine that locomotor activity was not different between groups) was recorded by a video camera, using a tracking system (EthoVision, Noldus, The Netherlands).

2.2.1. Pre-conditioning

On day 1 (Habituation), rats were placed in the neutral chamber and allowed to explore both chambers for 20 min in order to adapt to the environment. This test was repeated on day 2; the amount of time spent in each of the three chambers was recorded and the initial preference was calculated. If the animal spent more than 60% of the time on day 2 for either side (initial side preference) it was eliminated from the experiment.

2.2.2. Conditioning

Rats were treated with morphine at the dose of 5 mg/kg, s.c. on days 3, 5, and 7 and saline on days 4, 6, and 8 during the conditioning phase. Drug and saline administration alternated daily such that, in any group, half of the rat received drug in the chamber A and the other half received drug in the chamber B. Conditioning sessions lasted 15 min.

2.2.3. Post-conditioning

On day 9, rats were placed in the neutral chamber and allowed to explore both chambers again for 20 min. Preference or conditioning score is calculated by subtracting the amount of time spent in the drug-paired chamber before conditioning (on day 2) from the amount of time spent after conditioning (Fig. 1).

2.3. Measurement of locomotor sensitization

We have used the open field chamber consisted of a transparent cylinder 25 cm in diameter and 30 cm high, as described previously [36]; to assess the morphine-induced locomotor sensitization. The floor of the cylinder was divided into four equal quadrants by two intersecting lines. First, baseline locomotor activity was evaluated. Then, to induce locomotor sensitization, morphine (5 mg/kg, s.c.) was injected once daily for 3 consecutive days without placing in the open field chamber (for the last 2 days). However, the acute locomotor response of morphine was then evaluated only after the first (acute) injection of morphine. Then, the rats did not receive any treatment for the next 5 days. All rats were challenged with morphine (1 mg/kg, s.c.) on the sixth day after a 5-day drug-free period and evaluated for locomotor activity (post- morphine challenge), so that rats allowed to explore the chamber for 10 min in order to adapt to novel environments. Then, the number of lines crossed for each rat with all four legs were measured for the next 10 min. The test room was illuminated with soft white light (Fig. 1). The data expressed as the mean ± standard error of the mean (S.E.M.). These data were analyzed by using three-way analyses of variance (ANOVA) with the fixed factors maternal care × housing condition × sex followed by Tukey’s test for individual between-group comparisons. Statistical differences were considered significant at P < 0.05.

3. Results

The results of the conditioned place preference (CPP) are illustrated in Fig. 2A. Three-way ANOVA revealed a significant effect of maternal care (F1, 48 = 20.15, P = 0.0001), exercise (F1, 48 = 16.94, P = 0.0001) and sex (F1, 48 = 11.63, P = 0.001). Also, three-way ANOVA indicated a significant interaction between maternal care × sex (F1, 48 = 6.06, P = 0.017) and maternal care × exercise × sex (F1, 48 = 3.34, P = 0.027). Post hoc comparisons showed that the morphine-induced place preference score in MS/No Swim group was higher than No MS/No Swim group (P = 0.003, P = 0.046; for male and female rats, respectively). Also, morphine-induced place preference score in MS/Swim group was less than MS/No Swim group (P = 0.040, P = 0.004; for male and female rats, respectively) (Fig. 2A). Also, preference score was higher in MS/No Swim male rats than female (P = 0.039). There were a significant effect of sex in the frequency of visits to the conditional and non-conditional chambers, respectively in pre-conditioning (F1, 48 = 42.9, P = 0.0001) and post-conditioning (F1, 48 = 11.89, P = 0.001) periods using a three-way ANOVA, but no effect of maternal care and exercise or any interaction (Fig. 2B).

The results of the open field chamber using a three-way ANOVA are illustrated in Fig. 3. There were no significant effect of sex, maternal care and exercise and no interaction of
maternal care × exercise × sex in the number of lines crossed after baseline and the acute injection of morphine. However, the results in behavioral sensitization to morphine in post-morphine challenge revealed a significant effect of maternal care (F1, 48 = 170, P = 0.0001), sex (F1, 48 = 5.74, P = 0.02) and no significant effects of exercise (F1, 48 = 0.1, NS). Also, three-way ANOVA indicated significant interaction between maternal care × exercise × sex (F1, 48 = 13.87, P = 0.001), maternal care × sex (F1, 48 = 15.27, P = 0.0001), exercise × sex (F1, 48 = 6.27, P = 0.016) and maternal care × exercise × sex (F1, 48 = 9.94, P = 0.003). Post hoc comparisons showed that the number of lines crossed in MS/No Swim group was higher than No MS/No Swim group in both sexes (both, P = 0.0001). Also, the number of lines crossed among male rats in MS/Swim group was less than MS/No Swim group (P = 0.0001). Also, Between-group comparisons showed that the number of lines crossed were higher in MS/No Swim male rats after morphine challenge than the acute response of morphine and the baseline response (P = 0.0001, both), while was less in MS/Swim male rats after morphine challenge than the acute response of morphine (P = 0.0001). The number of lines crossed was less in MS/Swim female rats after morphine challenge than the acute response of morphine (P = 0.016). The number of lines crossed were higher in MS/No Swim male rats after morphine challenge than female rats (P = 0.0001).

4. Discussion

The results of our study indicated that maternally separated pups for only 3 h/day increased the morphine-induced conditioned place preference in both sexes. Previous studies have shown that 3-h separation from mother increases the sensitivity to morphine’s rewarding effects [42,43] and amphetamine [4] in the CPP paradigm. It may be due to a decreased dopamine transporter expression [3,29], a lower activity in enkephalinergic system, and the more sensitivity of opioid receptors [42] following maternal separation.

Also, we found that male and female swimmer rats separated from the mother showed a decrease in place preference for morphine. In the swimmer maternally separated rats was observed a greater preference for the compartment associated with saline. This finding is in accordance with a previous study showing that treadmill exercise decreases rewarding impacts of morphine [38], and self-administration of cocaine [7] and amphetamine-induced CPP [12]. Also, it has been shown that long-term voluntary exercise decreases sensitivity to the positive reinforcing effects of cocaine in female rats [39]. It may be due to increase in the release of endogenous opioid peptides in the brain [26], activity of serotonergic, dopaminergic, and noradrenergic systems [6,11,30,39] in response to maternal separation [1] and or the adaptive changes produced by exercise in the mesolimbic reward pathway [15]. Also, given the lack of group differences in locomotor activity; so, a greater preference for morphine in the maternally separated rats was not directly due to an increase in the number of visits to the chambers.

A further analysis showed that the increase in morphine challenge-induced locomotor activity in the male and female MS/No Swim pups were respectively higher and lower than the acute response of morphine, which was reduced by swimming in male pups. Also, morphine challenge-induced locomotor activity in the female MS/No Swim pups was very nearly the same as that obtained in the basic response. These findings indicated that maternally separated male rats become sensitized to repeated morphine treatments, supporting previous findings [10,21], which is proba-
bly due to increased hypothalamic corticotrophin-releasing factor (CRF) in maternally separated rats [34]. While, female rats became tolerant to the locomotor activity effects of morphine which have not found in similar studies. Also, we found that place preference for morphine was higher in MS/No Swim male rats than females. These sex related differences in morphine-induced reward and behavioral sensitization may reflect differences in stress-reactivity following maternal separation [34], the faster onset of corticosterone secretion in female rats [46], sex differences in glucocorticoid receptor, dopamine transporter expression [23] and higher levels of norepinephrine in the hippocampus [28], emphasizing the need for further research. Swimming exercise decreased locomotor sensitivity to morphine in maternally separated male pups, but had no effect on the morphine challenge-induced locomotor activity in female pups. No study with the same nature has been conducted thus far. However, wheel running decreased behavioral sensitization to cocaine in male rats [14,35]. It has been suggested that swimming exercise results in a decreased pituitary-adrenal activity and transient alterations in the gene transcription of CRF [33], which in turn lead to increases in dopaminergic reward signaling [24] that regulate motor activity.

5. Conclusions

This study provides novel evidence that maternal separation enhanced morphine-induced CPP in both sexes and locomotor sensitization in male pups, while tolerance to morphine developed in female pups. Swimming attenuated morphine-induced CPP in both sexes and locomotor sensitization in male pups. Thus, swimming exercise may have utility for prevention of drug abuse following neonatal maternal separation during adolescence.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the article.

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