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Research article

Effects of environmental enrichment during abstinence in morphine dependent parents on anxiety, depressive-like behaviors and voluntary morphine consumption in rat offspring

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HIGHLIGHTS

- The offspring of morphine treated parents exhibited depression, anxiety and drug craving.
- EE in morphine treated parents decreased anxiety, depression in their offspring.
- EE in morphine treated parents reduced voluntary morphine consumption in their offspring.

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ABSTRACT

Chronic morphine exposure during puberty increased morphine-induced rewarding effects and sensitization in the next generation. Given the well-known beneficial effects of environmental enrichment on the severity of physical and psychological dependence on morphine, we examined effects of enriched environment during morphine abstinence in morphine dependent parental rats before mating on the anxiety and depressive-like behaviors, and voluntary morphine consumption in their offspring. Paternal and/or maternal rats were injected with bi-daily doses (10 mg/kg, 12 h intervals) of morphine for 14 days followed by rearing in a standard environment (SE) or enriched environment (EE) during 30 days of morphine abstinence before mating. The pubertal male and female rat offspring were tested for anxiety (the elevated plus maze- EPM) and depression (sucrose preference test-SPT), and voluntary morphine consumption using a two-bottle choice (TBC) paradigm. The results showed that EE experience in morphine-dependent both parents result in an increase in the percentage of time spent into open arms/time spent on both arms using EPM in male offspring, higher levels of sucrose preference in female offspring and lower levels of voluntary morphine consumption in male and female offspring. Thus, EE experience in morphine-dependent both parents reduced anxiety, depressive-like behavior and also the voluntary morphine consumption in their offspring during puberty which may prevent the vulnerability of the next generation to drug abuse.

1. Introduction

Chronic morphine exposure during pregnancy causes behavioral and neurobiological alterations in offspring, including anxiety [9]- and depressive [11] like behavior and voluntary consumption of morphine [9], the brain reward circuitry [5], the expression of morphine-induced conditioned place preference (CPP) [6], the vulnerability to drug [22]. The transgenerational consequences of parental morphine exposure have been observed in systems regulating reward and stress, even in the absence of prenatal exposure to morphine [2,19]. It has been shown that morphine exposure of adult male and female rats increased anxiety-like behavior and hippocampal dendritic retraction [13] and morphine sensitization [1] in the next generation. Thus, the reversal or prevention of the brain alterations induced by parental morphine exposure could be a useful method for the prevention and treatment of behavioral deficits and drug abuse-related behaviors in the next generation.

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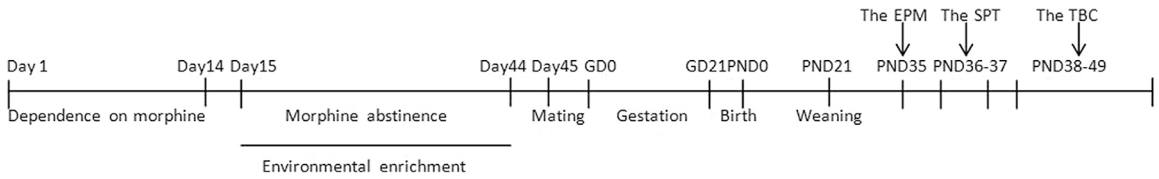

Fig. 1. Timeline of experiment (see Section 2 for details).

The beneficial effect of enriched environment (EE) on drug abuse-related behaviors has been previously demonstrated in our animal lab, including a reduction in physical dependence on morphine, anxiety/depressive-like behavior and voluntary morphine consumption [8], the spontaneous morphine withdrawal signs and the self-grooming behavior [7] in morphine-withdrawn rats. In another study, the rearing of offspring born from morphine dependent parents in the EE after weaning decreased anxiety-like behavior and hippocampal dendritic retraction [13]. It also has been shown that EE decreased sensitivity to morphine reward [23], depressive-like behavior and morphine-induced CPP induced by prenatal chronic stress [24] in rats. Thus, a more important question would be whether EE during morphine abstinence could blunt the deleterious effects of parental morphine exposure in the next generation. Therefore, the aim of this study was to investigate whether exposure of rats to an EE during morphine abstinence in morphine dependent parents before mating would attenuate the anxiety/depressive-like behavior, and voluntary morphine consumption in the pubertal male and female rat offspring.

2. Materials and method

2.1. Animals, induction of morphine dependence and housing conditions

Adult Wistar rats of each sex (200 + 20 gr) were housed in cages with a 12-h light/dark cycle at 22–24 °C and had *ad libitum* access to food and water. All of the experimental procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory. Paternal and/or maternal rats were made dependent on morphine (morphine sulfate; Temad, Iran) subcutaneously at a dose of 10 mg/kg, twice per day at 12 h intervals for 14 days [8]. The control rats were treated similarly with an injection of saline. Then, rats were group housed in their home cages (SE or EE) over a 30 day period of morphine abstinence. Enriched rats were placed in large cage (96cm × 49cm × 38 cm) with plastic tunnels, rope, swing, balls, ramp, ladder, shelters, step, cube and a running wheel, toys, which were changed every 2 day to maintain its novelty. Control rats were placed in standard laboratory cages (42 × 34 × 15 cm)[8]. At day 45, male Wistar rats (n = 24) were allowed to mate with female virgin Wistar rats (n = 24) during a 24 h period. Female rats were checked for the presence of a vaginal plug twice at mid-night and at 5 a.m. the next day. Observation of vaginal plug in each pregnant dam was considered as gestational day 0 (GD0) [9]. Born offspring were weaned at 21 days of age from the dams and housed together in their cages with same-sex littermates up to puberty. To exclude any possible effects of genetic and prenatal factors, one or two pups of each sex from each litter randomly assigned for each group. The pups (n = 7-8/sex/experiment/rearing group) were randomly divided into eight groups according to sex as follows:

1. Saline exposed paternal-Saline exposed maternal/SE (P Sal-M Sal/SE)
2. Saline exposed paternal-Saline exposed maternal/EE (P Sal-M Sal/EE)
3. Morphine exposed paternal-Morphine exposed maternal/SE (P Mo-M Mo/SE)
4. Morphine exposed paternal-Morphine exposed maternal/EE (P Mo-M Mo/EE)
5. Morphine exposed paternal-Saline exposed maternal/SE(P Mo-M Sal/SE)
6. Morphine exposed paternal-Saline exposed maternal/EE (P Mo-M Sal/EE)
7. Saline exposed paternal-Morphine exposed maternal/SE (P Sal-M Mo/SE)
8. Saline exposed paternal-Morphine exposed maternal/EE (P Sal-M Mo/EE)

All behavioral tests started 14 days after weaning during pubertal stages of male and female offspring (see Fig. 1. timelines of experiments).

2.2. Anxiety measurement using the elevated plus maze (EPM)

To assess the level of anxiety, the offspring were individually placed in the center of the EPM with two open (50 × 10 cm) and two closed (50 × 10 × 40 cm) arms, and a central platform (10 × 10 cm), and allowed to explore the apparatus for 5 min as described previously [8]. Time spent in, and entries into, open and closed arms were measured during each 5 min test by a tracking system (EthoVision, Noldus, The Netherlands). The apparatus was cleaned with water after each trial

2.3. Depressive-like behavior using the sucrose preference test (SPT)

All offspring were kept individually in cages for 24 h before testing. Then, the offspring had free access to two bottles in each cage for 48 h, one with 200 ml of 32% sucrose (w/v) and the other also with 200 ml of tap water. The positions of the bottles were changed every 12 h to avoid learning. Fluid intake and sucrose were measured every day. At the end of 48 h, the bottles were removed and sucrose preference was calculated as: 100% × sucrose solution consumption (ml)/total fluid consumption (ml) [8].

2.4. Voluntary morphine consumption using two-bottle choice (TBC) procedure

Each offspring was individually housed in cages with two bottles for a period of 12 days of testing. In one bottle, morphine sulfate was dissolved in 3% sucrose solution and also 3% sucrose solution was in control bottle as follow; on days 1–4 (0.3 mg/ml morphine); 5–8 (0.5 mg/ml morphine) and 9–12 of test (0.7 mg/ml morphine). Offspring were allowed continuous access to both bottles. The positions of the bottles in the cage were changed at the time of daily bottle weighing to avoid learning. Fluid intake was measured by weighing the bottles between 9:00 and 10:00 am daily. Body weights of the offspring were measured in the start of each period. The average morphine consumption was evaluated during a 4-day period [8,9].