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Effects of treadmill running exercise during the adolescent period of life on behavioral deficits in juvenile rats induced by prenatal morphine exposure

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HIGHLIGHTS

- Morphine exposure during pregnancy induces behavioral deficits in young rat offspring.
- The influence of adolescent period of the life is essential in the adulthood behaviors.
- Exercise has lots of beneficial effects on brain health including cognitive functions.
- Treadmill running exercise alleviates deficits induced by prenatal morphine exposure.

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ABSTRACT

Prenatal exposure to morphine throughout pregnancy results in an array of prolonged or permanent neurochemical and behavioral deficits, including deficits in learning and memory in children of addicted mothers. This study investigated the effects of forced exercise on behavioral deficits of pups born to mothers addicted to morphine in rats. After mating and ensuring of pregnancy of female Wistar rats, they were divided into morphine or saline groups and in the second half of pregnancy (on days 11–18 of gestation) were injected subcutaneously with morphine or saline, respectively. Pups were weaned at postnatal day (PND) 21 and trained at mild intensity on a treadmill 20 days. On PND 41–47, the behavioral responses were studied. Light/dark (L/D) box and elevated plus maze (EPM) apparatus were used for investigation of anxiety, shuttle box and forced swimming tests were used to assess passive avoidance learning and memory and depression behavior, respectively. The results showed that prenatal morphine exposure caused reductions in time spent in light compartment of L/D box and EPM open arm, while postnatal exercise reversed these effects. We also found that prenatal morphine exposure caused a reduction in step through latency in passive avoidance memory test and exercise counteracted with this effect. Performance in the forced swimming test did not affected by prenatal morphine exposure or postnatal exercise. Exercise seems to be one of the strategies in reduction of behavioral deficits of children born to addicted mothers to morphine.

1. Introduction

In prenatal life, the central nervous system (CNS) exhibits notable plasticity and can be changed greatly by a variety of influences. In humans, drug exposure during gestation causes general retardation of development and growth. A great percentage of children born to drug-addicted mothers exhibit disturbances in the development of the CNS, which result in an array of prolonged or permanent neurochemical and behavioral deficits such as intellectual and social impairment and cognitive and language deficits [1]. Opiates, such as morphine, are able to traverse the placenta to influence the growth of the CNS and thus result in neurobiological alterations in young offspring such as impairment of learning and memory processes [2], or alterations in social and play behaviors [3].

Postnatal life and influences of adolescent period are also essential in the adulthood behaviors. Some studies have investigated the effects of different experiences, such as handling, enriched environment and physical exercise in adolescent animals [4–6]. The effects of physical exercise on the CNS and behaviors of animals have been well documented [7,8].

The beneficial effects of exercise are remarkable in that they are almost the diametric opposite of the pattern of structural and functional deficits seen following prenatal morphine exposure. For example, exercise has been shown to modify the expression of neurotrophic factors [8], the development of neuronal processes [9] and neurogenesis in the hippocampal formation [7], a greatly plastic area of the brain

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essential for memory, learning and emotional processes [10]. Exercise has also been shown to enhance performance in the Morris water maze [11], performance in the radial arm maze [12] and long-term potentiation [13] in rodents. Previously, we have shown that exercise reduces the anxious behaviors induced by morphine dependence and attenuates naloxone-precipitated withdrawal signs in adult rats [14]. We also have demonstrated that exercise could ameliorate the spatial memory deficits in morphine-dependent rats through a brain-derived neurotrophic factor (BDNF)-mediated mechanism [15]. BDNF levels are elevated through exercise which plays a significant role in mediating exercise-induced enhancement of learning and memory [8].

Since both prenatal morphine exposure and physical exercise in postnatal adolescent period have been reported to alter animal behaviors, exercise may have the capacity to modify some behavioral deficits of prenatal morphine exposure. The purpose of the present study was to determine if treadmill running exercise can rescue the behavioral deficits induced by prenatal morphine exposure in juvenile rats.

2. Materials and methods

2.1. Animals and prenatal treatment

Adult female and male Wistar rats were obtained from breeding colony of Semnan University of Medical Sciences and maintained on a 12 h light/dark cycle (6:00–18:00 h) with free access to food and water. All animal treatments were conducted in accordance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals and were approved by the local ethical committee. After a 2-week period of acclimatization to the novel animal holding room, in order to facilitate of mating, female and male rats were kept together one-by-one in a cage at night. The presence of vaginal plug or sperm cells in vaginal smear the next morning and a high rate of body weight gain in the following 10 days were used as the indices for pregnancy. On gestational days 11–18, pregnant rats were randomly injected with morphine sulfate (Temad company, Tehran, Iran) twice daily at 6:00 AM and 18:00 PM, while the rats in control group were injected with saline instead. The dose of the first three morphine injections was 5 mg/kg and the dose for all following injections was 10 mg/kg [16]. All injections were administered subcutaneously (s.c.). No necrotic skin lesions were detected at the injection sites.

2.2. Postnatal treatment

A summary of the experimental design is displayed in Fig. 1. The day of delivery was designated postnatal day (PND) 0. On PND1, pups were weighed and sexed. The injection paradigm did not change the number of pups per litter and body weight of the offspring as described in previous studies [16]. Saline- and morphine-exposed litters were cross-fostered such that every mother raised half of her own and half of the adopted pups receiving the different prenatal treatment [16]. All litters were reduced to a maximum of 10 pups.

On PND 21, animals were weaned and were distributed into 4 experimental groups (n = 8 in each group) saline/sedentary (Sal/Sed): pups from saline exposed dam and placed on stationary treadmill; morphine/sedentary (Mor/Sed): pups from morphine exposed dam and placed on stationary treadmill (sedentary); saline/exercise (Sal/Exc): pups from dams prenatally exposed to saline and did running exercise from PND 21 to PND 40; and morphine/exercise (Mor/Exc): pups from dams prenatally exposed to morphine as previously described and did running exercise from PND 21 to PND 40. The rat pups in the exercise groups initially were habituated to a motor-driven treadmill at a speed of 2 m/min for 10 min/day during first and second days of exercise. Then they were made to run on treadmill for 30 min once a day for 18 consecutive days. The exercise load used for the exercise groups consisted of running at a speed of 2 m/min for the first 5 min, 5 m/min for the next 5 min and then at a speed of 8 m/min for the last 20 min, at a 0° inclination [17]. Control rats were placed on stationary treadmills for the same duration.

2.3. Behavioral tests

Behavioral assessments began at the PND 41 after the termination of exercise procedure. All behavioral tests were conducted in a noiseless room during the light period (between 10:00 and 15:00 h) under bright and modest illumination, and the rats were kept in the room for at least 1 h before the assessment. At the end of each test session, the equipment carefully cleaned with 70% ethanol, and the cage was relocated back to the colony room.

2.3.1. Light–dark box (L/D box)

On PND 41, juvenile rats were tested on L/D box. The L/D box consisted of a white–black Plexiglas rectangular box (length 46 cm, width 27 cm and height 30 cm), which separated into two compartments (light and large: 27 cm × 27 cm, dark and small: 18 cm × 27 cm) by a partition. These areas connected by a small central open door (7.5 cm × 7.5 cm) located in the center of the partition at floor level. To start the test, each pup was placed at the center of the light compartment, facing away from the door and the animal was allowed to discover liberally both compartments for 5 min and their behavior was automatically recorded using computer-controlled detection equipped with infrared beam sensors in the chamber. The following parameters were recorded: the percent of time spent in light compartment (TLC), the numbers of entries into light compartment (ELC) (all four paws) and the number of exploratory rearing in light compartment (RLC), defined as directed sniffing with the forepaws extended vertically upon the sides of the light compartment [18]. A decrease or an increase in the TLC and ELC, respectively, were indicative of an anxiogenic- or an anxiolytic-like effect [19].

2.3.2. Elevated plus maze (EPM)

On PND 42, juvenile rats were tested on an EPM which consists of two enclosed arms (50 cm × 10 cm, surrounded by 40 cm high wooden walls) and two open arms (50 cm × 10 cm) elevated 50 cm above the floor. The walls spread from a central neutral area (10 × 10 cm). To avoid falls, the open arms were bordered by a 0.5 cm high Plexiglas edge. All sessions were videotaped by a camera placed 1.80 m above the device. Rats were gently placed in the neutral area facing one of the open arms and given 5 min to discover the maze. The duration and number of entries into open and enclosed arms were recorded. A total of four paws inside of an arm were used as criteria for entrance. Anxiolytic influences

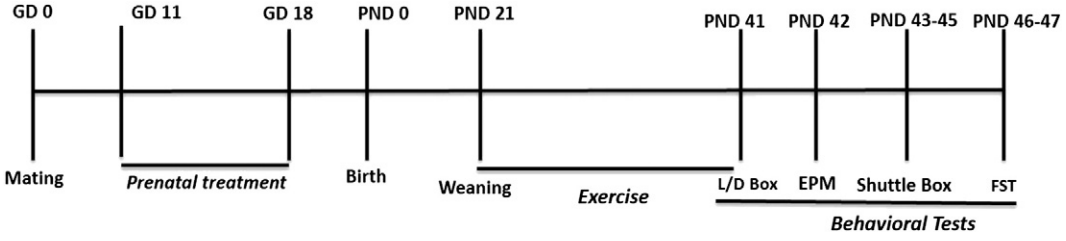

Fig. 1. Timeline of prenatal treatment, treadmill exercise and behavioral tests (see Materials and Methods for details).