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Dopamine D3 receptor knock-out mice display deficits in locomotor sensitization after chronic morphine administration

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ABSTRACT

Locomotor sensitization is the progressive and enduring enhancement of locomotion induced by stimulants such as drugs, which alter rodent locomotion in a long-standing manner. The dopamine D3 receptor has been reported to play a role in morphine addiction. The aim of the present study was to investigate the role of dopamine D3 receptor in the morphine induced locomotor sensitization using dopamine D3 receptor knock-out mice. The dopamine D3 receptor knock-out mice did not display an enhanced behavioral response to acute morphine administration or develop an increased rate of locomotor sensitization to intermittent morphine administration. When 2 mg/kg naloxone was co-administered with 10 mg/kg morphine, morphine-induced locomotion sensitization in wild-type mice was significantly blocked while the locomotion in the D3 receptor knock-out mice was decreased. Then the wild-type mice were administered with dopamine D3 antagonist nafadotride. It was found that co-administration of morphine with nafadotride could effectively suppress the level of morphine induced behavioral sensitization. It was concluded that a loss of the dopamine D3 receptor gene may inhibit acute morphine induced hyperlocomotor activity and chronic morphine induced behavioral sensitization.

Drug abuse disorder is a chronic brain disease [20] characterized by the compulsion to seek and take a drug, the loss of control in limiting intake and the emergence of a negative emotional state such as dysphoria, anxiety and irritability when access to the drug is deprived [17]. Addictive stimulants such as cocaine and morphine can induce pleasant states (euphoria in the initiation phase) or relieve distress; however, continued use of such stimulants will induce adaptive changes in the central nervous system and ultimately lead to tolerance, physical dependence, sensitization, craving, and relapse [2].

Recent research indicates that adaptations in dopamine and glutamate transmission in the nucleus accumbens are critical for expression of behavioral sensitization [31]. Among the five dopamine receptors (D1R- D5R), the dopamine D3 receptor (D3R) is identified [29] and shown to display distinct features suggesting its involvement in the effects of abused drugs [10,18]. A growing body of evidence strongly suggests that the D3R is significantly involved in mechanisms of drug dependence and abuse [12–14]. Pharmacological studies indicate that D3R antagonist SB-277011-A may attenuate the cue-controlled cocaine-seeking behavior and the rewarding effects of cocaine [9,32] and could block cue-induced reinstatement of cocaine and alcohol self-administration [14]. In vivo study of the efficacy of the partial D3R agonist BP-897 also demonstrate that administration of BP-897 (1 mg/kg) can reduce cocaine-seeking behavior [4]. Several recent discoveries suggest a potential role of the D3R in genesis of behavioral sensitization [5,27]. Pharmacological studies suggest that dopamine D3 receptor-preferring agonists such as 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) could inhibit the development of hyperlocomotion in rodents by stimulating the D3R [8,30]. The dopamine D3 receptor antagonist NGB 2904 could increase spontaneous and amphetamine-stimulated locomotion [25]. Other studies find that the dopamine D3 receptor antagonist could inhibit the development of locomotion sensitization to amphetamine [6,26].

Obviously, D3R plays an important role in mediating incentive motivational effects of stimuli such as cocaine or amphetamine. However, little has been known about the role of the dopamine D3 receptor in the morphine-induced locomotion sensitization. The present study was, therefore, designed to investigate the role of

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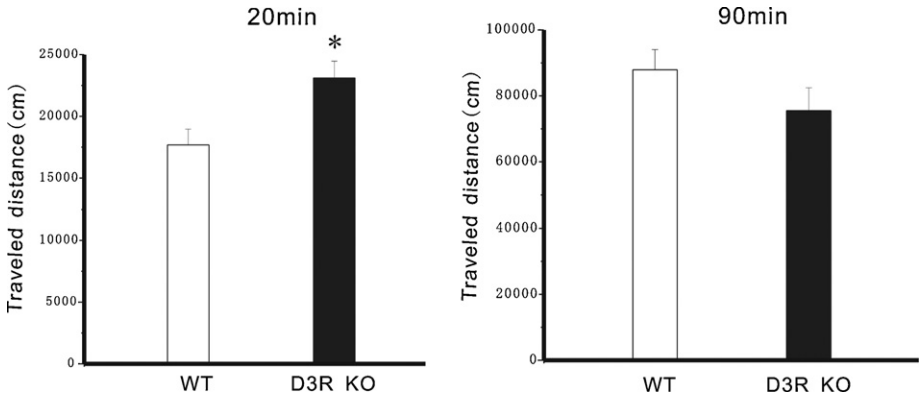

Fig. 1. The comparison of basal locomotor activity between wild-type mice and D3R knock-out mice ($n = 12$) in a novel environment. Left: locomotor activity was monitored for the first 20 min of the total 90 min in the novel environment. Right: locomotor activity was monitored for the total 90 min in the novel environment. Mean (±S.E.M.) was used to evaluate the distance traveled during each recording session. D3R knock-out mice displayed a significant increase in the traveled distance in the first 20 min, compared with wild-type mice; however, there was no significant difference in the traveled distance between the two genotypes in the whole 90 min period (*unpaired Student's *t*-tests, $p < 0.05$).

dopamine D3 receptor in the morphine-induced locomotion sensitization using dopamine D3 receptor knock-out mice.

Animals. Dopamine D3 receptor knock-out mice and their corresponding wild-type counterparts used in this study were offspring of the mice used in a previous study [33]. Genotypes of breeder pairs and all the mouse offspring used in experiments were confirmed by PCR as previously described [24]. Male D3R knock-out and their corresponding wild-type littermates (8 weeks, 25–30 g) were housed in separate cages in groups of four per cage at constant temperature (20–22 °C) and humidity (50–55%) under a 12 h light/dark cycle with food and water provided ad libitum. Mice were allowed to habituate in the colony room for one week before experimental manipulations were undertaken. All animal protocols were approved by the Animal Care and Use Committee of Xi'an Jiaotong University.

Drug treatments and behavioral testing. Morphine HCl (the First Pharmaceutical Factory of Shenyang, China) and naloxone HCl (Huasu Pharmaceutical Factory of Beijing, China) were dissolved in 0.9% sodium chloride solution, respectively. Homozygous mutant and wild-type littermates were randomly divided into chronic morphine sensitization group, saline-treated group, naloxone-treated group and naloxone-morphine group. The acute effects of morphine were also evaluated.

The sensitization protocol was based on intermittent administration of morphine at a dose of 10 mg/kg/10 ml intraperitoneal injection (i.p.) every 72 h, as previously described [15]. Behavioral experiments were preformed between 8:00 and 13:00. The basal activities of wild-type or D3R knock-out mice were evaluated by measuring their horizontal locomotion on day 0 and the five injections of morphine were administered at an interval of 72 h within the following 13 days. The controls were treated similarly with 0.9% saline (10 ml/kg) or opiate receptor antagonist naloxone (2 mg/kg). The naloxone-morphine mice were administered with an injection of naloxone (2 mg/kg) 30 min before each morphine injection.

Individual mice were tested in an animal activity measurement system (Jiliang Software Technology Co., Ltd. JLBehv-LAM-1, ShangHai, China). The system consists of four testing boxes (42 cm × 42 cm × 42 cm) with a TV camera. The interior bottoms of the testing boxes were painted white while the interior sides were painted black. The boxes were set in an isolated dark room and illuminated by two standard laboratory lamps. The experimental animals were placed into the boxes and their locomotor activities were recorded by the TV camera for 90 min and analyzed off line by a PC. Horizontal trajectories of the mice were video recorded and analyzed to determine their traveled distances in 90 min or per 10-min period. Recording commenced within 2–5 min after each injection. Mice were sacrificed 24 h after the last morphine injection.

Statistical data analysis. The data were expressed as means ± S.E. Statistical significance was assessed by one-way or two-way ANOVA analysis. Individual comparisons were performed with the unpaired Student's *t*-tests. The significance level was set at $p < 0.05$.

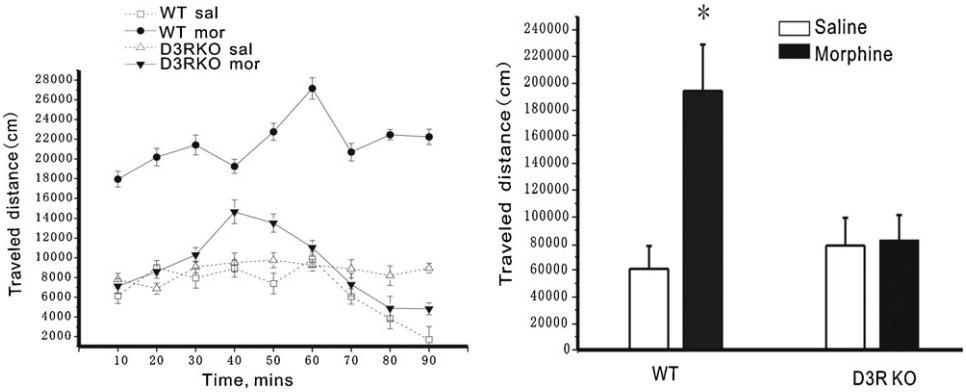

Fig. 2. Effect of acute morphine administration on the locomotor activity in wild-type and D3R knock-out mice. Mice were acutely treated with saline ($n = 8$), or 10 mg/kg morphine ($n = 8$). Locomotor activity was monitored for 90 min following each injection using a video camera. Left: time course of morphine- or saline-induced locomotor activity. Data points represent the mean (±S.E.M.) distance traveled during the 10-min periods on each recording session. Right: mean (±S.E.M.) was used to evaluate the distance traveled during the whole 90-min recording session (*two-way ANOVA, $p < 0.05$ compared with saline-treated group).