International Journal of Developmental Neuroscience 69 (2018) 49–59

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Adult rat morphine exposure changes morphine preference, anxiety, and the brain expression of dopamine receptors in male offspring

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ARTICLE INFO

Keywords:
Anxiety
Dopamine receptor expression
Morphine preference
Offspring

ABSTRACT

Addiction to drugs, including opioids is the result of an interplay between environmental and genetic factors. It has been shown that the progeny of addict people is at higher risk for drug addiction. However, the mechanisms of such trans-generational effects of drugs are not so clear. Here we have evaluated the effects of parental morphine consumption on anxiety, morphine preference, and mRNA expression of dopamine receptors in F1 and F2 male offspring. Morphine was chronically administered to adult male and female Wistar rats followed by 14-day abstinence before mating. Morphine preference and anxiety-like behavior in the offspring were measured by two-bottle-choice paradigm and elevated-plus maze, respectively. Real-time PCR was used to measure the mRNA expression level of dopamine receptors in the striatum, nucleus accumbens, prefrontal cortex, and hippocampus of F1 animals. The results indicated that F1 but not the F2 male progeny of morphine-exposed parents had a greater preference for morphine, and more anxiety-like behavior compared to the offspring of saline-treated parents. In F1 male progeny of morphine-treated parents, D1 and D5 dopamine receptors were significantly increased in the prefrontal cortex and nucleus accumbens. D5 and D2 receptors were decreased in the hippocampus. D4 dopamine receptor was augmented in striatum and hippocampus and decreased in the prefrontal cortex. Adulthood exposure to chronic morphine in male and female rats before conception leads to higher morphine preference and increased anxiety in F1 but not F2 male progeny. Alterations of dopamine receptor expression in the reward system may be one mechanism responsible for observed changes in F1 offspring.

1. Introduction

Addiction to opioids such as morphine is a chronic disorder that affects the mesocorticolimbic dopaminergic pathway in the brain. Activation of the pathway ultimately leads to dopamine release in the nucleus accumbens (NAc) (Di Chiara et al., 1998) and stimulation of dopamine receptors. These receptors are considered as two main categories: D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors, each of which is generated from a different gene (Sibley et al., 1993). Activation of D1-like and D2-like dopamine receptors decreases motivation to seek reward or facilitate the reinforcing properties of addictive drugs, respectively (Li et al., 2006).

Both genetics and environmental factors are involved in an individual's vulnerability to addiction (Cloninger et al., 1981). It is estimated that genetic variation accounts for nearly half of a person's risk to develop substance abuse disorders (Goldman et al., 2005). Besides, aggregation of drug addiction among family members may develop as far as fifth-degree relatives (Tyrfingsson et al., 2010). In addition to genetics, epigenetic factors also contribute to the inheritance of a variety of disorders, including drug addiction. In fact, epigenetics may provide the missing link between environmental stimuli and genetic heritability of the disorders (Bohacek and Mansuy, 2013; Danchin et al.,

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https://doi.org/10.1016/j.ijdevneu.2018.06.008
Received 26 January 2018; Received in revised form 27 May 2018; Accepted 24 June 2018
Available online 30 June 2018
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2011). Data indicate that approximately 50% of addicted parents' children are hospitalized for psychiatric disorders. Besides, parental alcohol abuse could lead to some long-term concerns for their 15- to 27-years-old kids such as augmented mortality and self-destructive actions (e.g. suicide or drug abuse) (Christoffersen and Soothill, 2003). In animal studies, exposure of female rats to morphine in the adolescent period could alter the response of their offspring to morphine (Byrnes, 2005). At the molecular level, histone acetylation and DNA methylation as two major epigenetic events have been shown to occur in the NAc and striatum after drug exposure (Anier et al., 2010; Maze et al., 2010; Sheng et al., 2011).

In almost all the researches that have investigated the effect of parental morphine addiction on their offspring, the female rat was exposed to morphine either during the adolescent period (Byrnes et al., 2011, 2013) or in pregnancy (Riley and Vathy, 2006; Szutorisz et al., 2014; Chen et al., 2015; Shen et al., 2016). Adolescent period could affect the development of neural and endocrine systems, and pregnancy studies make the researcher unable to exclude the direct effects of morphine on the fetus. Thus, an important question was remained to be answered: does the induction of opioid addiction in *adult* rats (which excludes the effects of morphine on developmental events) several days before gestation (which means that animals are abstinent at the time of mating, and fetus is not directly exposed to the drug) influences the behavior and addiction vulnerability of their offspring? Therefore, here we have evaluated the effect of parental morphine exposure before gestation on anxiety behavior and morphine preference in F1 and F2 male generations. Besides, in order to investigate the involved mechanisms, we have assessed the gene expression of dopamine receptors in different parts of the reward pathway in the rat brain.

2. Experimental procedures

2.1. Animals

Male adult Wistar rats weighing 220–250 gm and female adult Wistar rats weighing 180–220 gm (Pasteur Institute, Tehran, Iran) were used in the study. The animals were maintained four in standard rat cages under a 12/12-h light-dark cycle (light beginning at 7 a.m.) and constant temperature (22 ± 2 °C) with free access to food and water. Experiments were performed in the light cycle. All processes were carried out in accordance with institutional guidelines for animal care and use. Experimental protocols were approved by the Research and Ethics Committee of Tehran University of Medical Sciences.

2.2. Drugs

The drugs used in this study were morphine sulfate (Temad, Tehran, Iran), naloxone hydrochloride (Tocris, Bristol, UK), and sucrose (Merck). Morphine sulfate was consumed orally after solving in 2% sucrose solution in order to remove the bitter taste of the drug. In addition, for the parental administration of morphine, the drug was dissolved in sterile 0.9% saline to a volume of 1 ml/kg. Naloxone hydrochloride was solubilized in sterile 0.9% saline for intraperitoneal (i.p.) injection at a volume of 1 ml/kg.

2.3. Experimental design

2.3.1. Parental drug treatment

Ten male and ten female adult Wistar rats received subcutaneous injections of morphine sulfate (10 mg/kg) twice daily (at 7 a.m. and 7 p.m.) for 14 days (Craig and Bajic, 2015). Control animals (10 males and 10 females) received saline injections at the times and intervals similar to the morphine group. After 14 days, development of dependence to morphine in the drug-administered group was confirmed by observing withdrawal symptoms such as wet-dog shake, penile liking, paw tremor, and diarrhea after i.p. injection of naloxone (3 mg/kg). It should be mentioned that injection of naloxone was done in an extra group of animals (six males, six females). Therefore, our main study animals did not receive naloxone at the end of the duration of morphine administration. Two weeks after the last injection of morphine, animals were allowed to mate. Control animals mated with each other, and morphine administered rats coupled separately. Male and female adult offsprings were used for further procedures.

2.3.2. Offspring groups and mating protocol

The offsprings were divided into four groups: group 1 were 20 male rats from parents with addiction histories (F1-MEP), group 2 was composed of 20 male rats from control parents (F1-SEP), group 3 were five female rats from parents with addiction history, and group 4 were five female rats from control parents. Five male rats from group 1 and Five male rats from group 2 were randomly selected and sacrificed for further analysis of brain gene expression. Ten male animals in these two groups (1 and 2) were first subjected to the elevated plus maze (EPM) and then morphine preference tests with details described below. Each test was performed only once for each animal. Five remaining male animals in group1 were allowed to mate with five female rats of group 3 (one male and one female rat in each box). Similarly, five remaining male animals in group 2 and five female animals of group 4 were also coupled (one male and one female rat in each box). Thus, the male and female offspring of addicted parents mated with each other, and the male and female offspring of control animals coupled separately to create second-generation (F2) litter. The F2 adult male rats (F2-MEP and F2-SEP) were also tested respectively, for anxiety level with EPM and morphine preference with the two-bottle-choice paradigm. The summary of experimental design has been shown in Fig. 1.

It should be mentioned that in all the processes of the study, assignment of animals to different groups was completely random. It means that the selection of animals for decapitation or behavioral tests were randomly performed using the code number of each animal and the experimenter was blind to group assignments.

2.4. Elevated Plus-Maze test (EPM)

2.4.1. Apparatus

The EPM apparatus was a gray plastic cross-shaped maze containing four arms (two open and two closed arms) organized in the form of a plus sign. The open arms possessed no walls (50 cm × 10 cm); however, a rim of Plexiglas (0.5 cm high) bordered the perimeter of the open arms to avoid the rats from falling. The closed arms were bordered by 40 cm high walls (50 cm × 10 cm × 40 cm). A square platform of 10 cm × 10 cm without any walls was present at the place where the four arms intersected. The entire apparatus was 50 cm higher from the base.

2.4.2. Behavioral testing

The animals were handled for 3 min for two days before the test. All experiments were performed in a noiseless room with dim light separated from the colony room. Animals were transferred to the test room 1 h before beginning of experiments in order to adapt to the new environment. Each rat was put in the midpoint of the maze fronting a closed arm and was allowed to explore freely for the period of 10 min of testing. The trials were recorded by a video camera connected to a monitor and a video camera recorder in a nearby room. Records were evaluated and scored by an observer who was blind to the experiments. The number of entries (with all four paws) into and the total time spent in open and closed arms were recorded. The percentage of open arm time (% OAT) (the ratio of the total time spent in the open arms to the total time spent in four arms × 100) and open arm entries (% OAE) (the ratio of the total entries into the open arms to the total entries in four arms × 100) were used as the standard anxiety indices. General locomotor activity was calculated by evaluating total closed arm entries (Rodgers and Johnson, 1995).

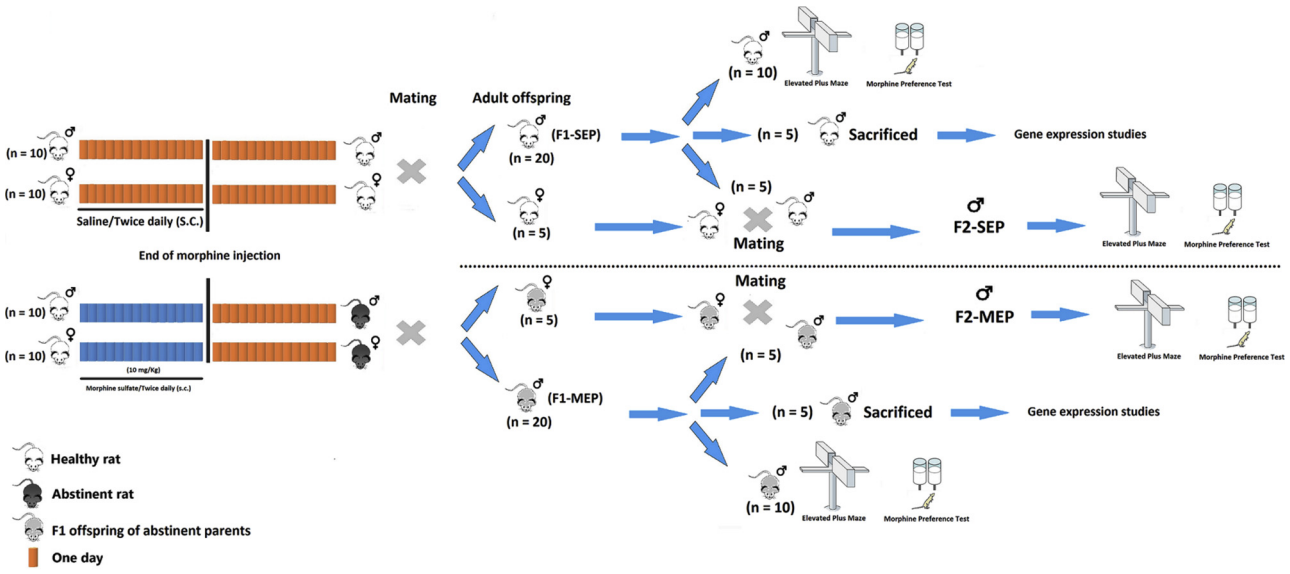

Fig. 1. The schematic diagram of experimental design.

2.5. Morphine preference and consumption

In order to investigate morphine preference in animals, two-bottle-choice paradigm (Planeta, 2013) was used in the study. It is a non-operant self-administration method limited to the oral route of administration. Each rat could choose between two bottles, which one of them contains a solution of 2% sucrose, and the other one is filled with a solution of 2% sucrose plus 25 mg/L morphine. At first, the animals had to learn the possibility of free choice in the mentioned model. Thus, they were maintained individually in standard cages and trained to consume water from both flasks. After one week, one of the bottles was substituted by a bottle containing sucrose solution (2%), and the other one was exchanged by morphine solution (25 mg/L in 2% sucrose) for eight weeks. Fluid intake was measured twice a week, and all drunk solutions were totally replaced each week. In order to avoid side preference, the place of bottles was reversed every other day. Morphine consumption has been calculated as mg of morphine per kg for 24 h. Morphine preference has been defined as the percentage of the volume of consumed morphine (ml per 24 h) divided by the total fluid intake (ml per 24 h).

2.6. Brain tissue collection

With the purpose of studying the effect of parental morphine exposure on the gene expression level of dopamine receptors in the offspring's brain, 5 randomly selected rats from F1-MEP and 5 male rats from F1-SEP groups were anesthetized with i.p. injection of 3.5% sodium-pentobarbital (3 ml/kg) and decapitated after 5 min by quick guillotining. Then, the whole brain was extracted and immediately immersed in chilly phosphate-buffered-saline (PBS) for about 3 min. After that, the brain was placed on a sterile dish which was put on ice, and a sagittal cut was performed to divide the hemispheres. One of the hemispheres was returned to the cold PBS, and the other one was cut coronally 1 mm in front of the forceps minor of the corpus callosum (cut 1) using the rat brain atlas of Paxinos and Watson (2007) in order to separate the PFC. After that, another cut was performed in front of the column of the fornix (cut 2). The tissue existing between cut 1 and cut 2 includes NAc and striatum areas. The NAc was isolated by a proper punch and then the striatum was separated. The hippocampus was smoothly isolated by cutting up between the visual cortex and superficial gray layer of the superior colliculus. Brain tissues were put immediately in individual micro-tubes and immersed in liquid nitrogen. After a while, micro-tubes were moved to the −80 °C freezer.

2.7. Total RNA extraction and reverse transcription

Total RNA was extracted from four brain regions (PFC, NAc, striatum, and hippocampus) using RNeasy Lipid Tissue Mini Kit (Qiagen, USA) according to the manufacturer's protocol. Quantity, purity, and integrity of RNA were determined by spectrophotometry and gel electrophoresis (1.2% agarose; Gibco/BRL). Using Prime Script First Strand cDNA Synthesis Kit (Takara, Japan), 1 µg of extracted RNA was converted into cDNA by reverse transcription in a final volume of 20 µl.

2.8. Real-time PCR primers

Real-time PCR amplification of dopamine D1, D2, D3, D4, and D5 receptors, and beta-actin (as the housekeeping gene) were performed using oligonucleotide primers purchased from the Qiagen company primer bank (see Table 1).

2.9. Real-time PCR

All Real-Time PCR amplifications were done using 2 µl cDNA, specific primers and SYBR Green Master Mix (Takara, Japan) according to the manufacturer's protocol on a StepOnePlus Real-Time PCR System (Applied Biosystems) in a final volume of 25 µl. The annealing temperature for all primer pairs was adjusted in 60 °C. For each gene, the specificity of the PCR product was accepted by confirming a single peak

Table 1
list of the primers used in real-time PCR reactions of the study.

Gene name	Product name	Cat. No.	Company
Rat dopamine D1 receptor	Rn_Drd1_1_SG QuantiTect Primer Assay	QT00386631	Qiagen
Rat dopamine D2 receptor	Rn_Drd2_1_SG QuantiTect Primer Assay	QT01081990	
Rat dopamine D3 receptor	Rn_Drd3_1_SG QuantiTect Primer Assay	QT00182392	
Rat dopamine D4 receptor	Rn_Drd4_1_SG QuantiTect Primer Assay	QT00493591	
Rat dopamine D5 receptor	Rn_Drd5_1_SG QuantiTect Primer Assay	QT00459466	

in melting curve analysis. For additional length confirmation, PCR products were observed on 2% agarose gel with ethidium bromide in gel documentation. The amount of target gene of each sample was calculated from the cycle at which the sample fluorescence came across a predetermined threshold (Ct) significantly beyond the background. After that, the Ct was referred to a standard curve present on each reaction run, which was depicted using standard 10-fold serial dilutions of a cDNA sample with unknown absolute concentration. Thus, the concentrations of the serial dilutions were only relatively assumed. Samples were tested in duplicate, and the mean was used for further analysis. All the data of sample and control groups of the study were normalized using beta-actin as the housekeeping gene.

2.10. Statistical analysis

Behavioral tests were evaluated by SPSS statistic software (version 21). The mean number of each group was defined, and the data were stated as the mean ± standard error of the mean (SEM). Variances among groups in behavioral experiments (EPM and morphine intake tests) were evaluated by one-way analysis of variance (ANOVA) followed by a post-hoc test of Tukey. $P < 0.05$ was defined as statistically significant. Analysis of gene expression data was performed by Relative Expression Software Tool (REST)-XL version 2 (Pfaffl et al., 2002) which is used to compare sample and control group in terms of significant variations of relative expression state. The software uses pairwise fixed reallocation randomization test to define the significance of the results. One of the advantages of this software is that gene quantification and normalization are performed simultaneously. Data are shown as fold differences of mean normalized expression values ± SEM. $P < 0.05$ is considered statistically significant.

3. Results

3.1. Litters survival

The total number of F1 litters in morphine-treated parents were 72 animals, of which 21 died (29.16%). F1 litters in saline-treated parents were 69 of which 9 animals died (13%). Most of the deaths occurred in the first week after birth. We practically observed that maternal care was deficient in morphine-treated mothers compared to the saline-treated ones. Control mothers kept their litters close to themselves and cared more about feeding and licking the litters. However, the litters of morphine-treated mothers were scattered in the cage and were less fed and licked compared to the control litters. Although the total number of litters was not significantly different between two groups (72 in morphine-treated and 69 in control females), the consequence of motherhood deficiency in morphine-treated females was reflected in the number of dead litters in both groups (21 in morphine-treated and 9 in control animals). This finding is in agreement with previous studies reporting that maternal care is insufficient in morphine-exposed female animals (for example, see Johnson et al., 2011).

3.2. Measurement of anxiety level by elevated plus maze test (EPM)

Fig. 2(A) is the comparison of %OAT between F1-MEP, F1-SEP, F2-MEP and F2-SEP groups. One-way ANOVA showed that a significant difference exists between experimental groups ($F(3, 28) = 3.112$, $P < 0.05$). Post-hoc analysis revealed that %OAT was reduced in the F1-MEP group compared to F1-SEP animals ($P < 0.05$). However, the percentage of OAT of F2-MEP rats was not statistically different from the F2-SEP group.

Fig. 2(B) presents the data of percentage of open arm entries (OAE %) in the experimental groups. One-way ANOVA showed that % OAE did not change significantly between groups ($F(3, 28) = 0.086$, $P > 0.05$).

Fig. 2(C) demonstrates total locomotion in all four groups of the study. One-way ANOVA showed that experimental groups are significantly different from each other ($F(3, 28) = 3.699$, $p < 0.05$). Post-hoc analysis showed that total locomotion was decreased in the F1-MEP group compared to F1-SEP animals ($P < 0.05$). However, total locomotion of F2-MEP animals was not statistically different from F2-SEP rats.

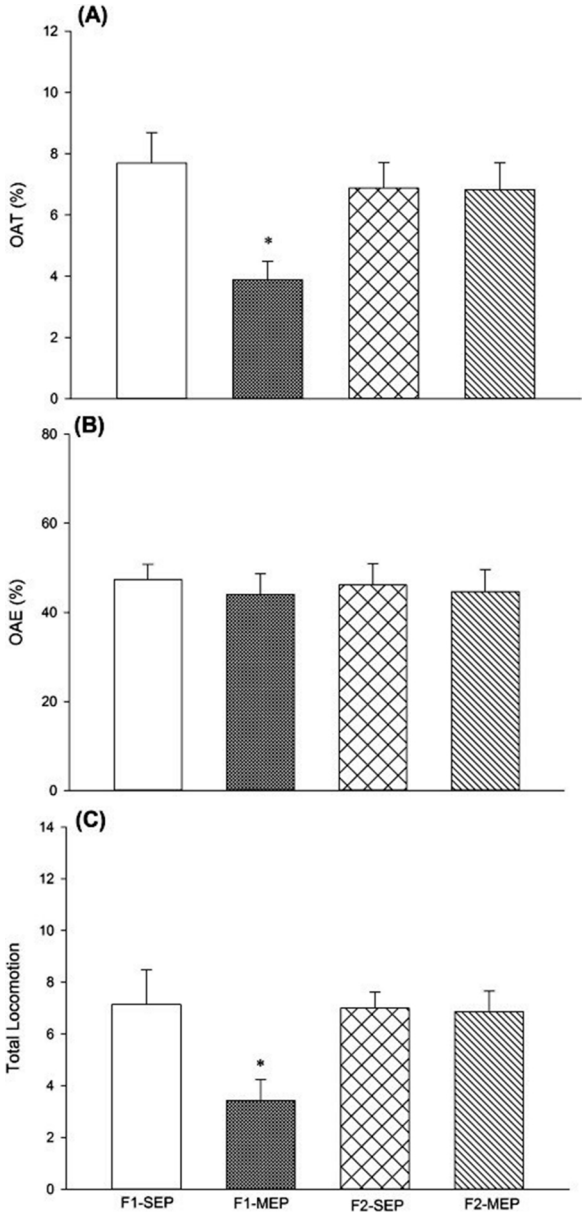

Fig. 2. The anxiety level measured by % OAT (A), % OAE (B) and total locomotion (C) in F1-SEP, F1-MEP, F2-SEP, and F2-MEP groups in EPM test. The bars represent mean ± SEM of eight animals in each group. *$P < 0.05$ different from F1-SEP group.

3.3. Evaluation of morphine solution intake

Fig. 3(A) demonstrates morphine preference (%) in experimental groups in a duration of 8 weeks. One-way ANOVA showed that rats in the F1-MEP group had more preference for morphine in comparison to other groups [$F(31, 288) = 47.21$, $P < 0.001$]. According to the result of post-hoc analysis, it was found that at the end of each week except week 1, morphine preference in the F1-MEP animals was significantly greater than the F1-SEP rats ($P < 0.01$ for weeks 2, 4, and 5; $P < 0.001$ for weeks 3, 6, 7 and 8). The data also revealed that

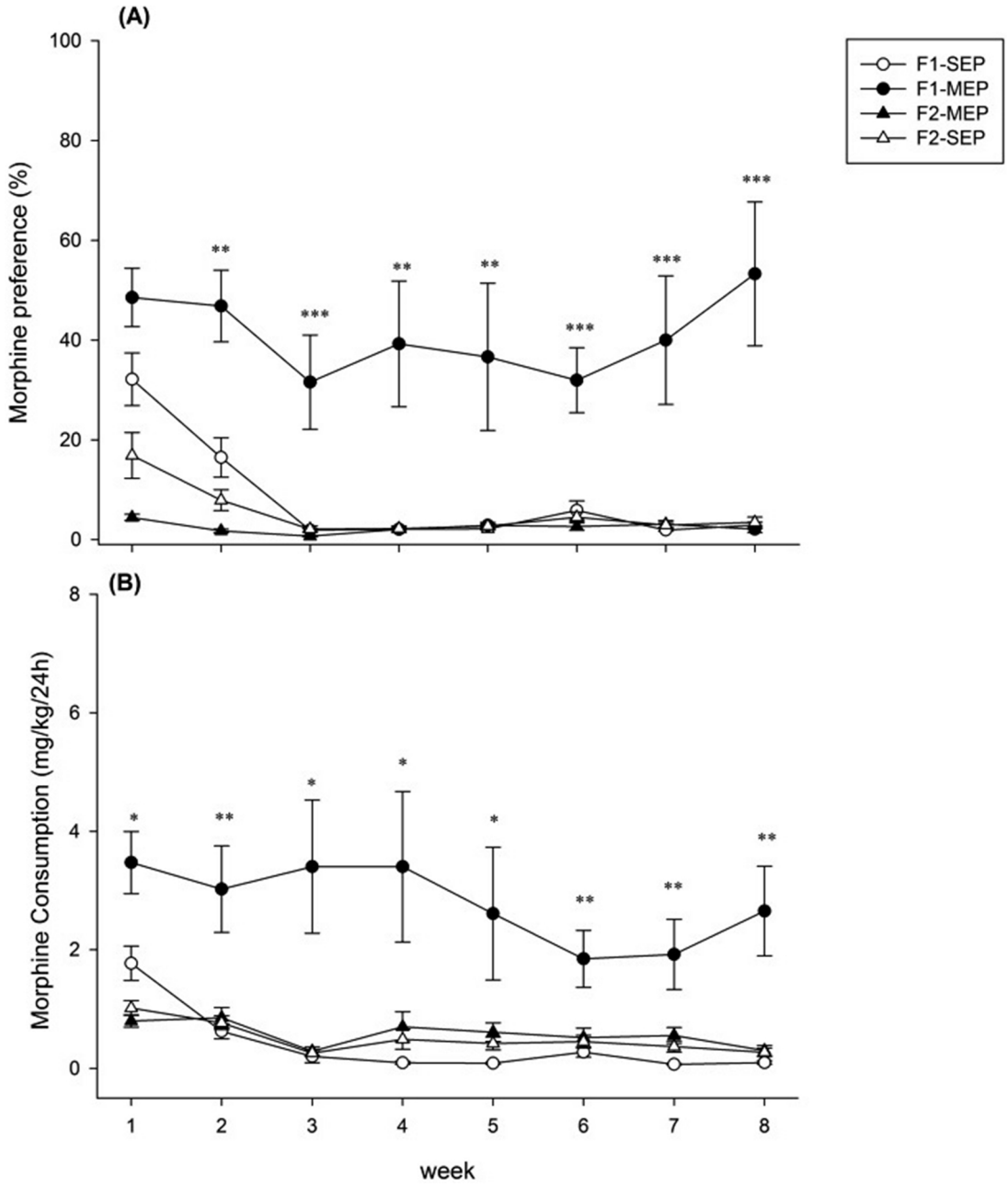

Fig. 3. Oral morphine preference (A) and consumption (25 mg/L) (B) measured by the two-bottle-choice paradigm in F1-SEP, F1-MEP, F2-SEP, and F2-MEP rats for eight weeks (n = 10). The results are expressed as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in comparison to F1-SEP group.

morphine preference was not statistically different between F2-MEP and F2-SEP animals.

Fig. 3(B) shows morphine intake (mg/kg/24 h) in experimental groups through a period of eight weeks. One-way ANOVA revealed that F1-MEP rats consumed more drug in comparison to other animal groups [F (31, 288) = 46.46, $P < 0.001$]. Post-hoc analysis clarified that morphine intake at the end of each week in F1-MEP rats was significantly higher than F1-SEP animals ($P < 0.05$ for weeks 1, 3, 4, and 5; $P < 0.01$ for the rest of the weeks). However, morphine consumption in the F2-MEP group was not statistically different from F2-SEP animals. It should be stated that total liquid consumption through the experiment and water intake at the start of the experiment was not significantly different between study groups (data not shown).

3.4. Expression of dopamine receptors in the offspring's PFC

Fig. 4 shows the data for comparison of the mRNA expression level of D1 (A), D2 (B), D3 (C), D4 (D) and D5 (E) dopamine receptors in the PFC between F1-MEP and F1-SEP animals. The D1 dopamine receptor was up-regulated in F1-MEP animals by the factor 1.75 which was statistically significant from the F1-SEP group ($P < 0.05$). D2 and D3 dopamine receptors were also up-regulated in the F1-MEP group (by the factor 1.1 and 1.56, respectively) which were not statistically different from F1-SEP rats ($P > 0.05$). The data also showed that the expression level of D4 dopamine receptor in F1-MEP animals was significantly reduced in comparison to F1-SEP rats by the factor 4.16 ($P < 0.01$). Similar to the D1 dopamine receptor, the D5 receptor was up-regulated in F1-MEP rats compared to the F1-SEP group by the factor 3.26 ($P < 0.01$). Beta-actin expression was the same in all tested groups (data not shown).

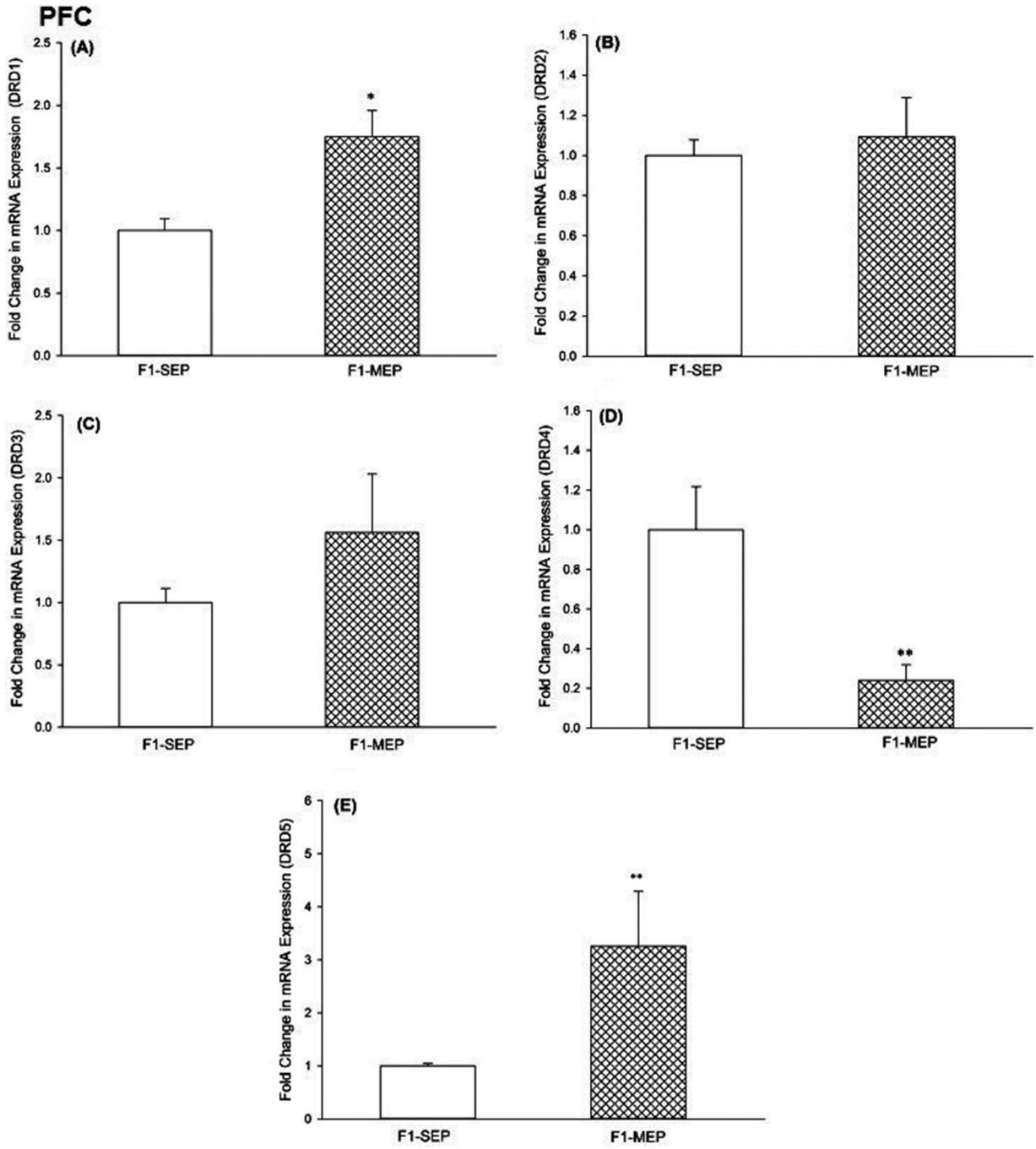

Fig. 4. The mRNA expression of D1(A), D2 (B), D3 (C), D4 (D), and D5 (E) dopamine receptors in the PFC of F1-SEP and F1-MEP groups. Bars represent fold differences of mean normalized expression values ± S.E.M. (n = 5). * $P < 0.05$, ** $P < 0.01$ from the F1-SEP group.

3.5. Expression of dopamine receptors in the offspring's NAc

Fig. 5 represents the mRNA expression level of D1 (A), D2 (B), D3 (C), and D5 (D) dopamine receptors in the NAc of F1-MEP and F1-SEP animals. D1 Dopamine receptor was up-regulated by the factor 1.404 ($P < 0.05$) in the F1-MEP group; however, the decrease of the D2 Dopamine receptor (by the factor 1.247) and the increase of D3 receptor (by the factor 1.33) in the F1-MEP group was not statistically significant compared to F1-SEP animals ($P > 0.05$). Like D1 dopamine receptor, the D5 receptor was up-regulated in F1-MEP animals compared to the F1-SEP group by the factor 6.5 ($P < 0.001$).

It should be mentioned that D4 dopamine receptor was not found in the NAc which is similar to the previous reports indicating that the level of this receptor is inconsiderable in this brain region (Khan et al., 1998; Oak et al., 2000).

3.6. Expression of dopamine receptors in the offspring's hippocampus

The mRNA expression level of D1 (A), D2 (B), D3 (C), D4 (D), and D5 (E) dopamine receptors in the hippocampus of F1-MEP and F1-SEP animals has been depicted in Fig. 6. The expression level of D1 dopamine receptor in the hippocampus of F1-MEP rats was up-regulated by the factor 1.1 which was not different from the F1-SEP group ($P > 0.05$). D2 dopamine receptor was significantly decreased in F1-MEP by the factor 2.892 ($P < 0.001$). D3 and D4 dopamine receptors were both up-regulated in F1-MEP rats (by the 1.53 and 3.21, respectively)

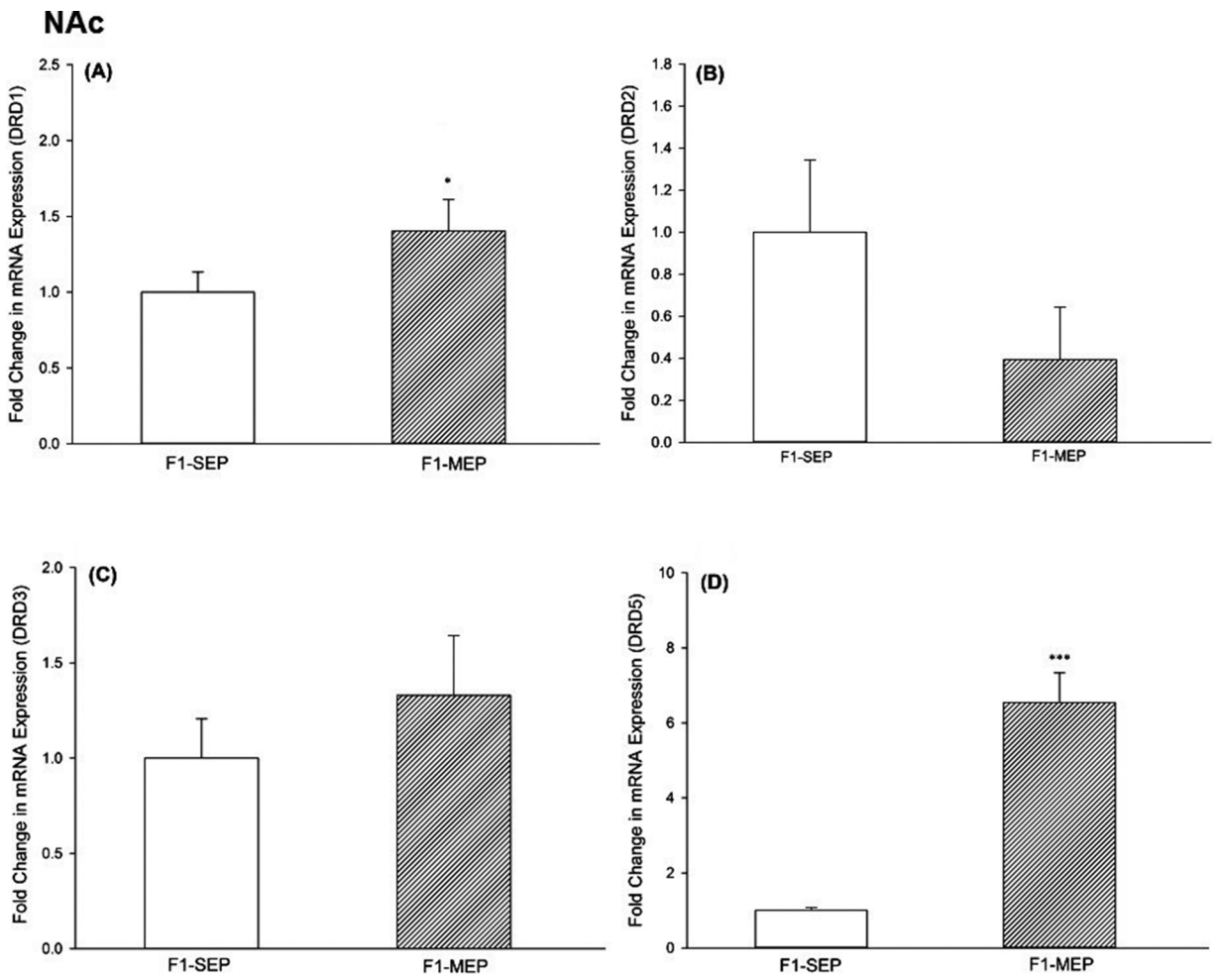

Fig. 5. The mRNA expression of D1(A), D2 (B), D3 (C), and D5 (D) dopamine receptors in the NAc of F1-SEP and F1-MEP groups. Bars represent fold differences of mean normalized expression values ± S.E.M. (n = 5). * $P < 0.05$, *** $P < 0.001$ from the F1-SEP group.

which was only significant for D4 dopamine receptor ($P < 0.001$). The mRNA expression of the D5 receptor in F1-MEP rats was significantly down-regulated compared to F1-SEP animals by the factor 3.1 ($P < 0.05$).

3.7. Expression of dopamine receptors in the offspring's striatum

The mRNA expression level of D1 (A), D2 (B), D3 (C), D4 (D), and D5 (E) dopamine receptors in the striatum of F1-MEP and F1-SEP animals has been shown in Fig. 7. D1, D2, and D3 dopamine receptors were all reduced in the striatum of F1-MEP rats by the factor 1.12, 1.2, and 1.37, respectively, which none of them was statistically different from their control groups ($P > 0.05$). D4 and D5 dopamine receptors were both increased in the F1-MEP group (by the factor 3 and 1.24, respectively) which was only significant for D4 dopamine receptor ($P < 0.001$).

4. Discussion

Both genetic and environmental factors are involved in drug addiction. Recent evidence has shown that epigenetics may potentially influence the trans-generational effects of drug abuse. Hypermethylation of mu opioid receptors in the sperm samples of opioid-addicted individuals may be an example of the effects of drugs that could be transferred to the next generation and influence their addiction susceptibility (Chorbov et al., 2011). Besides, mu, kappa and delta opioid receptors are found in oocytes, which may be involved in the trans-generational inheritance of addiction (Yohn et al., 2015). Furthermore, previous studies have shown that altered response to morphine is transmittable to the next generation of morphine administered rats (Akbarabadi et al., 2018; Byrnes, 2005; Byrnes et al., 2013). Physiological and organic alterations in the brain of the progeny of morphine exposed parents have also been reported (Cicero et al., 1991; Sarkaki et al., 2008).

Our study showed that parental morphine exposure in the adulthood is associated with an increased tendency toward morphine consumption in F1 but not F2 male offspring. Thus, it seems that trans-generational effects of parental morphine consumption disappear in the second generation. In support of our data, it has been suggested previously that exposure of adolescent female rats to opioids may increase the risk of substance use in their offspring (Vassoler et al., 2014). Furthermore, studies have shown that offspring of alcohol-preferring rats shows greater nicotine consumption and reinstatement after extinction compared to the offspring of alcohol-non preferring animals (He et al., 2006). In the addicted subjects' children, higher rates of psychopathologic disorders such as depression, substance abuse, and suicidal behaviors have also been observed (Corte and Becherer, 2007). The reasons for the above-mentioned effects are not much understood; However, it has been shown that morphine exposure during the adolescent period may change maternal care (Johnson et al., 2011). Furthermore, both female and male adolescence opiate exposure may lead to neurodevelopmental alterations that increase the risk of substance abuse in the offspring (Byrnes et al., 2013).

Here we found that in the first generation of morphine-exposed

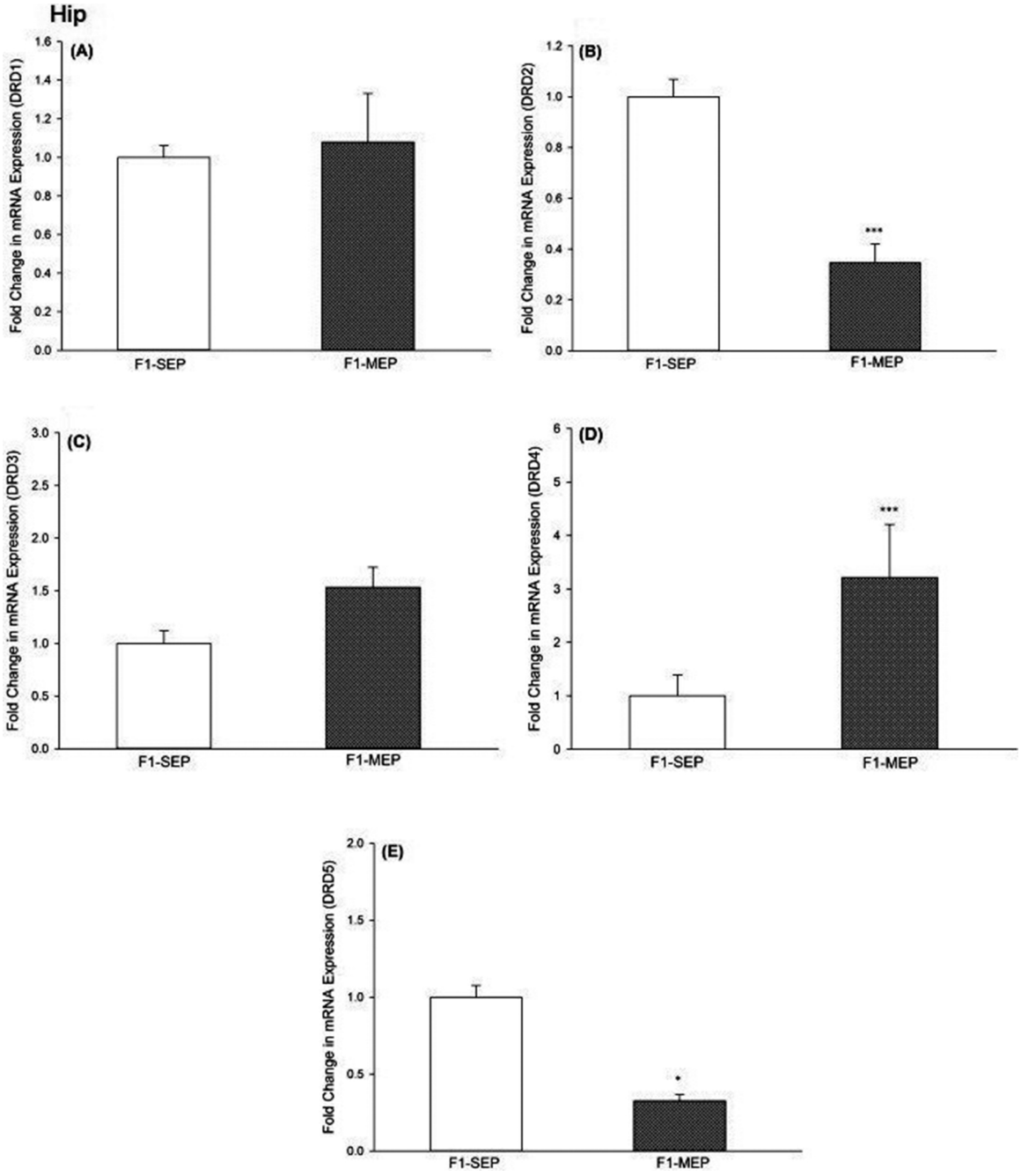

Fig. 6. The mRNA expression of D1(A), D2 (B), D3 (C), D4 (D), and D5 (E) dopamine receptors in the hippocampus of F1-SEP and F1-MEP groups. Bars represent fold differences of mean normalized expression values ± S.E.M. (n = 5). * $P < 0.05$, *** $P < 0.001$ from the F1-SEP group.

parents (F1-MEP), open arm time and total locomotion were decreased while open arm entry was not altered in the EPM test. Thus, it seems that anxiety-like behavior is increased in the male offspring of morphine-exposed parents. In support of our data, previous studies have demonstrated that prenatal morphine exposure increases anxiety in EPM test in the F1 offspring (Chen et al., 2015).

In order to clarify the possible mechanisms involved in morphine preference in the offspring of morphine exposed parents, we evaluated the pattern of gene expression of dopamine receptors in four important reward sites of the brain (PFC, NAc, hippocampus, and striatum). Gene expression study was not performed in the F2 offspring because their morphine preference and anxiety level were not different from their control group. We found that D1-like dopamine receptors were significantly increased in the NAc and PFC of the F1-MEP group. D1 dopamine receptor is found in all dopaminergic neurons projecting areas of the nigrostriatal and mesocorticolimbic pathways (Fremeau et al., 1991). The receptor modulates opioid-induced neuroadaptations, reward, and reinforcement. Polymorphisms of D1 dopamine receptor gene may affect susceptibility to opioid dependence (Zhu et al., 2013). Alcohol consumption and preference and similarly self-administration of cocaine are reduced in D1-like dopamine receptors knockout mice (El-Ghundi et al., 1998). Thus, the up-regulation of D1 dopamine receptors in the brain reward pathway of the F1-MEP group may be a mechanism for higher preference to morphine in these animals.

D5 dopamine receptors are mostly expressed in the thalamus, hippocampus, and on the dopaminergic neurons in the ventral tegmental area (Ciliax et al., 2000). The role of these receptors in the brain is not completely determined. However, D5 receptors may participate in

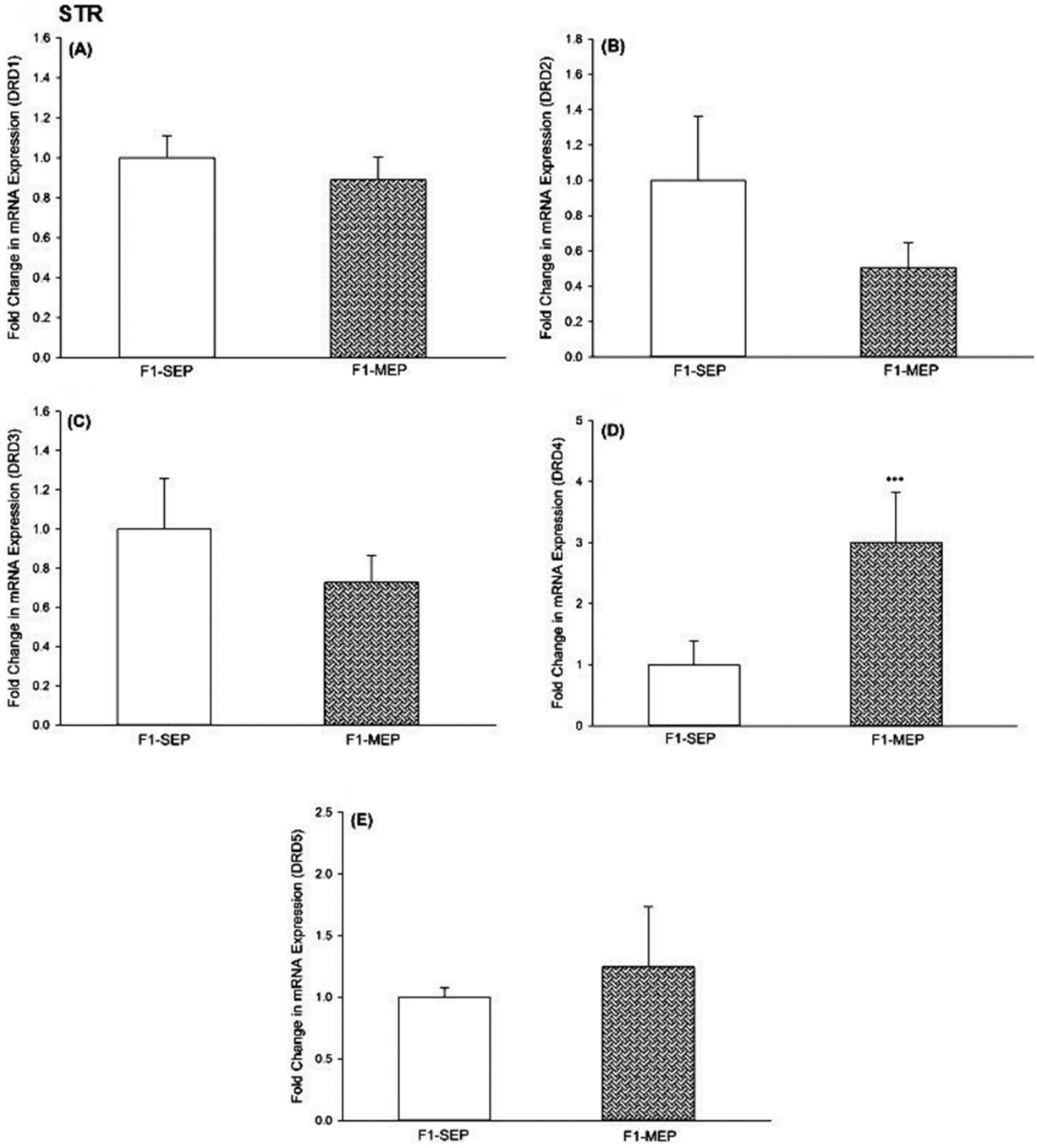

Fig. 7. The mRNA expression of D1(A), D2 (B), D3 (C), D4 (D), and D5 (E) dopamine receptors in the striatum of F1-SEP and F1-MEP groups. Bars represent fold differences of mean normalized expression values ± S.E.M. (n = 5). *** $P < 0.001$ from the F1-SEP group.

emotion regulation, memory, and response to novel stimuli (Knight, 1996). It has been shown that intra-NAc injection of D5 receptor antisense oligonucleotide could lead to the selective defeat of cocaine recognition (Filip et al., 2000). Lack of LTP development in DRD5 +/- mice proposes that the receptor is needed for the normal formation of synaptic plasticity (Swant et al., 2010). We found that D5 dopamine receptor was increased in the NAc, PFC, and striatum that was significant in the NAc and PFC. Besides, the receptor was considerably down-regulated in the hippocampus. Clinical studies have suggested that D5 dopamine receptor may be a candidate gene for the beginning of smoking and development to nicotine addiction (Sullivan et al., 2001). A very limited genetic association and linkage studies propose that D5 receptor gene may be a candidate gene for drug abuse (Li et al., 2006; Straub et al., 1999; Vanyukov et al., 1998). Thus, the overexpression of D5 receptor in our F1-MEP rats may be another mechanism for increased morphine preference in these animals that needs to be further evaluated in the future.

Our data also showed that D2 dopamine receptor was down-regulated in the hippocampus, NAc and striatum of F1-MEP animals, which was only significant in the hippocampus. Both agonists and antagonists of D2 dopamine receptor are able to decrease heroin self-administration (Hemby et al., 1996; Rowlett et al., 2007). Knockout studies have suggested a critical role for D2 dopamine receptor in mediation of morphine-induced CPP (Dockstader et al., 2001; Maldonado et al., 1997) or self-administration behavior (Elmer et al., 2002). It has been reported that heroin reinforcement in rhesus monkeys is blocked by D2

receptor agonists (Rowlett et al., 2007). Thus, it seems reasonable that down-regulation of D2 dopamine receptor in the F1-MEP rat brain may be a risk factor toward morphine preference and dependence.

In F1-MEP animals, the D3 dopamine receptor was increased in the NAc, hippocampus, and PFC and decreased in the striatum but the changes were not statistically significant. Studies have suggested that D3 dopamine receptor plays a considerable role in heroin approach behaviors motivated by conditioned stimuli (Galaj et al., 2015). The receptor up-regulates after alcohol (Vengeliene et al., 2006) and nicotine (Le Foll et al., 2003) exposure in the rat striatum and after chronic opioid consumption in human peripheral blood lymphocytes (Goodarzi et al., 2009). Researchers have reported that antagonists of D3 dopamine receptor reduce drug cue-induced reinstatement of drug seeking (Cervo et al., 2005; Galaj et al., 2014; Gilbert et al., 2005). Therefore, it is not surprising that the receptor was up-regulated (although not significantly) in our F1-MEP rats that had a higher preference for morphine compared to their control group.

We also found that D4 dopamine receptor was significantly up-regulated in hippocampus and striatum and down-regulated in the PFC. The receptor is found in especially brain areas that are involved in reward and reinforcement such as striatum (Rivera et al., 2002), cerebral cortex, and hippocampus (Suzuki et al., 1995). It should be mentioned that we did not find D4 receptor expression in the NAc of study animals. This is in agreement with previous experiments reporting the lack of effect of intra-accumbal administration of L-750,667 (a selective antagonist of D4 dopamine receptor) on cocaine-seeking behavior (Anderson et al., 2006). Other experiments have also reported that the expression of D4 dopamine receptor in the NAc is too low to modify the reinforcing effects of drugs of abuse (Khan et al., 1998; Oak et al., 2000). It has been suggested that insufficient expression of this receptor in the reward system can increase the susceptibility toward drug addiction (Czermak et al., 2004). In addition, a famous polymorphism exists in the exon III of D4 dopamine receptor gene, which is expressed as a 48-bp variable number of tandem repeats (Ding et al., 2002). When the repeat number reaches 7 (7R), the response of the resulted receptor to dopamine is diminished (Rao et al., 1994). Human studies have reported that the 7R allele is a risk factor for drug addiction (Li et al., 1997; Shields et al., 1998) and is related to decrease of gene expression in human brain tissue (Simpson et al., 2010). Earlier, we have shown that the expression of D4 receptor is considerably reduced in peripheral blood lymphocytes of opioid addicts (Goodarzi et al., 2009).

The reason behind the observed up- and down-regulation of dopamine receptors in the brain in the F1-MEP group may reside in epigenetic mechanisms such as DNA methylation or histone acetylation. For example, it has been shown that chronic administration of morphine increases acetylation of histone H3 lysine 14 in the NAc (Sheng et al., 2011) and basolateral amygdala in rats (Wang et al., 2015). Therefore, it is reasonable to hypothesize that histone acetylation may be involved in D1-like dopamine receptor up-regulation in F1-MEP animals. Similarly, DNA methylation may be the reason for down-regulation of dopamine receptors in reward sites of the brain. All of these hypotheses need to be examined in future studies.

5. Conclusion

The data of the present study revealed that chronic parental morphine exposure of adult rats before gestation results in higher morphine preference and increased anxiety in their F1 but not F2 male offspring. It is important to highlight that there was no gestational exposure to opioids in the F1 animals because parents were remained drug-free for two weeks prior to mating. Furthermore, our results showed that alterations in the expression profile of dopamine receptors in the reward pathway might be one of the mechanisms involved in observed changes in F1 animals, which could account for susceptibility to opioid abuse in the litter of morphine-exposed animals.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors acknowledge the grant support of the Tehran University of Medical Sciences (94-02-49-29647).

References

Akbarabadi, A., Niknamfar, S., Vousooghi, N., Sadat-Shirazi, M.S., Toolee, H., Zarrindast, M.R., 2018. Effect of rat parental morphine exposure on passive avoidance memory and morphine conditioned place preference in male offspring. Physiol. Behav. 184, 143–149.

Anderson, S.M., Schmidt, H.D., Pierce, R.C., 2006. Administration of the D2 dopamine receptor antagonist sulpiride into the shell, but not the core, of the nucleus accumbens attenuates cocaine priming-induced reinstatement of drug seeking. Neuropsychopharmacology 31, 1452–1461.

Anier, K., Malinovskaja, K., Aonurm-Helm, A., Zharkovsky, A., Kalda, A., 2010. DNA methylation regulates cocaine-induced behavioral sensitization in mice. Neuropsychopharmacology 35, 2450–2461.

Bohacek, J., Mansuy, I.M., 2013. Epigenetic inheritance of disease and disease risk. Neuropsychopharmacology 38, 220–236.

Byrnes, E.M., 2005. Transgenerational consequences of adolescent morphine exposure in female rats: effects on anxiety-like behaviors and morphine sensitization in adult offspring. Psychopharmacology 182, 537–544.

Byrnes, J.J., Babb, J.A., Scanlan, V.F., Byrnes, E.M., 2011. Adolescent opioid exposure in female rats: transgenerational effects on morphine analgesia and anxiety-like behavior in adult offspring. Behav. Brain Res. 218, 200–205.

Byrnes, J.J., Johnson, N.L., Carini, L.M., Byrnes, E.M., 2013. Multigenerational effects of adolescent morphine exposure on dopamine D2 receptor function. Psychopharmacology 227, 263–272.

Cervo, L., Burbassi, S., Colovic, M., Caccia, S., 2005. Selective antagonist at D3 receptors, but not non-selective partial agonists, influences the expression of cocaine-induced conditioned place preference in free-feeding rats. Pharmacol. Biochem. Behav. 82, 727–734.

Chen, H.H., Chiang, Y.C., Yuan, Z.F., Kuo, C.C., Lai, M.D., Hung, T.W., Ho, I.K., Chen, S.T., 2015. Buprenorphine, methadone, and morphine treatment during pregnancy: behavioral effects on the offspring in rats. Neuropsychiatric Dis. Treat. 11, 609–618.

Chorbov, V.M., Todorov, A.A., Lynskey, M.T., Cicero, T.J., 2011. Elevated levels of DNA methylation at the OPRM1 promoter in blood and sperm from male opioid addicts. J. Opioid Manage. 7, 258–264.

Christoffersen, M.N., Soothill, K., 2003. The Long-term consequences of parental alcohol abuse: a cohort study of children in Denmark. J. Subst. Abuse Treat. 25, 107–116.

Cicero, T.J., Adams, M.L., Giordano, A., Miller, B.T., O'Connor, L., Nock, B., 1991. Influence of morphine exposure during adolescence on the sexual maturation of male rats and the development of their offspring. J. Pharmacol. Exp. Therap. 256, 1086–1093.

Ciliax, B.J., Nash, N., Heilman, C., Sunahara, R., Hartney, A., Tiberi, M., Rye, D.B., Caron, M.G., Niznik, H.B., Levey, A.I., 2000. Dopamine D(5) receptor immunolocalization in rat and Monkey brain. Synapse (New York, N.Y.) 37, 125–145.

Cloninger, C.R., Bohman, M., Sigvardsson, S., 1981. Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. Arch. Gen. Psychiatry 38, 861–868.

Corte, C., Becherer, M., 2007. Differential effects of maternal and paternal alcoholism and gender on drinking, alcohol-related self-cognition, and psychopathology. J. Addict. Nurs. 18, 175–185.

Craig, M.M., Bajic, D., 2015. Long-term behavioral effects in a rat model of prolonged postnatal morphine exposure. Behav. Neurosci. 129, 643–655.

Czermak, C., Lehofer, M., Wagner, E.M., Prietl, B., Lemonis, L., Rohrhofer, A., Schauenstein, K., Liebmann, P.M., 2004. Reduced dopamine D4 receptor mRNA expression in lymphocytes of Long-term abstinent alcohol and heroin addicts. Addiction (Abingdon, Engl.) 99, 251–257.

Danchin, É., Charmantier, A., Champagne, F.A., Mesoudi, A., Pujol, B., Blanchet, S., 2011. Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. Nat. Rev. Genet. 12, 475–486.

Di Chiara, G., Tanda, G., Cadoni, C., Acquas, E., Bassareo, V., Carboni, E., 1998. Homologies and differences in the action of drugs of abuse and a conventional reinforcer (food) on dopamine transmission: an interpretative framework of the mechanism of drug dependence. Adv. Pharmacol. 42, 983–987.

Ding, Y.C., Chi, H.C., Grady, D.L., Morishima, A., Kidd, J.R., Kidd, K.K., Flodman, P., 2002. Evidence of positive selection acting at the human dopamine receptor D4 Gene Locus. Proc. Natl. Acad. Sci. U. S. A. 99, 309–314.

Dockstader, C.L., Rubinstein, M., Grandy, D.K., Low, M.J., Kooy, D.V., 2001. The D2 receptor is critical in mediating opiate motivation only in opiate-dependent and withdrawn mice. Eur. J. Neurosci. 13, 995–1001.

El-Ghundi, M., George, S.R., Drago, J., Fletcher, P.J., Fan, T., Nguyen, T., Liu, C., Sibley, D.R., Westphal, H., O'Dowd, B.F., 1998. Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. Eur. J. Pharmacol. 353, 149–158.

Elmer, G.I., Pieper, J.O., Rubinstein, M., Low, M.J., Grandy, D.K., Wise, R.A., 2002. Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. J. Neurosci. 22, RC224.

Filip, M., Thomas, M.L., Cunningham, K.A., 2000. Dopamine D5 receptors in nucleus accumbens contribute to the detection of cocaine in rats. J. Neurosci. 20, RC98.
Fremeau, R.T., Duncan, G.E., Fornaretto, M.G., Dearry, A., Gingrich, J.A., Breese, G.R., Caron, M.G., 1991. Localization of D1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. Proc. Natl. Acad. Sci. U. S. A. 88, 3772–3776.
Galaj, E., Ananthan, S., Saliba, M., Ranaldi, R., 2014. The effects of the novel DA D3 receptor antagonist SR 21502 on cocaine reward, cocaine seeking and cocaine-induced locomotor activity in rats. Psychopharmacology 231, 501–510.
Galaj, E., Manuszak, M., Babic, S., Ananthan, S., Ranaldi, R., 2015. The selective dopamine D3 receptor antagonist, SR 21502, reduces cue-induced reinstatement of heroin seeking and heroin conditioned place preference in rats. Drug Alcohol Depend. 156, 228–233.
Gilbert, J.G., Newman, A.H., Gardner, E.L., Ashby, C.R., Heidbreder, C.A., Pak, A.C., Peng, X.Q., Xi, Z.X., 2005. Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: role of dopamine D3 receptors. Synapse 57, 17–28.
Goldman, D., Oroszi, G., Ducci, F., 2005. The genetics of addictions: uncovering the genes. Nat. Rev. Genet. 6, 521–532.
Goodarzi, A., Vousooghi, N., Sedaghati, M., Mokri, A., Zarrindast, M.R., 2009. Dopamine receptors in human peripheral blood lymphocytes: changes in mRNA expression in opioid addiction. Eur. J. Pharmacol. 615, 218–222.
He, F., Lidow, I.A., Lidow, M.S., 2006. Consequences of paternal cocaine exposure in mice. Neurotoxicol. Teratol. 28, 198–209.
Hemby, S.E., Smith, J.E., Dworkin, S.I., 1996. The effects of eticlopride and naltrexone on responding maintained by food, cocaine, heroin and cocaine/heroin combinations in rats. J. Pharmacol. Exp. Therap. 277, 1247–1258.
Johnson, N.L., Carini, L., Schenk, M.E., Stewart, M., Byrnes, E.M., 2011. Adolescent opiate exposure in the female rat induces subtle alterations in maternal care and transgenerational effects on play behavior. Front. Psychiatry 2, 29.
Khan, Z.U., Gutiérrez, A., Martín, R., Peñafiel, A., Rivera, A., Calle, A.D., 1998. Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. J. Comp. Neurol. 402, 353–371.
Knight, R., 1996. Contribution of human hippocampal region to novelty detection. Nature 383, 256–259.
Le Foll, B., Diaz, J., Sokoloff, P., 2003. Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. Synapse 47, 176–183.
Li, T., Xu, K., Deng, H., Cai, G., Liu, J., Liu, X., Wang, R., et al., 1997. Association analysis of the dopamine D4 gene exon III VNTR and heroin abuse in Chinese subjects. Mol. Psychiatry 2, 413–416.
Li, Y., Shao, C., Zhang, D., Zhao, M., Lin, L., Yan, P., Xie, Y., Jiang, K., Jin, L., 2006. The effect of dopamine D2, D5 receptor and transporter (SLC6A3) polymorphisms on the cue-elicited heroin craving in Chinese. Am. J. Med. Genet. Part B, Neuropsychiatric Genet. 141B, 269–273.
Maldonado, R., Saiardi, A., Valverde, O., Samad, T.A., Roques, B.P., Borrelli, E., 1997. Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. Nature 388, 586–589.
Maze, I., Covington, H.E., Dietz, D.M., LaPlant, Q., Renthal, W., Russo, S.J., Mechanic, M., et al., 2010. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. Science (New York, N.Y.) 327, 213–216.
Oak, J.N., Oldenhof, J., Van Tol, H.H., 2000. The dopamine D (4) receptor: one decade of research. Eur. J. Pharmacol. 405, 303–327.
Paxinos, G.A., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates, 6th ed. Academic Press, London, UK.
Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative Expression Software Tool (REST) for group-wise comparison and statistical analysis of relative expression results in Real-time PCR. Nucleic Acids Res. 30, e36.
Planeta, C.S., 2013. Animal models of alcohol and drug dependence. Rev. Bras. Psiquiatr. 35, S140–146.
Rao, P.A., Pickar, D., Gejman, P.V., Ram, A., Gershon, E.S., Gelernter, J., 1994. Allelic variation in the D4 dopamine receptor (DRD4) gene does not predict response to clozapine. Arch. Gen. Psychiatry 51, 912–917.
Riley, M.A., Vathy, I., 2006. Mid- to late gestational morphine exposure does not alter the rewarding properties of morphine in adult male rats. Neuropharmacology 51, 295–304.
Rivera, A., Cuéllar, B., Girón, F.J., Grandy, D.K., De la Calle, A., Moratalla, R., 2002. Dopamine D4 receptors are heterogeneously distributed in the Striosomes/matrix compartments of the Striatum. J. Neurochemistry 80, 219–229.
Rodgers, R.J., Johnson, N.J., 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol. Biochem. Behav. 52, 297–303.
Rowlett, J.K., Platt, D.M., Yao, W.D., Spealman, R.D., 2007. Modulation of heroin and cocaine self-administration by dopamine D1- and D2-like receptor agonists in rhesus monkeys. J. Pharmacol. Exp. Therap. 321, 1135–1143.
Sarkaki, A., Assaei, R., Motamedi, F., Badavi, M., Pajouhi, N., 2008. Effect of parental morphine addiction on hippocampal long-term potentiation in rats offspring. Behav. Brain Res. 186, 72–77.
Shen, Y.L., Chen, S.T., Chan, T.Y., Hung, T.W., Tao, P.L., Liao, R.M., Chan, M.H., Chen, H.H., 2016. Delayed extinction and stronger drug-primed reinstatement of methamphetamine seeking in rats prenatally exposed to morphine. Neurobiol. Learn Mem. 128, 56–64.
Sheng, J., Gang Lv, Z., Wang, L., Zhou, Y., Hui, B., 2011. Histone H3 phosphoacetylation is critical for heroin-induced place preference. Neuroreport 22, 575–580.
Shields, P.G., Lerman, C., Audrain, J., Bowman, E.D., Main, D., Boyd, N.R., Caporaso, N.E., 1998. Dopamine D4 receptors and the risk of cigarette smoking in African-Americans and Caucasians. Cancer Epidemiol. Biomarkers Prevent. 7, 453–458.
Sibley, D.R., Monsma, F.J., Shen, Y., 1993. Molecular neurobiology of dopaminergic receptors. Int. Rev. Neurobiol. 35, 391–415.
Simpson, J., Vetuz, G., Wilson, M., Brookes, K.J., Kent, L., 2010. The DRD4 receptor exon 3 VNTR and 5' SNP variants and mRNA expression in human post-mortem brain tissue. Am. J. Med. Genet. Part. B Neuropsychiatric Genet. 153B, 1228–1233.
Straub, R.E., Sullivan, P.F., Ma, Y., Myakishev, M.V., Harris-Kerr, C., Wormley, B., Kadambi, B., et al., 1999. Susceptibility genes for nicotine dependence: a genome scan and follow up in an independent sample suggest that regions on chromosomes 2, 4, 10, 16, 17 and 18 merit further study. Mol. Psychiatry 4, 129–144.
Sullivan, P.F., Neale, M.C., Silverman, M.A., Harris-Kerr, C., Myakishev, M.V., Wormley, B., Webb, B.T., Ma, Y., Kendler, K.S., Straub, R.E., 2001. An association study of DRD5 with smoking initiation and progression to nicotine dependence. Am. J. Med. Genet. 105, 259–265.
Suzuki, T., Kobayashi, K., Nagatsu, T., 1995. Genomic structure and tissue distribution of the mouse dopamine D4 receptor. Neurosci. Lett. 199, 69–72.
Swant, J., Chirwa, S., Stanwood, G., Khoshbouei, H., 2010. Methamphetamine reduces LTP and increases baseline synaptic transmission in the CA1 region of mouse hippocampus. PloS One 5, e11382.
Szutorisz, H., DiNieri, J.A., Sweet, E., Egervari, G., Michaelides, M., Carter, J.M., Ren, Y., Miller, M.L., Blitzer, R.D., Hurd, Y.L., 2014. Parental THC exposure leads to compulsive heroin-seeking and altered striatal synaptic plasticity in the subsequent generation. Neuropsychopharmacology 39, 1315–1323.
Tyrfingsson, T., Thorgeirsson, T.E., Geller, F., Runarsdóttir, V., Hansdóttir, I., Bjornsdottir, G., Wiste, A.K., et al., 2010. Addictions and their familiality in Iceland. Ann. N.Y. Acad. Sci. 1187, 208–217.
Vanyukov, M.M., Moss, H.B., Gioio, A.E., Hughes, H.B., Kaplan, B.B., Tarter, R.E., 1998. An association between a microsatellite polymorphism at the DRD5 gene and the liability to substance abuse: pilot study. Behav. Genet. 28, 75–82.
Vassoler, F.M., Byrnes, E.M., Pierce, R.C., 2014. The impact of exposure to addictive drugs on future generations: physiological and behavioral effects. Neuropharmacology 76 (Pt B), 269–275.
Vengeliene, V., Leonardi-Essmann, F., Perreau-Lenz, S., Gebicke-Haerter, P., Drescher, K., Gross, G., Spanagel, R., 2006. The dopamine D3 receptor plays an essential role in alcohol-seeking and relapse. FASEB J. 20, 2223–2233.
Wang, Y., Lai, J., Cui, H., Zhu, Y., Zhao, B., Wang, W., Wei, W., 2015. Inhibition of histone deacetylase in the basolateral amygdala facilitates morphine context-associated memory formation in rats. J. Mol. Neurosci. 55, 269–278.
Yohn, N.L., Bartolomei, M.S., Blendy, J.A., 2015. Multigenerational and transgenerational inheritance of drug exposure: the effects of alcohol, opiates, cocaine, marijuana, and nicotine. Prog. Biophys. Mol. Biol. 118, 21–33.
Zhu, F., Yan, C., Wen, Y.C., Wang, J., Bi, J., Zhao, Y.L., Wei, L., Gao, C., Jia, W., Li, S.B., 2013. Dopamine D1 receptor Gene variation modulates opioid dependence risk by affecting transition to addiction. PLoS One 8, e70805.