Addiction & Health

https://ahj.kmu.ac.ir
10.34172/ahj.2023.1396
Vol. 15, No. 3, 2023, 169-176

Original Article

Does Morphine Exposure Before Gestation Change Anxiety-Like Behavior During Morphine Dependence in Male Wistar Rats?

Saba Sabzevari[1], Kiyana Rohbani[1], Mahsa Sadeghi-Adl[1], Solmaz Khalifeh[2], Mitra-Sadat Sadat-Shirazi[1*], Mohammad-Reza Zarrindast[1,2,3*]

[1]Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran
[2]Cognitive and Neuroscience Research Center (CNRC), Tehran Medical Sciences, Amir-Almomenin Hospital, Islamic Azad University, Tehran, Iran
[3]Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: Anxiety is one of the comorbid disorders of opioid addiction, which leads to opioid abuse or persuades people to engage in opioid abuse. Evidence revealed that morphine exposure before conception changes the offspring's phenotype. The current study aimed to investigate the influence of morphine dependence and abstinence on anxiety-like behavior in morphine-exposed and drug-naïve offspring.

Methods: Adult male and female rats were treated with morphine or vehicle for 21 days. Then, all rats were left without drug treatment for 10 days. A morphine-exposed female rat was mated with either a vehicle-exposed or morphine-abstinent male. According to parental morphine exposure, the offspring were categorized into four distinct groups: (1) control (both drug-naïve parents), (2) paternal morphine-exposed, (3) maternal morphine-exposed, and (4) biparental morphine-exposed. The anxiety-like behavior was measured in adult male offspring using open field and elevated plus-maze tests before morphine exposure (naïve), 21 days after morphine exposure (dependence), and ten days after the last morphine exposure (abstinence).

Findings: The results indicated that anxiety-like behavior increased before morphine exposure in maternal and biparental morphine-exposed offspring ($P<0.05$). However, after morphine exposure, the anxiety level did not change among the groups. Ten days after the last morphine exposure, anxiety-like behavior increased only in biparental morphine-exposed offspring ($P<0.05$).

Conclusion: The offspring of morphine-abstinent parents exhibited an anxious phenotype. Disruption of the HPA axis was seen in the progeny of maternal and biparental morphine-exposed rats. Indeed, morphine exposure for 21 days did not change anxiety-like behavior in these offspring which might be correlated to disruption of HPA axis in them.

Keywords: Intergeneration, Morphine, Anxiety-like behavior, Offspring

Citation: Sabzevari S, Rohbani K, Sadeghi-Adl M, Khalifeh S, Sadat-Shirazi MS, Zarrindast MR. Does morphine exposure before gestation change anxiety-like behavior during morphine dependence in male wistar rats? *Addict Health*. 2023;15(3):169-176. doi:10.34172/ahj.2023.1396

Received: August 28, 2022, **Accepted**: December 3, 2022, **ePublished:** July 29, 2023

Introduction

Substance use disorder is one of the most critical health problems in the world. In opiate abusers, physical dependence comes after the euphoria, and both positive and negative reinforcing effects develop relapse.[1] Dysphoria, somatic signs of withdrawal, and physical distress occur during morphine abstinence. Consequently, these negative signs (negative reinforcement) lead to craving and relapse.[2] Like humans, chronic exposure to morphine increases reward and decreases pain thresholds in rodents, followed by dysphoria and anxiety-like behaviors.[2]

There are social differences in the pattern of drug abuse among countries. Evidence shows that drug addiction is an inherited disorder, and it is claimed that genetics influences substance use disorder approximately by 0.07-0.30%.[3] However, it should be noted that the effect of hereditary factors might vary depending on gender, age, and cultural characteristics.[3] Recent evidence reveals that the interaction between genes and the environment determines the risk of drug addiction.[4-7] Meanwhile, epigenetic changes (histone acetylation, DNA methylation, and miRNA) in different brain regions play an important role in both drug addiction and anxiety.[8,9] Since epigenetic changes pass through generations, they might cause molecular and behavioral alterations in the offspring.

Anxiety disorder has a crucial role in opioid addiction.[10] An animal study revealed that chronic morphine treatment caused an enhancement in anxiety levels in

*Corresponding Authors: Mitra-Sadat Sadat-Shirazi, Email: mitra.sadat-shirazi@tufts.edu and Mohammad-Reza Zarrindast, Email: zarinmr@ams.ac.ir

the open field test.[11] Furthermore, animal studies showed that maternal morphine exposure during adolescence influences anxiety levels in female litters depending on the estrous cycle.[12] Besides, it was reported that parental morphine exposure before mating leads to changes in anxiety-like behavior in the offspring.[13,14]

The results of previous studies indicated that parental (maternal, paternal, and biparental) morphine treatment ten days before conception dramatically affects the behavior of the offspring. Along with behavior, molecular changes were also detected in different regions of the brain in the offspring of morphine-exposed rats.[13,15-17] Accordingly, the main objective of this study was to assess the anxiety-like behavior during dependence and abstinent phases of morphine addiction in the offspring of morphine-exposed parent(s).

Methods

Animals

In this study, a total of 32 female (200-220 g, 8 weeks old) and 32 male (220-250 g, 8 weeks old) Wistar rats were used as F0 (parents). The animals were kept in Plexiglas cages (n = 4) under a 12/12 light and dark cycle with lights on at 6:00 AM. Animals had free access to food and water. The temperature was constant during the experiment (22 ± 2 °C).

Morphine exposure protocol

Morphine sulfate (Faran Shimi, Iran) was administrated to 16 male and 16 female rats for 21 days. They received morphine orally, as was described previously.[18] The protocol of oral morphine exposure contains a different dosage of morphine during days 1-6 dissolved in water. Rats received 0.1 g/L on days 1 and 2, 0.2 g/L on days 3-4, and 0.3 g/L on days 5 and 6. Then, the amount of morphine was set at 0.4 g/L from day 7 till 21. By adding sucrose (2%, Merck, Germany) to the water, the bitter taste of morphine was eliminated. It should be noted that the control group received a solution of water and sucrose (2%) as a vehicle. Figure 1 shows the experimental procedures.

Mating protocol

On the 10th day after the last morphine exposure, the rats were prepared for mating. The mating procedures were as follows:

1. Vehicle-exposed male and female rats: male offspring included in the study as the control group
2. Morphine-abstinent male and vehicle-exposed female: male offspring included in the study as paternal morphine-exposed offspring (P.ME)
3. Vehicle-exposed male and morphine-abstinent female: male offspring included in the study as maternal morphine-exposed offspring (M.ME)
4. Morphine-abstinent male and female: male offspring included in the study as biparental morphine-exposed offspring (P + M-ME)

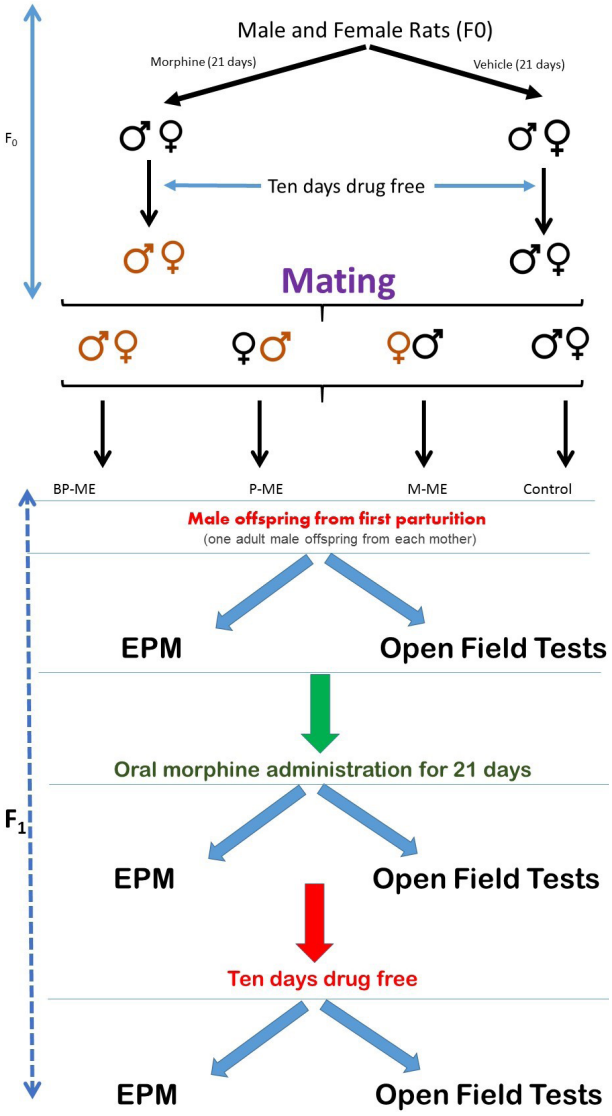

Figure 1. The summarized diagram of the experimental procedures

Female rats were examined daily (between 8:00-10:00 AM) for pregnancy, and once the vaginal plug was formed, they were separated from the male. Eight male offspring of the first parturition (one animal from one female rat) were included in behavioral studies. Animals were subjected to behavioral tests before and after morphine exposure. In addition, after ten days of abstinence, they were subjected to behavioral tests again.

Behavioral tests

Each animal was handled before the test (3 days before the test for 5 minutes each day) and transferred to the test room one hour before the behavioral test to habituate.

Elevated plus-maze (EPM)

The EPM apparatus is an equal-armed cross-shaped wooden maze. It has two open arms (50 cm × 10 cm × 0.5

cm) and two closed arms (50 cm × 10 cm × 40 cm). In the center of the apparatus, there is a square-shaped platform. The height of the EPM apparatus from the floor was 50 cm. Each rat was placed gently in the middle of the maze facing the open arm and was allowed to explore the apparatus for 10 minutes. A camera recorded all movements of the rats during the test; after that, an investigator who was blind to the grouping calculated the open and closed arm time and entries. The percentage of open arm time (OAT) and entries was calculated as follows:

$$\text{OAT (\%)} = \left(\frac{\textit{total time spent in open arms}}{\textit{total time spent in open and closed arms}} \right) \times 100$$

$$\text{OAE (\%)} = \left(\frac{\textit{total entries in open arms}}{\textit{total entries in open and closed arms}} \right) \times 100$$

Open field test

A box (1 × 1 × 1 m) was used for the open field test. The arena within the walls was divided into squares. Each animal was gently put in the middle of the open field apparatus, and a video camera recorded all movements for 5 minutes. Total locomotor activity (horizontal activity), the total number of rearing (vertical activity), and the total number of grooming were recorded by observers who were blind to group data. It should be noted that between each test, the apparatus was cleaned with ethanol (70%).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 24 was used to analyze the results. At first, all data were analyzed by the Kolmogorov-Smirnov test for normal distribution. Then, repeated measures analysis of variance (ANOVA) was used to find the differences between groups. In addition, in all analyses, a significant difference was considered when $P < 0.05$.

Results

Anxiety-like behavior changes in the offspring of morphine-exposed parents in EPM test

Figure 2 illustrates the effect of morphine exposure on anxiety-like behavior in the offspring of morphine-exposed parents compared with the drug-naïve offspring. As is shown in Figure 2A, the open arm entries (OAE) percentage decreased in the M.ME and P + M.ME before morphine administration. After 21 days of morphine exposure (dependence phase), no significant changes were detected in OAE between groups. However, 10 days after the last morphine exposure (abstinence phase), OAE decreased in P + M.ME group compared to the drug-naïve offspring (Tests of within-subject effects: parental morphine exposure × morphine exposure (Greenhouse-Geisser): $F_{1,84} = 5.01$, $P = 0.028$, Tests of between-subject effects: parental morphine exposure: $F_{1,84} = 5.44$, $P = 0.022$).

The percentage of OAT also decreased in M.ME and P + M.ME in comparison to the control group before the administration of morphine. Morphine exposure for 21 days did not alter OAT between groups, although OAT decreased in P + M.ME 10 days after last morphine exposure (Figure 2B, Tests of within-subject effects: parental morphine exposure × morphine exposure (Greenhouse-Geisser): $F_{1,84} = 7.78$, $P = 0.007$, Tests of between-subject effects: parental morphine exposure: $F_{1,84} = 9.05$, $P = 0.004$).

In Figures 2C and 2D, groups are categorized according to parental morphine exposure. As is shown, in the dependence phase, the anxiety-like behavior increased only in drug-naïve and P.ME groups. Thus, the results showed maternal and biparental morphine exposure ten days before mating enhanced the levels of anxiety in male offspring. However, after 21 days of morphine exposure (dependence phase), there was no difference between the offspring of morphine-exposed parents and drug-naïve offspring. In the abstinence phase (ten days after the last morphine exposure), anxiety-like behavior increased only in P + M.ME compared with the drug-naïve offspring (Figure 2).

Open-field test in the offspring of morphine-exposed parents

Figure 3 shows the total number of grooming (A), the total number of rearing (vertical activity, B), and total locomotion (horizontal activity, C) in the open field test. Before the administration of morphine, the total number of grooming increased in the morphine-exposed offspring; however, in the dependence and abstinence phases, there were no significant differences in grooming among groups (Figure 3A, Tests of within-subject effects: parental morphine exposure × morphine exposure (Greenhouse-Geisser): $F_{1,84} = 0.76$, $P = 0.38$, Tests of between-subject effects: parental morphine exposure: $F_{1,84} = 0.55$, $P = 0.36$). The vertical activity also increased in the offspring of morphine-exposed parents only before the administration of morphine. It should be noted that during the dependence and abstinence phases, there were no differences among groups (Figure 3B, Tests of within-subject effects: parental morphine exposure × morphine exposure (Greenhouse-Geisser): $F_{1,84} = 7.92$, $P = 0.006$, Tests of between-subject effects: parental morphine exposure: $F_{1,84} = 7.99$, $P = 0.006$). Like other parameters measured in the open field test, locomotion was not affected during the dependence and abstinence. Before the administration of morphine, the locomotor activity decreased in M.ME and P + M.ME groups compared with the drug-naïve offspring (Figure 3C, Tests of within-subject effects: parental morphine exposure × morphine exposure (Greenhouse-Geisser): $F_{1,84} = 16.145$, $P < 0.001$,

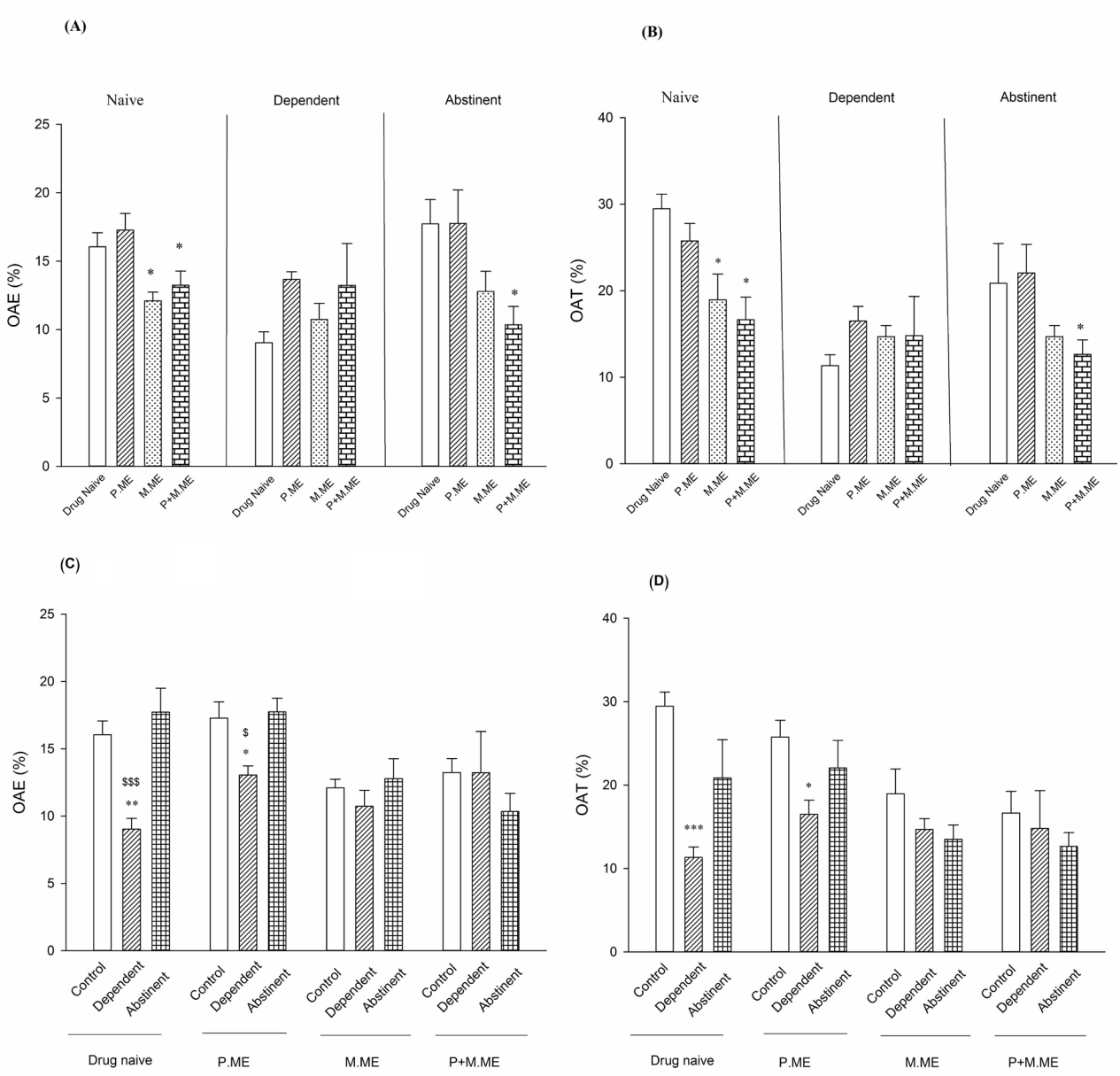

Figure 2. Effect of morphine exposure on anxiety-like behavior in the offspring of morphine-exposed rats in the EPM test. (A) the percentage of open arm entries (OAE) and (B) the percentage of open arm time (OAT) in drug naïve (control), paternal (P.ME), maternal (M.ME), and biparental (P + M.ME) morphine-exposed animals are shown before morphine exposure (naïve), 21 days after morphine treatment (dependence), and ten days after last morphine treatment (abstinence). (C) OAE and (D) OAT percentages before, during, and after morphine exposure in control, paternal, maternal, and biparental morphine-exposed offspring. Data are expressed as mean ± SEM. * $P<0.05$ vs control, $^{\$}P<0.05$, $^{\$\$\$}P<0.001$ vs abstinence, (A-B) * $P<0.05$ vs drug-naive group, (C-D) * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs control group

Tests of between-subject effects: parental morphine exposure: $F_{1,84} = 18.07$, $P < 0.001$).

Figure 3D-F shows the effect of morphine exposure (naïve, dependence, and abstinence phases) in parental morphine exposure. The enhancement in the total number of rearing and grooming was only observed in drug-naïve offspring. There were no statistically significant changes in other groups before and after morphine exposure (Tables 1 and 2).

Discussion

The results of this study implicated that anxiety enhanced in the maternal and biparental morphine-exposed male offspring. Besides, the anxiety-like behavior in the offspring that were treated chronically (21 days) with morphine did not change between groups. The results of the present study are in line with previous studies[13,14,16] showing that chronic morphine exposure prior to mating enhances anxiety-like behavior in male offspring (F1).

It has been indicated that genetics can play a role in an individual's susceptibility to addiction. However, simple Mendelian inheritance did not explain the transgenerational effect of abused drugs.[19] Obviously, genetic and non-genetic (such as developmental and environmental factors) factors are mutually involved in the susceptibility of substance use disorder.[20] There are

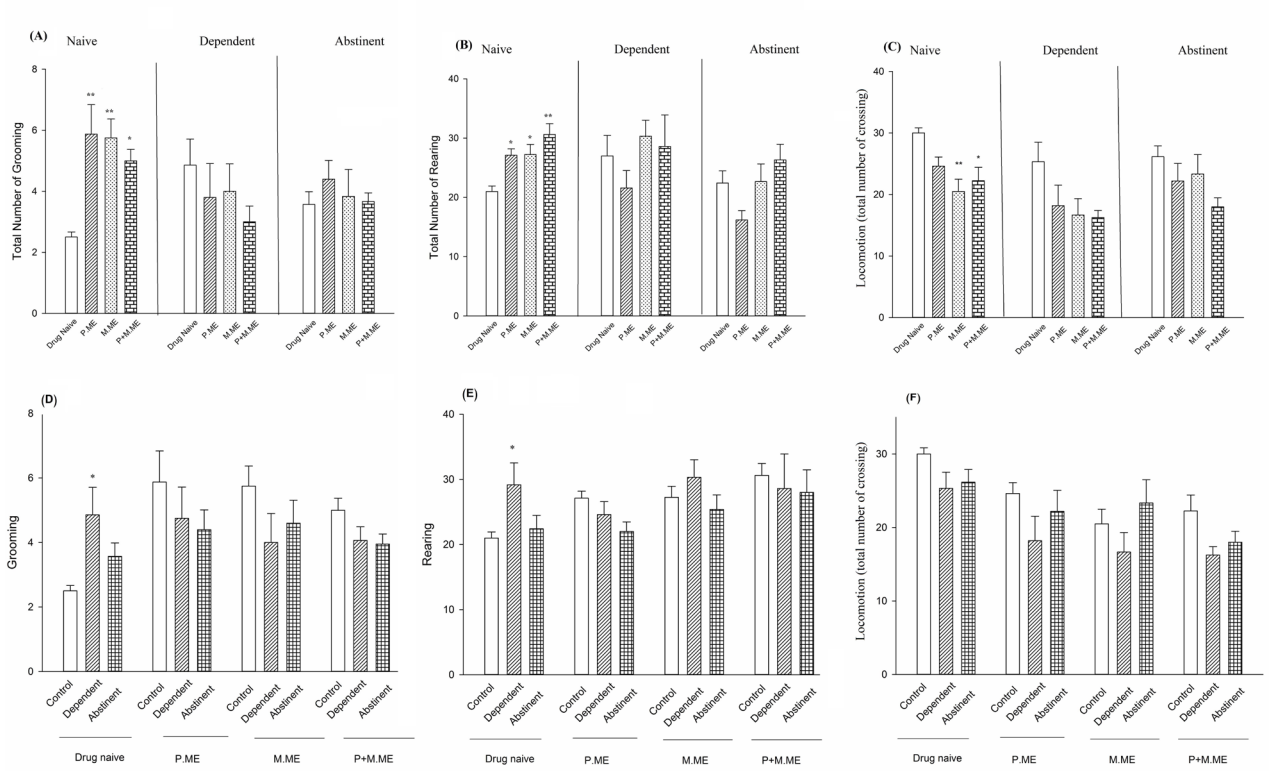

Figure 3. Effect of morphine exposure in the open field test. (A) the total number of grooming, (B) the total number of rearing, and (C) the total locomotor activity in drug naïve (control), paternal (P.ME), maternal (M.ME), and biparental (P + M.ME) morphine-exposed animals are shown before morphine exposure (naïve), 21 days after morphine treatment (dependence), and ten days after last morphine treatment (abstinence). (D) grooming, (E) rearing, and (F) locomotor activity before, during, and after morphine exposure in control, paternal, maternal, and biparental morphine-exposed offspring. Data are expressed as mean ± SEM. * $P<0.05$, ** $P<0.01$

Table 1. The statistical results of the study according to morphine exposure phases (naïve, dependence, abstinence)

Test	Phase	One-way ANOVA		Repeated measures (Greenhouse-Geisser)	
		F	*P*		
OAE	Naïve	5.37	0.049	5.01	*P*=0.028*
	Dependence	1.64	0.21		
	Abstinence	3.83	0.026		
OAT	Naïve	6.85	0.013	7.78	*P*=0.007**
	Dependence	0.73	0.54		
	Abstinence	1.58	0.04		
Grooming	Naïve	6.66	0.0013	0.76	*P*=0.38
	Dependence	0.77	0.52		
	Abstinence	0.37	0.77		
Rearing	Naïve	3.77	0.01	7.92	*P*=0.006**
	Dependence	0.99	0.41		
	Abstinence	2.76	0.06		
Locomotion	Naïve	5.87	0.0026	16.14	0.001***
	Dependence	2.17	0.12		
	Abstinence	1.88	0.16		

*$P<0.05$, **$P<0.001$, and ***$P<0.001$ diffrent of behavior (OAE, OAT, Rearing, and locomotion) between groups.

Table 2. The statistical results of the study according to the parental morphine exposure (drug naïve (control), paternal (P.ME), maternal (M.ME), and biparental (P + M.ME) morphine exposed animals)

Test	Group	One-way ANOVA	
		F	*P*
OAE	Control	11.78	0.0004 ***
	P.ME	5.54	0.014
	M.ME	0.88	0.43
	P + M.ME	0.62	0.55
OAT	Control	9.41	0.001***
	P.ME	3.93	0.042
	M.ME	1.59	0.23
	P + M.ME	0.37	0.69
Grooming	Control	4.92	0.017*
	P.ME	0.78	0.47
	M.ME	1.57	0.23
	P + M.ME	2.59	0.101
Rearing	Control	3.89	0.03*
	P.ME	3.23	0.06
	M.ME	1.18	0.31
	P + M.ME	0.18	0.82
Locomotion	Control	2.66	0.09
	P.ME	1.78	0.19
	M.ME	1.56	0.23
	P + M.ME	2.63	0.1

*$P<0.05$, **$P<0.001$, and ***$P<0.001$ vs control

studies showing that drug exposure before gestation induces phenotype changes in the next generations.[21] It has been shown that exposing males to some kinds of stress, such as maternal separation, social instability stress, and some drugs during their lifetime, affects the levels of some mi-RNA in the sperm. Thus, it passes through generations and affects the phenotype of the offspring.[22,23] In addition, exposure to stress and drugs also affect the epigenome of ovules, which are involved in the phenotype changes in the next generation.[24-26] Then, all epigenetic modifications, including histone acetylation, DNA methylation, and changes in mi-RNAs, might be implicated in changes in the offspring's anxiety-like behavior. Since epigenetic changes are stable, it can be proposed that these changes act as the primary modulator of phenotype changes in the offspring of morphine-exposed rats.

Robust data indicate anxiety disorders and opioid abuse correlate with each other.[10,27] The anxiogenic effect of chronic morphine exposure in rodents was also previously reported. It has been declared that chronic morphine administration increases corticotropin releasing factor (CRF), consequently enhancing anxiety-like behavior.[2] Besides, in the EPM and light/dark box tests, male rats exposed to morphine for ten days exhibited more anxiety-like behavior than saline-exposed groups.[28] Indeed, morphine-dependent rats displayed more anxiety-like behavior.[11] Furthermore, it has been declared that the levels of CRF in the plasma and cerebrospinal fluid (CSF) increase after 21 days of morphine exposure in drug-naïve male rats.[14] All these might lead to enhancing anxiety-like behavior in morphine-dependent animals. In this study, parents were exposed to morphine or saline (21 days exposure) before mating, then, after 10 days free period, each gender was allowed to mate with a saline-treated or a morphine-treated rat. As far as morphine exposure causes stress and anxiety-like behavior, especially in abstinence (as mentioned above), it can influence the development of offspring. Thus, morphine exposure in parents before gestation leads to a higher rate of developing addictive behaviors (psychological stress is more important than physical stress in developing addictive behaviors in the offspring). Since maternal care was affected by anxiety and poor maternal care caused disruption in HPA in the offspring,[13] it might involve in the occurrence of the anxiety-like phenotype observed in M.ME and P + M.ME.

In addition, as previous research showed,[13,14] the anxiety levels enhanced in the offspring of maternal and biparental morphine-exposed rats in comparison to the control group. Exposure of female rats to morphine during adolescence caused an anxious phenotype in the female offspring.[29] In line with the results of the present study, Li et al[30] indicated that maternal and biparental morphine exposure three weeks prior to mating enhanced anxiety levels in the offspring. Besides, a study conducted by Farah Naquiah et al[31] demonstrated that paternal heroin (diacetylmorphine) exposure 14 days prior to gestation increased anxiety levels in the first and second generations. In contrast, recent evidence showed that maternal morphine exposure 20 days before gestation decreased anxiety-like behavior in the female offspring (F1, and F2); this reduction occurred during diestrus.[12]

Parental morphine exposure before gestation disrupts the HPA axis in the offspring.[14,32] The levels of CRF in the plasma and CSF increased in both male and female rats after 21 days of chronic morphine treatment. Interestingly, the levels of CRF remained higher than the control group after ten days of abstinence. It is speculated that the increase in CRF levels is transferred to the offspring, and in the case of male offspring, the levels of CRF remain elevated.[14] Current results indicated that anxiety did not change among morphine-exposed and drug-naïve offspring after chronic morphine exposure (21 days). However, after ten days of abstinence, the levels of anxiety-like behavior increased only in biparental morphine-exposed offspring. The anxiety-like behavior did not change after chronic morphine exposure and ten days after withdrawal in the maternal and biparental morphine-exposed offspring. Thus, it could be suggested that as these offspring had a disruption in the HPA axis and showed anxiety-like behavior more than the drug-naïve offspring, morphine exposure could not affect anxiety-like behavior anymore. It should be noted that higher mortality rates among morphine-exposed offspring were one of the limitations of this study, which is also in line with previous studies.[13]

Conclusion

In conclusion, the administration of morphine before gestation increased anxiety-like behavior in the first generation (F1). Moreover, in F1, chronic morphine exposure did not change the anxiety levels in male rats. However, in abstinence (10 days after the last morphine exposure), anxiety-like behavior increased only in biparental morphine-exposed offspring. In other words, morphine exposure for 21 days increased anxiety-like behavior in control and P.ME; after ten days of abstinence, anxiety-like behavior decreased. Nevertheless, in M.ME and P + M.ME, morphine dependence and abstinence did not affect anxiety-like behavior. It has been suggested that morphine exposure even before conception disrupts the HPA axis and increases anxiety-like behavior in F1 males, but morphine exposure for 21 days did not influence it again. Accordingly, it is proposed that epigenetic mechanisms might be involved in this phenomenon. However, more studies should be conducted to determine the exact epigenetic changes in parents confronting drug abuse.

Acknowledgments

This research project was supported by the Tehran University

of Medical Sciences. We are immensely grateful to Dr. Sedighe Nikzat for her help in data analysis.

Authors' Contribution

Conceptualization: Mitra-Sadat Sadat-Shirazi.
Data curation: Mitra-Sadat Sadat-Shirazi, Solmaz Khalifeh, Mohammad-Reza Zarrindast.
Formal analysis: Mitra-Sadat Sadat-Shirazi, Solmaz Khalifeh.
Funding acquisition: Mohammad-Reza Zarrindast.
Investigation: Saba Sabzevari, Kiyana Rohbani, Mahsa Sadeghi-Adl.
Methodology: Saba Sabzevari, Kiyana Rohbani, Mahsa Sadeghi-Adl.
Project administration: Mohammad-Reza Zarrindast.
Software: Mitra-Sadat Sadat-Shirazi.
Supervision: Mohammad-Reza Zarrindast, Mitra-Sadat Sadat-Shirazi.
Validation: Mohammad-Reza Zarrindast, Mitra-Sadat Sadat-Shirazi, Solmaz Khalifeh.
Visualization: Solmaz Khalifeh.
Writing–original draft: Mitra-Sadat Sadat-Shirazi.
Writing–review & editing: Mitra-Sadat Sadat-Shirazi.

Competing Interests

The authors declare no conflict of interest.

Ethical Approval

All procedures on animals were in agreement with national guidelines for animal care (NIH guidelines; Guide for the Care and Use of Laboratory Animals, NIH Publication 86-23) and had the approval of the Tehran University of Medical Sciences ethics committee (IR.TUMS.VCR.REC.1396.3149).

References

1. Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. Am J Psychiatry. 2007;164(8):1149-59. doi: 10.1176/appi.ajp.2007.05030503.
2. Koob GF. Neurobiology of opioid addiction: opponent process, hyperkatifeia, and negative reinforcement. Biol Psychiatry. 2020;87(1):44-53. doi: 10.1016/j.biopsych.2019.05.023.
3. Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, et al. Familial transmission of substance use disorders. Arch Gen Psychiatry. 1998;55(11):973-9. doi: 10.1001/archpsyc.55.11.973.
4. Agrawal A, Lynskey MT. Are there genetic influences on addiction: evidence from family, adoption and twin studies. Addiction. 2008;103(7):1069-81. doi: 10.1111/j.1360-0443.2008.02213.x.
5. Mistry CJ, Bawor M, Desai D, Marsh DC, Samaan Z. Genetics of opioid dependence: a review of the genetic contribution to opioid dependence. Curr Psychiatry Rev. 2014;10(2):156-67. doi: 10.2174/1573400510666140320000928.
6. Zheng B, Fletcher J, Zheng F, Lu Q. Gene-by-peer-environment interaction effects on cigarette, alcohol, and marijuana use among US high school students of European ancestry. Soc Sci Med. 2022;309:115249. doi: 10.1016/j.socscimed.2022.115249.
7. Zhang H, Khan A, Rzhetsky A. Gene-environment interactions explain a substantial portion of variability of common neuropsychiatric disorders. Cell Rep Med. 2022;3(9):100736. doi: 10.1016/j.xcrm.2022.100736.
8. Zhu C, Liang M, Li Y, Feng X, Hong J, Zhou R. Involvement of epigenetic modifications of GABAergic interneurons in basolateral amygdala in anxiety-like phenotype of prenatally stressed mice. Int J Neuropsychopharmacol. 2018;21(6):570-81. doi: 10.1093/ijnp/pyy006.
9. Browne CJ, Godino A, Salery M, Nestler EJ. Epigenetic mechanisms of opioid addiction. Biol Psychiatry. 2020;87(1):22-33. doi: 10.1016/j.biopsych.2019.06.027.
10. Martins SS, Fenton MC, Keyes KM, Blanco C, Zhu H, Storr CL. Mood and anxiety disorders and their association with non-medical prescription opioid use and prescription opioid-use disorder: longitudinal evidence from the National Epidemiologic Study on Alcohol and Related Conditions. Psychol Med. 2012;42(6):1261-72. doi: 10.1017/s0033291711002145.
11. Raghav R, Jain R, Jacob TG, Dhawan A, Roy TS. Co-administration of nalbuphine attenuates the morphine-induced anxiety and dopaminergic alterations in morphine-withdrawn rats. Psychopharmacology (Berl). 2021;238(4):1193-211. doi: 10.1007/s00213-021-05765-3.
12. Vassoler FM, Byrnes EM. Transgenerational effects on anxiety-like behavior following adolescent morphine exposure in female rats. Behav Brain Res. 2021;406:113239. doi: 10.1016/j.bbr.2021.113239.
13. Akbarabadi A, Sadat-Shirazi MS, Kabbaj M, Nouri Zadeh-Tehrani S, Khalifeh S, Pirri F, et al. Effects of morphine and maternal care on behaviors and protein expression of male offspring. Neuroscience. 2021;466:58-76. doi: 10.1016/j.neuroscience.2021.04.011.
14. Sabzevari S, Rohbani K, Sadat-Shirazi MS, Babhadi-Ashar N, Shakeri A, Ashabi G, et al. Morphine exposure before conception affects anxiety-like behavior and CRF level (in the CSF and plasma) in the adult male offspring. Brain Res Bull. 2019;144:122-31. doi: 10.1016/j.brainresbull.2018.11.022.
15. Ashabi G, Sadat-Shirazi MS, Akbarabadi A, Vousooghi N, Kheiri Z, Toolee H, et al. Is the nociception mechanism altered in offspring of morphine-abstinent rats? J Pain. 2018;19(5):529-41. doi: 10.1016/j.jpain.2017.12.268.
16. Ahmadian-Moghadam H, Akbarabadi A, Toolee H, Sadat-Shirazi MS, Khalifeh S, Niknamfar S, et al. Correlation among the behavioral features in the offspring of morphine-abstinent rats. Addict Health. 2019;11(4):262-75. doi: 10.22122/ahj.v11i4.253.
17. Sadat-Shirazi MS, Nouri Zadeh-Tehrani S, Akbarabadi A, Mokri A, Taleb Zadeh Kasgari B, Zarrindast MR. Exercise can restore behavioural and molecular changes of intergenerational morphine effects. Addict Biol. 2022;27(2):e13122. doi: 10.1111/adb.13122.
18. Sadat-Shirazi MS, Karimi F, Kaka G, Ashabi G, Ahmadi I, Akbarabadi A, et al. Parental morphine exposure enhances morphine (but not methamphetamine) preference and increases monoamine oxidase-B level in the nucleus accumbens. Behav Pharmacol. 2019;30(5):435-45. doi: 10.1097/fbp.0000000000000465.
19. Goldman D, Oroszi G, Ducci F. The genetics of addictions: uncovering the genes. Nat Rev Genet. 2005;6(7):521-32. doi: 10.1038/nrg1635.
20. Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci. 2001;2(2):119-28. doi: 10.1038/35053570.
21. Sadat-Shirazi MS, Sadeghi-Adl M, Akbarabadi A, Ashabi G, Mokri A, Zarrindast MR. Inter/transgenerational effects of drugs of abuse: a scoping review. CNS Neurol Disord Drug Targets. 2023;22(4):512-38. doi: 10.2174/1871527321666220429122819.
22. Dickson DA, Stohn P, Saavedra Rodriguez L, Hernandez A, Harrington A, Liaw L, et al. Involvement of early embryonic miR-409-3p in the establishment of anxiety levels in female mice. Dev Neurobiol. 2020;80(5-6):160-7. doi: 10.1002/dneu.22756.

23. Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, et al. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci. 2014;17(5):667-9. doi: 10.1038/nn.3695.
24. Di Emidio G, D'Aurora M, Placidi M, Franchi S, Rossi G, Stuppia L, et al. Pre-conceptional maternal exposure to cyclophosphamide results in modifications of DNA methylation in F1 and F2 mouse oocytes: evidence for transgenerational effects. Epigenetics. 2019;14(11):1057-64. doi: 10.1080/15592294.2019.1631111.
25. López-Rodríguez D, Aylwin CF, Delli V, Sevrin E, Campanile M, Martin M, et al. Multi- and transgenerational outcomes of an exposure to a mixture of endocrine-disrupting chemicals (EDCs) on puberty and maternal behavior in the female rat. Environ Health Perspect. 2021;129(8):87003. doi: 10.1289/ehp8795.
26. Khan N, Peters RA, Richardson E, Robert KA. Maternal corticosterone exposure has transgenerational effects on grand-offspring. Biol Lett. 2016;12(11):20160627. doi: 10.1098/rsbl.2016.0627.
27. Nazeri M, Nezhadi A, Shabani M. Role of opioid system in empathy-like behaviours in rats. Addict Health. 2019;11(4):216-22. doi: 10.22122/ahj.v11i4.243.
28. Miladi-Gorji H, Rashidy-Pour A, Fathollahi Y. Anxiety profile in morphine-dependent and withdrawn rats: effect of voluntary exercise. Physiol Behav. 2012;105(2):195-202. doi: 10.1016/j.physbeh.2011.08.010.
29. Byrnes EM. Transgenerational consequences of adolescent morphine exposure in female rats: effects on anxiety-like behaviors and morphine sensitization in adult offspring. Psychopharmacology (Berl). 2005;182(4):537-44. doi: 10.1007/s00213-005-0122-4.
30. Li CQ, Luo YW, Bi FF, Cui TT, Song L, Cao WY, et al. Development of anxiety-like behavior via hippocampal IGF-2 signaling in the offspring of parental morphine exposure: effect of enriched environment. Neuropsychopharmacology. 2014;39(12):2777-87. doi: 10.1038/npp.2014.128.
31. Farah Naquiah MZ, James RJ, Suratman S, Lee LS, Mohd Hafidz MI, Salleh MZ, et al. Transgenerational effects of paternal heroin addiction on anxiety and aggression behavior in male offspring. Behav Brain Funct. 2016;12(1):23. doi: 10.1186/s12993-016-0107-y.
32. Vassoler FM, Johnson-Collins NL, Carini LM, Byrnes EM. Next generation effects of female adolescent morphine exposure: sex-specific alterations in response to acute morphine emerge before puberty. Behav Pharmacol. 2014;25(2):173-81. doi: 10.1097/fbp.0000000000000032.