

Research Paper: Environmental Enrichment Ameliorates Psychological Dependence Symptoms and Voluntary Morphine Consumption in Morphine Withdrawn Rats Under Methadone Maintenance Treatment



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ABSTRACT

Introduction: Previous studies have shown that physical and psychological dependence and the vulnerability to relapse are still present during MMT. Thus, this study examined whether Enriched Environment (EE) would attenuate anxiety, depressive, and obsessive-compulsive-like behaviors, as well as voluntary morphine consumption following Methadone Maintenance Treatment (MMT) in morphine withdrawn rats.

Methods: The rats were injected bi-daily doses (10 mg/kg, 12-h interval) of morphine for 14 days. Then, the rats were reared in a Standard Environment (SE) or EE for 30 more days during morphine withdrawal, simultaneous with receiving MMT. The rats were tested for anxiety (the Elevated Plus Maze [EPM]) and depression (Sucrose Preference Test [SPT]), Obsessive-Compulsive Disorder (OCD) as grooming behavior, and voluntary morphine consumption using a Two-Bottle Choice (TBC) paradigm.

Results: The findings revealed that EE experience in morphine withdrawn rats under MMT significantly increased the EPM open-arm time and higher sucrose preference than SE rats. Also, we found that the EE decreased the self-grooming behavior and morphine preference ratio in morphine withdrawn rats receiving MMT compared to the SE group.

Conclusion: We conclude that exposure to EE decreased methadone-induced anxiety, depressive and OCD-like behaviors, and voluntary morphine consumption in morphine withdrawn rats under MMT. Thus, the EE seems to be one of the strategies for reducing MMT-induced behavioral dysfunction and the risk of relapse induced by morphine withdrawal.

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Highlights

- Methadone Maintenance Treatment (MMT) exacerbates psychological dependence in morphine-withdrawn rats.
- An Enriched Environment (EE) decreases MMT-induced anxiety, depression, and obsessive-compulsive behavior in morphine-withdrawn rats.
- EE decreases morphine preference ratio in morphine-withdrawn rats under MMT.

Plain Language Summary

Chronic administration of morphine is associated with increased physical and psychological dependence signs, including anxiety, depression, OCD, and vulnerability to relapse, which are still permanent under MMT. Previous findings indicate that exposure to Environmental Enrichment (EE), including physical, social, and cognitive enrichment, can produce a range of plastic responses in the brain's reward system. Our findings showed that exposure to EE decreased methadone-induced anxiety, depressive and OCD, and voluntary morphine consumption in morphine withdrawn rats under MMT. Our results may have a potential therapeutic application to prevent physical and psychological dependence and relapse in opiate use disorder patients under MMT.

1. Introduction

Methadone-Maintenance Treatment (MMT) is a well-known approach to treat opioid addiction (Saxon, Hser, Woody, & Ling, 2013; Soyka et al., 2011) and to improve addicts' health and social communication (Joseph, Stancliff, & Langrod, 2000). Despite its ubiquitous use, considerable challenges have remained, such as the severity of physical dependence signs, depression and anxiety (Alizadeh, Zahedi-Khorasani, & Miladi-Gorji, 2018; Rounsaville, Weissman, Kleber, & Wilber, 1982), Obsessive-Compulsive Disorder (OCD) (Peles, Adelson, & Schreiber, 2009), voluntary morphine consumption (Alizadeh, Zahedi-Khorasani, et al., 2018), locomotor sensitization (Alizadeh, Khorasani, et al., 2018; Taracha, Chrapušta, Lehner, Skórzewska, & Płaźnik, 2009) and the vulnerability to relapse (Curran, Bolton, Wanigaratne, & Smyth, 1999; Fareed et al., 2010). These issues have been studied in animal and clinical studies. They may reflect persistent changes induced by opioids in the brain reward and motivational system (Koob & Le Moal, 2008), which may hinder the effectiveness of MMT in addicted individuals (Kauer & Malenka, 2007). Thus, the reversal or prevention of MMT-induced behavioral and mood disorders could be a helpful method for the treatment of relapse in addicts during MMT. It seems that the EE models by directly acting on the brain's reward system (Thiel, Pentkowski, Peartree, Painter, & Neisewander, 2010) may be effective in treating addicts under MMT. An enriched cage is larger and contains

toys and running wheels (Hammami-Abrand Abadi & Miladi-Gorji, 2016). We have previously shown that EE attenuates the spontaneous morphine withdrawal signs, the self-grooming behavior (Hammami-Abrand Abadi & Miladi-Gorji, 2016), anxiety/depressive-like behavior, and voluntary morphine consumption (Hammami-Abrand Abadi, Miladi-Gorji, & Bigdeli, 2016) in morphine withdrawn rats.

Given the well-known beneficial effects of EE on morphine-induced behavioral deficits, it is expected that exposure to the EE during MMT improves the behavioral deficits and relapse in addicted individuals. Thus, the present study aimed to investigate whether EE would reduce anxiety, depressive, and obsessive-compulsive-like behaviors, as well as voluntary morphine consumption in morphine withdrawn rats under MMT.

2. Methods

Animals and induction of morphine dependence and housing conditions

Adult male Wistar rats (average weight: 170±10 g) were group-housed in cages (35×15×24 cm), 4 rats per cage, under 12/12-h light/dark cycle (6 AM lights on – 6 PM lights off) at 22 °C –24 °C and had access to food and water ad libitum. All experimental procedures were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Morphine sulfate (Temad, Iran) was injected subcutaneously at a dose of 10 mg/kg, twice a day at 12 h intervals for 14

days, and all injections were performed with a quantity of 1 mL/kg. The control rats were injected similarly with saline. Then, the rats were group reared over 30 days under MMT during morphine withdrawal (n = 8 rats per cage) in a Standard Environment (SE) or EE. Enriched rats were placed in a large cage (96×49×38 cm) with plastic tunnels, rope, swing, balls, ramp, ladder, shelters, step, cube and a running wheel, toys, which were changed every two days to maintain its novelty. Control rats (SE) were placed in standard laboratory cages (42×34×15 cm), as previously described by our laboratory (Hammami-Abrand Abadi & Miladi-Gorji, 20117) (Figure 1; Timeline of the experiment).

Methadone-maintenance treatment

Methadone hydrochloride (Temad, Iran) was injected subcutaneously once per day for four consecutive 2-day periods with doses of 0.5, 1, 2, and 3 mg/kg, respectively. The maintenance dose of 3 mg/kg was then administered 6 days per week (to avoid toxicity) until day 30 of morphine withdrawal, as previously described by our laboratory (Alizadeh et al., 2018). None of methadone overdose symptoms (apnea, cyanosis, motionlessness, and irritability) were observed during the first 72 h after the injection in all our experiments.

Experimental protocol

Sixty-four rats were divided randomly into 8 groups (n=8 rats per group) based on our experimental protocols (Figure 1; timeline of experiment) as follows: saline/saline/standard environment (Sal/Sal/SE), Sal/Sal/enriched environment (Sal/Sal/EE), Sal/methadone/SE

(Sal/Meth/SE), Sal/Meth/EE, dependent/Sal/SE (D/Sal/SE), D/Sal/EE, D/Meth/SE, and D/Meth/EE. To avoid the bias of circadian rhythms, we conducted all behavioral tests between 10:00 AM to 1:00 PM.

Anxiety Measurement in the Elevated Plus Maze Test (EPM)

On day 46, all rats were tested with the EPM. The rats were individually placed in the center of the EPM with two open (50×10 cm) and two closed (50×10×40 cm) arms, and a central platform (10×10 cm). They were allowed to explore the apparatus for 5 min as described previously (Alizadeh et al., 2018). Time spent in and entries into open and closed arms were recorded during each 5 min test by a tracking system (EthoVision, Noldus, The Netherlands). The apparatus was cleaned with water after each trial.

OCD-Like Behavior

Grooming behavior as an innate behavior in animals is similar to OCD in humans. On day 47, the rats were individually placed in a Plexiglas box (41 cm length×33 cm height×41 cm width). Components of grooming behavior included vibration, face and head washing, body grooming, scratching, paw licking, head shaking, and genital grooming. Grooming behavior was recorded at 15-s intervals for 30 min. Thus, the maximum available score was 120 during the observation period, as previously described (Hammami-Abrand Abadi & Miladi-Gorji, 2017).

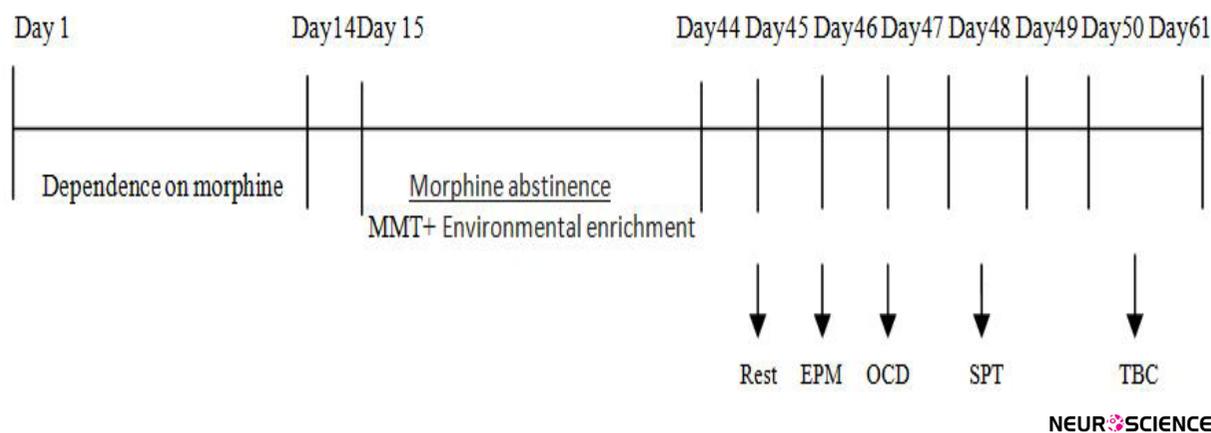


Figure 1. Timeline of Experiments (see section 2 for details)

Rats were made dependent on morphine for 14 days. Then, the rats under Methadone Maintenance Treatment (MMT) were reared in an Enriched Environment (EE) for 30 days of morphine abstinence. The rats were tested for the elevated plus maze (EPM), OCD-like behavior, Sucrose Preference Test (SPT), and voluntary morphine consumption using a Two-Bottle Choice (TBC) paradigm.

Depressive-Like Behavior Using the Sucrose Preference Test (SPT)

All rats were kept individually in cages for 24 h before testing. Then, they had free access to two bottles in each cage for 48 h on days 48 and 49: one with 200 mL of 32% sucrose (w/v) and the other with 200 mL of tap water. The positions of the bottles were changed every 12 h to avoid learning. Fluid intake and sucrose were measured every day. At the end of 48 h, the bottles were removed, and sucrose preference was calculated as $100\% \times \text{sucrose solution consumption (mL)} / \text{total fluid consumption (mL)}$ as previously described (Alizadeh et al., 2018).

Two-Bottle Choice (TBC) Paradigm

Each rat was housed in cages with two bottles over 12 days (from day 50 to 61). In one bottle, morphine sulfate was dissolved in 3% sucrose solution and also 3% sucrose solution was in the control bottle as follows: on days 1–4 (0.3 mg/mL morphine); 5–8 (0.5 mg/mL morphine) and 9–12 (0.7 mg/mL morphine). The rats were allowed to have continuous access to both bottles. The positions of the bottles in the cage were changed at the time of daily bottle weighing to avoid learning. Fluid intake was measured by weighing the bottles between 9:00 and 10:00 AM every day. The body weights of rats were measured at the start of each period as previously described (Alizadeh et al., 2018). The average morphine

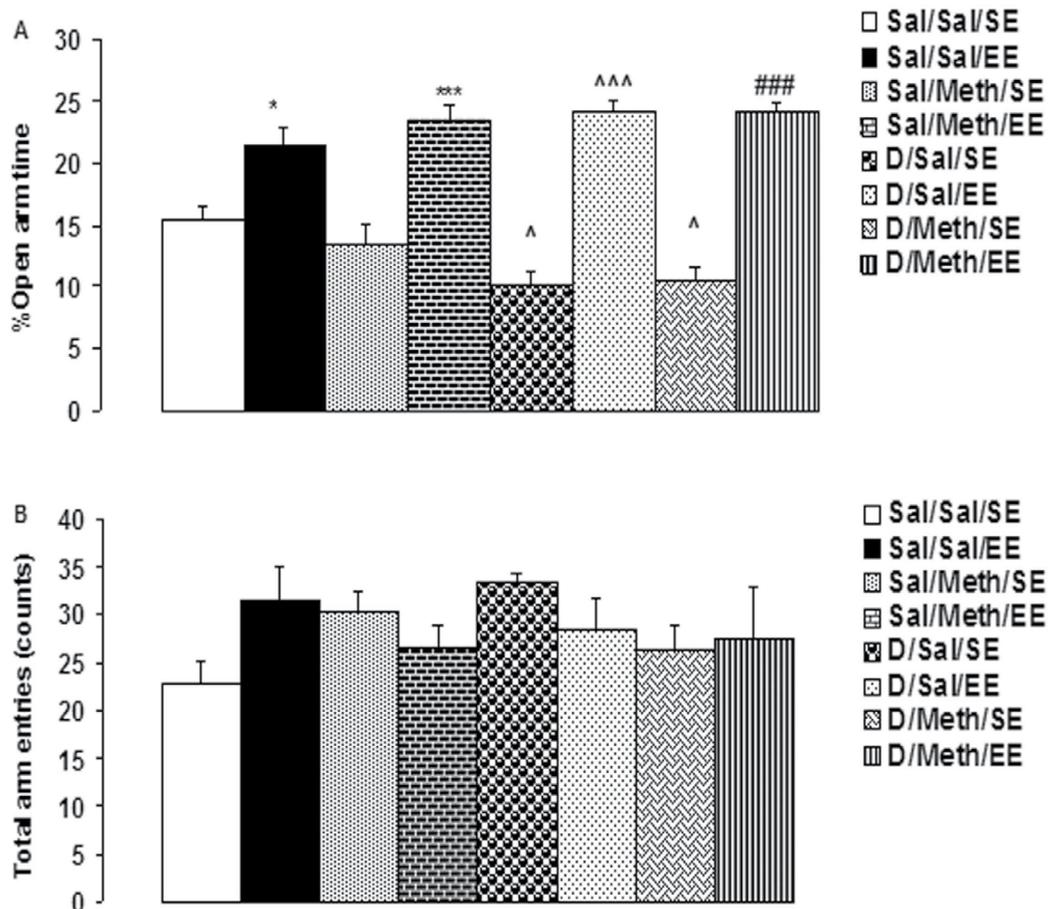


Figure 2. Effect of Environmental Enrichment (EE) on the Anxiety-Like Behavior in Morphine Withdrawn Rats Receiving MMT

A: The Percentages of Time Spent in Open Arm; and B: The Total Arm Entries (counts) of the EPM (n=8 rats per group).

Data are expressed as Mean \pm SEM. EE rats spent significantly more time in the open arms in the Sal/Sal/EE, D/Sal/EE, and D/Meth/EE groups.

*P<0.05 vs the Sal/Sal/SE group; ***P<0.0001 vs the Sal/Meth/SE group; ^^^P<0.0001 vs the D/Sal/SE group; ###P<0.0001 vs the D/Meth/SE group.

consumption and preference ratios (morphine solution consumed [mL]/total consumed from both bottles [mL]) were evaluated during a 4-day intake period.

Statistical analysis

The data were expressed as the mean±SEM. The data were analyzed using a 3-way Analysis of Variance (ANOVA) with the fixed factors of dependence×methadone×housing and with repeated measures as required. The post-hoc study included

Tukey's test. Statistical differences were considered significant at $P < 0.05$.

3. Results

Anxiety-Like Behavior

The data of EPM are shown in Figure 2-A and B. Three-way ANOVA showed the significant effects of methadone ($F_{1,55}=3.01$, $P=0.05$), housing ($F_{1,55}=169.38$, $P=0.0001$), methadone×housing ($F_{1,55}=5.29$, $P=0.025$), and dependence×methadone×housing ($F_{1,55}=13.02$,

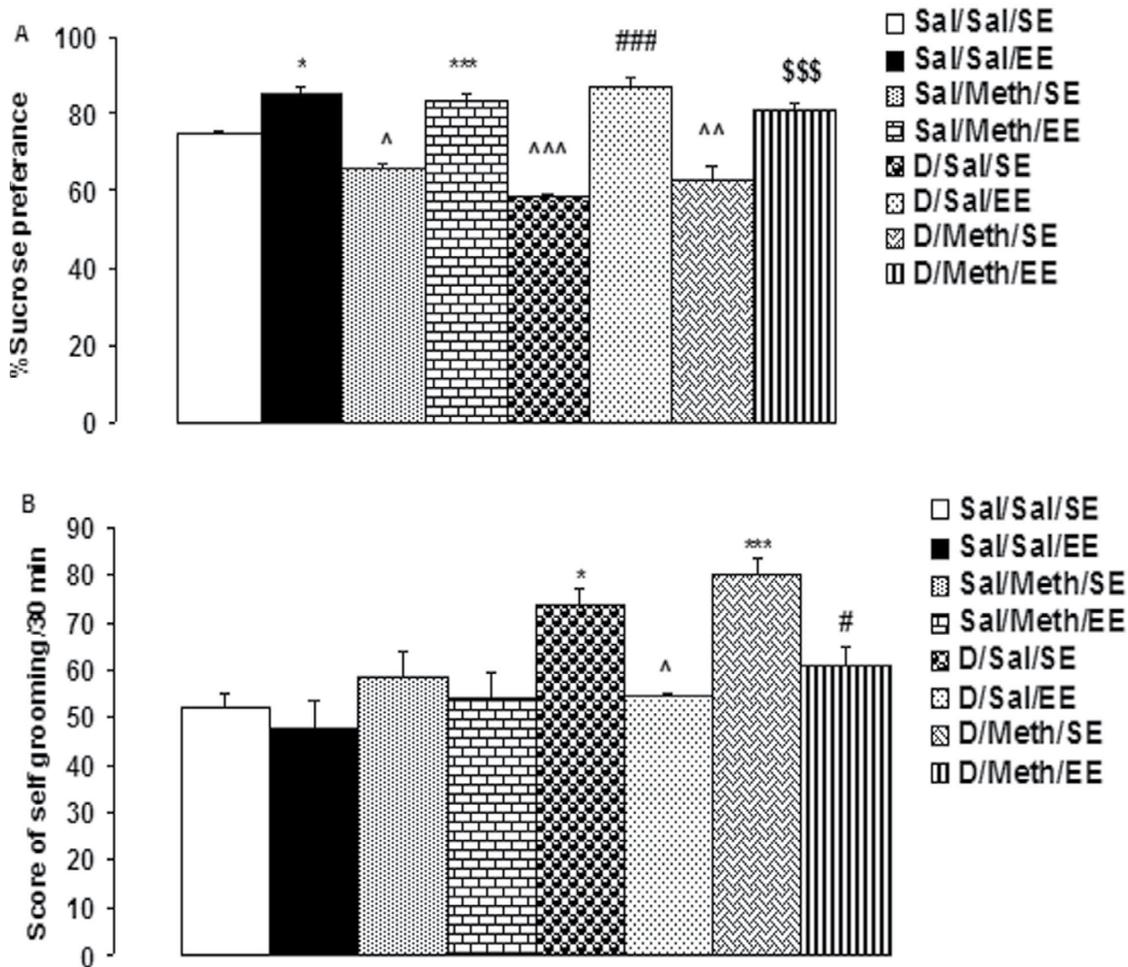


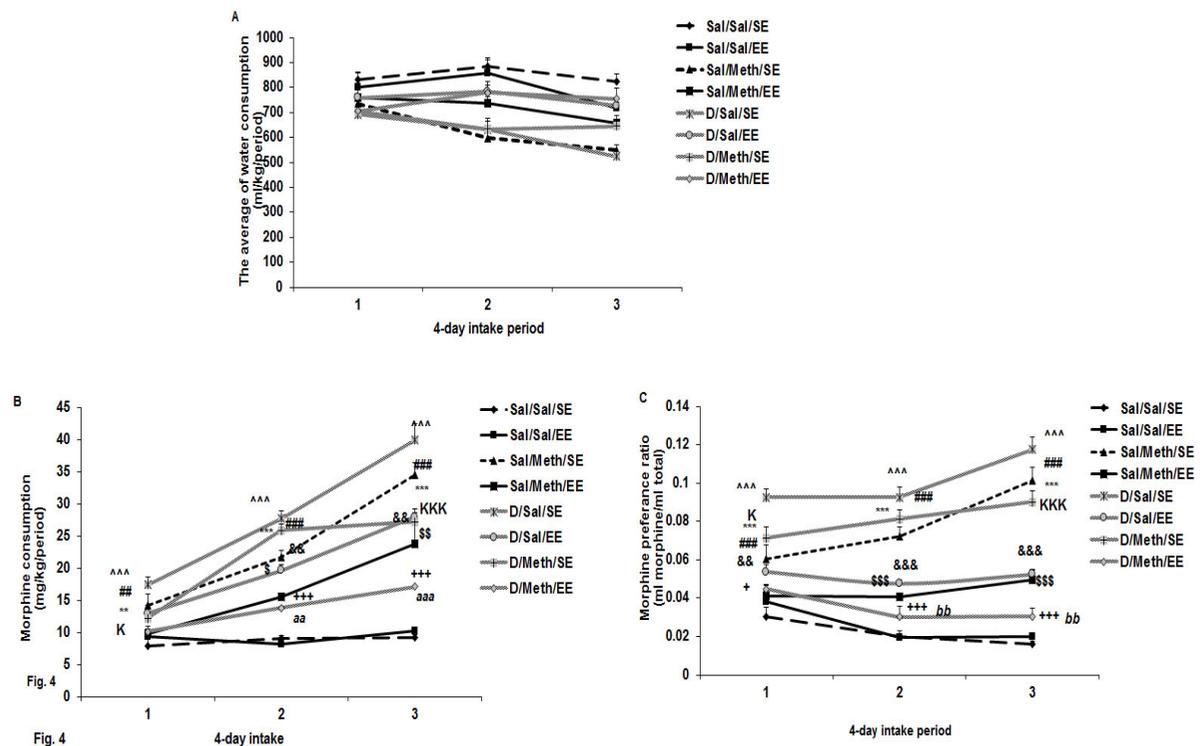
Figure 3. Effect of Environmental Enrichment (EE) on the Depression and OCD-Like Behaviors in Morphine Withdrawn Rats Receiving MMT

A: The Percentages of Sucrose Preference Using the SPT; and B: The Self-Grooming Sign (n=8 rats per group).

Data are expressed as Mean±SEM. The percentages of sucrose preference were lower in the D/Sal/SE and D/Meth/SE rats, while the percentages were higher in the D/Sal/EE and D/Meth/EE groups. Also, the self-grooming scores were lower in the D/Sal/EE and D/Meth/EE rats.

In A: [^] $P < 0.05$; ^{^^} $P < 0.0001$; ^{^^^} $P < 0.003$ vs the Sal/Sal/SE group; ^{***} $P < 0.0001$ vs the Sal/Meth/SE group; ^{###} $P < 0.0001$ vs the D/Sal/SE group; ^{\$\$\$} $P < 0.0001$ vs the D/Meth/SE group.

In B: * $P < 0.02$; ^{***} $P < 0.0001$ vs the Sal/Sal/SE group; [^] $P < 0.047$ vs the D/Sal/SE group; [#] $P < 0.04$ vs the D/Meth/SE group.



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Figure 4. Effect of Environmental Enrichment (EE) on Voluntary Morphine Consumption in Morphine Withdrawn Rats Under MMT

A: Water Intake; B: Morphine Consumption; C: The Morphine Preference Ratio (n=8 rats per group).

Data are expressed as Mean±SEM. The Sal/Meth/EE, D/Sal/EE, and D/Meth/EE groups exhibited a decrease in morphine preference ratios during three periods.

In B and C: $^{AAA}P < 0.0001$, $^{AA}P < 0.011$, $^{AAA}P < 0.0001$, $^{AA}P < 0.011$, $^{AAA}P < 0.0001$ vs the Sal/Sal/SE group; K $P < 0.047$, KKK $P < 0.0001$, KKP < 0.003 , KKKP < 0.0001 vs the D/Sal/SE group; $^SP < 0.04$, $^SSP < 0.011$, $^SSSP < 0.0001$ vs the Sal/Meth/SE group, $^*P < 0.031$, $^{+++}P < 0.0001$ vs the D/Meth/SE group, $^{aa}P < 0.005$, $^{aaa}P < 0.0001$, $^{aa}P < 0.005$ vs the D/Sal/EE group.

$P=0.03$) in the percentage of time spent in open arms (Figure 2-A). Between-group comparisons revealed that the percentages of time spent in the open arms were significantly lower in the D/Sal/SE and D/Meth/SE (both, $P=0.049$) groups compared to their control groups. While the times were significantly longer in the Sal/Sal/EE ($P=0.013$), Sal/Meth/EE ($P=0.0001$), D/Sal/EE ($P=0.0001$), D/Meth/EE ($P=0.0001$) groups than their control rats. There was no significant difference in the number of total arm entries among the groups as a measure of locomotion (Figure 2-B). Thus, EE completely reversed the anxiogenic effects of MMT.

Depression and obsessive-like behavior

In the SPT test, 3-way ANOVA revealed the significant effects of dependence ($F_{1,54}=10.54$, $P=0.002$), methadone ($F_{1,54}=4.9$, $P=0.032$), housing ($F_{1,54}=158.19$, $P=0.0001$), dependence×housing ($F_{1,54}=11.1$, $P=0.002$), and dependence×methadone×housing ($F_{1,54}=8.65$, $P=0.005$). Comparisons between groups showed that sucrose pref-

erence in the Sal/Meth/SE ($P=0.05$), D/Sal/SE ($P=0.003$), and D/Meth/SE ($P=0.0001$) groups was less than that in the Sal/Sal/SE group. While the sucrose preferences of the Sal/Sal/EE ($P=0.016$), Sal/Meth/EE ($P=0.0001$), D/Sal/EE ($P=0.0001$), D/Meth/EE ($P=0.0001$) groups were significantly higher than that in the control group (Figure 3-A). The results of the self-grooming behavior test for assessing OCD-like behavior (Figure 3-B) using a 3-way ANOVA revealed the significant effects of dependence ($F_{1,56}=21.57$, $P=0.0001$), methadone ($F_{1,56}=4.5$, $P=0.039$), housing ($F_{1,56}=16$, $P=0.0001$), dependence×housing ($F_{1,56}=5.81$, $P=0.019$), and dependence×methadone×housing ($F_{1,56}=3.3$, $P=0.049$). Between-group comparisons showed that the self-grooming scores in the D/Sal/SE ($P=0.02$) and D/Meth/SE groups ($P=0.001$) were more than that in the Sal/Sal/SE group, while rats of D/Sal/EE ($P=0.047$) and D/Meth/EE groups ($P=0.04$) got significantly lower scores than the control rats. Thus, exposure to EE decreased depressive/OCD-like behaviors in morphine withdrawn rats under MMT.

Assessment of voluntary morphine consumption

Data of the voluntary morphine consumption during three periods of the TBC test are shown in Figure 4. There was no significant difference between groups in water consumption (Figure 4-A). Three-way ANOVA with repeated measures for the voluntary consumption of morphine during three periods exhibited the significant effects of day ($F_{2,110}=148.67$, $P=0.0001$), dependence ($F_{1,55}=88.24$, $P=0.0001$), methadone ($F_{1,55}=14.59$, $P=0.0001$), housing ($F_{1,55}=78.51$, $P=0.0001$), dependence×methadone ($F_{1,55}=108.74$, $P=0.0001$), dependence×housing ($F_{1,55}=21.25$, $P=0.0001$), methadone×housing ($F_{1,55}=15.5$, $P=0.0001$) and day×dependence×methadone×housing ($F_{2,110}=3.42$, $P=0.036$). Also, 3-way ANOVA with repeated measures for the morphine preference ratio during three periods of intake revealed significant effects of day ($F_{2,110}=6.11$, $P=0.003$), dependence ($F_{1,55}=146.4$, $P=0.0001$), methadone ($F_{1,55}=35.79$, $P=0.0001$), housing ($F_{1,55}=230.45$, $P=0.0001$), dependence×methadone ($F_{1,55}=110.51$, $P=0.0001$), dependence×housing ($F_{1,55}=70.1$, $P=0.0001$), methadone×housing ($F_{1,55}=31.12$, $P=0.0001$), dependence×methadone×housing ($F_{1,55}=8.4$, $P=0.005$), and day×dependence×methadone×housing ($F_{2,110}=4.1$, $P=0.019$). Between-group comparisons showed that morphine preference ratios during three periods of intake in morphine withdrawn rats receiving saline and MMT housed in EE were significantly lower than those in the D/Sal/SE ($P=0.003$, $P=0.0001$, and $P=0.0001$), D/Meth/SE groups ($P=0.031$, $P=0.0001$ and $P=0.0001$, respectively) (Figure 4-C). Also, the morphine preference ratio of the Sal/Meth/SE group was higher than that in the Sal/Sal/SE group during three periods of intake ($P=0.0001$), which was lower than in the Sal/Meth/EE group during periods 2 and 3 of intake (both, $P=0.0001$). Data related to the voluntary consumption of morphine (Figure 4-B) were similar to that observed in the morphine preference ratio and followed the same pattern. Also, between-group comparisons showed that the morphine consumption and preference ratio of the D/Meth/EE rats were lower than those in the D/Sal/EE group during periods 2 ($P=0.007$ and $P=0.005$, respectively) and 3 ($P=0.0001$ and $P=0.005$, respectively) of intake. Thus, EE decreased voluntary morphine consumption as an animal model of relapse.

4. Discussion

The results of our study showed that morphine-withdrawn rats receiving saline and MMT reared in the SE exhibited anxiety/depressive/OCD-like behaviors after 30 days of abstinence from morphine. It seems that the effects of morphine withdrawal are much longer, indicating that the anxiety, depression, and OCD are persistent behaviors after 30 days of abstinence, even though

the rats simultaneously were under MMT. This finding was consistent with previous studies' results, showing the anxiety/depressive-like behaviors in morphine withdrawn rats (Alizadeh et al., 2018) and patients (Brienza et al., 2000; Lin et al., 2012) under MMT. Also, the Sal/Meth/SE group showed an increase in depression-like behavior.

It is likely that the mild persistent of MMT-induced locomotor sensitization in morphine-withdrawn rats (Alizadeh et al., 2018) and also, grooming behavior as a driver of drug-seeking behavior (Lubman, Yücel, & Pantelis, 2004) in turn may intensify drug craving and relapse in addicted individuals (Boileau et al., 2006). The observed behavioral dysfunction as a part of psychological dependence can increase the risk of relapse and drug-seeking after protracted abstinence (Lubman et al., 2004).

In line with this assumption, we saw that the D/Sal/SE, D/Meth/SE, and Sal/Meth/SE groups exhibited an increase in the voluntary morphine consumption and morphine preference ratios during three intake periods with the Sal/Sal/SE group. Thus, the rats under MMT are still at risk for relapse, although the morphine preference ratio was significantly lower in the D/Meth/SE group than the D/Sal/SE group. A heightened response to drug cues probably occurs in the medial prefrontal cortex and the extended limbic system in patients under MMT (Langleben et al., 2008), which drives further drug-seeking behavior.

We found that exposure to EE decreased anxiety/depressive/OCD-like behaviors. These findings are in line with previous studies showing that exposure to EE reduces the severity of physical and psychological dependence on morphine (Hammami-Abrand Abadi & Miladi-Gorji, 2017; Hammami-Abrand Abadi et al., 2016; Pooriamehr, Sabahi, & Miladi-Gorji, 2017), behavioral sensitization (Bardo, Robinet, & Hammer, 1997) and rewarding and reinforcing properties of morphine (Xu, Hou, Gao, He, & Zhang, 2007). Thus, EE, because of its positive life experiences and anti-stress effects, may prevent the development of drug addiction (Solinas, Thiriet, Chauvet, & Jaber, 2010). This finding is supported further by previous studies showing that EE decreased repetitive behavior (Lewis, Tanimura, Lee, & Bodfish, 2007), stereotyped behavior (Turner, Lewis, & King, 2003), stress level, and locomotor activity (Xu, Sun, Xue, & Li, 2014) in animal studies.

We also showed that the morphine consumption and preference were lower in the D/Meth/EE group than the D/Sal/EE group during periods 2 and 3 of intake,

which was consistent with an earlier study that MMT may improve craving for heroin in drug abusers (Wang et al., 2014). Therefore, exposure to EE in the morphine withdrawal rats receiving MMT may exert an adjunctive protective role on the risk of relapse after protracted abstinence. Our finding may suggest some additive effects between MMT and EE on the craving and drug-seeking behavior in morphine withdrawn rats.

Presently, it is unclear how the EE can reduce MMT-induced behavioral dysfunction and the risk of relapse in morphine-withdrawn rats. Our findings cannot answer this question. Previous results indicate that exposure to an EE enhanced hippocampal neurogenesis (Simpson & Kelly, 2011) and Insulin-Like Growth Factor 2 (IGF-2) (Li et al., 2014), higher serotonin levels in the frontal cortex (Leger et al., 2015), and metabolic activity in the cortex and striatum (Turner, Yang, & Lewis, 2002) are probably involved in reducing anxiety/depression and OCD. Also, it seems that the modulation of neurotransmitter systems and plastic changes resulting from the EE in the hippocampus, the frontal cortex, and the striatum (Simpson & Kelly, 2011) may account for the decrease in the morphine preference ratio and behavioral dysfunction in morphine-withdrawn rats under MMT. Future studies should examine the neurobiological mechanisms caused by EE in morphine-withdrawn rats under MMT.

5. Conclusion

This study indicates that morphine dependence and MMT are associated with increased anxiety/depression/OCD-like behaviors in parallel with morphine preference in rats. It provides novel evidence that exposure to the EE attenuates anxiety/depression/OCD-like behaviors and morphine preference ratio in morphine withdrawn rats under MMT. Therefore, exposure to the EE during morphine withdrawal and receiving MMT may be a proper therapeutic strategy for preventing relapse in addicts.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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