Effect of environmental enrichment on physical and psychological dependence signs and voluntary morphine consumption in morphine-dependent and morphine-withdrawn rats

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This study was designed to examine the effect of environmental enrichment during morphine dependency and withdrawal on the severity of naloxone-precipitated withdrawal signs, anxiety, and depressive-like behaviors and voluntary morphine consumption in morphine-dependent rats. The rats were injected with bi-daily doses (10 mg/kg, 12 h intervals) of morphine for 14 days following rearing in a standard environment (SE) or enriched environment (EE) during the development of morphine dependence and withdrawal. Then, rats were tested for withdrawal signs after naloxone injection, anxiety (the elevated plus maze) and depression-related behavior (sucrose preference test), and voluntary consumption of morphine using a two-bottle choice paradigm, in morphine-dependent and morphine-withdrawn rats. The results showed that EE decreased naloxone-precipitated withdrawal signs, but not anxiety or sucrose preference during dependence on morphine. The EE-withdrawn rats showed an increase in the elevated plus maze open arm time and entries and higher levels of sucrose preference than SE rats. Voluntary consumption of morphine was lower in the EE-withdrawn rats than in the SE groups in the second period of drug intake. Thus, exposure to EE reduced the severity of morphine dependence and voluntary consumption of morphine, alongside reductions in anxiety and depression-related behavior in morphine-withdrawn rats. *Behavioural Pharmacology* **27:270–278**

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Introduction

Chronic administration of morphine is associated with an increase in physical and psychological dependence signs including autonomic-somatic symptoms, anxiety, and depression (Janiri *et al.*, 2005; Miladi-Gorji *et al.*, 2012), reflecting plastic changes in neuronal circuitry including the brain's reward-processing system (Kauer and Malenka, 2007), which eventually lead to drug-seeking behaviors and relapse in addicted individuals (Weiss, 2005).

Thus, the reversal or prevention of the plastic changes induced by morphine dependence could be a useful method for the treatment of relapse to drug seeking. It seems that environmental enrichment, including physical, social, and cognitive enrichment, is an effective method that can produce a range of plastic responses in the brain's reward system (Xu *et al.*, 2007; Thiel *et al.*, 2010), including neurogenesis and improvements in learning and memory (Petrosini *et al.*, 2009; Sale *et al.*, 2009), and recovery from neurodegenerative disease and psychiatric disorders (stress, anxiety, depression, schizophrenia, and drug addiction) (van Praag *et al.*, 2000; Will *et al.*, 2004; Nithianantharajah and Hannan, 2006; Laviola *et al.*, 2008; Simpson and Kelly, 2011; Nader *et al.*, 2012).

In EE models, animals are placed in large cages containing physical stimuli, such as toys and running wheels, that allow animals to explore, play, and exercise, and can be allowed more control over the environment, which is much richer than under the standard conditions (Simpson and Kelly, 2011). EE rearing has been shown to reduce the rewarding and reinforcing effects of opioids, drug-induced early gene expression in the striatum, vulnerability to develop drug-seeking behaviors (Xu *et al.*, 2007; Solinas *et al.*, 2008, 2009), and locomotor activity and behavioral sensitization to morphine (Bardo *et al.*, 1997; Xu *et al.*, 2007). Thus, an important question would be whether EE could also blunt the deleterious effects of chronic administration of morphine during morphine dependence and withdrawal. Therefore, the aim of this study was to investigate whether exposure of rats to an enriched environment (EE) during induction of morphine dependence and spontaneous withdrawal would attenuate the severity of the naloxone-precipitated withdrawal syndrome, the anxiety and depressive-like behaviors, and voluntary consumption of morphine.

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Methods

Subjects and housing conditions

Male Wistar rats (170 ± 20 g) were housed in cages with a 12-h light/dark cycle at 22–24°C and had free access to food and water. All of the experimental procedures were performed in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. In addition, care was taken to use the minimum number of animals in each experiment. Rats were housed 7–8 per cage. The standard environments (SEs) consisted of standard plastic cages (42 × 34 × 15 cm), whereas the EE consisted of larger cages (96 × 49 × 38 cm) containing plastic tunnels, rope, swing, balls, ramp, ladder, shelters, step, cube, and a running wheel that were cleaned and changed or moved every 2–3 days to maintain the novelty of the environment. Rats were handled during cage cleaning every 2–3 days (Brenes *et al.*, 2008; Hajheidari *et al.*, 2015).

Induction of morphine dependence

Morphine sulfate (Temad Company, Tehran/Karaj, Iran) was injected subcutaneously at a dose of 10 mg/kg, twice per day at 12 h intervals (06:00 and 18:00 h), as described previously (Miladi-Gorji *et al.*, 2011, 2012) for 14 days. The control rats were injected with saline.

Withdrawal rating scale

Immediately after injection of naloxone hydrochloride (Sigma-Aldrich, Germany) (1 mg/kg, intraperitoneal), withdrawal signs were recorded and scored according to a modified version of the Gellet–Holtzman scale as described previously (Gellert and Holtzman, 1978; Skelton *et al.*, 2007; Miladi-Gorji *et al.*, 2011, 2012) for 30 min. Graded signs including jumps, wet dog shakes, and abdominal contractions were counted as the number of events occurring during the test time. Body weights were recorded immediately before and 60 min after naloxone injection, and the percentage change in body weight was calculated. Checked signs, including diarrhea, ptosis, erection or genital grooming, teeth chattering, writhing, and irritability, were counted as positive if the sign occurred at any time over a 30 min period. The overall severity of withdrawal was calculated by summing the proper weighting factor of somatic signs.

Anxiety measurement

To assess the level of anxiety, the rats were individually placed in the center of the elevated plus maze (EPM) with two open (50 × 10 cm) and two closed (50 × 10 × 40 cm) arms, and a central platform (10 × 10 cm), and allowed to explore the apparatus for 5 min as described previously (Miladi-Gorji *et al.*, 2012). Time spent in, and entries into, open and closed arms were measured during each 5 min test. The apparatus was cleaned with water after each trial.

Depression-related behaviors

Sucrose preference test

A modified version of the sucrose preference test (SPT) as described previously (Brenes *et al.*, 2006; Casarotto and Andreatini, 2007) was used. All rats were maintained in individual cages for 24 h before testing (to reduce isolation stress during the test). Rats were allowed access for 48 h to two bottles: one containing 200 ml of 32% sucrose (w/v) solution and the other containing 200 ml of tap water. The positions of the bottles were changed every 12 h to avoid potential location bias. Fluid intake and sucrose were measured each day. At the end of 48 h, the bottles were removed and sucrose preference was calculated as 100% × sucrose solution consumption (ml)/ total fluid consumption (ml).

Two-bottle choice procedure

Voluntary morphine consumption and preference were quantified using a modified two-bottle choice (TBC) procedure, as described previously (Belknap, 1990; Berrettini *et al.*, 1994; Ferraro *et al.*, 2005; Haydari *et al.*, 2014). Following the end of the SPT, each rat was housed individually in cages with continuous access to two bottles for a period of 12 days of testing. One bottle contained a 3% sucrose solution and the other contained morphine sulfate dissolved in 3% sucrose solution at the following concentrations: days 1–4, 0.3 mg/ml; days 5–8, 0.5 mg/ml; and days 9–12, 0.7 mg/ml. Sucrose 3% w/v was added to morphine sulfate solution to make the morphine solution less bitter. To minimize position effects, the position of the bottles in the cage was changed at the time of daily bottle weighing. Fluid intake was measured by weighing the bottles between 9:00 and 10:00 h daily. Body weights were measured at the start of each period. The average morphine and water consumption, and preference ratios (ml morphine solution consumed/total ml consumed from both bottles), were evaluated during each 4-day period.

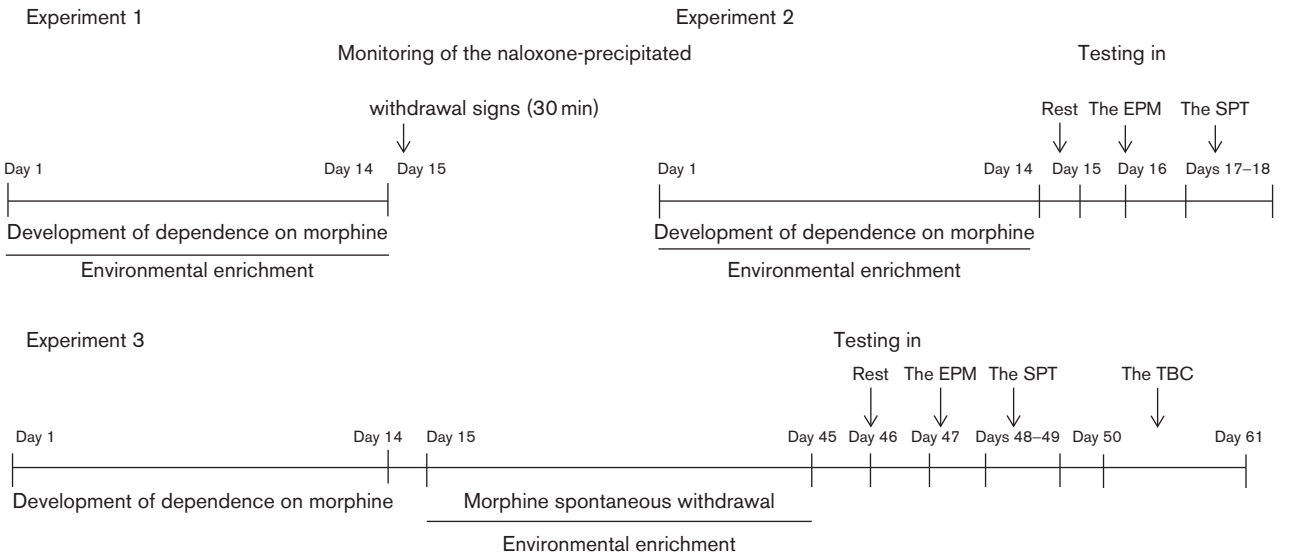
Experiment 1

This experiment examined the effects of EE on the severity of morphine dependence. Naive rats were divided into two groups ($n = 7$–8 rats per group): dependent-standard environment (D/SE) and dependent-enriched environment (D/EE). The EE group was allowed to freely exercise, play, and explore their environment for 14 days during the development of morphine dependence. On day 15, the global severity of morphine withdrawal behaviors induced by naloxone (1 mg/kg, intraperitoneal) was measured 2 h after a morphine injection (see Fig. 1 for timeline).

Experiment 2

This experiment examined the effect of EE on anxiety and depressive-like behaviors in morphine-dependent rats. Naive rats were divided into four groups ($n = 7$–8 rats per group): saline-standard environment (Sal/SE),

Fig. 1



Timelines of experiments (see the Methods section for details). EPM, elevated plus maze; SPT, sucrose preference test; TBC, two-bottle choice.

saline-enriched environment (Sal/EE), D/SE, and D/EE. The EE group was allowed to explore their environment and received simultaneously injections of saline or morphine for 14 days. Then, all rats were rested in standard cages on day 15 with continued injection. On days 16–19, 2 h after injection of saline or morphine (to prevent the development of morphine withdrawal), all animals were tested in the EPM and SPT, respectively (see Fig. 1 for timeline).

Experiment 3

This experiment examined the effect of EE on anxiety and depressive-like behaviors and voluntary morphine consumption during spontaneous withdrawal in morphine-dependent rats. Thirty naive rats were divided into four groups ($n = 7$–8 rats per group): Sal/SE, Sal/EE, D/SE, and D/EE. In each group, saline or morphine was injected for 14 days. Then, the EE groups were placed in their home cages for 30 days during spontaneous morphine withdrawal and all rats were rested in standard cages on day 46.

All animals were tested in the EPM and SPT, respectively, on days 47–49. Then, morphine-withdrawn rats were housed individually in cages at the end of SPT for a period of 12 days to evaluate the voluntary consumption of morphine on days 50–61. On testing days, the rats were housed in standard cages (see Fig. 1 for timeline).

Statistical analysis

The data were expressed as the mean ± SEM and analyzed using two-way analyses of variance (ANOVAs) with the between-subjects factors treatment (saline or morphine) and housing condition (SE or EE) and with repeated measures as required. Post-hoc analyses were carried out using Tukey's test. Graded somatic signs of opiate withdrawal and preference ratio were analyzed using Student's *t*-test. Checked somatic signs of opiate withdrawal were analyzed using the Mann–Whitney *U*-test and expressed as the percentage of rats in a particular experimental group that showed that sign during the observation period. Statistical differences were considered significant at P value less than 0.05.

Results

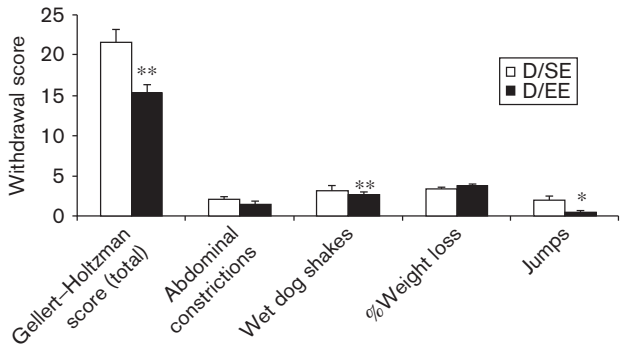
Experiment 1

The overall Gellert–Holtzman scores were significantly lower in morphine-dependent rats exposed to the EE than in the D/SE rats ($t_{13} = 2.45$, $P < 0.01$). Among the graded signs, jumping ($t_{13} = 20.87$, $P < 0.05$) was lower in the D/EE rats compared with the D/SE rats (Fig. 2). Among the checked signs (Table 1), the numbers of rats per group with diarrhea ($U = 11$, $P < 0.025$) and ptosis ($U = 14$, $P < 0.005$) were decreased among the D/EE rats compared with the D/SE rats. There were no statistically significant differences in other withdrawal signs between the two groups.

Experiment 2

In morphine-dependent rats, two-way ANOVA of the EPM results (Fig. 3) showed a significant effect of treatment ($F_{1,26} = 6.34$, $P < 0.02$) for closed arm entries and of housing ($F_{1,26} = 4$, $P < 0.05$) for closed arm time. Also, two-way ANOVA indicated significant treatment × housing interactions on closed arm entries ($F_{1,26} = 4.79$, $P < 0.05$) and closed arm time ($F_{1,26} = 5.42$, $P < 0.05$). Comparisons between groups showed that the number of

Fig. 2



Effect of environmental enrichment on naloxone-precipitated morphine withdrawal signs in dependent animals ($n=7$ for SE rats and $n=8$ for EE rats). Environmental enrichment decreased the graded signs of morphine withdrawal. $^{**}P<0.01$, and $^{*}P<0.05$, versus the D/SE group. D/EE, dependent-enriched environment; D/SE, dependent-standard environment; EE, enriched environment; SE, standard environment.

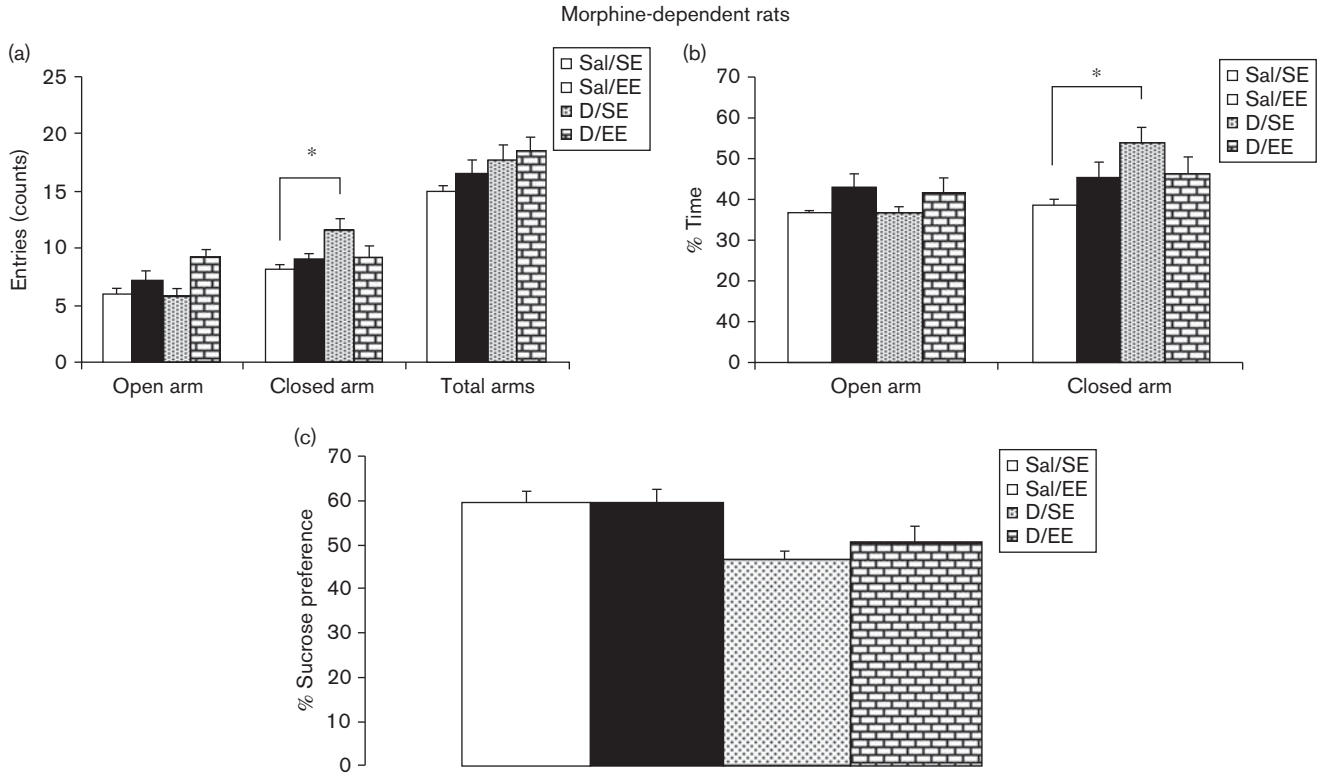
Table 1 Effect of environmental enrichment on checked signs of naloxone-precipitated morphine withdrawal

Withdrawal signs	D/SE (%)	D/EE (%)
Diarrhea	85.71	25*
Ptosis	100	50^
Writhing	100	87.5
Erection, genital grooming	71.43	62.5
Teeth chattering	57.4	25
Irritability	28.57	25

Each of the withdrawal signs was checked if present in a given rat at any point during a 30 min observation ($n=7$ for SE rats and $n=8$ for EE rats). Each of these signs was then converted into the percentage of rats in each experimental group that showed that sign during 30 min. Environmental enrichment produced significant decreases in the percent of checked signs of naloxone-precipitated morphine withdrawal, such as diarrhea and ptosis.
D/EE, dependent-enriched environment; D/SE, dependent-standard environment; EE, enriched environment; SE, standard environment.
$^{*}P<0.05$, $^{\wedge}P<0.005$, versus D/SE.

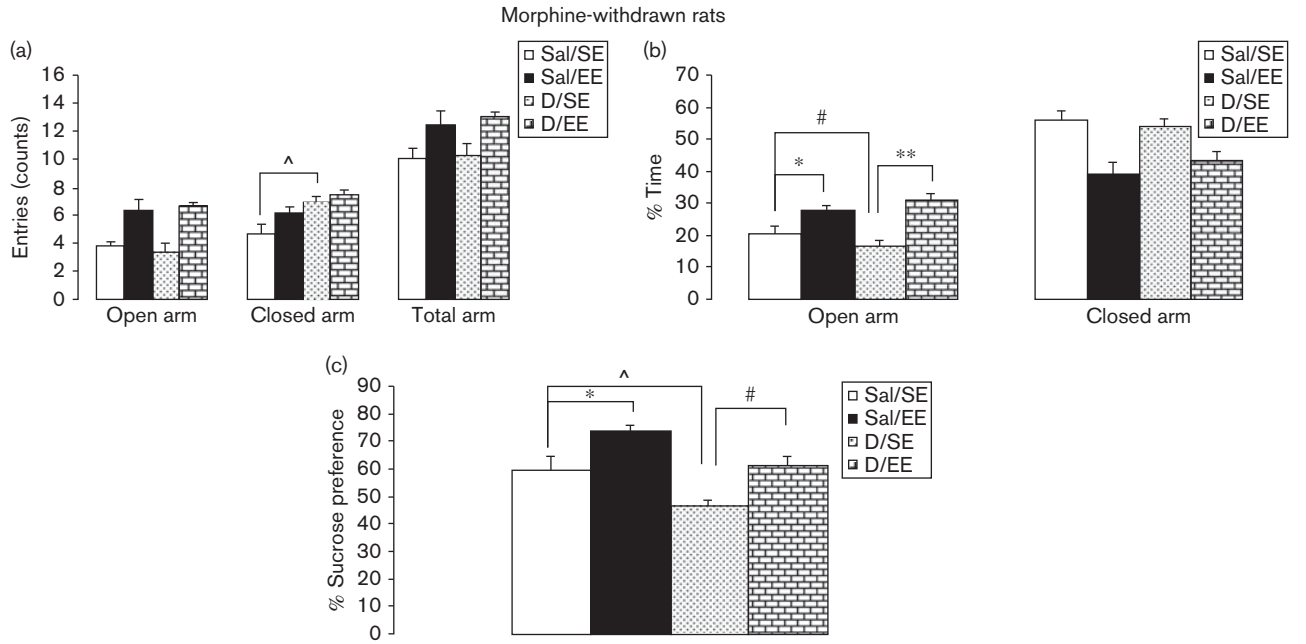
entries into closed arms in the D/SE group was significantly more than that in the Sal/SE group ($P<0.02$; Fig. 3a). Also, the time spent on the closed arms was significantly greater in the D/SE group than in the Sal/SE group ($P<0.041$; Fig. 3b). There were no statistically significant changes in the percentage of time spent in and entries into the EPM open arms and in the number of total arm entries between the four groups.

Fig. 3



Effect of environmental enrichment on anxiety and depressive-like behaviors in morphine-dependent rats ($n=7$ for SE rats and $n=8$ for EE rats). (a) The number of entries into and (b) the time spent in open and closed arms, (c) sucrose preference (%). The number of entries into and the time spent on the closed arms were significantly higher in the D/SE group than the Sal/SE group. $^{*}P<0.01$ (a) or $^{*}P<0.05$ (b), versus the Sal/SE group. D/SE, dependent-standard environment; EE, enriched environment; Sal/EE, saline-enriched environment; Sal/SE, saline-standard environment; SE, standard environment.

Fig. 4



Effect of environmental enrichment on anxiety and depressive-like behaviors in morphine-withdrawn rats ($n=7$ for SE rats and $n=8$ for EE rats). (a) The number of entries into and (b) the time spent in open and closed arms, (c) the sucrose preference (%). The number of entries into the closed arms was significantly more in the D/SE group than the Sal/SE group. EE groups spent significantly more time in the open arms than the SE groups, and had a significantly greater sucrose preference. (a) $^{\wedge}P<0.05$, versus Sal/SE group. (b) $^{*}P<0.05$, versus the Sal/SE group; $^{\#}P<0.02$, versus the Sal/SE group, $^{**}P<0.001$, versus the D/SE group. (c) $^{*}P<0.05$, versus the Sal/SE group; $^{\wedge}P<0.02$, versus the Sal/SE group, $^{\#}P<0.005$, versus the D/SE group. D/SE, dependent-standard environment; EE, enriched environment; Sal/EE, saline-enriched environment; Sal/SE, saline-standard environment; SE, standard environment.

The results of the SPT, using a two-way ANOVA, showed a significant effect of treatment ($F_{1,26}=11.8$, $P<0.002$), and no significant effects of housing ($F_{1,26}=0.34$, NS), and no significant interaction ($F_{1,26}=0.35$, NS; Fig. 3c).

Experiment 3

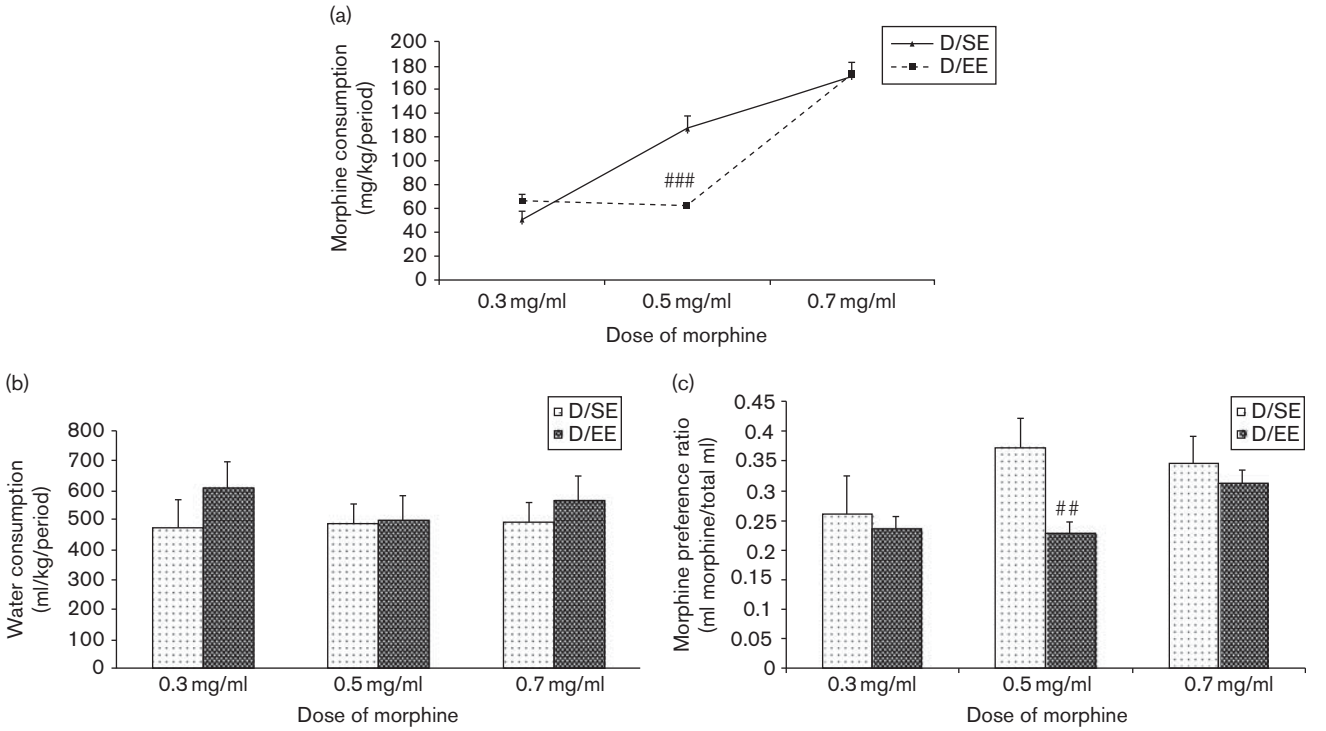
The results of the EPM testing in morphine-withdrawn rats are shown in Fig. 4. Two-way ANOVA indicated a significant main effect of treatment ($F_{1,26}=21.79$, $P<0.001$) for closed arm entries and of housing for open arm entries ($F_{1,26}=17.826$, $P<0.001$), open arm time ($F_{1,26}=30.96$, $P<0.001$), closed arm entries ($F_{1,26}=4.93$, $P<0.05$), and closed arm time ($F_{1,26}=23.39$, $P<0.001$). There were no significant treatment × housing interactions on open arm entries and the percentage of time spent of closed arm. Thus, the latter comparisons were not examined separately. However, two-way ANOVA indicated significant treatment × housing interactions on closed arm entries ($F_{1,26}=3.114$, $P<0.05$) and open arm time ($F_{1,26}=10.1$, $P<0.005$). Comparisons between groups showed that the number of closed arm entries of the D/SE was significantly higher than that of the Sal/SE group ($P<0.05$). Comparisons between groups showed no significant differences in the number of closed arm entries of the Sal/EE and D/EE groups. No significant differences were found in the number of total arm entries between the four groups (Fig. 4a).

Also, the percentage of time spent in the open arms was significantly higher ($P<0.001$) in the D/EE group compared with the D/SE group. The percentage of time spent in the open arms was significantly higher in the Sal/EE ($P<0.036$) and lower in the D/SE ($P<0.012$) groups than that of the Sal/SE groups (Fig. 4b).

The results of the SPT are shown in Fig. 4c. Two-way ANOVA indicated significant effects of housing ($F_{1,26}=23.76$, $P<0.001$) and treatment ($F_{1,26}=15.67$, $P<0.001$), and a significant treatment × housing interaction ($F_{1,26}=4.35$, $P<0.05$). Comparisons between groups showed that sucrose preference in D/SE rats was less than that in the Sal/SE group ($P<0.02$). The sucrose preferences of the Sal/EE ($P<0.05$) and the D/EE ($P<0.005$) groups were significantly higher than those of the Sal/SE and D/SE groups, respectively (Fig. 4c).

Voluntary morphine consumption using a TBC is shown in Fig. 5. Two-way repeated-measures ANOVA showed significant effects of days ($F_{2,28}=90.2$, $P<0.001$) and groups ($F_{1,14}=4.87$, $P<0.034$), and a significant days × groups interaction ($F_{2,28}=10.23$, $P<0.001$). There were

Fig. 5



Effect of environmental enrichment on voluntary morphine consumption during morphine spontaneous withdrawal ($n = 7$ for SE rats and $n = 8$ for EE rats). (a) Morphine consumption, (b) water intake, and (c) preference for the different doses of morphine. Note the different units in (a) (mg/kg/period) and (b) (ml/kg/period). D/EE rats showed a lower consumption and preference ratio compared with the D/SE group during the second period of drug intake (i.e. only at dose of 0.5 mg/ml morphine). $^{\#\#}P < 0.002$, $^{\#\#\#}P < 0.001$, versus D/SE. D/EE, dependent-enriched environment; D/SE, dependent-standard environment; EE, enriched environment; SE, standard environment.

no significant differences in voluntary water intake between groups in any of the three periods (Fig. 5b). However, D/EE rats showed a lower voluntary consumption of morphine ($t_{14} = 4.63$, $P < 0.001$; Fig. 5a) and also a lower preference ratio ($t_{14} = 2.62$, $P < 0.002$) compared with the D/SE group, but only at the dose of 0.5 mg/ml morphine (Fig. 5c).

Discussion

Enriched environment decreases the severity of withdrawal signs in morphine-dependent rats

The present study provides novel evidence that rats exposed to an EE during the development of morphine dependence showed fewer withdrawal signs as assessed by overall Gellert–Holtzman scores. This finding is supported by previous studies showing that an EE leads to decreases in behavioral sensitization and in the rewarding and reinforcing properties of morphine (Xu *et al.*, 2007; Solinas *et al.*, 2009; Hofford *et al.*, 2010). Also, enriched rats were more sensitive to the antinociceptive effects of μ-opioid agonists (Smith *et al.*, 2005; Stairs and Bardo, 2009). At present, the neurobiological mechanisms underlying the decreased morphine withdrawal signs after exposure to EE are still unclear. Previous findings indicate that exposure to an EE reduced the expression of FosB-like proteins in the nucleus accumbens (Xu *et al.*, 2007), in addition to decreasing substance P and attenuating pain (Vachon *et al.*, 2013). Animals raised in EEs also show increased levels of GABAergic transmission (He *et al.*, 2010) and opioid signaling (Lee *et al.*, 2013). Subsequent studies have confirmed that exposure to EEs decreased morphine-induced conditioned place preference (Xu *et al.*, 2007; Solinas *et al.*, 2009), possibly through functional changes in mesolimbic dopamine transmission, and altered sensitivity to the effects of dopamine agonists (Smith *et al.*, 2009, Simpson and Kelly, 2011). Taken together, these findings indicate that the reduced rewarding properties of morphine and neurotransmitter changes following exposure to an EE could contribute toward reducing some morphine withdrawal signs, including the decreased overall Gellert–Holtzman scores, jumping, diarrhea, and ptosis in rats exposed to the EE in our study.

Enriched environment reduces the anxiety and depressive-like behaviors during morphine spontaneous withdrawal, but not during morphine dependence

We found that morphine-dependent and morphine-withdrawn rats raised under standard housing conditions showed anxiogenic-like behaviors as assessed by

the EPM. The SE morphine-dependent rats made significantly more closed arm time and entries than control rats (Sal/SE). Also, morphine-withdrawn rats raised in the SE made significantly more closed arm entries and spent less time in the open arms than control animals (Sal/SE). Our finding is in agreement with our previous study showing that morphine dependence and withdrawal has been associated with an increase in anxiogenic-like behavior in rats (Miladi-Gorji *et al.*, 2012). We also found that exposure to an EE is associated with a reduction in anxiety during spontaneous morphine withdrawal, but not during the development of morphine dependence. Similar changes were observed in depressive-like behavior: sucrose preference was reduced in morphine-dependent and morphine-withdrawn rats raised under standard housing conditions, which can be interpreted as an indicator of anhedonia. Exposure to the EE ameliorated this depressive-like behavior following withdrawal of morphine, but not during dependence. Our finding is in agreement with previous studies showing that EE can alleviate the stress and locomotor activity induced by acute morphine administration (Xu *et al.*, 2014), and ameliorate stress-induced depressive-like (Veena *et al.*, 2009; Zhang *et al.*, 2011) and anxiety-like (Ravenelle *et al.*, 2013) behaviors. The lower level of sucrose preference in morphine-withdrawn rats raised under standard housing conditions is also in accordance with a previous study showing that rats showed a reduced preference to sweet solutions for 6 days after morphine withdrawal (Lieblich *et al.*, 1991). However, in our study, morphine withdrawal lasted 30 days, showing that depression-like and anxiety-like behaviors are persistent in morphine-withdrawn rats. Also, we found no significant difference in the number of the total arm entries between groups, suggesting that reduced open arm activity in the morphine-withdrawn rats raised under standard housing conditions was because of increased anxiety and not hypoactivity or motor impairment. Our findings are in agreement with a study showing that a 3-week exposure to EE is required to reduce anxiety-like behavior and might be related to higher serotonin levels in the frontal cortex (Leger *et al.*, 2014). These results suggest that the duration of exposure to environmental enrichment is important for inducing the behavioral and neurobiological effects. It seems that rats raised in EEs have lower emotional reactions; thus, they can explore their environment more efficiently. Probably, the enhanced serotonin levels (Brenes *et al.*, 2008, 2009), high level of glucocorticoid receptors, and lower hypothalamic–pituitary axis reactivity (Larsson *et al.*, 2002; Simpson and Kelly, 2011) observed in rats exposed to EEs could facilitate their coping responses. At present, the neurobiological mechanisms by which EEs reduce anxiety or depression levels are still not known. Identified impacts of EE show direct and indirect involvement of a variety of neurotransmitter systems including serotonin (Brenes *et al.*, 2008, 2009), noradrenaline (Naka *et al.*, 2002), GABA (He *et al.*, 2010), BDNF (Simpson and Kelly, 2011), neurogenesis (Veena *et al.*, 2009), and behavioral plasticity (Urakawa *et al.*, 2013) in the various regions of the brain. These mechanisms may underlie the ability of 30 days of exposure to the environmental enrichment to reduce the anxiety-like and depression-like behaviors in morphine-withdrawn rats.

Enriched environment partially reduces voluntary morphine consumption during morphine spontaneous withdrawal

Our findings have shown that EE morphine-withdrawn rats had a lower voluntary consumption of morphine during the second period of the intake of drug using a TBC paradigm as an animal model of relapse, reflecting an incentive demand for the drug in morphine-withdrawn rats. It seems that stressful events (Ferguson *et al.*, 2004; Weiss, 2005), anxiety, and depression (Self and Nestler, 1998; Aston-Jones and Harris, 2004) may be important in drug craving and vulnerability to relapse.

The effect of the EE on the susceptibility to drug addiction and intake of morphine is still not understood. It was reported recently that an EE can trigger long-term modification in neural functions, and hence, may have a beneficial effect against relapse following a period of extinction (Xu *et al.*, 2007; Loewinger *et al.*, 2012; Hofford *et al.*, 2014). It was also found that EE eliminated drug addiction-related memory (Xu *et al.*, 2014) and decreased sensitivity to morphine-induced reward and conditioned place preference (Xu *et al.*, 2007), and cocaine-induced craving (Chauvet *et al.*, 2009), but did not confer lasting protection against drug-elicited relapse (Thiel *et al.*, 2009, 2011). In this respect, there was no difference in the third period of drug intake in our study, which is consistent with other studies showing that EE decreased self-administration of methylphenidate (Alvers *et al.*, 2012) and amphetamine responses in drug-seeking behavior (Stairs *et al.*, 2006) at low drug doses, but not at high doses. Also, exposure to an EE increased reinstatement of sucrose-seeking following a larger sucrose prime and the reinstatement threshold to the amphetamine prime (Stairs *et al.*, 2006). However, rearing under enriched housing conditions had no effect on cocaine-primed reinstatement (Thiel *et al.*, 2009, 2011). It seems that higher doses of morphine reinstate drug-seeking behavior in EE morphine-withdrawn rats, which might be because of incentive motivational effects of morphine at higher doses. Also, it seems that reinforcement was enhanced by EE, given the increasing sucrose preference in saline-treated and morphine-treated rats reared under enriched housing conditions. In this study, the rats had free access to water and morphine and SE morphine-withdrawn rats had a greater preference for the intermediate dose of morphine over water than EE morphine-withdrawn rats. Given that there was no significant difference between the two groups in water consumption,

this may reflect an incentive demand for the drug in the TBC. Thus, we conclude that exposure to EE partially decreases the rewarding effects of morphine, which can reduce the risk of sensitivity and drug seeking after withdrawal. Therefore, EE may decrease the incentive motivation for morphine intake at the intermediate dose, relative to SE morphine-withdrawn rats.

It is suggested that the EE may not be an effective treatment for countering craving elicited by morphine, perhaps because of the short duration of EE rearing, for only 30 days, in our study. Future studies need to examine the neurobiological mechanisms impacted by an EE. Also, a limitation of our study was the lack of a saline control group in the TBC experiment, and this should be considered in future studies.

Conclusion

This study provides novel evidence that access to EE during the development of morphine dependence can decrease the severity of physical dependence. It also reduced anxiety and depression-like behaviors and led to a relatively smaller reduction in the voluntary consumption of morphine after protracted periods of abstinence. Our findings may have potential therapeutic application in the prevention of morphine-induced behavioral deficits and relapse.

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Conflicts of interest

There are no conflicts of interest.

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