

## Accepted Manuscript

**Title: Environmental enrichment attenuates morphine-induced conditioned place preference and locomotor sensitization in maternally-separated rat pups**

**Authors: Soheyla Khalaji<sup>1</sup>, Imanollah Bigdeli<sup>2</sup>, Raheb Ghorbani<sup>3</sup>, Hossein Miladi-Gorji<sup>\*4</sup>**

<sup>1</sup> Faculty of Psychology and Educational Sciences, University of Semnan, Semnan, Iran.

<sup>2</sup> Faculty of Educational Sciences and Psychology, Ferdowsi University of Mashhad, Mashhad, Iran.

<sup>3</sup> Social Determinates of Health Research Center, Semnan University of Medical Sciences, Semnan, Iran.

<sup>4</sup> Laboratory of Animal Addiction Models, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran.

*\*Corresponding author at: Hossein Miladi-Gorji, (PhD) Associate Professor of Physiology, Laboratory of Animal Addiction Models, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, P.O. Box 35131-38111, Semnan, Iran. **Email:** [Miladi331@yahoo.com](mailto:Miladi331@yahoo.com) (H. Miladi-Gorji) **Mobile phone no:** +98 9125313069, Fax: +98 23 33354186*

To appear in: Basic and Clinical Neuroscience

**Received date:** 20.02.2017

**Revised date:** 11.09.2017

**Accepted date:** 08.11.2017

This is a “Just Accepted” manuscript, which has been examined by the peer-review process and has been accepted for publication. A “Just Accepted” manuscript is published online shortly after its acceptance, which is prior to technical editing and formatting and author proofing. Basic and Clinical Neuroscience Journal provides “Just Accepted” as an optional and free service which allows authors to make their results available to the research community as soon as possible after acceptance. After a manuscript has been technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Please note that technical editing may introduce minor changes to the manuscript text and/or graphics which may affect the content, and all legal disclaimers that apply to the journal pertain.

**Please cite this article as:**

Khalaji, S., Bigdeli, I., Ghorbani, R., & Miladi-Gorji, H. (In Press). Environmental enrichment attenuates morphine-induced conditioned place preference and locomotor sensitization in maternally-separated rat pups. *Basic and Clinical Neuroscience*. Just Accepted publication Nov. 8, 2018. doi:

***Environmental enrichment attenuates morphine-induced conditioned place preference and locomotor sensitization in maternally-separated rat pups***

Soheyla Khalaji<sup>1</sup>, Imanollah Bigdeli<sup>2</sup>, Raheb Ghorbani<sup>3</sup>, Hossein Miladi-Gorji<sup>\*4</sup>

<sup>1-</sup> *Faculty of Psychology and Educational Sciences, University of Semnan, Semnan, Iran.*

<sup>2-</sup> *Faculty of Educational Sciences and Psychology, Ferdowsi University of Mashhad, Mashhad, Iran.*

<sup>3-</sup> *Social Determinates of Health Research Center, Semnan University of Medical Sciences, Semnan, Iran.*

<sup>4-</sup> *Laboratory of Animal Addiction Models, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran.*

*\*Corresponding author at: Hossein Miladi-Gorji, (PhD) Associate Professor of Physiology, Laboratory of Animal Addiction Models, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, P.O. Box 35131-38111, Semnan, Iran.*

***Email:*** [Miladi331@yahoo.com](mailto:Miladi331@yahoo.com) (H. Miladi-Gorji)

***Mobile phone no:***

+98 9125313069

Fax: +98 23 33354186

### ***Research highlights***

- ▶ Maternal separation increased morphine preference and locomotor sensitization.
- ▶ Environmental enrichment decreased CPP in maternally separated pups.
- ▶ Environmental enrichment attenuated locomotor sensitization in maternally separated pups.

### ***Abstract***

***Introduction:*** This study investigated effect of the environmental enrichment during adolescence on morphine-induced conditioned place preference (CPP) and locomotor sensitization in maternally separated male and female rat pups.

***Methods:*** Male Wistar rats were allowed to mate with female virgin Wistar rats. Pups were separated from the dam daily for 3h during postnatal days 2–14. All pups were weaned at postnatal (PND) 21 and reared in standard environment (SE) or environmental enrichment (EE) from PND21 to 50 with litter-mates of the same sex. The CPP and behavioral sensitization to morphine were assessed by an un-biased place conditioning paradigm and open field method.

***Results:*** The results showed that the maternal separation enhanced morphine-induced CPP in both sexes, locomotor sensitization in male pups and tolerance to morphine-induced motor activity in female pups during adolescence. While, male and female pups reared in an EE exhibited a decrease in morphine-induced CPP, locomotor sensitization and tolerance induced by maternal separation **than their control pups.**

***Conclusion:*** Our findings have shown that access to EE during adolescence may exert a protective effect against morphine-induced reward, locomotor sensitization and tolerance in adolescent male and female rats following maternal separation.

***Keywords:*** Maternal separation, Enriched environment, Conditioned place preference, Morphine, Behavioral sensitization.

## 1. Introduction

Previous studies have shown that exposure to negative early life events such as maternal separation increases the vulnerability to behavioral and physiological deficits in adulthood (Champagne, Francis, Mar, & Meaney, 2003), disruption of the enkephalinergic system (Vazquez et al., 2005). Also, it reported that maternal separation increases vulnerability to substance use (Dube, Cook, & Edwards, 2010; Sinha, 2008), amphetamine (Campbell & Spear, 1999) - and morphine (Vazquez, Weiss, Giros, Martres, & Daugé, 2007)-induced conditioned place preference (CPP), morphine-induced sensitization and tolerance (Vazquez et al., 2005).

It seems that the environmental enrichment (EE) models can be prevented maternal separation-induced functional brain alterations.

Our previous findings suggest that EE is probably a useful method for the prevention of the brain alterations induced by maternal separation. The EE contains physical stimuli, running wheel, rubber balls, tunnels, toys which stimulate exploration behavior in laboratory animals (Hajheidari, Miladi-Gorji, & Bigdeli, 2015a; Simpson & Kelly, 2011). We have previously shown that EE reduced severity of drug dependence, the voluntary consumption of drug and anxiety/depressive-like behavior in morphine (Hammami-Abrandabadi, Miladi-Gorji, & Bigdeli, 2016) and methamphetamine withdrawn rats (Hajheidari et al., 2015a; Hajheidari, Miladi-gorji, & Bigdeli, 2015b).

Some evidence indicated that EE reduced expression of morphine-induced CPP in male C57BL/6 mice (Xu, Hou, Gao, He, & Zhang, 2007), vulnerability to cocaine addiction (Nader et al., 2012), and sensitization to morphine (Bardo, Robinet, & Hammer Jr, 1997; Xu et al., 2007), cocaine (Solinas, Chauvet, Thiriet, El Rawas, & Jaber, 2008) and maternal separation induced- stress reactivity (Francis, Diorio, Plotsky, & Meaney, 2002). Thus, the aim of the present study was to investigate whether exposure to EE during adolescence can

attenuate morphine-induced CPP and locomotor Sensitization in maternally separated male and female rat pups.

## **2. Materials and method**

### **2.1. Animals**

Male Wistar rats ( $250 \pm 10$  g) were allowed to mate with female virgin Wistar rats ( $250 \pm 10$  g) ( $n=14$ ) during a 24 h period as described previously by our laboratory (Akhavan et al., 2013; Haydari, Miladi-Gorji, Mokhtari, & Safari, 2014) with a 12 h light/dark cycle, at temperature  $22 \pm 4^{\circ}\text{C}$  and food and water were available *ad libitum* throughout the experiment. Postnatal 0 (PND 0) was the day of birth and maternal separation was conducted from PND2 to PND14 for 180 min that mothers were separated daily from their pups. The pups were carried to an adjacent room with their original cage while room temperature was maintained at  $32 \pm 0.5^{\circ}\text{C}$  (days 2–5) or  $30 \pm 0.5^{\circ}\text{C}$  (days 6–14) for 3 h (Taghavi-Khalil Abad, Miladi-Gorji, & Bigdeli, 2016; Francis et al., 2002; Geuzaine & Tirelli, 2014). Control pups were reared under standard conditions. All pups were weaned on PND 21 and housed with litter-mates of the same sex.

To attenuating litter-size-induced variability in body weight of pups and to homogenize possible effects of genetic and prenatal factors, one or two pups of each sex from each litter randomly assigned for each group. The pups ( $n=6-8/\text{sex}/\text{experiment}/\text{rearing group}$ ) were randomly divided into four groups according to sex into two separate experiences; pups that were not separated from mothers and housed in a standard environment (SE) (No MS/SE); pups that were not separated from mothers and housed in an EE (No MS/EE); pups separated from mothers and housed in a SE (MS/SE); and pups separated from mothers and housed in an EE (MS/EE). Pups were reared in EE or SE for 4 weeks from PND21 to PND50. Then, all pups were rested in standard cages on PND51. All animals were tested in the CPP and the open field chamber from PND52 to PND60 (see Fig. 1 for timeline).

## **2.2. Enriched environmental conditions**

The EE consisted large cages (96cm×49cm×38 cm) containing plastic tunnels, rope, swing, balls, ramp, ladder, shelters, step, cube and a running wheel, which were cleaned and changed every 2–3 days to maintain its novelty, with food and water *ad libitum* as described previously by our laboratory (Hajheidari et al., 2015a). The control animals were placed in standard cages as the SE condition. The SE consisted of standard plastic cage (42cm×34cm×15 cm). The pups were housed 6-9 per cages in both of EE and SE housing.

## **2.3. Assessment of CPP**

The place preference apparatus was made from wood and consisted of two distinct compartments A and B (30×30×30 cm) with a black background or white and different pattern of white or black stripes (vertical or horizontal). It separated from each other by a neutral area (30×15×30 cm) with a red background and having guillotine gates. This study was performed for 9 days as described previously (Taghavi-Khalil Abad et al., 2016; Xu et al., 2007), and behavioral activity (time spent and the number of visits to the each chamber with back-and-forth motions during pre- and post-conditioning test sessions, to determine that locomotor activity was not different between groups) was recorded by a video camera, using a tracking system (EthoVision, Noldus, The Netherlands):

**Pre-conditioning:** On day 1 (Habituation), rats were placed on the neutral area and given free access to the entire chamber for 20 min in order to adapt to the environment. This test was repeated on day 2, the amount of time spent in each of the three compartments was recorded and the initial preference was calculated. If the animal spent more than 60% of the time on day 2 for either side (initial side preference) it was eliminated from the experiment.

**Conditioning:** Rats were treated with morphine at the dose of 5 mg/kg, s.c. on days 3, 5, and 7 and saline on days 4, 6, and 8 during the conditioning phase. Drug and saline administration

alternated daily such that, half of the rat received drug in the chamber A and the other half received drug in the chamber B. Then each rat placed into one of the two choice compartments for 15 min.

**Post-conditioning:** On day 9, rats were placed on the center compartment and allowed to explore the entire chamber for 20 min. Preference or conditioning score is calculated by subtracting the amount of time spent in the drug-paired chamber before conditioning (on day 2) from the amount of time spent after conditioning.

#### **2.4. Assessment of locomotor Sensitization**

Morphine-induced locomotor sensitization was assessed by the open field chamber. It consisted of a clear glass cylinder 25 cm in diameter and 30 cm high on a wooden plate with the same diameter. It was divided into 4 equal zones by two intersecting lines as described previously (Taghavi-Khalil Abad et al., 2016; Sahraei et al., 2006). First, baseline locomotor activity was evaluated. Then, to induce locomotor sensitization, morphine (5 mg/kg) was subcutaneously injected once daily for 3 consecutive days. However, the acute locomotor response of morphine was then evaluated only after the first (acute) injection of morphine. Then, the rats did not receive any treatment for the next 5 days. All rats were challenged with morphine (1 mg/kg, s.c.) on the sixth day after a 5-day drug-free period, and evaluated for locomotor activity (post- morphine challenge), so that rats were placed on the open field chamber and allowed to explore the chamber for 10 min in order to adapt to novel environments. Then, the number of lines crossed for each rats with all four legs were counted manually for the next 10 min. After each test the arena was cleaned with 90% alcohol solution.

#### **Fig. 1**

#### **2.5. Statistical analysis**



The data expressed as the mean  $\pm$ standard error of the mean (S.E.M.). These data were analyzed by using three-way analyses of variance (ANOVA) with the fixed factors maternal care (No MS and MS), housing condition (SE and EE) and sex (male and female). Post-hoc analyses included Tukey's test. Statistical differences were considered significant at  $P < 0.05$ .

### 3. Results

#### 3.1. Environmental enrichment decreased morphine-induced CPP in maternally separated pups.

The results of the CPP are illustrated in Fig. 2A. The analysis revealed a significant effect of maternal care ( $F_{1, 47} = 52.16, P = 0.0001$ ), and housing ( $F_{1, 47} = 121.49, P = 0.0001$ ) and sex ( $F_{1, 47} = 6.01, P = 0.018$ ), maternal care  $\times$  sex interactions ( $F_{1, 47} = 23.4, P = 0.0001$ ), maternal care  $\times$  housing interactions ( $F_{1, 47} = 14.17, P = 0.0001$ ) and maternal care  $\times$  housing  $\times$  sex interactions ( $F_{1, 47} = 5.95, P = 0.018$ ). Between groups comparisons showed that the morphine-induced place preference score were lower in the No MS/EE male group ( $P = 0.003$ ) and higher in the MS/SE group than the No MS/SE group in male and female rats (both,  $P = 0.0001$ ). While the place preference score for the morphine-paired compartment in the MS/EE group was significantly less than the MS/SE group in male and female rats (both,  $P = 0.0001$ ). Also, preference score was higher in MS/SE male rats than female ( $P = 0.003$ ). In summary, our study demonstrates that maternal separation enhanced morphine-induced CPP and EE attenuated the rewarding effect of morphine in both sexes than their control pups.

Also, EE also severely attenuated morphine-induced CPP in the No MS/EE male group than the No MS/SE group.

Also, there were no significant sex ( $F_{1, 47} = 1.23, NS$ ), ( $F_{1, 47} = 2.3, NS$ ), maternal care ( $F_{1, 47} = 0.28, NS$ ), ( $F_{1, 47} = 0.92, NS$ ) and housing ( $F_{1, 47} = 3.13, NS$ ), ( $F_{1, 47} = 0.75, NS$ ) effects and interaction among them ( $F_{1, 47} = 1.32, NS$ ), ( $F_{1, 47} = 1.95, NS$ ) in frequency of crossing from

conditional and non-conditional part in pre-conditioning and post-conditioning situation, respectively. Thus, there was no difference in locomotor activity between groups in pre-conditioning and post-conditioning.

### Fig. 2

### 3.2. Environmental enrichment decreased morphine-induced locomotor sensitization in maternally separated pups.

The results of the open field chamber using a three-way ANOVA are illustrated in Fig. 3.

There were no significant effect of sex, maternal care and housing and no interaction of maternal care  $\times$  housing  $\times$  sex in the number of lines crossed after baseline and the acute injection of morphine.

However, the analysis of morphine challenge-induced locomotor activity revealed a significant effect of maternal care ( $F_{1, 52} = 27.2$ ,  $P = 0.0001$ ), and housing ( $F_{1, 52} = 35.4$ ,  $P = 0.0001$ ) and no significant effects of sex ( $F_{1, 52} = 0.32$ , NS), significant maternal care  $\times$  housing interactions ( $F_{1, 52} = 34.9$ ,  $P = 0.0001$ ), maternal care  $\times$  sex interactions ( $F_{1, 52} = 11.55$ ,  $P = 0.001$ ), housing  $\times$  sex interactions ( $F_{1, 52} = 5.47$ ,  $P = 0.023$ ) and maternal care  $\times$  housing  $\times$  sex interactions ( $F_{1, 52} = 6.35$ ,  $P = 0.015$ ). Comparisons between groups showed that the number of lines crossed in the MS/SE group was significantly higher after morphine challenge than No MS/SE group in male ( $P = 0.0001$ ) and female ( $P = 0.049$ ). While the number of lines crossed in the MS/EE group was less than the MS/SE groups in male ( $P = 0.0001$ ) and female rats ( $P = 0.007$ ) after morphine challenge.

Also, Between-group comparisons showed that the number of lines crossed were higher in MS/SE male rats after morphine challenge than the baseline response and the acute response of morphine ( $P = 0.007$ ,  $P = 0.013$ ; respectively), while was less in MS/EE male rats after morphine challenge than the acute response of morphine ( $P = 0.003$ ). The number of lines crossed was less in MS/SE and MS/EE female rats after morphine challenge than the acute

response of morphine ( $P=0.004$ ,  $P=0.03$ ). In summary, our study demonstrates that maternal separation enhanced locomotor sensitization in male pups and tolerance to morphine-induced motor activity in female pups during adolescence and EE decreased locomotor sensitization and morphine tolerance than their control pups (MS/SE).

### Fig. 3

#### 4. Discussion

We found that the maternally separated rats enhanced morphine-induced CPP in both sexes, which was more evident in male than female pups; probably due to high basal levels of corticosterone in female rats (Rubio et al., 1998) in response to stress. Our finding is consistent with previous studies showing that the maternal separation enhanced morphine-induced CPP (Taghavi-Khalil Abad et al., 2016; Vazquez et al., 2007) and cocaine self-administration (Moffett et al., 2006). It may be due to a decreased dopamine transporter expression (Brake, Zhang, Diorio, Meaney, & Gratton, 2004; Meaney, Brake, & Gratton, 2002), GABA<sub>A</sub> receptor levels (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000) and serotonin reuptake transporter expression (Lee et al., 2007), oxytocin (Amini-Khoei et al., 2017) and BDNF levels (Roceri, Hendriks, Racagni, Ellenbroek, & Riva, 2002) following maternal separation. This study provides novel evidence that exposure to EE for 30 days after maternal separation decreased morphine-induced CPP in male and female pups during adolescence. Although, previous studies have shown that EE reduced morphine-induced CPP (Xu et al., 2007) and self-administration of cocaine and amphetamine (Gipson, Beckmann, El-Maraghi, Marusich, & Bardo, 2011; Green, Gehrke, & Bardo, 2002). Given that maternal separation plays a major role in dysregulation of reward function (Ploj, Roman, & Nylander, 2003a, 2003b). Therefore, EE can activate the same pathways that are activated by morphine and may also lead to neuroplastic changes in the mesolimbic reward pathway (Thiel, Pentkowski, Peartree, Painter, & Neisewander, 2010).

Also, it may be due to increase in brain serotonin levels following EE (Koh, Magid, Chung, Stine, & Wilson, 2007). Future studies need to examine the neurobiological mechanisms. Also, in present study, there was no significant difference between the male and female rats in the number of visits to the conditional and unconditional chambers during pre-conditioning and post-conditioning measurement. Thus, a higher preference score for morphine in the maternally separated rats was not directly due to an increase in the number of visits to the chambers. We also found that the EE decreased morphine-induced CPP in the No MS male rats. Thus, the observed effects of EE in the MS male group did not merely nullify the impact of maternal separation. This finding indicates that rearing of No MS male rats in an EE before the onset of the CPP conditioning made morphine less effective to induce a CPP. It implies that the repeated activation of morphine reward system by EE decreased the rewarding effect of morphine, possibly through functional changes in mesolimbic dopamine transmission. In this regard, we have previously shown that exposure to EE partially decreases the incentive motivation for morphine intake, which can reduce the risk of sensitivity and drug seeking after withdrawal (Hammami-Abrandabadi et al., 2016). Therefore, this is not to say that might occur aversion in maternally separated pups. For example, it was shown that cocaine concomitantly activates neural circuits producing both reward and aversive behaviors (Kim, Pollak, Hjelmstad, & Fields, 2004). Also, it has shown that the neural circuits involved in drug reward are distinct from those involved in drug aversion (Bardo & Bevins, 2000). Thus, the expression of both CPP and conditioned place aversion (CPA) reflect drug-experience dependent plasticity (Kim et al., 2004). Therefore, unlike previous work (Briellmaier, McDonald, & Smith, 2012), there is no requirement that a negative score of the CPP indicates aversion. Because previous studies (Bardo & Bevins, 2000; Lima et al., 2017) have suggested that both CPP and CPA have distinct mechanisms.

Future studies need to examine whether exposure to EE can produce CPA which has not yet been examined.

We also found that morphine challenge-induced locomotor activity were higher and lower in the male and female MS/SE pups than the acute response of morphine which was reduced by EE in both sexes which have not found in similar studies. These findings indicated that maternally separated male and female pups become sensitized and tolerant to repeated morphine treatments; respectively, supporting previous findings (Taghavi-Khalil Abad et al., 2016; Kalinichev, Easterling, & Holtzman, 2001, 2002). It may be due to increase in anxiety-like behaviors and oversecretion of corticosterone (Kalinichev, Easterling, Plotsky, & Holtzman, 2002), activation of the HPA axis (Pihoker, Owens, Kuhn, Schanberg, & Nemeroff, 1993) during adolescence.

Therefore, these findings indicate that greater sensitivity to the reinforcing properties of morphine, modulation of neurotransmitter release and stressor-induced corticosterone responses following maternal separation could contribute to the enhanced CPP and locomotor sensitization induced by morphine in both males and females pups during adolescence.

These sex related differences in morphine-induced reward and locomotor sensitization may reflect differences in the effects of maternal separation on stress-reactivity (Plotsky & Meaney, 1993), the faster onset of corticosterone secretion in female rats (Young, Altemus, Parkison, & Shastry, 2001), sex differences in glucocorticoid receptor, dopamine transporter expression (Kikusui, Faccidomo, & Miczek, 2005), emphasizing the need for further research.

## ***5. Conclusion***

Our results have been shown that access to EE following maternal separation can decrease the morphine-induced CPP and prevents the development of locomotor sensitization or tolerance to morphine in male and female rat pups during adolescence. Thus, access to EE could be exploited in the development of new therapeutic approaches for drug abuse prevention following negative early life events including maternal separation.

### **Conflicts of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

### **Acknowledgments**

This work was supported by grants from University of Semnan (Semnan, Iran) and also, we would like to thank the Research Center of Physiology, School of Medicine, Semnan University of Medical Sciences for providing research facilities.

### **References**

- Taghavi-Khalil Abad, A., Miladi-Gorji, H., & Bigdeli, I. (2016). Effects of swimming exercise on morphine-induced reward and behavioral sensitization in maternally-separated rat pups in the conditioned place preference procedure. *Neuroscience Letters*, *631*, 79-84 .
- Hammami-Abrandabadi, A., Miladi-Gorji, H., & Bigdeli, I. (2016). Effect of environmental enrichment on physical and psychological dependence signs and voluntary morphine consumption in morphine-dependent and morphine-withdrawn rats. *Behavioural Pharmacology*, *27*, 270-278.
- Akhavan, M. M., Miladi-Gorji, H., Emami-Abarghoie, M., Safari, M., Sadighi-Moghaddam, B., Vafaei, A. A., & Rashidy-Pour, A. (2013). Maternal voluntary exercise during pregnancy enhances the spatial learning acquisition but not the retention of memory in rat pups via a TrkB-mediated mechanism: the role of hippocampal BDNF expression. *Iranian journal of basic medical sciences*, *16*(9), 955 .
- Amini-Khoei, H., Mohammadi-Asl, A., Amiri, S., Hosseini, M.-J., Momeny, M., Hassanipour, M., . . . Sanjarimoghaddam, H. (2017). Oxytocin mitigated the

- depressive-like behaviors of maternal separation stress through modulating mitochondrial function and neuroinflammation. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 76 ,169-178 .
- Bardo, M., & Bevins, R. A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology*, 153(1), 31-43 .
- Bardo, M., Robinet, P., & Hammer Jr, R. (1997). Effect of differential rearing environments on morphine-induced behaviors, opioid receptors and dopamine synthesis. *Neuropharmacology*, 36(2), 251-259 .
- Brake, W. G., Zhang, T. Y., Diorio, J., Meaney, M. J., & Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *European Journal of Neuroscience*, 19(7), 1863-1874 .
- Brielmaier, J., McDonald, C. G., & Smith, R. F. (2012). Effects of acute stress on acquisition of nicotine conditioned place preference in adolescent rats: a role for corticotropin-releasing factor 1 receptors. *Psychopharmacology*, 219(1), 73-82 .
- Caldji, C., Francis, D., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology*, 22(3), 219-229 .
- Campbell, J., & Spear, L. P. (1999). Effects of early handling on amphetamine-induced locomotor activation and conditioned place preference in the adult rat. *Psychopharmacology*, 143(2), 183-189 .
- Champagne, F. A., Francis, D. D., Mar, A., & Meaney, M. J. (2003). Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiology & behavior*, 79(3), 359-371 .
- Dube, S. R., Cook, M. L., & Edwards, V. J. (2010). Peer Reviewed: Health-Related Outcomes of Adverse Childhood Experiences in Texas, 2002. *Preventing chronic disease*, 7(3) .(
- Francis, D. D .,Diorio, J., Plotsky, P. M., & Meaney, M. J. (2002). Environmental enrichment reverses the effects of maternal separation on stress reactivity. *The Journal of Neuroscience*, 22(18), 7840-7843 .

- Geuzaine, A., & Tirelli, E. (2014). Wheel-running mitigates psychomotor sensitization initiation but not post-sensitization conditioned activity and conditioned place preference induced by cocaine in mice. *Behavioural brain research*, 262, 57-67 .
- Gipson, C. D., Beckmann, J. S., El-Maraghi, S., Marusich, J. A., & Bardo, M. T. (2011). Effect of environmental enrichment on escalation of cocaine self-administration in rats. *Psychopharmacology*, 214(2), 557-566 .
- Green, T., Gehrke, B., & Bardo, M. (2002). Environmental enrichment decreases intravenous amphetamine self-administration in rats: dose-response functions for fixed-and progressive-ratio schedules. *Psychopharmacology*, 162(4), 373-378 .
- Hajheidari, S., Miladi-Gorji, H., & Bigdeli, I. (2015a). Effect of the environmental enrichment on the severity of psychological dependence and voluntary methamphetamine consumption in methamphetamine withdrawn rats. *Neuroscience letters*, 584, 151-155 .
- Hajheidari, S., Miladi-gorji, H., & Bigdeli, I. (2015b). Effects of environmental enrichment during induction of methamphetamine dependence on the behavioral withdrawal symptoms in rats. *Neuroscience letters*, 605, 39-43 .
- Haydari, S., Miladi-Gorji, H., Mokhtari, A., & Safari, M. (2014). Effects of voluntary exercise on anxiety-like behavior and voluntary morphine consumption in rat pups borne from morphine-dependent mothers during pregnancy. *Neuroscience letters*, 578, 50-54 .
- Kalinichev, M., Easterling, K. W., & Holtzman, S. G. (2001). Early neonatal experience of Long-Evans rats results in long-lasting changes in morphine tolerance and dependence. *Psychopharmacology*, 157(3), 305-312 .
- Kalinichev, M., Easterling, K. W., & Holtzman, S. G. (2002). Early neonatal experience of Long-Evans rats results in long-lasting changes in reactivity to a novel environment and morphine-induced sensitization and tolerance. *Neuropsychopharmacology*, 27(4), 518-533 .
- Kalinichev, M., Easterling, K. W., Plotsky, P. M., & Holtzman, S. G. (2002). Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. *Pharmacology Biochemistry and Behavior*, 73(1), 131-140 .



- Kikusui, T., Faccidomo, S., & Miczek, K. A. (2005). Repeated maternal separation: differences in cocaine-induced behavioral sensitization in adult male and female mice. *Psychopharmacology*, *178*(2-3), 202-210 .
- Kim, J. A., Pollak, K. A., Hjelmstad, G. O., & Fields, H. L. (2004). A single cocaine exposure enhances both opioid reward and aversion through a ventral tegmental area-dependent mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(15), 5664-5669 .
- Koh, S., Magid, R., Chung, H., Stine, C. D., & Wilson, D. N. (2007). Depressive behavior and selective downregulation of serotonin receptor expression after early-life seizures: reversal by environmental enrichment. *Epilepsy & Behavior*, *10*(1), 26-31 .
- Lee, J.-H., Kim, H. J., Kim, J. G., Ryu, V., Kim, B.-T., Kang, D.-W., & Jahng, J. W. (2007). Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation. *Neuroscience research*, *58*(1), 32-39 .
- Lima, B. F., Ramos, D. C., Barbiero, J. K., Pulido, L., Redgrave, P., Robinson, D. L., . . . Da Cunha, C. (2017). Partial lesion of dopamine neurons of rat substantia nigra impairs conditioned place aversion but spares conditioned place preference. *Neuroscience*, *349*, 264-277 .
- Meaney, M. J., Brake, W., & Gratton, A. (2002). Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology*, *27*(1), 127-138 .
- Moffett, M. C., Harley, J., Francis, D., Sanghani, S. P., Davis, W. I., & Kuhar, M. J. (2006). Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. *Journal of Pharmacology and Experimental Therapeutics*, *317*(3), 1210-1218 .
- Nader, J., Claudia, C., El Rawas, R., Favot, L., Jaber, M., Thiriet, N., & Solinas, M. (2012). Loss of environmental enrichment increases vulnerability to cocaine addiction. *Neuropsychopharmacology*, *37*(7), 1579-1587 .
- Pihoker, C., Owens, M. J., Kuhn, C. M., Schanberg, S. M., & Nemeroff, C. B. (1993). Maternal separation in neonatal rats elicits activation of the hypothalamic-pituitary-adrenocortical axis: a putative role for corticotropin-releasing factor. *Psychoneuroendocrinology*, *18*(7), 485-493 .

- Ploj, K., Roman, E., & Nylander, I. (2003a). Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. *Neuroscience*, *121*(3), 787-799 .
- Ploj, K., Roman, E., & Nylander, I. (2003b). Long-term effects of short and long periods of maternal separation on brain opioid peptide levels in male Wistar rats. *neuropeptides*, *37*(3), 156-149 ,(
- Plotsky, P. M., & Meaney, M. J. (1993). Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Molecular brain research*, *18*(3), 195-200 .
- Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B., & Riva, M. (2002). Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Molecular psychiatry*, *7*(6), 609 .
- Rubio, P., De Fonseca, F. R. g., Martín-Calderón, J. L., Del Arco, I., Bartolomé, S., Villanúa, M. a. A., & Navarro, M. (1998). Maternal exposure to low doses of  $\Delta$  9-tetrahydrocannabinol facilitates morphine-induced place conditioning in adult male offspring. *Pharmacology Biochemistry and Behavior*, *61*(3), 229-238 .
- Sahraei, H., Faghieh-Monzavi, Z., Fatemi, S. M., Pashaei-Rad, S., Salimi, S. H., & Kamalinejad, M. (2006). Effects of Papaver rhoeas extract on the acquisition and expression of morphine-induced behavioral sensitization in mice. *Phytotherapy Research*, *20*(9), 737-741 .
- Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats—behavioural and neurochemical aspects. *Behavioural brain research*, *222*(1), 246-264 .
- Sinha, R. (2008). Chronic stress, drug use ,and vulnerability to addiction. *Annals of the New York Academy of Sciences*, *1141*(1), 105-130 .
- Solinas, M., Chauvet, C., Thiriet, N., El Rawas, R., & Jaber, M. (2008). Reversal of cocaine addiction by environmental enrichment. *Proceedings of the National Academy of Sciences*, *105*(44), 17145-17150 .
- Thiel, K. J., Pentkowski, N. S., Peartree, N. A., Painter, M. R., & Neisewander, J. L. (2010). Environmental living conditions introduced during forced abstinence alter cocaine-seeking behavior and Fos protein expression. *Neuroscience*, *171*(4), 1187-1196 .
- Vazquez, V., Penit-Soria, J., Durand, C., Besson, M. J., Giros, B., & Daugé, V. (2005). Maternal deprivation increases vulnerability to morphine dependence and disturbs the

enkephalinergic system in adulthood .*The Journal of neuroscience*, 25(18), 4453-4462 .

Vazquez, V., Weiss, S., Giros, B., Martres, M.-P., & Daugé, V. (2007). Maternal deprivation and handling modify the effect of the dopamine D3 receptor agonist, BP 897 on morphine-conditioned place preference in rats. *Psychopharmacology*, 193(4), 475-486 .

Xu, Z., Hou, B., Gao, Y., He, F., & Zhang, C. (2007). Effects of enriched environment on morphine-induced reward in mice. *Experimental neurology*, 204(2), 714-719 .

Young, E. A., Altemus, M., Parkison, V & ,Shastry, S. (2001). Effects of estrogen antagonists and agonists on the ACTH response to restraint stress in female rats. *Neuropsychopharmacology*, 25(6), 881-891 .

### **Legends:**

**Fig. 1.** Timeline of experiments.

Maternal separation was conducted from PND2 to PND14. All pups were weaned on PND 21 and housed in a standard environment or environmental enrichment. The pups divided into two separate experiences (Experiment 1: Morphine-induced CPP and Experiment 2: Morphine-induced locomotor sensitization). All pups were tested in the CPP and the open field chamber from PND52 to PND60.

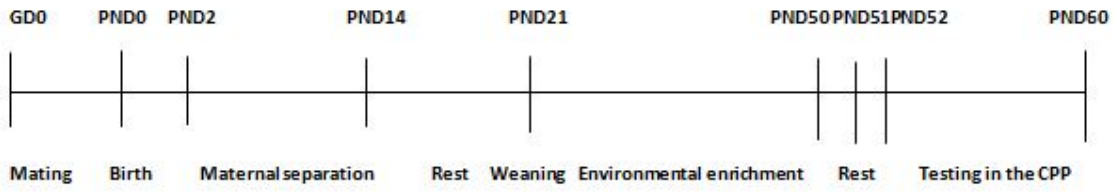
PND; Postnatal day, GD; Gestational day.

**Fig. 2.** Effect of environmental enrichment on morphine-induced CPP in adult male and female rats following maternal separation. A) Morphine-induced place preference. The number of visits to the conditioning compartments in male (B) and female (C) rats. In this experiment, morphine-induced CPP was assessed by the place preference apparatus in male and female maternally separated rats. Preference score were lower in the No MS/EE male group and higher in the male and female MS/SE groups than No MS/SE group, while preference score was less in MS/EE group than MS/SE group in both sexes. There was no

significant difference in locomotor activity between both sexes of rats **on the pre-conditioning and post-conditioning days.** Data was expressed as mean  $\pm$ SEM.  $^{\wedge}$ P=0.003,  $^{***}$  P= 0.0001 vs. No MS/SE,  $^{\wedge\wedge}$ P=0.0001 VS. MS/SE.  $^{\#\#}$ P=0.003 vs. MS/SE male rats. No separated from mothers and housed in a standard environment (SE) (No MS/SE); No separated from mothers and housed in an environmental enrichment (EE) (No MS/EE); Separated from mothers and housed in a SE (MS/SE); Separated from mothers and housed in an EE (MS/EE).

**Fig. 3.** Effect of environmental enrichment on morphine-induced locomotor sensitization in male and female maternally separated rats. In this experiment, morphine-induced locomotor sensitization was assessed by the open field chamber in male and female maternally separated rats. We found that the numbers of lines crossed were higher and lower in MS/SE male and female rats, respectively after morphine challenge; while it was less in rats of both sexes in MS/EE groups. Data was expressed as mean  $\pm$ SEM. In A;  $^{**}$ P=0.007,  $^{*}$ P=0.013 and  $^{***}$ P=0.0001 vs. No MS/SE,  $^{\wedge\wedge}$  P=0.0001 vs. MS/SE  $^{\wedge}$  P=0.003 vs. acute morphine. In B;  $^{*}$ P=0.049 vs. No MS/SE,  $^{\wedge}$ P=0.007 vs. MS/SE,  $^{***}$  P=0.004,  $^{\wedge}$ P=0.03 vs. acute morphine. No separated from mothers and housed in a standard environment (SE) (No MS/SE); No separated from mothers and housed in an environmental enrichment (EE) (No MS/EE); Separated from mothers and housed in a SE (MS/SE); Separated from mothers and housed in an EE (MS/EE).

### Experiment 1



### Experiment 2

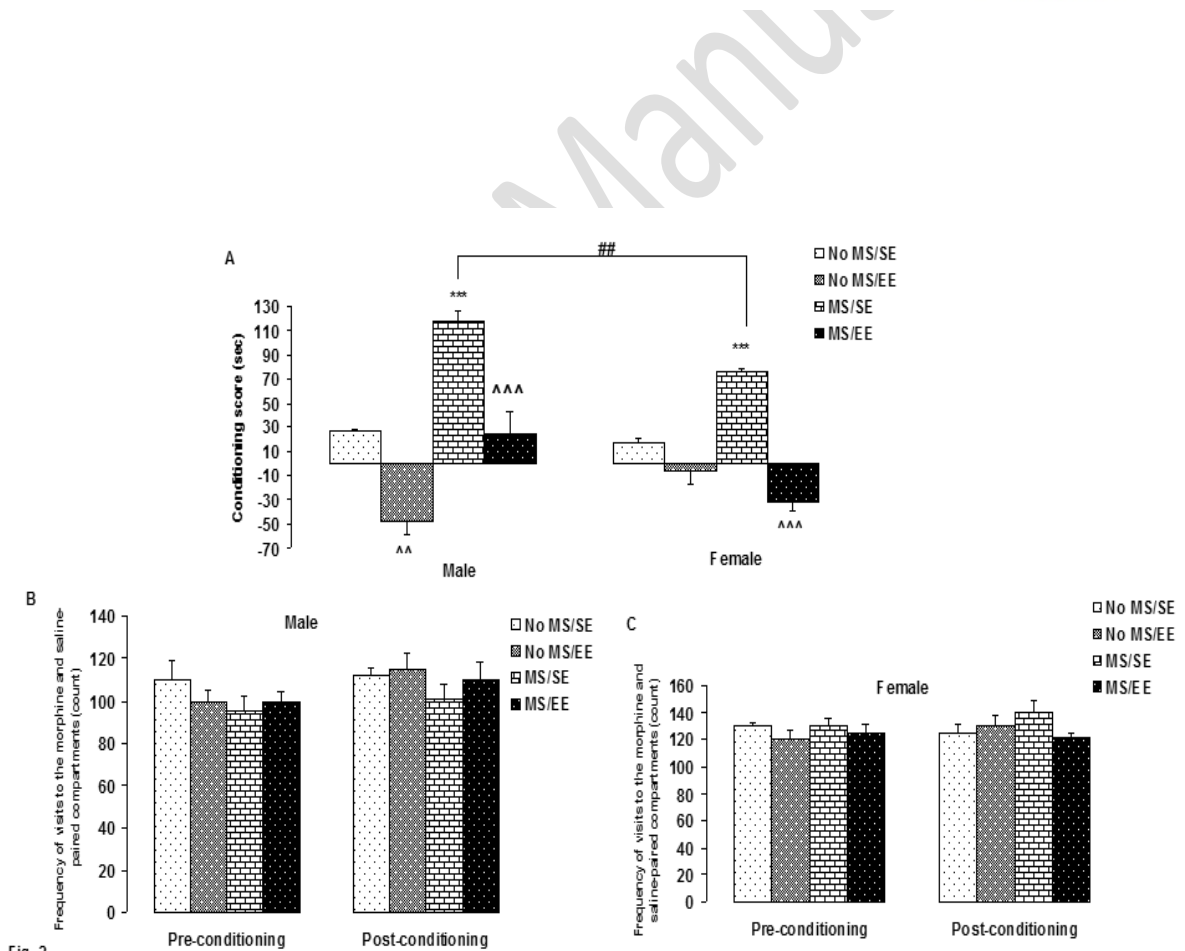
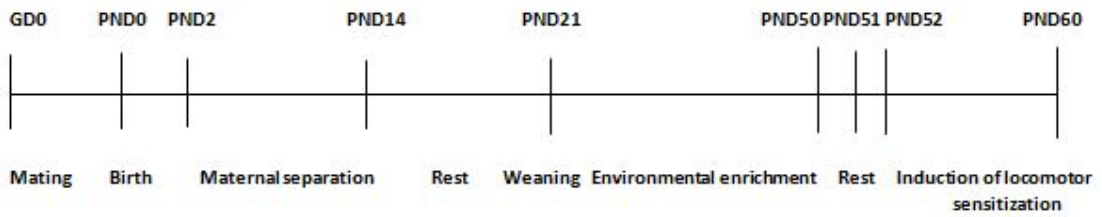


Fig. 2

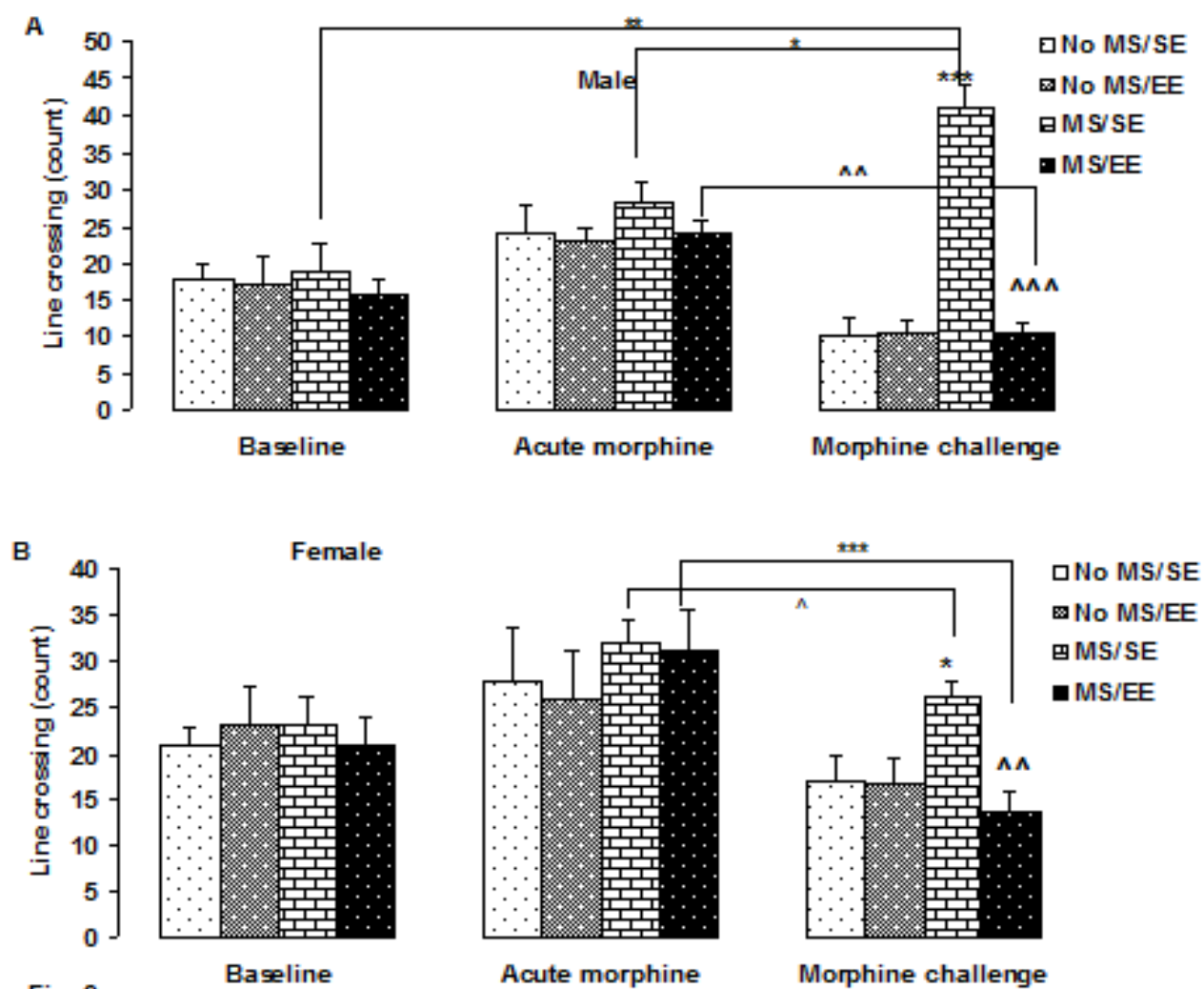


Fig. 3

ACCEPT