Title: Morphine Exposure Causes to Enhance Depression-like Behaviour in Confront with Chronic Stress in Adult Male Offspring Rat

Running title: Depression increased in litter of morphine exposed

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Abstract:

Background: Opioid addiction is an important issue in our World. Data demonstrated that substance use disorder could influence genetic and environmental factors and children of addicts have a higher rate of psychopathology. In this study, we investigated depression-like behavior among offspring of morphine-exposed parents.

Material and Methods: Adult male and female Wistar rats received morphine for 21 consecutive days and let them were free of drugs for ten days. Offspring of these rats divided into three distinct groups: maternal morphine-exposed; paternal morphine-exposed and maternal and paternal morphine-exposed. We used sucrose preference and Force Swim Test (FST) to measure depression-like behavior. Also, we induced chronic mild stress using repeated corticosterone injection and evaluated depression-like behavior in offspring of morphine-exposed parents compared with offspring of healthy parents.

Results: Results indicated that depression-like behavior in the litter of morphine-exposed was higher than offspring of the control group in confronting with chronic mild stress. Additionally, mild chronic stress can produce an exaggerated effect on depression-like behavior in offspring of the morphine-exposed parent(s) compared with control group.

Conclusion: Our data approved the previous hypothesis that showed depression rate increased in children of addicts. We verified that even mother or father was clean of opioid in the time of gestation, but children are susceptible to depression. Dysregulation of HPA axis and changing in neuronal features in hippocampus increased depression-like behavior in offspring of morphine exposure parents

Keywords: Depression; Chronic Stress; Corticosterone; Parental Morphine Exposure

1. Introduction:

There is no doubt today that the origin of addiction is multifactorial including genetic, neurobiological and psychosocial correlates (Goodman, 2008; Hurd, 2006). Drug addiction is a severe, chronically relapsing neuro-psychiatric disorder. It can characterize according to the definition of Koob and LeMoal by the compulsion to seek and take drugs, the loss of control in limiting the intake and by the occurrence of a negative emotional state reflecting a withdrawal syndrome when there is no access to the drug (Koob & Le Moal, 1997). Depression is comorbid with addiction; drugs used as self-medication for depression, but chronic drug use can exacerbate the severity of depression and increase drug taking (Bruijnzeel, Repetto, & Gold, 2004; Crist et al., 2013). Stress is a major environmental factor which is imperative in etiology of mood disorder (Charney & Manji, 2004; Paykel, 2003).Stress strongly implicated in both precipitation of depressive episodes (Kendler, Thornton, & Gardner, 2000; Willner, Scheel-Kruger, & Belzung, 2013) and relapse to drug abuse (Shaham, Shalev, Lu, De Wit, & Stewart, 2003). There is considerable overlap in the neurobiological substrates of depression and addiction (Bruijnzeel et al., 2004; Markou, Kosten, & Koob, 1998).

Epigenetic changes described as an alteration in gene expression without altering in DNA sequences which include changes in DNA methylation, histone modifications, and micro-RNA. Factors such as stress and abusing drugs could induce epigenetic changes and affect neuropsychiatric behaviors (Bagot, Labonte, Pena, & Nestler, 2014; E. J. Nestler, 2014; Robison & Nestler, 2011). There is some evidence that 50% of hospitalized children for psychiatric disorder had addicted parents. Alcohol abusing parents increase the risk of mental health problems such as depression, suicidal attack, antisocial behavior and drug abuse in children (Balsa, Homer, & French, 2009; Christoffersen & Soothill, 2003).

Cortisol level increased during stress and in a depressed patient (Dinan, 1994; Gold, Goodwin, & Chrousos, 1988; Reus & Miner, 1985; Sachar et al., 1973). Johnson and colleague used repeated corticosterone injection as a chronic stressor to for obtaining rat model of depression in consequence of stressful events (S. A. Johnson, Fournier, & Kalynchuk, 2006). Previous investigations revealed that chronic stress led to anhedonia-like symptoms such as reduced preference in sucrose (Wallace et al., 2009; Wook Koo et al., 2016). Our previous work established that parental morphine exposure before mating could affect anxiety-like behavior in the first generation but not in the second generation (submitted data).

In this study, we investigated the effect of parental (maternal and/or paternal) morphine exposure -10 days before gestation- on depression state of offspring. Also, we evaluated chronic mild stress-induced depression in offspring by using chronic corticosterone injection as a model of mild chronic stress.

2. Material and Methods:

2.1.Animals:

Adult male and female Wistar rat (7 weeks old) purchased from Pasteur Institute and kept four rats in each cage. All animals maintained in a room with controlled light-dark cycle (12/12 hours, light begin from 7 a.m.). The temperature kept on 23±1 $^{\circ C}$ and humidity was constant. They had free access to food and water except during the behavioral test. After two weeks of familiarization, animals randomly divided into four groups. All experiments were approved by the Ethics Committee of Tehran University of Medical Sciences (project number: 94-01-159-28023).

2.2. Experimental Procedure:

2.2.1. Morphine Exposure protocol:

Twenty-four male and twenty-four female Wistar rats exposed to oral morphine sulfate (Temad, Iran) administration. Oral morphine administration protocol performed as described before (Moini Zanjani & Sabetkasaei, 2010). Morphine sulfate was given in increasing concentrations as mentioned in Table 1. Sucrose (2%) added to diminish the bitter taste of morphine. Control group containing eight male and eight female rats, only received sucrose (2%). Morphine administration and mating protocol are shown in Fig 1.

2.2.2. Mating protocol:

As shown in Fig 1-A, ten days after last morphine intake, animals prepare for mating as described below:

1. Healthy male and healthy female.
2. Healthy female and morphine-abstinent male.
3. Healthy male and morphine-abstinent female.
4. Morphine-abstinent male and female.

In each group, one male and one female rat put in one cage. After parturition, the number of birth and number of death recorded. During lactation, maternal care and weight of infant monitored. Male offspring arranged into four groups (a) animals with both healthy parents (control) (b) paternal morphine-exposed offspring (PME) (c) maternal morphine-exposed offspring (MME) and (d) offspring with both morphine-exposed parents (PME+MME).

2.3. Behavioral tests:

2.3.1. Sucrose preference test:

In laboratory rodents, two-bottle choice paradigm usually used for sucrose preference as an index of anhedonia, particularly in stress-based models of depression (Overstreet, 2012). Preference calculated as a sucrose intake (volume of liquid consumption) divided to all fluid intake.

All animals should deprive of water and food 20 hours before the behavioral test. In this time each rat placed in the cage individually. Animals transfer to testing room 30 min before starting the test. In test day, each rat can choose between two bottles: (1) sucrose dissolved in water (1%) and (2) tap water. Bottles put across left and right sides of the cages. At the end of 1 h, the volume of sucrose and water consumption measured for each rat and sucrose preference calculated as the sucrose preference (%) = [sucrose consumption/ (sucrose consumption + water consumption)] *100.

2.3.2. Forced Swimming Test (FST):

Forced swimming test is a behavioral test to assess depression-like behavior in rodents (Porsolt, Le Pichon, & Jalfre, 1977). Briefly, rat placed in Plexiglas cylinder (60 cm height, 30 cm in diameter) which filled with water heightening 30 cm. Water temperature adjusted to 25°c. Each rat allowed swimming for 5 min and a video camera recorded from above the cylinder. The latency time to immobility and total time of immobility (lack of motion of the whole body) used as an index of depression-like behavior.

2.3.3. Mild Chronic Stress Inducing:

We used chronic corticosterone injection as a chronic stressor. All animals were handled once a day seven days before corticosterone injection. As shown in Fig 1-B &C, rats treated with corticosterone daily during 21 days. All behavioral tests performed 24h after the last injection.

Corticosterone (Sigma-Aldrich, UK) dissolved in 0.9% saline with 2% (x)-sorbitan mono-9-octadecenoate poly (oxy-1, 2-ethanediol) (Tween-80; Sigma-Aldrich) and injected subcutaneously (s.c) once per day between 9:00 A.M and 11:00 A.M. All animals weighed every day. Corticosterone injected in 5, 10 and 20 mg/kg in drug naïve rats (Fig 1-B) and non-effective dose chosen for offspring with morphine-exposed parent(s) (Fig 1-C).

2.4. Statistical analysis:

We used Statistical Package for the Social Sciences (SPSS) version 21 for data analyzing. Data analyzed with one-way ANOVA. Tukey's multiple comparison tests used for finding differences between groups. Data expressed as mean ± SEM of eight animals.

3. Results:

3.1. The mortality rate increased in offspring of the morphine-abstinent parent(s):

As shown in Table 2, mortality rate increased among litter of morphine-exposed parent(s) ($X^2 = 31.889$, $p<0.001$). There were no differences in weight or number of infants per parturition among groups.

3.2. Depression-like behavior increased in offspring of morphine-abstinent rats:

Fig 2 showed depression-like behavior in offspring of paternal and/or maternal morphine-exposed parent(s) compared with offspring of drug naïve parents. As shown in fig 2 A, offspring of morphine-abstinent parents have low sucrose preference compared with offspring of drug

naïve parent(s) [F (3,28)=3.766, p=0.02]. In FST, both latency to immobility and total time of immobility changed in offspring of parental and/or maternal morphine-abstinent parent(s) (panel B and C). Latency time decreased in offspring of one and/or two morphine-abstinent parent(s) in comparison to the litter of drug naïve animals [F(3,28]=42.833, p<0.001]. Also, animals with both morphine-abstinent parents showed a significant reduction in latency time compared with maternal morphine-exposed animals (p<0.01). Total time of immobility increased among litter of morphine-exposed parent(s) [F(3,28)= 19.686, p<0.001]. Total time of immobility increased in morphine-exposed parents in comparison with one morphine-exposed parent (p<0.01).

3.3. Chronic Corticosterone administration increased depression-like behavior dose-dependently:

Fig 3 demonstrated the chronic corticosterone administration increased depression-like behavior in drug naïve animal dose dependently. Fig 3A revealed that sucrose preference decreased in corticosterone received rats compared with saline-treated rats [F(3,28) = 6.025, p=0.003]. Latency to immobility and total time of immobility decrease and increase in chronic corticosterone administration dose-dependently in FST, respectively [F(3,28)= 5.260, p=0.005, F(3,28)= 5.386, p<0.005],(Figure 3B and 3C). The weight of each rat recorded daily; there were no changes in weight loss between groups (data not shown).

3.4. Chronic mild stressor increased depression-like behavior in the litter of morphine-exposed:

Fig 4 revealed that non-effective dose (5 mg/kg) of corticosterone led to increasing depression-like behavior in offspring of paternal and/or maternal morphine-exposed animals. Fig 4A revealed that sucrose preference in confronting with mild chronic stressor only changed in

the litter of both morphine-abstinent parents compared with offspring of drug naïve rats [F (3, 28) = 4.312, p=0.013]. Forced swim test established that offspring of the morphine-exposed parent(s) are more susceptible to depression in opposing mild chronic stress compared with control group [latency to immobility: $F(3,28)= 12.499$, $p<0.001$ and total time of immobility: $F(3,28)= 20.848$, $p<0.001$]. There was no statistical difference between weights lost among groups (data not shown.

4. Discussion:

Previous studies indicated parental morphine exposure could disrupt neurochemical and neurophysiological features in offspring (Cicero et al., 1991; Sarkaki, Assaei, Motamedi, Badavi, & Pajouhi, 2008). Also, our previous work claimed that parental morphine exposure before gestation led to memory deficit, changing in pain perception and increasing tolerance to morphine in male offspring (data not published yet). In this study, we found that parental morphine exposure could impress depression in offspring. Accumulating data declared mu opioid receptor and beta-endorphin expressed in the male reproductive organ (Albrizio, Guaricci, Calamita, Zarrilli, & Minoia, 2006). Furthermore μ, κ and δ opioid receptors expressed in the oocyte (Agirregoitia et al., 2012), so it is not impossible that morphine could induce the trans-generational effect.

As mentioned previously, a variety kind of psychopathologies like depression, substance abuse, and suicidal attacks are common among children with addicted parents (Balsa et al., 2009; Christoffersen & Soothill, 2003). In the base of Fuller-Thomson study's, children with alcoholic or addicted parents, experience depression 69% more than other children (Fuller-Thomson, R, V, J, & Brennenstuhl, 2013). Other investigation on laboratory animals verified morphine exposure

before gestation alters the endogenous opioid system in offspring (Cicero et al., 1991). Otherwise, paternal morphine exposure led to increasing beta-endorphin in hypothalamus and corticosterone level in serum in the female litter (Cicero et al., 1991). Moreover, maternal morphine exposure can attenuate quinpirole-induced corticosterone in both first and second generation male offspring (Byrnes, Johnson, Carini, & Byrnes, 2013). As approved in human studies (Fuller-Thomson et al., 2013), we confirmed that depression rate is higher among offspring with morphine-exposed parent(s) in comparison to offspring of control parents. Additionally, low sucrose preference in the litter of both morphine-exposed parents, decreasing in latency to immobility and increasing in the total time of immobility in FST proven our hypothesis.

Johnson et al. evaluated that repeated corticosterone injections increase depression-like behavior dose-dependently and they suggested that repeated corticosterone injections dysregulated HPA axis and consequently caused to depressive disorder (S. A. Johnson et al., 2006). We also found that offspring of morphine-exposed rats are more vulnerable to depression in response to mild chronic stressor like low doses of corticosterone. Obtained data indicated administration of corticosterone with low doses (5 mg/kg) did not induce depression-like behavior in offspring of the healthy rat but could cause depression-like behavior in offspring of the morphine-exposed rat. This effect may have occurred following HPA axis dysregulation (Cicero et al., 1991). Mild stressor might increase corticosterone level in offspring of morphine-exposed rats more than offspring of healthy rats and probably such effect mediated via enhanced level of corticosterone in plasma. A study by Houshyar and colleagues revealed that chronic morphine administration gives rise to the persistent elevation of basal plasma corticosterone level

approximately ten times in comparison with control group (Houshyar, Galigniana, Pratt, & Woods, 2001).

Hippocampus is an important area involved in depression (Sheline, Mittler, & Mintun, 2002). Sarkaki and coworkers showed that paternal morphine exposure disrupted hippocampal synaptic plasticity in male offspring (Sarkaki et al., 2008). Paraventricular nucleus (PVN) of the hypothalamus-contain neurons with corticotropin-releasing factor and these neurons integrate information pertinent to stress. In PVN, excitatory afferent from the amygdala and inhibitory afferent from hippocampus are prominent. Moreover, ascending monoamine inputs, inputs from the periphery and inhibitory inputs from circulating glucocorticoids are convergent in PVN. Chronic stress and chronic elevating of glucocorticoids caused to decrease expression of Brain-Derived Neurotrophic Factor (BDNF) in the hippocampus. BDNF has a significant role to neurons survival and decreasing in its level may contribute to CA3 atrophy. Losing of CA3 neurons increased susceptibility to psychiatric diseases such as depression and anxiety (E. Nestler, Hyman, & Malenka, 2015). Likewise, prenatal morphine exposure decreases long-term depression (LTD) in CA1 pyramidal neurons (Yang et al., 2003). These changes in neuronal plasticity in the hippocampus may influence on depression-like behavior in offspring of the morphine-exposed parent(s). Evidence proved that morphine exposure before parturition or during mothering change maternal care (Bridges & Grimm, 1982; Miranda-Paiva, Nasello, Yin, & Felicio, 2001; Slamberova, Szilagyi, & Vathy, 2001). Other investigations revealed that morphine administration in adolescence could alter maternal care like nursing and contact time during lactation (N. L. Johnson, Carini, Schenk, Stewart, & Byrnes, 2011).

Finally, in addition to the role of HPA axis psychological changes in offspring of addict rats, another possible reason for such depressive disorder is an epigenetic transmission.

Morphine can directly affect epigenetic germ cells. These changes impress developmental processes in the embryo. The mechanisms which cause such trans-generational effect are unknown, and other investigation should be designed to find exact mechanisms of such effects.

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Conflict of interests:

There is no conflict of interests.

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Figures Legends:

Fig 1. Flow chart of study design

Fig 2. Sucrose preference (A), latency to immobility (B) and total time of immobility (C) in morphine-abstinence-derived offspring. (PME: Paternal Morphine Exposure, MME: Maternal Morphine Exposure). Each value represents the mean ± SEM of eight rats per group. * $p<0.05$, **$p<0.01$ and ***$p<0.001$ compared to control group. # $p<0.05$ and ## $p<0.01$ campared to MME+PME groups in panel B and C respectively. & $p<0.05$ compared MME+PME group.

Fig 3. Sucrose preference (A), latency to immobility (B) and total time of immobility (C) in 21 days corticosterone-treated (5, 10 and 20 mg/kg) and saline-treated rats (n = 8, for each group). Values are the means ± SEM. * $p<0.05$, **$p<0.01$ vs. control group.

Fig 4. Sucrose preference (A), latency to immobility (B) and total time of immobility (C) in MME, PME, MME+PME and control group which received 21 days corticosterone (5 mg/kg). Values presented as the means ± SEM for eight rats in each group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared to control group. $p<0.05$ and $$$p<0.001$ compared to PME groups in panel B and C respectively. ## $p<0.01$ compared to MME group.

Table legend:

Table 1. Morphine concentration for oral morphine administration

Table 2. Parental morphine administration before gestation increased mortality rate in offspring. Data represented as ratio [(number of dead litters/total number of litters) *100] . PME: Paternal Morphine Exposure, MME: Maternal Morphine Exposure