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Effects withdrawal acute and chronic morphine on anxiety behavior in male rats under isolation and socially rearing

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ABSTRACT

The opioid medicines administration affects anxiety processes inconsistently in an acute and chronic form. On the other hand, isolation and socially rearing is not obvious on behavioral differences in the dependency and withdrawal process. This study examines isolation and socially rearing rats on the anxiety behavior in the chronic and acute dependency on the morphine or withdrawal of it. In this experimental study, 64 male Wistar rats were divided into 8 groups of eight each: isolation and socially

rearing, then these groups were divided into control (saline) and morphine sulfate (10 mg/kg) and each one of them includes the acute and chronic subgroups. The injection was conducted intraperitoneally for 10 days in acute groups and in chronic groups for 60 days. The rats were quitted after the end of the period of dependence with withdrawal drug, and their anxiety and locomotors activity indexes were measured by elevated plus maze (EPM). The results of this study indicate that, 5 days after morphine withdrawal, the percentage of the time spent on the open arm of EPM was significantly decreased compared to the control group. Also, the percentage of the time spent on the open arm in isolation-reared groups compared to the corresponding group in socially reared groups has significantly decreased ($P<0.05$). However, locomotor activity in the isolation and socially reared groups does not show significant differences. The findings of this study indicate that life in isolation-reared rats can increase the level of anxiety and increase the chance of addiction recurrence.

Keywords: Anxiety, morphine, isolation rearing, socially rearing, acute, chronic, rats.

1. INTRODUCTION

Addiction is a complicated phenomenon with serious social-economic and healthy consequences, economic and health consequences. In addition, morphine is a common opioid drug that used widely as a pain killer among cancer patients and for acute pain (Motaghinejad et al., 2016). Chronic administration of morphine is associated with increasing psychological and physical dependence, anxiety and depression symptoms (Hammami-Abrand Abadi & Miladi-Gorji, 2016). Anxiety is one of the most commonly reported cases of withdrawal of several opiate drug groups and it is thought that ending emotional symptoms of the withdrawn such as anxiety, numbness, excitement, restlessness and fatigue is a stimulus for continuing the drug use, and finally, it leads to dependence on drugs (Grasing et al., 1996; Harris & Gewirtz, 2004). Withdrawal from opioids is associated with anxiety-like symptoms in humans and animals (Harris & Gewirtz, 2004). There are similarities in animal models between dependence on drug and sedation in behavioral and neurochemical levels (Grasing et al., 1996; Muhammad Farhan et al. 2017). Mu Opioid receptors are activated directly with morphine and indirectly with alcohol, cannabinoids and nicotine and they play a central role in the development of addiction therapies, and their transmission and regulation depend on to agonist strongly (Contet et al., 2004; Nweze Chibuzo Carole & Nweze OtitoAdaeze, 2018). The effect of opioidergic on social behaviors is mediated through Mu and kappa opioid receptor systems, while Delta opioid receptor function has no effect on social behaviors (Vanderschuren et al., 1995). Kappa opioid receptor antagonists can reduce social function disorders in addicted individuals (Lalanne et al., 2017). The chronic morphine withdrawal induces anxiety-like and disruptions in the behaviors of the social function, which is along with increased of the connection to the metabotropic glutamate receptor 5 (mGlu5R) (Zanos et al., 2016). Changes in the operation of the neural circuit in the brain reward system involve in creating anxiety during the drugs withdrawal (Hammami-Abrand Abadi & Miladi-Gorji, 2016; Grasing et al., 1996), and Mu opioid receptors in the nucleus accumbens mediate the reward system and interact in the social interactions of adolescent rats (Trezza et al., 2011). Generally, in rodents, social motivation has been enhanced by the Mu opioid receptor agonists and reduced by its antagonists (Loseth et al., 2014). Therefore, the rehabilitation or prevention of neurological changes through the dependence on morphine can be a useful way of treating the recurrence of drugs seeking. The blockage of opioid receptors by naloxone has also improved the development of social behaviors in rhesus monkeys (Trezza et al., 2011). On the other hand, the early life's bad experience in the social environment such as separation from parents or isolation affects the structural and functional development of the brain and puberty behaviors in rodents (Koike et al., 2009). Induced behavioral changes by rearing in isolation, such as aggressive behaviors (Ibi et al., 2008; Amiri et al., 2015), anxious, and anxious-like behaviors (Wei et al., 2007) have been known. Environmental enrichment, including cognitive, physical and social richness, seems to be an effective method that can provide a broader range of plastic responses in the brain reward system (Haydari et al., 2014; Miladi-Gorji et al., 2012; Thiel et al., 2010), which apparently plays a role in the anxiety caused by the drug withdrawal. It has been observed that life in isolation rearing mice more susceptible to alcohol consumption and anxiety-like behaviors. The change in the Kappa opioid receptor function in isolation rearing plays a role in creating an inclination to alcohol (Karkhanis et al., 2016). However, there is little information about the effect of isolation on the drug withdrawal symptoms and recurrence of drugs-related. In this study, we intend to study the effect of socially and isolation rearing on the occurrence of anxiety behaviors in rats during the acute and chronic morphine withdrawal.

2. MATERIALS AND METHODS

Animals

In this experimental study, Sixty four 21-day-old Wistar rats (250-300 g) were randomly allocated groups under-controlled conditions of 23 ±1°C temperature and 12 h light/dark cycle (lights on at 08:00), with ad libitum access to food and water. All experimental procedures were carried out between 09:00-13:00 h and were approved by the Animal Experimentation Ethics Committee of the University by this Code: IR.REC.1396.1544 After this allowance, the researcher has conducted this project. Every effort was made to minimize animal suffering and the number of animals used.

Medicines

Morphine sulfate (Sigma-Alderich, GmbH, Germany) was physiologically dissolved in saline. Morphine sulfate was injected intraperitoneally(ip) at a dose of 10 mg/kg. All injections were conducted in 0.1 ml /100gr of the body weight.

Treatment

Animals were randomly were divided into 8 groups of eight each: isolation and socially rearing, then these groups were divided into control (saline) and morphine sulfate and each one of them includes the acute and chronic subgroups. Each socially reared group was kept in a cage as well as one rat was kept in a separate cage with the size of 40 x 25 x 22 cm in each isolated-reared group. The injection was conducted intraperitoneally for 10 days in acute groups and in chronic groups for 60 days (Miladi-Gorji et al., 2012; Tzschentke et al., 2018). The Maze test was performed after the drug withdrawal in the acute group on 11^{th} and 15^{th} days, and for the chronic groups on 61^{th} and 65^{th} days.

Anxiety Measurement

Elevated Plus Maze (EPM) Test

The EPM was measured anxiety behaviors and to examine the general locomotor activity of the rats.

The EPM comprised two open arms (50×10 cm, with a ledge of 5 mm) and two closed arms (50×10×40 cm). The apparatus was placed at a height of 50 cm from the floor. As rats have an innate fear of elevated open places, they entered the open arms less frequently and stayed in the open arms for less time compared to the closed arms when allowed to freely explore the maze (Pellow et al., 1985). Before the test on the test day, the animals for 5 minutes in a Plexiglas box with black walls with dimensions of 40 × 40 × 30 cm was transferred to investigative activity increased animal. The following variables were measured during each 5 min test: (a) time spent in open and closed arms as a percentage of the total time spent exploring both the open and closed arms; (b) the number of entries into the open and closed arms as percentage of the total number of entries into both open and closed arms. Percent time spent in, and entries into, the open arms were used as measure of anxiety (Pellow et al., 1985). In addition, the total numbers of arm entries were used to determine the rate of generality activity (Ookawa et al., 2007). The apparatus was cleaned after each trial with water.

Statistical Analysis

The data were expressed as mean ± standard error of the mean. The parameters were evaluated by analysis of variance (two-way ANOVA), followed by Tukey's test. The differences among the groups were assessed by Student's t test, with a significance level of $P<0.05$.All statistical analyzes were performed by using version 16 of the spss software.

3. RESULTS

The results of this study indicate that, 5 days after morphine withdrawal, the percentage of the time spent on the open arm of EPM was significantly decreased compared to the control group. Also, the percentage of the time spent on the open arm in isolation-reared groups compared to the corresponding group in socially reared groups has significantly decreased ($P<0.05$)(Figure 1A). Also, the percentage of the time spent in the closed arm was significantly increased only in the chronic socially group compared to the control (Figure 1B).

The percentage of entries on the open arm of EPM increased significantly only in the chronic socially reared compared to the control. Also, the percentage of the number spent on the open arm in isolation groups compared to the corresponding group in socially reared groups decreased significantly (Figure 1C). The total number of entries to open and closed arms in the acute groups decreased significantly compared to the control and was not significant compared with the socially rearing and isolation-reared groups (Figure 1D).

The percentage of time spent on open arm on the first day after drugs withdrawal (in the acute group on the 11th day and in the chronic group on 61th day) increased only significantly in the acute isolation reared group compared to the control. Also, in the control groups, the isolation reared groups have significantly decreased compared to the socially control (Figure 2).

Comparison between the acute and chronic groups, it was found that, 5 days after drug withdrawal, the percentage of time spent on the open arms in chronic groups decreased significantly compared to the corresponding acute group but not significantly (Figure 3).

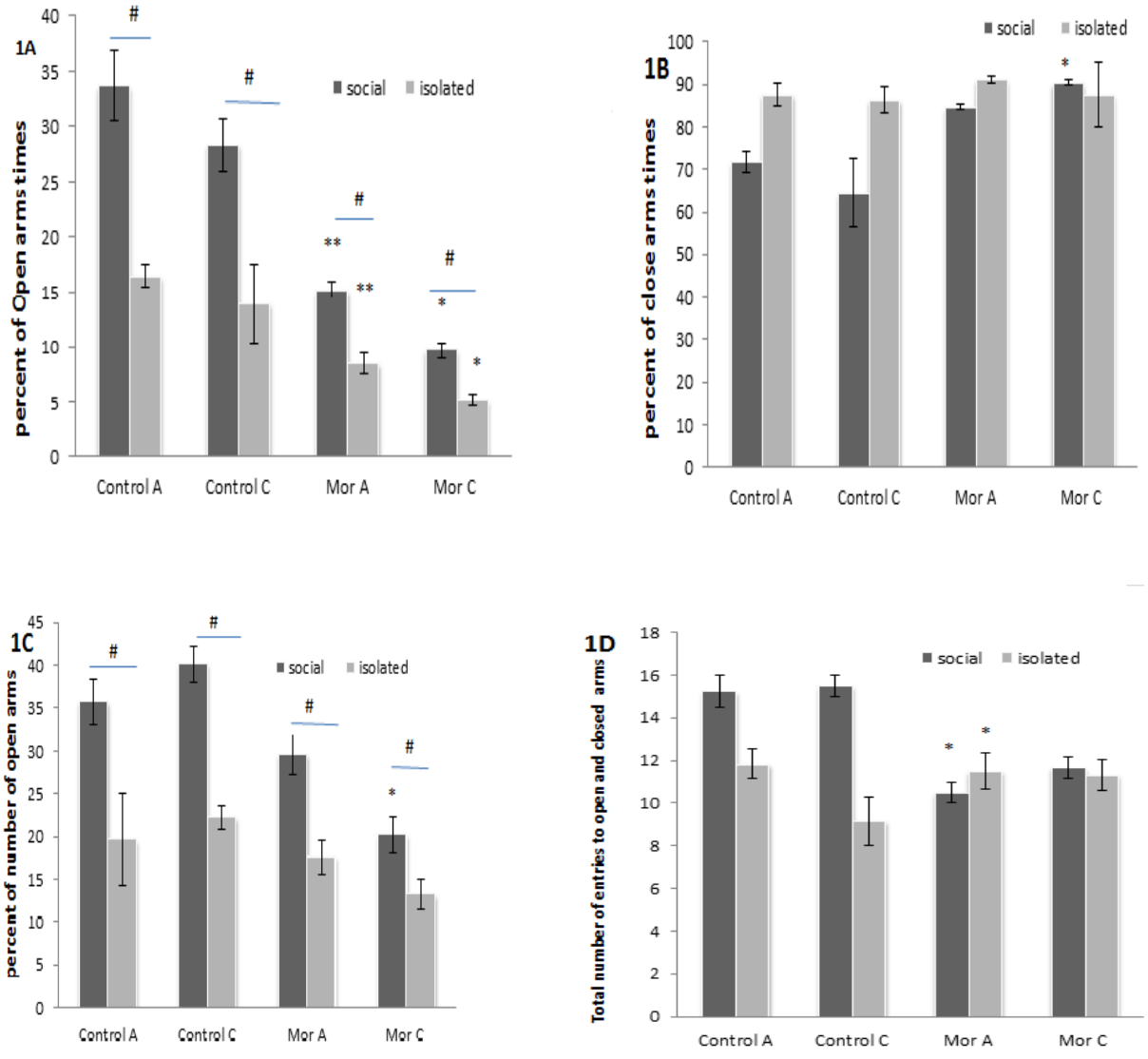

Figure 1 The effect of morphine withdrawal (Mor, 10Mg/kg) on the percentage of spent time on the open arm (A), the percentage of spent time on the closed arm (B), the percentage of entries' number on the open arm (C), and the total number of entries on open and closed arms (D) in the EPM for rats in socially and isolation rearing compared to the control group, Morphine and saline were injected intraperitoneally. Data represent means ± SEM of 8 rats per group. *: P<0.05. ** P<0.01. Significant differences for saline recipients #P <0.05. Significant differences between socially reared groups and corresponding isolation groups. A: Acute, C: Chronic.

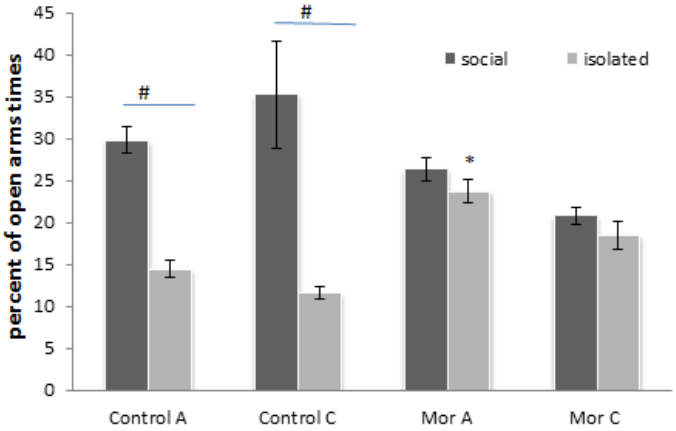

Figure 2 Percentage of the time spent on the open arm on the first day after morphine withdrawal (Morphine, 10mg/kg) in the EPM for rats in socially-reared and isolation rearing compared to the control. Morphine and saline were injected intraperitoneally. Data represent means ± SEM of 8 rats per group. *: P<0.05. Significant differences for saline recipients #P<0.05, Significant differences between socially reared groups and corresponding isolation-reared groups. A: Acute, C: Chronic.

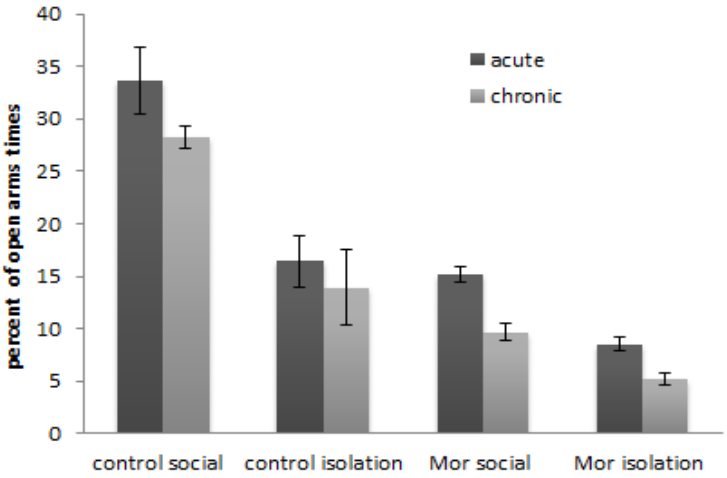

Figure 3 Comparison of acute and chronic withdrawal of morphine (Mor, 10mg/kg) on the percentage of time spent on the EPM open arms for rats under socially and isolation rearing, Data represent means ± SEM of 8 rats pergroup. *: P<0.05.

4. DISCUSSION

The results of this study showed that rats under withdrawal that are dependent-morphine show more anxiety behaviors compared to the control, and anxiety in chronic addiction was often more than acute under withdrawal. Also, the animals under isolation rearing show more anxiety in the test.

On the other hand, studies have shown that dependence on morphine and its withdrawal in general activity have significant effects only on acute groups and reduce them. However, locomotor activity in the isolation and socially reared groups does not show significant differences.

It is thought that HPA axis may able to modify the effect of some drugs, such as morphine, and as a result with increase of releasing the corticosterone, occurrence of anxiety responses of rats increase by EPM (Iredale et al., 2000). Anxiety increases the tendency to get drugs among the addicts and also people under withdrawal treatment; as a result, with treatment of the anxiety, one can reduce the tendency of taking morphine in addicted animals or animals under withdrawal treatment. In a study, adult female rats exposed to chronic morphine during the postnatal period and eventually, it was observed that their children spent less time and less seeking activity in the open arm of the EPM apparatus (Byrnes, 2005). The acute and chronic administration of

morphine to rats induces anxiety in addicted rats (Sanaie et al., 2017; Hamilton et al., 2013). In this study, anxiety was also obvious in the groups under treatment of chronic and acute morphine regardless of the type of rearing conditions by decreasing the number of entries and time spent on the open arm of the EPM apparatus.

It was shown in this study that animals after withdrawal under acute and chronic treatment exhibit anxiety behaviors as well. Castilho (2008) reported similar results and they attributed the occurrence of anxiety-like symptoms in mice under morphine withdrawal to Sensitization of dorsal periaqueductal gray (Castilho et al., 2008). On the other hand, enriched environments can improve the anxiety symptom of young rats that were exposed to chronic morphine on embryonic period (Ahmadalipour et al., 2015). It has also been shown that rearing in isolation can create many behavioral abnormalities.

The results of this study indicate that generally, the level of anxiety in isolation rearing rats was higher, so that, in some cases, the level of anxiety of the rats under treatment in isolation control group was higher than the addicted rats in the socially reared group.

Amiri et al., (2015) showed that isolation can lead to the occurrence of depression and anxiety in mice simultaneously. In another study, it has been shown that rearing in the isolation and the stress arising from it, increases the responsiveness of kappa opioid receptors, and resulting in a negative adjustment of the dopamine system that leads to a tendency toward alcohol drinking (Karkhanis et al., 2016). Similar results have been reported in the present study on occurrence of the anxiety for rearing rats in the isolation conditions. These rats exhibited higher levels of anxiety than their counterparts in socially rearing, and even in some cases the anxiety caused by these conditions is more than the anxiety caused by morphine withdrawal. Several studies have shown the effect of social, environmental enrichment on the modification of anxiety behaviors in mice under drugs withdrawal (Amiri et al., 2015; Hajheidari et al., 2015; Hajheidari et al., 2015; Nobre, 2016). In this study, it was also observed that breeding rats in isolation exhibited a high level of anxiety; occurrence of anxiety could be one of the main reasons for recourse to narcotic drugs. Hence, the reduction of anxiety through rearing in social conditions can reduce the rate of anxiety caused by taking or quitting drugs and help to a successful withdrawal. Environmental and social enrichment can lead to functional changes in the population of opioid receptors (Smith et al., 2005). Local changes observed in the connection of opioid receptors in the medial prefrontal cortex and parafascicular areas show the role of opioid systems in the consequences of isolation and adjusting social behavior in rats (Vanderschuren et al., 1995) and neurochemical changes in the brain areas in the mice under alcohol withdrawal and aggressive behavior have been observed in these mice (Hwa et al., 2015), and an impairment of the development of prefrontal cortex and behavioral changes has been observed in mice that were isolated from adolescence (Medendorp et al., 2018). These connective changes and even changing the spread of the receptor in the brain development during isolation rearing can play a role to occur different anxiety behaviors in response to taking morphine or its withdrawal compared with social mice. The beta-Adrenergic receptor antagonists are able to reduce the anxiety arising from the withdrawal in rats of dependence on morphine or cocaine (Harris & Aston-Jones, 1993). In the brain of the rats, the nucleus accumbens is one of the richest parts of this type of receptor that also involve to occurrence of social behaviors (Palacios & Kuhar, 1982).

In this study, there is no significant difference in the rate of general motion and locomotor activity in addicted groups and under withdrawal ones and isolation rearing as well as socially reared groups, which indicates the lack of impact of these conditions on the locomotor activity of the laboratory models. These results are confirmed by Hodgson et al., (2009), they did not find the difference in the locomotor activity of adolescent rats, but in adult mice the rate of locomotor activity decreases with increasing withdrawal time. This difference can be caused from the time of locomotor activity's review in this study, which only carried out on the 5th day after leaving the test.

5. CONCLUSION

Our research showed that withdrawal chronic morphine could increase the anxiety behavior in rats, and this rate of the anxiety behavior increases in the isolation reared. The occurrence of anxiety behaviors was significantly lower in acute administration than in chronic administration at the time of dependence and after withdrawal. Dependence, withdrawal and also social conditions have not had any effect on the general activity of mice. However, Socialization in rats can improve tolerance during the course of withdrawal and reduce its harmful effects.

Conflict of interest

The authors declare no conflict of interest related to the present manuscript.

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