ORIGINAL ARTICLE

Morphine Addiction is Tolerated Better in Socialized Male Rats

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ABSTRACT

Drug abuse in some addicts progresses to compulsive drug seeking and taking behaviors. High-risk addictive behaviors tend to make interactions between an addict and the community more complex. Socialization is a necessary process that needs to be developed to accommodate to social life. The aim of this study was to evaluate the benefits of social interaction during addiction. Forty two (42) male Sprague-Dawley rats were randomly divided into four groups: social, isolated, socialized morphine-treated (SMT) and isolated morphine-treated (IMT). At the end of the study, neurogenesis, corticosterone, nitrite/nitrate, anhedonia with forced swim test and sucrose and salt craving were examined. Neurogenesis was reduced in IMT group compared to SMT group. SMT animals and control demonstrated better forced swim test performance compared to IMT animals. Furthermore, sucrose and salt (NaCl 3%) consumption was found to be higher in IMT animals compared to SMT animals. Corticosterone was higher in IMT rats compared to SMT rats. Nitrite/nitrate was higher in IMT rats compared to SMT rats. Socialization preserves brain functions necessary for life. Hence, socialized addicts can better tolerate addiction-induced adverse effects.

Keywords: Neurogenesis, morphine, forced swim test, sucrose, nitrite/nitrate, salt, craving

INTRODUCTION

Addiction (drug abuse) is a complex brain disease with devastating consequences. It is associated with a wide variety of economic, financial, and social problems (Strassels, 2009). 180 million people are addicted to various illegal drugs all over the world. Illicit drug dependence directly accounted for about 20 million disability-adjusted life years (DALYs) in 2010, accounting for about 0.8% of global all-cause DALYs. Worldwide, more people were dependent on opioids and

amphetamines than other drugs. Opioid dependence was the largest contributor to the direct burden of DALYs (about 9.2 million) (Degenhardt et al., 2013). The level of economic development in countries also plays an important role. The burden from psychoactive substance use is higher in the developed countries than especially in the high mortality developing countries (Terzic, Senta, & Ahel, 2010). The co-occurrence of comorbid psychiatric disorders such as anxiety, depression, and schizophrenia highlights the importance of comorbid psychiatric disorder as the cause of relapse to drug abuse (Tate, Brown, Unrod, & Ramo, 2004). This co-occurrence motivates to find the underlying mechanism to prevent relapse. Addictive behaviors result in complex social interactions between an addict and the society. As the texture of society become more complex these interactions become more complicated. So it is very useful to understand and diagnose these complexities.

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Drug abuse adversely affects brain function and disturbed brain that in return contributes to the development of drug abuse side-effects. These side effect vary in degree of severity and personal traits (Giorgi, Piras, & Corda, 2007). After drug abuse, there is a period of psychiatric comorbid. These comorbid can contribute to relapse to the drug of abuse. Treatment of these comorbid can reduce relapse to drug abuse (Ramo, Prince, Roesch, & Brown, 2012). Of these comorbid conditions are depression and personality changes (Ramo, Prince, Roesch, & Brown, 2011). So by promoting these behavioral changes to proper traits we can reduce relapse to the drug of abuse. Delineating brain mechanism for curing of this comorbid can help establishing a good treatment.

Social isolation has many adverse effects on brain and behavioral processes such as learning, memory and dopamine metabolism in nucleus accumbens (F. S. Hall et al., 1998). Also in one study social isolation has increased vulnerability to addiction (Whitaker, Degoulet, & Morikawa, 2013). In the other hand, socialization that is describing in animal studies two rats putting together in one cage (pair) is associated with positive effects (Peitz et al., 2013). Socialization is a necessary habit that helps to be fit in society and adjusts behavioral norms for living. Without socialization, it seems comorbid psychiatric symptoms develop. Timely managing drug-abused people is important because by timely intervention for comorbid conditions that increase the risk of relapse, relapse to drugs decreases. These comorbid conditions are depression and emotional disturbances.

Generation of new neurons after birth in some certain brain regions is called neurogenesis. The nature of neurogenesis is quiescent phenomena. It means that neurogenesis should be activated in response to certain stimuli. It is regulated by intracellular and extracellular factors (Liu & Song, 2016). New neurons integrate to preexisting circuits and modulate them (Neves, Cooke, & Bliss, 2008). Stroke, opioids, and ischemia alternate of neurogenesis (A. J. Eisch, Barrot, Schad, Self, & Nestler, 2000; Marlier, Verteneuil, Vandenbosch, & Malgrange, 2015). It occurs primarily in the subventricular zone of the lateral ventricles and subgranular zone of the dentate

gyrus in the hippocampal formation. However, some studies have also reported neurogenesis occurring in (Ming & Song, 2005) striatum (Ernst et al., 2014) and nucleus accumbens (Roberts et al., 2014). Overall increase in neurogenesis is associated with better brain function. In this study, it is also believed that reduction of neurogenesis decreases brain reserves capability to overcome the stress.

Addiction can attribute to some conditions and does not have a unique definition (Amelia J Eisch & Harburg, 2006). Addiction can develop in more than one way, so in this study, because we want to understand the devastating effects of morphine, we have used both conceptions for it, because adverse effects to the brain can happen through dependence to morphine (addiction) or from morphine toxicity (Di Chiara et al., 2004). So in this study, we have used both addiction and morphine-treated rats for showing that it is possible that both conditions have caused the adverse condition.

Hippocampus as the part of reward circuit is of great importance for studying addiction. The reward circuit is consisting of nucleus accumbens, ventral tegmental area (VTA), amygdala and hippocampus (Adinoff, 2004). Neurogenesis in hippocampus can be considered as a good potential candidate for studying relapse to addiction because the hippocampus is one of the parts of reward circuit. It is thought in this study that hippocampal neurogenesis regulates the other areas in reward circuit. It is known that psychiatric disorders improve with improvement in neurogenesis (Malberg, Eisch, Nestler, & Duman, 2000). In this study forced swim test has been used for assessing mood balance, the salt appetite for assessing sensitization and sucrose preference test for assessing tendency to consume more drugs. Based on previous studies depression (Tate et al., 2004), sensitization (Souza-Formigoni et al., 1999) and sucrose consumption (Coudereau et al., 1999) have been associated with more relapse and poor prognosis in addiction period.

Interestingly, the reward system is mandatory for the formation of social bonds that is necessary for both childhood and adulthood. Formation of successful bonds will happen in the presence of oxytocin and vasopressin

(Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). These hormones increase and strengthen social bonds through their effects on the reward system. Psychologically, pair bond formation is necessary for desired object stage (Bartels, 2012).

Animal models of addiction include injection of morphine and assessing addictive behavior with self-administrator box (Abrous et al., 2002). In this study injection of a constant dose of morphine has been used as a model to induce dependence (Di Chiara et al., 2004). Assessing of drug dependence has been done by related behavioral experiments.

Addiction is a stress that exposes the body to oxidative stress (Cunha-Oliveira, Cristina Rego, & R Oliveira, 2013) and various kind of vitamin deficiencies such as C, E and A (Miller, 2010). All these vitamins are necessary for defense against oxidative stress. It is believed that increase in nitric oxide synthesize activity as assessed by nitrate/nitrite level would help addicts to overcome the adverse effects of substance abuse.

Corticosterone is a hormone that is elevated in some forms of relapse (Erb, Shaham, & Stewart, 1998). As we know the elevation of this hormone is accompanied by elevation of CRF. It can also affect brain structures such as the hippocampus (Brummelte & Galea, 2010; Dorey, Pierard, Chauveau, David, & Beracochea, 2012). In this study, we assess it if its elevation is associated with adverse effects. Corticosterone elevation reduces antioxidant defense (Şahin & Gümüşlü, 2007).

As mentioned above socialization is a mandatory part of life that is necessary for every stage of life. Neurogenesis in the hippocampus is thought based on previous studies regulates both socialization process and reward system. These effects are partly regulated by hormones. Also, oxidative stress happens in addicted people by different mechanisms such reduced vitamins. Comorbid psychiatric disorders can both initiate and can deteriorate addiction process. So we hypothesize that socialization of rats can increase neurogenesis and along with it brain functions improve and prevents the development of the comorbid condition in addition period and reduce the risk of relapse.

METHOD AND MATERIAL

Animal care

All the experiments were performed in accordance with the guidelines for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996). The experimental protocol was approved by the institutional care and use committee of Tehran University of Medical Sciences (Tehran, Iran).

Animals

Forty two male Sprague-Dawley rats weighing 200-250g were randomly divided into four groups: (1) social (control) (2) isolated (3) socialized morphine-treated (SMT) group and (4) isolated morphine-treated (IMT) group. Animals in the isolated group were housed individually in cages covered with black plastic. In the socialized group, animals were randomly paired and housed in transparent cages. Housing took place under standard 12 hour day/night cycle at room temperature 22°C and animals had free access to water and chow ad libitum.

Morphine treatment (addiction)

After one-week adaptation to the environment, animals in isolation (IMT) and socialization (SMT) groups received 0.75 mg morphine sulfate for two consecutive weeks intraperitoneally (i.p).

Social (control)

In the control, group rats received saline intraperitoneally (i.p).

Socialization

In this study, we put two rats in each cage (42x15x21) for modeling of socialization (Peitz et al., 2013).

Isolation

In this study, we put one rat in each cage (27x15x21) for isolation which covered by black plastic and the roof of the cage was open, so the day-night cycle would not be disturbed (Yorgason, Espana, Konstantopoulos, Weiner, & Jones, 2013).

Figure 1: Schematic diagram of experiment has been delineated. In the first phase of the experiment rats were treated with morphine and BrdU and in second phase behavioral tests were completed by rats. Then immunohistochemistry and biochemical analysis were completed.

Plan of Experiment

Forty two rats were divided into four groups: social (control), isolated, morphine treated in isolation and morphine-treated in socialization. After one week for adaptation rats receive in control group BrdU and saline (i.p) for 14 days and in morphine-treated groups they received morphine and BrdU (i.p) also for 14 days. In day 13 rats treated sucrose solution for adaptation and after that forced swim test was performed. In the two next day, sucrose preference test and salt craving test was performed. These behavioral tests based on previous studies are indicators of development of dangerous addictive behaviors that eventually leads to poor prognosis addiction in which compulsive drug seeking and taking behaviors appear. After those rats were anesthetized and a blood sample was got from the heart and brain was fixed via cardiac perfusion and sections prepared from the hippocampus of rat's brain for immunostaining (Figure 1).

Forced swim test

This test has been used in animal studies for assessing depression. In this experiment, the rats were forced to swim in a water-filled opaque cylinder (30 cm x 50 cm), without any possibility to escape. The water was 40 cm deep, and with temperature 25°C. Two trials were performed on two subsequent days. The first trial (pre-test) lasted for 15 min, followed by another trial (test) after 24 hr. which lasted for 5 min. The water was changed after every swimming test to eliminate urine, excrement,

and fur. The test sessions were videotaped for scoring the movements and behavior of the animals. When animals ceased all movements except those necessary for survival (keeping the head above the water), the behavior was considered to be immobile. Immobility was defined as the absence of movement, with the body inclined forward, passively floating, and the paws immobile. Immobility (in a sec) along with the number of times the animal stopped swimming (number of stops) was measured. The increase in a score of the mentioned parameters is indicative of the presence of depressive-like behavior in a rat (Menezes et al., 2008).

Sucrose preference test

This test has been used in animal models in addiction studies with different protocols. In some increase consuming indicated that the rats have less anhedonia, and in some studies, increase consumption has been associated with increased tendency for substance abuse that probably means seeking for more pleasure. In this study, the second one has been investigated. It means isolated morphine treated (IMT) rats expected to have the most consumer. Animals were tested for the preference of a 1% sucrose solution (Sucrose, Sigma-Aldrich), using a two-bottle choice procedure. Before the test, animals were adapted to 2% sucrose solution and water for 48h. Animals were then housed individually without any access to sucrose solution or water for the next 3h. Subsequently, animals were provided with two bottles; one of sucrose (1%) and one of tap water. The

amount of sucrose solution and water intake was measured in one hour. Fluid intake was measured by weighing the bottles after a 60-min period and percentage of sucrose preference was calculated (volume of sucrose intake x 100/total fluid intake) (Romieu et al., 2008).

Salt appetite assessment

The increase in salt consumption is indicative of the appearance of sensitization phenomena. When sensitization develops in a rat, it predicts that the substance abuse will follow a poor process which means this rat will develop compulsive drug-seeking and taking behavior. To evaluate salt appetite, animals were placed on food and water restriction plan for 24 hr. The animal was offered NaCl 3% (Sigma-Aldrich) for the next one hour. Total NaCl 3% consumption was calculated (Roitman, Na, Anderson, Jones, & Bernstein, 2002).

Assessment of corticosterone

Corticosterone in addiction studies is used to assess the initiation of relapse to drugs. More corticosterone level is associated with more relapse and occurrence of high-risk behaviors. It is related to CRF (corticotropin-releasing hormone) axis. In this experiment for assessing the level of hormone, blood samples were obtained from the heart with syringe 5 after anesthesia before heart perfusion for brain fixation. It was collected in an experimental tube and then kept for 15 minutes for coagulation. Then it was centrifuged at 50000 rpm for 15 minutes, and serum was collected in a microtube and was kept immediately in -70 ° C. The level of corticosterone was assessed by Elisa kit (sigma) in Noor laboratory.

Nitrite/Nitrate assessment

Nitrite/nitrate was the result of nitric oxide synthase activity. It means more activity of the enzyme results in more production of antioxidant defense. So for assessing the activity of the enzyme indirectly nitrite/nitrate is being measured. Total Nitrite/Nitrate was assessed according to Griess reaction. Simply for performing this experiment n-1 (naphthyl) ethylenediamine (NEDD), sulfanilamide and acid phosphoric all were mixed together. 100

microliter of serum was added to the above mixture. The final solution developed pink color, and maximum absorbance was measured at 540 nm with the spectrometer (Miranda, Espey, & Wink, 2001).

Immunohistochemistry

After the completion of adaptation period, Bromo-deoxyuridine (BrdU; 50 mg/kg, Sigma-Aldrich Co.) was injected for 14 days intraperitoneally. BrdU is an analog of thymine base which is incorporated in DNA of newly proliferated neurons in the dentate gyrus of the hippocampus. At the end of day 14, animals were anesthetized with Ketamine (100 mg/kg) and Xylazine (10 mg/kg) and then sacrificed. It shall be noted that Ketamine was used as an anesthetic drug and Xylazine as a sedative drug to prevent stress in animals. After thoracotomy, all animals were first perfused with 120 cc normal salines and then, with 120 ccs paraformaldehyde 4% via intracranial infusion. After fixation, the brains were removed from the skull. The brains were kept in graded sucrose solution plus PBS. The cryosections (30 µm) were prepared from dentate gyrus of the hippocampal region. Immunohistochemistry was performed for ten sections from each brain, five of which were stained for BrdU-positive neurons with anti-BrdU antibody kit (5-Bromo-2'-dU Labeling and Detection Kit II; Roche, Germany, Cat. No. 11299964001-en-17). BrdU-positive cells in dentate gyrus were counted directly under a light microscope (Zeiss Co.) in original magnification, 400X BrdU-positive neurons had been colored brown and they were in some forms such as isolated and clusters. Clusters are counted according to the size of isolated neurons (Spritzer, Ibler, Inglis, & Curtis, 2011).

Statistical analysis

Data were analyzed using SPSS version 22 and GraphPad prism. ANOVA was done for equality of variance and if the inequality was significant, post hoc test of Tukey was done for assessing of equality of means. ANOVA was performed for two variables: morphine treatment and isolation. This was done to analyze the effect of the above two variables on related parameters such as immobility time, a number of stops, sucrose and

Figure 2: A. Number of the stops in forced swim test. B. Immobility time (s) in forced swim test (n=10). Data are represented as Mean $\pm$ SEM. SMT: Socialized Morphine-Treated and IMT: Isolated Morphine-Treated. Complementary information about mean values and the significant differences between the groups is available in Table 1 and 2.

salt consumption, neurogenesis, corticosterone and nitrite/nitrate. It should be noted that because one rat has been used for modeling socialization, about 28 rats have been considered for statistical analysis. Data were as represented as mean $\pm$ SEM and $P < 0.05$ considered significant. Significance difference of mean data among different groups was analyzed for the level of statistical significance of $P < 0.05$.

RESULTS

Forced swim test

Statistical analysis with ANOVAs showed variance is significant difference among four groups for number of stops (NS) and immobility time (IT) in forced swim test (Number of stops: $F: 6.048$, $R\text{-square}: 0.4410$, $DF: 3, 23$ and $P: 0.0034$ and time of immobility: $F: 8.091$, $R\text{-square}: 0.5483$, $DF: 3, 20$ and $P: 0.0010$). Compared to SMT rats and social rats, immobility time and number of stops were significantly higher in the IMT rats (NS: $P: 0.0078$ and IT: $P: 0.0046$). Also, isolated rats stopped more frequently and had more immobility time while swimming as compared to social group (NS: $P: 0.0044$ and IT: $P: 0.0073$). In these experiments, IMT rats had a worse outcome than social rats (NS: $P: 0.0294$ and IT: $P: 0.0250$). Also, in these experiments isolated rats had a worse outcome than SMT rats (NS: $P: 0.0028$ and IT: $P: 0.0011$) (Figure 2, A and B).

Sucrose preference test

Statistical analysis with ANOVAs showed variance is significant difference among four groups for sucrose consumption in sucrose preference test ($F: 14.37$, $R\text{-square}: 0.6621$, $DF: 3, 22$ and $P: 0.0001$). The percentage

Figure 3: Percentage of sucrose consumption in sucrose preference test. Data are represented as mean $\pm$ SEM. SMT: Socialized Morphine-Treated and IMT: Isolated Morphine-Treated. Complementary information about mean values and the significant differences between the groups is available in Table 1 and 2.

of sucrose consumption was higher in IMT rats compared to SMT (P: 0.0028). In this experiment isolated rats had a worse outcome than social rats (P: 0.0095). Also, isolated rats had a better outcome than IMT (P: 0.0092) (Figure 3).

Salt appetite

Statistical analysis with ANOVAs showed variance is significant difference among four groups for NaCl (3%) consumption in salt appetite test (F: 15.34, R-square: 0.6970, DF: 3, 23 and P: 0.0001). NaCl (3%) consumption in one hour was higher in IMT rats than SMT rats (P: 0.0009). The isolated group had a worse outcome than social rats in control groups (P: 0.0078). Also, isolated rats had a better outcome than IMT rats (P: 0.0435) (Figure 4).

Figure 4: Salt intake (NaCl 3%) (ml) in one hour. Data are represented as mean $\pm$ SEM. SMT: Socialized Morphine-Treated and IMT: Isolated Morphine-Treated. Complementary information about mean values and the significant differences between the groups is available in Table 1 and 2.

Corticosterone

Statistical analysis with ANOVAs showed variance is significant difference among four groups for corticosterone level when it was assessed corticosterone in serum (F: 3.657, R-square: 0.3922, DF: 3, 17 and P: 0.0336). It was higher in IMT rats compared to SMT rats (P: 0.0190). In other groups, it does not change significantly (Figure 5).

Figure 5: Plasma level of corticosterone (pg/dl). Data are represented as mean $\pm$ SEM. SMT: Socialized Morphine-Treated and IMT: Isolated Morphine-Treated. Complementary information about mean values and the significant differences between the groups is available in Table 1 and 2.

Figure 6: Plasma level of nitrite/nitrate (μ mol/L). Data are represented as mean $\pm$ SEM. SMT: Socialized Morphine-Treated and IMT: Isolated Morphine-Treated. Complementary information about mean values and the significant differences between the groups is available in Table 1 and 2.

Nitrite/Nitrate in serum

Statistical analysis with ANOVAs showed variance is a significant difference among four groups for nitrite/nitrate when it was assessed in serum ($F: 47.36$, $R\text{-square: } .09660$, $DF: 3, 20$ and $P: 0.0004$). Nitrite/nitrate was higher in social rats compared to isolated rats ($P: 0.0249$). In IMT rats nitrite/nitrate was higher compared to isolated and SMT rats ($P: 0.004$ and $P: 0.0093$, respectively). Also, social rats had a higher level of nitrite/nitrate compared to SMT rats ($P: 0.0151$) (Figure 6).

Neurogenesis

Statistical analysis with ANOVAs showed variance is a significant difference among four groups for a number of BrdU-positive neurons after immunostaining ($F: 104.3$, $R\text{-square: } 0.7746$, $DF: 3, 20$ and $P: 0.0001$). The Number of BrdU-positive cells in dentate gyrus of the hippocampus was significantly higher in SMT rats compared to IMT rats ($P: 0.0001$). Neurogenesis was better in a social group than isolated rats ($P: 0.0001$). Also, isolated rats had better neurogenesis than IMT rats ($P: 0.0117$) (Figure 7 and 8).

Figure 7: BrdU-positive cells in cryosections prepared from dentate gyrus of the hippocampus (No). Data are represented as mean $\pm$ SEM. SMT: Socialized Morphine-Treated and IMT: Isolated Morphine-Treated. Complementary information about mean values and the significant differences between the groups is available in Table 1 and 2.

Figure 8: Micrograph of cryosections stained for BrdU antigen; labeling with anti-BrdU antibody for newly proliferated neurons in the dentate gyrus of the hippocampus. BrdU-positive cells are indicated by arrows. They may be in single or cluster forms. It should be noted that all BrdU-positive cells have not been marked by arrows.

DISCUSSION

In the present study, we demonstrated that drug abuse (morphine-treated) in isolated rats reduces neurogenesis, emotional reactivity (depression) and increases sucrose and salt consumption. Furthermore, isolated morphine-treated rats consumed more sucrose than socialized and control rats as observed in sucrose preference. Also, isolated morphine-treated rats have been shown to have more salt appetite. Therefore, our study indicates that morphine-abuse in socialized (pair) group is better tolerated accompanied by less adverse

Table 1: The below complementary data shows values of variables (Mean $\pm$ SEM) in the experiments

Experiments	Groups of Experiments			
	Social	Isolated	SMT	IMT
Number of Stops	1.500 $\pm$ 0.8660 (NO) n=4	8.250 $\pm$ 1.250 (NO) n=4	2.333 $\pm$ 0.8660 (NO) n=9	7.900 $\pm$ 1.567 (NO) n=10
Immobility Time	1.750 $\pm$ 0.8539 (NO) n=4	10.75 $\pm$ 2.097 (NO) n=4	2.000 $\pm$ 0.9063 (NO) n=8	8.750 $\pm$ 1.790 (NO) n=8
Sucrose Preference Test	8.250 $\pm$ 8.250 (ml) n=4	57.25 $\pm$ 10.14 (ml) N=4	53.43 $\pm$ 8.927 (ml) N=9	90.36 $\pm$ 5.431 (ml) N=9
Salt Appetite	0.2500 $\pm$ 0.2500 (ml) n=4	2.250 $\pm$ 0.2500 (ml) n=4	2.444 $\pm$ 0.3768 (ml) n=9	6.300 $\pm$ 0.8439 (ml) n=10
Corticosterone	290.4 $\pm$ 6.071 (pg/dl) n=5	282.4 $\pm$ 28.95 (pg/dl) N=5	215.7 $\pm$ 15.92 (pg/dl) N=6	297.0 $\pm$ 24.85 (pg/dl) N=5
Nitrite/Nitrate	48.50 $\pm$ 2.836 (μ mol/L) n=6	32.20 $\pm$ 1.800 (μ mol/L) n=6	23.85 $\pm$ 4.350 (μ mol/L) n=6	73.43 $\pm$ 2.075 (μ mol/L) n=6
Neurogenesis	232.2 $\pm$ 34.04 (NO) n=6	28.67 $\pm$ 3.798 (NO) n=6	158.2 $\pm$ 20.63 (NO) n=6	14.59 $\pm$ 3.837 (NO) n=6

Note. SMT = Socialized morphine-treated; IMT = isolated morphine-treated

Table 2: The below complementary data shows exact differences among different groups

Experiments	comparisons
Number of Stops (NS)	Isolated=IMT>SMT>Social
Time of Immobility (TI)	Isolated=IMT>SMT>Social
Sucrose Preference Test (SPT)	IMT>Isolated=SMT>Social
Salt Appetite	IMT>Isolated=SMT>Social
Corticosterone	Social=Isolated=IMT>SMT
Nitrite/Nitrate	IMT=Social>SMT=Isolated
Neurogenesis	Social=SMT>Isolated>IMT

Note. SMT = Socialized Morphine-treated; IMT = Isolated morphine-treated

effects. In almost all experiment control group had the best performance, so disturbances can all contributed to the effect of morphine and isolation.

Substance abuse is a complex brain disease accompanied by negative physical, personal, or social consequences. This study shows that morphine-induced reduction in neurogenesis underlies many adverse effects of addiction.

Similar to previous studies, this study shows that neurogenesis can influence learning, mood and emotional state of animals (Imayoshi et al., 2008).

Neurogenesis is necessary for learning capabilities (Sudai et al., 2010) and learning abilities is essential for survival and life. A study has shown that better learning ability can be associated with better tolerance and successfully withdrawing it because successful withdrawal needs proper memory (Schoenbaum & Shaham, 2008).

Furthermore, adequate neurogenesis is also beneficial in preventing depression (Wang, David, Monckton, Battaglia, & Hen, 2008). We have shown that isolated morphine-treated (IMT) rats with reduced neurogenesis are at a greater risk of depression and it helps for further withdrawing from society. Improving neurogenesis may improve depression and prevents personality deterioration and more social isolation. Also in several human studies, substance abuse relapse has been associated with the more comorbid psychiatric disorder such as depression and schizophrenic-like symptoms such as isolation (Horsfall, Cleary, Hunt, & Walter, 2009). In this study, the positive correlation of neurogenesis with behavioral functions was well illustrated, especially salt appetite and sucrose consumption. In IMT rats that had the lowest rate of neurogenesis salt and sucrose consumption was the highest. Also in forced swim test isolated and IMT rats had the weak performance. Enough neurogenesis is needed to avoid development of high-risk behavior. Control rats, on the other hand, had the lowest salt and sucrose consumption and immobility time in forced swim test.

Sucrose preference test is performed for assessment drug craving (compulsive taking or abusing more drugs despite adverse effects without control on it) (Pastor, Kamens, McKinnon, Ford, & Phillips, 2010) and depression (Ren et al., 2013). It should be noted that assessment of

craving is indirectly, and of course, we cannot assess it for sure with this experiment. But in some studies drugs, treatment had altered sucrose consumption. So we preferred to conclude in accordance with few studies that it can indirectly assess craving (Pastor et al., 2010; Romieu et al., 2008). Also, the other evidence is that disturbance in taste (may be sucrose) sensation has been associated with drug abuse. It has been indicated that morphine abuse can augment sucrose consumption. A study has documented common genes for both, addiction and taste sensations. Furthermore, it can be explained through morphine effect on pleasure center. Furthermore, a depressed state may cause one to be isolated which in turn may result in a decline of ego defense mechanisms (Nesil, Kanit, & Pogun, 2015; Pastor et al., 2010). Morphine - induced increase in salt appetite results in locomotor sensitization and increased morphine craving (Na, Morris, & Johnson, 2009). Conditions which alter serotonin (5-HT) and noradrenaline levels, such as anxiety or depression, are associated with taste disturbances. This may indicate the importance of these neurotransmitters in the determination of taste thresholds in health and disease (Heath, Melichar, Nutt, & Donaldson, 2006). In addition, the increased expression of stress hormones receptors, specifically glucocorticoids receptors, on sweet buds may augment sucrose consumption in stressful situations (Parker, Feng, Chamuris, & Margolskee, 2014). It should be noted that neuronal mechanisms for proving that increase in sucrose consumption are associated with more craving have not been established.

Sensitization is vague phenomena which explaining the nature of this phenomena is hard. However, for explaining this phenomenon different paradigms of experiments have been used. These paradigms include inducing sensitization with salt and changing the frequency of drug administration (Sakai, Frankmann, Fine, & Epstein, 1989). Drug sensitization usually occurs after repeated administration of drugs such as nicotine, amphetamine, cocaine, morphine, and caffeine and is behaviorally expressed as increased locomotion in an open field test or increased self-administration of a drug (D. A. Hall, Stanis, Marquez Avila, & Gulley, 2008).

Behavioral sensitization to opiates and psychostimulants

is characterized by the progressive increase in ambulatory activity and in the frequency of more focused, non-ambulatory behaviors such as sniffing, rearing, licking, and gnawing, following repeated drug treatments (Giorgi et al., 2007). It should be noted that assessment of above signs is not an issue of this study and in previous studies, it has been studied by various dosages including our dosages (Becker, Gerak, Li, Koek, & France, 2010).

Age can also influence the mechanism of sensitization. This reflects different mechanisms of neurobehavioral adaptations in different groups of ages (Abrahao & Souza-Formigoni, 2012; Stevenson, Besheer, & Hodge, 2008). The activation of accumbal D2 receptors is essential for the expression of signs of behavioral sensitization (Abrahao, Quadros, Andrade, & Souza-Formigoni, 2012). In mice, repeated administration of addictive substance may induce behavioral sensitization a process of progressive potentiation of its stimulant effects, associated with neuroadaptations in the brain reward system (Koob & Volkow, 2010). Different manifestations of sensitization involve different mechanisms. Cross-sensitization is associated with more NMDAR activation (Abrahao & Souza-Formigoni, 2012). Locomotor sensitization to cocaine is associated with increased FOS expression in the accumbens (Crombag, Jedynak, Redmond, Robinson, & Hope, 2002).

We assessed mood balance (anhedonia) by forced swim test. Depression decreases socialization and the resultant isolation decreases neurogenesis reduces. Amygdala, a part of the limbic system, are primarily involved in memory formation, emotional reaction, and decision making (Phelps, 2006). Reduced neurogenesis and deterioration of brain functions resulting from inadequate neurogenesis may affect the functioning of the amygdala. In addition, striatum and cingula also regulate memory formation and emotions (Duval, Javanbakht, & Liberzon, 2015). The increase in neurogenesis may promote pleasure center to recover its reduced function that is one of the markers of poor prognosis in drug-abused people. Also, other mechanisms may activate through another effect of socialization.

However, in this study, it is postulated that increase in salt consumption and sucrose consumption increases

relapse to opioids. Since isolated morphine-treated (IMT) rats consume more sucrose and salt, it is postulated that isolation in addition period increases the tendency to opioids. Isolated rats in control group consume less sucrose and salt than isolated morphine-treated (IMT) rats. It indicates that isolation and morphine increase the likelihood of relapse than one of them alone. In forced swim test this effect is less evident. Along with these behavioral outcomes neurogenesis in isolated morphine treated (IMT), rats has the least score. So it can be concluded that neurogenesis reduces relapse in a direct manner. Since neurogenesis in isolated rats in control group is more than isolated morphine-treated (IMT) rats. These positive effects are due to the positive effects of social interaction. The positive effects of social interaction in this study are related to increasing etherate of neurogenesis. This increase may happen through mechanisms in the brain itself or through hormonal effects.

According to our study, social interaction promotes neurogenesis, decrease depression, and drug craving. It is well-known that social interaction influences psychological, physiological and behavioral functions. Furthermore, social interaction has also seen to reduce anxiety and depression (Neumann & Landgraf, 2012). On the other hand, negative social interactions have adverse effects on behavior and brain function. These effects may involve in increase anxiety and depression. The positive effects of social interaction are dependent on reward center (Krach, Paulus, Bodden, & Kircher, 2010). Of hormonal factors, oxytocin is the most putative hormone. According to a study, the beneficial effects of social interaction are related to oxytocin (Uvnas-Moberg & Petersson, 2005). Also, it seems that rewarding learning reduces anhedonia and depression and CRF needs for it (Bogdan, Santesso, Fagerness, Perlis, & Pizzagalli, 2011). Therefore, improving social interactions may help in preventing adverse effects of morphine such as reduced neurogenesis. Other options for improving neurogenesis in addicts may include; DBS (deep brain stimulation) (Warner-Schmidt, Madsen, & Duman, 2008), antidepressant treatment (Elsayed et al., 2012; Encinas, Vaahtokari, & Enikolopov, 2006) and exercise (Creer,

Romberg, Saksida, van Praag, & Bussey, 2010).

Social interaction also reduces the negative effects of stress. Adult-born neurons appear to have a role in the regulation of stress (Schloesser, Manji, & Martinowich, 2009; Surget et al., 2011). Antidepressant improves depression symptoms by increasing neurogenesis and studied in animals with impaired neurogenesis suggest this effective treatment (Malberg et al., 2000; Manev, Uz, Smalheiser, & Manev, 2001; Santarelli et al., 2003). Interestingly, newborn neurons express GABA receptors more than the older ones and this makes these cells more excitable (Chancey et al., 2013). Loss of these cells prone body to response to stress more than usual and attenuates amygdala response to fearful stimuli and resultant decreased the ability of the body to respond to stress (Schloesser et al., 2009). This hypothesis support this theory that increase in neurogenesis is associated with decreasing stress and increase in stress such as isolation decrease newborn neurons. Besides, antidepressant exerts their effect through enhancing these newborn neurons under chronic stress conditions (Surget et al., 2011).

In this experiment level of corticosterone in serum was also assessed. In our experiment, it was elevated in isolated morphine-treated (IMT) rats. It is in accordance with previous studies, that it is elevated in conditions with relapse and more adverse effect of substance abuse. It is well proved that some forms of relapse to drug abuse are associated with elevated level of corticosterone and it is associated with negative prognosis (Erb et al., 1998). Also in another study elevated levels of corticosterone prone rats to aggressive behavior and reduced social interaction (Veenit, Cordero, Tzanoulidou, & Sandi, 2013). Another possible explanation is a reduction of neurogenesis by an elevated level of corticosterone (Brummelte & Galea, 2010). In some studies, it has been proposed that chronic administration corticosterone reverses depression (Waters & McCormick, 2011). So it seems an increase in corticosterone may have an adverse effect or is not enough for the restoration of mood disorder. Also, reduce in neurogenesis may have serious adverse effect on mood and also neurotransmitter deregulation like serotonin is responsible for depression (Sundar et al., 2014). As

noted, reduction of memory is also responsible for relapse. In some studies, it has been proved that reduction of memory is associated with elevated level of corticosterone (Dorey et al., 2012).

Oxidative stress is correlated with brain function and psychological disorders (Salim, 2014). In this study it was higher in isolated morphine-treated (IMT) rats. It is highly indicative of brain demand for proper function under stress status. In this study, it has been affected by socialization. Neurogenesis is also affected by oxidative stress. In different brain diseases, especially neurodegenerative brain diseases such as those occur as the result of aging and neuroinflammation, oxidative stress is one of the factors that inhibits neurogenesis (Yuan, Gu, Shan, Machado, & Arias-Carrion, 2015). Neurogenesis that is inhibited as the result of morphine as

a toxic agent is anticipated to be increased as the result of improvement in oxidative defense.

Our results suggest that addiction in the socialized group is better tolerated and brain functions are preserved in these animals. Furthermore, socialization seems to help animals against depression and more drug abusing.

CONCLUSION

Social interactions during addiction may play an important role in preventing morphine-induced adverse effects and promote better functioning of the brain.

Conflict of Interest Statement

None to declare

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