La Prensa MedicaArgentina

Research Article

# Improve in Neurogenesis with Socialization can Reduce Behaviors that are Related to Poor Prognosis in Withdrawal Period in Male Rats

**Famitafreshi H[1], Karimian M[2*], Hassanzadeh G[3] and Fatima S[1]**


## Abstract

**Introduction:** Morphine withdrawal period is associated with a wide variety of problems. Neurogenesis seems necessary for brain functions. Socialization is a lifelong process that helps getting behavioural norms from society. The aim of this study is to investigate if better neurogenesis in socialization can alleviate problems encountered during withdrawal period.

**Method and material:** Rats were randomly divided to: control rats, isolated rats, withdrawal isolated rats and withdrawal socialized rats. Animals received morphine 7 days and 14 days Brdu was injected. At the end of experiment, memory, neurogenesis, emotional reactivity and sucrose, NaCl consumption was assessed.

**Results:** Rats in withdrawal socialized group had better reference memory and working memory, and they decrease relapse. Furthermore, neurogenesis was higher in withdrawal socialized rats as compared to isolated rats. Enough neurogenesis is necessary for proper brain function. Sucrose preference test and force swim test were well performed in withdrawal socialized rats. These indicate lower incidence of co morbid psychiatric disorder in withdrawal period. Salt consumption as indicator of more relapse to drug abuse was seen to be higher in withdrawal isolated group of animals.

**Conclusion:** Socialization during withdrawal period can help to better tolerate withdrawal period and its adverse effects. So socialization reduces relapse and establishes behavioural norms for successful healthy life.



## Keywords

Neurogenesis; Sucrose; Salt; Withdrawal; Isolation; Socialization; Emotional reactivity; Memory


## Introduction

Substance abuse is a complex and devastating disorder accompanied by many adverse effects. Addiction cannot be always managed successfully which results in enormous financial and social burden. Relapse to substance abuse is often attributed to mesolimbic dopamine system whose efferent and afferent connections are the neural substrate for the rewarding effects of drugs of abuse [1]. Studies using brain imaging have shown that neuronal activity in the orbit frontal cortex, a brain area thought to promote the ability to control behavior, is disturbed in drug addicts [2].

Drug abuse adversely affects brain function and disturbed brain in return contributes to development of drug abuse sides-effects. These sides effect vary in degree of severity and personal traits [3]. After drug abuse there is a period of psychiatric co morbid. Theses co morbid can contribute to relapse to drug of abuse. Treatment of this co morbid can reduce relapse to drug abuse [4]. Of this co morbid conditions are depression and personality changes [4]. So by promoting these behavioral changes to proper traits we can reduce relapse to drug of abuse. Delineating brain mechanism for curing of this co morbid can help establishing good treatment. It should be noted that assessment of above signs is not issue of this study and in previous studies it has been studied by various dosages including our dosages [5].

Previous studies has shown that environmental enrichment (EE) can affect the process of brain diseases and processes such as depression, Alzheimer, drug addiction, learning neurogenesis and emotions. During EE, a large group of rats are put together in larger cage than the standard cage and are given toys and equipment, enabling more social contact, and providing a greater surface area per rat, and a more stimulating environment [6]. In other extreme is social isolation with many adverse effects on many brain and behavioral processes such as learning, memory and dopamine metabolism in nucleus accumbens [7]. Also in one study social isolation has increased vulnerability to addiction [8]. In the middle of this extreme is socialization that is describing in animal studies two rats putting together in one cage [9]. Socialization is a lifelong process that helps to be fit in society and adjust behavioral norms for living. Without socialization it seems co morbid pschychiatric symptoms develop. Managing people in withdrawal period is important because by timely intervention for co morbid conditions that increase risk of relapse, relapse to drugs decreases. These co morbid conditions are depression and emotional disturbances.

Neurogenesis in some regions of brain like dentate gyrus of hippocampus and sub ventricular zone [10] has been linked to learning and memory and preventing psychiatric disease [11]. Increase in neurogenesis can improve symptoms of brain diseases such as depression [12]. So because neurogenesis better in social state, withdrawal severity can be reduced in social state, because it improves brain functions such as memory [13]. Considering that neurogenesis can positively affect brain functions like cognition, successful withdrawal can be linked to improved rate of neurogenesis. In addition, subject's emotional state may also influence cognitive functions [14].

Some studies have proposed that disturbance in taste sensations can be associated with addiction and its relapse [15]. It has been recently documented that common genes mediate addiction and taste [16]. Thus, altered taste sensations may increase individual's susceptibility towards drug abuse and can predict likelihood of drug relapse.

***Corresponding author:** Morteza Karimian, Professor of Physiology, Tehran University of Medical Sciences, Tehran, Iran, Tel: +98 216405 3269; E-mail: karimian@tums.ac.ir

**Received:** July 12, 2015 **Accepted:** August 31, 2015 **Published:** September 05, 2015

So successful withdrawal from drugs is not easy in withdrawal period in every person, and many addicted people relapse to abuse with different mechanisms. Co morbid psychiatric conditions such as depression seem to accelerate it. Also socialization is an important part of adaptive behavior for social life and brain functions works better in part with proper neurogenesis in socialization.

Considering that strengthening of some behaviors is accompanied by worse prognosis and can possibly predict worse outcome it can be great help for diagnosis of such poor prognosis patient. But if these symptoms have common pathologies has not been well understood. If these symptoms occur in a person we can predict those with poor prognosis and by implementing proper treatment such as improving neurogenesis can help to have less sever withdrawal period. Based on previous studies some behaviors such as salt consumption [17], sucrose consumption [18] and low mood state and impaired memory were associated with poor prognosis. Therefore, these tests were selected to assess severity of withdrawal period. So, we designed this study to investigate if withdrawal in socialized group with mechanism of improving neurogenesis lessens behaviors associated with poor prognosis and this can reduce relapse risk and induce better tolerance towards successful drug withdrawal.

## Materials and Methods

### Animal care

The experimental protocols followed in this study were conformed to the Guidelines for the Care and Use of Laboratory Animals published by National Institutes of Health (NIH Publication No.85-23, revised 1996) and was further approved by the institutional ethical committee at Tehran University of Medical Sciences (Tehran, Iran). Twenty male Sprague-Dawley rats weighting 200 to 250 grams were housed in an air-conditioned colony room on a 12 hours light-dark cycle at 21–23°C. The animals were provided with free access to food and water. The animals were divided into 4 groups (n= 10); isolated group and socialized group, withdrawal isolated and withdrawal socialized.

### Experimental design

Animals were injected morphine sulphate (Temad Co., Iran) with increasing dose of 5 mg/ kg with base dose of 5 mg/kg interaperitoneally for 7 days and then in day 8 naltrexone was injected 3 mg/kg for avoidance of acute signs of withdrawal from morphine and better tolerance of withdrawal period to the end of experiments. In should be noted that acute signs include chattering, salivation, diarrhea, ptosis, irritating, writhing, dog shake, jump, tremor, vocalization, body weight loss and so on. Injection of naltrexone in low dose has been shown to improve tolerance of withdrawal from drugs especially less than 10 days. Also Brdu (5-bromo-2'-deoxy-uridine, Sigma-Aldrich Co.) was injected with the dose of 50 mg/kg for 14 days. Rats in control groups (isolation and pair) received saline and BrdU. After performing Morris water maze and other behavioral test (force swim test, sucrose preference test and salt consumption test) the rats were sacrificed and sections prepared for Brdu staining. For other behavioral test like force swim test, sucrose preference test and salt appetite different group of animals were used for avoidance of interference in behavioral tasks (Figure 1).

Isolation and socialization of animals: Animals in the isolated group were housed individually in cages covered with black plastic (27×15×21). In socialized group, two rats were housed in each cage and the cages were left transparent (42×15×21). Animals were caged for 1-week adaptation period followed by two weeks of experimental period (Figure 2).

### Morris water maze

The Morris water maze was used to assess learning and spatial working memory in rats. This setup consisted of a large circular black tank (diameter 183 cm) placed in the center of the room. After performing the visible test with clear water, the tank was filled with water (27°C) which was made opaque with non-toxic black tempera paint and divided into four quarters (North, East, West and South). An escape platform (10 cm in diameter) was submersed 0.5 cm below the surface of water in the southeast quadrant. Furthermore, non-motile visual cues were placed around the water tank. Since the animals were kept in opaque water and could not see the platform, they had to rely on external maze cues to find their route of escape. During probe trials, the platform was retracted from the bottom of the maze. For the visible platform task, the water level was slightly reduced such that the escape platform was visible 2 cm above the water surface. Large red geometrical shapes with white backgrounds were place around the maze to provide external cues. During the entire experiment, the animal's performance was videotaped using a video camera mounted above the maze and interfaced with a computerized tracking system. In this 3-day experiment, 2 trials were performed each day. However, on the 3rd day the probe trial was performed. In this experiment, two types of memory were tested; reference memory and working memory. First trial of each session was considered as the test for reference memory and the later one for working memory [19]. In addition, swimming speed was also recorded to consider the emotional state of the animal.

### Spatial acquisition test

In these trials, the rats were trained to find the hidden platform using extra-maze cues. A transparent lucite platform (10×10 cm) was submerged 0.5 cm beneath the surface of water in the southeast quadrant of the water tank. Each rat participated in 16 spatial trials and the position of platform was not changed in any of these trials. The animal was given 60 sec to reach the platform and if the platform was not located within 60 sec, the animal was placed on it by the

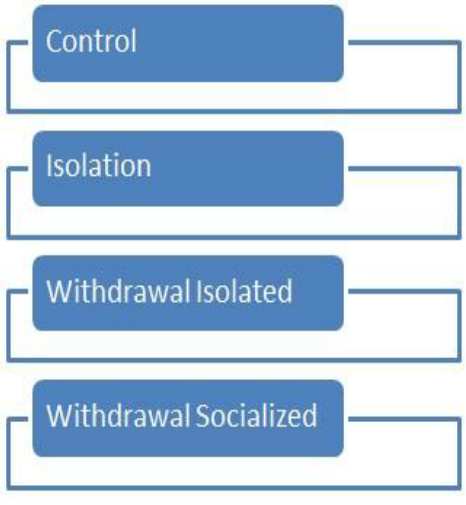


**Figure 1:** Groups of Experiments.

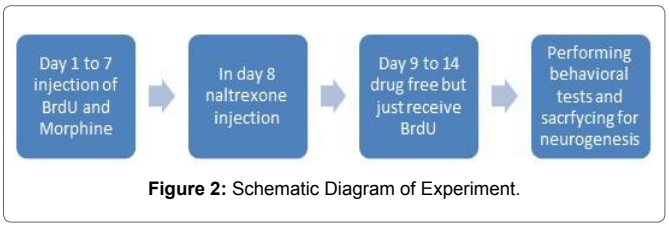


**Figure 2:** Schematic Diagram of Experiment.

experimenter. Between each trial, a 20-sec rest period was given to the animal. Escape latency (sec), distance traveled (cm), and swimming speed (sec/cm) were recorded. For escape latency and distance travelled, lower numbers were indicative of better performance conversely, for swimming speed, higher numbers were indicative of good speed.

Escape latency is the time taken to reach the submersed platform.

Distance traveled (cm) is the total distance swum to find the submersed platform.

Swimming speed (cm/ s) is calculated as distance traveled (cm) divided by escape latency (s).

### Spatial probe trial

This trial was conducted to examine if the animal has learnt the location of the platform. During this trial, the escape platform was removed and the animal was allowed to swim freely for 60 sec. In contrast to swim speed, for the time spent and the swim distance in the fourth quadrant, higher numbers were indicative of better performance.

Fourth quadrant is a quadrant that platform had been located in spatial acquisition phase, and in probe trial phase were removed.

### Force swim test (FST)

In this task, the animals 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, maintained at 25°C. Two trials were performed. 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 referred to the absence of movement, with the body inclined forward, passively floating, and the paws immobile. Immobility (in sec) along with the number of times the animal stopped swimming (number of stops) was measured [20].

### Sucrose preference test (SPT)

Animals were tested for the preference of a 2% sucrose solution (Sucrose, Sigma-Aldrich), using a two-bottle choice procedure. Each animal was housed individually during the 2-day test period. Animals were given two bottles, one of sucrose (2%) and one of tap water. The amount of sucrose solution and water intake was measured daily. To prevent potential location preference of drinking, the position of the bottles was changed every 24 h. Food and water were available ad libitum prior to the SPT. The preference for the sucrose solution was determined as the percentage of sucrose solution ingested relative to the total intake [18].

### Evaluation of NaCl appetite

To evaluate salt appetite, animals were placed on food and water restriction plan for 24 hr. Animal were offered NaCl 3% (Sigma-Aldrich) for one hour. Total NaCl consumption was calculated [21].

Immunohistochemistry: Bromo–deoxyuridine (BrdU; 50 mg/kg) was injected for 14 days interaperitoneally (IP). BrdU is an analogue of thymine base which is incorporated in DNA of newly proliferated neurons in dentate gyrus of hippocampus. At the end of day 14, animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). The animals' brains were first perfused with normal saline and then with paraformaldehyde 4% via intracardial infusion. After fixation, the brain removed from skull. For the first 2 days brains were kept in PBS + paraformaldehyde 4% and then at day 3 in sucrose 10% + paraformaldehyde 4% + PBS. Throughout day 4, the brains were kept in sucrose 20% + paraformaldehyde 4% + PBS and for the rest of the days they were kept in sucrose 30% + paraformaldyde 4% + 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 antiBrdu antibody kit (5-Bromo-2′-dU Labeling and Detection Kit ll; Roche, Germany, Cat. No.11299964001-en-17). Some solutions should be prepared before staining brain sections. First of them is anti-BrdU working solution that we made it by dilution of primary anti body 1:10 with incubation buffer (It is differ from washing buffer and 100 ml solution consist of 66 mM Tris buffer + 0.66 $MgCl_2$ + 1 mM 2-mercaptoethanol). This antibody binds to BrdU that is already incorporated to newly proliferated neuron in dentate gyrus. The second was anti-mouse-Ig-AP working solution that we made it by dilution of anti-mouse-Ig-AP solution 1:10 with PBS. This antibody binds to primary antibody. The third was washing buffer that we made it by diluting washing buffer (PBS) 1:10 with double still water. Finally the forth was color substrate solution that we made it by adding 13 μl of NBT and 10 μl BCIP-solution to 3 ml substrate buffer (100 mM TrisHCl-buffer + 100 mM NaCl + 50 mM $MgCl_2$ (PH = 9.5 at 20°C)). This color solution binds to secondary antibody and gives brown color to staining. The procedure briefly contains rehydrating glass slides with washing buffer (washing three times with washing buffer) then adding primary antibody (anti-BrdU working solution) and giving 30 minutes time for binding to BrdU. Then again washing three times and adding secondary antibody (anti-mouse-Ig-AP working solution) and giving 30 minutes time in 37°C in humid atmosphere. Then again washing three times and this time we add substrate color and incubate it 15-30 minute at 15 to 25°C. Then the glass slides cover-slipped with mounting medium (Canada balsam) and BrdU-positive cells in dentate gyrus were counted directly under 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 size of isolated neurons [22].

## Statistical Analysis

Data were analyzed using SPSS version 22 and Graphpad prism 5. Two-way ANOVAs was performed for 4 groups and if variance significantly different post-hoc Tukey t-test was performed for mean difference. Data are as represented as mean ± SEM and $P<0.05$ considered significant. *, # and $ represent significance difference of mean data among different groups for level of statistical significance

of P< 0.05. *was used for groups that are besides together (control × isolation and withdrawal isolated × withdrawal socialized) and for those apart from each other, other symbols (# and $) were used.

## Results

### Morris water maze

**Reference memory and working memory:** Our results showed that during withdrawal, reference memory and working memory performance were markedly improved in socialized group as compared to the isolated group. Also withdrawaled animals had better memory than isolated animals (Figure 3).

**Speed:** Our analysis showed that during withdrawal, speed of swimming in socialized rats was higher than isolated ones. The speed of swimming is indicative of emotional reactivity. Also withdrawaled rats had higher emotional level than control rats (Figure 4).

**Probe trial:** During probe trials, socialized rats spent more time and traveled longer distance in fourth quadrant, as compared to the isolated group. Withdrawaled rats also had better performance than control groups (Figure 5).

### Sucrose preference test

During withdrawal, percentage of sucrose consumption was higher in isolated group than socialized group. Also rats in withdrawaled groups had increased consumption (Figure 6).

### Forced swim test

Compared to socialized rats, immobility time was significantly higher in the isolated group during withdrawal. Also, isolated rats stopped more frequently while swimming as compared to the socialized rats and control. Also rats in withdrawaled group had lower number of stops than control rats (Figure 7).

### Salt appetite test

During withdrawal period, NaCl (3%) consumption was higher in isolated group as compared to the socialized group. Also rats in control groups had lower consumption than withdrawaled rats (Figure 8).

### Neurogenesis

During withdrawal period, neurogenesis in dentate gyrus of hippocampus was significantly lower in isolated rats as compared to socialized rats. Also neurogenesis was higher in withdrawaled rats compared to control rats (Figures 9 and 10).

## Discussion

In the current study, we demonstrated that withdrawal from morphine is better tolerated in socialized group characterized by reduced adverse psychiatric co morbid symptoms that result in reduce morphine seeking and compulsive abusing (craving) behaviors. Better performance in Morris water maze task indicates that learning may be essential for better withdrawal. In addition, neurogenesis essentially promotes learning which in turn enables better tolerance during withdrawal period. Emotional reactivity is also required for better tolerance of withdrawal signs and may provide other benefits like improving neurogenesis. Overall the results in almost all control groups support the hypothesis that isolation and morphine potentiates their adverse effects, in the rest we just discuss about the experimental group. But in detail there are some points that should be addressed. We hypothesized that addiction in general has more adverse effect than isolation. But results showed that rats in withdrawal socialized group has better outcome than control group. This can be explain through this reason that after withdrawal there is an increase in neurogenesis and this reactive increase, also increase outcome result of behavioral experiments. This is in consistence with Nixon et al. study [23]. Also results showed that withdrawal isolated rats had worse outcome than isolation alone. This means that reactive neurogenesis in isolated group has not been useful.

In this study, we used Morris water maze to assess learning and memory (working memory and reference memory) [24]. In this study working memory both in spatial task acquisition and probe trial was impaired. Impaired working memory in withdrawal isolated group indicated that these animals were unable to temporary store and manages information required to carry out complex cognitive tasks such as learning. Therefore, impaired learning may result in relapse to drug use, as good memory is crucial for a successful withdrawal. In this study isolation had more effect on memory than morphine

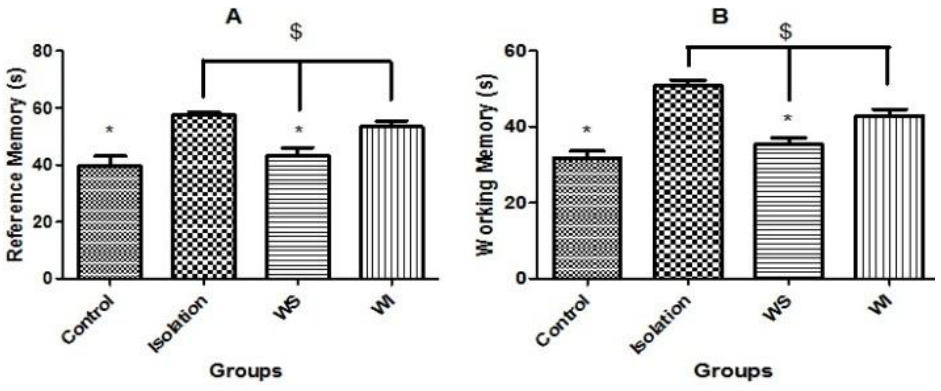


**Figure 3:** A. Reference memory performance at day 13 and 14 day of experiment in Morris water maze test. It was better in socialized groups, although withdrawal rats overall all had worse performance. Better memory is associated with reduce risk of relapse (n= 10) B. Working memory performance at day 13 and 14 day of experiment in Morris water maze. It was better in socialized group, although withdrawal rats overall all had worse performance. Better memory is associated with reduce risk of relapse (n= 10). Data are represented as Mean ± SEM. *means significant difference adjacent groups and $ between those apart from each other. WS: withdrawal Socialized and WI: withdrawal Isolated.

and withdrawal from it but overall isolation and morphine impaired working memory.

Further, our results indicate that reference memory was also impaired in withdrawal isolated animals and they were unable to retain past events (probe test). In this experiment as the figures shows withdrawled rats had better performance than isolation state. This along with best performance in control group indicates that reference memory had been affected equally from drugs and state of animals in isolation or socialization.

Socialized group showed a higher a speed of swimming during spatial acquisition task trials which indicates that these animals had greater emotional reactivity as in human is described by positive emotional symptoms and this needed for better withdrawal. It seems that emotional state of an animal modulates learning which is indicative of involvement of amygdale via sertonergic receptors in prefrontal cortex. In this experiment as the figures shows withdrawled rats had better performance than isolation state. This along with best performance in control group indicates that emotional state had been affected equally from drugs and state of animals in isolation or socialization.

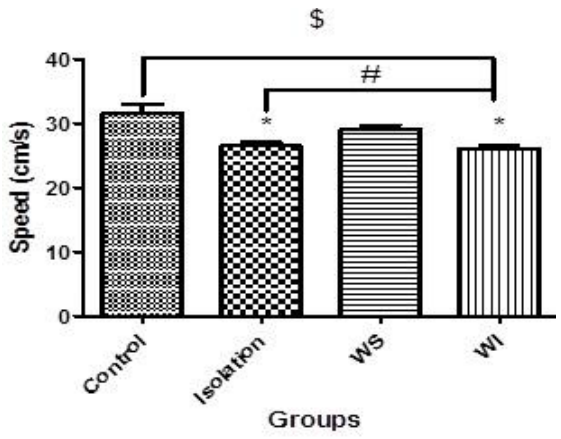


**Figure 4:** Speed of swimming of rats (n= 10). In this task socialized rats overall had better performance and withdrawal rats had the higher performance may be because of reactive neurogenesis. Speed in morris water maze is indicator of emotional reactivity. Higher emotional reactivity is associated with improvement of good habits that help successful withdrawal. Data are represented as Mean ± SEM. *means significant difference adjacent groups and $ and # between those apart from each other. WS: withdrawal Socialized and WI: withdrawal Isolated.

Furthermore, we performed sucrose preference test to assess behaviors that support drug- seeking and craving behaviors. It should be noted that sucrose preference test is not directly assess craving and in previous studies in has been used for anhedonia. In some studies it has been proposed that taste disturbances have accompanied with addiction and in some sucrose consumption has been increased in withdrawal period. So we can indirectly come to this conclusion that it can be an indicator of craving (drug seeking and compulsive abuse). Increased sucrose consumption in withdrawal isolated animals can be explained by increased sensitization that increase appetite responses for non-drug substance that is considered as natural reinforce. High sucrose consumption besides signifying morphine seeking and craving behaviors, may suggest presence of depression. Increase in depression can cause more isolation which in turn may result in addiction. Increased appetite for natural sweeteners augments dopamine release in hypothalamus and this may potentiate addictive's effects of morphine in nucleus accumbens. Addiction and compulsive drug abuse may be attributed to disturbances in taste sensation. Also, it has been documented that reward system does not necessarily rely on dopamine release, suggesting involvement of other brain circuits [25]. In this experiment as the figures shows withdrawal rats had better performance than isolation state. This along with best performance in control group indicates that sucrose consumption had been affected equally from drugs and state of animals in isolation or socialization.

In withdrawal isolated animals, impaired forced swim test indicates impaired emotional reactivity. Accordingly, isolation results in depression followed by drug replace [26]. Impaired emotional reactivity may also be a contributing factor for reduced neurogenesis. It can also potentiate development of devastating behaviors that can result in abusing drugs. In addition, it has been shown that neurogenesis is essential for appropriate functioning of amygdale and hypothalamus, thereby controlling behavior [27]. Depression can also change nutrition behaviors, and altered nutrition can affect neurogenesis and bodily defense mechanisms. Not only this, reduced neurogenesis decreases glutamate in hippocampus,

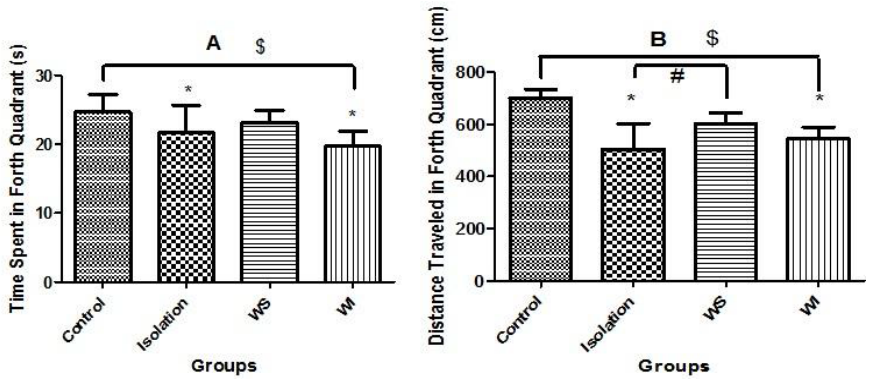


**Figure 5:** A. Time spent in fourth quadrant in probe trial of Morris water maze test (n= 10). B. Distance traveled in fourth quadrant in probe trial of Morris water maze test (n= 10). In these two tests withdrawal rats had worse performance than control rats. These parameters in Morris water maze were indicative of reference memory. Good memory is needed for successful withdrawal. This is unknown which type of memory is more important. Data are represented as Mean ± SEM. *means significant difference adjacent groups and $ and # between those apart from each other. WS: withdrawal Socialized and WI: withdrawal Isolated.

thereby affecting memory [28]. Also in Kirby et al study stimulation of amygdala enhances neurogenesis in hippocampus [29]. Thus, considering that learning impairments and drug seeking craving behaviors are significantly higher in isolated animals, it can be stated that neurogenesis is an essential element of successful withdrawal. In this experiment as the figures shows withdrawal rats had better performance than isolation state. This along with best performance in control group indicates that mood state had been affected equally from drugs and state of animals in isolation or socialization.

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. A growing number of studies suggest that the development of compulsive drug seeking and taking depends on dorsostriatal mechanisms.

Wang study suggests that the up regulation of NR2B-NMDAR activity within the DMS by alcohol contributes to the maladaptive synaptic changes that lead to excessive alcohol intake and relapse [30].

Different age shows difference in sensitization. Adolescent mice are less sensitive to ethanol sensitization, and this blunted behavioral response in adolescents might reflect differential ethanol-induced neurobehavioral adaptations [31,32]. The activation of accumbal $D_2$ receptors is essential for the expression of EtOH behavioral sensitization [33]. In mice, repeated ethanol administration may induce behavioral sensitization a process of progressive potentiation of its stimulant effects, associated with neuro adaptations in the brain reward system. More sensitization associated with more NMDA activation. Locomotor sensitization to cocaine is associated with increased FOS expression in the accumbens, but not in the caudate [34].

It has been hypothesized that sodium appetite sensitization is similar to drug sensitization. After repeated exposures to amphetamine or the state of sodium deficiency, changes in behavioral responses can last at least 4 months [35]. 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 [36]. In this experiment as mentioned salt consumption increased, and also sucrose increased. May be the above mechanisms are responsible for both of them. In this experiment as the figures shows withdrawal rats had better performance than isolation state. This along with best performance in control group indicates that salt consumption had been affected equally from drugs and state of animals in isolation or socialization.

Potential mechanism involving reduced neurogenesis remains unclear to date. However, in certain regions of brain it may be attributed to some hormonal and paracrine factors. Toner et al., has shown that prolactin can reduce neurogenesis under stressful conditions [37]. Corticosterone one of the putative factors has shown to markedly increase drug craving [38].

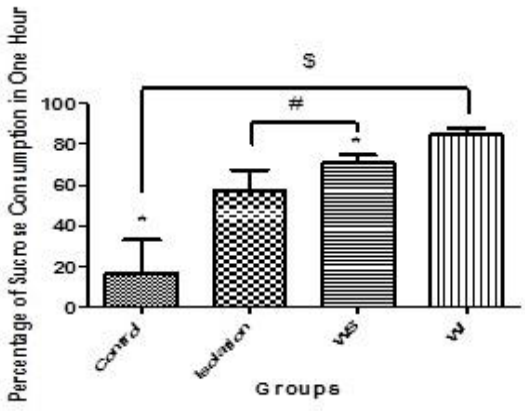


**Figure 6:** Percentage of sucrose consumption in sucrose preference test (n=10). In this test data were in consistence with hypothesis that isolation can precipitate adverse outcome of addiction and also addiction can augment adverse effects. Increased sucrose consumption is associated with increased drug-seeking and taking behaviors. It acts through epigenetic methylation. Sucrose consumption overall is more in withdrawal rats that is indicative of effect of drugs, and isolation had made it more. Data are represented as Mean ± SEM. *means significant difference adjacent groups and $ and # between those apart from each other. WS: withdrawal Socialized and WI: withdrawal Isolated.

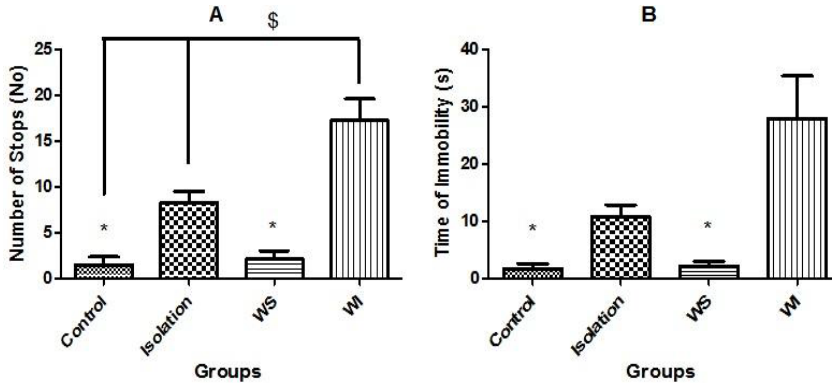


**Figure 7:** A. Number of stops in forced swim test. In this test in withdrawal rats had worse outcome. This means that addiction and isolation had synergistic effect in adverse outcome (n= 10). B. Immobility time in forced swim test. In these tests socialized rats had better outcome (n= 10). Low mood state is associated with more risk of relapse. Data are represented as Mean ± SEM. *means significant difference adjacent groups and $ between those apart from each other. WS: Withdrawaled Socialized and WI: Withdrawaled Isolated.

Addiction often involves some regulatory brain areas which may result in symptoms common to other conditions. One study documents similar pattern of learning impairments in obesity and addiction. This can suggest that controlling obesity may be beneficial in improving brain functions and tolerance of drug withdrawal.

Addiction often involves some regulatory brain areas which may result in symptoms common to other conditions. One study documents similar pattern of learning impairments in obesity and addiction. This finding suggests that controlling obesity may improve brain functions and tolerance for drug withdrawal [39]. In this experiment as the figures shows withdrawled rats had better performance than isolation state. This along with best performance in control group indicates that neurogenesis had been affected equally from drugs and state of animals in isolation or socialization.

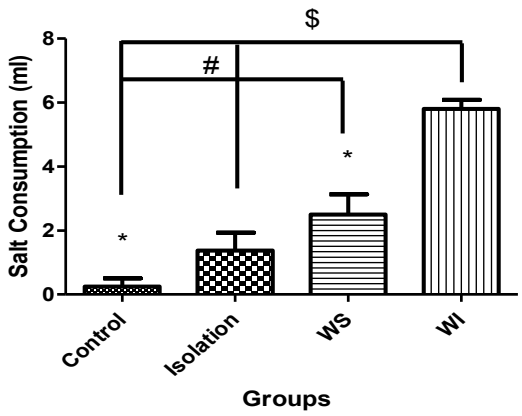


**Figure 8:** Rate of NaCl consumption in one hour after 24 hour of food deprivation (n= 10). This test was completely proved that isolation and addiction can augment adverse effect of each other. Increase in salt consumption is associated with sensitization and strengthening of behaviors that increase relapse to drugs. Salt consumption is more in withdrawal state that can best show it is the result of drugs and isolation. Data are represented as Mean ± SEM. *means significant difference adjacent groups and $ and # between those apart from each other. WS: withdrawal Socialized and WI: withdrawal Isolated.

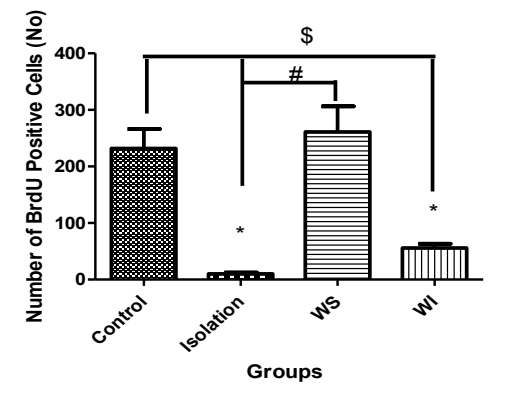


**Figure 9:** Number of Brdu positive labeled cells in dentate gyrus of hippocampus. Neurogenesis was reactively was higher in withdrawal socialized rats and in some performance test it was happened in accordance with higher rate of neurogenesis. Increase in neurogenesis can improve poor prognosis behaviors. As you can see higher neurogenesis in withdrawal rats shows reactive neurogenesis and more than control group (n= 6). Data are represented as Mean ± SEM. *means significant difference adjacent groups and $ and # between those apart from each other. WS: Withdrawal Socialized and WI: Withdrawal Isolated.

There is some evidence that application of naltrexone in withdrawal period decreases abstinence and also craving [40,41]. In this study naltrexone has been used for at least two reasons 1) for decreasing the effect of the last heavy dose (35 mg/ kg) shortly after withdrawal 2) ameliorating acute withdrawal signs 3) decreasing mortality of unknown reason. It should be noted that application of naltrexone in many studies in abstinence period is controversy, and in this study it was used for exerting its useful effect and that may be known and may be unknown. Just according to previous concepts we thought it is useful, although it may has harmful effects such as precipitating withdrawal signs that in low doses (3 mg/ kg) this effect has not been seen.

In an unofficial study that has not been published acute withdrawal sighs such as chattering, salivation, diarrhea, ptosis, irritating, writhing, dog shake, jump, tremor, vocalization, and body weight loss reduce in severity in social state. But this study was not designed in such a way that severity these symptoms assessed, because of threat of mortality of rats after injection of naloxane and disturbance of rats after these severe symptoms that can make interpretation of the results of this study difficult. This study has been designed in such a way that can predict relapse to drugs and predict severity of withdrawal period in long term. So improvement in neurogenesis can prevent relapse directly by improving behaviors or indirectly through socialization. These symptoms show more acute involvement but if poor prognosis last for longer periods is not well demonstrated by these symptoms. Thus for assessment and delineation of these differences in this experiment these behaviors were examined. Also it should be noted that other experiment for assessment of such behaviors can be used, and no obvious extinction is obvious for us to choose which of them.

Social interaction, such as adult-adult and adult-offspring interactions are an integral part of human society and affect psychological, physiological, and behavioral functions. Strong adult-adult and adult offspring interactions also play a protective role on the vulnerability to substance abuse [42]. Social connectedness, defined as internal sense of social belonging, reduces the likelihood of experiencing anxiety and is a protective factor against depression [43]. In contrast, negative social interactions, such and disruptions of social bonds, confrontation, and isolation, or neglect, can cause psychological stress, posing a risk to mental and physical health [43]. In addition, lack of social interaction leading to feeling of loneliness has been correlated with the experience of depression [43] further highlighting the importance of social interactions. Finally, the inability to form social bonds is often used to diagnose psychological disorders, including autism, social anxiety, and schizophrenia [43].

So, it seems that social interaction and its maintenance is important for physical and mental health. It is evident that oxytocin has prominent role in formation and maintenance of strong social bonds [44]. Oxytocin also needed for social motivation [45]. In a study, it has been revealed that oxytocin reverses amphetamine-induced deficits of social-bonding. Consequently, during substance abuse oxytocin should be secreted to combat the negative effects of isolation [47]. Social interaction seems to bear a rewarding nature. It means that it needs society's interaction. So its beneficial effects

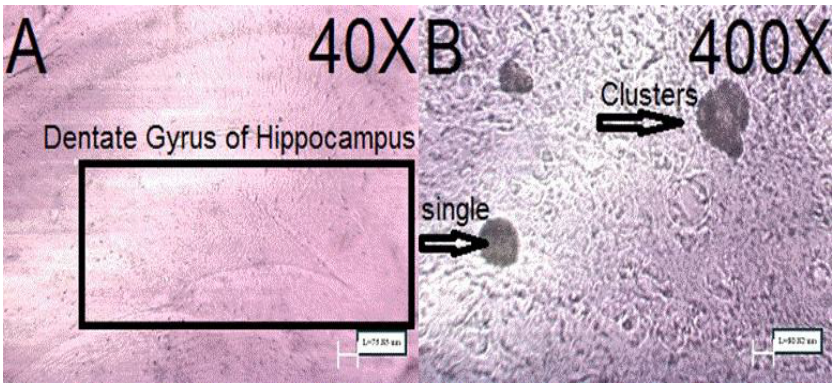


**Figure 10:** Cryosections stained for BrdU-positive neurons; labeling with anti-Brdu antibody for newly proliferated neurons in dentate gyrus of hippocampus A. Marked region shows dentate gyrus of hippocampus and BrdU- positive neurons have been counted in this region (40 X). B. BrdU-positive neurons have been colored brown and have been shown by arrows. They may be in clusters or single forms (400X).

gains in society and it imply that addicted person better be in contact with other persons. Reward learning is a behavior that we need for avoidance of anhedonia, CRF needs for it [48]. Thus hormonal factors favors better benefits of social interactions. In one study it has proved that decrease in stress is associated with decrease self-administration of cocaine [49]. Oxytocin produces different pro-social behaviors in both men and women. In men it improves the ability to identify competitive relationships whereas in women it facilitates to improve kinship. So because oxytocin acts different in men and women, socialization in men and women can act in different mechanisms [50].

Olfactory cues are important for social interaction [51]. On the other hand, olfactory functioning needs neurogenesis [52]. In our study, isolation of animals reduced neurogenesis which in turn may have affected olfaction and social interaction [53]. Hence, we can consider that social interaction increases neurogenesis and this can overcome the adverse effects of isolation. More importantly, olfactory stimulation may also help with isolation.

As state drug addiction has wide varieties of problems. This study is designed in such a way that basic mechanism of this problems defined and good treatment establishes for abstinence period. For example by promoting neurogenesis with antidepressant like fluoxetine these co morbid condition can be reduced especially in isolated addicted in withdrawal period [54]. Also by delineating symptoms like increase in salt intake, people in an increased risk of higher sides-effects can be identified and rigorous and purposeful treatment and rehabilitation can be employed. Also psycho logic treatment in withdrawal period for ameliorating these adverse effects can be helpful.

## Conclusion

As we saw neurogenesis in withdrawal period was higher in withdrawaled socialized rats. Also along with it co morbid psychiatric disorders increased in rats with lower neurogenesis. Also salt consumption as an indicator of low prognostic value for relapse was higher in withdrawal isolated rats. Thus socialization improves brain functions associated with better tolerance of withdrawal period from morphine.

### References

1. Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30: 215-238.
2. Schoenbaum G, Shaham Y (2008) The role of orbitofrontal cortex in drug addiction: a review of preclinical studies. Biol Psychiatry 63: 256-262.
3. Giorgi O, Piras G, Corda MG (2007) The psychogenetically selected Roman high- and low-avoidance rat lines: a model to study the individual vulnerability to drug addiction. Neurosci Biobehav Rev 31: 148-163.
4. Ramo DE, Prince MA, Roesch SC, Brown SA (2012) Variation in substance use relapse episodes among adolescents: a longitudinal investigation. J Subst Abuse Treat 43: 44-52.
5. Becker GL, Gerak LR, Li JX, Koek W, France CP (2010) Precipitated and conditioned withdrawal in morphine-treated rats. Psychopharmacology (Berl) 209: 85-94.
6. Westwood JA, Darcy PK, Kershaw MH (2013) Environmental enrichment does not impact on tumor growth in mice. F1000Res 2: 140.
7. Hall FS, Wilkinson LS, Humby T, Inglis W, Kendall DA, et al. (1998) Isolation rearing in rats: pre- and postsynaptic changes in striatal dopaminergic systems. Pharmacol Biochem Behav 59: 859-872.
8. Whitaker LR, Degoulet M, Morikawa H (2013) Social deprivation enhances VTA synaptic plasticity and drug-induced contextual learning. Neuron 77: 335-345.
9. Peitz GW, Strickland JC, Pitts EG, Foley M, Tonidandel S, et al. (2013) Peer influences on drug self-administration: an econometric analysis in socially housed rats. Behav Pharmacol 24: 114-123.
10. Ming GL, Song H (2005) Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28: 223-250.
11. Canales JJ (2007) Adult neurogenesis and the memories of drug addiction. Eur Arch Psychiatry Clin Neurosci 257: 261-270.
12. Sahay A, Hen R (2007) Adult hippocampal neurogenesis in depression. Nat Neurosci 10: 1110-1115.
13. Perry JL, Joseph JE, Jiang Y, Zimmerman RS, Kelly TH, et al. (2011) Prefrontal cortex and drug abuse vulnerability: translation to prevention and treatment interventions. Brain Res Rev 65: 124-149.
14. van der Staay FJ, Schuurman T, van Reenen CG, Korte SM (2009) Emotional reactivity and cognitive performance in aversively motivated tasks: a comparison between four rat strains. Behav Brain Funct 5: 50.
15. Pastor R, Kamens HM, McKinnon CS, Ford MM, Phillips TJ (2010) Repeated ethanol administration modifies the temporal structure of sucrose intake patterns in mice: effects associated with behavioral sensitization. Addict Biol, 15: 324-335.
16. Nesil T, Kanit L, Pogun S (2015) Bitter taste and nicotine preference: evidence for sex differences in rats. Am J Drug Alcohol Abuse 41: 57-67.
17. Sakai RR, Fine WB, Epstein AN, Frankmann SP (1987) Salt appetite is enhanced by one prior episode of sodium depletion in the rat. Behav Neurosci

101: 724-731.

18. Romieu P, Host L, Gobaille S, Sandner G, Aunis D, et al. (2008) Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. J Neurosci 28: 9342-9348.
19. Anisman H, McIntyre DC (2002) Conceptual, spatial, and cue learning in the Morris water maze in fast or slow kindling rats: attention deficit comorbidity. J Neurosci 22: 7809-7817.
20. Menezes HS, Bueno BB, Ciulla L, Schuh A, Luz Fde F, et al. (2008) Antidepressant behavioral effects of duloxetine and amitriptyline in the rat forced swimming test. Acta Cir Bras 23: 447-450.
21. Roitman MF, Na E, Anderson G, Jones TA, Bernstein IL (2002) Induction of a salt appetite alters dendritic morphology in nucleus accumbens and sensitizes rats to amphetamine. J Neurosci 22: RC225.
22. Spritzer MD, Ibler E, Inglis W, Curtis MG (2011) Testosterone and social isolation influence adult neurogenesis in the dentate gyrus of male rats. Neuroscience 195: 180-190.
23. Nixon K, Crews FT (2002) Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem 83: 1087-1093.
24. Snyder JS, Hong NS, McDonald RJ, Wojtowicz JM (2005) A role for adult neurogenesis in spatial long-term memory. Neuroscience 130: 843-852.
25. Cannon CM, Palmiter RD (2003) Reward without dopamine. J Neurosci 23: 10827-10831.
26. Salomé N, Taube M, Egecioglu E, Hansson C, Stenström B, et al. (2011) Gastrectomy alters emotional reactivity in rats: neurobiological mechanisms. Eur J Neurosci 33: 1685-1695.
27. Fowler CD, Liu Y, Wang Z (2008) Estrogen and adult neurogenesis in the amygdala and hypothalamus. Brain Res Rev 57: 342-351.
28. Hlinák Z, Gandalovicová D, Krejcí I (2005) Behavioral deficits in adult rats treated neonatally with glutamate. Neurotoxicol Teratol 27: 465-473.
29. Kirby ED, Friedman AR, Covarrubias D, Ying C, Sun WG, et al. (2012) Basolateral amygdala regulation of adult hippocampal neurogenesis and fear-related activation of newborn neurons. Mol Psychiatry 17: 527-536.
30. Wang J, Lanfranco MF, Gibb SL, Yowell QV, Carnicella S, et al. (2010) Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. J Neurosci 30: 10187-10198.
31. Stevenson RA, Besheer J, Hodge CW (2008) Comparison of ethanol locomotor sensitization in adolescent and adult DBA/2J mice. Psychopharmacology (Berl) 197: 361-370.
32. Abrahao KP, Souza-Formigoni ML (2012) Behavioral sensitization to ethanol results in cross-sensitization to MK-801 but not to NMDA administered intra-accumbens. Behav Brain Res 235: 218-224.
33. Abrahao KP, Quadros IM, Andrade AL, Souza-Formigoni ML (2012) Accumbal dopamine D2 receptor function is associated with individual variability in ethanol behavioral sensitization. Neuropharmacology 62: 882-889.
34. Crombag HS, Jedynak JP, Redmond K, Robinson TE, Hope BT (2002) Locomotor sensitization to cocaine is associated with increased Fos expression in the accumbens, but not in the caudate. Behav Brain Res 136: 455-462.
35. Sakai RR, Frankmann SP, Fine WB, Epstein AN (1989) Prior episodes of sodium depletion increase the need-free sodium intake of the rat. Behav Neurosci 103: 186-192.
36. Hall DA, Stanis JJ, Marquez AH, Gulley JM (2008) A comparison of amphetamine- and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. Psychopharmacology (Berl), 195: 469-478.
37. Torner L, Karg S, Blume A, Kandasamy M, Kuhn HG, et al. (2009) Prolactin prevents chronic stress-induced decrease of adult hippocampal neurogenesis and promotes neuronal fate. J Neurosci 29: 1826-1833.
38. Graf EN, Wheeler RA, Baker DA, Ebben AL, Hill JE, et al. (2013) Corticosterone acts in the nucleus accumbens to enhance dopamine signaling and potentiate reinstatement of cocaine seeking. J Neurosci 33: 11800-11810.
39. Martin-Fardon R, Boutrel B (2012) Orexin/hypocretin (Orx/Hcrt) transmission and drug-seeking behavior: is the paraventricular nucleus of the thalamus (PVT) part of the drug seeking circuitry? Front Behav Neurosci 6: 75.
40. Comer SD, Sullivan MA, Yu E, Rothenberg JL, Kleber HD, et al. (2006) Injectable, sustained-release naltrexone for the treatment of opioid dependence: a randomized, placebo-controlled trial. Arch Gen Psychiatry 63: 210-218.
41. Kaminski BJ, Duke AN, Weerts EM (2012) Effects of naltrexone on alcohol drinking patterns and extinction of alcohol seeking in baboons. Psychopharmacology (Berl) 223: 55-66.
42. Bell NJ, Forthun LF, Sun SW (2000) Attachment, adolescent competencies, and substance use: developmental considerations in the study of risk behaviors. Subst Use Misuse 35: 1177-1206.
43. Lieberwirth C, Wang Z (2012) The social environment and neurogenesis in the adult Mammalian brain. Front Hum Neurosci 6: 118.
44. Uvnas-Moberg K, Petersson M (2005) Oxytocin, a mediator of anti-stress, well-being, social interaction, growth and healing. Z Psychosom Med Psychother 51: 57-80.
45. Gordon I, Martin C, Feldman R, Leckman JF (2011) Oxytocin and social motivation. Dev Cogn Neurosci 1: 471-493.
46. Krach S, Paulus FM, Bodden M, Kircher T (2010) The rewarding nature of social interactions. Front Behav Neurosci 4: 22.
47. Young KA, Liu Y, Gobrogge KL, Wang H, Wang Z (2014) Oxytocin reverses amphetamine-induced deficits in social bonding: evidence for an interaction with nucleus accumbens dopamine. J Neurosci 34: 8499-8506.
48. Bogdan R, Santesso DL, Fagerness J, Perlis RH, Pizzagalli DA (2011) Corticotropin-releasing hormone receptor type 1 (CRHR1) genetic variation and stress interact to influence reward learning. J Neurosci 31: 13246-13254.
49. Goeders NE (2002) Stress and cocaine addiction. J Pharmacol Exp Ther 301: 785-789.
50. Fischer-Shofty M, Levkovitz Y, Shamay-Tsoory SG (2013) Oxytocin facilitates accurate perception of competition in men and kinship in women. Soc Cogn Affect Neurosci 8: 313-317.
51. Ryan BC, Young NB, Moy SS, Crawley JN (2008) Olfactory cues are sufficient to elicit social approach behaviors but not social transmission of food preference in C57BL/6J mice. Behav Brain Res 193: 235-242.
52. Heron PM, Stromberg AJ, Breheny P, McClintock TS (2013) Molecular events in the cell types of the olfactory epithelium during adult neurogenesis. Mol Brain 6: 49.
53. Feierstein CE, Lazarini F, Wagner S, Gabellec MM, de Chaumont F, et al. (2010) Disruption of Adult Neurogenesis in the Olfactory Bulb Affects Social Interaction but not Maternal Behavior. Front Behav Neurosci 4: 176.
54. García-Cáceres C, Tschöp MH (2014) The emerging neurobiology of calorie addiction. Elife 3: e01928.

## Author Affiliations

Top

*[1]Department of Physiology, International campus, Tehran University of Medical Sciences, Tehran, Iran*
*[2]Professor of Physiology, Tehran University of Medical Sciences, Tehran, Iran*
*[3]Anatomy Department, Tehran University of Medical Sciences, Tehran, Iran*