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Original Article

Assessment of increase in citrate and KCl consumption and probable underlying mechanisms in morphine-dependent socially isolated rats

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Abstract

Introduction: Abusing drugs such as morphine continues to be a serious medical and social problem. Defining a habit that increases the devastating effect such as compulsive use and craving is necessary.

Methods: This experiment was designed in four groups 1) group-housed (GH) 2) isolation 3) group-housed morphine-treated (GHMT) (4) isolated-housed morphine-treated (IHMT). Rats were received morphine (0.75 mg/rat/day) for three weeks for inducing morphine dependence. BrdU (50 mg/kg/day) injection begins from the first day of the experiment and lasted for 21 days for assessing neurogenesis. At the end of experiment sensitization with open field, copper in serum with an atomic spectrophotometer, taste disturbance for bitter (potassium chloride, KCl) and sour (citrate), mood disturbance with tail suspension test, brain-derived neurotrophic factor (BDNF) in CSF with Elisa, malondialdehyde (MDA) in serum with thiobarbituric acid (TBA) and neurogenesis with BrdU staining were assessed.

Results: Copper was higher in GHMT rats. Sensitization was higher in IHMT rats. Citrate and KCl consumption were higher in IHMT rats. Time of immobility was higher in IHMT rats. BrdU-positive cells and BDNF were lower in IHMT rats. MDA increased in IHMT rats.

Conclusion: Avoiding isolation in a period of morphine injection decreases behaviors that favor morphine abuse. Also avoiding isolation increases neurogenesis that has a positive effect on rewarding center.

Keywords:
Morphine;
Socially isolation;
Sensitization;
Copper;
Open field;
Citrate and KCl

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Introduction

Drug addiction continues to be a serious medical and social problem. Vulnerability to develop an addiction to drugs is dependent on genetic, environmental, social and biological factors. Among these establishing a habit that improves brain functions necessary for better tolerance and reduces relapse to drug abuse is helpful.

Neurogenesis is a phenomenon that occurs in some brain regions such as hippocampus and sub ventricular zone (Ming and Song, 2005). Neurogenesis in some studies considered as neuroplasticity and this view give it a functional role for regulating brain circuits. In this study, we

hypothesized that neurogenesis can modulate brain receptor expression and in this way, it can exert its neuroplasticity effect.

Mood regulation is a mandatory part of life. Without it, depression and anxiety develop and this may cause more drug seeking behavior. Revest et al., divided hippocampus into two regions: ventral for mood and rostral for memory (2009). The higher rate of neurogenesis probably is associated with more balance of mood state and co-morbid conditions. So, with promoting the health of hippocampus good behavior maintains.

In previous experiments increase appetite for salt along with locomotor activity and rearing in open field have been used for assessing poor prognosis and relapse to drug abuse (Clark and Bernstein, 2004; Valjent et al., 2010). However, in this study, two novel tastes including bitter and sour as tested by potassium chloride (KCl) and citrate respectively were assessed. There is a question about the nature of sensitization as it is a primary taste disturbance or it is something else. Some experts believe that it is due to taste disturbances as in one study genes for this hypothesis have been identified (Nesil et al., 2015). In this experiment, KCl and citrate were used for assessing the nature of sensitization as it is taste disturbance or not.

Copper is an essential element that is necessary for differentiation of new neurons during embryonic development (Watanabe and Tezuka, 2006). One of the organs in the brain that needs copper is hippocampus that regulates cognitive behaviors through NMDA receptors (Schlief et al., 2005). The copper increase in the sub ventricular zone and choroid plexus during elderly is responsible for the decline in cognitive abilities (Fu et al., 2015a). Copper plays an important role in Alzheimer disease along with deterioration of oxidative –stress status (Dong et al., 2008). Also, neurogenesis can be affected by alternations of the copper level and bioavailability (Fu et al., 2015b).

Brain-derived neurotrophic factor (BDNF) is one of the major neurotrophic factors that primarily support the growth and survival of cholinergic, dopaminergic and motor neurons. BDNF is synthesized by sensory neurons and glia and may have both autocrine and paracrine functions in mediating activity-dependent plasticity. It is highly expressed in brain areas that are known to regulate cognitive and emotional behaviors such as the hippocampus and amygdala (Ichisaka et al., 2003).

Stress-oxidative (SO) status is one of the main determinants of body health. Malondialdehyde (MDA) production rate is one of the predictors of SO status. MDA overproduction causes lipid peroxidation of membrane lipids. We know that many enzymes and cellular components including signaling molecules are made of lipids (Ayala et al., 2014). In this way, disturbance of MDA production and degradation cause destructive effects including disruption of neurogenesis. In this experiment, MDA level was assessed for estimating its role for reduction of neurogenesis besides possible reduction of BDNF.

Isolation is a condition with adverse effects to the brain. In several studies, social isolation has impaired brain function. On the other hand, socialization has been associated with better brain functions. Socialization can be modeled by two paradigms: two animals in one cage or three animals in one cage. In this study, two rats were put in one cage (Peitz et al., 2013). Also for isolation, one rat was put in one small cage (Yorgason et al., 2013).

This study designed for the purpose of defining behaviors that employ and strengthening such good behaviors and weakens behaviors associated with poor prognosis and discovering some new behaviors. The aim of this study was to prove that brain functions that are necessary for better tolerance and reduce the risk of relapsing work well in the socialized state. So this study designed in isolation and social state to prove the above hypothesis.

Materials and methods

Animals and experimental procedure

The experimental protocols followed in this study were conformed to the guidelines for the care and use of laboratory animals published by the national institution of health (NIH publication No. 85-23, revised 1996) and was further approved by the institutional ethical committee at Tehran University of Medical Science (Tehran, Iran).

In this study male Sprague-Dawley rats weighting 200 to 250 grams were used. In each group, 9 rats were used. In socialized groups, one rat was used for modeling socialization. For social isolation, animals were isolated in cages covered with black plastic for 14 days plus one week for adaptation to the

environment (Ibi et al., 2008). Morphine-treated animals in the group-housed groups were housed with healthy rats.

For induction of morphine addiction and dependence, based on previous experiments, we used a daily injection of morphine (0.75 mg/day/ip for three weeks). However because assessing addiction needs experiment, and this has proved to us by previous protocols, we did not assess withdrawal signs for assessing addiction (Di Chiara et al., 2004). For assessing neurogenesis, BrdU (50 mg/kg/day) was injected from the first day of the experiment and lasted for 21 days.

In the end of the experiments, rats were anesthetized and then sacrificed. Before sacrificing serum was obtained for assessing copper, MDA and CSF were collected for BDNF assessment, and then the brain removed from the skull for sectioning and labeling.

Open field test

The experiment was performed in open field arena with a diameter of 96×96×25. 25 squares of 15.5 cm are inside of the arena. Rats were placed individually in the center of the arena. The total duration of the test was 5 minutes. Video-tap was taken to score locomotors frequency (i.e. number of lines crossed with all four paws), rearing frequency (i.e. number of times rat stood erect on its hind legs with its forelegs in the air) and measure duration of immobility (i.e. total time in the second with spontaneous movement) (Reis Ede et al., 2014).

Citrate preference test

In this experiment citrate (Sigma-Aldrich) at a concentration of 50 mM was prepared. The two-bottle test was performed for 48 hours. 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 citrate preference test. At the end of the experiment, the preference for the citrate was determined as the percentage of citrate solution ingested relative to the total intake (Tordoff and Bachmanov, 2002).

Twenty-four hour two-bottle test for KCl

We measured overnight KCl intake to gain insight if rats show an appetite for KCl in long-term. In this experiment, rats were given a 24-h two-bottle choice test with dH2O and 200 mM KCl (Merck). The relative positions of the two bottles were counterbalanced across rats. Relative intake of potassium bottle to total intake was calculated (Guenthner et al., 2008).

Tail suspension test

This test is applied for assessing mood state. In this test, rats are suspended by their tail with tester's hand and time of immobility is considered as an index of low mood state or anhedonia (Gu et al., 2014).

Copper assessment

For obtaining serum, after thoracotomy before paraformaldehyde perfusion, five-milliliter blood was taken from left heart. After coagulation and centrifugation serum was collected in microtubes and stored at -70 °C. For preparing serum for analysis of copper level first, they were incubated with 65% citric acid for 2 hours. Then for one hour they were incubated with 65% perchloric acid. The final solution was examined with atomic spectroscopy (Varian-220-FS-aa). After obtaining absorbed wavelength it was adjusted with calibration curve and expressed as p.p.m (Gümüş et al., 2011).

BDNF measurement in CSF

For obtaining CSF, an apparatus that is made up of 1 ml syringe, polyethylene 50 and disposable needle of syringe 5 was used. They were inserted to each other sequentially for making the CSF apparatus collector. Briefly, syringe 5 needle was inserted to cisterna magna, and with syringe, CSF was pulled gently over 3 to 8 minute to the syringe. After collection of CSF (0.4- 1 µl/rat), BDNF level was assessed by ELISA kit (Promega, USA), according to the manufacturer's instructions.

MDA measurement in serum

Serum sample (300 µl) was mixed with 30% trichloroacetic acid and 5N HCl followed by the addition of 2% thiobarbituric acid in 0.5 N NaOH. The mixture was heated at 90˚C for 15 min and centrifuged at 12,000 g for 10 minutes. The final product was measured at 532 nm using a UV-visible spectrophotometer. It was assessed by applying standard curve and expressed as µmol/L.

Immunohistochemistry

At the end of 14th day, animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg).

The brains were perfused with 100 ml normal saline and then, fixed with 100 ml paraformaldehyde (PFA) 4% via intra-cardiac infusion. After fixation, the brains were removed from the skull. For the first 2 days, the brains were kept in PBS + PFA 4% and then at day 3, in sucrose 10% + PFA 4% + PBS. Throughout day 4, the brains were kept in sucrose 20% + PFA 4% + PBS and for the rest of the days, they were kept in sucrose 30% + PFA 4% + PBS. The cryosections (30 µm) were prepared from the hippocampal region and five sections per animal were selected and stained for BrdU-positive neurons using a commercially available anti-BrdU antibody kit (5-Bromo-2-dU Labeling and Detection Kit ll; Roche). BrdU-positive cells in the dentate gyrus (Fig. 8) were counted directly under a light microscope (Zeiss Co.) at 400X magnification. BrdU-positive neurons appeared colored brown were observed as single cells or in clusters (Spritzer et al., 2011).

Quantification of BrdU positive cells

Every fifth section throughout the hippocampus (total 10 sections for each rat) was processed for BrdU immunohistochemistry. All BrdU-positive cells in the sub granular zone (SGZ), hilus and granular cell layer (GCL) (Zeiss, Germany) were counted in a blinded manner bilaterally by applying a light microscope. It should be noted that counting of the BrdU-positive cell was done directly under a light microscope without using software. BrdU-positive cells were counted in dentate gyrus rostrocaudal fashion. As shown in Fig. 9 regions that were counted in the hippocampus were whole dentate gyrus. BrdU-positive neurons appeared much bigger than usual and appeared as singles or cluster cells. Averages were determined for every five sections in this study and neurons were not multiplied by each section count (Ibi et al., 2008).

Statistical analysis

Data were analyzed using SPSS version 22 and Graph pad prism. Univariate ANOVA with two factors (morphine treatment × group-housing) 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. Data were represented as mean ± SEM and $P<0.05$ considered significant. Symbols (*, # and $) represent significance difference of mean data among different

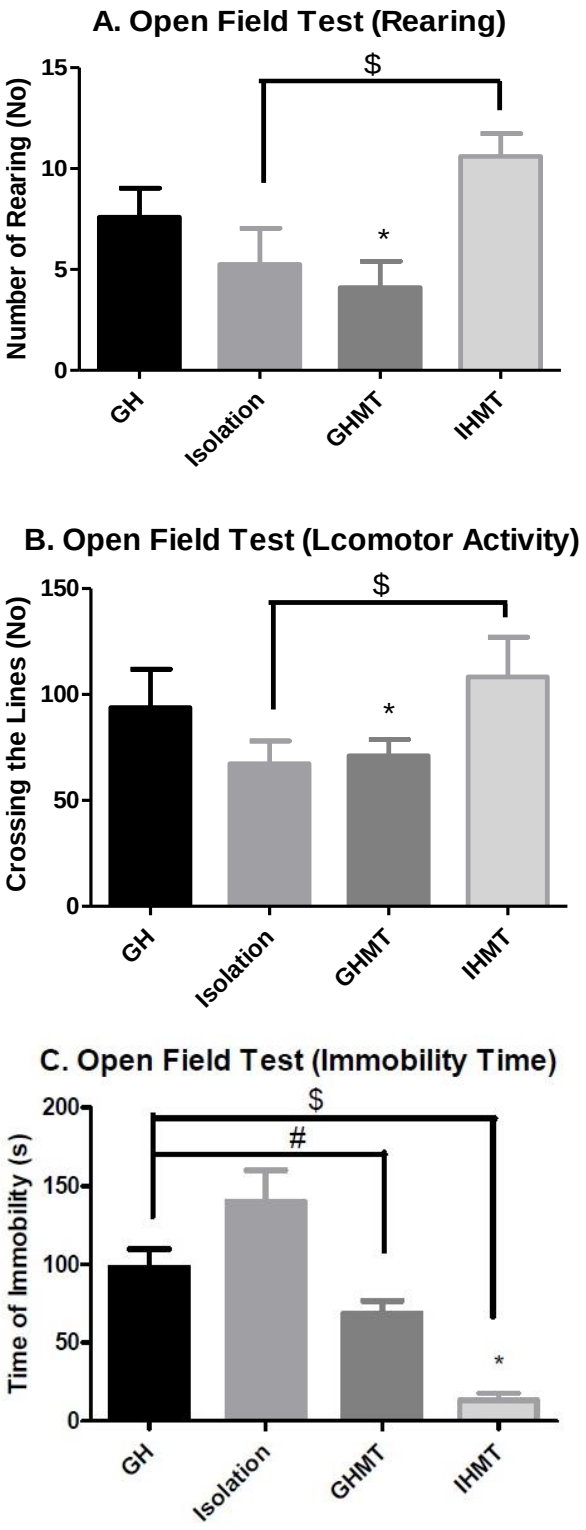

Fig.1. A: number of rearing in open field test (n=9). B: scores of locomotor activity in open field test (n=9). C: immobility time in open field test (n=9). Data are represented as Mean ± SEM. * was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols ($ and #) were used.

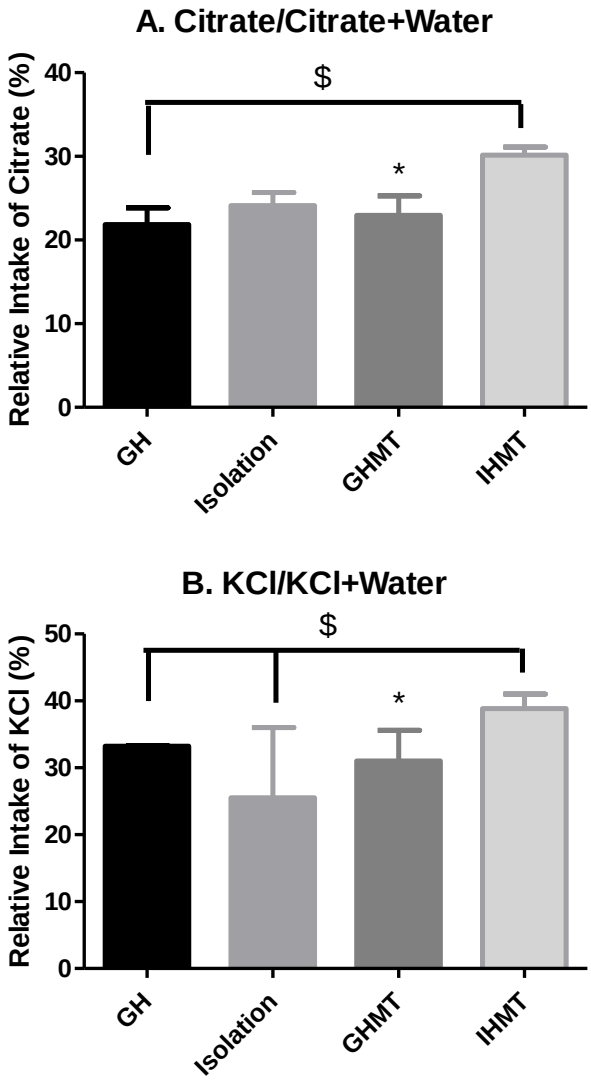

Fig.2. A: percentage of citrate consumption to total fluid intake (n=9). B: percentage of KCl consumption to total fluid intake (n=9). Data are represented as Mean ± SEM. * was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols ($) were used.

groups for the level of statistical significance of $P<0.05$. The star symbol (*) was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols (# and $) were used.

Results

Open field test

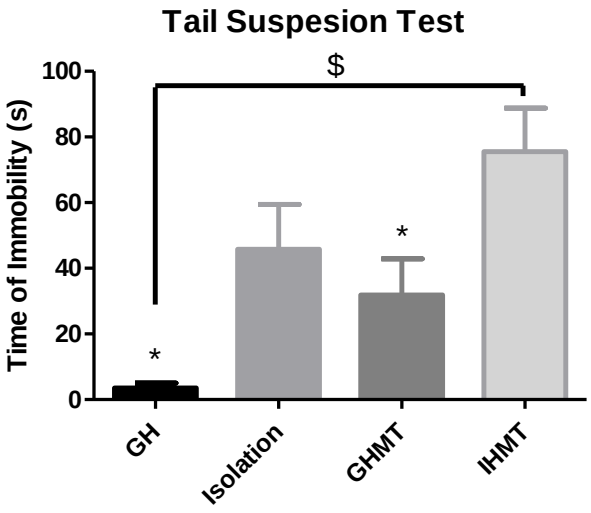

Fig.3. Time of immobility in tail suspension test (n=9). Data are represented as Mean ± SEM. * was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols ($) were used.

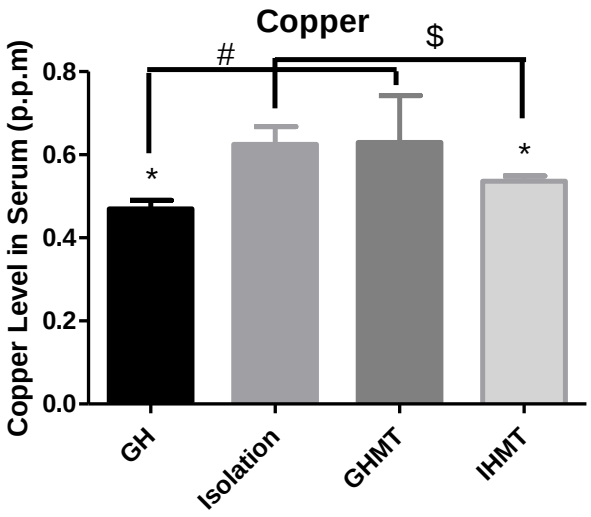

Fig.4. Level copper in serum assessed by atomic spectrophotometer (n=6). Data is represented as mean ± SEM. * was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols ($ and #) were used.

Rearing and locomotor activity: number of rearing was higher in IHMT rats as compared to GHMT rats; also locomotor activity had higher scores in IHMT rats as compared to GHMT rats. Immobility time was lower in IHMT rats as compared to GHMT rats (Fig. 1).

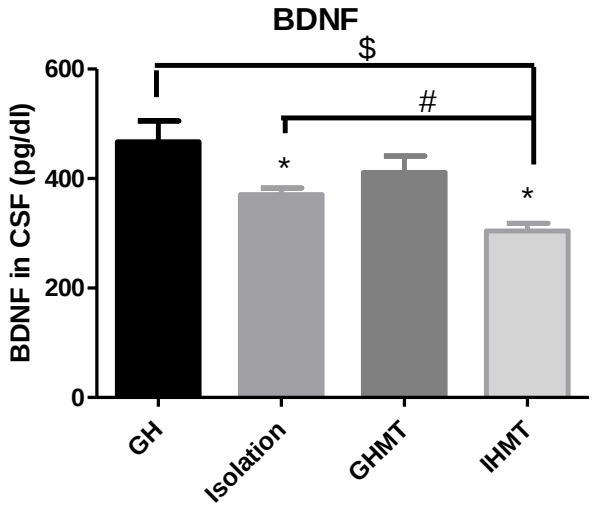

Fig.5. CSF level of BDNF (pg/ml) (n=6). Data are represented as mean ± SEM. * was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols ($ and #) were used.

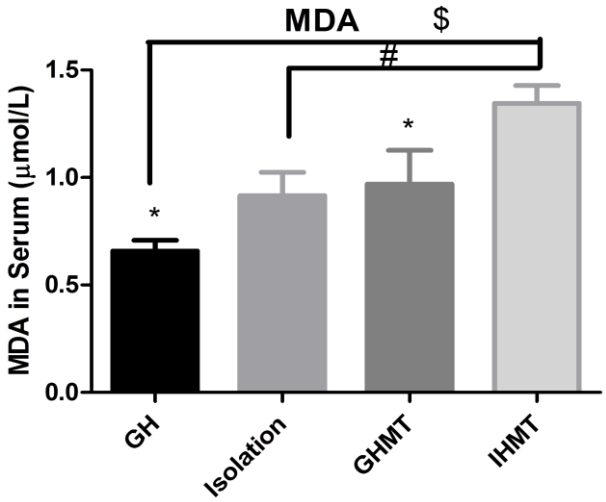

Fig.6. MDA level of serum (µmol/L) (n=6). Data are represented as mean ± SEM. * was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols ($ and #) were used.

Taste

Sour and bitter taste: citrate consumption was higher in IHMT rats as compared to GHMT rats, also it was higher in IHMT rats as compared to GH rats; KCl consumption was higher in IHMT rats as compared to GHMT rats and isolated rats. Also, it was higher in IHMT rats as compared to GH rats (Fig. 2).

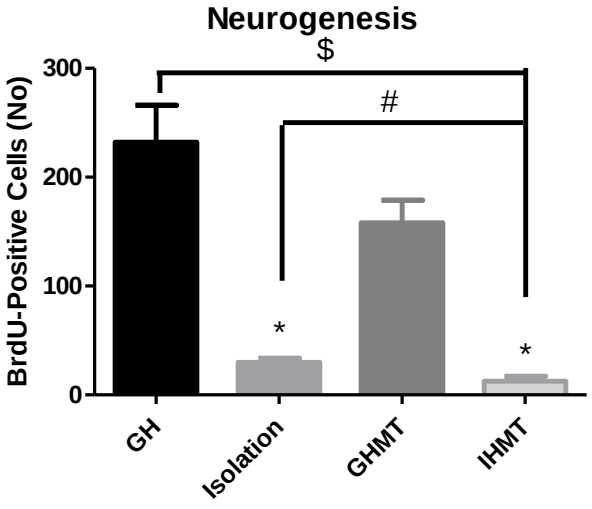

Fig.7. Number of BrdU-positive cells in dentate gyrus of the hippocampus (n=6). Data are represented as mean ± SEM. * was used for groups that are besides together (group-housing (GH) × isolation and group-housed morphine-treated (GHMT) × isolated-housed morphine-treated (IHMT)) and for those apart from each other, other symbols ($ and #) were used.

Tail suspension

Time of immobility in tail suspension test was higher in IHMT rats as compared to GHMT rats; also it was higher in IHMT rats as compared to GH rats (Fig. 3).

Copper level

In isolated rats, copper increased as compared to GH rats. Also in GHMT rats, copper increased as compared to IHMT rats. In group-housed morphine-treated rats, copper increased significantly compared to GH rats. Also in isolation period, copper increased as compared to IHMT rats (Fig. 4).

BDNF level

BDNF was higher in GH rats compared to isolation rats. Also, it was higher in GHMT rats as compared to IHMT rats. GH and isolated rats had higher BDNF level than IHMT rats (Fig. 5).

MDA level

MDA was lower in GH rats compared to isolated rats. Also, it was lower in GHMT rats compared to IHMT rats. GH and isolated rats had lower MDA level than IHMT rats (Fig. 6).

Neurogenesis

The number of BrdU-positive cells was lower in IHMT rats compared to GHMT rats. Also, rats in isolation

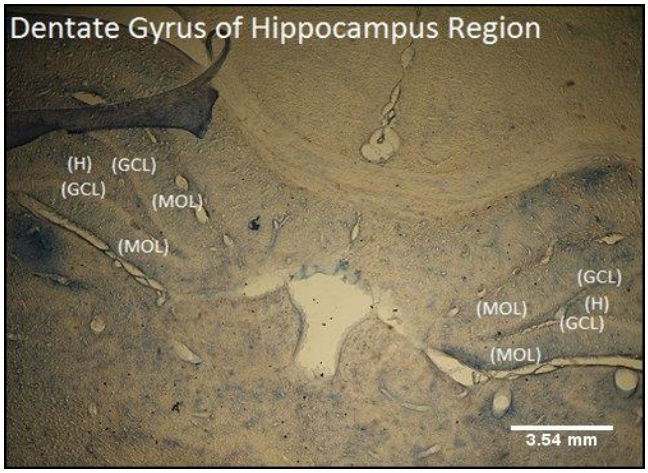

Fig.8. Different parts of the dentate gyrus of hippocampus have been marked in the picture. Counting of the BrdU-positive cell has been done in these areas. It has three parts: molecular layer (MOL) (outer [OML], middle [MML] and inner [IML]), granular cell layer (GCL) and hilus (sub granular zone [SGZ] and deep hilus).

had more cells than IHMT rats. Rats in the GH group had more positive cells than IHMT group (Fig. 7, 8 and 9).

Discussion

In the present study it is assigned that 1) group-housing improved sensitization in open field test 2) reduced the risk of craving for KCl and citrate 3) reduced depression-like behavior and also 4) improved neurogenesis. Hippocampal neurogenesis might be the main cause of change in adjacent areas such as nucleus accumbens, amygdala and parts of the limbic system.

The neurons that mediate rewarding properties of drug addiction are mainly located in the ventral tegmental area that affects dopaminergic neurons in the nucleus accumbens. So drugs that cause addiction primarily affect neurons in nucleus accumbens (Pierce and Kumaresan, 2006). Sensitization is a process that occurs after repeated administration of drugs and manifest itself through an increase in locomotor activity and other behaviors such as sniffing. Brain regions that responsible for initiation and maintenance of sensitization are a dopaminergic system in mesolimbic areas, medial prefrontal cortex, hippocampus, ventral tegmental area and nucleus accumbens (Kalivas et al., 2005). Sensitization manifests itself as drug craving,

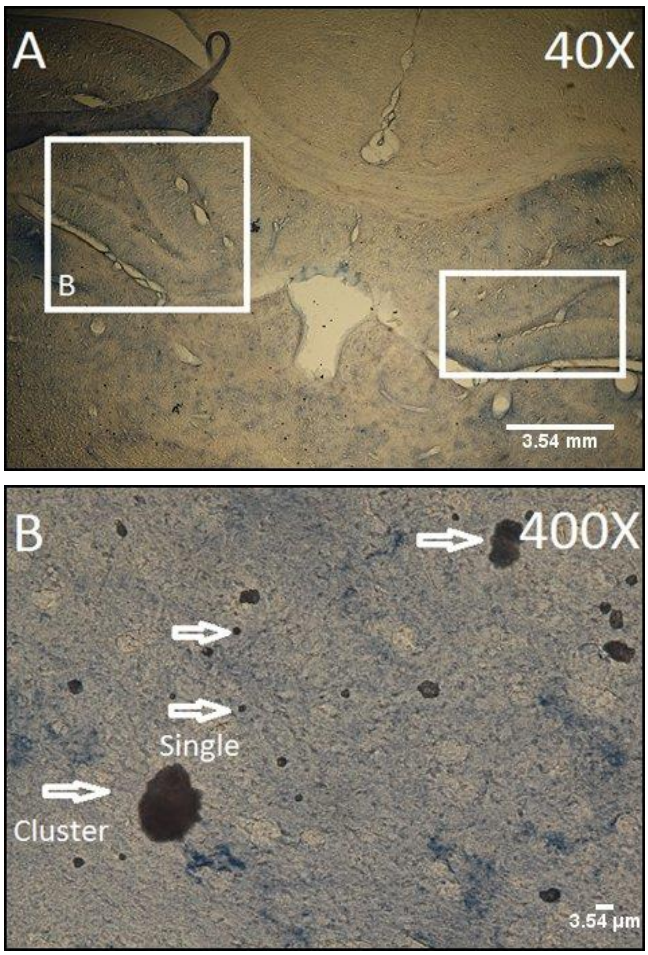

Fig.9. A: dentate gyrus of hippocampus (shown bilaterally) B: arrows shows BrdU-positive cells. It should be noted that all BrdU-positive cells have not been showed by arrows. They may occur as single or cluster.

compulsive drug seeking behavior and propensity to relapse (Everitt and Wolf, 2002).

Sensitization manifests itself as an increase in locomotor activity and increase in consumption of drugs after repeated administration (Morgan et al., 2006; Lodge and Grace, 2008). For assessing sensitization in open field test usually locomotor activity and rearing is used. Other variables such as immobilization, time in center and stool weight are assessed for assessing anxiety. In this study, locomotor activity and rearing were increased in isolated and isolated-housed morphine-treated rats. It means sensitization increased. Also variables those used for assessment of anxiety showed a reduction of anxiety in isolation and isolated-housed morphine-treated rats. Another behavior that uses for assessment of sensitization is salt appetite. Previous studies had evaluated salt appetite. For the first time in this study citrate and KCl appetites were assessed.

In this study citrate and KCl consumption increased in isolated-housed morphine-treated rats. Previous studies support this hypothesis that salt sensitization is similar to drug sensitization. In one study both repeated amphetamine abuse and the state of sodium deficiency, has been associated with changes in behavioral responses for at least 4 months (Sakai et al., 1989). Repeated administration of drugs needed for development of sensitization and in the open field test observed increased locomotion but if the same mechanisms cause salt sensitization has not been studied. There is a question how the group-housed condition has decreased appetite for salt, citrate and KCl.

For the probable role of adult neurogenesis both in an increase in the open field test and salt-like consumption there are not many studies, however in this study higher rate of consumption was associated with low rate of neurogenesis. For relating neurogenesis to these phenomena we can say that addiction in some studies has been proposed as a disease of neuroplasticity (Abrahao and Souza-Formigoni, 2012). If neurogenesis considered as a type of neuroplasticity that may regulate rewarding center and peri-hippocampal area, we can say that neurogenesis directly affects and regulates behaviors that regulate drug-abuse behaviors and also rewarding centers that affect taste sensation and sense of joy of life. But if these behaviors are related to reward center and new neurons alters receptor expression in these areas, this study support this hypothesis. On the other hand, if the increase in citrate and KCl consumption is related to the function of rewarding center or other different mechanisms, there is not clear evidence. According to one study hippocampus is necessary for taste learning without a study of neurogenesis (Chinnakkaruppan et al., 2014).

For assessing mood involvement along with other impairments, we performed tail suspension test and immobility time in the open field test. There is accumulating evidence that the nucleus accumbens plays an important role in the pathophysiology of depression. Given that clinical depression is marked by anhedonia (diminished interest or pleasure), dysfunction of the brain reward pathways have been suggested as contributing to the pathophysiology of depression (Jiang et al., 2013). Since the nucleus accumbens is the center of reward and learning, it can be hypothesized that anhedonia might be produced by changing the function of the nucleus accumbens. Indeed, it has been reported that stress, drug exposure and drug recovery, all of which produce depressive-phenotype, alter various functions within the nucleus accumbens andleading to inhibited dopaminergic activity in the nucleus accumbens (Giorgi et al., 2007). Again here we think that neurogenesis helps proper function of the rewarding center by regulating these dopaminergic neurons in the adjacent area, especially limbic system. Also, hippocampus neurogenesis regulated by proper function of rewarding center in a positive manner (Kirby et al., 2011). Decreased anxiety also predisposes rats to uncontrolled behavior such as drug-taking as assessed by the open field test.

In this study depression behavior was increased in an isolated group. It can happen through above mentioned mechanisms or something different. Depression behaviors can augment the adverse effects of drug abuse may be with more isolation from the society. We think neurogenesis in hippocampus directly affects rewarding center. Some studies support this hypothesis. Ideas that support the role of the hippocampus for drug addiction stem from studies that support changes in addictive behaviors along with alternation of hippocampal neurogenesis and related structures such as subiculum. Since it is important for learning and contextual conditioning we can come to this idea that it can affect craving to drugs by deregulating affections associated with memories. As in one study relapse to drug abuse associated with cue-induce relapse has been associated with an increase in hippocampal blood flow (Volkow et al., 2004). Disturbances of taste and mood in one study have had the same mechanism (Heath et al., 2006). In this study, we also proposed the same mechanism that is a reduction of neurogenesis.

Neurogenesis in the hippocampus can regulate mood independently of other areas such as nucleus accumbens. However, whether if neurogenesis can affect brain regions involve in mood regulation is not clear. But in this study and other studies, it seems that it can affect mood regulations both in the hippocampus and maybe other areas. Also in this study increased in neurogenesis positively affected mood.

To date, there is no study for proving the role of

hippocampal neurogenesis in regulating rewarding center. However in this study hippocampal neurogenesis indirectly affect reward center by positively regulating mood and reducing negative symptoms. We really do not know if the rewarding center is necessary for the maintenance of mood balance. NMDA receptors are necessary for withdrawal period. It seems neurogenesis positively regulates these receptors in different parts of the brain and BDNF helps this function (Pandey et al., 2006). In one study neurogenesis has been linked to serotonin receptors, since neurogenesis in adult hippocampus regulates stress in anterior and ventral part and antidepressant actions in this area and serotonin is regulated by stress, so adult hippocampal neurogenesis involves in stress regulation (Mahar et al., 2014). Reduction in neurogenesis may occur through epigenetic changes or it may occur through an increase in microRNAs (Hollander et al., 2010). MiRNAs are small RNA molecules that trans-regulated gene expression, degrading target mRNAs and in some cases, repressing translation. MiR-212 was reported to be up-regulated in response to cocaine in the striatum of rats, leading to an amplification of the stimulatory effects of cocaine on cAMP response element-binding protein signaling.

Socialization is a process that can have six forms 1) primary socialization 2) secondary socialization 3) reverse socialization 4) developmental socialization 5) anticipatory and 6) resocialization. In this study developmental and secondary socialization (group-housed) were used. It means learning habits for developing one's skills in a group at a present time and developing primary skills for adult life. It should be pointed out that this action is involuntary and the rats getting these benefits unconsciously.

In this study, it was seen that in group-housed rats, better recovery occurs. Some hormonal factors such as oxytocin have been implicated to assert social interaction benefits (Uvnas-Moberg and Petersson, 2005). Also in one study amphetamine, adverse effects have been reversed by oxytocin treatment (Young et al., 2014). Prolactin is another hormone that is important for beneficial effects of social interaction (Torner et al., 2009). Further studies for assessment of these hormones are recommended.

In this study, copper increased in isolated rats, also group-housed morphine-treated rats had a higher level of copper compared to isolated and control rats. This emphasizes on the role of pair state (socialization) on the balance of copper level. Copper is a necessary element may be for enough level of neurogenesis (Suh et al., 2009). Also, neurogenesis in the hippocampus may directly or indirectly through rewarding center regulates addictive behaviors. Changes in neurogenesis can be resulted in some ways by an altered level of copper such as lower level of enzyme activity (Desai and Kaler, 2008).

In previous studies reduction of BDNF has been associated with a reduction of brain functions and decline in cognitive functions (Laske et al., 2011). In this study in consistence with previous studies, isolation and morphine reduce BDNF level and along with it cognitive functions and adaptive behavior decreased (Koo et al., 2015). BDNF in this study seems to exert its effects through reduction of neurogenesis.

In this study MDA, production increased in isolated animals. This was consistent with other studies. Also in isolated-housed morphine-treated rats it reduced. We know that neurogenesis is a phenomenon that develops in several stages. We mean that neurons have different compositions in these stages. We cannot exactly estimate which types of neurons destroys more but we can say younger neurons destroys more because of loss of enough maturation. The increase in MDA production impairs signaling for both maturation and proliferation of young neurons for enough neurogenesis. In one study MDA and BDNF have been proposed for predicting outcome in withdrawal period from crack (Sordi et al., 2014).

Overall drug addiction is a disease that when initiated some factors favors it to develop it to compulsive abuse. This may be in frequency or amount of usage. Some studies propose some diagrams and hypothesis that support our study that increase in co-morbid conditions such as increase arousal, tension, anxiety and depression predisposes individuals to more abuse. The hypothesis states that increase in a co-morbid condition such as increase arousal causes impulse control disorder and this can lead to a tense desire for immediate gratification or compulsive disorder that anxiety and stress predispose it. Our study supports this hypothesis that decreases in these states by socialization and social interaction is associated with good prognosis. We can say that with on occasion abuse isolation can favor its progression

to more devastating states such as more abuse. The mechanism is changes of neuroplasticity. In this study, if neurogenesis is considered as a form of neuroplasticity, these mechanisms support previous studies (Koob and Volkow, 2010). It acts through its action on amygdala as negative reinforcement. As in one study support that neurogenesis affects amygdala function (Kirby et al., 2011).

Conclusion

Isolation can adversely affect drug abuse prognosis by at least two ways: 1) decreasing neurogenesis and the resultant effect on nucleus accumbens and VTA 2) affects behaviors that increase drug abuse sides-effects such as memory and mood disturbance. Also citrate and KCl intake reduce in socialized state. So with promoting neurogenesis with socialization or at least be together and avoiding isolation addiction can be tolerated better.

Acknowledgments

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Conflict of interest

Conflict of interest statement: None to declare

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