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Susceptibility of Drug-seeking and Taking Behaviors Increases through Dysregulation of Copper and Zinc and Impaired Prefrontal Function in Addiction Period in Male Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author HF designed the study, wrote the protocol, performed experiments and performed statistical analysis with the assistant from author MK. Author MA mainly contributed in this study in assessing of oxidative-stress status. Author HF managed the literature searches, wrote the first and final draft of the manuscript and edited the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Drug addiction is a condition that in some occasions occurs with relapsing episodes. Reducing these relapsing episodes slows progression of recreational abuse with least adverse effects to everyday abuse with the most debilitating adverse effects.
Materials and Methods: In this study 16 male Sprague-Dawley rats weighing 200 to 250 gram were divided into 2 groups: Control and morphine-received. At the end of experiment sucrose consumption, salt appetite, novelty-seeking behavior, zinc in serum, copper in serum, glutathione in serum and prefrontal cortex stress-oxidative status for MDA (Malondialdehyde) were assessed.
Results: Sucrose and salt consumption were increased in morphine-receiving rats compared to control rats. Also, Novelty-seeking behavior was increased in morphine-receiving rats compared to

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control rats. Copper in serum was increased in morphine-receiving rats compared to control rats. Zinc in serum was increased in morphine-receiving rats compared to control rats. Oxidative-stress status as assessed for MDA in the prefrontal cortex was increased in morphine-receiving rats compared to control rats. Glutathione in serum was increased in morphine-received rats compared to control rats.

Conclusion: Involvement of prefrontal cortex in morphine-receiving period can be responsible for the occurrence of drug-seeking and taking behavior. In this sense, copper, zinc and antioxidant defense can play a pivotal role.

Keywords: Morphine; sucrose; salt; MDA; prefrontal; glutathione; copper and zinc.

1. INTRODUCTION

There is a wide range of variability for the outcome of addiction in different people. The adverse effects vary from mild cognitive and emotional disturbances to compulsive drug seeking and taking behavior [1]. One of the main problems with these adverse effects is that addicted person seeks for more abusing despite its adverse effects [2]. Applying effective treatments for avoiding entrance of addicted person to compulsive drug taking during abusing drugs such as morphine can be an effective strategy.

Addiction is a multistage process that finally develops to compulsive-drug taking behavior in a proportion of abusers [3]. The main problem is why some abusers develop such devastating behavior. In this study for answering this question, rats received morphine every day for inducing addiction with a normal dose of morphine. According to previous studies for inducing addiction constant dose can induce addiction and high dosage application makes no difference [4]. This protocol induces every day abuse [4].

The prefrontal cortex is the brain region responsible for personality and performing planable executive function [5,6]. Also, memory function is dependent on the proper function of the prefrontal cortex [7]. In recent studies, prefrontal cortex dysfunction has been associated with relapse and compulsive drug-seeking behavior [8]. However, the role of oxidative-stress in this regard has not been studied.

In animal studies, the experiments that are applied for assessing for relapse and drug seeking and taking behavior is increasing in salt appetite [9], sucrose preference test [10] and novelty seeking behavior [11].

Therefore this study was designed for investing the devastating effects of morphine on normal Prefrontal function, and assessing the resultant increase in more abuse with the above behavioral test. Also, the importance of copper and zinc were investigated in this regard.

2. MATERIALS AND METHODS

2.1 Animal Care

The experimental protocols and performing of experiments in this study were conducted in accordance with the guidelines for the care and uses of laboratory animals published by national institution of health (NIH Publication No. 85-23, revised 1996) and were further approved by the institutional ethical committee at Tehran University of medical science (Reference code: 91-01-159-18022, Tehran, Iran). The minimum number of animals required to obtain consistent data was used.

2.2 Animals

In this study male Sprague-Dawley rats weighing about 200-250 grams were used. In each group, 8 rats were used. Samples sizes were calculated based on pilot studies (for zinc and copper), other experiments (for behavioral experiments and oxidative –stress markers) and statistical formula for sample size estimation. It should be noted that for copper and zinc assessments, there is little studies. However, first, 4 rats were examined and then 4 rats were added to get the desired results.

2.3 Addiction

For induction of addiction rats were received 0.75 mg/rat/intraperitoneally/day morphine [4]. Based on previous studies there was no difference between applying a high dose of morphine and medium dose of morphine for addiction induction [4]. During behavioral experiments, morphine injection was done as described.

2.4 Sucrose Preference Test

The rats were submitted to a 48 h period drinking both sucrose and water for adaptation to sucrose solution (2%) taste. For 3 days water was the only available fluid. In the day of experiment rats were placed individually in the test cage, first with no access to fluid for 3 h, and then they had access to the two bottles, one containing tap water and the other a 1% sucrose solution. Fluid intake was measured by percentage sucrose consumption to total fluid intake [10].

2.5 Evaluation of NaCl Appetite

In this experiment, the food was removed for 24h. After that NaCl, 3% was offered for one hour. Total consumption was calculated [9].

2.6 Social Interaction Test

The social interaction test was first introduced by File and Hyde (1978). This test involves placing a pair of animals in an arena and measuring the amount of time engaged in such behaviors such behavior as grooming, sniffing, and boxing. Social interaction has been validated repeatedly as an index of anxiety related disorder [12]. A modification of the standard social interaction test was used to reduce the number of animals needed for the experiment. According to file (1993), the most sensitive procedure is to match up pairs of rats that have the same treatment on the basis of their body weights and then treat the number of interactions by pairs as the unit of measure. During the 5 minute session time spent in social interaction (grooming, sniffing, following) were scored individually for each rat [13].

2.7 Copper and Zinc Assessment

For obtaining plasma, after thoracotomy before paraformaldehyde perfusion, five-milliliter blood was taken from left heart. After processing 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 [14].

2.8 MDA (Malondialdehyde) Assessment

Total MDA was measured according to thiobarbituric acid (TBA) reaction. Simply for performing this analysis TBA 1% in HCl 5N and trichloroacetic acid (TCA) 20% in NaOH 1N were mixed together. Then 100 microliter of homogenized sample was added to the above mixture. After 90 minutes of boiling, the final solution developed brown color, and maximum absorbance was measured at 532 nm with the spectrometer [15].

2.9 Glutathione Assessment

DTNB (5, 5'-Dithio-Bis (2-Nitrobenzoic Acid)) was used as the reaction substrate for estimating the amount of reduced glutathione. For performing this experiment Tris buffer, DTNB and methanol were used. 100 micro liter of serum was added to the above mixture. The final solution developed yellow color, and maximum absorbance was measured at 412 nm with the spectrometer [16].

2.10 Statistic

Data were analyzed using SPSS version 22 and Graph pad Prism 5. An independent samples two-tailed t-test was performed for all experiments. Data are as represented as mean ± SEM and $P<0.05$ considered significant.

3. RESULTS

3.1 Sucrose Consumption

Sucrose consumption was increased in morphine-receiving rats compared to control rats (Fig. 1).

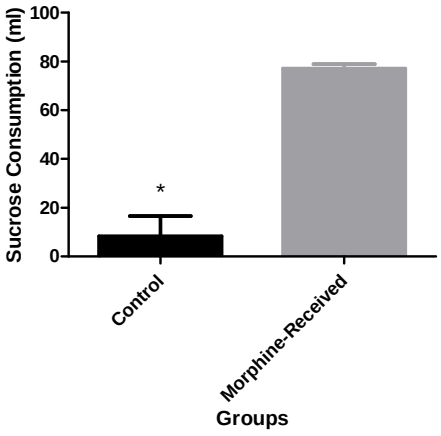

Fig. 1. Sucrose consumption was assessed in sucrose preference test

Data is represented as mean ± SEM (n=8)

3.2 Salt Consumption

Salt consumption was increased in morphine-receiving rats compared to control rats (Fig. 2).

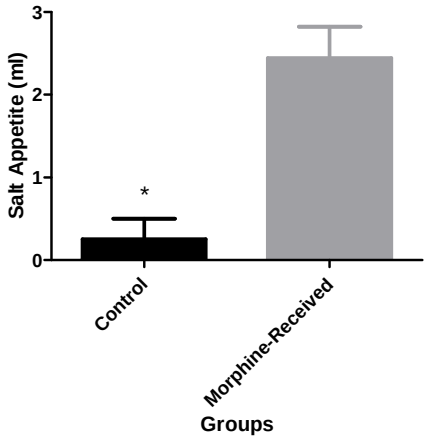

Fig. 2. Salt consumption was assessed in salt appetite test
Data is represented as mean ± SEM (n=8)

3.3 Social Interaction Test

Novelty seeking behavior was increased in morphine-receiving rats compared to control rats. Also this experiment can be used as an indicator of anxiety (Fig. 3).

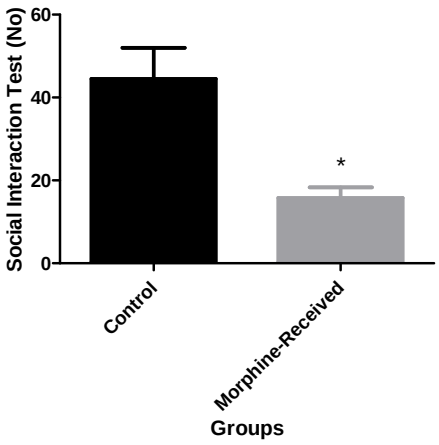

Fig. 3. Novelty seeking behavior was assessed in social interaction test
Data is represented as mean ± SEM (n=8)

3.4 MDA in Prefrontal Cortex

MDA was increased in prefrontal cortex of rats exposed to morphine compared to rats that have not received morphine (control) (Fig. 4).

3.5 Glutathione in Serum

Glutathione was increased in serum of rats exposed to morphine compared to rats that have not received morphine (control) (Fig. 5).

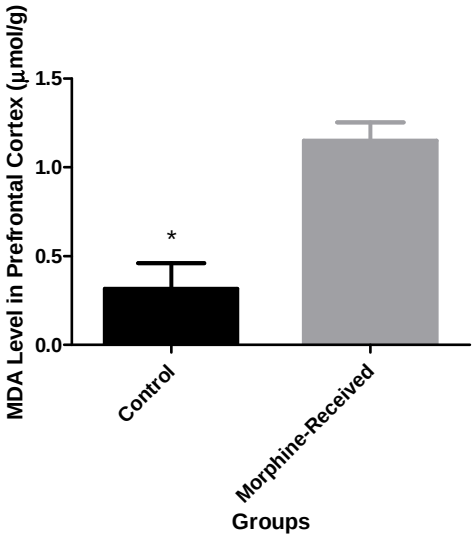

Fig. 4. MDA was assessed in prefrontal cortex
Data is represented as mean ± SEM (n=8)

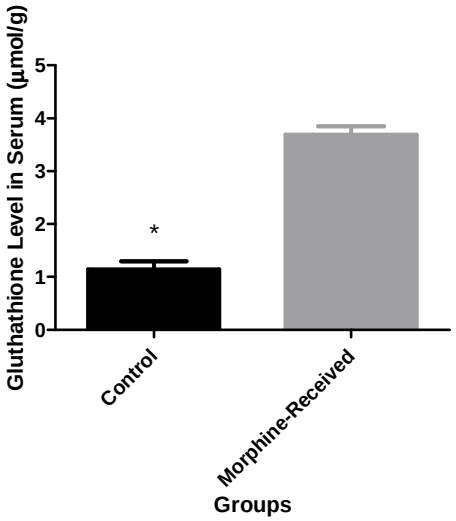

Fig. 5. Glutathione was assessed in serum
Data is represented as mean ± SEM (n=8)

3.6 Zinc in Serum

Zinc in serum was increased in the serum of rats exposed to morphine compared to rats that have not received morphine (control) (Fig. 6).

3.7 Copper in Serum

Copper in serum was increased in serum of rats exposed to morphine compared to rats that have not received morphine (control) (Fig. 7).

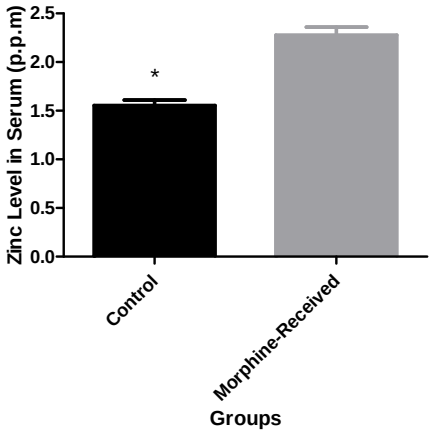

Fig. 6. Zinc was assessed in serum
Data is represented as mean ± SEM (n=8)

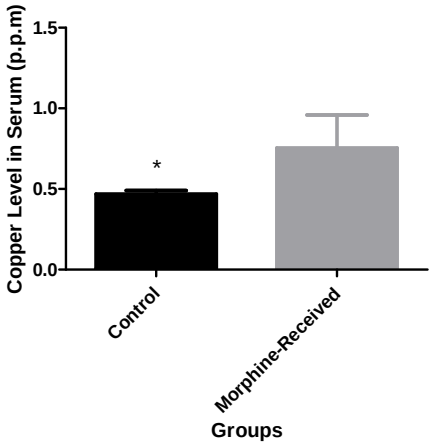

Fig. 7. Copper was assessed in serum
Data is represented as mean ± SEM (n=8)

4. DISCUSSION

The conventional research on relapse to drugs and development of drug-seeking behavior in abstinence period has focused on the dopaminergic system. The new approach to such behaviors in recent research has been focused on prefrontal cortex.

The underlying mechanism and behaviors that dysfunction of prefrontal cortex causes relapse in recent studies say some brain dysfunctions is necessary. Brain capabilities necessary for stopping relapse are self-control, proper emotional regulation, motivation, interoception, cognitive flexibility, learning and memory and salient attributions.

In this study, some novel behaviors investigated to find out a new mechanism for prefrontal cortex for regulation of addictive behaviors. During abstinence period three maladaptive behaviors develop that causes relapse: Excessive salient to drugs and drug-related cues, decreased sensitivity to non-drug reinforces and decreased the ability to inhibit maladaptive behaviors.

Sucrose preference test in previous studies has been used for two reasons. First for assessing craving for drugs and the second for assessing behaviors that are respond to non-drugs reinforces. Prefrontal cortex regulation of these behaviors has been established in previous studies, but the underlying mechanism has not been fully understood.

Salt appetite in previous studies has been used for assessing the probability of relapse in abstinence period. However, in this study, this experiment was used also for assessing if addiction itself causes a relapse or something unrelated to direct effects of abuse drugs to brain causes such behaviors.

Novelty seeking behavior in this experiment has been assessed with social interaction test. This test also can be used for assessing anxiety and mood balance that is important as co-morbid psychiatric disorder important for abstinence period for controlling relapse to drug abuse.

Prefrontal cortex involves in learning and memory [17], categorization [18], inhibitory control [19] and cognitive flexibility [20]. There should be many interconnections to other brain areas for accomplishing these tasks. Also, prefrontal cortex receives inputs from other parts of the brain that are part of the rewarding center including cingulate, prelimbic and infralimbic cortices [5]. In previous studies, it was well demonstrated that prefrontal cortex gives afferents to the hippocampus. Hippocampus is of known areas that regulate addictive behaviors. For investigating other mechanisms that prefrontal cortex may regulate addictive behavior, recent studies suggest it can regulate addictive behavior directly [8]. In this sense learning ability deficiency causes relapse to drug abuse. Also, prefrontal area modulates VTA, one of the main areas that its disturbance causes relapse to drug abuse [21]. Of known behaviors that cause relapse to drug abuse is impulse control that as said prefrontal actually play as an inhibitor to impulsive behaviors [19]. The most region of prefrontal cortex responsible for drug-seeking behavior is prelimbic prefrontal cortex area [22].

Co morbid psychiatric disorders such as depression and anxiety cause relapse to drug abuse with some known and unknown mechanisms [23]. In this study in social interaction test rats in the morphine-received group showed dysregulations in mood balance and this can cause an increase in drug-taking and seeking behavior.

Oxidative stress (OS) disturbance has been known in previous studies to cause mood dysregulations. In this study, OS occurred in the prefrontal cortex. In previous studies, it well approved that OS disturbance can disturb neuronal function through different mechanisms [24-26]. The exact neuron type that is responsible for the development of such maladaptive behaviors is not well demonstrated. In one study pyramidal neurons has been shown to exert most effects [27]. These neuronal damages have been well proved the occurrence of addictive behaviors through different mechanisms. However in this study novel mechanisms with different behaviors were experimented.

OS also acts through different neurotransmitter. In one study GABA and in another GLU neurotransmitters has been implicated [28,29]. Hormones in this regard in some studies have been proved to have positive effects. Melatonin and estrogen have been proved to enhance brain cognitive abilities through improvement of inflammation and acetyl cholinesterase activity [30,31]. Improper prostaglandin production also is a factor that works in concert to MDA elevation [32]. Disturbances of immune system function through improper cytokines production such as TNF-α and IL-10 is another contributing factor that may cause elevation of MDA [33]. Adverse effects of psychoactive drugs such as haloperidol also act through MDA dysregulation [34].

MDA elevation in previous studies has been associated with many disturbances. In brain research MDA elevation and glutathione reduction have been associated with Alzheimer and some psychiatric diseases such as depression and autism [35,36]. In this study glutathione elevation in serum may be is a response to distorted OS in the prefrontal cortex and the resultant development of addictive behaviors.

Metals are necessary for many brain functions. For reasons that are not completely understood brain, neurons need metals to sustain enough spontaneous activity. There are two types of metals necessary for brain function 1) alkali and alkaline for proper signaling 2) transition metals for tolerating OS injury. In one study copper has been shown to be essential for normal spontaneous activity [37].

Copper is essential element necessary for many cellular functions. In brain, copper is necessary for the function of a verity of enzymes. Copper is of known elements that are known to works in parallel with OS status for maintaining neuronal proper function [38].

Zinc is an essential part of many different proteins involve in different cellular function. Zinc also is necessary for the function of zinc-finger proteins that is located in the genome. Zinc-containing neurons are glutamatergic neurons. In these neurons, zinc is responsible for storage, release, re-uptake and modulation of glutamate receptors. The most prominent parts of the brain that contain zinc are cortex and amygdala [39].

5. CONCLUSION

Copper and zinc dysregulation in addiction period increases drug-seeking and drug-taking behaviors through impairment of oxidative-stress status in the prefrontal cortex. In this study, these behaviors were experimented through sucrose preference test, salt appetite, and social interaction test. The mechanisms that these behaviors support increase the risk of relapse are reward center inadequacy, increasing sensitization and novelty seeking behavior. Thus regulation of copper and zinc and antioxidant treatment can reduce drug-seeking and take behavior.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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