

## Effect of Memory Attenuation on Creating Morphine Dependency in Male Mature Mice

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Article information	Abstract
<p>Article history: Received: 6 Feb 2012 Accepted: 8 June 2012 Available online: 10 Apr 2013 ZJRMS 2013; 15(10): 49-53</p> <p>Keywords: Memory Morphine dependency Scopolamine Mice</p> <p>*Corresponding author at: Department of Biology, Science and Research Branch, Islamic Azad University, Fars, Iran. E-mail: ebrahim.hosseini@yahoo.com</p>	<p><b>Background:</b> Morphine and other addicting drugs induce an uncontrolled desire in man to consume the drugs overtly and stimulate the brain compensative systems such that the neuron sensitivity produced is not desensitized for some time after the consumption and detoxification of the drug. Therefore, the aim of this study is to examine the effect of memory attenuation in creating morphine dependency.</p> <p><b>Materials and Methods:</b> In this study, 60 male mature mice (85 days old) with a weight of 30-35 grams were enrolled as the experimental and the control groups. The experimental groups included 3 subgroups treated with morphine, scopolamine or morphine+scopolamine, respectively. Morphine was used for dependency and scopolamine for memory attenuation. Conditioned place preference (CCP) method was used to estimate dependency.</p> <p><b>Results:</b> Results showed no meaningful difference between the control and the witness groups and between the control and scopolamine groups in preferring a special location to receive the drug. However, there was a meaningful difference (<math>p&lt;0.05</math>) between the control and the morphine groups in preferring a location to receive morphine, and a meaningful attenuation was observed in the scopolamine+morphine group in preferring the location for receiving the drug compared with the group receiving morphine alone.</p> <p><b>Conclusion:</b> The results show that through memory attenuation, scopolamine decreases morphine dependent CPP. Binding of scopolamine to muscarinic receptors and blocking them affects the opioid receptors which together with reduced nitric oxide synthesis and decreased intracellular calcium, reduces the morphine-induced CPP.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

### Introduction

Activity of brain cholinergic systems plays a major role in memory and learning process [1, 2]. Scopolamine is considered as an antagonist of muscarinic receptors with a strong tendency toward  $M_1$  receptors, which is used for the memory-related experiences [3, 4]. Antagonists of muscarinic receptors, such as scopolamine, reduce cognition and learning, especially functional and declarative memories, visual acuity (VA) and psychomotor speed considerably [4-6]. The major side effects of this drug are related to its anticholinergic effects on parasympathetic post-synaptic receptors, some of them are xerostomia, heartthrob, blurred vision, and speech disorders [7]. By impairing the activity of the central nervous system (CNS), morphine reduces ability to respond to stimuli and it is effective in changing temperament and reducing anxiety. There are plenty of abuses related to heroin and it imposes its effects through connecting to opioid receptors [8].

Morphine is considered as a strong stimulatory source, which creates an uncontrollable desire in an individual to consume the drug [9]. Morphine over-stimulates brain reward systems, including mesolimbic dopaminergic system and in fact, drug ecstatic may be created due to the severe activity of this section of CNS [10]. By repeating drug consumption, brain's pleasure systems become ever

resistant against stimulation to find their natural balance; to reach pleasure, there would be no way out but the increase the dose of drug [11]. In case the drug consumption stops, brain pleasure systems that have become resistant needs redoubled desire for continuing consumption [12]. Reward mesolimbic dopamine system seems to deal with reward enjoyment quality, which is less than its stimulation qualities. Excessive activity of the system in an addict makes him/her have a great desire to consume the drug [13].

Nucleous accumbens (NAC), which is considered as a part of brain reward system, plays an important role in reward, competition, pleasure and the effects of drugs and placebos [14]. NAC and Ventral Tegmental Area (VTA) are of the initial areas that are affected by narcotic drugs, including morphine. Through dopamine neural modulation in processing reinforcement signals, the drug continuous dopamine activity in NAC by mediating neuronal activity in VTA [15].

Conditioning is a kind of associative learning, which is seen in two completely distinguished forms -classic and operant- and they are of the simplest forms of learnings [16]. Conditioned place performance (CPP), which shows conditioned place preferred process, is considered as the most famous experimental method to achieve rewarding

effects of different drugs [17]. Therefore, as explained above, the present research aims to study the effect of memory attenuation on creating dependency to morphine through CPP method in male mature mice.

## Materials and Methods

This is an experimental study carried out in 2011 in collaboration with Biology Department of Kazeroon Islamic Azad University and Shiraz University of Medical Sciences on 60 mature male mice of Wistar race having approximate weights of 30-35 grams aged 85 days. They were divided into 12-member groups, including control or without control and sham groups that only received distilled water as hypodermic injection. Three experimental groups were controlled by morphine, scopolamine, morphine and scopolamine as hypodermic injection. The protocol of this research was performed according to the international laws to protect lab animals and it was approved by the ethics committee of the university. The drugs used in the present research included morphine and scopolamine which were prepared by Iran Daru Company.

In addition, a box in 15×30×15 cm was used for conducting CPP experiment. The box was divided into two equal parts and there was also a middle part, which played the role of a corridor. In the box, there was a section with white walls and floors and another section with black walls and floors made of Plexiglas. There are three steps to carry out CPP experiment, which included pre-conditioning, conditioning and post-conditioning. In pre-conditioning stage, the mice were put inside the box one by one for one whole day and kept there for 10 minutes without any injection. They could have access to both parts of the box without any limitation. The presence duration of each mouse in each part was recorded. Normally, the mice did not show any preference. Statistically speaking, 50% were present in one section and 50% were present in the other. If a mouse preferred one section of the box more than 90%, it would be excluded. At the beginning of the experiment, each mouse was selected randomly in either white or black section of the box. Their presences were recorded so that the mice are put in the same section on the experiment day.

During the conditioning step, which lasted for 8 days, animals of the control group did not receive any drugs and those in sham group were received 1 cc distilled water as hypodermic injection. Ten mg/kg morphine, which is the best dose for creating psychological dependency through the CPP method, was injected as hypodermic injection to the group, which was received morphine. Immediately, the animals were randomly placed on the white or black sides of the box for 30 minutes. In this condition, the doors of either side of the box are closed and the animals have no access to the other sections. Such procedure was repeated for 8 days. Care was also taken to put the animals necessarily in the same color until the final day of experiment to avoid conditioning.

The group that received scopolamine were injected hypodermically a drug with the optimum dose of 2 mg/kg. After 15 minutes, which is the time required for maximum effectiveness of the drug, the animal was transferred to the section paired with the drug and kept there for 30 minutes. In the group that received scopolamine+morphine, first, the animals of the group were injected scopolamine with 2 mg/kg dose hypodermically. After 15 minutes, the animals were injected morphine with 10 mg/kg dosage hypodermically. Then the animals were immediately transferred to the section paired with the drug to stay there for 30 minutes. This group was compared with the group received morphine and control group. In all the groups, the time the section paired with the medicine for each animal was recorded using a chronometer to measure the tendency of the animal to the same section on the experiment day.

In the post-conditioning step, which included the fourth and the ninth day, each animal was put in the section where it was placed on the pre-conditioning day. The animal was allowed to access all the sections of the box the same as the post-conditioning day for ten minutes. The time the animal stayed in each section was recorded by a chronometer to specify whether injection of the drugs effective in memory had any effect on the dependency of the animal to morphine, with respect to the training of the animal. Finally, the data obtained through the ANOVA statistical tests, Tukey follow-up test, and independent *t*-test were analyzed using SPSS-18.  $p < 0.05$  was considered as the significance level of the data difference.

## Results

The results showed no preference toward some specific place in the mice pre-conditioning step in the CPP method. Statistically speaking, 50% of them are present in one section and the other 50% are in the other section (Table 1). In addition, the results show that there is no difference between the mean of elapsed time in the section paired with stimulus in both control and sham groups, which were injected distilled water hypodermically during the experiment days (fourth and ninth days), as compared with the first day. It means that neither of the groups showed any preference to stay in the section where they were put during conditioning step (Table 2).

According to table 2, no significant difference is seen in the mean of the elapsed time in pertinent section between the experimental group controlled by scopolamine and the control group within (fourth and ninth) experiment days, as compared with the first day (pre-conditioning).

**Table 1.** Mean and standard error of the presence of animals on both sides of the box on the pre-conditioning day (in seconds)

Number	Animal presence in the box			
	Black side		White side	
	Mean	SEM	Mean	SEM
96	303	4.23	294	6.02

**Table 2.** Mean and standard error of the time elapsed during different days among the groups under study (in seconds)

Groups	Number	Time elapsed in stimuli conjugated part in different experimental days (Mean±SEM in seconds)		
		Pre-conditional day	Test (fourth day)	Test (ninth day)
Control	12	240±4.23	249±2.17	239±1.45
Sham	12	248±2.47	247±2.13	248±2.15
Scopolamine	12	238±1.56	241±3.97	246±2.11
Morphine	12	238±1.74	381±4.64*	376±4.71*
Scopolamine+morphine	12	247±2.05	301±4.75*†	304±4.70*†

Symbol (\*) shows the difference at of  $p<0.05$  level between the relevant group and control group

Symbol (†) shows the difference at  $p<0.05$  level between the relevant group and morphine group

The statistical results indicate that there is a significant increase at  $p\leq 0.05$  level between the experimental group that received morphine on the (fourth and ninth) experiment days as compared with the first day (pre-conditioning) and the control group in terms of the time elapsed in the relevant section (Table 2). The statistical results indicate that there is a significant increase ( $p\leq 0.05$ ) level between the experimental group controlled by scopolamine+morphine and the control group in terms of the mean of the elapsed time in the relevant section (Table 2).

## Discussion

The results obtained from the present research showed that morphine intensifies CPP and scopolamine has no effect on CPP while simultaneous consumption of morphine and scopolamine decreases CPP caused by morphine.

Acetylcholine released in synaptic spaces in brain plays a major role in memory and learning and electrophysiological studies also showed that cholinergic interneuron reply to learning-initiating sensory stimulation and rewarding behaviors [1, 18]. Chronic consumption of morphine creates many changes in central nervous system, which causes drug-dependant behavioral changes [19]. It also activates dopaminergic neurons in VTA, which plays an important role in neurologic formation of addiction [20]. The dopaminergic pathway, which extends from VTA to NAC constitutes a critical area for initiating psychological dependency to narcotic drugs [21]. CPP is usually used to measure drugs reinforcement properly, measuring memory and learning, and simple relationship between a simulation and reward [22].

The studies showed that morphine creates a significant and dose-dependent CPP [23]. The intracranial studies on CPP show engagement of several neural pathways of central nervous system. They engage with brain reward mechanisms [24]. Having effect on mesolimbic dopaminergic system and VTA communications, amygdala might affect NAC and mediate narcotics rewards [15, 25, 26]. Dopaminergic  $D_2$  receptors in amygdala play an important role in central amygdala to create CPP by morphine [21].

Some studies strongly recommend that narcotics impede GABAergic mediatory neurons in VTA and reduce release of GABA, which lead to activating dopaminergic neurons and increasing dopamine release [27]. The use of

olmuscium injection as the agonist of GABA A receptors and injection of baclofen as the agonist of GABA B receptors to anterior hippocampus reduce rewarding effects of substances such as morphine, heroin, cocaine through modulating of dopamine transfer. By reducing dopamine level of extracellular in VTA and NAC, they distinctively reduce CPP of morphine [28, 29]. In addition, the use of GABA antagonists can activate VTA dopaminergic neurons and increase release of dopamine in NAC, as bilateral injection of antagonist Phaclofen of GABA B receptor to the posterior section of hippocampus may significantly increase morphine CPP [30]. Scopolamine, which is considered an antagonist of muscarinic acetylcholine receptors, is one of the most successful drugs to treat addiction; it removes the harmful effects of narcotics withdrawal [31, 32]. Scopolamine might affect through the direct effect in blocking muscarinic acetylcholine, which interact with opioid receptors [33, 34]. The subgroups of  $m_1$  and  $m_2$  receptors of muscarinic acetylcholine have many functions, including neural formation, memory learning and brain development that affect transfer and identity of opioid receptors [35, 36].  $\kappa$  and  $\sigma$  opioid receptors and muscarinic receptors regulate calcium ion, which is an important secondary messenger in transferring opioid signals. [35, 37, 38] Scopolamine might reduce intercellular calcium concentration slightly and decrease morphine withdrawal symptoms [36, 37, 39].

Scopolamine may reduce morphine dependency through indirect effect on mesolimbic dopaminergic pathways. Morphine withdrawal follows reduction of dopaminergic activity in VTA, which increases noradrenergic and cholinergic activity that basically interferes with morphine withdrawal symptoms [40]. In addition, scopolamine might reduce dependency to morphine through inducing amnesia and the effect on knowledge function [34]. It was proposed that central cholinergic neurons would be the mediator or many symptoms of narcotic withdrawal [41]; therefore, pharmacological blocking of central muscarinic receptors by the drugs such as scopolamine, which passes through brain-blood barrier (BBB) is extensively used in treating addiction and reducing the effect of narcotic drugs [32]. Nitric oxide (NO) in NAC interferes with controlling the release and increase of dopamine in this area [42]. Therefore, scopolamine might be effective in morphine by decreasing CPP; this is done through reducing NO synthesis [43]. With respect to the results obtained, it seems that memory attenuation may lead to reducing

psychological dependency toward addictive substances in animals. It is recommended to conduct similar studies on human samples.

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### Authors' Contributions

Author had equal role in design, work, statistical analysis and manuscript writing.

### Conflict of Interest

The authors declare no conflict of interest.

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