ORIGINAL ARTICLE

Role of music in morphine rewarding effects in mice using conditioned place preference method

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Submitted: 2012-06-05 Accepted: 2012-10-08 Published online: 2012-12-17

Key words: **music; morphine; CPP; mice; NAc; VTA**

Act Nerv Super Rediviva 2012; **54**(3): 127–130 ANSR540312A04

Abstract

This research aims at studying the neuroendocrine effects of music on creating morphine dependence in mice using conditioned place preference (CPP).

The mice treated with 10 mg/kg morphine subcutaneously, fast music and slow music. Morphine was used to create dependence. In order to recognize the morphine rewarding effects, CPP technique was used. In the conditioning stage that lasted for 8 days, different groups of mice, after receiving the treatment were randomly placed in compartment for 30 minutes. The post-conditioning stage included the fourth day, the ninth day, the 12th day and the 16th day.

Comparing place preference between morphine group and the control group, a significant increase ($p<0.05$) was observed in the place preference of morphine group, while a significant decrease ($p<0.05$) was demonstrated in the place preferences of morphine + taxi girl music group compared with morphine group alone. In addition morphine + alone in the rain music group demonstrated a significantly increased conditioned place preference ($p<0.05$) compared with the morphine group.

Alone in the rain music acts as a positive pleasant emotion increasing the dopaminergic activity in the Nucleus Accumbens (NAc) and Ventral Tegmental Area (VTA) and through associated learning mechanisms of reward-related behavior increases morphine addiction. However, taxi girl music may act as unpleasant experiences producing negative emotions and reducing morphine addiction.

Introduction

Music is an important part of most people's lives (Trehub 2003).Music has been shown to be capable of inducing strong positive and negative emotional responses among subjects (Huron 2006).

It is hypothesized that pleasant music as a reward system, activates area involved in emotional responses (Brown *et al* 2004). Studies indicate that music may not produce addiction and it has no control over behavior. In contrast, artificial rewards such as opiates (e.g. morphine) exert control over behavior (Yorulmaz *et al* 2002).

Opioids are drugs for creating addictive states. Morphine, as a member of this group, activates opioid receptors, which in turn modulate various signaling cascades and cellular functions. The cellular pathway and the neuroendocrine circuitry leading to opioid reward and addiction remain obscure (Bailey & Connor 2005).

CPP is a technique used in animal studies to evaluate preferences for environmental stimuli that have

been associated with a positive or a negative reward. The technique is often used to determine the addiction potential of drugs (Derea *et al* 2010).

Nowadays physicians agree that the addiction caused by addictive drugs including morphine is the most dangerous disease in both developed and developing countries. The most significant pathophysiologic process in the addiction process is the interference of these drugs with the action of the nervous system. Investigators try to discover the physiological and neuroendocrine relations of different drugs to justify the fast effect of such compounds on the nervous system and also the long-term need to use the drugs even after withdrawal (Kelley & Berridge 2002).

Considering the aforementioned facts, the aim of the present study was to find the neuroendocrine effects of music on creating morphine dependence in male mice using CPP technique.

Materials and methods

Animals, drug and music

Sixty mature male mice, weighing about 30–35 g with an average age of 90 days, were used in the study. The mice were divided into 6 groups of 10 including the control group and five experimental groups which were treated either by morphine, taxi girl music, alone in the rain music, taxi girl music + morphine or alone in the rain music + morphine groups. The study protocol was made based on the international laws protecting lab animals and was confirmed by the ethics committee of the Fars Science and Research Branch, Islamic Azad University. Morphine was bought from Iran Daru drug companies (Tehran, Iran). The mice receive 10 mg/kg morphine subcutaneously.

Conditioned place preference

To do the CPP experiment, a plexiglass box (15 cm × 37 cm × 15 cm) was used. The box was divided into two separate compartments by a central section which were connected by a guillotine window (Masahiko *et al* 2002). The walls and the floor of one of the compartments were white, and the walls and the floor of the other were black.

To study the effect of each music, the following protocols were performed on each group of mice during an eighty day period (Thiel *et al* 2008): (a) the pre-conditioning period or the time needed for the animals to adapt to experimental conditions was for one day. During this period, after removing the guillotine window, every animal was placed in either white or black compartment for 10 minutes. Their locations were recorded in order to place the animals in the same compartment on the day of the actual experiments. The length of the time each animal spent in each compartment was also recorded separately. The mice usually did not show any preference at this period and spent 50% of the time in each of the two compartments. If any mouse stayed in a compartment more than 90% of the time, it was excluded from the study; (b) in the conditioning period that lasted 8 days, prior to their placement in either white or black compartments, animals in the control group did not receive any treatment, and the morphine group received 10 mg/kg morphine subcutaneously. According to several investigations, this dose of morphine is optimum for the induction of CPP (Vargas-Perez *et al* 2009). The animals in each music group were randomly placed in one of the compartments and were then exposed to the respective music. The various music + morphine groups received a forementioned dose of morphine prior to their random placement in one of the compartments and their exposure to the specific music; (c) the post-conditioning stage or the periods of the actual experiments included the fourth day, the ninth day (a day after the last day of treatments), the 12th day (four days after the last day of treatments) and the 16th day (eight days after the last day of treatments). The purpose of post conditioning stage on the 12th and 16th day was to find out if after four and eight days of the last treatment, the effects of the music and morphine were still evident.

In the post conditioning stage, each animal was placed in the same compartment, as in the preconditioning day, for 10 minutes. The animals were free to move between the two compartments as in the pre-conditioning day. The time each animal spent in the compartment was measured by a chronometer to observe how different music affects morphine dependence of the animals.

Data analysis

The data were analyzed by one way ANOVA, Duncan test and Student's t-test, using SPSS software. A p-value of <0.05 was considered as statistically significant.

Table 1. mean and standard deviation of the time animals spent in either compartments on the preconditioning day (in seconds)

Animal presence in the box(in seconds)				
White side		Black side		Number
S.D.	mean	S.D.	mean	
0.55	249.9	0.56	248.8	60

S.D. - Standard deviation

Results

The results demonstrate that the animals had no preference for either compartment during the pre-conditioning period. They spent approximately 50% of the time in either compartment (Table 1).

As shown in Table Π, there were no statistically significant difference in the time spent in the stimuli paired chamber during the experimental periods (4th, 9th, 12th or 16th day) and the preconditioned period com-

Table 2. Mean and standard deviation of the time spent on different experimental days by different test groups (in seconds)(N=10)

Mean and standard deviation of the time spent on different experimental days by different test groups(in seconds)					group
Test(16th)	Test(12th)	Test(9th)	Test(4th)	Pre-conditional	
248.7±0.9	246.5±1.4	247.4±1.9	250±0.9	250.7±1.1	control
248.7±1.4	245.9±1.5	249.3±0.9	247.4±1	249.3±0.8	Taxi girl music
250.2±0.7	248.8±0.8	247.5±1.1	249.5±1.2	251.2±0.9	Alone in the rain music
400.1±2.1*	393.4±2.9*	391.3±4*	396.4±2.4*	242.5±1.6	Morphine
318.6±1.7*	320.9±1.8*	312.1±2.7*	313.5±3.1*	250.1±1.6	Morphine + taxi girl music
439.4±2*	445.9±2*	441.4±2.8*	442.2±2.5*	250.2±3	Morphine + Alone in the rain music

*The mean difference is significant at the .05 level

pared with that of the control group. Similarly no statistically significant differences were observed among various music groups and the control during the various experimental periods and the preconditioned ones. However, there was a statistically significant increase (p<0.05) in the time spent by the morphine group in the stimuli paired chamber during the experimental periods compared to the preconditioned day and also in comparison with that of the control group. However, there were no statistically significant difference among the time spent in the stimuli paired chamber by the morphine group at various experimental periods. Table Π also reveals that the morphine +taxi girl music group showed a statistically significant increase in the time spent in the stimuli paired chamber during various experimental periods compared to the preconditioned day or in comparison with the control group (p<0.05), in contrast to a significant reduction in the specified time compared to the morphine group (p<0.05). It is noteworthy to mention that the morphine+ alone in the rain music showed a statistically significant elevation in the time spent in the stimuli paired chamber during the experimental periods compared to those of the preconditioned or the control values. However, in contrast to the other taxi girl music group + morphine, they showed a statistically significant increase (p<0.05) in the specified times compared to those of the morphine groups (Table 2).

Discussion

We have demonstrated a significant increase in the time spent during the experimental days by the morphine + alone in the rain music group compared to the morphine group.

Mesolimbic dopaminergic system that projects from the VTA to the NAc is critical for the initiation of opioid reinforcement. Morphine, upon stimulating μ-opioid receptor in VTA, induces morphin addiction (Narita *et al* 2001).

Dopamine neurons within the VTA project throughout the limbic, forebrain and the Prefrontal Cortex (PFC). Dopamine plays an important role in reward-related learning. In addition, dopamine motivates behaviors aimed at obtaining rewards (Hyman 2005).

Listening to pleasant music activates NAc, olfactory cortex (OFC) and ventromedial PFC involved in the rewarding emotions (Barbas *et al* 2003). Some studies suggest that interactions in the NAc, VTA and hypothalamus may mediate brain responses to a reward and affect certain aspects of pleasant music (Esch & Stefano 2004).

The rewarding and reinforcing aspects of listening to a pleasant music are mediated through increased dopamine levels in VTA and the NAc (Barbas *et al* 2003). Furthermore, the pleasurable aspect of reward is thought to be modulated by endogenous opioid transmission within the NAc (Kelley & Berridge 2002).

In addition researchers indicate that pleasant music can help store and recall information. Positive emotions are increased rapid learning of both predictive cues and efficient behavioral sequences increase rewards (Larkin 2001).

Considering the excitatory effects of dopamine on morphine induced CPP in VTA (Di Angelantonio *et al* 2004), it can be concluded that alone in the rain music acting as positive emotion increases morphine induced CPP through its excitatory effect on dopaminergic neurons of VTA and on learning.

The mean of the time spent in the stimuli paired chamber by morphine + taxi girl music group compared with the morphine group during the experimental days, showed a significant decrease ($p \leq 0.05$).

Studies indicate that unpleasant music inhibits dopamine neurons in VTA (Valenti *et al* 2011). Music can be increased endogenous opioids. Activation of the Dynorphin K receptor system produces actions that are opposite to those of μ receptors. Dynorphin induces unpleasant states. In addition K receptor agonists decrease dopamine release or dopamine neuron activity in VTA, NAc and PFC (Bruchas *et al* 2010).

It can be concluded that taxi girl music acting as negative emotion decreases morphine – CPP through its inhibitory effect on dopaminergic neurons in VTA.

The results show no significant difference among the means of the time spent in stimuli paired chamber during the experimental days in each experimental group separately (Table 2).

Memory plays an important role in drug addiction. The expression of postsynaptic density-PSD-95 protein in the Hippocampal CA1 region, influences addiction memory (Han *et al* 2008). PSD-95 acts through increasing the plasticity of dendritic spine and the synapse. After morphine withdrawal, the expression of PSD-95 decreases, but the plasticity of dendritic spine has been steady (Zhu *et al* 2006).

In addition when opiate withdrawal occurs, c-fos mRNA expression also increases in the GABAergic neurons of amygdala, VTA and hippocampus (De Vries & Shippenberg 2002).This reflects an activitation of the dopaminergic neurons. These dopaminergic activities are mediated addictive memory after withdrawal (Cardinal *et al* 2002).

After morphine withdrawal, corticotropin releasing factor1 (CRF1) receptor's activity increases the transcription of dynorphin in NAc. Upregulation of dynorphin increases μ-opioid receptor (MOP) mRNA levels in the NAc. It then plays an important role in the reinforcement effects of opiates (Leri *et al* 2006).

Thus, taxi girl music and alone in the rain music might decrease and increase the negative effects of compulsive morphine-seeking behaviors and relapse to opiate abuse respectively.

Acknowledgement

The authors would like to extend their special thanks and appreciations to Islamic Azad University, Fars Science and Research Branch and Islamic Azad University, Kazerun Branch for their cooperation in performing this study.

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