Roles of the Nucleus Accumbens (Shell) in the Acquisition and Expression of Morphine-Induced Conditioned Behavior in Freely Moving Rats

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Original Article

ABSTRACT

Background: The nucleus accumbens (NAc) is a part of the rewarding cortico-mesolimbic dopamine (DA) pathway. This is a heterogeneous structure divided in two sub regions termed core and shell. DA function in the NAc is critical for goal-oriented behaviors, including those motivated by drug and brain stimulation reward. In the conditioned-place preference (CPP) paradigm, a test assessing animal's ability to associate drug-induced effects with environmental cause to quantify drug reward for example morphine.

Methods: In the present study, we investigated the influence of electrical stimulation with different current intensities on (25 and 100 μA) with and without an effective dose of morphine (0.5 and 5 mg/kg) on CPP.

Results: Subcutaneous administration of morphine 5 mg/kg produced significant CPP in comparison with saline group. Our findings also showed that electrical stimulation of NAc (100 μA) significantly ($P < 0.01$) suppressed morphine-induced CPP that reveals impaired learning and memory formation in the process of conditioning. We found that morphine-induced CPP can be successfully suppressed by current intensity (100 μA). It was probably due to decreasing of dopamine contents and its metabolites in the NAc. Current intensity (100 μA) in combination with ineffective dose of morphine (0.5 mg/kg) increased morphine-induced CPP probability via the prove reward system.

Conclusions: Since stimulation of dopaminergic neurons increases tendency to dependence to morphine, therefore in the present study, the stimulation of the NAc suppressed morphine-induced CPP that this shows impairment of learning and memory formation.

Keywords: Conditioned-place preference, morphine, nucleus accumbens, rat

INTRODUCTION

In human drug addicts, re-exposure to a drug of abuse often induces drug-seeking behavior and precipitates relapse even after long-term periods of abstinence. It has been made clear that the

administration of opiates increases the craving for opioids in drug-free addicts and may reinstate drug-seeking behavior after prolonged periods of extinction in opiate-experienced animals.[1] Conditioned-place preference (CPP) paradigm has been used widely to study the rewarding effects of various drugs of abuse, since it involves the drug-associated conditioned cue, which may be responsible for relapse in drug free former addicts. This property makes the CPP paradigm a useful tool for testing medications or other approaches for their effects of anti-craving and anti-relapse to drugs of abuse. Chronic morphine administration induces functional and morphological alterations in the mesolimbic dopamine system (MLDS), which is believed to be the neurobiological substrate of opiate addiction.[1] Dopaminergic neurons located in the ventral tegmental area (VTA) and their anterior projections to the limbic forebrain, for example, the nucleus accumbens (NAc) and the frontal cortex.[1]

Evidence for the involvement of the NAc in reward related mechanisms comes mainly from 2 types of studies CPP and deep brain stimulation. Morphine microinjections into the NAc can produce place conditioning similar to that after systematically administering morphine. NAc may play important roles in the formation of drugs-context association in CPP paradigm.[2] In recent years, CPP has been considered as an efficient method in order to evaluate the extent of reward caused by drugs. For example, researches show that morphine and nicotine both can cause significant and dose dependent CPP.[2] In the research these effects VTA and accumbens core have important role. A crucial matter in the creation of CCP resulting from morphine is the straight form of these two areas. In addition, some other areas of the brain are directly involved, especially award-dependent ones as memory and learning areas. It was also emerged that nitric oxide is effective in gaining and expression of CCP resulting from morphine.

The NAc can be divided into two major sub-regions: The shell - the ventro - medial part and core the dorsolateral part, which have different connectivity.[3] The shell sends efferent projections to the ventromedial ventral pallidum, extended amygdala - including the bed nucleus of stria terminalis, central amygdaloid nucleus and interconnecting sublenticular area., lateral pre-optic area, lateral hypothalamus, entopeduncular nucleus, VTA, mediodorsal substantia nigra pars compacta, mesopontine reticular formation and periaqueductal gray. The core sends major efferent projections to the dorsolateral ventral pallidum, entopeduncular nucleus, lateral part of VTA and substantial nigra. There are numerous functions of NAc, DA in a variety of behavioral such as: (i) Its role in appetitive behavioral arousal, (ii) its role as a facilitator as well as an inducer of reward processes and (iii) role in aversive contexts.[4]

Rewarding properties of addictive drugs are predominantly attributed to the increasing levels of synaptic dopamine (DA) in MLDS, including the VTA and NAc.[5] Chronic administration of morphine produces a number of adaptive changes in the MLDS.[1] DA release in terminal regions in the NAc by inhibiting gamma amino butyric acid ergic neurons in the VTA, which provide tonic inhibition of DA neurons, resulting in increased DA release in terminal regions. Thus the overwhelming actions of DA in the NAc lead to neural adaptation that underlies addiction of drugs.[3] A lot of investigators showed the effect of electrical or chemical stimulation on different parts of the brain and its effect on animal's behaviors,[6] for example peripheral electrical stimulation (PES) can suppress both morphine withdrawal syndrome and morphine-induced CPP expression in rats, as well as heroin craving in the addicts. Multiple 100 Hz PES could accelerate the recovery of morphine-induced morphological changes of dopaminergic neurons in the VTA.[1,2] Inhibition of morphine CPP produced by 100 Hz PES, suggesting an increased synthesis of dynorphin in the NAc. This is in line with previous findings that 100 Hz PES could increase the abundance of prepro dynorphin messenger ribonucleic in rat brain.[7] Other investigations showed that morphine-induced CPP can be successfully suppressed by PES, an effect accompanied by a reversal of the increased tissue contents of DA and its metabolites in the NAc of morphine-induced CPP rats.[7] Chronic high frequency stimulation of the rat NAc can block CPP induced by morphine and attenuate morphine reinforcement.[2] In the study, we used a directional electrical current for simulation with a freely moving method of stimulation with least human intervention.

Therefore, the present study was designed to evaluate the effect of electrical stimulation with different current intensities of NAc on effective and ineffective dose of morphine-induced CPP. In the present study, we investigated the influence of electrical stimulation with different current intensities on NAc (25 and 100 μA) with and without an effective dose of morphine (0.5 and 5 mg/kg) on CPP during conditioning and post-conditioning phases.

METHODS

Animals

Male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 250-350 g, at the time of surgery, were used. The animals were kept in an animal house with a 12-h light/12-h dark cycle (light on 6:30) and controlled temperature (20-22°C). They had *ad libitum* access to food and water. For familiar animals to laboratory they took to laboratory 2 days before experiments. Each animal was used once only. A total of 8 animals were used in each group of experiments (There are 4 groups and 8 numbers in each groups). All procedures were carried out in accordance with institutional guidelines for animal care and use.

Drugs

The drugs used in this study were chloral hydrate (350 mg/kg, intra peritoneal) for anesthetized and morphine sulfate (Temad, Tehran, Iran) dissolved in 0.9% saline just before the experiments. Morphine was injected subcutaneously. Control animals received vehicle (saline).

Surgical procedures

The animals were anesthetized with chloral hydrate (350 mg/kg intra peritoneal) and placed in a stereotaxic apparatus. A stimulating electrode was stereotaxically implanted into the NAc of each animal. Coordinates for the electrode implantation according to the atlas of Paxinos and Watson were as follows: Anterio-posterior, 3. Mediolateral, 1.3 dorsoventral, 6.5 relative to bregma and the skull surface and were fixed with dental acrylic and used jewelers screw for the holding of dental acrylic cements.[8] In this study, we use unipolar stimulation electrodes and our electrodes were staying during experiments (near 14 days). Following surgery, animals were housed individually in Plexiglas cages immediately after surgery for 72 h and then they were housed in group of 4 for 5-6 days prior to behavioral testing and began to recover from surgery and the effect of anesthesia.[6]

Apparatus

Apparatus consist of two square base compartments (height 38 cm × 30 cm × 30 cm): Two compartment apparatus for conditioned place preference white and the other with gray walls (except for the front wall facing the lamp) separated by a guillotine door to match the respective wall. The door has to be kept closed during the conditioning period while it is open during the pretest and the test.

Behavioral testing

The CPP paradigm took place on 5 consecutive days by using a biased procedure. The experiment consisted of the following three phases.[6]

Pre-test

In the pre-test investigators estimate the preference of the experimental animal, for each of two different environments of CPP apparatus that can be recognized for visual cues. This estimation is expressed as the time spent in each environment while the animal is moving freely between the two.

Conditioning

In the conditioning phase, the animal is paired alternately, in one of the two environments (no preferred one), with the drug under investigation for its potential motivational effects or other unconditioned stimulus and in the other environment, without any specific stimulus. Number and length of conditioning periods may vary.

Test

Phase after the conditioning, the animal without any treatment, is tested by placing it in the apparatus where can freely move between the two environments. An increase in the time spent in the environment in which the animal has experienced the rewarding stimulus is considered CPP.[9]

Experimental design

After recovery from the surgery, animals were divided into two surgical groups: Morphine-control and morphine-stimulation group. Morphine-control group was given effective and ineffective dose of morphine without any stimulation while

the morphine-stimulation group trained with stimulation before effective and ineffective dose of morphine injection. The effects of different electrical current intensities in combination with ineffective dose of morphine on CPP. In the pilot study for obtaining optimal current intensity, each animal was stimulated with two stimulating current intensities (25, 100, μA) with a constant stimulation frequency at 25 Hz) just 20 min prior to morphine administration (0.5 mg/kg) during the 3-day conditioning phase and before starting post-conditioning phase for 10 min period during 1 s every 5 s (Stimulator Isolator A36O, WPI, USA) in the separate box which was connected to the stimulator in the next room.

Effects of different current intensities on NAc in combination with effective dose of morphine on CPP

In this part of study, four stimulation current intensities same as the last section was given to animals just 20 min prior to morphine administration (5 mg/kg) during the 3-day conditioning phase and before starting post-conditioning phase for 10 min period during 1 s every 5 s. We used these stimulation current intensities 20 min prior to saline administration in the next group as the same. Stimulation currents were adjusted to the intensity at which no motor side effects were produced (A36O, WPI, USA). Conditioning score is calculated for each animal on the test day.

Histology

After completion of behavioral testing, each animal was sacrificed with an overdose of chloral hydrate and transcardially perfused with 0.9% saline, followed by 10% buffered formalin. The brains were removed and placed in a 10% formalin for at least 3 days before sectioning. Sections were examined to determine the location of the electrode aimed for the NAc. The electrode placements were verified using the atlas of Paxinos and Watson [Figure 1]. Data from 3 animals with improper placements of the electrode in the NAc region were not used in the analysis.

Statistical analysis

For analyzing data one-way analysis of variance (ANOVA) following Tukey's *post-hoc* test was used. All results were expressed as mean ± standard error of the mean and difference with $P < 0.05$ between experimental groups was considered to be statistically significant. Calculations were performed using SPSS 19 software (SPSS Inc., Chicago, Illinois, USA).

RESULT

Effect of NAc stimulation with 25 μA current intensity in combination with ineffective doses of morphine on CPP paradigm

One-way ANOVA with Tukey test shows that low current intensity of NAc stimulation causes to decrease acquisition phase and increase expression phase of CPP in combination with 0.5 mg/kg dose of morphine relative to sham group, but this change not significant [Figure 2a].

Effect of NAc stimulation with 25 μA current intensity in combination with effective doses of morphine on CPP paradigm

Statistical analysis of ANOVA with Tukey test showed that (25 μA) current intensity of NAc stimulation causes to increase acquisition phase and decrease expression phase of CPP in combination with 5 mg/kg dose of morphine relative to sham group, but this change was not significant [Figure 2b]. Electrical stimulation of NAc (25 μA) combination with other doses of morphine did not significant changes in CPP.

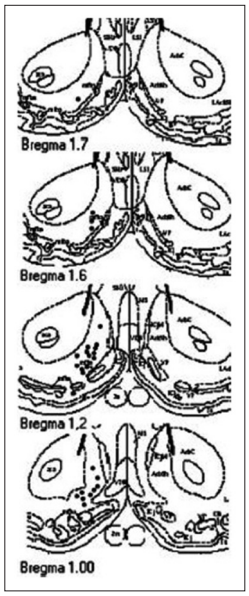

Figure 1: The placements of probes implanted in the nucleus accumbens (NAc) of rats included in statistical analysis, AcbC; NAc, core: AcbSh; NAc, shell. We compare location of electrode in NAc (shell) with this form

Effect of NAc stimulation with 100 μA current intensity in combination with ineffective doses of morphine on CPP paradigm

One-way ANOVA analysis in the acquisition and expression phase indicated electrical stimulation of NAc with ineffective dose of morphine causes to increase in compare with sham group but this change not significant. Electrical stimulation of NAc (25-100 μA) combination with ineffective doses of morphine did not significant changes in CPP [Figure 3a].

Effect of NAc stimulation with 100 μA current intensity in combination with effective doses of morphine on CPP paradigm

Statistical analysis of ANOVA with Tukey test showed that high current intensity of NAc stimulation combination with 5 mg/kg dose of morphine causes to suppress in the acquisition and expression phase of CPP relative to the sham group significantly reinforces and causes to aversion. But this electrical stimulation of NAc before saline injection had no significant effect.

Our results also showed that NAc electrical stimulation with current intensity (100 μA) in combination with 5 mg/kg dose of morphine causes to aversion and electrical stimulation (100 μA) in combination with 0.5 mg/Kg dose of morphine increase in acquisition and expression phase of CPP relative to sham group thus the suggest that 0.5 mg/Kg dose of morphine with combination different current intensity chosen for subsequent experiments [Figure 3b].

Effect of different dose of morphine on conditioned place preference paradigm

ANOVA statistical analysis showed that different dose of morphine (0.5, 2.5, 5, 7.5 and 10 mg/Kg) increased the time spent in the drug-paired compartment compared with saline compartment. Further Tukey test demonstrated 2.5 mg/kg and 5 mg/kg injection of morphine increased in time spent in the drug-paired compartment compared with that spent in the saline-paired compartment (*$P < 0.05$, ***$P < 0.01$) and other doses of morphine had not significant ($P > 0.05$) effect on CPP [Figure 4].

DISCUSSION

Drug addiction is primarily characterized by uncontrollable drug-seeking behaviors and chronic drug administration. Drug addiction is also known to be associated with dysfunction of many brain systems, including the memory, control and motivational systems. Brain dysfunction may contribute to the high rates of relapse in addicted individuals, even after long periods of abstinence are achieved.[10]

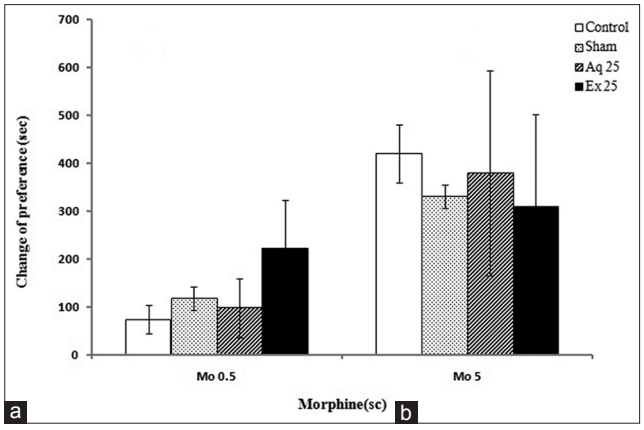

Figure 2: (a) Electrical stimulation of nucleus accumbens (NAc) (25 μA) combination with ineffective doses of morphine on conditioned-place preference (CPP) showed that low dose of morphine with this current intensity increase expression phase of CPP; (b) Electrical stimulation of NAc (25 μA) combination with effective doses of morphine on CPP showed to increase acquisition phase of CPP. The data were analyzed using one-way ANOVA followed by Tukey test $P > 0.05$

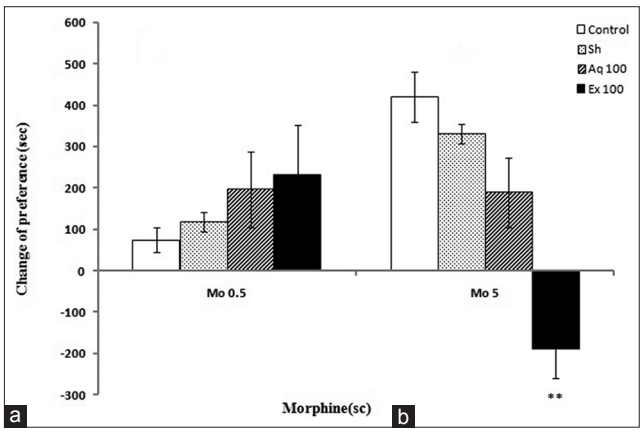

Figure 3: (a) Electrical stimulation of nucleus accumbens (NAc) (100 μA) combination with ineffective dose of morphine increase acquisition and expression phases; (b) Electrical stimulation of NAc (100 μA) combination with effective dose of morphine, showed significant effect NAc electrical stimulation in expression phase of CPP. The data were analyzed using one-way ANOVA followed by Tukey test compared with morphine group **$P < 0.01$

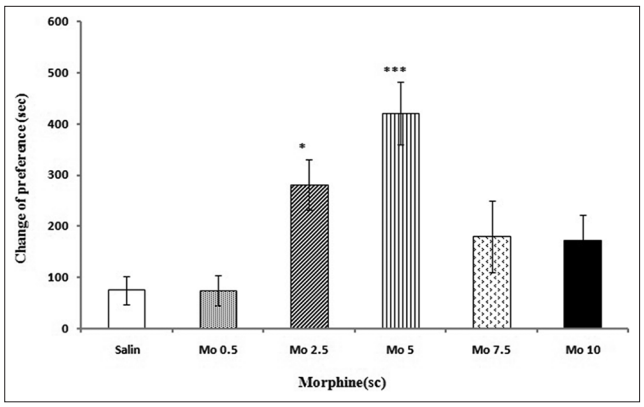

Figure 4: Place preference produced by morphine. Doses of morphine (0.5, 2.5, 5, 7.5 and 10, mg/kg) and saline (1 ml/kg) were administered in a 3-day schedule of conditioning. On the test day, the animals were tested for a 15-min period. The change of preference was calculated as the difference between the time spent on the day of testing and the time spent on the day of the pre-conditioning session. Data are expressed as mean ± standard error of the mean (n = 4-6). The data were analyzed using one-way ANOVA followed by Tukey test compared with the saline group *$P < 0.05$; ***$P < 0.001$

The present study investigated the effects of electrical stimulation with different current intensities of NAc on morphine induced-CPP. The animal were injected with morphine (0.5 and 5 mg/kg SC) using an unbiased CPP paradigm. Our findings showed that the administration of morphine (2.5 and 5 mg/kg) induced conditioned place preference [Figure 4]. These findings supported previous studies demonstrating that administration of opiates increases the craving for opioids in drug-free addicts and may reinstate drug-seeking behavior after prolonged periods of extinction in opiate-experienced animals.[3,10]

Moreover, in order to obtain the influence of different currents intensities on NAc, we used 25 and 100 μA current intensities in combination with the effective and ineffective doses of morphine during conditioning and post-conditioning phases. These results showed that NAc stimulation with a high current intensity (100 μA) in combination with ineffective dose of morphine (0.5 mg/kg) can induce both acquisition and expression of morphine-CPP while NAc stimulation with high current intensity (100 μA) in combination with effective dose of morphine (5 mg/kg) could suppress morphine-induced CPP [Figure 3b]. In agreement with these results, previous studies indicated that PES at 100-Hz during 30 min a day for 3 days suppressed both the expression of morphine-induced CPP and the reinstatement of extinguished CPP.[3] Injections of morphine or amphetamine into the NAc stimulate food intake therefore feeding stimulation induced by both drugs is related to their ability to engage reward systems at the level of the NAc.[11]

The current study showed that high intensity electrical stimulation of the NAc complete blocks morphine-induced CPP. 100 μA current intensity in combination with 5 mg/kg dose of morphine can suppress the morphine induced CPP in the rat [Figure 3b] which may help in reducing the craving for opiates in drug addicts.[12] Morphine failed to induce DA increase and was devoid of rewarding effects evidenced by CPP.[13] Chronic morphine administration induces functional and morphological alterations in the MLDS, which is believed to be the neurobiological substrate of opiate addiction. Moreover, current present investigation showed that different doses of morphine combination with low current intensity (25 μA) can make different degrees of effect on CPP in the acquisition and expression phase. In addition, our results showed that effective or ineffective electrical stimulation had no significant effect on ineffective doses of morphine (0.5 mg/kg) in the expression and acquisition phase [Figure 2a], but electrical stimulation with high current intensities (100 μA) combination with 0.5 mg/Kg dose of morphine can increase in acquisition and expression phase [Figure 3a]. Therefore, our suggestion that 100 μA current intensity in combination with different doses of morphine examining in a subsequent study because can make different changes in CPP. Furthermore, electrical stimulation with low current intensities (25 μA) combination with 0.5 mg/Kg dose of morphine produce non-significant expression phase of CPP [Figure 2a].

DA in the NAc is critically involved in the process of reinforcement.[7,14] The mesolimbic dopaminergic projection from the VTA to the NAc seems to be of central importance for reinforcement-related effects of drug abuse. Morphine microinjections into the NAc can produce place conditioning similar to that after systematically administering morphine. Intra accumbens injections of the DA receptor antagonist

or lesion of the NAc decrease the reinforcing effect of drug abuse. In support of this, lesion this pathway or blocking dopaminergic transmission in the NAc would attenuate the reinforcing effect of drugs.[14] Hence, it is possible that activation of mesolimbic DA system is critically related to link to the expression of morphine-induced place preference in mice.[2]

CONCLUSIONS

Our results revealed that electrical stimulation of NAc with high current intensities in combination with 5 mg/Kg dose of morphine blocked morphine induced-CPP which is due to disruption in CPP process. In contrast, using high current intensities in combination with 0.5 mg/Kg dose of morphine cause the increase in the expression and acquisition phase of CPP. It is possible that stimulation of NAc with 100 μA leads to activate the reward system and produce pleasure, like the effect of morphine.

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