Original Article

Electrical stimulation of prelymbic with different currents intensities on morphine induced spatial memory deficit in rats

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Abstract

Background: The medial prefrontal cortex (mPFC) is a part of brain reward system involved in cognitive functions such as learning and memory. Previous studies showed that electrical stimulation of prelymbic produced different effects on morphine-induced condition place preference. In this study, we investigated the electrical stimulation with different current intensities on spatial memory in rats.

Materials and Methods: In this study, male Wister rats weighing approximately 200–300 g were used. The effect of prelymbic electrical stimulation with 25 and 150 μA currents intensities in healthy and addicted rats on spatial memory was studied. Spatial memory was investigated using the Morris water maze test in addicted rats after 9 days of electrical stimulation.

Results: Our findings have shown that morphine reduces the memory and learning, whereas the present results indicated that electrical stimulation of prelymbic area with current intensity of the 25 μA shortened the time and distance to reach to platform that indicated improvement in spatial memory on addicted rats. Whereas the electrical stimulation of prelymbic area with the current intensity of 150 μA has special weakening effects on spatial memory and prolongs the time and distance to reach the platform.

Conclusions: The electrical stimulations of prelymbic with 25 μA current intensity improved the spatial memory in addicted rats while with 150 μA current intensity weakened spatial memory in rats. It is possible that increase in the release of some neurotransmitters reverses the effect of morphine on spatial memory.

Key Words: Electrical stimulation, morphine, prelymbic, spatial memory

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INTRODUCTION

The medial prefrontal cortex (mPFC) consists of four main parts: Dorsal to ventral are the medial agranular, the dorsal and ventral divisions, the prelymbic (PL) cortex, and the infralimbic (IL) cortex. The various subdivisions of the mPFC may have been different and have distinct functions. For example, dorsal regions of the mPFC area are linked to various motor behaviors, while the ventral regions of mPFC (PL and IL) are associated with diverse emotional and cognitive processes.[1] The PL cortex of mPFC primarily projects to limbic sites associated with cognitive behaviors, supporting its role in cognitive functions and in turn receives the noticeable dopaminergic input from the ventral tegmental area (VTA) and is a terminal region of the mesolimbic dopaminergic system.[2-4]

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Previous studies indicated that the effect of electrical stimulation on different nuclei of the brain and its effect on animal's behaviors. Previous researchers showed that the electrical stimulation of VTA modified persistent nociceptive behavior in rats.[2,5-7] Other research in this field suggested that the effect of electrical stimulation on condition place preference (CPP) induced morphine abuse and increase neurotransmitters. Therefore, many researchers have tried to understand the function of this region and its contribution to the cognitive and other behavioral performances.[3,8] Some researchers were performed to study special effects of electrical stimulation on CPP and different results were obtained.[9,10] But in some investigated electrical stimulation with a frequency of 100 Hz, a change in memory has been reported. It has been shown that electrical stimulation of prelymbic area with 25 μA current intensity facilitates the creation of reward through other mechanisms. The aim of this study was that because the prelymbic area sends glutamatergic projection to VTA and the nucleus accumbens (NAc), which are considered the main part of the brain reward system.[4,11]

MATERIALS AND METHODS

Animals

The animals were randomly allocated to different experimental groups. The animals were kept in an animal house with a 12 h light/dark cycle (light on 6:30) and controlled temperature (20–22°C). They had labium access to water and food. All animals were adapted to the laboratory conditions for at least 1-week before surgery and were handled for 5 min/day during this adaptation period. Each animal was used once; only eight animals were used in each group of experiments. All procedures were carried out in accordance with institutional guideline information for animal care and use. In this study, morphine sulfate (Temad Co., Tehran, Iran) was dissolved in sterile saline (0.9%), just before the experiments. It was injected subcutaneously. Saline groups received vehicle (saline).

Rats are grouped as follows:

- Saline (control)
- Morphine
- 25 μA + saline
- 25 μA + morphine
- 150 μA + saline
- 150 μA + morphine.

Drugs

The drugs used in this study are morphine sulfate (Temad, Tehran, Iran) dissolved in 0.9% normal saline before the experiments. Three doses of morphine 10, 20, and 40 mg/kg are injected intraperitoneally (i.p) consecutively in 9 days. Control animals received 0.9% saline.

Surgical procedures

Surgical protocol

The animals were anesthetized with chloral hydrate (Merck, alman 350 mg/kg, i.p) was at the 1.2 g/kg dose and placed in a stereotaxic (Stoelting Co., USA) apparatus. A stimulating electrode was stereo toxically implanted into the PL cortex part of the right mPFC (PL) of each animal. Coordinates for the electrode implantation according to Paxinos and Watson atlas were as follows: (AP) 3.2 (ML), 0.6, and (DV) 3.5 from the skull surface[12] and were fixed with dental acrylic. Following surgery, the animals were housed individually in PLEXIGLAS cages immediately after surgery. Animals were allowed for 1-week to recover from the surgery and anesthesia.[11]

Apparatus (Morris water maze test)

After the doses injection 10, 20, and 40 mg/kg morphine for 9 consecutive days, rats are placed in Morris water maze then be assessed spatial memory. The circular tank (180 cm in diameter) was filled with water (22 ± 2°C) made opaque and was surrounded by a variety of extra-maze cues. The tank was divided into four quadrants and four start positions were located at the interactions of the quadrants. Data were recorded using custom software (Radiab1) 24 h before water maze testing; all rats were habituated to the water and apparatus.

In the spatial acquisition phase, the rats learned to find a submerged platform using extra-maze cues. A transparent Lucite platform (10 cm) was submerged 2 cm underneath the water in the North-East quadrant of the tank, where it remained for all spatial trails. Each rat participated in 16 trails, which were organized into a daily block of for trails (1 trail, start position within a block), for 4 consecutive days. For each trail, the rat was given a maximum time of 60 s to locate the platform, after the rat remained there for 30s. If the rat did not locate the platform within 60 s, it was guided to it by the experimenter. The next trail started immediately after removal of rat from the platform. Escape latencies (s) and swim distance (cm) were recorded.[1] In the retention phase, 60 s probe trail was conducted to examine how well the rats had learned the exact location of the platform. During this trail, the platform was removed from the tank. The quadrant time (percent time spent in the training quadrant) was recorded during the probe trial.

To test the possible deficits in sensory motor processes, rats were tested in the water maze with

a visible platform on a new location on the final day of training.[1] Transforming data (square root) were considered when differences between the variances of the groups were significant. Probabilities <0.05 were considered significantly different. In Morris water maze test, escape latencies, path length, and swim speed were analyzed statistically by two-way repeated measures ANOVA followed by least significant difference (LSD) test for between subjects differences and within effects across the blocks. The probe trial data for the percentage of time spent in each of the four zones were analyzed by multivariate ANOVA followed by LSD test.

Electrical stimulation pattern

In order to obtain optimal current intensity, each animal is stimulated with two stimulating current intensities (25 and 150 μA) with a constant stimulation frequency at 60 Hz for 20 min period during 1 s every 5 s (Stimulator Isolator A360, WPI, USA).[12] For electrical stimulation of the brain, we used low currents with low frequency. These currents do not cause injury, but they can increase the electrical activity of neurons around the electrode. The electrical currents used in the central nervous system consist of a pulse wave with low current intensity under the threshold and have frequencies between 20 and 200 Hz. In this study, for implementing the electrical stimulation, the socket is fixed in the PL with dental acrylic [Figure 1].

Histology

After the completion of behavioral testing, all animals were sacrificed with an overdose of chloral hydrate and received a transcardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked, and placed in 10% formalin for at least 3 days before sectioning and cut coronary in 60 μm sections for determining the location of the electrode aimed for the NAc. Only the animals with correct electrode placements were included in the data analysis.

Statistical analysis

Data were analyzed using the SPSS version 16 for windows. In Morris water maze test, escape latencies, path length, and swim speed were analyzed statistically by two-way repeated measures ANOVA followed by LSD test for between subjects differences and within effects across the blocks. The probe trial data for the percentage of time spent in each of the four zones were analyzed by multivariate ANOVA followed by LSD test. The significant level was set at $P < 0.001$. Results are given as mean ± standard error of the mean.

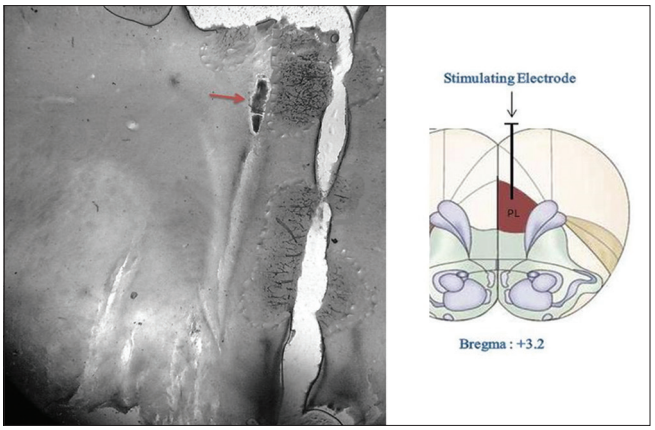

Figure 1: The following illustration shows the location of the effect of electrical stimulation on prelymbic

RESULTS

The effect of electrical stimulation prelymbic on spatial memory with 25 and 150 μA currents intensities in swimming distance to platform

Figure 2a shows that electrical stimulation PL with 25 μA shortened distance to platform in addicted rats that indication improves the spatial memory. In this figure, ANOVA statistical analysis showed the effect of electrical stimulation PL on distance on spatial memory significantly F (5, 42) =262.732; Figure 2a were different between the groups. The saline, the saline + 25 μA and the morphine + 25 μA groups found the platform more quickly than morphine and morphine + 150 μA groups [699.64 ± 35.97 cm, 951.5 ± 35.97 cm, 468.79 ± 35.97 cm, $P < 0.001$; Figure 2a], respectively, [48.32 ± 35.97 cm and 1.50 ± 35.97 cm, $P < 0.001$; Figure 2a] and took shorter distance to the platform. These findings indicate that PL stimulation with effective current intensity 25 μA improves the spatial memory whereas the current intensity 150 μA takes long time to reach the platform in addicted rats that indication is ineffective on spatial memory. Current intensity of 25 and 150 μA differ significantly with morphine ($P < 0.001$).

The effect of electrical stimulation prelymbic on spatial memory with 25 and 150 μA currents intensities on swimming speed to platform

As shown in Figure 2b, the electrical stimulation with the current intensity 25 μA increase the swimming speed to reach the platform that indicated the improvement in spatial memory whereas the electrical stimulation with the current intensity 150 μA had no effect on destructive morphine effects of spatial memory in addicted rats and just as evidence of Figure 3 the injection of morphine lead to decrease the swimming speed to reach the platform and was weakened in spatial memory. For speed, there was no difference between the groups F (5, 42) = 80.62.

For speed, there was no difference between the groups [F (3, 35) = 0.63, P = 0.6; Figure 2c]. However, the swim speed increased across blocks [BLOCK effect, F (3, 105) = 9.88, P < 0.001; Figure 2c] and the pattern of change in swim speed differed between the groups [GROUP *

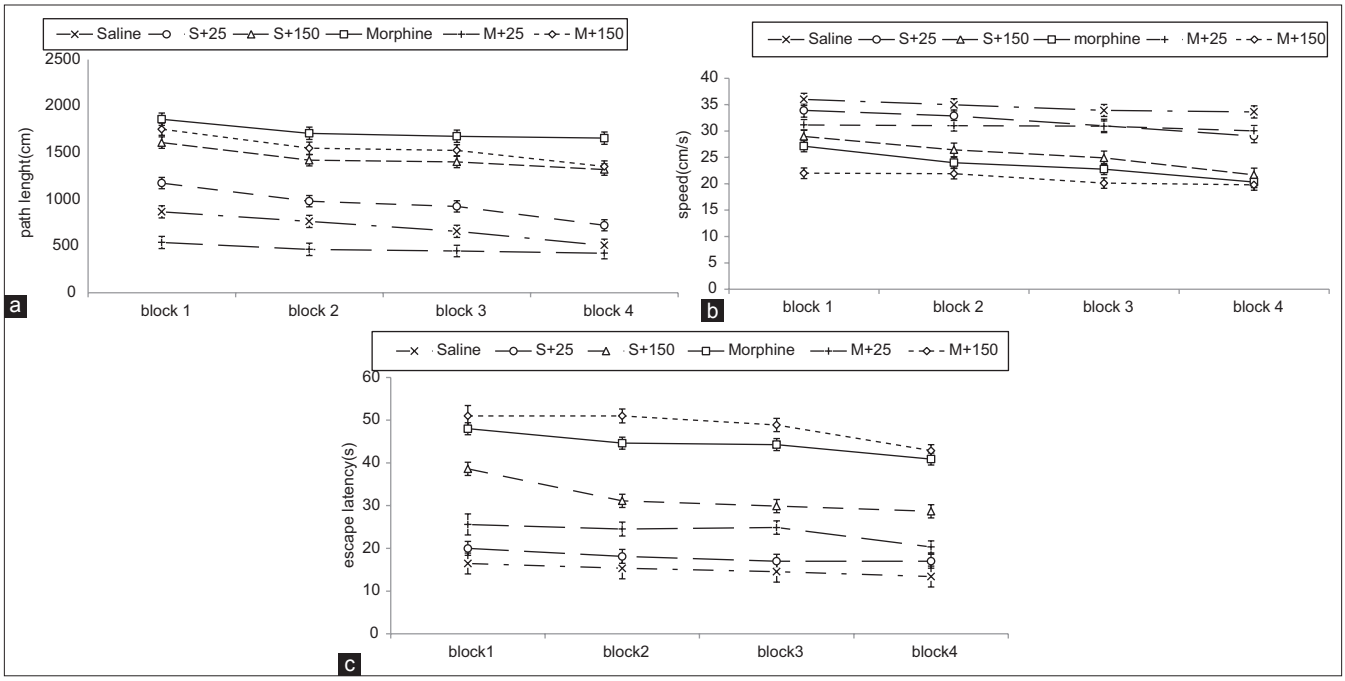

Figure 2: The effects of electrical stimulation with the current intensities (25 and 150 μA) of prelymbic area during the spatial acquisition of Morris water maze test in addicted rats. The path length (a) the swim speed at different days to reach the platform, (b) and escape latency, (c). Each point represents the day mean ± standard error of the mean of 4 swims. For latency and path length, the [Figure a] shows that distance to platform shorten in morphine + 25 μA and significant compare with morphine + 150 μA (P < 0.001). The [Figure b] shows that electrical stimulation with the current intensity 25 μA increase swimming speed to reach the platform that indicated improvement in the spatial memory (P < 0.001). The [Figure c] shows that electrical stimulation with the current intensity 25 μA improves the spatial memory, and repression shorted time to reach the platform (P < 0.001). Analyzed by two-way ANOVA followed by *post-hoc* least significant difference

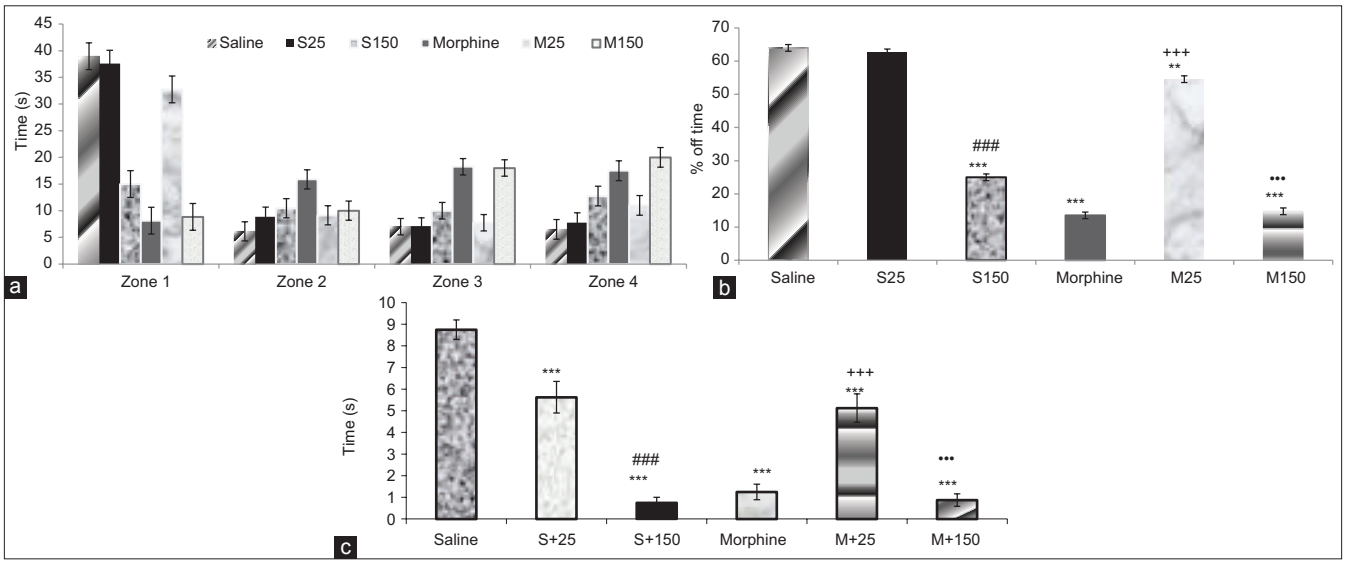

Figure 3: The effects of electrical stimulation with the currents intensities (25 and 150 μA) of medial prefrontal cortex area on spatial memory during the probe trial in rats, quadrant time, as measured by mean percentage (%) time spent in each of the four zones, 1-day after spatial acquisition phase (a), percent of time that spent in the training quadrant-zone 1 against chance 25%, (b) and the number of plate crossing, (c). ***P < 0.001 with respect to the saline group, ###P < 0.001 compared between saline + 25 μA and saline + 150 μA groups, +++P < 0.001 compared between morphine and morphine + 25 groups and •••P < 0.001 compared between morphine + 25μA and morphine + 150 μA groups. Zone 1 was the training quadrant that previously platform was located (P < 0.001 and P < 0.05 with respect to the morphine and morphine + 150 μA groups). Data are expressed as standard error of the mean ± mean of 8 animals per group, analyzed by two-way ANOVA followed by *post-hoc* least significant difference

BLOCK effect interaction, $F(9, 105) = 2.9$, $P < 0.01$; Figure 2c].

Electrical stimulation of prelymbic with 25 and 150 μA currents intensities on spatial memory on the time to reach the platform in rats

As shown in Figure 2c, the time to reach the platform in morphine + 25 μA after the electrical stimulation with the current intensity 25 μA to compare with morphine was shortened. Therefore, we conclude that the electrical stimulation with the current intensity 25 μA improve the spatial memory and repression effects of to get destructive morphine on memory whereas the time to reach the platform in the current intensity 150 μA was prolonged that represent the weakened in spatial memory in rats. The saline, the saline + 25 μA and the morphine + 25 μA groups found the platform more quickly than morphine and morphine + 150 μA groups [31.62 ± 1.18 s, 26.97 ± 1.18 s, 33.93 ± 1.18 s, $P < 0.001$; Figure 2c], respectively, [−33.93 ± 1.18 and – 31.27 ± 1.18 s, $P < 0.001$; Figure 2c] and showed a reduction in escape latencies [BLOCK effect, $F(5, 42) = 346.11$, $P < 0.001$; Figure 2c].

The effects of electrical stimulation with currents intensities (25 and 150 μA) of prelymbic area on spatial memory during the probe trial in rats

For the results of probe trial as measured by the time spent in each of the four zones, between group comparison indicated that the morphine (8.125) and morphine + 150 μA (8.846) groups spent less time in zone 1, where the platform was previously located, significantly than the saline (38.952) and morphine + 25 μA (32.75) and saline + 25 μA (37.589) groups; $P < 0.001$; $P < 0.05$. There was a significant difference in the morphine + 25 μA groups comparing to the morphine + 150 group μA [Figure 3a].

The effects of electrical stimulation with currents intensities (25 and 150 μA) of prelymbic area on spatial memory during the number of plat crossing

The results of plat crossing had showed a significant reduction in the all of the group relation to saline ($P < 0.001$). Comparisons between the saline and electrical stimulation groups showed that electrical stimulation with the current intensity 150 μA and morphine groups caused severe damage and impair memory. The electrical stimulation with the current intensity 25 μA promoted to spatial memory respect to morphine ($P < 0.001$). There was no significant between morphine and M + 150 μA.

DISCUSSION

Morphine is commonly used an analgesic for severe pains, but the rewarding effect of morphine represents a disadvantage in therapeutic settings due to its potential for abuse.[9,13] Some of the investigators indicated the effect of electrical or chemical stimulation on different parts of the brain and its effect on animal's behaviors.[2,5,6]

In this study, the effects of electrical stimulation of PL on spatial memory in healthy and addicted to morphine rats are examined. Several studies demonstrated that administration of opiates increases the craving for opioid in drug-free addicts and may reinstate the drug-seeking behavior after prolonged periods of extinction in opiate-experienced animals.[14,15] Regarding morphine injection, the findings of this study agree with the previous studies.[10,16] In order to obtain the influence of different currents intensities, PL 25 and 150 μA are applied. The findings here indicate that due to PL stimulation with the current intensity of 25 μA (effective current intensity); the injected morphine effect have improved the spatial memory; while the stimulation current intensity of 150 μA had no effect on spatial memory improvement. Our findings showed an optimum combination of the current intensity and the frequency of electrical stimulation will contribution memory and learning improved the results here have suggested that the effective or ineffective electrical stimulations contribute to the spatial memory significantly. The findings are an agreement with the previous studies of electrical stimulation of prelymbic activities glutamatergic, predict to the VTA activation capable of activating the mesolimbic dopamine system elevated dopamine.[7] Evidences indicate that all addictive drugs increase dopaminergic neurotransmission in the brain reward system and dopaminergic afferents caused by the VTA are crucial elements in the neural circuits that mediate the motivation and strength.[5,9,17] Thus, it is possible that the electrical stimulation of PL sub-region of mPFC produced emotional state and memory via the dopaminergic afferents which arose from VTA and terminate into the prelymbic area.[18] The effect of morphine administration on spatial learning in male rats show that, the morphine reduced the spatial learning because opiates such as morphine have high interest to opioid and morphine binding to these receptors may inhibit acetylcholine release. Acetylcholine is an important neural mediator that can increase learning and memorizing. Therefore, when its release is inhibited, the compound may cause the impairment of spatial learning and memorizing.[19,20] The mPFC has been implicated on learning and memorizing, these electrical stimulation of PL with different current intensities might lead to blocking the connection from the hippocampus to the PL cortex of mPFC or activation of these circuits.[21] It emphasizes the neural circuit linking of the hippocampus and

mPFC and provides a crucial pathway by which the spatial information can be integrated into the cognitive process in the future. The research stage is open for a comprehensive study to be conducted in this field of biology science through adopting a combination of electrical stimulation and opioids use for the enhancement of memory and learning.

CONCLUSION

In our data, it is revealed that the electrical stimulation of prelymbic with the current intensity 25 μA improves the spatial memory. It is possible that the stimulation of prelymbic with 25 μA intensity leads to activate the reward system and produce the pleasure like effect of morphine in prelymbic. It proposes that further research needs to determine the electrical stimulation of prelymbic with different dose of morphine and its mechanisms must be investigated.

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Conflicts of interest
There are no conflicts of interest.

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