Original Article

The effect of medial prefrontal cortex electrical stimulation on passive avoidance memory in healthy and addict 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. The mPFC receives strong dopaminergic innervations from ventral tegmental area (VTA) that comprises a portion of the mesolimbic dopaminergic system (MLDS), and sends glutamatergic projections to both the VTA and nucleus accumbens (NAc).

Materials and Methods: In this study, male Wister rats weighing 250-350 g were used. The effect of medial prefrontal cortex (mPFC) electrical stimulation with different current intensities (25, 50,100, and 150 μA) in healthy and addicted rats on passive avoidance memory was studied here.

Results: This study showed that 25 and 150 μA had no effect on improving avoidance memory in rats. Current intensities of 50 and 100 μA differ significantly with 25 and 150 μA. The PL of mPFC contributes to memory processing.

Conclusions: The electrical stimulations of prelimbic with 50 and 100 μA current intensities were improved avoidance memory in addicted rats while learning impairment is caused in healthy rats while the electrical stimulation with these used current intensities.

Key Words: Avoidance memory, electrical stimulation, medial prefrontal cortex, morphine

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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 prelimbic (PL) cortex, and the infralimbic (IL) cortex. The various subdivisions of the mPFC may have different and distinct functions. For example, dorsal regions of mPFC are linked to various motor behaviors, while ventral regions of mPFC (PL and IL) are associated with diverse emotional, cognitive, and mnemonic processes.[1] The PL cortex of mPFC primarily projects to limbic sites associated with cognitive behaviors, support its role in cognitive, and receives noticeable dopaminergic input from the ventral tegmental area (VTA) and other inputs from other subcortical basal ganglia via mediodorsal thalamus, and is a terminal region of the mesolimbic dopaminergic system. The mPFC sends glutamatergic projection to VTA and the nucleus accumbens (NAc) which are considered the main part of the brain reward system.[2-4] Investigators showed the effect of electrical stimulation on different nuclei of the

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brain and its effect on animal's behaviors.[3,5,6] Other previous researchers and investigations showed that the electrical stimulation of VTA modified persistent nociceptive behavior in rats.[7] Electrical stimulation of mPFC facilitates the creation of reward through other mechanisms. Therefore, many researchers have tried to understand the function of this region and its contribution to the cognitive other and behavioral performances.[4] In the current investigation we have studied the effect of current intensity 100 μA of mPFC on avoidance memory. In addition, we discussed studying the effect of different current intensities 25, 50, 100, and 150 μA on avoidance memory. The electrical current for simulation with a freely moving method of stimulation with the least human intervention is applied here. Since the activity of mesolimbic dopamine systems is considered as a central core in production reward, this study was designed to evaluate the effect of electrical stimulation of mPFC on avoidance memory in healthy and addict rats.

MATERIALS AND METHODS

Animals

Male wister rats (Pasteur Institute, Tehran, Iran) weighing 250-350 g were used. 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 libitum 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 six animals were used in each group of experiments. All procedures were carried out in accordance with institutional guideline information for animal care and use. Rats are grouped as follows

- Surgery, then saline, and morphine injection
- Electrical stimulation with current intensity 100 μA, then saline injection (stimulation + saline)
- Electrical stimulation with current intensity 100 μA, then morphine injection after electrical stimulation three doses of morphine 10, 20, 40 mg/kg are injected intraperitoneally consecutively in 9 days (stimulation + morphine)
- Electrical stimulation with current intensities 25, 50, 100, and 150 μA then three doses of morphine 10, 20, 40 mg/kg are injected intraperitoneally consecutively in 9 days (25 μA + morphine), (50 μA + morphine), (100 μA + morphine), (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. Morphine is injected intraperitoneally. Control animals received 0.9% saline.

Surgical procedures

The animals were anesthetized with chloral hydrate (80%, i.p.) and placed in a stereotaxic apparatus. A stimulating electrode was stereotaxically implanted into the PL cortex part of the right mPFC (PL) of each animal. Coordinates for the electrode implantation according to the of Paxinos and Watson atlas[8] were as follows: (AP) 3.2, (ML) 0.6, (DV) 3.5 and the skull surface[8] and were fixed with dental acrylic. Following surgery, animals were housed individually in plexiglass cages immediately for 3 day, and then they were housed in group of 4 for 5-6 days prior to behavioral testing.

Apparatus (Shuttle box)

Passive avoidance apparatus (Shuttle Box) includes both bright and dark chambers that are separated by a guillotine door; the chamber floor is covered with electrified bars.

Behavioral testing

After doses of injection 10, 20, 40 mg/kg morphine for 9 consecutive days, rats are placed in shuttle box and studied the passive avoidance learning. For this purpose, a rat was put in the light chamber and after 10 s, the guillotine door was opened and the rat entered the dark chamber. The guillotine door was closed and an electric shock of 1.5 μA was applied for 5 s to the rat's paws after the animal was out of the dark room and was placed in cages. After 1 h (the criterion evaluation speed learning), after 24 h (the criterion evaluation first long-term memory or primary), after 1 week (the criterion evaluation second long-term memory), and after 1 month (the criterion evaluation memory third long-term memory or long-lasting) after the shocks the passive avoidance memory is determined. The test period for each interval is 600 second. The delay time regarding the entrance to the dark chamber indicates weakness in avoidance memory.

Electrical stimulation pattern

In order to obtain optimal current intensity, each animal is stimulated with four stimulating current intensities (25, 50, 100, 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).[9] 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 electrical stimulation, the socket is fixed in the PL by dental acrylic.

Histology

After completion of behavioral testing, perfusion process is done. In this process an overdose of chloral hydrate 0.9% normal saline and 10% buffered formalin are is injected. The brain is removed and placed in a 10% formalin solution for at least 4 days before slicing. The sliced sections are examined in order to determine the location of the electrode aimed for the mPFC [Figure 1]. The electrode placements are verified using the Paxinos and Watson Atlas.[8] Data from animals with improper placements of the electrode in the mPFC region were not applied in the analysis.

Statistical analysis

The results of different stages of the experiments for behavior passive avoidance learning are analyzed using one-way ANOVA following *post hoc* tests LSD and *T*-test. All the results are expressed as S.E.M ± mean and difference of ($^*P < 0.05$) between data groups was considered statistically significant. Calculations are performed using SPSS statistical software.

RESULTS

The effects of mPFC electrical stimulation through effective current intensities on passive avoidance memory

The effects of different currents intensities (25, 50, 100 and 150 mA) on avoidance memory are shown in Figure 2. ANOVA statistical analysis showed the effect of PL electrical stimulation on avoidance memory significantly. These findings indicate that PL stimulation with effective current intensities 50 and 100 μA improve avoidance memory and prolonged delay entering the dark chamber in rats. Current intensities of 50 and 100 μA differ significantly with morphine ($^*P < 0.05$).

The effects of surgery on avoidance memory

According to Figure 2, surgery had no effect on avoidance memory in the sham group and a delay entry in the dark chamber was short due to received morphine. There were significant differences between saline and morphine groups in the delay entry to dark chamber. Indeed morphine weakened avoidance memory. It could be deduced that the surgery has no effect on avoidance memory.

The effects of mPFC electrical stimulation through ineffective currents intensity on passive avoidance memory

As shown in Figure 2, the electrical stimulation with current intensities 25 and 150 μA had no effect on improving avoidance memory in rats and the injection of morphine lead to shortening the latency in the rat to enter the dark chamber and was weakened in avoidance memory.

The comparison avoidance learning with current intensity 100 μA in healthy and addict rats

According to Figure 3, electrical stimulation with current intensity 100 μA in the morphine group led to improved avoidance memory and prolonged entry

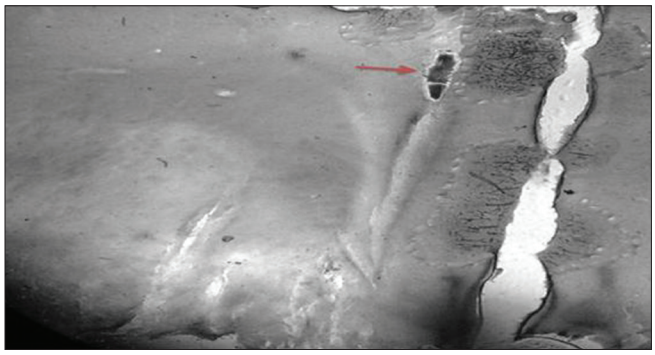

Figure 1: The arrow indicates the stimulating electrode site in the prelimbic

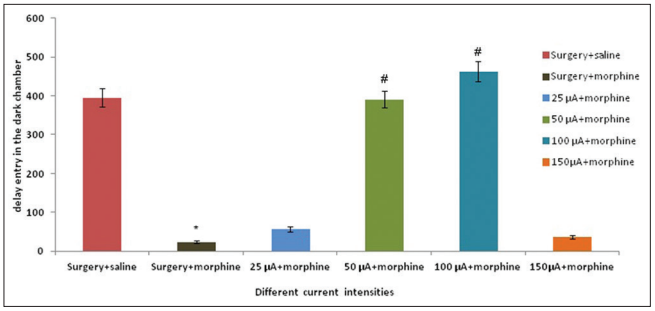

Figure 2: The effects of different current intensities of mPFC on the avoidance memory after 1 week. (*) Represent the difference between stimulation with morphine and with saline. (#) Represent the difference between 50 and 100 μA with the morphine group. Data are expressed as S.E.M ± mean of 6 animals per group, analyzed using one-way ANOVA followed by *post hoc* LSD ($^*P < 0.05$) comparison with the morphine group

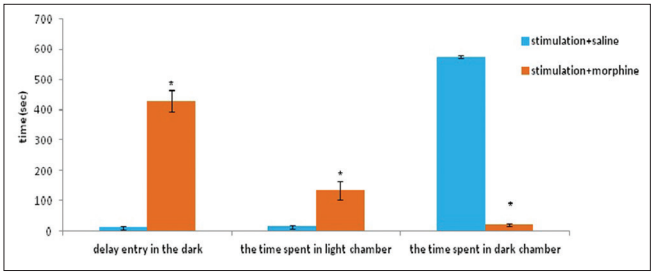

Figure 3: The Comparison of avoidance memory in the stimulation + morphine and stimulation + saline groups with current intensity 100 μA after 1 week. (*) Represent the difference between stimulation with morphine and with saline. Data are expressed as S.E.M ± mean of 6 animals per group analyzed using independent *t*-test ($^*P < 0.05$)

delay in the dark chamber, whereas in the saline group led to weakened avoidance memory and delay entry into the dark chamber was shortened. As the data indicate, there are significant differences between saline and morphine application.

The comparison avoidance learning with the criterion delayed entry in the dark chamber

One-way ANOVA analysis indicated that avoidance memory in the electrical stimulation + morphine group improved. As shown in Figure 4, the entry delay in the dark chamber in the electrical stimulation + morphine group was longer than the sham group [Figure 4], and this indicates that electrical stimulation of mPFC with current intensity 100 μA has improved the effects on avoidance memory with a significant effect on avoidance memory (*$P < 0.05$). According to Figure 4, the avoidance memory in the electrical stimulation + morphine group improved compared to the stimulation + saline group because entry delay in the dark chamber took longer than that of the stimulation + saline group. As the data indicate, there are significant differences between these groups.

The comparison avoidance learning with the criterion the time spent in the dark chamber

One-way ANOVA analysis indicates that avoidance memory in the electrical stimulation + morphine group improved. As shown in Figure 5, the time spent in the dark chamber in the electrical stimulation + morphine group was shorter than the morphine group [Figure 5], and this indicates that electrical stimulation of mPFC with current intensity 100 μA has improved the effects on avoidance memory with a significant effect on avoidance memory (*$P < 0.05$).

According to Figure 5, the avoidance memory in the electrical stimulation + morphine group improved compared to the morphine group because the time spent in the dark chamber took shorter than that of the stimulation + saline and morphine groups. As the data indicate there are significant differences between these groups.

DISCUSSION

Morphine is the most commonly used analgesic for severe pains although the rewarding effect of morphine represents a disadvantage in therapeutic settings due to its potential for abuse.[10,11] Previous studies have shown that nuclei in the brain can be affected directly or indirectly in the memory system.[12-14] In this study, the effects of electrical stimulation of mPFC on avoidance memory in healthy and addicted to morphine rats is examined. In this procedure, the rats were injected with morphine (10, 20, 40 mg/kg) on nine consecutive days. Regarding morphine injection, the findings of this study agree with the previous studies. In order to obtain the influence of different currents intensities on mPFC 25, 50, 100, and 150 μA are applied. The findings here indicate that due to mPFC stimulation with current intensities of 50 and 100 μA (effective currents intensities); the injected morphine effect had improved the avoidance memory; while the stimulation current intensity of 25 and 150 μA had no effect on avoidance memory improvement. Our suggestion is that finding an optimum combination of current intensity and frequency of electrical stimulation will contribution memory and learning. Improved results here show that the effective or ineffective electrical stimulations contribute to the avoidance memory significantly.

The findings are agreement with previous studies Electrical stimulation of mPFC activities glutamatergic, Predict to the VTA activation capable of activating the mesolimbic dopamine system elevated dopamine.[1] Evidences indicate that all addictive drugs increase dopaminergic neurotransmission in the brain reward

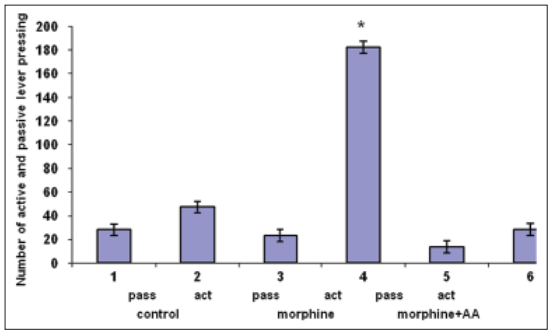

Figure 4: Comparison of avoidance memory with the criterion of avoiding entry delays in dark chamber between the stimulation + saline, stimulation + morphine, and morphine groups after 1 week. Data are expressed as S.E.M ± mean of 6 animals per group, analyzed using one-way ANOVA followed by *post hoc* comparisons LSD (*$P < 0.05$). (*) Represent the difference between stimulation + morphine and morphine group

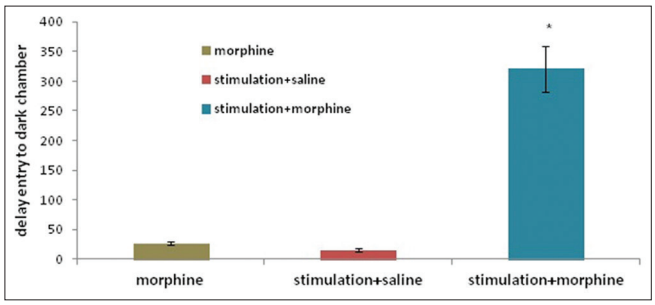

Figure 5: Comparison of avoidance memory with the criterion of avoiding the time spent in dark chamber between the stimulation + saline, stimulation + morphine, and morphine groups after 1 week. Data are expressed as S.E.M ± mean of 6 animals per group, analyzed using one-way ANOVA followed by *post hoc* comparisons LSD (*$P < 0.05$). (*) Represent the difference between stimulation + morphine and morphine

system and dopaminergic afferents caused by the VTA are crucial elements in the neural circuits that mediate motivation and strength.[5,15-18] Thus, it is possible that 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 mPFC area.[19] The effect of morphine administration on spatial learning in male rats show that, morphine reduced 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 impairment of spatial learning and memorizing[20,21] 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 (PL) or activation of these circuits. It emphasizes the neural circuit linking of the hippocampus and mPFC, and provides a crucial pathway by which 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 enhancement of memory and learning.

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