Original Article

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# An Evaluation of the Effect of Deprivation of Maternal Care on LTP Induction in Neurons of Hippocampal CA1 Region in Morphine-Dependent Rats

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## ABSTRACT

**BACKGROUND AND OBJECTIVE:** Deprivation of maternal care as a stressor causes disruption in cognitive and neurochemical activities of the brain. The aim of this study was to evaluation the effect of deprivation of maternal care on LTP induction in neurons of hippocampal CA1 region in morphine-dependent rats.

**METHODS:** This experimental study was conducted among 40 45-day-old male rats in control group, morphine-dependent group, and 3 groups of rats that were deprived of treatment for one, two and three weeks after birth for 3 hours daily. Except for the control group, the rest of the groups received 10 mg/kg body weight morphine sulfate subcutaneously every 12 hours for 10 days. On the eleventh day, the symptoms of deprivation syndrome were investigated by the Gellert-Holtzman method, and on the following day, the synaptic plasticity of neurons in CA1 region was studied.

**FINDINGS:** The Gellert-Holtzman score in the morphine-dependent group was 14.98±4.16 and increased to 31.79±5.12 in the group that was deprived of maternal treatment for 3 weeks ($p<0.001$). Although morphine dependence did not affect basic responses of CA1 region neurons and LTP induction, deprivation of maternal care reduced the range of basic responses from 1.01±0.04 in the morphine-dependent group to 0.68±0.09 mV in the group that was deprived of maternal care for 3 weeks ($p<0.001$) and prevented LTP induction ($p<0.001$) in a time-dependent manner.

**CONCLUSION:** The results of the study showed that deprivation of maternal care undermines the postsynaptic potential of the hippocampal CA1 region following morphine administration and disrupts the synaptic plasticity of the neurons in this region.

**KEYWORDS:** *Morphine, Deprivation Of Maternal Care, Long-Term Reinforcement, Hippocampus, Rat.* .

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## Introduction

Maternal separation, even for short periods of time (1), both in human samples and in animal models, is considered as a stressor and leads to disturbance in cognitive and neurochemical activities (2). It has been demonstrated that maternal separation within 5 – 9 days after birth for 8 hours causes disturbance in the conditioned place preference (CPP) in adult rats (3). Exposure to stressors at the first few days after birth causes changes in the function of the dopaminergic system in the mesolimbic brain regions, which itself can change the tendency towards opioid drugs (4). It has also been suggested that maternal separation increases the activity of the HPA axis and the production of corticosterone in children, which results in increased high risk behaviors (5). At the molecular level, maternal separation reduces the expression of BDNF and subunits of NR2A- and NR2B-containing NMDA receptors in the hippocampus (6).

The use of addictive drugs also causes permanent changes in the brain that ultimately lead to addictive behaviors such as tolerance and dependence (7). Although opioid drugs are clinically used as antinociceptive agents, they are highly addictive and their frequent use changes the activity of neural circuits and their plasticity in different regions of the nervous system, including the hippocampal region (8). The role of the hippocampus in the memory formation is crucial, and its function is necessary for the early stages of learning (9). Long-term potentiation (LTP) is a phenomenon that has been extensively studied and is now known as a key synaptic mechanism for memory formation (10). Some studies have shown that administration of 10 mg / kg morphine for 7 days weakens the synaptic plasticity in the CA1 region of the rat hippocampus (11).

Chronic use of morphine (two weeks) has also been shown to reduce the expression of MAPK and CamKII genes (involved in LTP) in rat hippocampus (12). Due to the role of the hippocampus in learning and memory processes and the two-way communication between this region and the reward circuitry, evaluating the effects of morphine on the synaptic plasticity of the neural circuits in the hippocampal region seems to be necessary (8, 13, 14). Considering that maternal separation can affect the maturation and activity of the circuits of different regions of the brain by changing the activity of the HPA axis, and on the other hand, since it has been shown that the use of opioid drugs can affect the activity of the neurons in the hippocampal regions, the present study was conducted to evaluate the effect of maternal separation on LTP induction in neurons of hippocampal CA1 region in morphine-dependent rats.

## Methods

This experimental study was carried out on 40 45-day-old male Wistar rats weighing 120 – 140 grams after approval at the Ethics Committee of Kashan University of Medical Sciences (IR.KAUMS.MEDNT.REC.1396.81). Animals were kept at the animal breeding center of Kashan University of Medical Sciences with a temperature of 22 ± 2 °C, 55 ± 5% humidity and 12 hours light/dark cycle, and had free access to water and food. Rats were divided into five groups (n=8) of control (CO), morphine-dependent (MD), and maternal separation for one week (MS1), two weeks (MS2), and 3 weeks (MS3) after birth. The maternal separation model (15) was as follows: every morning at 10 a.m., the mother was out of the cage and was returned to the cage at 1 p.m. At this interval, the cage of the infants was placed in a chamber at 35 to 37 °C. All animals were weaned on the 22$^{nd}$ day after birth. After reaching the age of 45 days that coincides with the period of brain maturation (16, 17), all animals except the control group received 10 mg / kg body weight morphine sulfate (TEMAD Co, Iran) subcutaneously every 12 hours for 10 days, and on the 10$^{th}$ day, the symptoms of morphine withdrawal syndrome were evaluated in these patients: Two hours after receiving morphine, 2 mg / kg naloxone hydrochloride (Tolidaro Co, Iran) was injected intraperitoneally and they were immediately transferred to a Plexiglas chamber with 30 cm diameter and 50 cm height. Deprivation symptoms were evaluated and scored for 30 minutes according to the modified model of Gellert-Holtzman (18). These symptoms include:

A. Graded symptoms (loss of weight within 24 hours after injection, number of jumps, abdominal contractions and 'Wet-Dog' shake [WDS] response);

B. Checked symptoms (diarrhea, bruxism, eyelid drooping, whole body contraction, penile licking and restlessness).

On the next day and half an hour before the experiment, the rats were anesthetized by intraperitoneal injection of urethane (1.5 g / kg). After fixing the animal's head in the Stereotaxic (Stoelting, USA), the location of the electrodes was specified on the skull by the Paxinos and Watson Atlas (19). The stimulatory electrode was located on the location of the axon of the neurons in the entorhinal cortex at the coordinates AP = -4.2 mm, LR = 3.8 mm, D = 2.4 mm, while the stability electrode was located on dendritic regions of CA1 neurons at the coordinates AP = -3.4 mm, LR = 2.5 mm, D = 2.5 mm. The electrodes were made of teflon-coated stainless steel and diameter of 0.005 inches (A-M Systems, USA). By applying Paired Pulse, the accuracy of the electrode location was examined. Higher range of the second response compared to the range of the first response by at least 20% indicated that the location of the electrodes was stable and stimulating. In response to the stimulation of entorhinal cortex neurons, excitatory postsynaptic potential (EPSP) was recorded by ELab system and eProbe 5.71 software (Parto-e Danesh, Iran). First, the Input / Output curve was plotted and the intensity of the stimulation, in which 60% of the maximum response range was received, was selected as the stimulation intensity for the rest of the test. The stimulations were applied with frequency of 0.1 Hz in 100 µs and a delay of 5 ms. Then, EPSP was recorded for 30 minutes. To induce LTP, high-frequency stimulation (HFS) was applied. The pattern of this stimulation included 10 trains with 10 stimulations with a frequency of 200 Hz and an interval of 2 ms. The duration of each stimulus pulse was 0.1 msec. After tetanic stimulation, the stimulation and recording process continued for 2 hours (20). In order to compare the groups, the percentage change in the range of the responses was evaluated (based on millivolt) before and after the application of the tetanic stimulation. An increase of at least 20% in the range of responses after tetanic stimulation was considered as a criterion for the incidence of LTP (21). Data were analyzed using two – way ANOVA and Tukey test, while $p < 0.05$ was considered significant.

## Results

In this study, the evaluation of deprivation symptoms showed that injecting morphine to rats led to their dependence. Data analysis showed that the severity of the symptoms of withdrawal syndrome was directly associated with the level of deprivation of the maternal care, i.e. Gellert-Holtzman score in rats that received normal maternal care during the lactation period and received morphine during puberty was 14.4 ± 98.16 and maternal separation increased the mean score up to 31.97 ± 5.12 in MS3 rats in a time-dependent manner. Post-test showed that the difference between MS2 and MS3 groups relative to MD group was significant ($p<0.001$, Fig. 1). Data analysis showed that receiving morphine by rats did not cause significant changes in the mean range of baseline responses. Meanwhile, receiving morphine by animals that experienced different periods of deprivation of maternal care caused a significant decrease in the range of their baseline responses in a time-dependent manner. The mean range of responses in animals in the control and morphine-dependent groups was 1.06±0.03 and 1.01±0.04, respectively, and maternal separation for 1, 2 and 3 weeks decreased the mean range of responses to 0.79±0.04, 0.66 ± 0.06 and 0.68 ± 0.09 mV, respectively ($p<0.001$, difference with control and morphine-dependent groups) (Fig. 2).

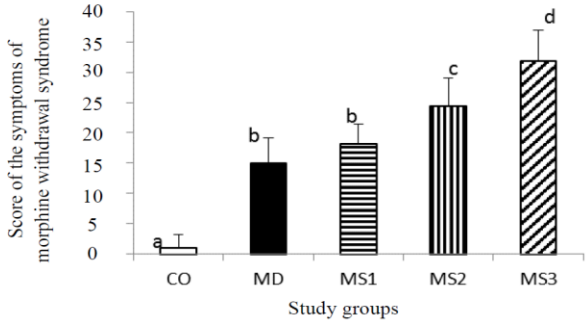


**Figure 1. Comparison of the mean score of the symptoms of morphine withdrawal syndrome based on the modified Gellert-Holtzman method in different study groups**

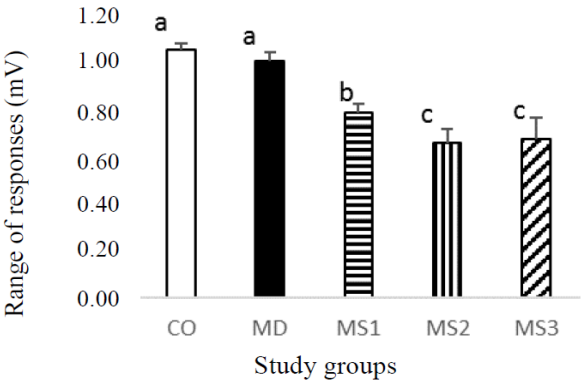


**Figure 2. The mean range of recorded postsynaptic potentials in hippocampal CA1 region of animals in different study groups. Although the use of morphine did not significantly change the range of responses, maternal separation caused morphine to have a reducing effect on the range of responses.**

LTP induction led to an increase of about 45% and 35% in the range of fEPSP recorded from animals in CO and MD groups, respectively (Fig. 3).

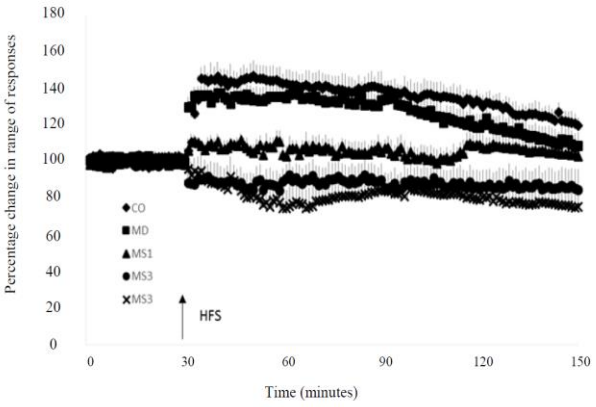


**Figure 3. LTP induction in the neurons of hippocampal CA1 region in rats. After induction, LTP was induced only in CO and MD groups. Maternal separation caused morphine to inhibit LTP induction in the neurons in the hippocampus region of rats in a time-dependent manner. P < 0.01: The difference in the mean range of response after LTP induction between CO and MD groups, and all three maternal separation groups. P<0.05: The difference in the mean range of response after LTP induction between MS1 and MS2 and MS3 groups.**

Given the 20% increase in the range of responses that was considered for induction, LTP was persistent for 2 hours in the CO group and up to about 1 hour in the MD group. However, the difference in the range of response after induction of LTP in CO and MD groups was not significant. Applying tetanic stimulation to the circuits in hippocampal CA1 region of the rats that experienced different periods of maternal separation was unable to induce LTP, and even reduced the range of response in MS2 and MS3 groups. Post-test results showed that the difference in mean range of response after induction of LTP between CO and MD groups, and all three deprived groups was significant ($p<0.01$). Moreover, the difference in mean range of response after LTP induction was significant between MS1 and MS2 and MS3 groups ($p < 0.05$).

## Discussion

The results of this study showed that maternal separation in lactation period increases the dependence of rats to morphine during puberty after exposure to morphine in a time-dependent manner. Moreover, the results showed that although the dependence of rats on morphine does not affect the postsynaptic potentials of neurons of hippocampal CA1 region by itself, history of maternal separation reduces the range of responses, and the longer the period of deprivation, the more the range of responses is reduced. In addition, the results showed that although morphine dependence does not inhibit the induction of LTP in these neuronal circuits, it reduces the stability by about half over time, and maternal separation prevents LTP induction in the circuits of the hippocampal CA1 region in a time-dependent manner and may even lead to the induction of LTD. Maternal separation is considered as a stressor and may lead to disruption of cognitive and neurochemical activities (2). Exposure to stressors at the first few days after birth causes changes in the function of the dopaminergic system in the mesolimbic brain regions, which itself can change the tendency towards opioid drugs (4). It has also been suggested that maternal separation increases the activity of the HPA axis and the production of corticosterone in children, which results in increased high risk behaviors (5). Maternal separation can also cause disturbances in brain reward systems to receive

stimulants and psychotropic drugs (22). Due to the role of the hippocampus in learning and memory processes and the two-way communication between this region and the reward circuitry, evaluating the effects of morphine on the synaptic plasticity of the neural circuits in the hippocampal region seems to be necessary (13).

Moreover, among opioid receptors, μ opioid receptors are expressed in the hippocampal region (23), and the involvement of Mu opioid receptors of the hippocampal region in the changes in the synaptic plasticity of the neural circuits after administration of morphine has also been proven (24); however, the results obtained from various studies are highly contradictory. On the one hand, it was stated that administration of 10 mg / kg of morphine for 7 days weakens the synaptic plasticity in the hippocampal CA1 region of the rats (11), and on the other hand, Miladi-Gorji et al. demonstrated that chronic use of morphine increases baseline responses and also induces LTP in the circuitry of dentate gyrus (25). In the present study, administration of morphine for 10 days proved to be ineffective both on the range of baseline responses recorded from the hippocampal CA1 region and on the induction of LTP in the circuits of the region, though the stability of LTP induction was reduced. Salmanzadeh et al. also demonstrated that morphine dependence does not affect the range of baseline responses recorded from the CA1 neurons in rat's brain slices and does not prevent LTP induction (26). Differences in these results may be associated with the type of studied animal, the location of evaluating synaptic plasticity, the LTP induction protocol (e.g. High frequency stimulation, Primed burst stimulation, or Theta burst stimulation), and the time interval between evaluation of synaptic plasticity and receiving the final dose of morphine. LTP is the long-term increase in the enhancement of synaptic transmission, which occurs after high-frequency synaptic activity and is considered as one of the intervening cellular processes in memory storage (27). In many types of synaptic plasticity, LTP induction requires a group of glutamate receptors called NMDA, and therefore, this kind of long-term enhancement is called NMDA receptor-dependent LTP (28); several studies have shown that LTP induced on the Schaffer Collateral Pathway to the hippocampal CA1 is of this type (29). It has been shown that maternal separation reduces the expression of BDNF protein (the protein that plays a major role in the health and life of the neurons) and subunits of NR2A- and NR2B-containing NMDA receptors in the hippocampus (6). According to Sousa et al., deprivation of maternal care from the second to the fourteenth days after birth caused plasma corticosterone levels in 70 weeks old rats to be significantly higher than that of the control group and the synaptic plasticity of the hippocampal CA1 neurons was impaired (30). Another study has shown that maternal separation during the second to ninth and fourteenth to twenty first days after birth decreases the synaptic plasticity of the hippocampal CA1 region of 40 – 50 – day – old rats (31). Shin et al. have shown that maternal separation from the second to the twentieth day after birth impairs LTP induction in the CA3 hippocampal mossy fiber pathway of mice (32). Given that both corticosteroid and opioid receptors are expressed in the hippocampus, it is likely that maternal separation enhance the destructive effects of the opioids on the neurons in this region of the brain by increasing the circulating corticosteroids.

In general, one can say that maternal separation in infancy may increase the dependence of rats on morphine, and undermine the post-synaptic potential of the hippocampal CA1 region after morphine administration. It also impairs the synaptic plasticity of neurons in this region.

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