Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

The Effect of Acute Stress on Formed Memory Recall after Prescription of Very Low to Regular Doses of Morphine in Rats

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Article information

Article history:
Received:4 January 2011
Accepted: 17 September 2011
Available online: 27 June 2011

Keywords:
Memory
Stress
Rat
Maze

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Abstract

***Background*:** Hippocampus is rich in corticosteroid and opioid receptors and is involved in memory. Both acute stress and morphine have a different effect on different stages of memory. Thus, the effect of acute forced swim stress on formed memory recall was investigated after prescription of morphine different doses.

***Materials and Methods* :** In this experimental study, adult male Wistar rats (200-250 g) were classified into three categories (for each group, n=7): 1) repeated morphine groups, 30 minutes before each training, they were intraperitoneally prescribed morphine for 4 days in Morris water maze, (1, 10,100 µg/kg and 1, 10 mg/kg) and the recall test was performed on 5th day. 2) Acute stress group, they were on training for 4 days without morphine and on the fifth day, 30 minutes before the recall test, they were subjected to forced swim stress for 5 min. 3) Repeated morphine plus acute stress groups, they were first treated same as first category and on the 5th day, they were behaved like the second category. On the day 12th, the same test as the 5th day was conducted, but the groups received no treatment.

***Results*:** In the repeated morphine groups, apparently only 10 mg/kg of morphine significantly disturbed learning ($p < 0.001$). But during the 5th and 12th day no significant difference was observed between all groups and the control group in memory recall. In the group treated with morphine plus acute stress, only 10 mg/kg of morphine significantly decreased memory recall during the 5th and 12th day compared to the control group ($p<0.05$).

***Conclusion*:** Repeated prescription of 10 mg/kg morphine plus acute stress intensifies the deleterious effects of stress on memory.

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Introduction

Hippocampus is one of the main areas involved in learning and memory. This area represents both opioid peptides and opioid receptors [1]. Opioid systems are involved in memory process. For example, β-endorphins and enkephalins disrupt the memory process [2]. In addition, a significant number of molecules involved in hippocampus Long-Term Potentiation (LTP) are affected by repeated prescription of opioids. Morphine may have effect on synaptic plasticity through direct and indirect mechanisms [3]. The effect of acute and repeated prescription of morphine has been studied on a variety of learning models and conflicting results are obtained [4]. However, the effects of opioids on cognitive function are still open to debate [1].

Repeated prescription of morphine makes learning slower in the water maze, but it does not disturb recall [1]. It is also shown that repeated prescription of oral morphine for 25 days has facilitating effects on learning and spatial memory in Morris water maze [5]. It is shown that prescription of 10 mg/kg dose of morphine half an hour before training for 10 days will led to the destruction of spatial memory in Morris water maze. However, the repeated prescription of 3 mg/kg dose has no effect [6]. On the other hand, the documents have shown that there may be a relationships or correlations between the phenomenon of opioid reward and some types of learning and memory processes [4]. Hippocampus is rich in corticosteroid receptors and will contribute to the termination of the stress response through negative feedback of glucocorticoid at the HPA (Hypothalamic - Pituitary - Adrenal) axis. It has been shown that in rat hippocampus, corticosterones regulate the metabolic, physiological and genomic functions of neurons [7].

Human and animal studies showed that acute stress and glucocorticoids have conflicting effects on different stages of memory. While they enhance learning and memory consolidation, they destroy spatial memory retrieval. The mechanisms involved in destructive effects of stress on memory retrieval are not well understood [8-11]. It is known that the effects of glucocorticoids on memory retrieval process are

too fast to be mediated by the genomic action and through intracellular receptors. Therefore, a non-genomic mechanism may be involved in creation of such responses [9]. Although the mechanism of such rapid non-genomic effects of glucocorticoids is not well identified, the membrane receptors of glucocorticoids, which are different from both glucocorticoid and mineralocorticoid receptors, have been identified on synaptic membrane [9, 12].

The effect of opioid receptors as well as the observed effects of stress on memory and hippocampus, abuse of exogenous opioids and its interaction with stress in human life, questions the combined effects of both factors on the memory formation and recall. Thus, in this study the effect of repeated prescription of very low to regular doses of morphine during the learning process in Morris water maze and the effect of forced swimming acute stress on the formed memory's recall have been studied.

Materials and Methods

Animals: in this experimental study, male Wistar rats weighing 200-250 g were used. 3-4 animals were put in each cage; being kept exposed to the 12 hour light-darkness cycle and at the controlled ambient temperature of (22±2^{o}C). All tests were performed between 10 to 14 o'clock and the animals had enough access to food and water. The tests were conducted under the ethical rules of working with experimental animals [13, 14]. All procedures of reproduction and breeding and the tests were conducted in the Animal Physiology Research Laboratory, Department of Biology, Faculty of Science; Ferdowsi University of Mashhad.

Morris water maze test: water maze consists of a circular pool of 150 cm in diameter and 80 cm in height. Platform is 10 cm in diameter. Walls, floor and platform of pool are black. The figures and instruments outside the maze were used as spatial signs for learning. The pool was filled with water of 23±1^{o}C so that the platform would be located 1.5 cm below the water surface. The computerized tracking system (manufactured by Mehad Sanat Shargh Company) and digital camera (Color CCD camera, Hivision) were used to record information. The Pool was divided into four quadrants (North, South, East and West) by the tracking system. In the first four days of testing, the platform was put in a quarter [5].

Test Method: In order to familiarize the animals with the laboratory environment conditions, the pool was filled with water, so there would be a visible platform in the center of the pool and each animal was put on the platform for 60 seconds. Whenever the animal entered the water from the platform, it was again placed on it. The days after the animal preparation, acquisition test was performed, which consists of 16 tests, which are 4 tests per day for 4 consecutive days. The rats were released into the water from all 4 positions (which were randomly determined by the computer) and they were allowed to swim for 60 seconds in search for the hidden platform [5, 15]. If the rats didn't find the platform after 60 seconds, they would be guided to the platform and they would be allowed to stay on the platform for 30 seconds. The spent time and the length of the path travelled to reach the hidden platform were recorded by the tracking system. In the day after the acquisition test, a search test was conducted on the animals to evaluate the accuracy and validity of the initial learning, in which the platform was removed. This stage has four tests. The spent time will be recorded in the target quadrant [5]. This test is also called the short-term memory recall stage. This test can also be repeated on the 12th day to investigate long-term memory [15].

Forced swim stress: each animal was forced to swim individually in a cylindrical tank with dimensions of 50cm in height and 35cm in diameter, containing 40cm water of 16±1^{o}C, for 5 minutes. At the end, the animal was dried with towel and was put into the subsequent assays [16].

Test and drug prescription groups: each of these groups, consisting of 7 animals, are divided into three categories:

a) repeated morphine group (control groups and groups of doses of 10 mg/kg and 1 mg/kg, 100 µg/kg, 10 µg/kg, and 1 µg/kg morphine). In the all four training days, morphine was intraperitoneally injected 30 min before the daily training and memory recall was measured with no treatment by Morris water maze on the 5th and 12th day.

b) The acute stress group (control and acute stress groups), that passed 4 training days without extra treatment and only on the 5th day; they were put under the forced swim stress 30 minutes before the test.

c) The repeated morphine - acute stress groups. In these groups, morphine treatment was conducted by the mentioned doses and on the training days and then, a stress session was conducted on the 5th day. In the all groups, the distance travelled and the time spent to find platform on the training days were recorded. On the 5th day, the time spent in the target quadrant was recorded. On the12th day, a test similar to that of the 5th day was conducted on all groups with the difference that the groups did not receive any treatment or training.

Data analysis: The results were presented as Mean ± SEM. Statistical analyses were conducted through Graphpad Instat software. The effectiveness of treatments is evaluated by one way ANOVA and consequently, T-test was estimated at minimum significance level of $p < 0.05$ and through Tukey post-hoc test.

Results

1-Results of repeated morphine groups: comparison of the time spent and the distance travelled to reach the platform on the training days between control group and groups of repeated morphine with different

doses: in control group and repeated morphine group, except the regular dose of repeated morphine (10 mg/kg), the time spent (Fig. 1-A) and the distance travelled (Fig. 1-B) to reach the platform have been significantly decreased day by day (p=0.00074) and (p=0.00638). These data show that the animals have learned the platform position during the training days. There is a significant difference in the time spent and the distance travelled to reach the platform between the control group and repeated morphine of 10 mg/kg group, (p=0.00022). Thus, it appears that this dose of morphine has totally disturbed the training process and has intensively increased the time and distance to reach the platform on the training days (Fig. 1).

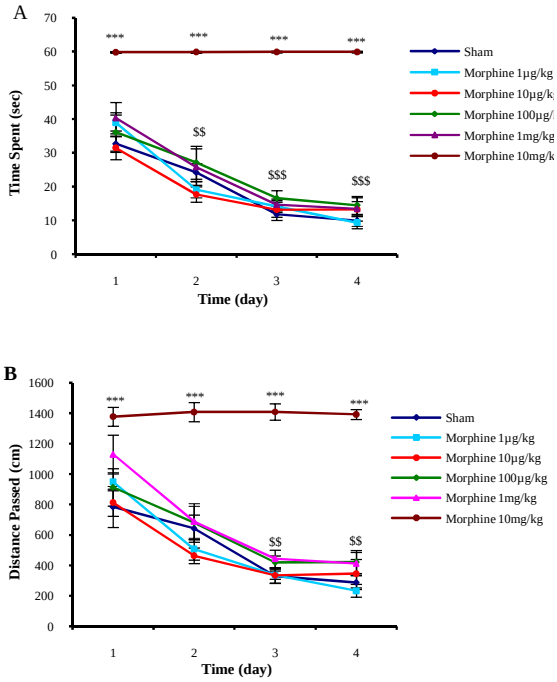

Figure 1. The comparison of (A) the time spent and (B) the distance travelled to reach the platform on the training days between the control group and repeated morphine group with doses of (1 µg/kg, 10 µg/kg, 100µg/kg, 1mg/kg, 10 mg/kg).

Learning process in the control group is so evident that the time spent and the distance travelled to reach the platform has been significantly decreased day by day. Only 10 mg/kg dose of repeated morphine has increased the time spent and the distance travelled to find the platform more than that of the control group on the same days. The results are expressed as Mean ± SEM. (p < 0.001 *** compared to the control group on the same days), (p < 0.01 $ $, p<0.001 $ $ $ compared to the first day of the control group), (n=7).

Comparison of the time spent by the animal in the target quadrant on the 5th and 12th day between the control group and repeated morphine groups with different doses: no significant difference was observed in the time spent by the animals in the target quadrant between the control group and repeated morphine groups on the 5th day (Fig. 2-A) and on the 12th day (Fig. 2-B). Therefore, the prescription of these doses of morphine during the learning process has had no effect on short-term (5th day) and long-term memory retention (12th day) (Fig. 2).

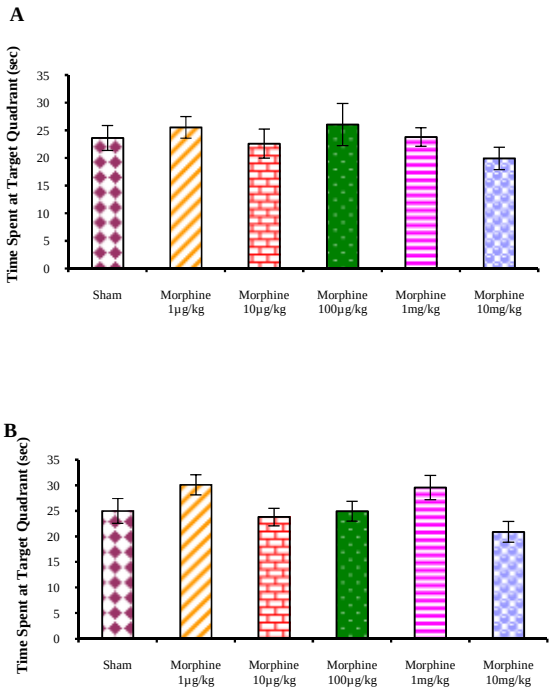

Figure 2. Comparison of the time spent by the animal in the target quadrant on the (A) 5th day and (B) 12th day between control group and repeated morphine groups. Prescription of these doses of morphine during the learning process has had no effect on the animal's presence time in the target quadrant. The results are presented as Mean ± SEM (n =7).

2-The results of acute stress group: no significant difference was observed in the time spent by the animal in the target quadrant between the control group and acute stress group on the 5th and 12th day. Thus, acute forced swimming stress applied in the fifth day after learning, has had no effect on short-term (5th day) and long-term memory retention (12th day).

3- The results of repeated morphine- acute stress groups: the time spent by the animal in the target quadrant in the group treated with repeated morphine (10 mg/kg) and then with acute stress was significantly decreased on the 5th day (Fig. A) and on the 12th day (Fig. B), compared to the control group (p=0.0354) and (p=0.0189).

It seems that in this group, repeated morphine has provided the way for inducing the deleterious effects of acute stress on memory recall; because in this case, neither acute stress nor morphine of 10 mg/kg had any effect on their own. However, no significant differences were observed on the 5th day (Fig. A) and on the 12th day (Fig. B), between control group and the groups which treated with repeated doses of morphine at (1 µg/kg, 10 µg/kg, 100µg/kg, 1 mg/kg) and then acute stress (Fig. 3).

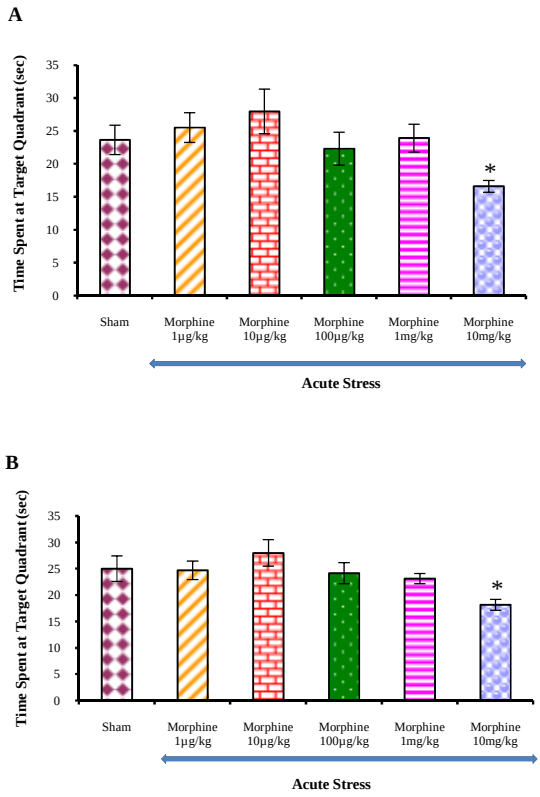

Figure 3. Comparison of the time spent by the animal in the target quadrant on the (A) 5th day and (B) 12th day between the groups treated with different doses of morphine during the learning process and then with acute stress on the fifth day and the control group. In the group treated with repeated morphine of 10 mg/kg with acute stress, the time spent by the animal in the target quadrant significantly decreased compared to the control group. The results are presented as Mean ± SEM ($p < 0.05$ * compared to the control group), (n=7).

Discussion

In summary the results showed that repeated prescription of very low to regular doses of morphine, except for dose of 10 mg/kg, in training days did not disrupt the learning process. Repeated prescription of very low to regular doses of morphine as well as to the treatment of acute forced swim stress did not cause any disturbance in the recall, but when the animals received morphine of 10 mg/kg dose during the training days and were under acute forced swim stress on the 5th day, memory recall was disturbed. The effect of morphine was studied in a variety of learning models and some conflicting results were obtained. On the other hand, the documents have shown there may be a relationship or correlation between the phenomenon of opioid reward and some types of learning and memory processes [4]. Human and animal studies have shown that acute stress and glucocorticoids have different effects on different stages of memory [8-11]. The effect of very low and regular doses of morphine on pain and analgesia has been different and mysterious [16]. Few studies are conducted on the combined effects of repeated prescription of very low to regular doses of morphine with stress on learning and memory process. In this study, the effect of acute forced swim stress on the formed memory recall has been analyzed after prescription of various doses of morphine. The results of these researches showed that there is no significant difference between the groups of repeated doses of morphine (1mg/kg and 1, 10 and100 µg/kg) and the control group in the time spent and the distance travelled to reach the platform in training days and the animal's presence time in the target quadrant on the 5th and 12th day. Thus, first it can be concluded that these doses of morphine have not had enough strength to have any effect on the learning process and memory recall (Fig 1 & 2); because in the group treated with repeated dose of 10 mg/kg morphine, the time spent and the distance travelled to reach the platform in training days was increased and showed a highly significant difference in comparison with the control group (Fig. 1). Thus, it seems likely that morphine (10 mg/kg) has totally disturbed learning process. But since on the 5th and 12th day no significant difference is observed with the control group in the animal's presence time in the target quadrant (Fig. 2), we can conclude that the animal has learned and remembered the platform position in training days. In other words, the dose of 10 mg/kg of morphine has caused the animal not to feel the need to find the platform from the first days; because in Morris water maze test, reaching the platform is considered as the reward. As we know, mesoaccumbens dopamine system is involved in the reward pathway [17]. Mesolimbic is a dopaminergic system, which is originated from the ventral tegmental area and terminates in the accumbens nucleus. This area is an important part of the reward system which is coupled with the effects of drugs. Opioids increase the release of dopamine in accumbens nucleus by activation of µ opioid receptor in the ventral tegmental and accumbens nucleus [18]. Morphine is probably connected to µ receptors on GABAergic interneurons and by controlling them, disinhibits the dopaminergic neurons. This will increase the release of dopamine in the accumbens nucleus [19]. Therefore, morphine prescription in high doses applies rewarding effects. Perhaps at least a part of the animal's behavioral effect in refraining from receiving bonuses (platform) is caused by morphine induction of feeling of reward by dopaminergic neurons in the animal. Thus, it seems plausible that the animal does not intend to find the platform and continues to swim. Previous studies have shown that the prescription of 10 mg/kg dose of morphine, half an hour prior to the test and for 10 days will lead to the destruction of spatial memory and the animal's performance impairment in Morris water maze [6]. This will at least confirm our results in part of the learning process, although in recall part we did not see any

significant disorder and its possible reason was presented.

The results obtained from these tests showed that in the acute stress group, acute forced swim stress cause no disturbance in the learning process on the 5th and 12th day. It is revealed that in the rates which received foot shock 30 minutes before the recall test in water maze, the presence time in the target quarter is decreased more than the control group [20]. This result is interpreted suggesting that the destruction of memory retrieval depends on time; so that glucocorticoid concentration reaches its highest level 30 minutes after the application of stress. Therefore, perhaps part of this occurred disturbance is dependent on glucocorticoid receptor activity [9, 20]. Another study showed that applying the acute restrain stress shortly before the recall test impairs long-term memory retrieval in the inhibitory avoidance test [9].

Our results are inconsistent with these findings. Perhaps this difference is due to the type of stress used in the tests. On the other hand, since swimming in water maze provides the necessary motivation to find the platform, swimming stress of a few minutes ago and swimming in the water maze are probably are in line to create the motivation to find the identified platform. In addition, if here stress has a disturbing effect on the recall; motivational linearity will cover the disordering effects of stress. The results showed that in the groups that have taken doses (1 mg/kg, 100 µg/kg, 10 µg/kg and 1 µg/kg) of morphine on training days and then were under acute forced swimming stress on the 5th day, the animal's presence time in the target quadrant on the 5th and 12th day was not significantly different with that of control group. But the animal's presence time in the target quadrant in the group treated with repeated morphine of 10 mg/kg in training days and then acute stress (in the 5th day) was decreased more significantly than that in control group on the 5th day (Fig. 3).

Previous studies have shown that LTP in hippocampus changes significantly by repeated prescription of morphine, which indicates the changes in hippocampal function after repeated prescription of opioids. After repeated opioid treatments, the capacity of LTP in hippocampus significantly decreases during the drug elimination. This shows that opioids cause changes in neuronal plasticity [1, 21]. In addition, previous studies have shown that acute stress applies their rapid effects through membrane glucocorticoid receptors and will lead to the deterioration of memory recall [9, 20]. Therefore, studies have shown that on the one hand, removal of repeated morphine will reduce LTP and on the other hand, acute stress through membrane glucocorticoid receptors leads to the degradation of memory recall. Therefore, it can be concluded that repeated morphine can facilitate the damaging effects of stress on short-term recall (the 5th day). Our results also confirm this (Fig 3-A). This will be further confirmed when it is mentioned that neither acute stress nor repeated morphine of 10 mg/kg alone had any effect on this case (Fig. 1). Furthermore, in the group treated with dose of 10 mg/kg of morphine in training days and acute forced swimming stress on the fifth day, the presence time in the target quadrant on the12th day showed a significant reduction more than that of control group (Fig 3-B). This long-term effect on the recall impairment may be justified with the above explanation. On the other hand, glucocorticoid can also cause long-term and late affects with help of the mechanisms dependent on glucocorticoid receptors. As we know, these receptors act by connection to DNA and protein synthesis [9]. It is shown that, long-term prescription of morphine reduces nerve regeneration in adult rat hippocampus [21]. Therefore, in this group, it can be concluded that the effect of repeated morphine to facilitate the deleterious effect of acute stress has been so that the reduction of presence in the target quadrant was observed in the 12th day (Fig. 3-B). This is while neither acute stress nor repeated morphine (10 mg/kg) alone had any effect on the same case (Fig. 2). As a general conclusion, although the regular dose of morphine (10 mg/kg) does not appear to disrupt the learning process alone, it does not play a role in memory recall. Therefore, in the training days, the animals learned and remembered the platform position.

As a result, we can say that the disorder observed in the learning process may be apparent and related to the other mechanisms including the reward pathway the dopamine effect. The regular dose of morphine (10 mg/kg) probably leads to the reward effect in the animal and the animal is not motivated enough to reach the platform (as a reward in the water maze); therefore, learning will be done without reference to the platform. When the repeated dose of morphine is associated with acute forced swim stress, it can practically cause damage to the memory recall. Therefore, the repeated morphine can probably pave the way for the induction of the deleterious effects of stress. While the ultra-low doses of morphine have no effect on the process of learning and memory in this battery of tests, either used alone or associated with acute stress. Thus, we suggest that some more decisive results can be obtained in this regard by contextual investigation of hippocampus area and study of important gene expressions in this area, including CamkII, CREB and the membrane glucocorticoid receptors.

Acknowledgements

This paper has been extracted from the thesis entitled "The Study of effect of stress on formed memory recall in very low to regular doses of morphine on white Norwegian Wistar rats (*Rattus norvegicus* albinus)" with the code 2012033 at Ferdowsi University of Mashhad, Faculty of Science, Department of Biology.

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Please cite this article as: Kavoosi Z, Fereidoni M, Moghimi A. The effect of acute stress on formed memory recall after prescription of very low to regular doses of morphine in rats. Zahedan J Res Med Sci (ZJRMS) 2012; 14(3): 1-6.