Facilitating effects of morphine dependence on spatial learning and memory in rat

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ABSTRACT

The effect of morphine on spatial learning and memory is controversial. In the present study, the male rats were used to evaluate the effect of morphine dependence and cold water swimming on spatial learning and memory. Dependent animals received morphine sulfate in drinking water for 25 days. Animals were divided into four groups in simple randomized manner. The first control and dependent groups, were studied in normal water (20± 2 °C), and the second control and dependent groups were studied in cold water (10-12° C). Morris Water Maze (MWM) experimentations were begun from 21st to 25th days of morphine administration. In each group of animals, spatial learning and memory parameters were analyzed. The results showed that morphine dependence may facilitates spatial learning and memory in MWM, and cold water reduces swimming speed but facilitating the formation of spatial memory. On the other hand, cold water swimming abolished the effects of morphine on spatial learning but facilitated its effect on spatial memory. The underling mechanism(s) to these phenomenon remains to be elucidated.

Keywords: Morphine dependence, Spatial learning and memory, Morris water maze.

INTRODUCTION

The role of opioid systems in learning and memory processes has been the focus of increasing experimental interests (1). Different studies indicate positive or negative effects of morphine on learning and memory processes in rodents. These differences may be due to different experimental parameters (1-3).There are several reports indicating the presence of opioidergic pathways and receptors in CA1 area and other regions of rat's hippocampus (4-6). Positive effects of opioids on hippocampal synaptic plasticity were reported previously (7,8). Also it has been reported that chronic oral administration of morphine augments long-term potentiation (LTP) in hippocampal CA1area in rats (7,9).

Morphine dependence is induced by several experimental models including "implant of morphine osmotic pump", "acute injection of morphine" and "chronic oral or injectional morphine administration"(2,10,11). In the chronic oral morphine treatment, morphine is added to drinking water; therefore, interferences of catecholamine and glucocorticoids secretions induced by stress are prevented. This model has more similarities to human addiction because animal (10) determines morphine consumption. On the other hand, chronic injection of morphine (s.c.), markedly reduces the capacity of hippocampal CA1 LTP during the period of withdrawal. Moreover, learning deficits after chronic exposure to morphine has been shown in Morris Water Maze (11). Dose-dependent impairing effects of morphine on avoidance learning have been also reported (12). Evidence indicates that administration of opioid agonists before or immediately after the task performances, impairs the learning process and Naloxone facilitated learning in MWM (1). There is a report of learning and memory impairments in passive avoidance model by intraseptal injection of morphine (13). in addition, In radial maze and Y maze models, it has been demonstrated that chronic morphine administration impairs learning in rodent (14).Injection of morphine to male rats impairs specifically spatial learning in MWM (1,3).

In MWM task the level of motivation can be manipulated by changes in water temperature (15). Injection of morphine induces deficits of place learning and it is later attenuated by colder water (10±1°C) in rat (1). These findings suggest that morphine impairs place and cue learning by reducing escape motivation rather than by impairing memory processes (1,3,16). Also, the effect of chronic oral administration of morphine

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in electrophysiological study has been shown an augmentation of LTP in male rat's hippocampus (7,9). Learning impairment in early stages of morphine withdrawal syndrome during MWM task performance has been also reported (17). Furthermore, hippocampal CA1 LTP in morphine dependent rats that were entered in withdrawal phase were reduced (11).
Consequently, the purpose of the present study was to determine the effects of morphine dependence through chronic oral administration method on spatial learning and memory in normal and cold water in rat.

MATERIALS AND METHODS

Animals
28 male N-MRI rats were studied. At the beginning of training the weight of animals were recorded (250g - 350 g). They were housed 2-3 per cage in a room with a 12:12 light/dark cycle and constant temperature (24±1 ° C). Food and water were provided *ad libitum*. All experiments were performed from 9 A.M to 6 P.M.

Chronic morphine administration
Rats were made dependent by chronic administration of morphine sulfate (Temad- Iran) at doses of 0.1, 0.2 and 0.3 $mg.ml^{-1}$ each for 48 hrs, and 0.4 $mg.ml^{-1}$ up to 25 days. Sucrose (4 $g.100ml^{-1}$) was added to drinking water to mask the bitter taste of morphine. In the control group, rats were administrated sucrose ($4g.100ml^{-1}$) in drinking water for the same duration of time (10). In our pilot study, the mean amount of water intake during the administration of highest dose ($0.4mg.ml^{-1}$) was $50mg.kg^{-1}.day^{-1}$. The withdrawal syndrome precipitated by naloxone ($4mg.Kg^{-1}$, i.p.) in dependent animals has been previously tested in this laboratory (9). Rats were investigated in 21st to 25th day in MWM.

Morris water maze
MWM was constructed from a circular black colored water tank, 140cm in diameter and 80cm in height that was located in the center of small room and was surrounded by numerous extramaze cues on the wall in the room. The tank was divided into four quadrants (N, E, W and S) was filled with water till it has reached 40 cm in depth. The experimenter stood in the southwest corner of the room. Invisible round disk platform (made of Plexiglas) 10cm in diameter were used and was located 1cm beneath the surface of the water. In the first 4 days of experiment, location of platform was constant throughout the sessions (see below). An automated infrared tracking system (CCTV B/W camera, SBC-300 (P), Samsung Electronic Co, Ltd, Korea) recorded the position of the rat in the tank. The camera was mounted 2.5m above the surface of the water.

A) Handling: Each rats received once daily, 10min handling period for three days, after which the animals were trained for two days to stand on the platform. On the first day, rats were placed on the platform which was at the center of the tank without water for 60 sec, and on the second day, the rats were placed again on the platform under the same conditions but the tank was filled with normal water, room temperature (20±2 ° C). When the rat climbed off the platform, the experimenter guided the rat to go back onto the platform (16).

B) Training procedure: Extramaze landmarks (window, door, etc.) in the room were spatial cues for learning of platform's position for animals. The position of the platform was fixed throughout the experiments. The platform was located in the north-west quarter of MWM tank with 20cm distance from the edge of the tank, and 1cm beneath the surface of water. Each rat was tested for 5 sessions. Each session consisted of 4 trials in a day.
In first sessions, a trial began by releasing the rat into the water facing the wall of the tank from one of the four quadrants (North, South, East or West). The sequence of starting location was chosen in a pseudorandom manner by computer in such a way that the starting location was different from the immediate preceding trial. The trial was concluded when the rat found the platform or at 60sec after start of the trial. If the rat could not reach the platform within 60 sec, the experimenter led the rat to the platform. The rat remained on the platform for 30sec and was then released into the water from the next starting location. After the last trial in each session, the rat was towel-wiped and placed in a drying chamber for 5 to 15min and then returned to the home cage. For evaluation of accuracy and validity of initial learning, probe trial was performed on the fifth day, in which, platform was expelled and animal during one session (consisting of 4 trials) was released into water exclusively from one of the above mentioned directions (East) that was determined by computer for all rats.

Experimental protocols
One group from control animals (control-normal) and one group from dependent animals (dependent-normal) (n=7 for each group) were studied in water at room temperature (20 ± 2°C) in MWM. Another two groups of animals

(control-cold and dependent-cold groups) (n=7 for each group) were studied in cold water(10-12 °C).

Data analysis

Data were subjected to analysis of variance (ANOVA) and followed by Tukey test for multiple comparisons. Differences were considered significant at $P<0.05$.

RESULTS

Evaluation of escape latency and swimming speed during training days

Results indicate that morphine administration reduces escape latency during training days. Also there were differences among experimental groups in second ($F(3, 24) = 9.56$, $P<0.0002$), third ($F(3, 24) = 8.83$, $P<0.0004$) and fourth ($F(3, 24) = 6.84$, $P<0.0017$) days of training. On these days, escape latency in the dependent-normal temperature group was less than that of control-normal temperature group. This difference was statistically significant in the second day of training($P<0.01$). Statistical assessments regarding results of dependent-normal temperature and dependent-cold temperature groups showed that escape latency in dependent-normal group were less than that of dependent-cold group, in all four training days. These differences were significant in the second ($P<0.001$), third and fourth ($P<0.01$) days of training (Fig. 1-A).

Results also indicates that there was a difference in swimming speed among experimental groups ($F(3, 12) = 48.66$, $P<0.0001$). Post-hoc analysis showed that differences between control-cold ($P<0.01$), dependent-normal ($P<0.001$) and dependent-cold ($P<0.01$) in comparison with control-normal were significant. Also differences in swimming speed between dependent-normal and control-cold groups was significant ($P<0.001$). On the other hand, the difference between dependent-cold and dependent-normal groups was significant ($P<0.001$) (Fig. 1-B).

Evaluation of percentage of presence in target quarter in probe trial

Percentage of the presence of animals in target quarter (quarter in which platform was located during training days) in probe trial session was investigated. Results indicates that, overall there was a significant difference among groups ($F(3, 24) = 2.87$, $P<0.05$). This difference was significant ($P<0.05$) between dependent-cold and control-normal groups (Fig. 2).

DISCUSSION

Results of the present study suggest that morphine dependence induced by chronic oral administration has facilitating effects on spatial learning and memory in Morris water maze. Morphine dependence, during training days, leads to decrease in escape latency and also an increase in the animals swimming speed as compared with the control group. These changes confirm strengthening of the spatial learning process with morphine as discussed earlier (15). Increase in the movement activity of animals by morphine has been reported previously (18). Similar to McNamara and Skelton study, in the probe trial, increase in the percentage of presence of animal in target quadrant confirms morphine's positive effects on spatial memory stabilization (1). Our results demonstrate that morphine dependence increases these parameters and provides with evidence to the positive effect of morphine dependency on spatial memory stabilization.

There are reports about the presence of opioidergic receptors and pathways in CA1 and other hippocampus areas (4,5,6). Also, it has been reported that opioids have positive effects on synaptic plasticity in hippocampus (8). Results of our previous study indicated that oral morphine dependence not only induced augmentation of LTP in CA1 area of hippocampus, but also slowed decay of LTP (9). It has also been reported that morphine facilitated remembering of the memory in passive avoidance task and this effect was completely antagonized by naloxone (19). Li et al. have also reported that acute morphine injections impaired memory performance in MWM and it can be recovered by repeated administration of equal daily dose of morphine (20). It is possible that opioid receptor's regulation are responsible for the inhibitory effects that may become tolerant to morphine (11,21). Those reports confirm findings of the present study based on positive effect of morphine dependence on the spatial learning and memory.

On the other hand, some reports have demonstrated that acute administration of morphine impairs learning and memory processes in MWM (1,3,22,23). McNamara and Skelton reported that repeated exposure to morphine slowe acquisition but do not impair memory retention in water maze task (1). It has been observed that in rats, chronic use of morphine produces residual working memory impairment and impairs the acquisition of both radial arm maze and Y maze choice escape, and with withdrawal of morphine–in subsequent weeks resulting in recovery of the morphine effects (14). In addition, it has been reported that opioid antagonists improves spatial learning of female rats in MWM (24). These results are in contrast with results of the present study. Some of the differences may be due to differences in method of morphine administration and differences in its dosing (2). Moreover, in the present study, morphine was added to the drinking water and

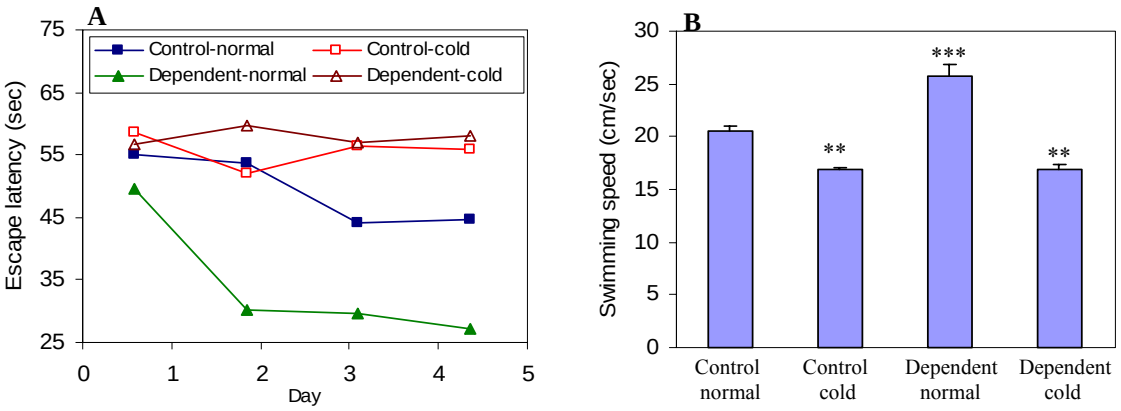

Figure 1. Effects of morphine and/or cold water on escape latency (A) and average of swimming speed (B) in the training days. Data are means ± SEM. ** P<0.01, *** P<0.001 vs. control-normal group.

therefore the level of drug reception was adjusted by animal itself. Decrease in water temperature which didn't decline central body temperature to provoke impairing memory stress, inducing reduction in motor activity of animals during training days and subsequently leading to facilitation of memory consolidation process (15). Based on the present findings, motor impairment caused by cold water not only suppressed positive effects of morphine dependence in spatial learning but also facilitated the effects of morphine and cold water in memory consolidation process, and integrated together to produce ultimate effects which was more than exclusive effects of each one. In addition, the average swimming speed of animals in "control – cold" group was decreased as compared with "control – normal" group. Decrease in movement activity due to the cold water has been previously reported (25,26). It seems that the cold water increases escape latency by decreasing the movement activity, especially in third and forth training days, and it has no destructive effects on spatial learning process. However in probe trial, the percentage of presence in target quadrant in "control – cold" group was higher than "control – normal" group. Better efficacy of animals for spatial learning in cold water versus normal water in Morris water maze has already been reported, and it has been suggested that the cold water increases the motivation for learning (1). In this study, escape latency in "dependent–cold" group was increased as compared with "dependent – normal" group, but swimming speed showed significant decreases, and we can speculate that the destructive effect is due to the damage of movement activity induced by cold water. Therefore, positive effects of morphine by increasing movement activity have been completely suppressed by negative effects of cold water. But in memory stabilization process, there is an increased efficiency in "control–cold" group rather than "control–normal" group. This increased efficiency is more obvious in "dependent–normal" group rather than "control–normal" group. Therefore, the percentage of the presence of the rats in target quadrant in "dependent–cold" group is significantly higher than "control–normal" group.

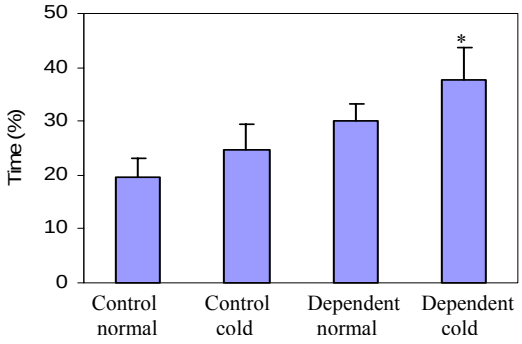

Figure 2. Effects of morphine and/or cold water on percentages of time that animals spent in target quarter in probe trial. Data are means ± SEM. * P<0.05 vs. control-normal group.

CONCLUSION

Results of the present study showed that inducing morphine dependence by chronic oral administration has a facilitating effect on spatial learning and memory in Morris water maze. Subsequently, decrease in water temperature which doesn't reduce central body temperature that induces impairing memory stress, leads to reduction of motor activity during training days which eventually leads to facilitation of memory consolidation process. Motor impairment caused by cold water sup-pressed positive effects of morphine dependence in spatial learning. However, facilitating effects of morphine with cold water in memory consolidation process is an integrating mechanism rather than dissociation of

each one's effect. Further studies are required to evaluate cellular and molecular mechanism(s) involved in these phenomena**.**

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