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Original Article

Comparison of Learning and Memory in Morphine Dependent Rats using Different Behavioral Models

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Abstract

There are several conflicting evidences showing the effect of morphine on learning and memory processes. In the present study the effect of chronic morphine administration on passive avoidance, active avoidance and spatial learning and memory of morphine dependent male rats using Passive Avoidance shuttle box and Morris Water Maze tasks were investigated, respectively. Male rats received morphine sulfate in their drinking water for 21 days. Morphine dependency was assessed by injection of naloxone HCl (2 mg/kg) showing the withdrawal signs. Our results showed that in the passive avoidance experiments although the learning of the morphine dependent group was lower than the sham and control groups, but was not statistically significant. Also no significant difference was observed between the memory retention of these groups. In the 2-way active avoidance task, learning was increased significantly in morphine dependent rats in the first day of training with respect to the sham and control groups. But, there was no significant difference in memory of these three groups. Our data in the Morris Water Maze showed that learning of the dependent group in the 3rd day of training decreased significantly with respect to the sham and control groups. But no significant difference was observed in their memory retention and also in their motor activity.

Our results showed that in the male rats, chronic morphine administration decreased spatial learning, but had no effect on spatial memory and motor activity. On the contrary, it facilitated 2-way active avoidance learning but had no effect on active and passive avoidance memory. In conclusion, it seems that the effect of morphine dependency on learning and memory in rats is task dependent and depends on the types of experimental learning and memory paradigms.

Keywords: Chronic morphine; Passive avoidance; Active avoidance; Morris water maze; Learning and memory

Introduction

Some studies suggest that endogenous opioids effect on learning and memory processes (1). Endogenous peptides are currently considered to be among the important neruomodulators in the central nervous system, being rich in the hippocampus and cerebral cortex (2, 3). The effect of morphine has been studied by several investigators in different models of learning. There are several conflicting evidences showing the effect of morphine on learning and memory. Morphine has induced deficit in spatial learning using Morris water maze task (4, 5). Also chronic opioids impair acquisition of both Radial and Y-maze choice escape in rats (6). Mc Namara and skelton (1992) reported that repeated exposure to morphine slowed acquisition of water maze task (7). In addition, the opiate antagonist naloxone enhances working memory based performance in the Radial–arm maze (8). It has been suggested that chronic administration of morphine

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impairs active avoidance learning (9, 10). Ukai and coworkers (2000) have demonstrated that opioids impair memory function on spontaneous alternation performance in mice (11).

On the other hand, evidance has indicated that there might exist some correlation between opiate reward and certain kinds of learning and memory processes. There are numerous studies in which morphine has been found to improve retention performance (12, 13).

Furthermore, it has been shown that morphine (high dose and average dose) facilitates memory in the post training of passive avoidance learning (14, 15). Nishimura and Coworkers have demonstrated that morphine has an effect on amnesia and reverses impairment of memory (16). Finally, McNamara and Skelton have reported that repeated exposure to morphine does not impair memory retention in water maze task (7). Also intra–amygdala morphine injections do not impair spontaneous alternation performance in rats (17). These controversial findings prompted us to investigate the role of chronic morphine administration on different learning tasks including active avoidance, passive avoidance (PA) and spatial learning and memory of morphine dependent male rats using shuttle box, passive avoidance apparatus and Morris water maze task respectively. In the present experiment chronic morphine administration was performed by adding morphine to drinking water so that the animal can adjust its own intake according to its need, which is similar to human addiction (18, 19).

Experimental

Animals

Adult male albino rats (Sprague-Dawley) weighing 150–200 gr were obtained from the breeding colony of the Pasteur institute of Iran. They were housed three to five rats per cage and maintained at constant temperature on a standard 12:12 light/dark cycle with light on at 7 am. Food and water were available ad libitum in the home cage.

Morphine administration and withdrawal

To achieve to the level of daily intake of morphine (48 mg/kg/24h), the drug was added by increasing concentration (48 h apart) of 0.1, 0.2, 0.3 and 0.4 mg/ml to drinking water. Sucrose %3 w/v was used to mask the bitter taste of morphine sulfate. The addicted group (n=30) received morphine sulfate for 21 days. This procedure has this advantage that morphine intake is determined by the animal itself and not by the experimenter. In the sham group only sucrose was added in their drinking water (n=30), and the control group (n=30) received only water. Morphine dependency was assessed by injection of naloxone HCl (2 mg/kg), showing the withdrawal signs (n=8) for 20 min including: wet-dog shakes, head shakes, diarrhea, ptosis, chattering teeth, writhing, ejaculation, chewing, paw tremor and irritability to touch and handling. Weight loss was measured 2h after administration of naloxone (19).

Passive avoidance response

A two compartment step–through passive avoidance (PA) apparatus was used as has been reported previously (20). In acquisition trial the animals were placed in the illuminated compartment and 5 s later a guillotine door was raised. After entering the dark compartment, the guillotine door was closed and immediately a 50 Hz, 1 mA constant current shock was applied for 1.5 s. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds. The number of trials (entries into the dark chamber) was recorded. After 48 hrs of PA training, the rat was placed in the illuminated chamber and 5s later, the guillotine door was raised for 5 min. The latency of entering the dark compartment (step-through latency = STL) was recorded (21).

Active avoidance response

The apparatus consisted of a shuttle box with two compartments (90 × 2 × 18) separated by a wall and equipped with an electrifiable grid floor (3 mm in diameter, 10 mm apart). The two compartments were connected by a guillotine door. A lamp (4w) in the shuttle box was used as the conditioned stimulus and presented for 10 s until the rat crossed to the opposite compartment. If the rat didn't cross to the opposite compartment, then it would receive a foot shock (0.5 mA constant current for maximum 5 s). When the rat crossed to the opposite compartment during the conditioned stimulus, an avoidance response was recorded. Animals were trained in two consecutive days,

each day 50 trials in the shuttle box apparatus, and their memory retention was tested one week later using 20 trails. Active avoidance learning was performed daily at 9 am to 11 am.

Morris water maze

The water maze was a dark circular pool, 140 cm in diameter and 80 cm high that was filled to a depth of 60 cm with 22±1^{o}C water. A clear plexiglass platform (11 cm diameter) stood 1 cm below the water surface, in the center of one of the arbitrarily designed orthogonal quadrants. The platform provided the only escape from the water. The position of the animal was monitored by a camera that was mounted above the center of the pool. The distance to reach the platform was measured. Rats received 4 trials per day (two blocks separated by a 3 min interval) for 5 consecutive days, and their memory was tested in the 6th day, during which the platform was removed (Probe trial).

Measurement of locomotor activity

Animals were tested for possible effects of chronic morphine administration on their locomotor activity using the activity monitor apparatus (Columbus). After placing the animal in the apparatus the total number of locomotor activity were counted.

Statistical analysis

All data were analyzed by one–way analyses of variance (ANOVA) followed by Tukey's test for multiple comparison. $p<0.05$ was accepted as significant.

Results and Discussion

There are different protocols to induce tolerance and dependence to morphine such as daily intraperitoneal injection (22), or using subcutaneous morphine pellets (23). In our experiment, morphine was administered in drinking water to avoid stress of handling and injection, or surgical procedure of morphine pellet implantation. The total morphine intake by this procedure is 48 mg/kg in 24 hr (18). The development of dependence by this protocol of morphine administration was checked in our pilot study using naloxone HCl and all treated rats showed signs of the withdrawal syndrome. This model of dependence is more similar to human dependence and addiction, because the animal can adjust the amount of drug received during the development of dependence.

Many reports have demonstrated that acute administration of opioids impairs learning and memory processes (10, 24, 25). On the other hand, the effect of chronic administration of morphine on memory processes is controversial.

Effect of chronic morphine administration on PA acquisition and retention.

In the passive avoidance experiments, our results showed that 30% of the addicted group learned the PA task slower than the control and sham groups, which was not statistically significant. Also, no significant difference was observed between the memory retention of these groups. The step through latency in the control, sham and addicted groups were 281±5.01, 272±7.2 and 282±3.7 seconds, respectively.

There are controversial reports about the effect of morphine on PA learning and memory. Shigii and coworkers have shown that acute administration of morphine (10 mg/kg) before retention test facilitated PA memory retrieval in mice (26). On the other hand, several investigators have reported that acute morphine administration (5-10 mg/kg) produced amnesia in PA learning in mice and rats (27, 28).

In another study Cestari and colleagues (29) reported that there are differences in the effect of morphine in different strains of mice on PA memory retention. They showed post training morphine administration (1-2.5 mg/kg) in C57 mice improved and in DBA mice impaired memory retention (29). The difference between these results and our observed data may come from the fact that in all of these experiments morphine was given acutely, while in our experiments, morphine was used chronically. It seems that in chronic situation, morphine may act differently on its receptors in the brain areas that are responsible for a simple learning task such as PA learning. Also, the dose of morphine seems to be a key issue in memory retention which might be due to the fact that different neurotransmitter systems are activated or inhibited with different doses of morphine as other studies have suggested (30, 31), which needs to be further elucidated.

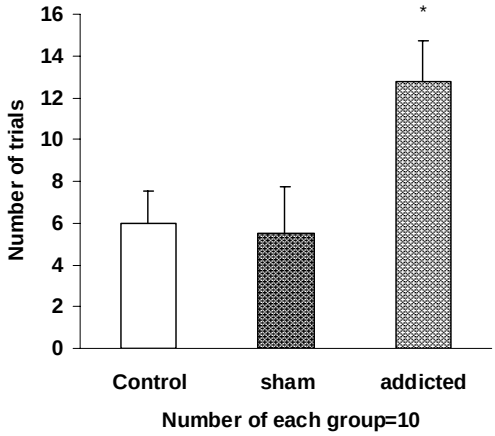

Figure 1. Comparison of active avoidance learning in lst.day between control, sham and addicted groups. Learning of addicted groups was significantly higher than the other groups. *P=0.02
Ordinate shows the mean ±SEM.

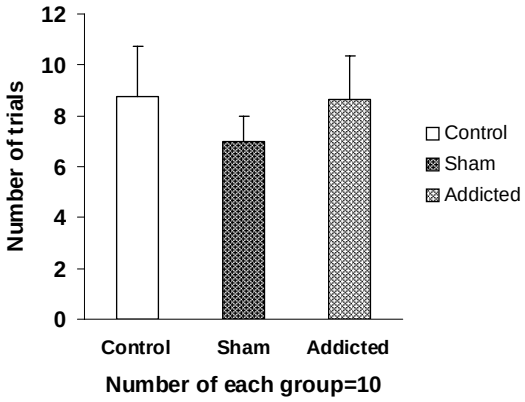

Figure 2. Comparison of retention of active avoidance response between control, sham and addicted groups after one week post training.
Ordinate shows the mean ±SEM.

Effect of chronic morphine administration on 2-way active avoidance learning

Our results showed that in 2-way active avoidance task, learning was increased significantly in morphine dependent rats in the first day of training with respect to the sham and control groups (p=0.02) (Figure 1). But there was no significant difference in memory of these three groups (Figure 2).

These results are in agreement with those obtained by some investigators, which have shown improvement of memory with chronic morphine administration (12, 13, 16). Previous studies in our labratory have also indicated that chronic morphine administration would enhance long term potentiation (LTP) induction which is indicative of learning processes in neurons of hippocampal slices (19, 32). On the other hand, some reports indicate the inhibitory role of morphine in active avoidance task (9, 29). Aguilar and colleagues have observed that morphine administration impairs dose dependent acquisition and performance of active avoidance in mice (9). Acquisition observed by some investigators may be due to the fact that the impairing effect of morphine has been masked by the stimulant effects of morphine on locomotor activity. But in our experiments, morphine had no significant effect on locomotor activity using activity monitoring apparatus.

Effect of chronic morphine administration on spatial learning and memory using Morris water maze task

Our data in the Morris water maze (MWM) showed that learning of dependent group in the third day of training decreased significantly with respect to the sham and control groups (p=0.01) (Figure 3). But there was no significant difference in their memory retention on the 6^{th} day of training (Figure 4).

Finally, no significant difference was observed between the motor activities of the experimental groups. The average locomotor activity over 45 min in the sham group was 580±72 count, and in addicted group was 552±48 count.

Some investigators have shown spatial learning deficit in chronic opiate administration (7, 33). It has been demonstrated that chronic use of opiate leads to impairment of performance in MWM task (22, 34). Also, a reduction in hippocampal LTP, which represents learning and memory, has been observed (34). In addition, it has been also shown, that morphine has memory impairment effects at escalating doses for 13 days in mice using MWM task (35). These results are in agreement with those obtained in our experiments. It has been shown that opioids inhibit the activity of cholinergic neurons in medial septal area, which project to the hippocampal formation. Ragozzino and Gold reported that morphine infusion in to medial septum causes spatial memory impairment (36).

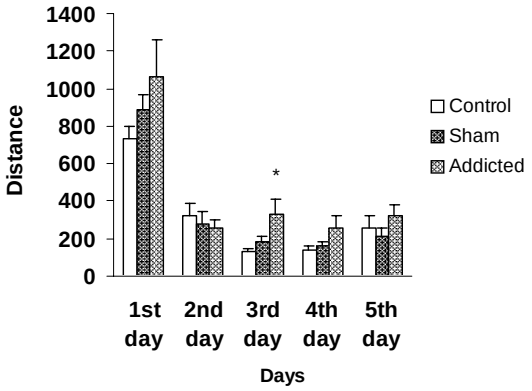

Figure 3. Comparison of distance to reach platform in days 1-5 between control, sham, and addicted groups in Morris water maze task. In addicted group the distance to reach the platform was significantly higher than the other two groups in 3rd day of training. *P=0.01
Ordinate shows the mean ± SEM of distance (cm) to reach the plattorm region.

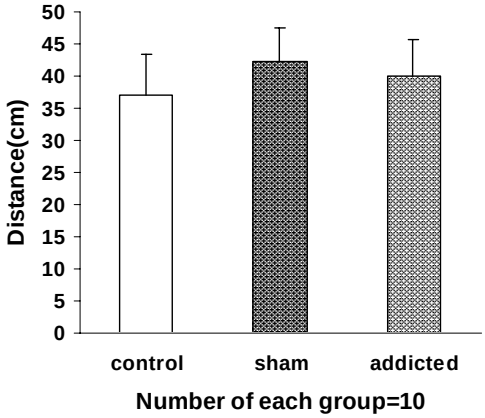

Figure 4. Comparison of memory retention in day 6, as represented by the distance to reach the platform region between control, sham and addicted groups in Morris water maze task.
Ordinate shows the mean ±SEM of distance (cm) to reach the platform region.

Moreover, Sandin et al. have shown that dynorphine B causes learning deficit in MWM and suggested that dynorphine peptides by acting on opioid k receptor has a modulatory effect on synaptic plasticity in hippocampus. (37).

The results of MWM show that morphine impairs learning, While they oppose the results of the active avoidance task. Given that the two types of memory models are very different, it is possible that in MWM, the animals were more anxious during testing on the water filled tank than in the closed compartmentalized active avoidance box, and therefore, they needed more time to complete the trials. It is also possible that the addicted animals needed more trials to learn and process environmental cues in the testing room to help them orient on the MWM, which was not a necessary strategy in the active avoidance shuttle box.

Finally it is concluded that the effect of morphine on learning and memory is task and state dependent, and depends on different parameters such as experimental paradigm, the dosage and route of morphine administration and the animal's strain.

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