Effects of morphine dependence on the performance of rats in reference and working versions of the water maze

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Abstract

Numerous studies have dealt with the role of opiate system in tasks aimed at measurement of cognitive behavior, but the role of morphine dependence on learning and memory is still controversial. In this study chronic exposure to morphine was employed to evaluate learning ability and spatial short-term memory (working memory) and long-term memory (reference memory) in the water maze task. Male albino rats were made dependent by chronic administration of morphine in drinking water that lasted at least 21 days. In Experiment 1, the performance of animals was evaluated in reference memory version of the water maze. Rats were submitted to a session of 6 trials for 6 consecutive days to find the submerged platform that was located in the center of a quadrant. Latency and traveled distance to find the platform were measured as indexes of learning. Memory retention was tested 24 h after the last training session in a probe trial (60 s) in which there was no platform and the time spent in each quadrant of the water maze was recorded. Results indicated that latency and traveled distance to find the platform were same in control and dependent rats during training days, but during the probe test morphine-dependent group spent significantly less time in the target quadrant. In Experiment 2, training on working memory version of the water maze task was started. Only two trials per day were given until the performance of animals was stabilized (at least 5 days). Final test was done at day 6. Acquisition–retention interval was 75 min. No significant differences were found on acquisition and retention trials between morphine and control groups. Our findings indicate that chronic exposure to morphine did not impair learning ability, but partially impaired retention of spatial long-term (reference) memory. Moreover, dependence on morphine did not affect either acquisition or retention of spatial short (working) memory.

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1. Introduction

Extensive evidence indicates that acute and chronic exposures to opiate drugs modulate memory process. When injected after training in a variety of tasks, opiate receptor agonists and antagonists impair and enhance memory consolidation, respectively [1]. However, the effects of chronic exposure to opiates on cognitive functions are still controversial. For instance, Spain and Newsom [2] demonstrated that chronic morphine treatment produced a residual working memory impairment in rats and impaired the acquisition of spatial long-term memory in both radial maze and Y-maze choice escape. Li et al. [3] observed that morphine administration impaired acquisition of spatial memory in the water maze at both equal daily dose and the escalating dose regimens. This impairment was restored by withdrawal. McNamara and Skelton [4] reported that repeated exposure to morphine slowed acquisition but did not impair memory retention in the water maze. Moreover, Pu et al. [5] found that chronic (subcutaneous injection) use of opiates in rats led to an impairment of long-term memory in the water maze, whereas re-exposure of animals to opiates restored the performances in the water maze. Dougherty et al. [6] found that morphine rats trained in the water maze during early, but not late withdrawal exhibited
deficits in performance in the water maze. The reasons for the observed discrepancies may be reflected in the experimental protocols such as type of behavioral tasks, procedure for chronic morphine treatment, etc.

In a recent study, we have shown that chronic exposure to morphine resulted in an impairment of long-term potentiation (LTP) in rat’s hippocampus in vitro [7]. Since it is suggested that LTP is a synaptic basis of long-term memory [8], it would be of interest to test whether the same protocol used for the dependence development in our previous LTP experiments can affect performances of animals in reference and working versions of the water maze. The important advantages of the present study are: 1) the protocol used for the dependence development was the same as before in this laboratory [7] by administration of morphine in drinking water. This procedure is more similar to human dependence and addiction, because the animal can adjust the amount of received drug during the development of tolerance [9]. Moreover, in comparison to other procedures (implantation or injection) this protocol does not induce stressful responses including release of glucocorticoids and catecholamines from the adrenal gland, which in turn, both affect memory processing [10]. 2) A variety of behavioral tasks are used to test the effect of acute or chronic administration of morphine on learning and memory, but the water maze task is very useful for the evaluation of the effect of chronic administration of drugs on spatial learning and memory. The main advantage of this task is that it allows the simultaneous evaluation of learning, spatial memory and working memory as well as motor activity by submitting the same animals to distinct and consecutive phases of training [11].

2. Materials and methods

2.1. Animals and induction of morphine dependency

Twenty male albino rats (250–300 g, Razi Institute, Iran) were housed 5 per cage, on a 12:12-h light/dark cycle. All efforts were made to minimize the number of animals used and their suffering. Rats were allowed free access (except at times of behavioral testing) to morphine solution as their sole source of fluid, and food. In order to make the morphine solution less bitter and more palatable for the rats to drink, sucrose 3% w/v was added to morphine sulfate solution (tap water with morphine). Rats (n = 10) were made dependent by chronic administration of morphine 0.1 mg/ml, 0.2 mg/ml and 0.3 mg/ml each for 48 h and 0.4 mg/ml during the following days [9]. The mean amount of water intake and morphine during the administration of the highest dose (0.4 mg/ml) were 342 ml/kg and 137 mg/kg per day, respectively. This procedure lasted at least 21 days to complete morphine dependence establishment. Control rats received only sucrose in their water. After termination of the behavioral experiments, morphine withdrawal was induced by an intra-peritoneal injection of naloxone HCL (2 mg/kg) and the behavioral signs of withdrawal including diarrhea, writhing, ejaculation, chewing, paw tremor, exploring and irritability to touch were monitored for a 30 min period. Weight loss also was determined 24 h after the administration of naloxone.

2.2. Testing spatial memory in a water maze

2.2.1. Apparatus

A detailed description of the apparatus and tracking system has been presented in our previous reports [12,13]. In brief, the water maze was a blue circular pool (140 cm in diameter and 50 cm high) filled to a 25 cm depth with 20±2 °C water.

2.2.2. Reference memory testing

For spatial training, rats were subjected to a session of 6 trials for 6 consecutive days. Twenty-four hours prior to the start of training, rats were allowed to swim 3 min in the pool containing no platform for habituation. On each trial, the rat was placed into the water from one of the four cardinal points of the compass (N, E, S, W), which varied from trial to trial in a quasi-random order. The rat had to swim until it found and climbed onto the escape platform. Rats were guided to the platform if they failed to locate it within 60 s. The rat was allowed to stay on the platform for 30 s as the inter-trial interval. After the last trial, the rat was towel dried and placed in a holding cage under a heating lamp before it was returned to the home cage.

The performance of each rat was tested 24 h after the final training day in a probe trial (60 s) during which the platform was removed. The parameters measured from probe test were time spent in each quadrant and total swim distance and swimming speed.

2.2.3. Working memory testing

Two days after reference memory testing, training on working memory version of the water maze task was started. Only two trials per day were given until the performance of animals in this task had stabilized (at least 5 days). Final test was done at day 6. In the first trial (acquisition), the rat had to find the platform in a new position and was allowed to stay there for 20 s before it was returned to the home cage. In the second trial (retrieval) 75 min later, the animal was stated from a different point for the same position of the platform.

2.3. Locomotor activity measurement

Immediately after probe test, locomotor activity of each animal was measured using an automated activity monitor system (TSE infraMot, TSE, Bad Homburg, Germany). The system uses so called “passive infrared sensors”. These sensors register the activity of animal by sensing the body heat image, i.e. infrared radiation, and its spatial displacement over time. The sensor unit (contained infrared sensors) was placed on the metal grid cover of a home cage. Locomotor activity of each rat was measured for six 2 min intervals. Only one animal was placed in each activity chamber per measurement time.

2.4. Statistical analysis

The data were analyzed by one-way and two-way analysis of variance (ANOVA) with a repeated measurement followed by Tukey test for multiple comparisons. Student’s t-test was used to compare two independent groups. Value of P<0.05 was accepted as significant.
3. Results

3.1. Test of dependence on morphine

Dependent rats (n=10) showed typical withdrawal syndrome induced by a single injection of naloxone during 30 min period observation as reported in our previous work [7]. We found that writhing was the most common withdrawal sign. Time-matched control rats did not show defined withdrawal signs.

3.2. Performance of control and dependent animals in reference memory

Fig. 1 shows the performance of control and dependent rats in reference memory version of the water maze task. Both groups learned this task well and spending less time each day to find the platform. ANOVA on escape latency data (Fig. 1a) revealed that the lack of a significant effects of treatment (F_{1,18}=0.37, P=0.847), a significant effects of days (F_{5,108}=43.861, P=0.000), and the lack of a significant interaction between both factors (F_{5,108}=1.857, P=0.108). Analysis of swim paths (Fig. 1b) revealed similar results. Again, no significant effects of treatment (F_{1,18}=0.196, P=0.659), a significant effects of days (F_{5,108}=40.376, P=0.000), and the lack of a significant interaction between them (F_{5,108}=1.122, P=0.353) were found.

Memory retention for the previously learned spatial information (platform location) was tested in both groups one day after the last training in a free probe trial for 60 s. Two-way ANOVA (Fig. 2) of the probe trial data (groups x quadrants) revealed that the lack of a significant effects of groups (F_{1,18}=0.48, P=0.827) but a significant effects of quadrants (F_{3,72}=38.67, P=0.000), and a significant interaction between groups and quadrants (F_{3,72}=5.077, P=0.003). There was a spatial bias to the target quadrant in both groups. In both groups, the time spent in the target quadrant was significantly longer the time they spent in the remaining quadrants (P<0.01) but the time spent by the morphine-dependent group in the target and opposite quadrants was significantly shorter and longer than that of control group, respectively (P<0.01 in both cases). This finding indicates that memory retention for spatial information is partially accessible in morphine-dependent rats.

3.3. Performance of control and dependent animals in working memory

Fig. 3 illustrates the mean escape latencies on the acquisition and retention trials for both control and morphine-dependent...
rats. Student’s t-test indicated that escape latencies of morphine and control groups were not significantly different in the acquisition ($t_{18}=1.543$, $P<0.14$) and retention ($t_{18}=0.676$, $P<0.5$) trials. This finding indicates that morphine dependence has no effect on either acquisition or retention or working memory in the water maze.

3.4. Locomotor activity

The locomotor activity results are shown in Fig. 4a and b. There were no significant differences between groups in the total activity recorded for a 12 min period ($t_{18}=0.492$, $P=0.629$). A two-way ANOVA (groups × times) of locomotor activity at each two min intervals of a 12 min period showed the lack of a significant effect of groups ($F_{1,12}=0.489$, $P=0.486$), a significant effect of times ($F_{5,108}=7.796$, $P=0.000$), and the lack of a significant interaction between the effects of groups and times ($F_{5,108}=0.214$, $P=0.956$). Post-hoc analysis indicated that in both groups the locomotor activity was increased during the first two min interval ($P<0.05$ for all comparisons). These results indicate that there were no significant differences in locomotor activity in control and dependent groups.

4. Discussion

The main finding of our study is that chronic exposure to morphine did not impair learning ability in reference memory (long-term memory) version of the water maze, but partially impaired retention of spatial long-term memory. Moreover, neither acquisition nor retention of working memory was affected by dependence on morphine. The protocol used for the dependence development was the same as before in this laboratory by administration of morphine in drinking water [7]. This procedure is more similar to human dependence and addiction, because the animal can adjust the amount of received drug during the development of tolerance [9].

Reference memory is a permanent (long-term) memory reflecting stable conditions in the environment. In Expt.1, we observed that all animals learned to locate the platform during the 6 successive days of training as indicated by decreasing escape latencies as training progressed. No significant differences were found between control and morphine-dependent groups in spatial memory acquisition in the water maze. Although the probe test data indicated that both groups showed a spatial bias to the target quadrant, the time spent in the target quadrant by dependent rats was significantly shorter than control group. Two important points can be made from these findings: 1) similar to control rats, dependent ones quickly learned to swim directly towards the platform from any starting position at the circumference of the pool, but during the probe test dependent rats partially failed to remember the exact position of the platform. 2) dependency did not disrupt a more general behavioral strategy directing their search to the most likely locations. The results of this study contrast with the results of Li et al. [3] showing that morphine administration impaired acquisition of spatial memory in the water maze at both equal daily dose and the escalating dose regimens, but they did not test retention of information in a free probe trial. The reasons for this discrepancy may be reflected in experimental procedures. For example, they administrated morphine daily (at the equal or escalating doses) and 20 min later tested animal in the task, but we first made animals dependent by administration of morphine in drinking water for at least 21 days and then tested the animals. Thus, the preserved ability of animals to acquire the task might be related to tolerance development to morphine after prolonged treatment as demonstrated in analgesia test [14]. Our results indirectly may confirm the findings of two other studies. First, Pu et al. [5] administrated morphine twice per day for 15 days. For the last 5 days, rats were trained and tested in the water maze 2 h before or 1 h after the second daily injection. They found that in the former condition rats displayed poorer performance (latency to find the platform) compared with control group, while in the later condition (re-exposure to morphine) rats showed normal performances compared with rats in the former condition. Results of the probe test indicated that rats in the former condition were impaired, whereas in the later condition showed normal performance compared with control. Second, Dougherty et al. [6] implanted morphine-treated rats with osmotic pumps containing morphine. Implants were removed after 7 days. Beginning 1 or 21 days following pump removal, the rats were tested for 8 days in the water maze. Morphine rats trained in the water maze during early, but not late withdrawal exhibited deficits in performance in the water maze.

Many nonspecific factors affecting sensory, attentional and motor processes influence place navigation. Our results revealed
that during training no significant differences were found between dependent and control animals in the traveled distance (Fig. 1b) to find the platform. However, morphine-dependent rats presented a tendency to a higher swim speed during training and the probe trial. Also when we tested motor activity in an activity box, the total amount of movement of control and dependent rats was the same, indicating the normal motor activity in both groups. Thus, the reasons for a tendency higher velocity in morphine-dependent rats in the water maze are not known.

The underlying mechanisms for a partial disruption of long-term memory retention in morphine-dependent animals are not known. The hippocampus is known to be one of the major area involved in spatial learning and memory [15,16]. This area expresses both opioid peptides [17] and opiate receptors [18,19]. Neurons in the hippocampus exhibit LTP, a long-lasting use-dependent modification of synaptic strength, which may be a cellular substrate of learning and memory [8]. There are some reports indicating that chronic exposure to morphine as well as morphine dependence can interact with the induction of LTP. Prenatal exposure of morphine shifts long-term plasticity of hippocampal synapses in favor of long-term depression [20]. In another study, it was found that chronic exposure of rats to morphine markedly reduced the capacity of hippocampal LTP during the period of drug withdrawal. The capacity of LTP could be restored to the normal level by re-exposure of the rats to opiates, indicating that synaptic function was already adapted to morphine. Moreover, testing in the water maze revealed that rats whose LTP was severely reduced exhibited poorer performance in hidden (spatial), but not visible versions of the water maze, indicating a selective impairment of spatial memory [5]. Hassi-son et al. [21] recently showed that chronic morphine treatment did not change LTP in hippocampal CA1. Our present behavioral findings may be in parallel with our electrophysiological findings. In the previous LTP experiments [7], using the same protocol for the dependence development, we have found that slices from morphine-dependent rats maintained in artificial cerebrospinal fluid (ACSF) with morphine exhibited significantly attenuated hippocampal CA1 LTP, but hippocampal slices from morphine-dependent groups in ACSF with either naloxone or just morphine free exhibited normal CA1 LTP. Such findings may explain the neural substrate of memory retention deficits seen in the present study as well as in opiate addicts. In addition to hippocampus, other brain regions such as ventral tegmental area and nucleus accumbens may be involved in cognitive deficits induced in dependent animals. These areas modulate emotional value and the strength of memories encoded in the hippocampus [22].

Working memory (short-term memory) stores information for correct solution of an ongoing task, i.e., remembering the coordinates of the new position of the escape platform. In Expt.2, we observed that chronic morphine administration has no effect on acquisition and retention of working memory test. This finding is consistent with results of study by Spain and Newsom [2] showing that chronic morphine dose not impair spatial working memory in the radial maze. However, other studies using an 8-arm radial maze have shown differential effects on working memory. Braida et al. [23] have demonstrated that acute administration of increasing dose of morphine impairs performance in working memory components of the task. On the other hand, Slamberova et al. [24] have shown that prenatal exposure reduces the time needed to complete the trials, but does not affect the accuracy of performance in male rats. In contrast, prenatal exposure had no effects on either the time or the accuracy of performance in female rats. Thus, the effects of morphine on working memory are complex and depend upon the type of task, gender of experimental subjects, and whether use is chronic versus acute.

In conclusion, our findings indicate that dependence on morphine did not impair learning ability in reference version of the water maze, but partially impaired memory retention for the previously learned spatial information. Moreover, dependence on morphine did not affect either acquisition or retention of spatial working memory (short-term memory). The underlying mechanisms for a partial disruption of long-term memory retention in morphine-dependent animals may be due to an impairment in hippocampal LTP as shown in our previous work [7]. Such electrophysiological and behavioral findings may explain the neural substrate of memory deficits seen in opiate addicts [25].

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References


