Original Article

The Time Profile of Morphine Effect on Different Phases of Inhibitory Avoidance Memory in Rat

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Abstract

Backgrounds: The amnesic effect of morphine is well known in the laboratory animals. But, it is unclear that morphine at what times can exactly affect different phases of memory, including acquisition, consolidation, and retrieval. Therefore, we investigated the time profile of morphine's amnesic effect on passive (inhibitory) avoidance learning and memory in male Wistar rats.

Methods: In order to evaluate the outcomes of pre- and post-training administrations of morphine, the animals were trained in a step-through type of passive avoidance task at various time points, and were tested 24 h after training to measure memory retrieval.

Results: The results showed that acquisition of memory was impaired in the animals that received a dose of 7.5 mg/kg of morphine (Intraperitoneally) at 0, 30 min, and 1 h before training, as evidenced by a decrease in step-through latency on the test day. Post-training administrations of morphine at 30 min and 1h, 4h except for the time immediately after training, did not impair memory consolidation. The results also showed that pre-test administrations of morphine at 0 and 30 min before the test, impaired retrieval of inhibitory avoidance memory.

Conclusion: Taken together, the results suggest that morphine, when injected at different time points before training, after training, or before testing affects different phases of inhibitory avoidance memory. With regard to the time of injections related to each phase, other experiments can be designed to investigate molecular mechanisms involved in the impairing effect of morphine in each phase.

Keywords: Amnesia, consolidation, inhibitory avoidance, morphine, retrieval

Cite this article as: Zarrindast MR, Ardjmand A, Rezayof A, Ahmadi S. The time profile of morphine effect on different phases of inhibitory avoidance memory in rat. *Arch Iran Med*. 2013; **16(1):** 34 – 37.

Introduction

Any approach focusing on the molecular mechanisms underlying memory encompasses three phases of memory including acquisition, consolidation, and retrieval or recall of memory.[1] Time of drug administrations at some points in relation to training or testing is used for determining whether a particular receptor or molecule is involved in the acquisition, consolidation, or retrieval phase of memory.[2] Pharmacological approaches offer the highest temporal specificity because they can be applied and removed from the system within a relatively short time span.[3] For this purpose, both the time of drug administrations in relation to training or testing (pre- or post-training and pre-test administrations) and different times in each phase should be considered for the evaluation of drug effects on acquisition, consolidation, and retrieval of memory. Manipulations before acquisition will affect early stages of consolidation, and manipulations after training will affect consolidation. Pre-retrieval manipulations, however, may affect not only the retrieval but also late stages of consolidation or retention.[4,5]

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Accepted for publication: 28 July 2012

Emotional memory in animals has extensively employed paradigms of aversive learning as tools for evaluating the neural mechanisms of memory acquisition and consolidation. Among the aversive learning paradigms, passive or inhibitory avoidance (IA) task is widely used.[3,6–8] Many reports have demonstrated that acute administration of opioids impairs learning and memory of behavioral tasks.[9–11] In particular, according to previous studies either pre- or post-training administrations of moderate doses of morphine (5 – 10 mg/kg) impaired IA memory.[12–18] Morphine-induced impairment may be due to the involvement of mu-opioid receptors, since the action of opioids could be inhibited by mu-opioid receptor antagonist.[12,16,19,20] Considering that morphine affects learning and memory processes and produces amnesia, the major aim of this study was to investigate the exact time of morphine's effect on various stages of IA memory. To approach this aim, morphine was injected before and after training and also before testing. The extent of memory retrieval was then examined to find out the exact stage and time point during which the effect takes place.

Patients and Methods

Animals

Male Wistar rats (Pasteur Institute, Tehran, Iran), weighting 220 – 240 g were used. All animals were housed upon their arrival in the laboratory (one week before the experiments) in groups of four in each cage with free access to food and water. Animals were maintained at a constant temperature of 22 ± 2 ºC, under a 12/12 h light-dark cycle (light began at 07:00 AM). The experiments were performed during the light phase between 08:00 and 12:00. Each experimental group consisted of eight animals; each animal was tested only once. All procedures were carried out in

accordance with international guidelines for animal care and use (NIH publication #85–23, revised in 1985).

Drugs

Morphine sulfate (Tolidaru, Tehran, Iran) was dissolved in sterile saline and injected intraperitoneally (IP) in a volume of 1 ml/kg. Morphine was injected at different times before or after training, and prior to the test.

Inhibitory avoidance apparatus

Inhibitory avoidance (IA) memory was evaluated in a step-through task which uses the natural preference of rats for a dark environment. The IA apparatus consisted of a dual chamber box separated by a wall divided into two equal sized compartments ($20 \times 20 \times 30$ cm^3). A guillotine-like door (7×9 cm^2) on the separating wall can be lifted manually. The walls and the floor of the light compartment were coated with white opaque resin and were illuminated by a 20 W electric bulb installed at a distance of ~50 cm above the floor of the apparatus. The walls of the dark compartment were dark and its floor consisted of stainless steel grids (3 mm in diameter and 1 cm apart from each other). Intermittent electric shocks (50 Hz, 3 s, and 1 mA intensity) were delivered to the grid floor of the dark compartment by an isolated stimulator (Borj Sanat CO, Tehran, Iran).

Behavioral procedures

Training

All animals were allowed to habituate to conditions for at least 30 min prior to the experiments in a room with light and sound attenuated condition. Then, each animal was gently placed in the lit compartment of the apparatus, after 5 s the guillotine door was lifted, and the animal was allowed to cross to the dark compartment. The latency of each animal to cross to the dark compartment was recorded, and those animals that waited more than 100 s to cross to the dark compartment were omitted from the experiments. When the animal crossed with all four paws to the dark compartment, the guillotine door was closed and the animal was placed into its home cage. After 30 min, the animal was placed into the apparatus, the guillotine door was again opened after 5 s, the door was closed as soon as the animal entered the dark compartment, and a foot shock (50 Hz, 1 mA, and 3 s) was immediately delivered to the grid floor (shock trial). After 20 s, the animal was taken from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in a way similar to the prior trials and its latency for crossing to the dark compartment was recorded. In the case of no entrance to the dark compartment within 120 s, a successful acquisition of IA memory was recorded for the animal. Otherwise, in the case of entrance to the dark compartment for the second time (before 120 s), the door was closed and the animal received another shock again.

Testing

The testing session was held 24 h after the training session. On the test day, no electric shock was applied. Each animal was gently placed in the light compartment, after 5 s the door was opened, and the step-through latency for crossing to the dark compartment was recorded for each animal. The testing session ended either when the animal entered the dark compartment or when it remained in the light compartment until the cut-off time (300 s).

Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA). Mean ± SEM of step-through latencies of eight rats per each experimental group on the test day were used in the analyses. Further analyses for between-groups comparisons were done using post hoc Tukey's test. In all comparisons, $P < 0.05$ was considered statistically significant.

Experimental design

Experiment 1

Effects of pre-training administration of morphine at different time points on acquisition of IA memory were evaluated. Six groups of animals received saline (1 ml/kg) or morphine (7.5 mg/kg) immediately or at different time points (0, 30, 60, 120, and 240 min) prior to the shock trial of training.

Experiment 2

Effects of post-training administration of morphine (7.5 mg/kg) at different time points on IA memory consolidation were examined. Six groups of animals received post-training saline (1 ml/kg) or morphine (7.5 mg/kg) at different time points (0, 15, 30, 120, and 240 min) after training.

Experiment 3

Effects of pre-test administration of morphine at different time points on IA memory retrieval were evaluated. Six groups of animals received pre-test administration of saline (1 ml/kg) or morphine (7.5 mg/kg) at different time points (0, 30, 60, 120, and 240) prior to the test.

Results

Effect of pre-training administration of morphine at different time points on acquisition of IA memory

Figure 1 shows the effect of pre-training administration of morphine (7.5 mg/kg) at different time points (0, 30, 60, 120, and 240 min) on acquisition of IA memory. One-way ANOVA indicated that pre-training administration of morphine altered the acquisition of IA memory [$F(5, 42) = 387.58$, $P < 0.001$]. Post hoc analysis with Tukey's test revealed that immediate pre-training administration of morphine and also its administration at 30 and 60 min before training impaired IA memory acquisition.

Effect of post-training administration of morphine at different time points on consolidation of IA memory

Figure 2 shows the effect of post-training administration of morphine (7.5 mg/kg) at different time points (0, 15, 30, 120, and 240 min) on consolidation of IA memory. One-way ANOVA indicated that post-training administration of morphine altered IA memory consolidation [$F(5, 42) = 118.75$, $P < 0.001$]. Post hoc Tukey's test showed that post-training administration of morphine, except for immediate post-training administration, did not impair IA memory consolidation.

Effect of pre-test administration of morphine at different time points on retrieval of IA memory

Figure 3 shows the effect of pre-test administration of morphine (7.5 mg/kg) at different time points (0, 30, 60, 120, and 240 min) on retrieval of IA memory. One-way ANOVA indicated that pre-test administration of morphine altered retrieval of IA memory [F

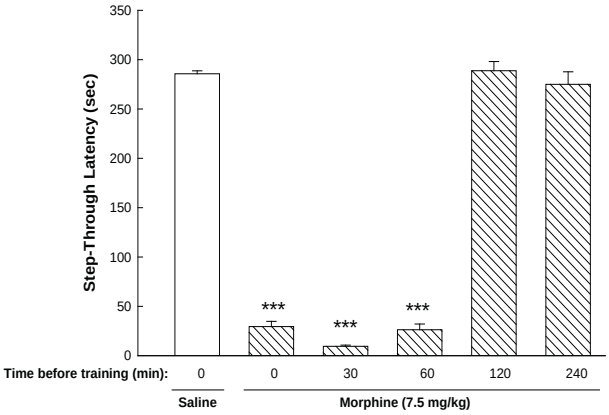

Figure1. Effects of pre-training administrations of morphine on acquisition of IA memory. Six groups of animals received saline or morphine (7.5 mg/kg) immediately or at different time points (0, 30, 60, 120, and 240 min) prior to training. All groups were tested 24 h after training session. Each bar represents mean±SEM of data from eight animals per group. ***P <0.001 compared to saline control group.

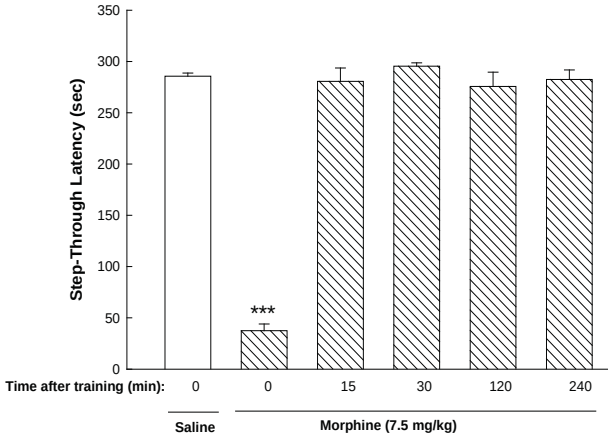

Figure 2. Effects of post-training administrations of morphine on consolidation of IA memory. Six groups of animals received post-training morphine (7.5 mg/kg) at different time points (0, 15, 30, 120, and 240 min) after training. All groups were tested 24 h after training session. Each bar represents mean±SEM of data from eight animals per group. ***P<0.001 compared to saline control group.

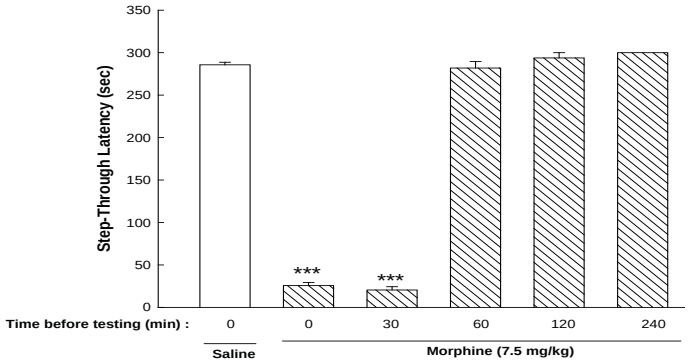

Figure 3. Effects of pre-test administrations of morphine on retrieval of IA memory. Six groups of animals received pre-test administration of morphine (7.5 mg/kg) at different time points (0, 30, 60, 120, and 240 min) prior to the test. Each bar represents mean±SEM of data from eight animals per group. ***P<0.001 compared to saline control group.

(5, 42) = 848.81, $P < 0.001$]. Post hoc Tukey's test also revealed that immediate pre-test administration of morphine and also morphine administration at 30 min before the test impaired retrieval of IA memory.

Discussion

Memory is often considered to be a process that has several stages including acquisition, consolidation, and retrieval.[1] Typically, some tasks that have been designed to modulate memory processes consist of a training session followed, after 24 or more hours, by a test session. It has been reported that manipulations before acquisition will affect not only the acquisition of memory but also the early stages of consolidation. Manipulations before retrieval may however affect late stages of consolidation or retention besides retrieval of memory.[1,5]

According to research from our laboratory, pre-training administration of morphine, 30 min before training, suppressed the learning of an IA task.[21–24] Other investigators have also reported that pre-training morphine impairs IA memory.[25–27] Pre-training administrations of morphine also inhibit the acquisition of memory in different paradigms such as y-maze discrimination,[28] active or passive avoidance[12], and operant tasks. [14] Other reports from our laboratory have also shown that post-training and pre-test administrations of morphine by themselves also impair IA memory.[15–17,20,21,29,30] An important question so far left unanswered by experiments such as those cited above is that: what stages of memory are in fact affected by morphine during morphine-induced impairment of IA memory? To address this and similar queries, we must consider the mechanisms under which the drug affects learning and memory. The drug should be administered at each of these time points: at different time points before training, immediately and at several time points after training, and immediately and at different time points before testing.

The results of the first experiment of this study showed that pre-training administrations of morphine at 0, 30, and 60 min before training impaired the acquisition of IA memory as revealed by a decrease in step-through latency on the test day. The results of the second experiment revealed that post-training administration of morphine only at immediately after training impaired the consoli-

dation of IA memory. Consistent with the present results, in our previous studies we have shown that pre-training administrations of morphine 30 min before training or immediate post-training administrations of the drug induced amnesia.[15–17,20,23] Similar experiments have also reported that either pre- or post-training administration of moderate doses of morphine (5 – 10 mg/kg) impairs passive avoidance tasks.[12]

The results of the third experiment of this study also showed that pre-test administration of morphine (7.5 mg/kg) at 30 min and immediately before testing impaired IA memory retrieval. In line with the present findings, we have previously reported that pre-test administrations of moderate doses of morphine at 30 min prior to the test could cause an impairment of memory retrieval.[30]

Other major findings of the present study are the confirmation of time dependence and also the detailed description of the time course for vulnerability to post-acquisition morphine-induced amnesia. In marked contrast to the amnesic effects of morphine when given before training and during the period (0 – 30 min) after the acquisition, the injection of morphine 4 h after the acquisition session did not produce disruption of 24 h retention. These observations have a twofold significance. First, it shows that the duration of this effect lasts for up to 4 h after treatment. In addition, these data highlight the fact that retention testing at periods up to 6 h after the treatment in post-trial morphine protocols may not eventually be contaminated by the presence of residual drug effects. In this context, some of the current controversies surrounding the putative mechanism of the amnesic action of morphine may ultimately be reconciled by taking into account the concept of a time-dependent, but differential role for the impairing effect of morphine on other neurotransmitter pathways in various subtypes of memory processes. Although we did not perform molecular experiments to show the detailed separate signaling pathways for these types of memory, our findings agree with the studies in which morphine selectively disrupted the working memory components of the radial maze task in a dose-dependent manner, thus suggesting that the opioid system plays a tonic role in functionally modulating cognition.[31]

In conclusion, the present results suggest that morphine, when injected IP at different time points before training, after training, or before testing, affects IA memory. Other experiments can be designed to investigate molecular mechanisms involved in the impairing effect of morphine in each phase.

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