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Research report

Antagonism of muscarinic M1 receptors by dicyclomine inhibits the consolidation of morphine-associated contextual memory

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Abstract

M1 muscarinic receptor has been shown to be involved in cognitive functions of the brain. Conditioned place preference (CPP) paradigm involves memory for the association between environmental stimuli and the rewarding properties produced by a treatment. Using a balanced CPP design, we studied the possible involvement of M1 muscarinic receptors on the acquisition, expression and consolidation of morphine place conditioning in male mice. Subcutaneous administration of morphine sulphate-induced CPP in a dose-dependent manner. Using a 6-day schedule of conditioning, it was found that dicyclomine, an M1 muscarinic antagonist, significantly reduced the time spent by mice in the morphine compartment when given immediately, but not 6 h, after each conditioning session (consolidation). It had no effect when administered 30 min before each conditioning session during CPP training period (acquisition) or 30 min before testing for place preference in the absence of morphine (expression). It is concluded that M1 muscarinic receptors may play a time-dependent role in the consolidation of reward-related memory of morphine.

Keywords: Memory; Consolidation; M1 muscarinic receptor; Dicyclomine; Morphine; Conditioned place preference; Mice

1. Introduction

Cholinergic receptors are widely expressed throughout the central nervous system (CNS). The importance of these receptors is well known in a widespread array of CNS functions including locomotor activity, emotional behavior, pain sensitivity, learning and memory [20,47,11,26,44]. It has also been shown that activation of both muscarinic and nicotinic acetylcholine receptors may show reinforcing and rewarding properties [21,40,46]. Recent evidence implicates the role of cholinergic receptors in rewarding properties of various drugs of abuse such as morphine and cocaine: intra-basolateral amygdala or dorsal hippocampal injection of physostigmine and nicotine potentiates the morphine-induced conditioned place preference (CPP) [49,30]. Administration of non-selective muscarinic antagonists such as scopolamine and atropine attenuates the rewarding properties of morphine and cocaine [50,13,17,29,49]. Moreover, deletion of the M5 muscarinic receptor subtype has the same effect [10,6].

Research examining the neurobiological basis of drug addiction has traditionally focused on mechanisms mediating the rewarding properties of various drugs of abuse [15]. Stimuli existing during drug administration, such as specific environmental cues, can acquire incentive motivational effects that may provoke drug craving and may contribute to relapse [9]. Drug-paired contextual cues are considered to acquire such incentive motivational effects through associative processes [39]. CPP paradigm has been widely used to assess the rewarding properties of drugs of abuse including opioids [42,5]. The test is based upon the principle that when a primary reinforcer is paired with a contextual stimulus, the contextual stimulus can acquire secondary reinforcing properties. These secondary reinforcing properties, which are presumably established due to a Pavlovian contingency, are thought to be capable of eliciting an operant approach response or place preference which results in a significant increase in the time

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spent in the drug-paired place [4,5]. However, as expression of CPP is inferred on a treatment-free test day, CPP behavior ultimately involves acquisition, consolidation, and retrieval of reward memory for an association between environmental stimuli and the affective state produced by a treatment [45,36].

Previous studies have investigated the neural mechanisms of reward-associated memory. In a previous study [37], results obtained with scopolamine administration suggest that cholinergic muscarinic receptors may play a role in the consolidation of food- and amphetamine-induced CPP; however, due to the lack of selectivity of scopolamine, it is not possible to infer which subtypes of muscarinic receptors would be involved with this type of memory. Rezayof et al. [30,31] reported that intra-hippocampal and intra-ventral tegmental area injection of physostigmine, an anticholinesterase, potentiated the morphine-induced place preference, while administration of non-selective muscarinic acetylcholine receptor antagonist, atropine to these brain areas inhibited the acquisition of morphine-induced CPP. The M1 muscarinic receptor is expressed in various regions of the forebrain including cerebral cortex, hippocampus, amygdala, nucleus accumbens, and striatum [43,33,1,19,28,8]. In a recent study, it has been reported that the acquisition of morphine- and cocaine-induced CPP is decreased in M1 receptor subtype deficient (M1 KO) mice and also after administration of an M1 antagonist, pirenzepine [8]. Besides its role in the reward system, some evidence points to an important function of the M1 muscarinic receptors in learning and memory processes [7,14,16,35]. Galeotti et al. [12] have shown that systemic administration of dicyclomine, an M1 muscarinic antagonist, after training sessions impairs the consolidation of inhibitory avoidance task. Soares et al. [38], however, have reported that the acquisition of inhibitory avoidance and contextual fear conditioning was impaired by pre-training administration of dicyclomine, while the consolidation of both tasks was not affected by dicyclomine administered immediately after training. They have also reported that pre-test injection of dicyclomine impaired the retrieval of contextual fear conditioning but not avoidance response of animals. Despite the evidence for M1 receptor involvement in morphine-induced place preference, the level of this interaction remains obscure (i.e., it is not yet clear that whether blocking of morphine rewarding effect or impairment of general learning and memory mechanisms is responsible for the observed decrease in morphine-induced CPP after M1 inhibition). Being administered an M1 antagonist before conditioning sessions, the animals might have been affected by the treatment in both training sessions and immediately after training; thus, one cannot conclude that whether M1 blockade specifically affects the acquisition or consolidation of learning in the place preference paradigm.

The aim of the present study is to evaluate the possible involvement of M1 muscarinic receptor in the morphine-associated contextual memory. Using CPP paradigm, the effects of pre-training, post-training and pre-test administration of dicyclomine, an M1 receptor antagonist, on the acquisition, consolidation and retrieval of morphine-associated contextual memory were assessed.

2. Materials and methods

2.1. Animals

A total of 220 male *NMRI* mice (Institute Pasteur of Iran, Tehran, Iran), weighing 20–30 g were used. The animals were housed 5–7 per cage in a temperature-controlled (22 ± 3 °C) colony room. They were maintained in a 12-h on and 12-h off light/dark schedule with ad libitum food and water, except during experimental procedures. All experiments were conducted between Zeitgeber time (ZT) 3 and ZT 5 (light onset is defined as ZT 0). Subjects were experimentally naïve. Animals were allowed 7 days to acclimatize to the laboratory environment before testing began. Each mouse was used only once and each treatment group consisted of 5–13 animals. Animals housed in the same cage were subjected to the same treatment. All procedures were carried out in accordance with institutional guidelines for animal care and use and possible measures were undertaken to minimize the number of animals used as well as animals' discomfort. The protocol was approved by the committee of ethics of the faculty of Sciences of Tehran University (357; 8 November 2000).

2.2. Drugs

Drugs used in the present study were morphine sulphate (Temad Pharmaceutical, Tehran, Iran) and dicyclomine hydrochloride (Toliddaru Pharmaceutical, Tehran, Iran). All drugs were dissolved in sterile normal saline solution. In all experiments, morphine was injected subcutaneously (s.c.), and dicyclomine was administered intraperitoneally (i.p.), in a volume of 10 ml/kg. Vehicle injections were of the appropriate volume of saline.

The doses and the interval between administration and training sessions were chosen based on a previous research that had used dicyclomine administration to examine inhibitory avoidance [38].

2.3. Place conditioning apparatus

The place preference apparatus were made of wood and consisted of two square-based wooden compartments (15 cm × 15 cm × 30 H cm each). In order to distinguish the two compartments, visual and sensory texture cues were used: the inner surface of one compartment was painted in black with a smooth floor, while the other was painted in white with a textured floor to create equally preferred compartments. During the conditioning phase, the two compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling.

2.4. Place conditioning paradigm

CPP was conducted using an unbiased procedure. It consisted of a 9-day schedule with three distinct phases: familiarization and pre-conditioning, conditioning, and post-conditioning.

2.4.1. Familiarization and pre-conditioning

On the first day of the trials (i.e., familiarization) and the second day (i.e., pre-conditioning), each mouse was placed separately into the apparatus for 10 min, while they could freely access both compartments. The time spent in each compartment was recorded on the pre-conditioning day to determine any individual innate preference for either of the two compartments. Placement in each compartment was defined as placement of the front paws and the head. Animals showing strong unconditioned preference for any compartment (i.e., time spent in either of the two compartments > mean + 2S.D.) were excluded from the experiments (a total number of five mice). Thus based on our unbiased method, in one of compartments, randomly chosen, the animals received morphine and in the other, they were administered with saline.

2.4.2. Conditioning

This phase consisted of six consecutive conditioning sessions, each 40 min in length and held on a separate day. Mice were confined to the considered compartment, by isolating the compartment using a removable partition. The mice received morphine on days 1, 3 and 5, and saline on days 2, 4 and 6 of the

conditioning phase immediately prior to placement into the apparatus. Treatment compartment and the orders of all administrations were counterbalanced for either group.

2.4.3. Post-conditioning

This phase was carried out on the ninth day of the trials (24 h after the last conditioning session, with no preceding injections) in a drug-free state. As in the pre-conditioning phase, the partition was raised and the animals were placed in the apparatus for 10 min, with free access to both compartments and the time spent in each compartment was recorded in real time by an observer blind to treatments and groups. Change in preference (CIP) was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the post-conditioning day and the time spent in this compartment in the pre-conditioning session.

2.4.4. Measurement of locomotor activity

Locomotor testing was carried out in both conditioning sessions (for acquisition experiments) and post-conditioning test (for expression experiments) as described in a previous study [23] with some modifications. To measure the locomotor activity, the ground areas of the compartments were divided into four equal-sized squares by two transverse lines and the number of squares that the mice entered (i.e., crosses) was measured by another observer and used as an index of locomotor activity.

2.5. Experimental design

2.5.1. Dose–response effects of place conditioning produced by morphine

In this experiment, we established a dose–response function for morphine place conditioning. Different doses of morphine (0.5, 2.5, 5 and 10 mg/kg, s.c.) were tested for their ability to produce CPP. Four groups of animals were injected with morphine and saline on alternate sessions. A separate group of animals was given saline (s.c.) in all sessions during the conditioning phase in order to confirm that the injections and conditioning schedule were not affecting the time allotment in the apparatus. This group was used as control. Animals were tested in a morphine free state. Locomotor activity was also measured in the testing phase to investigate that if morphine-induced motor effects influence the response [49].

2.5.2. Effect of pre-treatment with dicyclomine during conditioning sessions on the acquisition of morphine-induced CPP

Table 1 presents a timeline of the specific manipulations performed to evaluate acquisition, consolidation, and expression of morphine-induced CPP in the present study. Mice were randomly assigned to groups that received either dicyclomine (8, 16 and 32 mg/kg) or saline (10 ml/kg) injections prior to each of the six conditioning sessions. Mice received their assigned treatment 30 min prior to morphine (10 mg/kg, s.c.) or saline (10 ml/kg, s.c.) injections, which preceded placement into the appropriate compartment of the apparatus. Twenty-four hours after completion of the conditioning procedure, mice were tested for CIP. We also used a saline/saline control group in which mice received one saline injection 30 min before each conditioning session and another one immediately before placement into the appropriate compartment of the apparatus in all sessions during the conditioning phase. This control group was used to assess whether morphine 10 mg/kg induced CPP in the presence of dicyclomine or saline pre-treatment during conditioning sessions.

2.5.3. Effect of immediate post-treatment with dicyclomine during conditioning sessions on the consolidation of morphine-induced CPP

Mice were randomly assigned to groups that received dicyclomine (8, 16 and 32 mg/kg) or saline (10 ml/kg) injections within 3 min (referred to as immediate) after completing the 40-min session on each conditioning day. Following injection, mice were returned to their home cages in the colony. One saline/saline control group was used to evaluate whether morphine-induced CPP in the presence of immediate injections of dicyclomine or saline after training sessions. Animals in this group received saline injections in all six conditioning sessions and also immediately after each training session.

Table 1
Timeline of the specific manipulations performed to evaluate acquisition, consolidation, and retrieval of morphine-induced CPP

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Acquisition	Familiarization	Pre-test	Dic/saline → MS (after 30 min) → conditioning	Dic/saline → saline (after 30 min) → conditioning	Dic/saline → MS (after 30 min) → conditioning	Dic/saline → saline (after 30 min) → conditioning	Dic/saline → MS (after 30 min) → conditioning	Dic/saline → saline (after 30 min) → conditioning	Post-test	–
Early consolidation	Familiarization	Pre-test	MS → conditioning → Dic/saline (immediately)	Saline → conditioning → Dic/saline (immediately)	MS → conditioning → Dic/saline (immediately)	Saline → conditioning → Dic/saline (immediately)	MS → conditioning → Dic/saline (immediately)	Saline → conditioning → Dic/saline (immediately)	Post-test	–
Late consolidation	Familiarization	Pre-test	MS → conditioning → Dic/saline (after 6 h)	Saline → conditioning → Dic/saline (after 6 h)	MS → conditioning → Dic/saline (after 6 h)	Saline → conditioning → Dic/saline (after 6 h)	MS → conditioning → Dic/saline (after 6 h)	Saline → conditioning → Dic/saline (after 6 h)	Post-test	–
Retrieval	Familiarization	Pre-test	MS	Saline	MS	Saline	MS	Saline	Post-test 1	Dic/saline → post-test 2 (after 30 min)

MS: morphine sulphate; Dic: dicyclomine.

2.5.4. Effect of delayed post-treatment with dicyclomine during conditioning sessions on the consolidation of morphine-induced CPP

Mice were randomly assigned to groups that received dicyclomine (8, 16 and 32 mg/kg) or saline (10 ml/kg) injections 6 h (referred to as delayed) after completing the 40-min session on each conditioning day. We also used a saline/saline control group to assess whether morphine-induced CPP in the presence of delayed injections of dicyclomine or saline after training sessions. Animals in this group received saline injections in all six conditioning sessions and also 6 h after each training session.

2.5.5. Effect of pre-test injection of dicyclomine on the expression of morphine-induced CPP

Mice were randomly assigned to groups that received morphine (10 mg/kg, s.c.) and saline (10 ml/kg, s.c.) on alternate sessions during conditioning days. On day 9, animals were tested in a morphine free state. On day 10, only mice that exhibited CPP on the test day (three mice showed no CPP and were excluded from this experiment) were pre-treated with dicyclomine (8, 16 and 32 mg/kg, i.p.) or saline (10 ml/kg, i.p.) injections and then retested for CPP 30 min later. To evaluate whether morphine-induced CPP in the presence of pre-testing injection of dicyclomine or saline, we used a saline/saline control group. These animals were injected with saline in all 6 training sessions and also 30 min before the second test for CPP on day 10.

2.6. Data analysis

Data for CIP and locomotion were assessed by one-way analysis of variance (ANOVA). If a significant *F*-value was obtained, post hoc analyses (Bonferroni, Student–Newman–Keuls (SNK) or Dunnett's multiple comparison tests) were performed appropriately to determine the effects of various treatments on induction of place preference and changes in locomotion. *P*-Values less than 0.05 were considered as significant. Calculations were performed using the SPSS statistical package (Version 11.5).

3. Results

3.1. Dose–response curve for place preference conditioning produced by morphine in mice

Fig. 1A shows the dose–response curve for place conditioning induced by morphine in mice. One-way analysis of variance indicated a significant effect of morphine on producing place preference (one-way ANOVA; $F(4,33) = 10.599$, $P < 0.001$). Further analysis showed animals which received saline (10 ml/kg) daily during six sessions and morphine (0.5 mg/kg), exhibited no preference for either compartment. Administration of different doses of morphine (2.5, 5, and 10 mg/kg) during conditioning induced CPP ($P < 0.05$, SNK test). The Bonferroni-adjusted *P*-values showed that morphine 5 and 10 mg/kg induced a significant CPP ($P < 0.01$ and 0.001, respectively). The results (Fig. 1B) did not show any significant effect of morphine on locomotor activity in this experiment (one-way ANOVA; $F(4, 33) = 0.086$, $P > 0.05$).

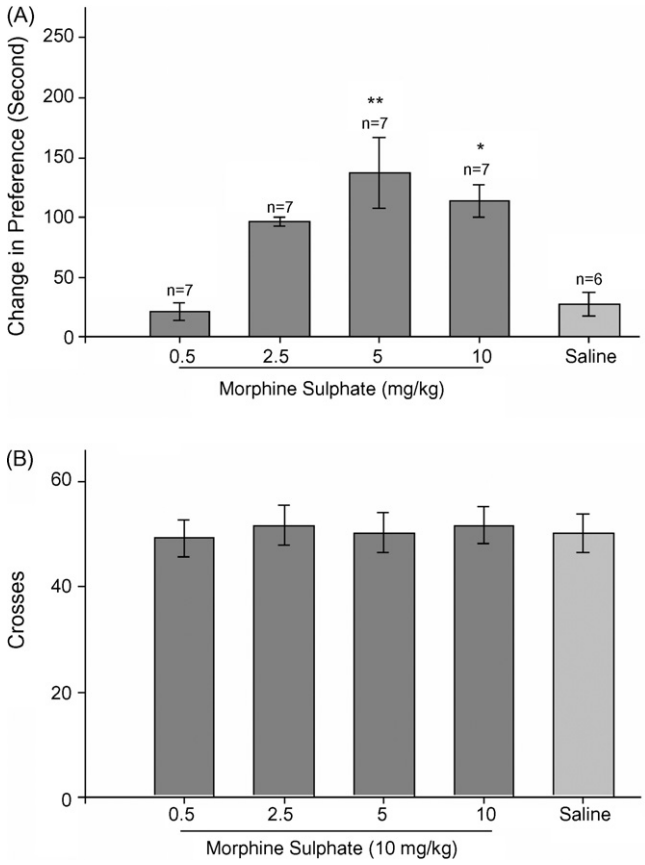

Fig. 1. Effects of morphine on (A) CPP induction in mice; in a 6-day schedule, animals received saline (10 ml/kg, s.c.) or morphine (0.5–10 mg/kg, s.c.) in the drug-paired compartment on the 1st, 3rd, and 5th day of conditioning. The data are shown as means of change in preference ±S.E.M. $^{*}P < 0.01$, $^{**}P < 0.001$ different from the group treated with saline (Bonferroni multiple comparison test), and (B) locomotor activity during testing for CPP; the data are shown as means of crosses ±S.E.M. Analysis revealed that no group showed a statistical significant difference.

3.2. Effect of pre-treatment with dicyclomine during conditioning sessions on the acquisition of morphine-induced CPP

Fig. 2A shows the effect of pre-treatment with different doses of dicyclomine on the acquisition of morphine-induced place preference. One-way ANOVA of CIP in mice receiving injections of dicyclomine or saline prior to each conditioning session revealed no significant effect of pre-treatment with dicyclomine during conditioning sessions on the acquisition of morphine-induced CPP (one-way ANOVA; $F(3, 38) = 1.982$, $P > 0.05$). Statistical analysis revealed a significant effect of morphine to induce CPP, regardless of dicyclomine or saline pre-treatment in this experiment (one-way ANOVA; $F(4, 48) = 24.451$, $P < 0.001$). The Dunnett's test revealed a significant difference between saline/saline control group and other groups ($P < 0.001$). The results (Fig. 2B) did not show any significant effect of dicyclomine on locomotor activity during conditioning sessions in this experiment (one-way ANOVA; $F(3, 38) = 0.173$, $P > 0.05$).

3.3. Effect of immediate post-treatment with dicyclomine during conditioning sessions on the consolidation of morphine-induced CPP

Fig. 3 shows the effect of immediate injections of dicyclomine in different doses on the consolidation of morphine (10 mg/kg) reward-related memory. Statistical analyses of CIP in mice undergoing 40-min conditioning sessions followed immediately

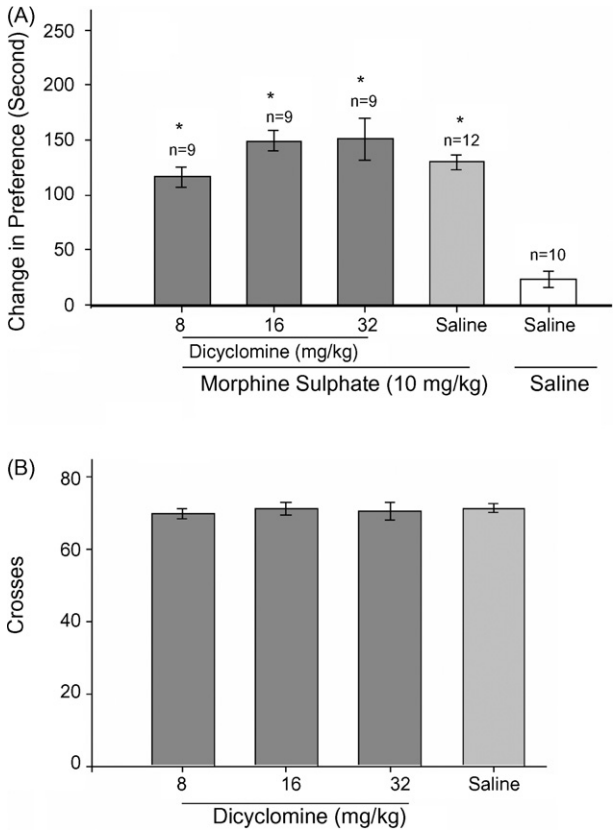

Fig. 2. Effect of pre-treatment with dicyclomine during conditioning sessions on (A) the acquisition of morphine-induced CPP. Values represent mean ± S.E.M. Respective dicyclomine (8, 16 and 32 mg/kg) and saline (10 ml/kg) injections occurred 30 min prior to all six conditioning sessions. *$P < 0.001$ different from the group received saline injections 30 min and immediately before placement into appropriate compartment during conditioning sessions (Dunnett's post hoc test), and (B) locomotor activity during conditioning sessions of training phase. The data are shown as means of crosses ±S.E.M. Analysis revealed that no group showed a statistical significant difference.

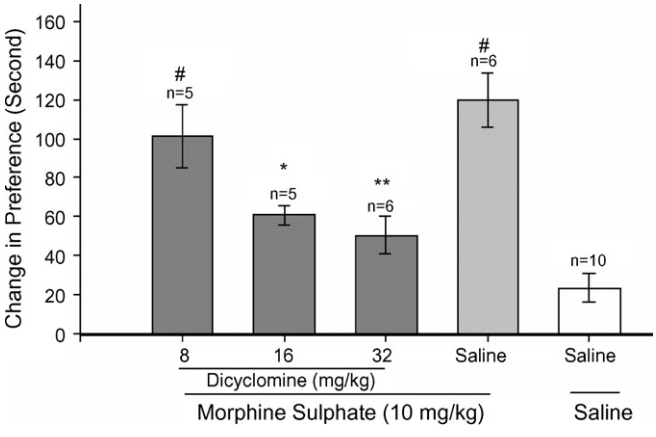

Fig. 3. Effects of immediate post-treatment with dicyclomine on the consolidation of morphine-induced CPP. Values represent mean ± S.E.M. Respective dicyclomine (8, 16 and 32 mg/kg) and saline (10 ml/kg) injections occurred immediately following all six conditioning sessions. *$P < 0.01$, **$P < 0.001$ different from the group receiving post-treatment with saline after either morphine or saline conditions (Bonferroni multiple comparison test); #$P < 0.001$ different from the group received saline injections in all six conditioning sessions and also immediately after each training session (Dunnett's multiple comparison tests).

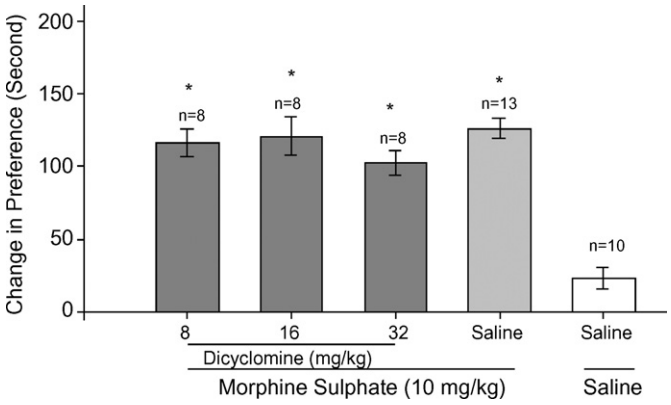

Fig. 4. Effects of delayed post-treatment with dicyclomine on the consolidation of morphine-induced CPP. Values represent mean ± S.E.M. Respective dicyclomine (8, 16 and 32 mg/kg) and saline (10 ml/kg) injections occurred 6 h after each conditioning session. *$P < 0.001$ different from the group received saline injections in all six conditioning sessions and also 6 h after each training session (Dunnett's multiple comparison test).

by dicyclomine or saline injections revealed a significant effect of dicyclomine (one-way ANOVA; $F(3, 21) = 7.97$, $P < 0.001$) on the consolidation of morphine-induced CPP. Dicyclomine 16 and 32 mg/kg disrupted consolidation of morphine-induced CPP ($P < 0.05$, SNK and Bonferroni tests), but post-treatment with saline and dicyclomine 8 mg/kg failed to alter it ($P > 0.05$). Also, statistical analysis revealed a significant difference between saline/saline control group and those groups which received dicyclomine 8 mg/kg or saline immediately after each conditioning session in this experiment ($P < 0.001$, Dunnett's test); groups administered dicyclomine 16 or 32 mg/kg immediately after training did not show CPP ($P > 0.05$).

3.4. Effect of delayed post-treatment with dicyclomine during conditioning sessions on the consolidation of morphine-induced CPP

Fig. 4 shows the effect of delayed injections of dicyclomine in different doses on the consolidation of morphine (10 mg/kg) reward-related memory. Statistical analyses of CIP in mice receiving dicyclomine or saline injections 6 h after conditioning sessions, revealed no significant effect of dicyclomine (one-way ANOVA; $F(3, 36) = 1.217$, $P > 0.05$) on the consolidation of morphine-induced CPP. One-way ANOVA revealed a significant effect of morphine to induce CPP regardless of dicyclomine or saline post-treatment in this experiment (one-way ANOVA; $F(4, 46) = 25.458$, $P < 0.001$). The Dunnett's test revealed a significant difference between saline/saline control group and other groups ($P < 0.001$).

3.5. Effect of pre-test injection of dicyclomine on the expression of morphine-induced CPP

Fig. 5A shows the effect of different doses of dicyclomine on the expression of morphine-induced place preference. Statistical analyses of CIP in mice receiving dicyclomine or saline injections prior to the CPP test, revealed no significant effect of

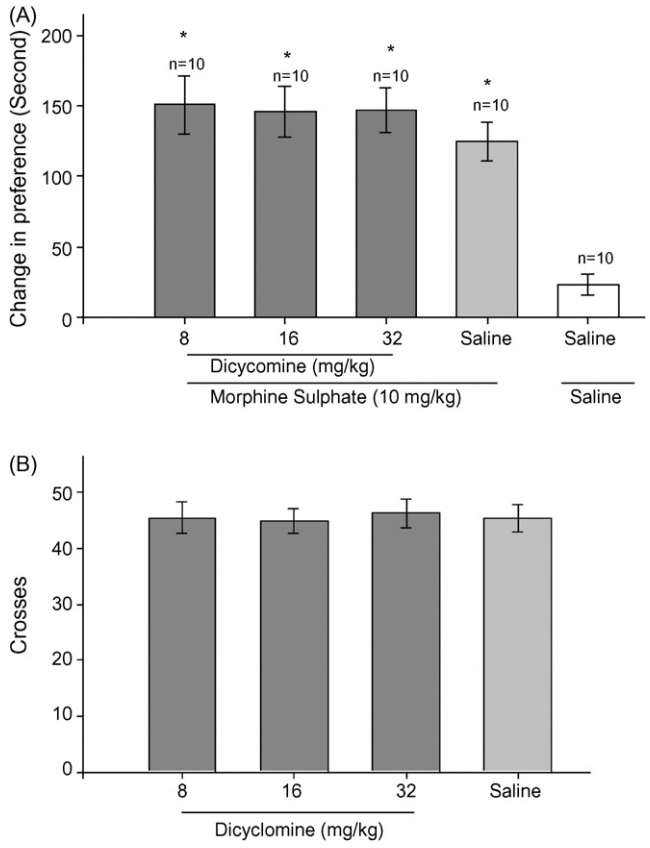

Fig. 5. Effect of pre-test injection of dicyclomine on (A) the expression of morphine-induced CPP. Values represent mean ± S.E.M. Mice received dicyclomine (8, 16 and 32 mg/kg) or saline (10 ml/kg), 30 min prior to testing for morphine-induced CPP. There was no significant difference between dicyclomine or saline treated groups ($P > 0.05$). *$P < 0.001$ different from the group received saline injections in all six conditioning sessions and also before testing for the CPP (Dunnett's multiple comparison test), and (B) locomotor activity during testing phase. The data are shown as means of crosses ±S.E.M. Analysis revealed that no group showed a statistical significant difference.

dicyclomine on the expression of morphine-induced CPP (one-way ANOVA; $F(3, 39) = 0.464$; $P > 0.05$). One-way ANOVA showed a significant effect of morphine to induce CPP regardless of pre-test injection of saline or dicyclomine (one-way ANOVA $F(4, 49) = 12.107$, $P < 0.001$). Further analysis showed a significant difference between saline/saline control group and other groups ($P < 0.001$, Dunnett's test). The results (Fig. 5B) did not show any significant effect of dicyclomine on locomotor activity in this experiment (one-way ANOVA; $F(3, 39) = 0.052$, $P > 0.05$).

4. Discussion

Muscarinic acetylcholine receptors (M1–M5) modulate the activity of the central nervous system and have a range of physiological functions. The M1 muscarinic receptor is expressed in various regions of the forebrain including cerebral cortex, hippocampus, amygdala, nucleus accumbens, and striatum [43,33,1,19,28,8]. There is evidence supporting the role of M1 receptor in learning, memory, locomotion, reinforcement, and antinociception [44]. In addition, a recent report revealed that M1 receptor is involved in the rewarding effects of morphine and cocaine [8].

The CPP paradigm used in this study represents an animal model to assess the rewarding properties of various drugs of abuse including opioids [4]. However, as expression of CPP is inferred on a treatment-free test day, CPP behavior requires memory for the association between environmental stimuli and the affective state produced by a rewarding treatment [45,37]. Memory acquisition occurs as the animal learns an association between a context and a reward. During consolidation, this memory is moved from a labile to a more fixed state. During retrieval, the animal is returned to the conditioning context, where memory for the context-reward association is assessed. The current study evaluates the effects of intraperitoneal administration of M1 receptor antagonist, dicyclomine, on the acquisition, consolidation, and expression of morphine-associated contextual memory. Here, we tried to show possible involvement of M1 in processes related to learning and memory for morphine-induced CPP.

Results showed that morphine induces a significant CPP in a dose-dependent manner in mice, which is consistent with previous studies [18,41]. The drug at the doses used in our experiments did not alter locomotor activity in comparison with the control group. The main findings from this study are: (i) pre-treatment with different doses of dicyclomine during conditioning sessions does not affect acquisition of morphine-induced CPP; (ii) administration of different doses of dicyclomine (16 and 32 mg/kg) immediately, but not 6 h, following each conditioning session appears to impair morphine-induced CPP; (iii) expression of morphine-induced CPP is not affected by the pre-testing treatment with dicyclomine.

These results suggest that dicyclomine, at least in the dose range used (8, 16 and 32 mg/kg) does not interfere with the acquisition of morphine-induced CPP. However, it has been reported that blockade of muscarinic receptor before administration of morphine, by atropine injection into basolateral amygdale, dorsal hippocampus or ventral tegmental area attenuates the acquisition of morphine-induced CPP [49,30,31]. Carrigan and Dycstra [8] reported that morphine- and cocaine-induced CPP were attenuated in the M1 KO mice and in the mice after the systemic administration of another M1 antagonist, pirenzepine. This discrepancy may reflect additional modulation of muscarinic mechanisms of morphine-induced reward by experimental factors; we used systemic administration of M1 muscarinic antagonist, dicyclomine prior to morphine 10 mg/kg and saline in NMRI mice; Carrigan and Dycstra [8] used systemic injections of another M1 antagonist, pirenzepine before morphine 5 mg/kg in C57BL6 mice; central injections of atropine, a non-selective muscarinic receptor antagonist, prior to morphine 5 or 6 mg/kg in Wistar rats were used in [30,31,49]. More studies are needed to clarify this discrepancy. Anagnostaras et al. [2], however, reported that M1 receptor mutant mice showed working memory and consolidation impairment without acquisition deficit in some learning and memory dependent tasks. Moreover, it would be interesting to evaluate the role of M1 muscarinic receptors in morphine-induced rewarding

properties using other reward-measuring paradigms like drug self-administration.

Post-training drug administration provided a method for investigating drug effects on memory consolidation avoiding the confounding effects on acquisition or retention performance [22]. Many studies of the post-training treatments, which modulate cholinergic function indicate that alterations in cholinergic functioning could influence memory consolidation [3,34,37]. Cholinergic function apart from other possible influences on cognitive processes, influences the consolidation of new memories. Studies reporting memory enhancement by post-training systemic or intracranial cholinergic treatments predict that endogenous acetylcholine release should be enhanced immediately after a learning experience in brain regions that are involved in consolidating the memory [27]. Previous findings revealed that muscarinic cholinergic activation may play an important role in the regulation of consolidation and coordination of memory processes [27]. It has been reported that post-training intra basolateral amygdala infusion of the general muscarinic receptor antagonist, scopolamine, impairs memory consolidation underlying initial acquisition of CPPs for both food and amphetamine [37]. In this study, M1 inhibition immediately after training sessions blocked morphine-induced CPP, suggesting that M1 is involved in memory consolidation processes which can modulate morphine-induced responses to environmental cues. This impairing effect of dicyclomine on memory consolidation is time-dependent; administration of dicyclomine 6 h after training sessions did not affect consolidation of morphine-induced CPP, suggesting that morphine-induced CPP reveals a temporally limited labile state during consolidation which the processes of memory storage can be modulated by muscarinic M1 receptors. Several, but not all of, previous studies showed that in inhibitory avoidance, another learning and memory dependent task, systemic or intra-cerebral administration of dicyclomine or other M1 antagonists after training sessions impaired the consolidation of this behavioral paradigm [12,32].

Alternative explanation is that dicyclomine does not impair the memory for morphine-induced CPP but inhibition of morphine-induced CPP is due to aversive properties of dicyclomine. This explanation is unlikely because in the present experimental design, mice received post-training dicyclomine in association with both conditioning compartments; considering the algebric summation of hedonic processes [48], if dicyclomine had aversive properties per se, exposure to the saline compartment would be expected to be more aversive than exposure to the morphine compartment. Therefore, aversive properties of dicyclomine should have little effect on the place preference. On the other hand, if dicyclomine blocked the primary rewarding properties of morphine, pre-training dicyclomine administration should have been more effective than its post-training administration.

We failed to find evidence for M1 involvement in expressing morphine-induced CPP following pre-testing dicyclomine administration. This finding suggests that the contribution of M1 is less essential when a CPP task is already well learned, so its inhibition would not impair retrieval of morphine-induced reward-associated memory. It has been recently reported [24,25] that cholinergic system in other behavioral paradigms modulates memory processes allowing normal acquisition of new learning, while it is less important in memory retrieval given that it does not severely affect memories once they are encoded. On the other hand, as M1 inhibition had no effect on expression of morphine-induced CPP, it can be inferred that this receptor dose not appear to be involved in the incentive motivational effects of the morphine-paired environment that underlie approach behavior during testing [5].

In conclusion, these results demonstrate that M1 receptor may play a time-dependent role in the consolidation of morphine-associated contextual memory. Further research is needed to explore the specific loci of the brain in which muscarinic system modulates memory for morphine-induced CPP.

Conflict of interest

None.

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