Full Paper

The Influence of Contextual Cue on Antinociceptive Tolerance and Facilitation of Memory With Morphine

Mototaka Nakama-Kitamura* and Nobutaka Doe

Department of Psychology, Kwansei Gakuin University, Uegahara, Nishinomiya 662-8501, Japan

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Abstract. The response latencies of the biting response in the tail-pinch test and step-through response in the passive avoidance test were measured in mice. 1) Scopolamine decreased the latency of the step-through response in the passive avoidance test, but diazepam did not. 2) Morphine (5 mg/kg, i.p.) was given 30 min before the test for 4 consecutive days in novel environments by sessions. The decrease in biting response latency was attenuated and delayed in the groups in which contexts were changed daily compared to the groups that were maintained in the same context throughout the conditioning period. The latency of step-through response was increased with morphine in both groups regardless of the change or lack of change in context. 3) Morphine was repeatedly administered to animals in the same environment and then the context was changed. The decreased latency in the tail-pinch test was significantly reversed by the change in context, but the response in the passive avoidance test maintained a longer latency. These findings indicate that morphine develops associative and nonassociative antinociceptive tolerance, indicating that antinociceptive tolerance to morphine has contextual specificity, but the facilitation of memory does not. The results indicate that the facilitation of memory with morphine may contribute to associative learning in antinociceptive tolerance to morphine.

Keywords: associative antinociceptive tolerance, contextual specificity, memory facilitation, tail-pinch test, passive avoidance test

Introduction

The development of tolerance has contextual specificity. The contextual change after the development of tolerance to morphine that had been administered in sessions of the same context shows the reappearance of the antinociceptive effect (e.g., refs. 1–4). Recently, it has been demonstrated that morphine administration in sessions with a novel context delayed the development of antinociceptive tolerance (5). Using the same experimental procedure in rats with chronic pain induced by repeated heterotopic injection of formalin, the development of antinociceptive tolerance was prominently attenuated (6). These findings indicate that the process of antinociceptive tolerance development involves an associative factor between drug effects and contextual cues. Thus tolerance is considered to develop as a compound phenomenon consisting of associative

and nonassociative factors. Most of these studies have been conducted by thermal stimulation methods such as the hot-plate test and tail-flick test, but a few investigators have used the pressure stimulation method (e.g., ref. 7).

In view of the associative tolerance developed during the course of morphine conditioning, we wanted to explore the relationship between associative tolerance and memory. Morphine (8, 9) and opioids peptides (10) were reported to inhibit learning and memory processes, and the effect is reversed by the opioid antagonist naloxone, which itself also facilitates memory (11). However, the administration of morphine 24 h after training or 30 min before a test facilitated memory retrieval (12, 13). Thus the effects of morphine on memory vary with the timing of administration, foot shock intensity, or dosage of the drug (14). To examine the effects of morphine on the relationship between the role of contextual cues and memory facilitation, morphine should be administered 30 min before the test in

*Corresponding author. FAX: +81-798-52-7353
E-mail: Kitamura@kwansei.ac.jp

animals that have already received passive avoidance training. If morphine facilitates memory and has little contextual specificity, the facilitated memory may contribute to the development of a learned associative tolerance of antinociception.

As a theoretical explanation of associative tolerance in nociception, a compensatory conditioned response (CCR) theory has been proposed (1, 2). According to this proposal, tolerance (conditioned response: CR) to morphine exposure (unconditioned stimuli: US) with a paired context (conditioned stimuli: CS) is developed as an opposing response to analgesia (unconditioned response: UR) to counteract or compensate for the primary analgesic effect. However, there are several contradicting arguments against the CCR theory: there is no evidence of CCR by paw-pressure, tail-shock, and tail-flick tests in rats (7); tolerance to morphine would be related to habituation and adaptation (15); CCR is found only in a tested activity, but not in the parameters to measure the analgesic effect (16); and homeostasis is fundamental (17, 18).

This study was designed to answer three main questions: 1) whether an associative tolerance to morphine is produced by the pressure stimulus method, 2) whether morphine facilitates memory, and 3) whether the facilitation of memory, if any, has context specificity or not. We used two methods to determine the effects of repeated injection of morphine and contextual cues: the tail-pinch test for antinociceptive tolerance and the step-through type passive avoidance learning test for memory function.

Morphine was injected in sessions of different contexts, with a novel context in each session or in the same context. We also confirmed the influence of a contextual cue on associative tolerance by another contextual procedure. According to these results, the relationship between associative, antinociceptive tolerance and memory function was discussed.

Materials and Methods

Animals

The animals were male ddY mice, approximately 55-day-old, with an average weight of 25.9 g at the beginning of the experiment. They were housed in plastic cages in groups of 7 or 8 with free access to water and food (Oriental MF Yeast Co., Ltd., Osaka). These cages were kept under conditions of 22–23°C room temperature, 55–60% humidity, and a 12-h light-dark cycle (lights on from 08:00 to 20:00).

All experiments were carried out in accordance with the National Institute of Health guide “Principles of Laboratory Animal Care (NIH publication No. 85-23,

revised 1985)”; and the care and use of animals adhered to the “Guide for the Care and Use of Laboratory Animals at the Department of Psychology, Kwansei Gakuin University (1992)”. All experiments were conducted between 10:00 and 14:00 every day to avoid any temporal factor (e.g., circadian rhythm).

Drugs

Morphine hydrochloride (10 mg/ml; Takeda Pharm Co., Ltd., Osaka) was diluted 1/20 with physiological saline (Mor) and intraperitoneally (i.p.) injected at a dose of 10 ml (5 mg)/kg into the mice. Diazepam (5 mg/ml) was diluted 1/25 with tragacanth gum (Dia) and i.p. injected at a dose of 10 ml (2 mg)/kg. Scopolamine hydrobromide (0.2 mg/ml; Kyorin Pharm Co., Ltd., Tokyo) was diluted 1/10 with physiological saline (Sco) and i.p. injected at a dose of 10 ml (0.2 mg)/kg. Control groups were injected with an equal volume of physiological saline (Sal).

Apparatus

Tail pinch clip: A 5-cm long artery clip was employed to measure the antinociceptive response of mice (19).

Passive avoidance box: A step-through type passive avoidance box (15-cm-wide × 16-cm-high × 30-cm-long) was used. It was divided into two parts by a vertically sliding door: a 9-cm-long light compartment and a 10-cm-long dark compartment. The box was made of white acrylic walls, a transparent ceiling, and a grid floor consisting of 3-mm steel rods spaced 10-mm apart center to center. In order to change the color of the inside of the dark compartment, a four-sided black chimney of acrylic board was vertically inserted into the compartment, and the lid of the dark compartment was covered with black nylon sheets. In the dark compartment, on the lower part of each side wall 40 mm from the border between the dark and light compartments, a sensor that could detect the animal entering the compartment was set. The apparatus was illuminated by a lamp (40 W), which shed light of approximately 155 lux in the light compartment.

Contexts

ABCD context sequence: Four different contexts were made and the context was changed in the session in the sequence of ABCD. These contexts were as follows: a quadratic box made by hard paper (8 × 18 × 8 cm), a translucent pink plastic box (10 × 15 × 7 cm), a cylindrical steel can (6-cm diameter × 12-cm-long) with sandpaper pasted on its inside wall, and a cylindrical black plastic box (10-cm diameter × 12-cm-long) with gravel spread over the floor. The sequence of the contexts was randomized in each animal.

AAAAB context sequence: To set the AAAAB context sequence, two different contexts were made that differed in size, material, and lighting condition. One context was a white acrylic box (30 × 45 × 25 cm) placed in an experimental room illuminated by fluorescent light. This box had a steel-latticed ceiling. The other context was a paper box (35 × 45 × 30 cm) placed in the adjacent dark room. It had a scent bag containing parabenzalchonium chloride suspended from the lid, and gravel was spread over the floor to provide olfactory and tactile cues.

Procedures

Evaluation of the passive avoidance learning method: Sixty mice were divided into four groups of 15 mice each. These four groups were designated as Groups Mor, Sco, Dia, and Sal, according to the drug they were administered. The acquisition training trial was performed on the first day without drugs. On the next day, the passive avoidance test trial was done. Mor, Sco, Dia, or Sal were administered intraperitoneally 30 min prior to the test, and the latency of the passive avoidance response was measured.

ABCD context procedure: The experiment using the ABCD context procedure was based on a 2 × 2 factorial design. The first factor was the drug, morphine (Mor) or saline (Sal). The second factor was the arrangement of contexts, different contexts (Diff) or the same context (Same). According to the experimental design, 42 animals were divided into four groups and labeled as Groups Mor-Diff, Mor-Same, Sal-Diff, and Sal-Same. Groups Mor-Diff and Mor-Same were injected with Mor. Groups Sal-Diff and Sal-Same were injected with saline instead of Mor. Groups Mor-Diff and Sal-Diff were injected with drug or Sal in a novel context by sessions for four successive days. Groups Mor-Same and Sal-Same were exposed to one of four contexts throughout the phase. Thirty minutes later, the analgesic response was measured in each context. Then, the animals were subjected to the passive avoidance test trial. After these tests, animals were confined to their contexts again, and 15 min later they were returned to their home cages. For Groups Mor-Diff and Sal-Diff, different context sequences were assigned to each animal, and the sequences were matched between these two groups. The contexts used for Groups Mor-Same and Sal-Same were also matched between groups.

AAAAB context procedure: The experiment using the AAAAB context procedure was also based on a 2 × 2 factorial design. The first factor was the drug, Mor or Sal. The second factor was the context sequence, Diff or Same. Fifty-two animals were divided into four groups of 13 mice each for the tail-pinch tests. Another 55 mice

were divided into four groups and employed for the passive avoidance test. The four groups in each experiment were labeled Groups Mor-Diff, Mor-Same, Sal-Diff, and Sal-Same according to the experimental design. All the animals were injected with a drug or saline for five consecutive days. After daily drug injection, half of the animals in each group were confined to one context, and the other half of the animals were confined to the other context. Thirty minutes later, the animals were taken out of their context, and each animal received the tail-pinch test or passive avoidance test. After the test, the animals were confined to their contexts again, and 15 min later, they were returned to their home cages. On day 5, for Groups Mor-Diff and Sal-Diff, the context in which the animals was kept after drug administration was changed to the alternate context. Groups Mor-Same and Sal-Same were kept in the same context throughout the five sessions.

Measurement of antinociceptive response

The antinociceptive response was measured by a modified tail-pinch method using a 500-g pressure clip described by Takagi et al. (19). The latency from the pinch at the base of the tail until the biting response was measured. The cut-off time was 12 s, and when the animal did not respond within 12 s, the latency was regarded as 12 s. The experimenter who measured the latency had no information about the group assignment of each animal being tested.

Measurement of passive avoidance learning

The training trial commenced by placing the animal in the light compartment. After leaving the animal in the compartment for 5 s, the door was opened. When the animal entered the dark compartment, the door was closed and two electric shocks (100 and 200 V, 3 s) were delivered through the grid floor. The timing of the first 100-V shock after the animal entered the dark compartment was 10 s. The interval between the first 100-V shock and the second 200-V shock was 5 s.

The passive avoidance test trial was identical to the training trial except that shocks were not presented. The latency until entering the dark compartment (maximum 120 s) was measured.

Statistical analyses

Data were analyzed by mixed-type ANOVA, and the differences among groups and days were confirmed by the LSD test.

Results

Evaluation of the passive avoidance learning method

In the passive avoidance training trial, the mean step-through latency in the pre-conditioning phase of Groups Mor, Sco, Dia, and Sal were 7.5, 10.4, 9.5 and 8.7 s, respectively, and the difference among the groups was not significant ($F < 1$). As a result of shock presentation in the dark compartment, the mean latency was greatly prolonged in all groups. As shown in Fig. 1, the mean latency of Group Sco was shorter than that of Group Sal. However, the mean latency of Group Mor was longer than that of Group Sal. The result in Group Dia was almost the same as that in Group Sal. A 4 (group) $\times$ 2 (trial) ANOVA on the data shown in Fig. 1 yielded significant main effects of group ($F(3, 56) = 3.50$, $P < 0.05$) and day ($F(1, 56) = 295.31$, $P < 0.001$), and their interaction ($F(3, 56) = 4.57$, $P < 0.01$). Subsequent Fisher's Protected LSD conducted for groups on the test day revealed significant differences between Groups Sco and Sal ($P < 0.01$). The difference between Groups Mor and Sal on the test day was marginally significant ($P = 0.072$).

ABCD context procedure

Antinociceptive tolerance: Figure 2 shows the results

Fig. 1. Effect of morphine, scopolamine, diazepam, and saline on the step-through type passive avoidance learning test. Each drug was administered intraperitoneally 30 min prior to the test trial for groups Mor ($n = 13$), Sco ($n = 13$), Dia ($n = 13$), or Sal ($n = 13$). Error bars represent S.E.M. ($n = 15$) Pre: Preconditioning. A 4 (group) $\times$ 2 (trial) ANOVA yielded significant main effects of group ($P < 0.05$) and day ($P < 0.001$), and their interaction ($P < 0.01$). The LSD test indicated a significant group difference between Sco and Sal ($P < 0.01$) in the test trial. The difference between Groups Mor and Sal on the test day was marginally significant ($P = 0.072$).

of the tail-pinch tests in the experiment using the ABCD context procedure. The mean latency on Day 0 (baseline level) of each group was about 2 s, and the difference among groups was not significant ($F < 1$). However, the mean latency on Day 1 (first administration) for Groups Mor-Diff and Mor-Same was clearly prolonged, indicating that morphine had a pain-attenuating effect. The latency gradually decreased as a function of the injections for the two groups, suggesting that antinociceptive tolerance had developed. Group Mor-Diff, however, still maintained a longer latency than Group Mor-Same. However, the mean latency for the two groups injected with saline was maintained at almost the same level throughout the 5 days. A 2 (drug) $\times$ 2 (context) $\times$ 5 (day) ANOVA on the data shown in Fig. 2 yielded significant main effects of drug ($F(1, 38) = 41.11$, $P < 0.001$) and day ($F(4, 152) = 13.52$, $P < 0.001$), and significant interactions of drug $\times$ day ($F(4, 152) = 10.01$, $P < 0.001$) and context $\times$ day ($F(4, 152) = 3.03$, $P < 0.05$). To confirm the difference between Groups Mor-Diff and Mor-Same, a 2 (context) $\times$ 5 (day) ANOVA on the data of these two groups was conducted. This analysis showed that the main effect of day ($F(4, 88) = 19.10$, $P < 0.001$) and interaction of context $\times$ day ($F(4, 88) = 4.27$, $P < 0.01$) were significant. Subsequent Fisher's Protected LSD revealed that the latency of Group Mor-Diff was longer

Fig. 2. Mean latencies of the biting-response by the tail-pinch test in the experiment using the ABCD context procedure. Error bars represent S.E.M. ($n = 9 - 12$). From Day 1 to Day 4, morphine or saline was administered 30 min prior to the tail-pinch test. Statistical analyses revealed that the latency of Group Mor-Diff was significantly longer than that of Group Mor-Same on Days 3 and 4 ($P < 0.05$).

than that of Group Mor-Same on Days 3 and 4. These results suggest that contextual change by sessions delayed the development of antinociceptive tolerance.

Passive avoidance learning: Figure 3 shows the results of passive avoidance learning investigated using the ABCD context procedure. Although there was a marked increase in latency from Days 0 to 1 for all groups, the mean latency in Groups Mor-Diff and Mor-Same were evidently longer than those of Groups Sal-Diff and Sal-Same, especially on Days 3 and 4. A 2 (drug) $\times$ 2 (context) $\times$ 5 (day) ANOVA on the data summarized in Fig. 2 yielded significant main effects of drug ($F(1, 38) = 5.11$, $P < 0.05$) and day ($F(4, 152) = 29.26$, $P < 0.001$) and significant interaction of drug $\times$ day ($F(4, 152) = 4.49$, $P < 0.01$). The subsequent LSD test revealed a significant difference between the two groups injected with morphine and the two groups injected with saline on Days 3 and 4 ($P < 0.05$). Neither the main effect of context ($F < 1$) nor any kind of its interaction ($F < 1$) was significant. Although Fig. 3 shows a slight difference between Groups Mor-Diff and Mor-Same, a 2 (group: Mor-Diff vs Mor-Same) $\times$ 5 (day) ANOVA failed to detect any significant group effect and interaction of group $\times$ day ($F < 1$).

Fig. 3. Mean latencies of the step-through response of passive avoidance training and tests in the experiment using the ABCD context procedure. Error bars represent the S.E.M. ($n = 9 - 12$). All animals were presented with foot shocks (100 V) in the dark compartment of the avoidance learning box without drug on Day 0. For the passive avoidance test, animals tested in the tail-pinch test were used. Statistical analyses supported significant differences between the two Mor groups and two Sal groups on Days 3 and 4 ($P < 0.05$). Neither the main effect of context ($F < 1$) nor any kind of its interaction ($F < 1$) was significant. A 2 (group: Mor-Diff vs Mor-Same) $\times$ 5 (day) ANOVA also failed to detect a significant group differences ($F < 1$).

AAAAB context procedure

Antinociceptive tolerance: Figure 4 shows the results of the tail-pinch tests conducted using the AAAAB context procedure. The mean latency of each group was about 2 s on Day 0, and the difference among groups was not significant ($F < 1$). However, the mean latency for the two Groups injected with morphine was clearly prolonged on Day 1 (first administration), indicating the nociception-attenuating effect of morphine. The latency for these two groups gradually decreased as a function of the number of injections, suggesting that antinociceptive tolerance had developed. However, the mean latency for the two groups injected with saline remained almost the same as that on Day 0 throughout all the sessions. A 2 (drug) $\times$ 5 (day) ANOVA on the data from Day 0 to Day 4 yielded significant main effects of drug ($F(1, 50) = 27.44$, $P < 0.001$) and day ($F(4, 200) = 22.72$, $P < 0.001$), and their significant interaction ($F(4, 200) = 20.12$, $P < 0.001$).

In addition, contextual change on Day 5 for Group Mor-Diff caused a prolongation of latency, suggesting that the antinociceptive effect of morphine was partially revived. A 2 (drug) $\times$ 2 (context) ANOVA on the data on Day 5 yielded significant main effects of drug ($F(1, 48) = 12.78$, $P < 0.001$) and context ($F(1, 48) = 6.35$, $P < 0.05$) and their significant interaction ($F(1, 48) = 10.62$, $P < 0.001$). Subsequent Fisher's Protected LSD revealed that the latency of Group Mor-Diff was longer than that of any of the other groups.

Fig. 4. Mean latencies of the biting-response in the tail-pinch test in experiment using the AAAAB context procedure. Error bars represent the S.E.M. ($n = 13$). From Day 1 to Day 5, morphine or saline was administered 30 min prior to the tail-pinch test. Statistical analyses showed that the latency of Group Mor-Diff was longer than those of the other groups on Day 5 ($P < 0.05$).

Passive avoidance learning: Figure 5 shows the results of passive avoidance learning in the experiment conducted using the AAAAB context procedure. As can be seen in Fig. 5, the mean latency of the two groups administered morphine was longer than that of the two groups injected with saline in every test trial. A 2 (drug) $\times$ 5 (day) ANOVA on the data from Days 0 to 4 yielded significant main effects of drug ($F(1, 53) = 15.85$, $P < 0.001$) and day ($F(4, 212) = 27.79$, $P < 0.001$). Although the context for Groups Mor-Diff and Sal-Diff was changed from A to B on Day 5, the mean avoidance latency in both groups did not change compared with the level on Day 4. A 2 (drug) $\times$ 2 (context) ANOVA on the data of Days 4 and 5 indicated that only the drug effect was significant ($F(1, 51) = 6.91$, $P < 0.05$). Neither the main effect of context nor the drug $\times$ context interaction was detected ($F < 1$).

Discussion

The first novel finding in the present study was that hindering the mouse's ability to associate with environmental cues under the effect of morphine delayed the development of antinociceptive tolerance by the ABCD contextual procedure using the tail-pinch test. The fact that the antinociceptive tolerance was delayed in the

ABCD contextual procedure indicates that the associative component is superimposed on the nonassociative component, and both components are undissociable (5). To verify this finding in the ABCD procedure, we employed the AAAAB procedure. In the AAAAB contextual procedure, tolerance that had been developed during Days 1 to 4 was diminished on Day 5 by the administration of morphine. The associative and non-associative factors involved may be a function of the experimental condition, such as inter dose interval, and dose (20, 21). The present results confirmed that the associative and nonassociative tolerance to morphine is measurable not only by the thermal stimulation test (5) but also by the pressure stimulation test. Previous studies indicated that thermal stimuli markedly increased the release of immunoreactive somatostatin in the perfusate from the spinal dorsal horn, but pressure stimuli increased the release of immunoreactive substance P in rabbits (22). These results may suggest that the pharmacological and physiological bases for the associative and nonassociative antinociceptive tolerance involves both somatostatin and substance P neuron systems.

The second novel finding in the present studies is that the facilitation of memory with morphine has no context specificity. The validity evaluation of the passive avoidance test confirmed that the test is a measure of memory performance rather than fear or anxiety. In Fig. 1, the latency with diazepam was similar to that observed with saline. If the main factor for retention of acquired passive avoidance response is fear or anxiety, the response latency after the injection of the optimal dose of diazepam should be decreased. However, diazepam, even at 4 mg/kg (i.p.) that produced a muscle relaxant effect, did not affect the latency. On the other hand, if the main factor is memory of contingency relation between moving to the dark room and electro-shock, the latency would be decreased with scopolamine as shown in the present results. For this reason, the latency in the passive avoidance test was considered to be the measure of memory performance. The result that the administration of morphine facilitates memory conflicts with those reported in some other studies (8, 9); however, the fact that the administration of morphine before test facilitates memory is in accord with the majority of the previously reported data (e.g., refs. 12, 13, 23, 24). In a preliminary study, we have confirmed that morphine injection in mice that had not received electroshock showed no effect on the step-through latency. This result supported the fact that morphine facilitates memory performance. In the ABCD contextual procedure, the mice subjected to the passive avoidance test after the tail-pinch test showed an increase in the step-through latency, suggesting that morphine facilitates memory of a contingency of

Fig. 5. Mean latencies of the step-through response in passive avoidance training and tests in the experiment using the AAAAB context procedure. Error bars represent the S.E.M. ($n = 12 - 15$). All animals were presented with foot shocks (200 V) in the dark compartment of the avoidance learning box without drug on Day 0. From Day 1 to Day 5, morphine or saline was administered 30 min prior to the passive avoidance test trial. Analyses of the data from Days 4 and 5 indicated that only the drug effect was significant ($P < 0.05$). The main effect of context and the interaction between the drug and context were not significant ($F < 1$).

step-into the dark compartment with grid shock. This increase in the latency in the passive avoidance response was maintained during the four sessions even when the context was changed to a novel one daily, indicating that the facilitation of memory has no context specificity. The latency in the passive avoidance response in the morphine-treated group in the different contexts was slightly higher than those in the group in the same context on Days 3 and 4. This slight difference might be due to a contingency of tail-pinch stimuli and manipulation. In the AAAAB contextual procedure, morphine facilitated memory, and there was no context specificity in memory performance. Thus the examination by the AAAAB contextual procedure reproduced the finding in the ABCD procedure. These results suggest that morphine facilitates memory retrieval. However, the mean latency of Group Sal in the ABCD context sequence and that of Group Sal in the AAAAB context sequence was different. One of the main reasons of this difference might be the different sensitivities to the conditioning by electroshock. The strain, days after birth, body weight, breeder, and housing conditions were identical in both experiments. The sensitivity to morphine in both context sequences was similar.

The learning modulation of morphine (25) and somatostatin (26) was indicated to be mediated in the hippocampus. Substance P has been reported to enhance memory via interaction with the endogenous opioid system in rats (27, 28). However, whether the injection of these peptides in animals shows context specificity seems to be unclear. The present results provide empirical support for our proposal that facilitation of memory, with no contextual specificity, with morphine could contribute to the development of the associative tolerance. On the contrary, the interference in memory retrieval by contextual change may disrupt the associative tolerance. This proposal may be implied by the recent report that the activation of the AMPA/kainite-glutamate and the non-competitive NMDA receptors within the central nucleus of the amygdale contribute to the negative affective component of morphine abstinence (29). Other data suggest that pretreatment with the non-competitive NMDA receptor antagonist MK-801 blocked the development of tolerance to morphine by the tail-flick test in mice (30). However, there are only a few reports about the effect of MK-801 on the contextual specificity in the development of tolerance by repeated morphine treatment. In a separate experiment, mice treated with MK-801 showed a dose-dependent delay in the development of tolerance to morphine (M. Nakama-Kitamura, manuscript in preparation). Memory retrieval in an organism requires that it be in a state similar to that in which the event has been

experienced (31). Tolerance to the antinociceptive effect of morphine in the present studies is considered to involve the associative learning between the environmental cues and the state in which the organism received morphine. The delay of the development of tolerance in the ABCD contextual procedure and the attenuation of tolerance in the AAAAB contextual procedure in the present studies indicate that the stated dependency was disrupted by the contextual change. The interaction between exteroceptive contextual and drug state changes has been studied, but there is no general conclusion about whether drug state dependent learning is predominant (32).

The prevention of associative learning may result in the diminution of antinociceptive tolerance as CR, which may be an anticipatory opposed response to UR (33). The present results may be a clue for exploring the mechanism of the model for the inner-process of memory in Pavlovian drug conditioning and the standard operation procedure model (34, 35) that postulates an operation process for the conditioning mechanism in the central nervous system.

In Pavlovian drug conditioning, the associative tolerance to morphine is produced by the pressure stimulus method and delayed by preventing the animal's ability to associate with environmental cues and the effect of morphine. Morphine facilitates memory and the drug has no context specificity. Thus, memory facilitation may contribute to associative learning in antinociceptive tolerance.

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