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Olfactory Cues and Morphine-Induced Conditioned Analgesia in Rats

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VALONE, J. M., C. K. RANDALL, P. J. KRAEMER AND M. T. BARDO. *Olfactory cues and morphine-induced conditioned analgesia in rats*. PHARMACOL BIOCHEM BEHAV **60**(1) 115–118, 1998.—In a Pavlovian conditioning procedure, rats were exposed to an odor conditioned stimulus (CS) and then were given morphine with its effect serving as the unconditioned stimulus (US). After four CS-US pairings, the CS was tested alone to assess the presence of an analgesic conditioned response (CR) using a hot-plate test. In Experiment 1a, two groups were conditioned by pairing either 10 mg/kg morphine or saline with an odor CS. In Experiment 1b, two groups were given an odor CS paired or unpaired with 10 mg/kg morphine. These results established that an odor cue can support a morphine-induced analgesic CR. Experiment 2 characterized the dose–effect curve (0, 3, 10, and 30 mg/kg morphine) using an odor conditioning procedure. The dose–effect curve showed an inverted U-shaped function, with the 10 mg/kg morphine group having significantly longer paw-lick latencies compared to all other groups. This finding contrasts with the monotonically ascending dose–effect curve for the analgesic unconditioned response (UR) to morphine. © 1998 Elsevier Science Inc.

Morphine Analgesia Conditioning Hot plate Odor Paw lick

HISTORICALLY, a great deal of research has been conducted to examine drugs and their various conditioned effects (7,17,18,27). In drug conditioning studies, a given CS may form selective associations with different properties of a drug US such as morphine. Working from this basic premise, it has been shown that different morphine-induced unconditioned responses (URs) can form selective associations with contextual or gustatory cues. For example, when a contextual CS such as a tactile or visual cue is paired with morphine, animals approach the drug-paired cue when it is presented alone (23,25). Paradoxically, many other studies using a gustatory cue as the CS and morphine as the US have demonstrated that rats will avoid a taste that has been paired with morphine (6,15,16,21).

Further studies examining morphine-induced analgesia have shown that the type of CS used in conditioning is important in defining the subsequent direction of the CR. Specifically, one study showed that when a contextual CS is paired with a morphine US the resulting CR is hyperalgesia (12). In contrast, other studies have shown that when a gustatory CS is paired with a morphine US the resulting CR is hypoalgesia (1,15). Taken together, these studies demonstrate that when morphine is used in conditioning procedures, the multiple properties of a single US can enter into selective associations with different CS types and that different CSs can affect the nature of the CR.

In contrast to pairing a contextual or gustatory CS with morphine, there is little known about the ability of olfactory cues to serve as a CS in morphine conditioning studies. In particular, it is not known if the CR to odor/morphine pairings is similar to those found using either context or taste paired with morphine. One group of researchers showed that rat pups developed preference for an odor paired with a low dose of morphine (10,11). Odor cues have also been used to condition rat pups to the dose-dependent aversive and appetitive effects of morphine (19). However, there is little information about the ability of odor cues to be conditioned to morphine effects in adult rats.

The most pertinent study investigating odor-cued conditioned morphine effects in adult rats directly demonstrated morphine-induced conditioned analgesia using a two-odor discrimination procedure (20). In that study, groups of animals were exposed to two different odors (orange and banana) on separate conditioning days. One group of animals had orange paired with morphine (10 mg/kg), while another group had banana paired with morphine (10 mg/kg). Both odors were then tested individually on 2 consecutive days for their ability to elicit conditioned analgesia. Although evi-

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dence for conditioning was obtained on the first odor test day, no conditioning was obtained on the second odor test day. Interpretation of the results from the second odor test day were problematic, however, because control paw-lick latencies were elevated on the second hot-plate test, thus potentially obscuring the conditioned analgesia. This increase in paw-lick latencies following repeated hot-plate tests replicates existing literature (9,26) and likely reflects a conditioned stress-induced analgesia. Because of this problem, the present study first sought to determine if a single-odor (no discrimination) procedure using a single analgesic hot-plate test would establish morphine-induced conditioned analgesia and to rule out a possible nonassociative interpretation by using an unpaired control. Second, because the previous study by Randall et al. (20) assessed only one dose of morphine (10 mg/kg), the present study also sought to characterize the dose–effect curve for morphine-induced conditioned analgesia using a single-odor conditioning procedure. If the dose response function for the CR to morphine parallels the dose–response function for the UR, this would suggest a similarity in the underlying neural mechanisms mediating the CR and UR.

METHOD

Subjects

In Experiment 1a, subjects were naive Sprague–Dawley male rats weighing 400–550 g. In Experiments 1b and 2, subjects were naive Sprague–Dawley male rats weighing 200–225 g at the beginning of each experiment. Animals were housed individually in standard wire cages in a climate-controlled colony room with a 12 hr light:12 hr dark cycle. All conditioning and testing was conducted during the light portion of the cycle. Free access to food and water was available in their home cage throughout the experiments.

Materials

Conditioning was conducted in a row of stainless steel hanging wire cages ($18 \times 17 \times 24$ cm each) located in a room separate from the colony room. Pine bedding was placed in stainless steel trays 7.5 cm below the conditioning cages for odor presentation. Odor used for conditioning was banana extract (Kroger brand) distributed over the pine bedding at approximately 1.5 mL per cage. A slide warming tray (Clinical Scientific Equipment Co., No. 26020) was used for the hot-plate test. A clear Plexiglas chamber ($15 \times 20 \times 28.5$ cm) with no floor and a removable top was used to contain animals on the hot plate. The hot plate and conditioning cages were located in a room separate from the colony room.

Drug

Morphine sulfate (National Institute on Drug Abuse, Rockville, MD) was mixed in 0.9% NaCl and administered subcutaneously in a volume of 1 mL/kg body weight. Dosage was based on the salt form of morphine.

Procedure

Animals were transported from the colony room to the conditioning/test room in their home cage. Banana odor was used on days 1, 3, 5, and 7. Subjects remained in the colony room on days 2, 4, 6, and 8, receiving no treatment or handling. In Experiment 1a, animals were assigned to one of two groups (n = 10–11): 0 or 10 mg/kg morphine. In Experiment 1b, animals were assigned to one of two groups (n = 10–11): odor paired or unpaired with 10 mg/kg morphine. In Experiment 2, animals were assigned to one of four different groups (n = 9–10 per group): 0, 3, 10, or 30 mg/kg morphine.

During the conditioning phase of Experiments 1a and 2, each conditioning trial began with a 15-min exposure to the banana odor, followed by an injection of the appropriate morphine dose according to group assignment. In Experiment 1b, rats in the paired group were given a morphine injection, while rats in the unpaired group received saline. After each injection, subjects were given an additional 30 min of exposure to the banana odor. At the end of the odor exposure period, all animals were placed on the inactive hot plate for 60 s to habituate them to the apparatus. They were then transported back to the colony room in their home cage. Also, for Experiment 1b, rats in the unpaired group received an injection of morphine, and rats in the paired group received a saline injection in the colony room 6 h following odor exposure.

On the test day (day 9), all subjects were given 15-min exposure to banana, followed immediately by an injection of saline. Thirty minutes after the injection, each subject was placed on the active (54 ± 0.5°C) hot plate. Because a variety of pain response measures are found in the literature, pain responsivity was assessed by taking two paw-lick latency measures. A "blind" observer recorded the latency to the first paw-lick response (front or hind paw), as well as the latency to the hind paw-lick response. When a hind paw-lick was observed, the animal was immediately removed from the hot plate. If a hind paw-lick was not observed within 60 s, the test was terminated. The apparatus was cleaned with a damp sponge between subjects. An increase in paw-lick latencies for the groups that had received odor/morphine pairings relative to the saline control group was taken as evidence of conditioned analgesia.

RESULTS

Because analysis for first and hind paw-lick latencies in each experiment yielded essentially identical results, only the results for the first paw-lick measure are presented here. In Experiments 1a and 1b, the hypothesis that conditioning would increase paw-lick latencies in morphine-treated animals compared to control animals was confirmed by one-tailed t-tests. In Experiment 1a, results showed that rats in the 10 mg/kg group had significantly longer first, $t(19) = 2.095$, $p < 0.05$, paw-lick latencies compared to saline controls (Fig. 1A). Likewise, in Experiment 1b, rats in the paired group had significantly longer first, $t(19) = 1.829$, $p < 0.05$, paw-lick latencies compared to unpaired control animals (Fig. 1B).

Mean paw-lick latencies for each group in Experiment 2 are illustrated in Fig. 2. Results from an ANOVA on the first paw-lick latency revealed a significant difference among the groups, $F(3, 38) = 4.98$, $p < 0.05$. Specific comparisons using the Newman–Keuls method showed that animals in the 10 mg/kg group had significantly longer first paw-lick latencies compared to saline controls. However, paw-lick latencies for subjects in the 3 and 30 mg/kg groups were not significantly different from saline controls. Further, tests showed that the mean first paw-lick latency in the 10 mg/kg group was significantly longer than the mean latency for both the 3 and 30 mg/kg morphine treated groups.

DISCUSSION

The present results support the hypothesis that an olfactory cue can be used to establish morphine-induced analgesia

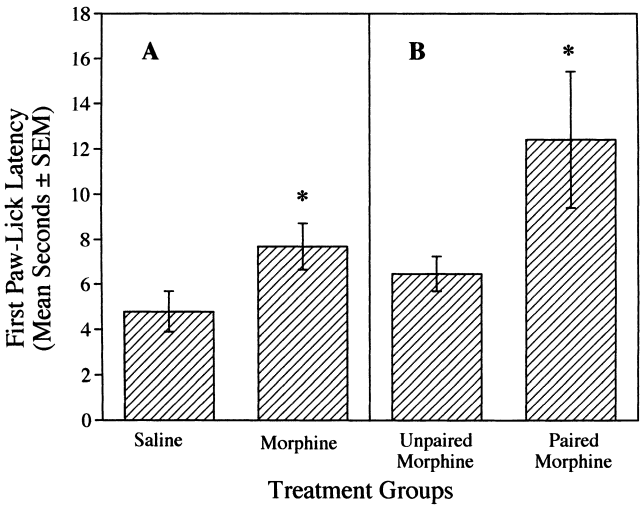

FIG. 1. (A) Mean first paw-lick latencies for each treatment group on the test day from Experiment 1a. (B) Mean first paw-lick latencies for each treatment group on the test day from Experiment 1b. An asterisk (*) represents a significant difference from the saline control group (A) or the unpaired control group (B), $p < 0.05$.

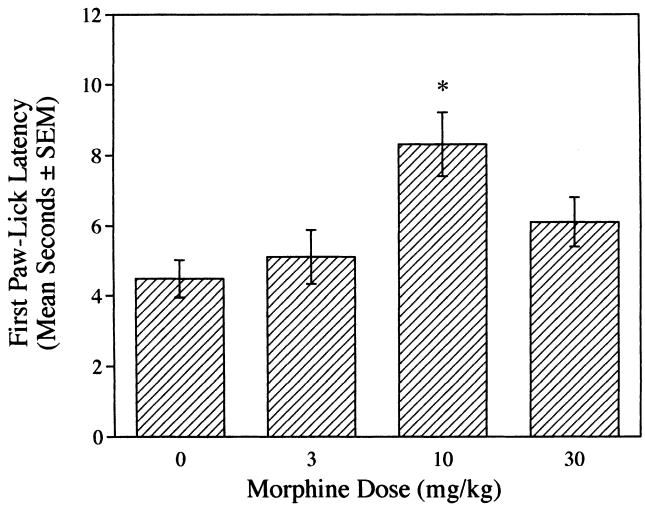

FIG. 2. Mean first paw-lick latencies for each treatment group on the test day from Experiment 2. An asterisk (*) represents a significant difference from the control group, $p < 0.05$.

using a Pavlovian conditioning procedure. Specifically, when a banana odor was repeatedly paired with morphine and subsequently tested in the absence of morphine, an analgesic CR was observed. This analgesic CR did not result from exposure to either the CS or US alone, because it was also evident relative to a control that received both the CS and US in an unpaired manner. We have no cogent explanation for the larger variability found in the paired morphine group in Experiment 1b compared to to the morphine group in Experiment 1a. However, one potential reason is that animals in Experiment 1a were older than those in Experiment 1b. Studies have shown there are age-dependent differences in locomotor activity in morphine-treated animals (24). In any case, these data are consistent with those of Randall et al. (20), and suggest that an odor can acquire stimulus control of the analgesic response to morphine. Although we cannot rule out the possibility that contextual cues other than odor controlled responding in the present experiment, this seems unlikely based on the results of Randall and his colleagues (20). Specifically, their study used two distinct odor cues presented on separate days during conditioning with morphine. If context had acquired stimulus control over responding, both groups that had exposure to either odor would have shown an analgesic CR on the first test day. Their results showed that the morphine-induced analgesic CR was evident only in animals conditioned and tested with the same odor. Taken together with our results, these data make the possibility of contextual control under these conditions unlikely.

Although an analgesic CR was found at the intermediate morphine dose (10 mg/kg), odor did not appear to control responding for animals receiving low (3 mg/kg) or high (30 mg/kg) doses of morphine. Consequently, a biphasic dose–effect curve was observed. In contrast to this apparent dose-dependent CR, the analgesic UR to morphine within this same dose range follows a monotonic, ascending dose–effect curve (5), suggesting that the CR and UR may involve different neural mechanisms. Further studies using an odor CS are necessary to complete the dose–effect curve for morphine doses on the ascending and descending portions of the inverted U-shaped function.

Although these results may be interpreted as evidence for morphine-induced conditioned analgesia produced by an associative process, there is at least one alternative nonassociative explanation that may account for the increase in paw-lick latencies obtained in the 10 mg/kg morphine group in the present experiments. That is, because animals were habituated to the nonfunctional hot plate on each conditioning trial, perhaps morphine prevented animals from becoming habituated to the hot-plate apparatus. Previous research has shown that exposure to a novel hot-plate apparatus increases paw-lick latencies (2,22), a finding usually referred to as novelty-induced analgesia. In a previous study that more directly investigated a novelty-induced interpretation using a paradigm similar to that used here, some evidence to support the notion of novelty-induced analgesia was obtained (4). Although we cannot rule out the possibility that novelty contributed to the increased paw-lick latency in the 10 mg/kg morphine group, the results evident from the 30 mg/kg morphine group militate against this possibility, as this higher dose should have also prevented the habituation process.

It is presently unclear why 30 mg/kg morphine, in contrast to 10 mg/kg morphine, failed to produce evidence for conditioned analgesia. The biphasic dose–effect curve obtained for conditioned analgesia is suprising because the dose–effect curve for the unconditioned analgesic effect of morphine increases monotonically within the dose range used in the present study (5). One possible explanation for loss of conditioned analgesia using the highest dose of morphine tested (30 mg/kg) may be related to the ability of morphine to disrupt memory processing. Previous studies have shown that morphine impairs learning and memory in a dose-dependent manner on various learning tasks (3,14).

Regardless of the interpretation, however, a previous study using a taste CS paired with morphine showed that an analgesic CR was obtained using 30 mg/kg morphine (1). It is unclear why an analgesic CR at higher doses was found using

a taste CS, but not found when using an odor CS. One possibility may be that the resulting CR for each different stimulus is mediated by a different neural system. For example, because morphine alters various neurotransmitter systems (i.e., endorphins, norepinephrine, and serotonin) to inhibit painful stimulation (13), it is possible that different systems are activated when different CSs are used for conditioning morphine effects. A second possibility is that the conditionability of a stimulus depends on the type of US to which it is associated (8). Perhaps the analgesic property of morphine across a wide dose range is more easily associated with a taste than an odor.

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