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Morphine-Induced Taste Avoidance Is Attenuated With Multiple Conditioning Trials

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SIEGEL, S., L. A. PARKER AND I. MOROZ. *Morphine-induced taste avoidance is attenuated with multiple conditioning trials.* PHARMACOL BIOCHEM BEHAV **50**(2) 299-303, 1995.—Morphine has paradoxical effects in learning experiments. The drug can serve as a reinforcer in several situations; yet rats avoid tastes paired with morphine, much as they avoid tastes paired with an emetic drug such as lithium chloride (LiCl). The results of the present experiment indicate that, in contrast with LiCl-induced taste avoidance, the strength of morphine-induced avoidance is nonmonotonically related to the duration of training. Although taste avoidances produced by both drugs are readily established, the morphine-induced avoidance (unlike the LiCl-induced avoidance) weakens with continued flavor-drug pairings. These results, together with prior findings, suggest that there are distinctive features of morphine-induced taste avoidance.

Conditioned taste avoidance Conditioned taste aversion Morphine Lithium Classical conditioning
Rats Psychopharmacology Drug reinforcement

ON THE BASIS of some assessments, morphine is a reinforcing drug. Rats will press a lever to obtain morphine (17) and also display a preference for a location paired with the drug (1,16), much as they respond with other reinforcers. Paradoxically, on the basis of other assessments, morphine is an aversive drug. Rats avoid a taste paired with morphine, much as they avoid a taste paired with a drug that induces gastrointestinal upset (3). This paradox has been the subject of extensive investigation and theoretical discussion (7).

Although the fact that morphine will motivate conditional taste avoidance (CTA) has been known for many years, more recent research has indicated several distinctive features of the CTA induced by morphine, compared with that induced by an emetic drug such as lithium chloride (LiCl). For example, when the doses of LiCl and morphine are equated to produce equivalent taste avoidance, the nature of the conditional responses (CRs) elicited by flavored solutions paired with the two drugs differ; although flavors paired with LiCl produce a pattern of aversive taste reactions that suggests that the solution has become conditionally unpalatable (6), flavors paired with morphine do not elicit this pattern of aversive reactions (13,14). In addition, a monotonically increasing dose-response curve has been demonstrated for LiCl-induced CTA (12), but not for morphine-induced CTA (4,15).

Especially relevant to the present experiment are reports that the morphine-induced CTA is transitory. That is, although the avoidance may be seen after only a few flavor-drug pairings (which typically is all that are administered in morphine CTA studies), continued pairings may lead to an attenuation (rather than a further augmentation) of the CTA (4,8). There is some suggestion that this "trend for the aversion to wane with continued injections" (4, p. 366) appears more pronounced with small (approximately 5 mg/kg) rather than larger doses of the opiate (8).

With the doses typically used, the CTA induced with morphine is relatively weak compared with that induced with LiCl. It is possible that the attenuation of a CTA with extended training is characteristic of weak CTAs, rather than CTAs established with morphine. The present experiment was designed to evaluate further the effect of extended acquisition on the morphine-induced CTA. A range of morphine doses was used. To evaluate the possibility that the aversion-

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attenuation reported with extended training may be a characteristic of weak CTAs, the design of the experiment also included groups trained with different doses of LiCl.

METHOD

Subjects

The subjects were 52 male Sprague-Dawley rats, purchased from Harlan-Sprague Dawley Breeding Laboratories (Indianapolis, IN), weighing 251-294 g on the first conditioning trial. They were housed individually in stainless-steel cages and maintained on ad lib Purina rat chow throughout the experiment. The colony room was maintained on a 12 h : 12 h light-dark schedule (lights on at 0800 h). The experimental procedures started 1 h after the lights were turned on.

Procedure

The rats were adapted to the laboratory for a 1-week period before the experiment commenced. They were handled on each of the first 4 days of this week.

During training, rats received one conditioning trial every 48 h. They were deprived of fluid for 18 h on the conditioning day, but received 24-h access to water on the intervening days (thus insuring fluid repletion between trials). This procedure was used to enhance the sensitivity of the assessment of the CTA (18). Rats were accommodated to the fluid access schedule during a 10-day adaptation session. During adaptation, water was removed at 1600 h every other day. Following 18-h deprivation, they received a graduated drinking tube of water for 15 min. Water bottles were returned at 1600 h that evening and were removed 24 h later at 1600 h.

Seventy-two hours after the final adaptation session, rats received their first conditioning trial while water-deprived for 18 h; they were presented with drinking tubes containing 0.1% saccharin solution during the 15-min drinking period that followed the 18-h deprivation period. Immediately upon removal of the drinking tubes, the rats received an intraperitoneal (IP) injection. Different groups were injected as follows: group 5 morphine (5 mg/kg morphine, n = 8); group 15 morphine (15 mg/kg morphine, n = 9); group 40 morphine (40 mg/kg morphine, n = 9); group 0.15 Lithium (0.15 mEq/kg, i.e., 6 mg/kg LiCl, n = 8); group 0.3 Lithium (0.3 mEq/kg, i.e., 12 mg/kg LiCl, n = 9); and group saline (n = 9). The morphine solutions were prepared to provide equivolume injections of 2 ml/kg. The lithium solution was prepared as a 0.15 M solution of LiCl and injected in a volume of 1 ml/kg (0.15 mEq/kg) or 2 ml/kg (0.3 mEq/kg). The LiCl doses are provided in terms of mEq/kg and the morphine doses in terms of mg/kg, to maintain consistent terminology with previous investigations that employed these drugs in the taste-avoidance paradigm. All procedures occurred in the home cage. The identical procedure was continued for each of 40 conditioning trials conducted over 80 days.

A two-bottle test was given 48 h after the final conditioning trial. The same water access schedule was maintained between

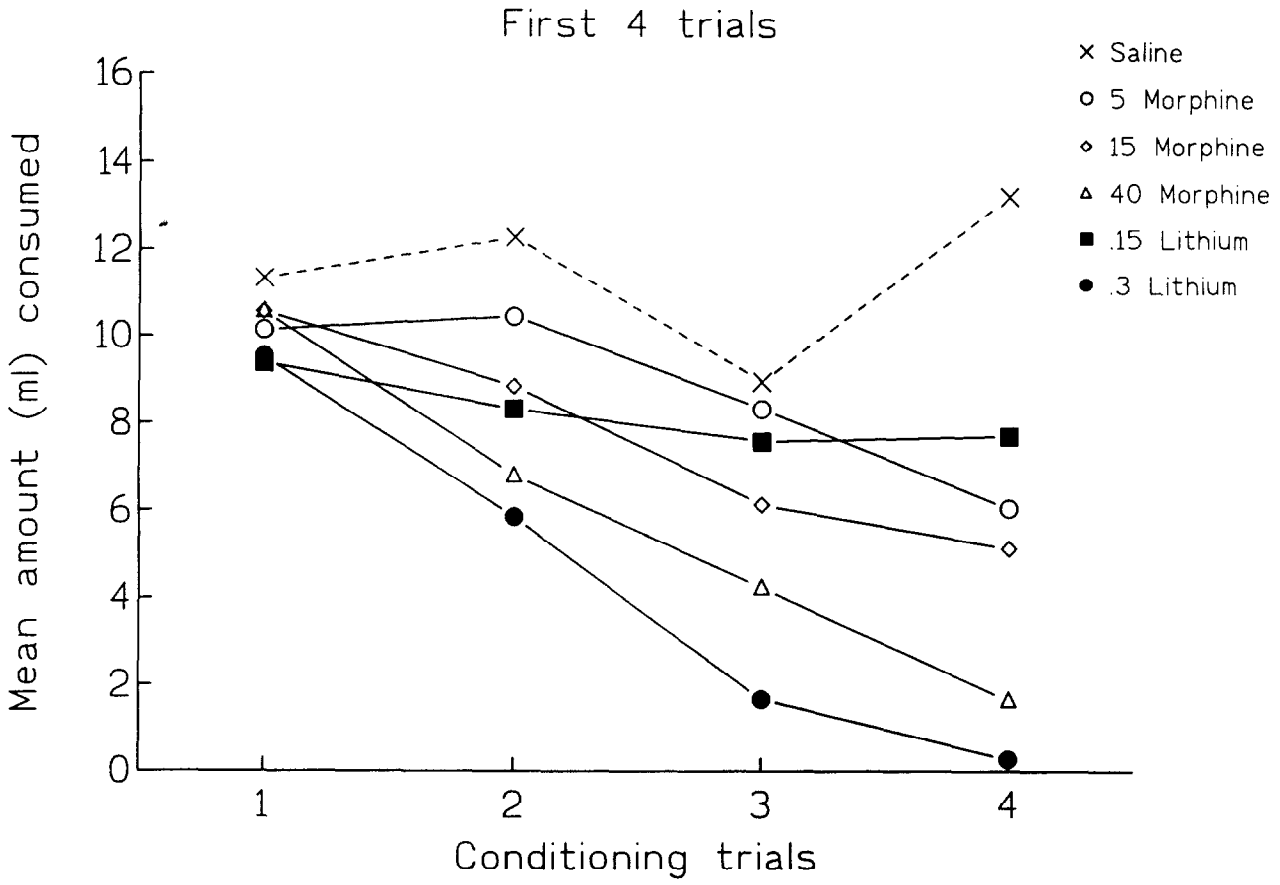

FIG. 1. Mean amount of 0.1% saccharin solution consumed by each group during the first four conditioning trials.

trial 40 and the two-bottle test; therefore, the rats were 18 h water-deprived at the beginning of the two-bottle test. For this two-bottle test, the rats were presented with two graduated tubes, one of which contained 0.1% saccharin solution and the other water. The spouts were 5 mm apart. The amount consumed after a 15-min test and the amount consumed after 24 h were measured.

RESULTS

For convenience, the acquisition data were analyzed in blocks of four trials. The present experiment involved extensive flavor-drug pairings. This contrasts with most CTA experiments, in which training is not continued beyond the minimal number of trials required to display avoidance. Thus, the early acquisition trials provide a basis for comparison of the results of the present experiment with typical CTA experiments. The mean saccharin consumed by subjects in each group on each of the first four acquisition trials is shown in Fig. 1. As may be seen in Fig. 1, lithium- and morphine-injected rats acquired CTAs. Higher doses of each drug induced more pronounced avoidance, and, for both drugs, the strength of the avoidance increased over the course of training.

A mixed-design analysis of variance (ANOVA) of the data summarized in Fig. 1 indicated a significant groups × trials interaction [$F(15, 138) = 5.75, p < 0.001$]. As is apparent in Fig. 1, this interaction resulted because the groups were similar on trial 1 (before the first injection), but subsequently diverged. Single-factor ANOVAs (followed by Newman-Keuls pairwise comparisons) indicated that although the groups did not significantly differ on trial 1, they did on each of the other trials. By trial 4, rats in group saline rats drank more saccharin than did rats in any other group ($p < 0.05$ for each). On trial 4, rats assigned to groups given the highest drug doses (0.3 lithium and 40 morphine) displayed similar avoidance ($p > 0.05$), but this avoidance was significantly greater than that seen in the other groups ($p < 0.05$ for each). The avoidance displayed by rats in group 15 morphine was significantly greater than that displayed by rats in group 0.15 lithium ($p < 0.05$).

The development of CTAs over the entire 40 conditioning trials is summarized in Fig. 2, which presents the mean saccharin consumed by subjects in each group in four-trial blocks. The strength of the avoidance remained proportional to the drug dose; however, the avoidance seen in morphine-injected rats, in contrast with that seen in lithium-injected ones, tended to weaken over the course of acquisition. This weakening was most apparent with lower doses of the opiate.

A mixed-design ANOVA of the data summarized in Fig. 2 revealed a significant interaction between groups and trial blocks [$F(45, 414) = 5.3, p < 0.001$]. Single-factor, repeated-measure ANOVAs were conducted across blocks of trials for each group, followed by Newman-Keuls pairwise comparisons ($p = 0.05$). With the exception of group 0.15 lithium, a significant effect of trials was seen in all groups [$F(9, 72) = 3.1, p < 0.01$ for each]. The rats in group saline drank less saccharin during block 1 than during any other

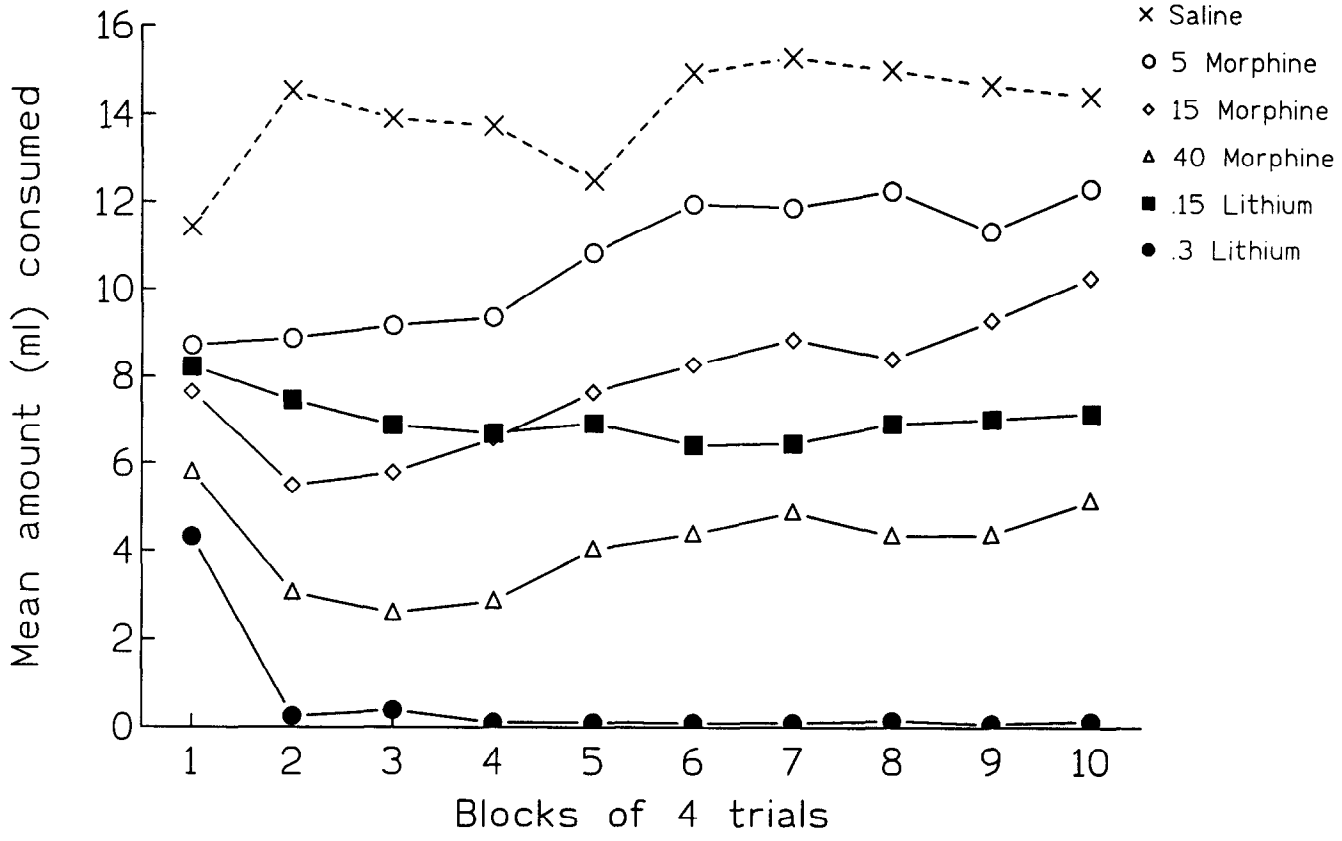

FIG. 2. Mean amount of 0.1% saccharin solution consumed by each group during each block of four conditioning trials.

block (except block 5). The rats given the highest dose of lithium (group 0.3 lithium) drank more saccharin during block 1 than during any other block ($p < 0.01$ for each) and did not differ in their intake across blocks two through 10. The rats in group 5 morphine displayed their strongest avoidance during the earlier blocks of trials; they drank less during each of blocks one through four than during each of blocks six through 10. The rats in the groups that received the higher morphine doses displayed a U-shaped function relating consumption to trials. Group 15 morphine drank less during blocks two and three than during block one or five through 10; in fact, this group displayed greater intake during the final block of testing than during blocks one through six or eight. Group 40 morphine drank less during blocks two through four than one, and less during block three than during block 10.

A single-factor ANOVA revealed that the groups differed in their saccharin intake during the final block of testing [$F(5, 46) = 19.8$, $p < 0.001$]. By subsequent Newman-Keuls pairwise comparisons (with $p = 0.05$), groups 0.3 lithium, 0.15 lithium, and 40 morphine drank less saccharin than did groups 5 morphine or saline. Group 15 morphine drank more saccharin than did groups 40 morphine or 0.3 lithium, but less saccharin than group saline. By this final block of training, the highest dose of lithium produced a stronger avoidance than any other group (including group 40 morphine).

After the 40th CTA acquisition trial, all subjects received a saccharin-water preference test. The amounts consumed of saccharin and water during the two-bottle preference test were converted to saccharin preference ratios. The saccharin preference ratios were obtained by dividing the amount of saccharin consumed by the total amount of fluid (saccharin + water) consumed by each rat.

The mean (±1 SEM) saccharin preference ratios for each group during the first 15 min and total 24 h of the two-bottle test are displayed in Fig. 3. Single-factor ANOVAs revealed a significant group effect for both measures [$F(5, 46) > 9.6$, $p < 0.01$ for each]. Subsequent Newman-Keuls analyses revealed that during the first 15 min of testing, group saline demonstrated significantly greater saccharin preference than did groups 0.3 lithium and 0.15 lithium ($p < 0.05$ for each), but no other groups significantly differed from each other. When the duration of the drinking period was similar to that employed during conditioning trials, the only groups that displayed a significant CTA in the two-bottle test were those conditioned with lithium; none of the morphine-conditioned groups displayed a CTA with this test, which was reported to be more sensitive than the single-bottle test (5). However, there was a trend for morphine-injected animals to display a saccharin aversion.

Evidence for morphine-induced aversion was apparent in the 24-h test (Fig. 3, bottom). When the rats were allowed to consume from both bottles for 24 h, Newman-Keuls analysis revealed that group saline differed from each of the other groups, except 5 morphine ($p < 0.05$ for each). The 24-h, two-bottle preference test appeared to be the most sensitive test of saccharin avoidance. When allowed to consume saccharin and water over a 24-h period, all drug-conditioned groups except group 5 morphine displayed significant CTAs.

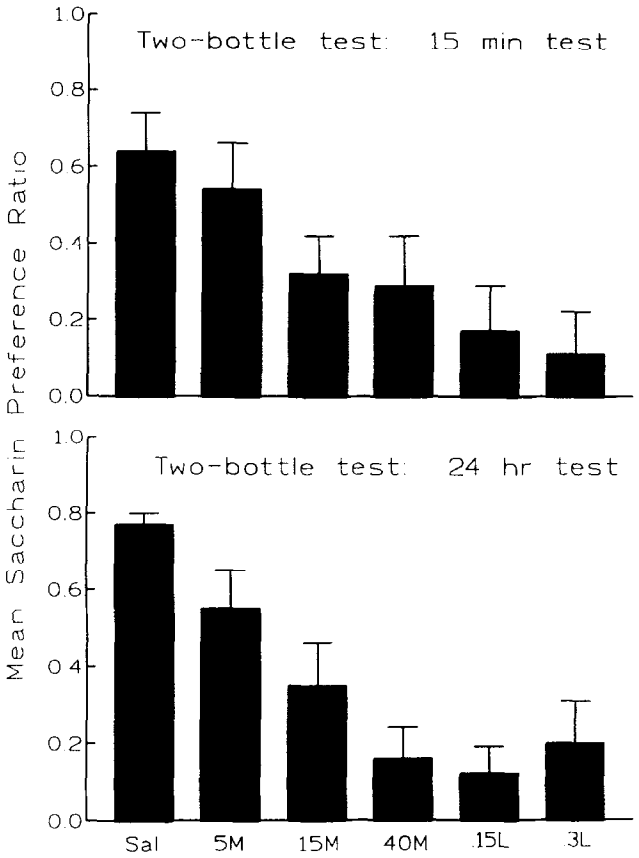

FIG. 3. Mean (±SE) saccharin preference ratio (Saccharin/Saccharin + Water) for each group during the first 15 min of testing and the total 24 h of testing during the two-bottle test.

DISCUSSION

Although both morphine and lithium induce CTAs, the pattern of intake across conditioning trials was different for rats trained with the two drugs. Once the CTA was established, it weakened with further training of rats assigned to morphine groups, but not of rats assigned to lithium groups. The difference in the pattern of intake was not a function of differential strengths of the initial CTA produced by the two drugs. A dose of 0.15 mEq/kg lithium produced a weaker CTA than did 40 or 15 mg/kg of morphine during early conditioning trials; yet, the strength of the CTA produced by 0.15 mEq/kg of lithium did not decrease across conditioning trials as did the strength of the CTA produced by 5, 15, and 40 mg/kg of morphine. By the final block of testing, a dose of 0.15 mEq/kg lithium produced a stronger CTA than did 5 mg/kg of morphine, and no longer differed from a dose of 40 mg/kg of morphine in the single-bottle conditioning/testing trials. By the two-bottle test, in the similar 15-min test, the only groups that displayed a significant CTA were the two lithium-conditioned groups.

One possible explanation for the nonmonotonic relationship between conditioning trials and the strength of the morphine-induced CTA is provided by data and theory suggesting that μ-opioids may have both rewarding and aversive effects, each mediated by separate mechanisms (2,9). In contrast with the CTAs seen following pairing of a taste with the doses of morphine used in this experiment, a conditioned taste preference (CTP) results if a flavor is followed by a very small dose of morphine (about 0.25 mg to 0.50 mg/kg) (10,11): "Presumably, CTP is produced by pairing a taste with the rewarding effect and CTA by pairing a taste with the aversive effect. When a taste is followed by both effects, CTA tends to

be dominant" (9, p. 238). It is possible that over the course of repeated morphine administrations, tolerance develops to a greater extent to the aversive than to the rewarding effects of the drug. Thus, the diminution of the CTA over the course of repeated trials results from the increase in the rewarding effect that is manifested as the aversive effect decreases.

Finally, the present findings do suggest that, like CTA produced with lithium, the strength of morphine-induced CTA is dose dependent, in contrast to previous reports (4,6,15). The present procedure provided a sensitive test of a CTA. The rats were conditioned and tested when water-deprived for 18 h after a 24-h period of free access to water on each trial. Using this sensitive assessment test of a CTA, a dose-dependent morphine CTA was apparent. These results again suggest (5), that the nature of the CTA test employed must be considered an important variable when assessing flavor–drug associations.

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