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# Role of Associative and Nonassociative Mechanisms in Tolerance to Morphine "Anorexia"

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WOLGIN, D. L. AND H. D. BENSON. *Role of associative and nonassociative mechanisms in tolerance to morphine "anorexia."* PHARMACOL BIOCHEM BEHAV **39**(2) 279–286, 1991.—To determine whether tolerance to morphine-induced anorexia involves associative mechanisms, rats were given chronic injections of morphine (Group 1, 10 mg/kg; Group 2, 20 mg/kg) in the presence of one compound cue on alternate days and injections of saline in the presence of another compound cue on the intervening days. After tolerance developed to the initial suppression of intake, three tests of Pavlovian conditioning were conducted. On the *compensatory response test*, in which saline injections were given in the presence of the morphine cue, only Group 2 showed a significant increase in milk intake. On the *explicit unpairing test* and the *environmental specificity test*, in which morphine injections were given in the presence of the saline cue or in an entirely different room, respectively, neither group showed a significant loss of tolerance. The failure to demonstrate cue-dependent tolerance in this paradigm may have been due in part to inadvertent temporal conditioning and in part to the rapid development of nonassociative tolerance.

Morphine anorexia Associative tolerance Pavlovian conditioning Compensatory response Explicit unpairing Environmental specificity Drug tolerance

A large literature attests to the importance of associative mechanisms in the development of tolerance to opiates [for recent reviews, see (1,9)]. For the most part, these studies have focused on the analgesic and thermic effects of morphine. To extend this line of research, the present study investigates the role of Pavlovian conditioning in the development of tolerance to the "anorexigenic" effect of morphine. As shown by Leshem (4,5), morphine has a triphasic effect on feeding. Following injection of 15 mg/kg, for example, morphine suppresses intake during the 1st h, enhances intake during the next 3 h, and then suppresses intake again for up to 24 h. We focused on the initial period of "anorexia." Although the precise mechanism of this effect is not known, it is assumed to be secondary to a motor impairment (5, 6, 8).

In a previous study (10), we found that tolerance to morphine-induced anorexia is not contingent on experience with food while the rats are intoxicated. Thus rats developed comparable levels of tolerance whether they received injections of morphine (10 or 20 mg/kg) before or after access to milk. During the course of that experiment, we also noted that rats given injections of saline on a regularly scheduled drug day show significantly higher milk intakes than they do when injected with saline on a nondrug day. Although such an effect might be viewed as a Pavlovian conditioned compensatory response, procedural considerations made this interpretation unlikely. For example, no explicit drug cues were provided, and the effect was greater in rats that had been given morphine after they had access to milk, a procedure that would not support Pavlovian conditioning. The present study, therefore, was designed to provide a more direct test of the potential role of Pavlovian conditioning in the development of tolerance to morphine-induced anorexia.

In brief, rats were given injections of morphine in the presence of one distinctive compound cue and injections of saline in the presence of another. After tolerance developed to the initial suppression of intake, three tests of Pavlovian conditioning were conducted. In the first, saline was injected in the presence of the morphine cue to unmask a putative compensatory response (i.e., increased intake). In the second and third tests, morphine was administered either in the presence of the saline cue (explicit unpairing test) or in an entirely different environment (environmental specificity test) in order to inhibit the expression of tolerance.

## METHOD

### *Subjects*

The subjects were 48 male Sprague-Dawley albino rats weighing 324–508 g at the start of the experiment. The rats were housed individually in stainless steel cages under a 12:12-h light-dark cycle (lights on at 6:00 a.m.) and maintained on three Purina Lab Chow pellets (about 15 g) and ad lib water daily.

### *Procedures*

*Baseline phase.* To establish baseline levels of milk intake, the rats were given daily 30-min tests in their home cages dur-

ing which water bottles were replaced with calibrated drinking tubes containing sweetened condensed milk diluted with water (1:2). These tests were conducted over a 6-week period. Preceding each test, the subjects were injected with isotonic saline (Sal; 1 cc/kg IP) 30 min before access to milk. At the end of each test, water bottles were replaced, and the rats were fed. For statistical purposes, the control level of milk intake for this phase of the experiment (*prescreen baseline*) was the mean of the last 7 trials.

The rats were then screened with a 10 mg/kg IP dose of morphine sulfate (Mor; Merck Chemical Division) injected 30 min before access to milk. Twenty-four rats whose intakes were decreased by at least 50% were designated Group 1. The remaining 24 rats were designated Group 2. Both groups were then given daily injections of Sal and access to milk for an additional 2.5 weeks to reestablish a stable baseline and to minimize the opportunity for tolerance to develop as a function of this initial uncued exposure to the drug. The mean of the last 7 of these trials was used for statistical purposes (*prechronic phase baseline*).

*Chronic phase.* The rats in each group were then assigned to one of two subgroups (n's = 12) equated for milk intake, body weight, and response to Mor. For each group, one subgroup received injections of Mor (Group 1, 10 mg/kg; Group 2, 20 mg/kg) and access to milk on alternate days (*drug days*) in the presence of a compound auditory/olfactory cue (the "Mor cue"). On the intervening days (*saline days*), this subgroup received injections of Sal and access to milk in the presence of a second auditory/olfactory cue (the "Sal cue"). The other subgroup was treated similarly except that Sal was injected on both "drug" and "saline" days (i.e., in the presence of both cues). To control for potential differences in milk intake on days in which Mor was administered, the intakes of the Sal subgroups were yoked to those of their respective Mor subgroups on the previous drug day. Thus the Sal subgroup received a restricted ration of milk on drug days and unlimited milk on the intervening saline days. A total of 24 trials was conducted with each drug/cue condition.

The auditory components of the compound cues consisted of a tape recording of either a repetitive percussion rhythm or synthesized complex instrumental ("New Age") music. The olfactory components were either banana or orange extract, which was poured into two shallow dishes containing several cotton balls. One dish was placed in front of a floor fan on one side of the room, and the other was placed on the opposite side of the room. An exhaust fan in the ceiling helped circulate the odors. The percussion tape was paired with the orange odor, and the "New Age" music with the banana odor. To control for potential differences in the salience of the cues, half of each Mor subgroup received drug injections in the presence of one of the compound cues, whereas the other half received these injections in the presence of the other compound cue. Because only one compound cue could be presented on any given day, this counterbalancing was achieved by testing half of each subgroup with Mor and the other half with Sal on each day. Because the intakes of the Sal subgroups were yoked to those of the Mor subgroups on drug days, the Sal subgroups were also subdivided, with half of each subgroup tested in association with one of the Mor subgroups. For each of these subgroups, one cue was designated the Mor cue and the other the Sal cue.

*Tests of Pavlovian conditioning.* At the conclusion of the chronic phase, three tests of Pavlovian conditioning were conducted. To determine whether tolerance to morphine was mediated by a conditioned compensatory response (hyperphagia), rats in the Mor subgroups were given injections of Sal in the presence of the Mor cue. Rats in the Sal subgroups were given injections of Sal, as in the chronic phase. On the following day, the associative nature of tolerance was assessed by giving the Mor subgroups injections of the drug in the presence of the Sal cue (i.e., the cue explicitly unpaired with Mor). Again, the Sal subgroups were given injections of Sal. Following this test, the rats resumed the chronic phase regimen of alternating compound cues and drug/saline injections to maintain the level of tolerance in the Mor subgroups. At weekly intervals during this period, the rats were transferred to an adjoining room, injected with saline, and given access to milk for 30 min to establish baseline levels of intake in a new environment. Neither compound cue was presented on these days. The adjoining room was also larger, brighter, and quieter than the usual test room. Following three such trials, the environmental specificity of tolerance was assessed by giving rats in the Mor subgroups injections of morphine and access to milk in the new environment. Rats in the Sal subgroups were again injected with Sal on this day.

*Dose-response determinations.* Following the test of environmental specificity, dose-response curves were established for each Mor subgroup by injecting a test dose of the drug at weekly intervals on a regularly scheduled drug day (i.e., in the presence of the Mor cue). Rats in the Sal subgroups were also tested with Mor on the same days as their corresponding Mor subgroup. During these tests, all groups were given access to unlimited milk. For Group 1, rats in the Mor subgroup were tested with 6.5, 10, 15, and 30 mg/kg Mor, whereas rats in the Sal subgroup were tested with 3, 6.5, 10 and 15 mg/kg. For Group 2, rats in the Mor subgroup were tested with 6.5, 10, 15, 20, and 40 mg/kg Mor, whereas rats in the Sal subgroup were tested with 6.5, 10, 15, 20, and 30 mg/kg. Each subgroup continued to receive its respective chronic phase treatment between test doses of the drug.

*Extinction of temporal conditioning.* Because Mor injections were administered on alternate days, the rats may have learned to anticipate the drug. Such temporal conditioning would tend to maintain tolerance even when Mor was administered in the absence of the compound cue associated with the drug, as during the tests involving explicit unpairing and environmental specificity. Consequently, an attempt was subsequently made to extinguish any temporal conditioning that may have developed during the course of the experiment.

Rats in both groups were given daily injections of Sal and access to milk in their home cages for 6 days, but in the absence of the compound cues. Each subgroup was then injected with Mor in the presence of one of the compound cues. For half the subjects, this was the Mor cue, whereas, for the other half, it was the Sal cue. The rats were then given saline injections and milk in the absence of cues for 6 more days, followed by a second Mor test in the presence of the other compound cue.

A summary of the experimental protocol is presented in Table 1.

### *Data Analysis*

Most of the data were analyzed with analyses of variance (ANOVA) for repeated measures. When warranted by significant F ratios, individual comparisons were made with Tukey's HSD test. Some of the data pertaining to the tests of Pavlovian conditioning involved planned comparisons. These analyses were made using the test of Dunn-Sidak (2). To analyze shifts in the dose-response curves, milk intakes were first converted to a percentage of intake under Sal and plotted against dose of Mor using a log scale. Doses that suppressed intake by 50% ($ED_{50}$s) were then calculated with linear regression analysis using commercially available software (Analytical Graphics, Version 3, Hu-

TABLE 1

SUMMARY OF EXPERIMENTAL PROTOCOL

1. Prescreen baseline (6 weeks)
2. Screen (10 mg/kg Mor)
3. Prechronic phase baseline (2.5 weeks)
4. Chronic phase (7 weeks)
   Morphine Group: Cue M + Mor injection
   Cue S + Sal injection
   Saline Group: Cue M + Sal injection
   Cue S + Sal injection
   (Chronic phase baseline = mean of last 7 "Cue S" trials)
5. Tests of Pavlovian conditioning
   *a) Compensatory response*
   Morphine Group: Cue M + Sal injection
   Saline Group: Cue M + Sal injection
   *b) Explicit unpairing*
   Morphine Group: Cue S + Mor injection
   Saline Group: Cue S + Sal injection
   *c) Environmental specificity*
   Morphine Group: New room + Mor injection
   Saline Group: New room + Sal injection
6. Dose-response determination
7. Extinction of temporal conditioning
   Morphine Group: No cue + Sal injections
   Saline Group: No cue + Sal injections
8. Retest conditioning
   Morphine Group: Cue M + Mor injection
   Cue S + Mor injection
   Saline Group: Cue M + Mor injection
   Cue S + Mor injection

Mor injections: Group 1 = 10 mg/kg; Group 2 = 20 mg/kg.

man Systems Dynamics, Northridge, CA). Shifts in $ED_{50}$ were expressed as ratios of the values of the Mor and Sal subgroups of each group.

During the course of the experiment, 1 rat from the Sal subgroup of Group 1 died of unknown causes. Its data were excluded from the statistical analysis. The data of 17 other rats were also excluded from the analysis. Four rats from the Mor subgroup of Group 2 were dropped because they failed to acquire tolerance by the end of the chronic phase. Five rats from the Mor subgroup of Group 1 were also dropped because they were found to be tolerant to the drug on the first conditioning trial of the chronic phase. Because such tolerance resulted from exposure to Mor on the screening test, it was clearly nonassociative in nature. (Although these data are pertinent to the conclusions drawn from this experiment, we felt that it was inappropriate to include them in the statistical analysis.) Because the original assignment of rats to groups involved matching of subjects on the basis of baseline intake and initial response to Mor, we also eliminated rats from each of the Sal subgroups that were matched to those dropped from the Mor subgroups. This procedure resulted in n's of 7 for subgroups of Group 1 and n's of 8 for subgroups of Group 2.

## RESULTS

Preliminary analyses revealed that there were no differences within subgroups as a function of which compound cue was paired with Mor. Consequently, the data for all rats in each subgroup were pooled.

### *Baseline Intakes*

*Group 1.* As shown in Fig. 1 (left panel), both subgroups of Group 1 ingested 23–25 cc of milk during the baseline periods preceding the screen (prescreen) and chronic phase of the experiment (prechronic). However, during the chronic phase, rats in the Mor subgroup showed a significant decrease in intake on the days they were given milk in the presence of the Sal cue, whereas rats in the Sal subgroup showed a slight increase on these trials (left panel, last 7 chronic). The baseline shift in the Mor subgroup was confirmed by a significant subgroup × day interaction, $F(2,24) = 4.89$, $p < 0.02$. Individual comparisons revealed that, for the Mor subgroup, intakes in the presence of the saline cue were significantly lower than those during the prescreen and prechronic periods. Intakes on the latter did not differ. In contrast, there were no significant differences in intake for the Sal subgroup.

*Group 2.* Figure 2 (left panel) shows that both subgroups of Group 2 also drank 23–25 cc of milk during the periods preceding the screen (prescreen) and chronic phase of the experiment (prechronic). Although rats in the Mor subgroup showed a significant decrease in intake during the first 7 Sal trials of the chronic phase (see data in Fig. 6), their intakes recovered to baseline levels by the last 7 Sal trials. Rats in the Sal subgroup showed stable intakes throughout the experiment.

### *Development of Tolerance*

*Group 1.* As shown in Fig. 1 (middle panel), Mor produced a marked suppression of intake in both subgroups of Group 1, followed by complete tolerance in the Mor subgroup by the last 7 trials of the chronic phase (middle panel, *last 7 chronic*). The degree of tolerance was derived from Fig. 3, which shows the dose-response data for the two subgroups. The $ED_{50}$s calculated from the regression lines yielded values of 13.53 and 8.72 for the Mor and Sal subgroups, respectively. The ratio of these values reveals a 1.5-fold rightward shift of the Mor subgroup's dose-response function.

*Group 2.* The intakes of Group 2 were not substantially reduced by the screen dose of 10 mg/kg Mor (Fig. 2, middle panel). However, injection of 20 mg/kg on the first day of the chronic phase produced a decrease in intake in the Mor subgroup comparable to that produced by 10 mg/kg in Group 1. By the last 7 trials, intake recovered to near baseline levels. The dose-response data are shown in Fig. 4. The $ED_{50}$s calculated from the regression lines yielded values of 21.94 and 14.27 for the Mor and Sal subgroups, respectively, indicating a 1.5-fold shift to the right in the dose-response curve of the Mor subgroup.

### *Tests of Pavlovian Conditioning*

*Group 1.* The results of the tests for Pavlovian conditioning for Group 1 are presented in Fig. 1 (right panel). In the compensatory response test, milk intake following injection of Sal in the presence of the Mor cue was compared to three measures of baseline intake: the prescreen baseline, the prechronic phase baseline, and the mean of the last 7 Sal trials of the chronic phase (during which injections of Sal were given in the presence of the *Sal* cue; see Fig. 1, left panel). Significant differences were found in the pattern of intake on these days for rats in the Mor and Sal subgroups, as revealed by a significant subgroup × day interaction, $F(3,36) = 4.70$, $p < 0.007$. For the Mor subgroup, milk intakes on the compensatory response test exceeded those on the last 7 Sal trials of the chronic phase, but did not differ from those on the prescreen or prechronic phase baselines. For the Sal subgroup, in contrast, intakes on the compensatory response test exceeded those on the prescreen and prechronic phase baselines, but did not differ from those during the

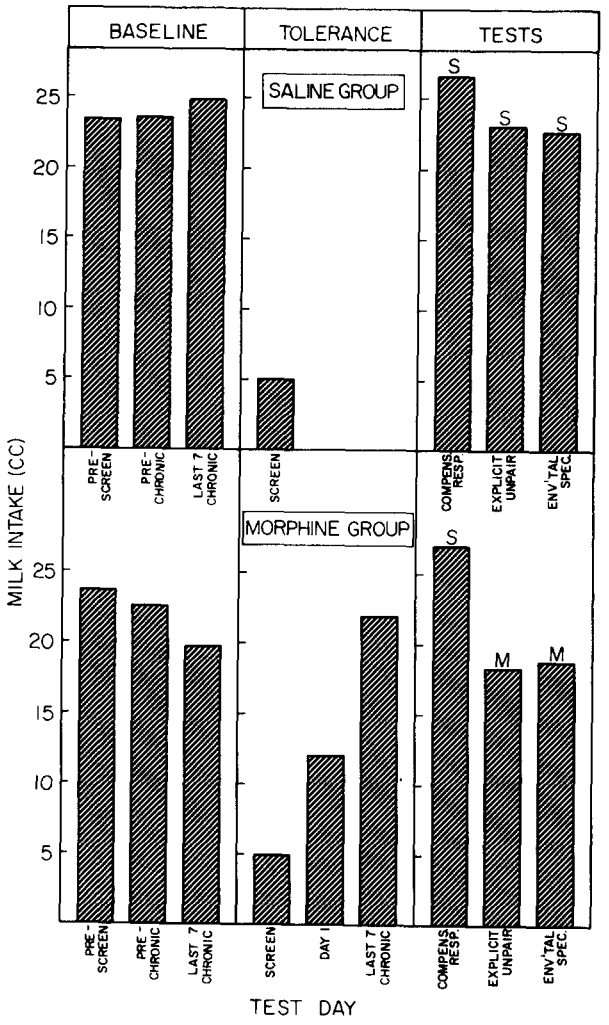


FIG. 1. Mean milk intakes of Mor and Sal subgroups of Group 1 during various phases of the experiment. [*Baseline*: Prescreen = mean of 7 trials preceding the initial test with Mor; prechronic = mean of 7 trials preceding the tolerance phase; last 7 chronic = mean of last 7 Sal trials during the tolerance phase. *Tolerance*: Screen = initial test with 10 mg/kg Mor; day 1 = 1st drug day with the Mor cue; last 7 chronic = mean of last 7 Mor trials. *Tests*: Compens. Resp. = intakes after injection of Sal in the presence of the Mor cue; explicit unpair = intakes after injection of Mor (Morphine Group) or Sal (Saline Group) in the presence of the Sal cue; env'tal spec. = intakes after injection of Mor (Morphine Group) or Sal (Saline Group) in a new environment.]

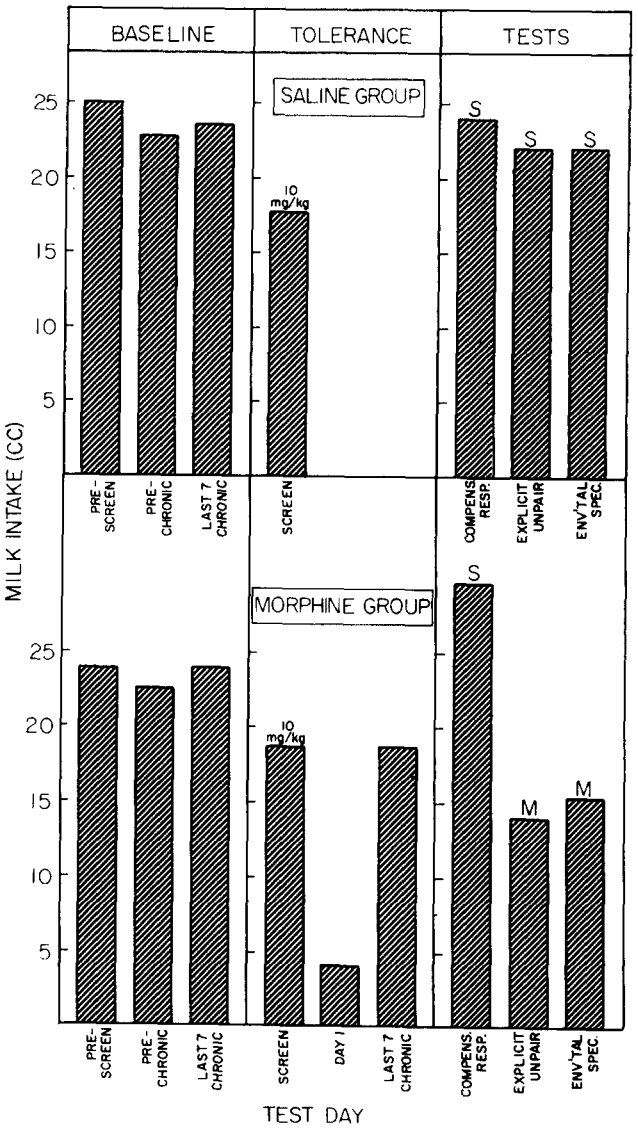


FIG. 2. Mean milk intakes of Mor and Sal subgroups of Group 2 during various phases of the experiment. [*Baseline*: Prescreen = mean of 7 trials preceding the initial test with Mor; prechronic = mean of 7 trials preceding the tolerance phase; last 7 chronic = mean of last 7 Sal trials during the tolerance phase. *Tolerance*: Screen = initial test with 10 mg/kg Mor; day 1 = initial test with 20 mg/kg Mor and the Mor cue; last 7 chronic = mean of last 7 Mor trials. *Tests*: Compens. Resp. = intakes after injections of Sal in the presence of the Mor cue; explicit unpair = intakes after injection of Mor (Morphine Group) or Sal (Saline Group) in the presence of the Sal cue; env'tal spec. = intakes after injection of Mor (Morphine Group) or Sal (Saline Group) in a new environment.]

chronic phase. Finally, the intakes of the Mor subgroup on the compensatory response test did not differ from those of the Sal subgroup.

The milk intakes of the Mor subgroup on the explicit unpairing and environmental specificity tests, in which Mor injections were given in the presence of the Sal cue or in a new environment, respectively, were not significantly different from those on the last 7 Mor trials. The intakes of the Sal subgroup on these tests did not differ from those during the baseline period.

The lack of a significant reduction in intake on the explicit unpairing and environmental specificity tests might signify that the cues were not discriminably different to the rats. To further evaluate this possibility, we compared the intakes of the subgroups on days in which Sal injections were administered in the presence of the Mor cue (compensatory response test, 0 mg/kg dose on the dose-response determination), the Sal cue (mean of the first 7 Sal trials, mean of the last 7 Sal trials), or neither cue (control trials for the environmental specificity test). The data are presented in Fig. 5. Statistical analysis revealed a significant subgroup × day interaction, $F(4,48) = 8.66$, $p < 0.001$. Individual comparisons confirmed that, for the Mor subgroup, intakes in the presence of the Sal cue were significantly lower than in the presence of the Mor cue or in the presence of neither cue.

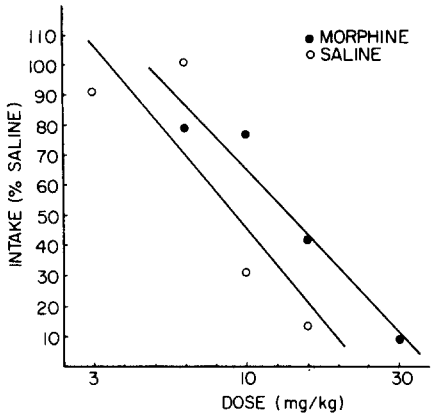


FIG. 3. Mean milk intakes of the Mor and Sal subgroups of Group 1 after injections of various doses of Mor. Doses are plotted on a log scale. Lines are derived from linear regression analysis of the data. (The data are expressed as percentages of intake after injection of Sal.)

For the Sal subgroup, intakes in the presence of the "Sal cue" did not differ from those in the presence of the "Mor cue," whereas intakes in the presence of neither cue were generally lower than any of the others.

*Group 2.* The results of the tests for Pavlovian conditioning for Group 2 are presented in Fig. 2 (right panel). In comparing the results of the compensatory response test with the three baseline measures (Fig. 2, left panel), significant differences between the subgroups were obtained, as confirmed by a significant subgroup × day interaction, $F(3,42)=5.05$, $p<0.004$. Rats in the Mor subgroup ingested significantly more milk during the compensatory response test than they did on the prescreen and prechronic phase baselines. Moreover, their intakes on this test exceeded those on the last 7 Sal trials of the chronic phase, in which Sal was injected in the presence of the Sal cue. Finally, the intakes of the Mor subgroup were significantly greater than those of the Sal subgroup, whose intakes on this test did not differ from their baseline levels. It should be noted, however, that the magnitude of the differences reported above was

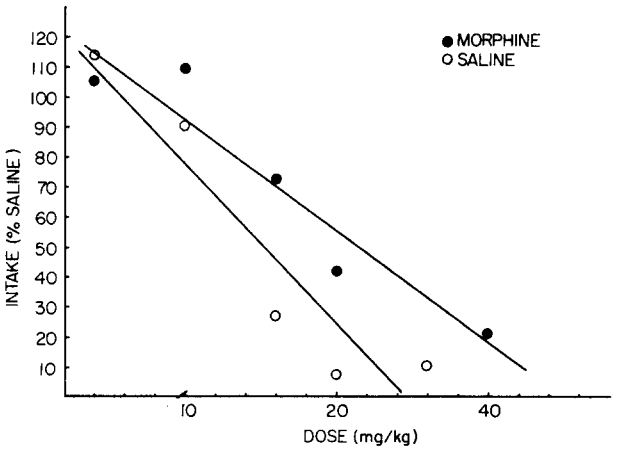


FIG. 4. Mean milk intakes of the Mor and Sal subgroups of Group 2 after injections of various doses of Mor. Doses are plotted on a log scale. Lines are derived from linear regression analysis of the data. (The data are expressed as percentages of intake after injection of Sal.)

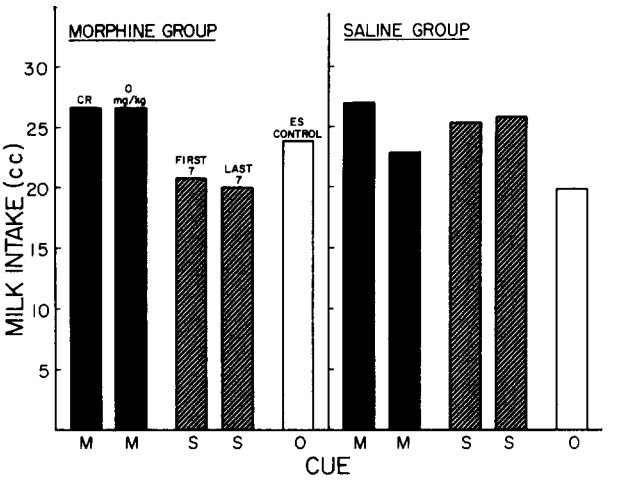


FIG. 5. Mean milk intakes of the Mor and Sal subgroups of Group 1 after injections of Sal in the presence of the Mor cue (M), the Sal cue (S), or neither cue (0). (Compens. resp. = compensatory response test; 0 mg/kg = Sal dose during the dose-response determination; first 7 = mean of 1st 7 Sal trials of the tolerance phase; last 7 = mean of last 7 Sal trials of the tolerance phase; ESC = mean of control trials for environmental specificity test.)

relatively small (5–7 cc).

The milk intakes of the Mor subgroup on the explicit unpairing and environmental specificity tests were not significantly different from those on the last 7 drug trials of the chronic phase. Similarly, the intakes of the Sal subgroup on these tests did not differ from those during the baseline period. As a further check on the discriminability of the cues, we compared the intakes of the subgroups on days on which Sal injections were given in the presence of the Mor cue, the Sal cue, and neither cue, as previously described. The data are shown in Fig. 6. Statistical analysis revealed a significant subgroup × day interaction, $F(4,56)=5.61$, $p<0.001$. Individual comparisons confirmed that, for the Mor subgroup, intakes in the presence of the Sal cues were significantly lower than in the presence of the Mor cues. Moreover, intakes with the Sal cues were higher on the last 7 trials than on the first 7. Intakes with neither cue present were significantly greater than those on the first 7 trials with the Sal cue, but they were not different from those on the last 7 trials with the Sal cue or from those with the Mor cue. For the Sal subgroup, intakes in the presence of neither cue were significantly lower than those in the presence of the "Mor" and "Sal" cues, but there were no differences in intakes between the latter.

### *Extinction of Temporal Conditioning*

*Group 1.* Milk intakes following chronic injections of Sal in the home cage, but with neither cue present, are shown in Fig. 7. For purposes of comparison, the means of the preceding 3 Sal trials, in which Sal injections were given in the presence of the Sal cue, are also included. (It should be noted that, on the drug day preceding the first extinction trial, rats in the Mor subgroup received their maintenance dose of 10 mg/kg Mor.) The Mor subgroup ingested significantly less milk during this 3-day baseline period than the Sal subgroup. However, during extinction, milk intakes recovered by the second trial and, with one exception, remained elevated until the Mor test. Following in-

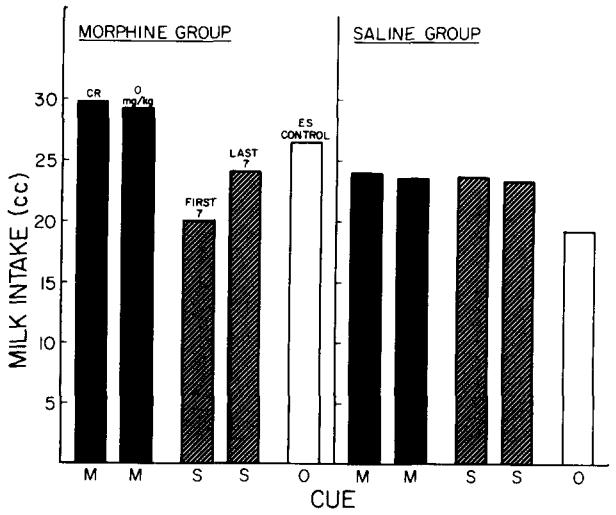


FIG. 6. Mean milk intakes of the Mor and Sal subgroups of Group 2 after injections of Sal in the presence of the Mor cue (M), the Sal cue (S), or neither cue (0). (Compens. resp. = compensatory response test; 0 mg/kg = Sal dose during the dose-response determination; first 7 = mean of 1st 7 Sal trials of the tolerance phase; last 7 = mean of last 7 Sal trials of the tolerance phase; ESC = mean of control trials for environmental specificity test.)

jection of Mor, milk intakes were suppressed for 2 days and then returned to control levels for the remaining 4 trials. For the Sal subgroup, milk intakes during the first 6 "extinction" trials did not differ significantly from the previous 3-day baseline period. However, following injection of Mor, intakes were suppressed for 2 days before returning to baseline levels. The differences between the Mor and Sal subgroups were confirmed by a significant subgroup × day interaction, $F(12,144) = 3.21$, $p<0.001$.

The effect of temporal extinction on the rats' response to Mor injections as a function of cue is presented in Fig. 8. For purposes of comparison, the milk intakes of the Mor subgroup prior to extinction are also shown. The latter consist of the mean of the last 7 drug trials of the chronic phase (Mor injection + Mor cue) and the results of the explicit unpairing test (Mor injection + Sal cue). For the Mor subgroup, milk intakes as a function of extinction condition and cue were analyzed with a 2 × 2 ANOVA. There were no significant effects of extinction (before vs. after), cue (Mor vs. Sal), or their interaction. Similarly, the

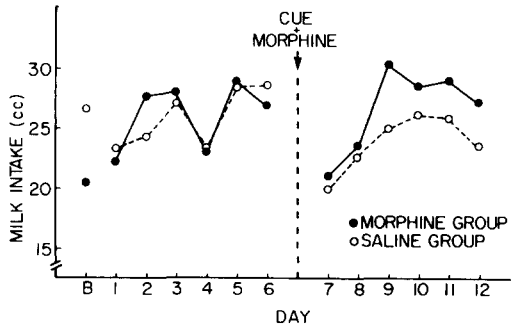


FIG. 7. Mean milk intakes of the Mor and Sal subgroups of Group 1 after injections of Sal in the home cage with neither cue present. (B = mean of preceding 3 Sal trials with the Sal cue present. Cued Mor injections and access to milk were given between trials 6 and 7.)

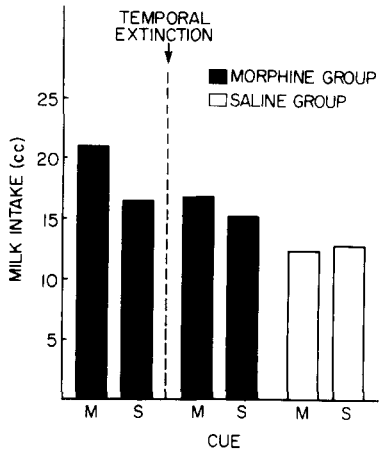


FIG. 8. Mean milk intakes of the Mor and Sal subgroups of Group 1 after injection of Mor in the presence of the Mor (M) and Sal (S) cues before (Mor subgroup) and after (both subgroups) extinction of temporal conditioning.

intakes of the Mor and Sal subgroups as a function of cue were analyzed with a 2 × 2 ANOVA. Again, there were no significant effects of group, cue, or their interaction.

*Group 2.* Milk intakes following chronic injections of Sal with neither cue present are shown in Fig. 9. The means of the preceding 3 Sal trials, in which Sal injections were given in the presence of the Sal cues, are also included. (It should be noted that, on the drug trial preceding the first extinction trial, rats in both subgroups received the last dose of the dose-response determination, 30 and 40 mg/kg for the Sal and Mor subgroups, respectively.) Statistical analysis of the data revealed significant effects of subgroup, $F(1,14) = 7.08$, $p<0.02$, and day, $F(12, 168) = 11.11$, $p<0.001$, but a nonsignificant subgroup × day interaction. Collapsing across subgroups, milk intakes were suppressed on the first extinction day, presumably due to the intervening injection of Mor on the preceding day. Intakes then increased over the following 5 trials. Following injection of Mor, milk intakes were again significantly suppressed for the next 2 days and then increased over the remaining trials. Overall, the intakes of the Mor subgroups exceeded those of the Sal subgroup.

The effect of temporal extinction on the rats' response to Mor injections as a function of cue is presented in Fig. 10. The milk intakes of the Mor subgroup prior to extinction are also shown for purposes of comparison. As previously explained, the latter consist of the mean of the last 7 drug trials of the chronic phase (Mor injection + Mor cue) and the results of the explicit unpairing test (Mor injection + Sal cue). For the Mor subgroup, milk intakes as a function of extinction condition (before vs. after) and cue (Mor vs. Sal) were analyzed with a 2 × 2 ANOVA. In contrast to the case for Group 1, there were significant effects of extinction, $F(1,7) = 6.48$, $p<0.04$, and cue, $F(1,7) = 9.30$, $p<0.02$, although the extinction × cue interaction was not significant. The significant effect of cue indicates that, across extinction conditions, intake was greater when Mor injections were given in the presence of the Mor cue than when they were given in the presence of the Sal cue (mean = 17.2 vs. 10.4).

Comparisons between the Mor and Sal subgroups during the postextinction period were also made with a 2 × 2 ANOVA. Preliminary inspection of the data revealed that the mean of the Sal subgroup on the test with the Mor cue was inflated by the intake of 1 rat that drank 25 cc. When the data were analyzed

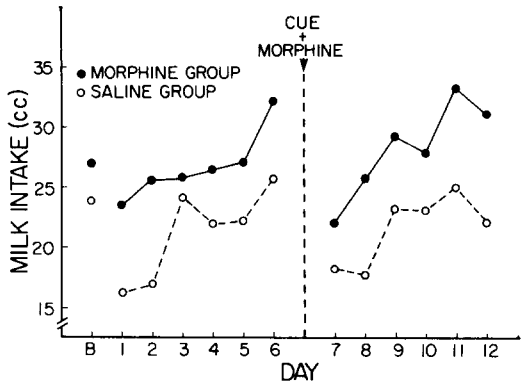


FIG. 9. Mean milk intakes of the Mor and Sal subgroups of Group 2 after injections of Sal in the home cage with neither cue present. (B = mean of preceding 3 Sal trials with the Sal cue present. Cued Mor injections and access to milk were given between trials 6 and 7.)

without this subject, a significant subgroup × cue interaction was obtained, $F(1,13)=6.30$, $p<0.025$. As shown in Fig. 10, rats in the Mor subgroup ingested significantly more milk when Mor injections were administered in the presence of the Mor cue than when they were given in the presence of the Sal cue, whereas the Sal subgroup ingested little milk in the presence of either cue.

## DISCUSSION

According to the Pavlovian conditioning model, tolerance is mediated by a compensatory response to the initial effects of drugs, which becomes conditioned to stimuli associated with drug administration (9). An alternative habituation model proposes that tolerance is mediated by habituation to the stimulus properties of the drug and does not involve a compensatory response. According to this latter model, both associative and nonassociative mechanisms can mediate tolerance. The probability of associative tolerance is thought to vary inversely with dose and directly with interdose interval (1). Based on the doses and interdose interval used in the present experiment, both models

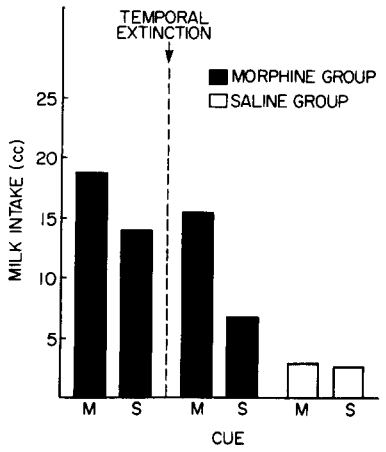


FIG. 10. Mean milk intakes of the Mor and Sal subgroups of Group 2 after injection of Mor in the presence of the Mor (M) and Sal (S) cues before (Mor subgroup) and after (both subgroups) extinction of temporal conditioning.

predict that tolerance should be contingent on the presence of drug-related cues, but only the Pavlovian model predicts a conditioned compensatory response.

Clearly, there was no evidence of a compensatory response for rats in Group 1. When saline injections were given in the presence of the morphine cue, milk intakes did not differ from baseline levels or from those of the control group. Although intakes on this test did exceed those on the last 7 Sal days of the chronic phase, this difference reflects a decrease in intake during the latter period. The data for Group 2, however, appear to be more consistent with the Pavlovian model. In this case, milk intakes on the compensatory response test exceeded those during the baseline period and during the last 7 Sal trials of the chronic phase. Moreover, their intakes exceeded those of the control group.

While these findings are consistent with the Pavlovian model, it is important to note that, in a previous study (10), we found that rats that received Mor injections *after* they had access to milk each day showed a comparable "compensatory response" when they were tested with Sal on a normally scheduled Mor day. Because posttest injections would not be expected to promote Pavlovian conditioning, it would appear that some other, as yet undiscovered, mechanism accounts for this phenomenon. It is also curious that, in both the previous experiment and this one, the effect was found only in rats given 20 mg/kg Mor (a dose that was equipotent with the 10 mg/kg dose given to the sensitive group). Because both sensitive and insensitive groups were exposed to the same stimuli and showed comparable shifts in their dose-response curves, it seems unlikely that this effect played a causal role in the development of tolerance.

A more important reason for questioning the role of associative mechanisms in this paradigm is that tolerance was not contingent on stimuli associated with drug administration. That is, neither group showed a significant loss of tolerance when Mor injections were given in the presence of either the Sal cue (explicit unpairing test) or neither cue (environmental specificity test). This finding, however, raises the question of whether the cues were sufficiently discriminable to the rats to support conditioning. To address this issue, we compared milk intakes on days in which Sal was administered in the presence of the Mor cue, the Sal cue, or neither cue. Intakes in the presence of the Mor cue were greater than those in the presence of the Sal cue, suggesting that the cues were discriminable. However, it is important to note that, in the previous experiment (10), in which Mor and Sal injections were also given on alternate days but not in the presence of explicit cues, intakes on the Sal days were also depressed relative to initial baseline levels. Thus, in the present study, lower intakes in the presence of the Sal cues may merely reflect a shift in baseline.

Although the discriminability of the Mor and Sal cues may be questioned, there can be little doubt that the cues associated with the home cage room were discriminably different from those of the room in which the environmental specificity test was conducted. Unlike the home cage room, in which an exhaust fan ran continuously and in which the compound cues were presented, the adjoining room was larger, brighter, and quieter. Despite these differences, however, there was no significant loss of tolerance when Mor was injected in this environment.

One possible reason that tolerance was not contingent on drug-related cues is that temporal conditioning may have occurred. Because Mor and Sal injections generally alternated, the rats may have come to anticipate Mor on the day after a Sal injection. If the rats expected Mor on the days they received the explicit unpairing and environmental specificity tests, then their levels of tolerance might not be severely disrupted. To examine this possibility, we attempted to alter the rats' expectancies by

injecting them with Sal with neither cue present for several days and then reassessed the effect of Mor in the presence of each of the cues. For Group 1, this procedure had no effect. For Group 2, however, the Mor subgroup showed a loss of tolerance when it was retested with Mor in the presence of the Sal cue, but retention of tolerance in the presence of the Mor cue. These findings clearly demonstrate that the cues were discriminable. Moreover, they are consistent with the hypothesis that, prior to extinction, temporal cues prevented the loss of tolerance, at least during the explicit unpairing test. Thus these data provide some support for the development of associative tolerance in Group 2. However, this explanation would not be applicable to Group 1.

Although the present findings do not permit a firm conclusion regarding the role of associative mechanisms in tolerance to Mor anorexia, it should be noted that there is considerable evidence that nonassociative tolerance readily develops at these doses, even at long interdose intervals. For example, in both this and the previous experiment (10), a significant number of rats developed at least partial tolerance to Mor after a single injection of the drug on the screening test. This occurred even though the second injection of the drug occurred 2.5 weeks later, following a series of interposed saline injections. In view of the absence of explicit drug-related cues on the initial day, such tolerance could not have been associative. Similarly, Leshem (4) reported tolerance to Mor anorexia after a single uncued injection given five months earlier. Moreover, some evidence suggests that the degree of single-dose tolerance to Mor increases the longer the interval between injections (3). It should also be noted that tolerance develops to Mor anorexia when rats are injected *after* access to milk (10), a procedure that is not compatible with Pavlovian conditioning. It is therefore possible that the development of nonassociative tolerance diminishes the impact of the cues that later accompany drug administration.

It is important to note that the basis for the anorexic effects of Mor is still unknown. Although the initial decreases in intake in both Group 1 and 2 were accompanied by immobility, the recovery of intake did not appear to be associated with any marked changes in gross locomotion. For the most part, tolerant animals remained immobile in front of the drinking tubes even when they were not drinking. It is possible that the initial suppression of intake was caused by sedation or by nausea, although there was no independent evidence for this latter effect (e.g., incipient retching).

Finally, in both this and the previous study (10), we found that milk intakes were suppressed on the day following Mor injections. Although Leshem (5) reported a delayed anorexic effect of Mor that lasted up to 24 h, the temporal extinction data from the present study demonstrate that a single Mor injection can suppress intake for up to 48 h. Curiously, however, this effect is not found if the injections are given after access to milk (10). Moreover, such delayed anorexia is not contingent on using cued Mor injections, and it does not seem to reflect withdrawal distress [cf, (10)]. Because Mor has a relatively short half-life [about 2 h; (7)], the suppression of intake is probably not the result of drug accumulation. As with the initial period of anorexia, the basis for the late anorexic effect of morphine is unknown. However, the fact that Mor exerted an effect on both Mor and Sal days may have contributed to the absence of associative tolerance in this paradigm.

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