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A Potential Role of Saline Trials in Morphine-Induced Place-Preference Conditioning[1]

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SCOLES, M. T. AND S. SIEGEL. *A potential role of saline trials in morphine-induced place-preference conditioning.* PHARMACOL BIOCHEM BEHAV **25**(6) 1169–1173, 1986.—The necessary conditions to alter rats' initial preferences for two sides of a shuttlebox were investigated, using procedures that are often used in the study of drug reinforcement. In Experiment 1, pairings of morphine sulfate (15 mg/kg, intraperitoneally) and either the nonpreferred side or a holding box was factorially combined with alternate-day pairings of saline and either the preferred side or a holding box. Pairings of saline and the preferred side were necessary and sufficient to increase preferences for the initially nonpreferred side. In Experiment 2, pairings of saline and the nonpreferred side, but not the holding box, strengthened the initial preference, regardless of whether morphine or saline injections preceded alternate-day holding-box placements. In Experiment 3, injection and placement in the preferred side in an unpaired manner, or placement only, decreased preferences for that side more than saline injections alone or no treatment. Paired saline injections and placement produced a greater change in preference than no treatment.

Classical conditioning Conditioned place preference Control groups Drug reinforcement
Exploratory behavior Habituation Morphine Rats

PLACE-PREFERENCE has become an increasingly popular means of studying the affective properties of drugs, primarily because of several advantages it has over traditional operant techniques [8,14]. A typical procedure [1, 5, 10, 12, 14, 17, 19] involves confinement of rats to one side of a two-compartment apparatus following injections of a drug and, on alternate sessions, confinement to the other side following injections of saline. A control group may be employed that receives saline [2, 11, 20] or drug [13] nondifferentially on both sides. Alternatively, one group of rats may be exposed to one "conditioning compartment" following drug injections whereas another group is exposed to the same compartment following saline injections [8, 9, 13, 15, 16, 18]. During a subsequent test, the rats are allowed access to both compartments, starting from one of the two compartments [1, 4, 5, 8, 9, 13] or from a small neutral area [2, 10–12, 15, 16, 18, 19]. The amount of time spent in the drug-paired side, relative to the saline-paired side, serves as an indication of the drug's affective value.

The drug to which these procedures have been applied most often is morphine. The consistent finding has been that rats spend more time in a compartment that has been paired with morphine than in one which has been paired with saline. This effect supposedly reflects the drug's rewarding properties [1, 2, 10, 12–14, 17, 18, 20]. The rationale is that during conditioning, location cues are established as secondary reinforcers as a result of their being paired with the primary reinforcing effects of morphine. The secondary reinforcing properties of these cues are revealed by the animals' tendency to approach them during the test [2, 4, 14, 16, 18]. However, in a preference procedure involving a choice among distinct locations, approach to one location necessarily involves withdrawal from another. Therefore, a rat's preference for a location where it has received morphine might not reflect a tendency to approach that location, but rather a tendency to avoid a location where it has received saline. Similarly, when between-groups comparisons are made, a difference between morphine and saline-treated rats in the amount of time spent in a conditioning compartment might not reflect a tendency for the morphine group to ap-

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proach that compartment, but rather a tendency for the saline group to avoid it. The first experiment provided an assessment of the relative contribution of these approach and withdrawal tendencies to the usual place-preference effect.

EXPERIMENT 1

METHOD

Subjects

The subjects were experimentally naive male Wistar rats (200–225 g, Charles River Canada, St. Constant, Quebec), housed individually in standard hanging wire cages for at least one week, and gentled prior to the experiment. The colony room lights were on from 0700–2300 hr and food and water was provided ad lib in the home cages. Initial preferences were determined for 37 rats, and the five with the most extreme biases were excluded from the experiment. Data were obtained from the remaining 32 rats.

Apparatus

Test apparatus. The test apparatus was divided by a solid aluminum barrier into two compartments, each measuring 22 cm long × 18 cm wide × 18 cm deep. During testing, the barrier was replaced by one which had a hole, 6 cm in diameter, centered 7 cm above the floor. Except for the barrier, the walls were constructed of clear acrylic, with the outer surfaces painted white for one compartment and black for the other. The lid was also clear acrylic, and was covered with black paper over the black compartment. A 28-VDC lamp (Type 304) was located 19.5 cm above the center of the lid. The two compartments shared a floor that was hinged below the barrier, allowing the rat's position to be indicated by a switch. The floor in each compartment consisted of 18 stainless-steel bars, 2.35 mm in diameter and spaced 11 mm apart (center-to-center). In the black compartment, the bars were covered by a 2 mm thick sheet of black vinyl. A drop pan beneath the floor contained Kitty Litter. The entire apparatus was enclosed in a light-proof and sound-attenuating chamber. A ventilation fan provided masking noise.

Holding boxes. Two clear plastic cages (36×30.5×16.5 cm), with wire lids and wood shavings covering the floor, were placed on top of the sound attenuating chamber.

Drugs

Injections of 0.9% saline or morphine sulfate dissolved in sterile water (15 mg/ml) were given intraperitoneally in a volume of 1 ml/kg.

Design and Procedure

Pretests. Each rat was given three preference tests, spaced approximately 48 hr apart. The rat was placed in the black or white compartment (randomly determined) and allowed to explore both sides of the apparatus for 20 min. For each subject, a pretest score was defined as the median percentage of time on the black side for the three pretests. The 32 rats were ranked on these scores and assigned to eight blocks of four subjects each. The four blocks of subjects that had pretest scores above the median for the 32 rats (53% time on the black side) had black designated as their preferred side, whereas the remaining subjects had white designated as their preferred side.

Conditioning. Subjects within each of the eight blocks were randomly assigned to four groups—SP/MN, SP/MH, SH/MN, and SH/MH. The first part of the group designation indicates placement following saline injections, in either the initially preferred side of the shuttlebox (SP) or a holding box (SH). The second part indicates placement following morphine injections, in either the initially nonpreferred side of the shuttlebox (MN) or a holding box (MH). The first of 24 injections was given 1 or 2 days following the last pretest. Subsequent injections were given at 1-day intervals, with the exception that the interval between the 12th and 13th injection was 5 days. Injections of saline alternated with morphine, with order counterbalanced across groups. Subjects were placed in the appropriate apparatus for 1 hr immediately following each injection.

Posttests. Each subject was given two preference tests, using the same procedures as for pretests. For half of the subjects in each group the first test took place 1 day following the 12th injection, and the second was given 2 days following the 24th injection. For the remaining subjects, the tests took place 2 days and 1 day following the 12th and 24th injections, respectively.

RESULTS AND DISCUSSION

Figure 1 depicts the mean percentage of time spent on the nonpreferred side following 0 (pretest scores), 12, or 24 days of conditioning for each group. As expected, an increase in time spent on the nonpreferred side was exhibited by subjects which had the preferred side paired with saline and the nonpreferred side paired with morphine (Group SP/MN). This increase was clearly greater than that exhibited by subjects that were placed in holding boxes following saline and morphine injections (Group SH/MH). However, a change in preference depended on placement in the preferred side following saline injections. Placement in the nonpreferred side following morphine injections was neither necessary (Group SP/MH) nor sufficient (Group SH/MN) to produce place-preference conditioning.

A randomized-block factorial ANOVA (Saline Placement × Morphine Placement × Posttest × Blocks), performed on the percentage of time on the nonpreferred side after 12 and 24 days of conditioning, revealed a significant main effect of Saline Placement, $F(1,7)=36.34$, $p<0.001$. No other main effect was significant, $F(1,7)<1$ for Morphine Placement, $F(1,7)=1.74$, $p>0.20$ for Posttest, nor were any interactions.

Rats avoided the saline-paired location but did not exhibit a change in preference for the morphine-paired location. The combination of these two factors produced the usual place-preference effect in Group SP/MN. Morphine apparently counteracted those processes that resulted in avoidance of the saline-paired location, perhaps through a reinforcement mechanism. However, it would be appropriate to determine the factors which result in avoidance of the saline location before speculating on how morphine counteracts this effect.

Experiment 2 was directed at determining whether experience with morphine was necessary to produce avoidance of the saline location. In addition, saline was paired with the initially nonpreferred side, to rule out the possibility that the results of Experiment 1 depended on the direction of training.

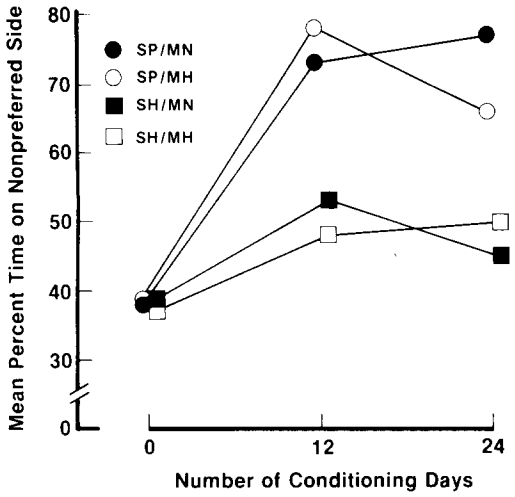

FIG. 1. Mean percentage of time spent on the initially nonpreferred side for each of the groups in Experiment 1 after 0, 12, and 24 conditioning trials. The means reported at 0 conditioning trials are based on pretest scores.

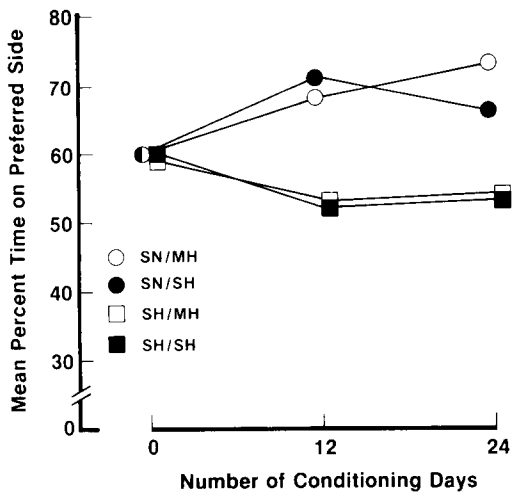

FIG. 2. Mean percentage of time spent on the initially preferred side for each of the groups in Experiment 2 after 0, 12, and 24 conditioning trials. The means reported at 0 conditioning trials are based on pretest scores.

EXPERIMENT 2

METHOD

Subjects

The subjects were similar to those in Experiment 1. Thirty-five rats were pretested and the three with the strongest biases were excluded from the experiment.

Apparatus and Drugs

The apparatus and drugs were the same as in Experiment 1, except that six holding boxes were used. These were placed in various locations in the experimental room.

Design and Procedure

Pretests. The procedures for pretesting, assignment of subjects to blocks, and designations of initial side preference were similar to those used in Experiment 1. The median pretest score for the 32 rats was 58% of the time in the black experiment.

Conditioning and posttests. Subjects within each of the eight blocks were randomly assigned to four groups—SN/MH, SN/SH, SH/MH, and SH/SH. The first part of the group designation indicates whether, at 2-day intervals, injections of saline were followed by placement in the nonpreferred side of the shuttlebox (SN) or a holding box (SH). The second part indicates whether, on intervening days, injections of morphine (MH) or saline (SH) preceded placement in a holding box. The remaining procedural details for conditioning and testing were the same as those described for Experiment 1.

RESULTS AND DISCUSSION

Figure 2 depicts the mean percentage of time spent on the preferred side following 0, 12, or 24 days of conditioning for each group. Subjects that were placed on the nonpreferred side following saline injections (Groups SN/MH and SN/SH) later spent more time on the preferred side than did subjects that were not placed on that side following saline injections (Groups SH/MH and SH/SH). There was no apparent effect of morphine administration on alternate days (SN/MH vs. SN/SH and SH/MH vs. SH/SH).

A randomized-block factorial ANOVA (Saline Placement × Morphine Treatment × Posttest × Blocks), performed on the percentage of time on the preferred side following 12 and 24 days of conditioning, revealed a significant main effect of Saline Placement, $F(1,7)=12.99$, $p<0.01$, but not of Morphine Treatment, Posttest, or any interactions ($ps>0.20$). It can be concluded that avoidance of saline-paired location, such as that observed in Experiment 1, does not depend on experience with morphine, nor is it restricted to situations in which rats are trained against their initial preference.

Avoidance of the saline-paired compartment might be attributable to an association between the conditioning compartment and some aversive aspect of the injection procedure. Support for this analysis would require use of control groups appropriate to a classical conditioning paradigm [7]. The change in preference would not be attributed to such an association if a procedure which fails to present these events in a paired manner (e.g., saline alone, placement alone, saline and placement unpaired, or neither treatment) produces as much of a change in preference as the paired procedure. Experiment 3 evaluated the effectiveness of these various procedures in producing place preferences.

EXPERIMENT 3

METHOD

Subjects

The subjects were similar to those in Experiments 1 and

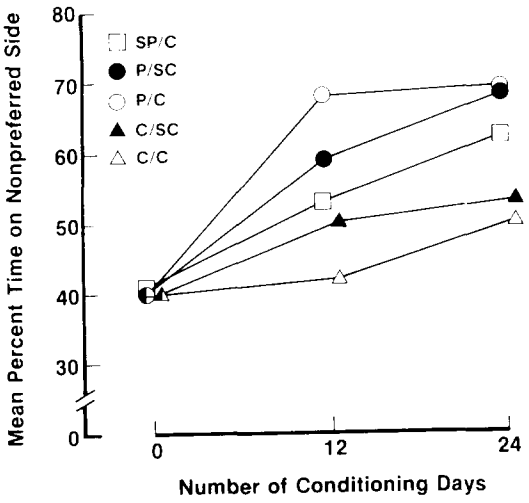

FIG. 3. Mean percentage of time spent on the initially nonpreferred side for each of the groups in Experiment 3 after 0, 12, and 24 conditioning trials. The means reported at 0 conditioning trials are based on pretest scores.

2. Forty-three rats were pretested and the three with the strongest biases were excluded from the experiment.

Apparatus and Drugs

The apparatus was the two-compartment shuttlebox used in Experiments 1 and 2. No holding boxes were used, and all injections were of saline.

Design and Procedure

Pretests. The procedures for pretesting, assignment of subjects to blocks, and designation of initial side preferences were similar to those used in Experiments 1 and 2, except that the interval between the second and third pretests was approximately 72 hr. On the basis of the ranked pretest scores, subjects were assigned to eight blocks of five subjects each. The median pretest score for the 40 rats was 37% of the time in the black compartment.

Conditioning and posttests. Subjects within each of the eight blocks were randomly assigned to five groups—SP/C, P/SC, P/C, C/SC, and C/C. The first part of the group designation indicates whether, at 2-day intervals, subjects were placed in the initially preferred side of the shuttlebox following a saline injection (SP), placed in the preferred side without an injection (P), or left in the home cage without an injection (C). The second part indicates whether on intervening days, subjects were given an injection of saline in the colony and returned to the home cage (SC) or left in the home cage without an injection (C). The interval between the third pretest and the first day of conditioning was 3 or 4 days. The remaining conditioning and testing procedures were as described for Experiment 1.

RESULTS AND DISCUSSION

Figure 3 depicts the mean percentage of time spent on the nonpreferred side following 0, 12, or 24 days of conditioning for each group. Subjects that were placed on the preferred side without an injection (Groups P/SC and P/C) spent more time on the nonpreferred side than did subjects that were never placed in the preferred side during conditioning (Groups C/SC and C/C). Subjects that were placed in the preferred side following saline injections (Groups SP/C), exhibited slightly less of an increase than did subjects that were not injected prior to placement.

A randomized-block factorial ANOVA (Groups × Posttest × Blocks), performed on the percentage of time on the nonpreferred side after 12 and 24 days of conditioning, revealed a significant main effect of Groups $F(4,28)=7.89$, $p<0.001$, and of Posttest, $F(1,7)=72.93$, $p<0.001$. Because the Groups × Posttest interaction was not significant, $F(4,28)<1$, the mean percentage of time on the nonpreferred side over both posttests was computed for each subject. Newman-Keuls tests were then performed on group means at the 5% significance level. These tests indicated that subjects that were placed in the preferred side (Groups SP/C, P/SC, and P/C) spent more time on the initially nonpreferred side than did the no-treatment control group (C/C). In addition, subjects that were not injected prior to placement (Groups P/SC and P/C) spent more time on the initially nonpreferred side than did the saline-only group (C/SC). These results indicate that a decreased preference for the saline-paired side, as observed in Experiments 1 and 2, was not due to an association between that side and the saline injections. Rather, exposure to one side in a nondrugged state was sufficient to produce a decreased preference for that side.

GENERAL DISCUSSION

The results of these experiments demonstrate that, following repeated exposure to a distinctive compartment, rats will avoid that compartment. This effect does not depend upon experience with morphine in another location (Experiment 2), nor is it based on an association between location cues and any consequence of saline injections (Experiment 3). However, the effect is eliminated if exposure to the compartment coincides with the effects of morphine, a result which produces the typical "morphine-induced" place-preference effect (Experiment 1).

The finding that rats prefer the compartment to which they have had the least amount of exposure is consistent with previous research on exploratory behavior. For example, it has been demonstrated that rats will approach and explore novel stimuli, and that this exploratory behavior habituates with repeated exposure to those stimuli [3]. Place-preference conditioning with morphine might be due to an attenuation of this habituation by morphine. During preference testing, subjects would be expected to spend more time exploring a location that has been paired with morphine than one which has been paired with saline. This nonassociative explanation of place-preference conditioning seems simpler than the widely accepted associative one, which requires that morphine has such powerful rewarding properties that it can establish diffuse location cues as secondary reinforcers in as little as one conditioning trial [8,14], and at doses as low as 0.08 mg/kg [14]. The nonassociative explanation is also supported by demonstration that low doses of morphine interfere with habituation of responses to phasic stimuli [6].

The explanation offered here should not be confused with one based on state-dependent habituation of exploratory behavior, which other investigators [13] have refuted by dem-

onstrating that the preference for morphine- over saline-paired cues is not reversed if rats are tested while drugged. Such a reversal would have been expected if attenuation of exploratory behavior depended on the animal being in the same drug state during training and testing. If, as suggested here, morphine directly interferes with habituation during training, the drug state at the time of testing would be irrelevant.

The present experiments are not the first attempt at comparing rats' preferences for morphine-paired and novel cues. Using a three-compartment box, it has been shown that rats will exhibit a preference for a morphine-paired compartment over both a novel compartment and one which has been paired with saline [13]. On the basis of the present results, one would have expected that both the morphine-paired and novel compartments would have been preferred over the saline-paired compartment, and that there would have been no preference for the morphine compartment over the novel one. The data of the present experiments are also contradicted by those of other investigators, who have reported a true increased preference for a morphine-paired location, and no change in preference for a saline-paired or otherwise familiar location [1, 4, 8, 13, 15]. On the other hand, it has been reported that, as in the present experiments, rats which have a conditioning compartment paired with saline decrease their preference for that compartment over the course of conditioning, while morphine-treated rats show no change [16]. Morphine-treated rats have also been observed to spend more time in a conditioning compartment than saline-treated rats, but no more time than rats that receive exposure to morphine and the conditioning compartment in an unpaired manner [13]. This last finding seems especially difficult to reconcile with a reinforcement interpretation, and suggests that nonassociative processes operating during saline trials are involved in place-preference conditioning.

It would be difficult to determine the factors responsible for the discrepancies between the various outcomes of the studies mentioned above, although route of injection [1,15], dose and duration of conditioning [4], and the type of apparatus employed [8,13] may be important. Perhaps more important, it is also difficult to determine whether the majority of the place-preference data obtained with morphine are attributable to a change in preference for saline- or for morphine-paired cues. In most of the published studies, pretest data are not collected [2, 9–12, 14, 17, 20] or, if they are collected, are not reported [16,18]. Given that place-preference conditioning supposedly reflects a tendency for rats to approach cues that have gained reinforcing properties as a result of their being paired with morphine [2, 4, 14, 16, 18], the routine reporting of initial preferences would seem appropriate.

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