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Conditioned Place Preference with Morphine: The Effect of Extinction Training on the Reinforcing CR[1]

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BARDO, M. T., J. S. MILLER AND J. L. NEISEWANDER. *Conditioned place preference with morphine: The effect of extinction training on the reinforcing CR.* PHARMACOL BIOCHEM BEHAV 21(4) 545–549, 1984.—Rats were injected with either morphine (5 mg/kg) or saline in association with one set of distinct environmental stimuli, and injected with saline in association with a different set of stimuli. After four conditioning trials, animals were given a 15-minute free-choice test to determine which stimulus environment was preferred. Animals displayed CPP as a significant increase in duration spent within the morphine-associated environment, but did not display any change in number of entries into that environment. In contrast, when extinction training was given following CPP, animals displayed a significant decrease in duration spent *per entry* into the morphine-associated environment, but did not display any change in total duration spent in that environment. These results suggest that assessment of the reinforcing conditioned response (CR) in the CPP model may require measurement of both duration spent in and number of entries into the drug-associated environment.

Conditioned place preference Morphine Drug reinforcement

CONDITIONED place preference (CPP) has been developed recently as an animal model of drug reinforcement. It is based on the notion that environmental stimuli which are paired reliably with an appetitive stimulus or drug may serve as conditioned reinforcers which facilitate operant behavior (e.g., [15]). To demonstrate CPP, animals are given a drug in association with distinct environmental stimuli, and then are given a free-choice to spend time in the presence of either drug-associated or non-associated stimuli. The strength of the reinforcing conditioned response (CR) is reflected presumably in the operant preference for drug-associated stimuli. Using this procedure, a variety of psychoactive drugs have been shown to produce CPP, including amphetamine [23,29], cocaine [18,30], heroin [4,25], and morphine [2, 11, 20, 24, 28, 34].

Several lines of evidence support the assumption that CPP involves the acquisition of a reinforcing CR in which the reinforcing properties of the drug become associated with environmental stimuli. First, it is clear that various drugs may serve as effective appetitive or primary reinforcers [9]. Second, environmental stimuli which are paired reliably with a drug may elicit a CR that mimics the unconditioned drug effect. For example, a low dose of morphine produces hyperthermia, and stimuli associated with this drug effect can elicit a similar hyperthermic CR [14, 17, 27]. Other CRs also mimic the unconditioned effects of morphine, including changes in catecholamine release into blood [16] and changes in cortical evoked potentials [31,36]. Third, and perhaps most important, evidence indicates that environmental stimuli associated with a reinforcing drug can direct operant behavior. For example, monkeys and rats injected with morphine in association with an environmental stimulus will perform an operant response which delivers the stimulus alone [5, 7, 26, 32].

Despite this evidence, however, there is presently little direct empirical support for the assumption that CPP reflects the acquisition of a reinforcing CR. In CPP, the CR is not observed directly, but is inferred from an operant choice response. Typically, CR strength is assumed to be reflected in the increased duration that an animal spends in the presence of drug-associated stimuli. If this assumption is correct, then CPP ought to be attenuated or extinguished when drug-associated stimuli are presented alone following conditioning. The present investigation examined that possibility, using morphine as the reinforcing stimulus.

EXPERIMENT 1

The first experiment was designed to examine the nature of the putative CR in morphine-induced CPP. Typically, CPP has been demonstrated by giving rats morphine in one distinct environment and saline in another environment. Later, when the animal is in a drug-free state, it is given a 15-minute free-access period to both environments simultaneously. While numerous reports demonstrate that rats spend more total time in the morphine-associated environment relative to saline-treated controls, it is unclear whether this preference is due to an increased *duration* spent during each entry into the environment or to an increased *number* of entries into the environment. Examination of these two alternatives may help characterize the nature of the CR.

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METHOD

Animals

The animals were adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing between 400–500 g at the start of the experiment. They were caged individually, and were supplied food and water continuously in their home cage.

Apparatus

Conditioning was conducted in a rectangular chamber with three different compartments separated by guillotine doors. The two end compartments measured 22×26×30 cm, while the middle compartment measured 22×14×30 cm. One end compartment had white walls, a wire-mesh floor, and pine wood chips under the floor. The other end compartment had black walls, a metal grid floor, and cedar wood shavings under the floor. The middle room had gray walls and a solid wood floor which was also gray. Preliminary pilot data indicated that naive animals had a slight preference for the black compartment.

Procedure

Each animal was acclimated to handling for one week prior to the start of the experiment. Following acclimations, animals were divided randomly into three separate groups (n=7 per group). All animals were given 2 daily injections, one immediately before and one immediately after being placed into the apparatus. For one group, morphine sulfate (5 mg/kg, SC) was given once every other day immediately before being placed into the white compartment for 30 min with the guillotine door closed. After removal from the white compartment, these animals were then injected with saline and returned to the home cage. On alternate days, these animals were injected with saline both before and after a 30-min exposure to the black compartment. This procedure continued until a total of 4 morphine-white pairings and 4 saline-black pairings were completed. The second treatment group was treated similarly, except that morphine was injected immediately *after* rather than immediately before exposure to the white compartment. The third treatment group was also treated similarly, except that all injections were saline. In sum, one treatment group experienced morphine simultaneously with exposure to the white compartment (group Morphine-Simultaneous or M-S); one experienced morphine delayed after exposure to the white compartment (group Morphine-Delayed or M-D); and one did not experience morphine, but served as a saline-injected control group (group Sal).

On the day after the last conditioning trial, all animals were assessed for CPP. Each animal was placed in the gray middle compartment with the guillotine doors open. An observer who was unaware of the animals' individual treatment recorded (1) the total duration spent in each compartment and (2) the number of entries into the white and black compartments. These readings were made in three consecutive 5-min blocks, for a total observation period of 15 min per animal.

Statistical Analyses

Data were analyzed by analyses of variance, using either split-plot, completely randomized, or factorial designs [12].

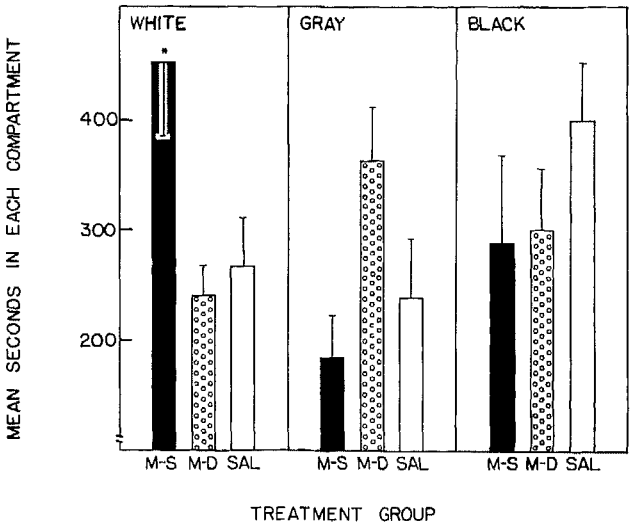

FIG. 1. Mean total duration (±S.E.M.) in each compartment on test day from animals in Experiment 1. Group M-S experienced morphine simultaneously with exposure to white; group M-D experienced morphine delayed after exposure to white; and group Sal received saline control treatment. Asterisk (*) represents significant difference from both M-D and Sal groups, $p<0.05$.

All subsequent pairwise comparisons were made using Tukey's HSD test of significance.

RESULTS

The data were analyzed initially across the three 5-min blocks for each animal. Across this repeated measure, there was a significant decline in the duration spent in the white compartment and a significant decline in the number of entries into the white compartment (data not shown). However, since this within-subjects repeated measure did not interact statistically with the treatment factor, the data were collapsed across the total 15-min observation period.

As shown in Fig. 1, there was a significant difference between treatment groups in the total duration spent in the white compartment, $F(2,18)=5.69$, $p<0.05$. Animals given morphine immediately *before* exposure to white (group M-S) spent significantly more time in white relative to animals given morphine immediately *after* exposure to white (group M-D; $t(18)=3.11, p<0.05$) and relative to saline-injected control animals (group Sal; $t(18)=2.71$, $p<0.05$). In contrast, animals given morphine immediately *after* exposure to the white compartment (group M-D) did not spend more time in white relative to saline controls. Thus, these results demonstrate that CPP may only be obtained when there is a temporal overlap between morphine and the to-be-conditioned stimulus environment, and that experience with morphine *per se* is ineffective in establishing CPP.

Analysis of the entry data revealed further that there were no significant treatment-related differences in the number of entries into either the white or black compartments. However, animals given morphine before exposure to white (group M-S) spent a longer duration *per entry* in white relative to saline-injected (group Sal) control animals (see Table 1). These results demonstrate that CPP evident in M-S animals in Fig. 1 is due to an increased duration spent in the white compartment during each entry, rather than an increase in the number of entries into white *per se*.

TABLE 1

TOTAL NUMBER OF ENTRIES AND MEAN DURATION PER ENTRY INTO WHITE AND BLACK COMPARTMENTS IN M-S, M-D AND SAL GROUPS IN EXPERIMENT 1

		Mean Number of Entries		Mean Duration per Entry		Mean Total Duration	
Group	N	White	Black	White	Black	White	Black
M-S	7	9.3	7.9	48.3*	34.0	449.2*	268.6
M-D	7	8.7	9.0	27.5	33.1	239.3	297.9
Sal	7	9.7	11.6	27.4	34.3	265.8	397.9

*Significant difference from Sal Group, $p<0.05$.

EXPERIMENT 2

The second experiment was designed to assess the effect of extinction training on CPP as assessed by the duration spent in the morphine-associated environment and by the number of entries into the morphine-associated environment.

METHOD

Animals

The animals (n=28) were similar to those described in Experiment 1, except that body weights ranged between 250–300 g at the start of the experiment.

Apparatus

The apparatus was that described in Experiment 1.

Procedure

Each animal was acclimated to handling prior to the start of the experiment. Following acclimation, animals were divided randomly into 4 separate groups (n=6–8 per group). Two groups were injected with morphine sulfate (5 mg/kg, SC) once every other day for a total of 4 morphine injections. Immediately after each morphine injection, these animals were placed into the white compartment for 30 min. On alternate days, these animals received saline and were placed in the black compartment for 30 min. The other two groups were treated similarly, except that all injections were saline.

Following conditioning, one-half of the morphine-treated and one half of the saline-treated animals were given extinction training. This consisted of placing the animal in the white compartment for 30 min daily on each of 6 consecutive days. During this period, the other animals were left in their home cages. In sum, the 4 treatment groups made up a 2×2 factorial design in which each animal was conditioned with either morphine or saline and was either given extinction training or not. On the day following the last extinction trial, all animals were tested for CPP as described before.

RESULTS

As in Experiment 1, the data were collapsed across three 5-min blocks, because this repeated measure did not interact statistically with either treatment factor. A factorial analysis of variance of the data summarized in Fig. 2 revealed that morphine-treated animals spent significantly more total time in the white compartment than the saline-treated animals when the data were collapsed across extinction groups, $F(1,24)=9.40$, $p<0.01$. Subsequent pairwise comparisons revealed that the morphine-induced increase in time spent in white was evident relative to saline controls, both in animals given extinction training, $t(12)=5.91$, $p<0.01$, and in animals given no extinction training, $t(12)=2.72$, $p<0.01$. Further, extinction training produced a clear decrease in the duration spent in white for both morphine- and saline-treated animals, $F(1,24)=15.91$, $p<0.001$. However, there was no significant interaction between the drug and extinction factors, indicating that extinction training attenuated the time in white for animals given either morphine or saline (see Fig. 2, left panel).

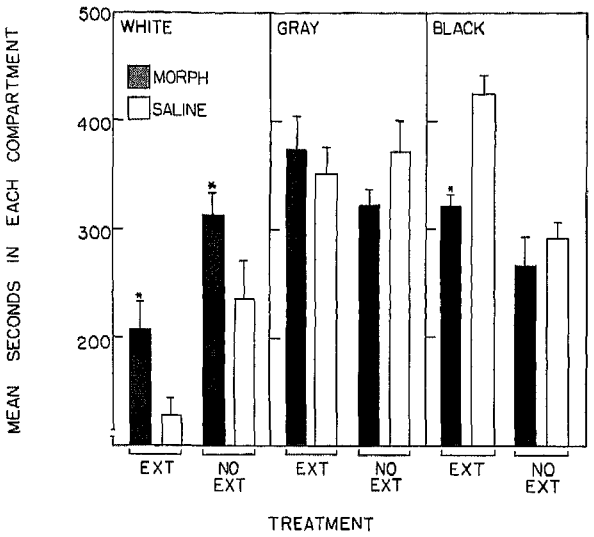

FIG. 2. Mean total duration (±S.E.M.) in each compartment on test day from animals in Experiment 2. Each animal was conditioned with either morphine or saline in white and then given either extinction training (Ext. groups) or no extinction training (No Ext. groups). Asterisks (*) represent significant difference from saline-injected control group, $p<0.05$.

Nonetheless, further analysis indicated that extinction training altered differentially the entry of morphine- and saline-treated animals into the morphine-associated environment. As shown previously in Experiment 1, morphine- and saline-treated animals (given no extinction training)

TABLE 2

TOTAL NUMBER OF ENTRIES AND MEAN DURATION PER ENTRY INTO WHITE AND BLACK COMPARTMENTS IN 4 TREATMENT GROUPS IN EXPERIMENT 2

				Mean Number of Entries		Mean Duration per Entry		Mean Total Duration	
Group	Drug	Extinction	N	White	Black	White	Black	White	Black
1	Morph	Ext	8	16.1*	17.0	12.9	18.8	207.7*	319.6*
2	Sal	Ext	6	10.8	17.0	11.8	24.9	127.4	423.3
3	Morph	No Ext	8	12.6	12.9	24.8*	20.9	312.5*	269.6
4	Sal	No Ext	6	12.1	13.3	19.6	21.9	237.2	291.3

*Significantly different from Sal control group, $p<0.05$.

entered the white compartment a similar number of times (see Table 2). This corroborates our earlier conclusion that CPP measured by total duration spent in the morphine-associated environment is due to an increase in the duration spent *per entry*, rather than an increase in the number of entries. More important, however, this conclusion did not hold following extinction training. Instead, morphine-treated animals given extinction training displayed significantly more entries into the white compartment relative to saline-treated control animals, $t(12)=4.34, p<0.001$, and relative to morphine-conditioned animals given no extinction training (see Table 2). Further, while there was a significant difference in duration per entry into white between morphine- and saline-treated animals given no extinction training, no significant difference was evident between morphine- and saline-treated animals given extinction training. In addition, extinction training *per se* attentuated the mean duration per entry into white for both morphine- and saline-pretreated groups.

DISCUSSION

The present results indicate that, in addition to measuring the duration spent in each compartment, the number of entries into each compartment may be an important dependent variable in the CPP model of drug reinforcement. In the typical CPP procedure, conditioned and non-conditioned animals display a similar number of entries into the drug-associated environment (Experiment 1). In this circumstance, the total duration and duration per entry measurements yield essentially identical outcomes. However, the present results also demonstrate that conditioned and non-conditioned animals may display a different number of entries into the drug-associated environment following extinction training (Experiment 2). In this instance, the total duration and duration per entry measurements yielded different outcomes.

Specifically, we found that extinction training following CPP produced differential effects on total duration spent in and number of entries into the drug-associated white compartment. Animals given extinction training decreased their total duration in the white compartment, regardless whether it was paired previously with either morphine or saline. This effect may reflect nonassociative habituation to cues which elicit exploratory approach behavior in that environment. In contrast, morphine-conditioned animals given extinction training displayed an increased number of entries into the drug-associated environment relative to saline-treated controls and relative to morphine-conditioned animals given no extinction training. This "extinction" effect may reflect a process similar to the transient increase in operant responding often seen during extinction of morphine-reinforced lever-pressing in rats [33]. However, regardless of the interpretation, when the results were expressed as duration per entry, significant CPP was evident in non-extinguished animals (cf., 24.8 vs. 19.6, Table 2), but not in extinguished animals (cf., 12.9 vs. 11.8, Table 2). Thus, when duration per entry is used as an index of CR strength, rather than total duration in the drug-associated environment, the present results are consistent with a classical conditioning interpretation of CPP.

Unfortunately, the present results do not rule out the possibility that morphine-induced CPP reflects the acquisition of a hypoactive CR, rather than a reinforcing CR. In rats, morphine doses of 5 mg/kg or higher produce a period of unconditioned hypoactivity which extends over 30 minutes [6, 13, 19]. Since morphine (5 mg/kg) was paired with environmental stimuli for 30 post-injection minutes, the hypoactive effect may have become conditioned to these stimuli. Consistent with this notion, conditioned animals displayed longer durations per entry into the morphine-associated environment relative to non-conditioned animals, suggesting that entry into that environment may have elicited a conditioned decrease in locomotor activity which reduced the likelihood of leaving. This possibility is further supported by the finding that conditioned locomotor effects are obtained with psychostimulant drugs [1, 3, 21, 22].

While acquisition of a hypoactive CR cannot be ruled out by the present results, several lines of evidence mitigate against this possibility. First, low doses of morphine (1 mg/kg) that produce an unconditioned *hyper*active effect in rats [10] can also produce CPP [11]. Second, stimulant drugs such as amphetamine and cocaine, which increase locomotor activity, produce CPP [18, 23, 29, 30]. Third, other agents such as lithium chloride and ethanol, which may produce an unconditioned hypoactive effect, do not produce CPP [18,35]. Taken together, these studies indicate that morphine-induced CPP reflects the acquisition of a reinforcing CR that is independent of drug-induced changes in locomotor activity. However, a direct measurement of locomotor activity during CPP assessment may be required to confirm this hypothesis.

Finally, it appears that the putative reinforcing CR in morphine-induced CPP requires some temporal overlap between the to-be-conditioned stimulus environment (CS) and the drug experience. Previous work indicated that adminis-

tration of morphine one hour before CS exposure produces CPP [24]. However, administration of morphine immediately *after* CS exposure is ineffective in establishing CPP [28]. The present results also demonstrate that once the putative CR is acquired, it is maintained for a substantial delay between conditioning and testing, i.e., 6 days. Similarly, in the self-administration model, the secondary reinforcing effects of morphine are obtained over a similar delay period [8], suggesting a parallel between CPP and self-administration procedures. Nonetheless, a number of procedural and theoretical differences exist between the CPP and self-administration models [18], and further work is required to determine whether these models reflect a common reinforcement process.

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