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Operant Place Conditioning Measures Examined Using Morphine Reinforcement

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CROWDER, W. F. AND C. W. HUTTO, JR. *Operant place conditioning measures examined using morphine reinforcement.* PHARMACOL BIOCHEM BEHAV **41**(4) 825-835, 1992.—A new place conditioning procedure for assessing drug reinforcement, based on drug administration following chamber entry, was examined in rats and compared to conditioned place preference (which is based on chamber exposure of drugged animals). Besides relative time subsequently spent in the morphine chamber, choices between the drug and nondrug chambers were recorded during the time test and during a discrete-trials choice test. In some experiments, either choices or latencies of chamber entry were recorded during training. Most choice and latency measures showed larger reinforcement effects than did time spent. Placement training was somewhat less effective than operant place conditioning overall, but produced significant time differences and discrete-trial choices in four of five groups. With intravenous morphine, the lengthy chamber exposures generally used in conditioned place preference were unnecessary.

Drug reinforcement Place conditioning Conditioned place preference Choice measures Latency Rats Morphine

STUDIES of the reinforcing properties of drugs in rats most frequently use continuous reinforcement drug self-administration methods in which the drug is administered through a venous catheter each time a lever is actuated (8,20,23). Although widely accepted, rate of self-administration has been criticized as being affected by variables other than reinforcement (7,8,15,22,24), and promising alternatives such as progressive ratio schedules of self-administration (13,19) are coming into use.

An alternative method often used is conditioned place preference (CPP), which consists of exposing rats to one compartment (positive compartment) in the presence of a possibly reinforcing agent—usually a drug—and to a different one (negative compartment) without the agent, generally for 15 min or longer. In a subsequent test with both compartments accessible, the relative times spent in the two compartments are used as the measure of reinforcement. Possible advantages of CPP have been pointed out (5,6,11,15,16) and various degrees of reservation concerning CPP have also been expressed (5,6,14,15,21).

Described here is a test for drug reinforcement combining certain features of self-administration with certain features of CPP. Operant place conditioning (OPC) is a spatial counterpart of drug self-administration in which the reinforced response is entering a chamber rather than pressing a lever. Like CPP, it employs discrete training trials spaced far enough apart to prevent the satiating and disrupting effects of drugs from interfering with conditioning or performance. It differs from CPP in three ways: 1) Its primary measures of reinforcement are based on entries into the positive chamber, not time spent there; 2) in OPC training, the drug is contingent on the subject's behavior, whereas in CPP training the animal is injected and placed into the chamber independently of its behavior; 3) in OPC, chamber stimuli always precede the onset of the drug effect; CPP employs a wide range of temporal relationship between placement and onset of the drug effect, with the drug effect sometimes being nearly maximal when the subject is placed into the chamber.

The present, largely exploratory, experiments were concerned with the first two of these characteristics of OPC, that is, its behavioral indices of reinforcement and its type of conditioning procedure. The questions were how each of several measures of spatial behavior are affected by morphine reinforcement and whether passive placement is as effective as active approach training in conditioning the spatial behavior. Information was also obtained on the effectiveness of training with brief exposures to the chambers.

METHOD

Subjects and Cannulation

Subjects, male Sprague-Dawley rats (Holtzman Company, Madison, WI, and Harlan Sprague Dawley, Indianapolis, IN), were 60-120 days old and housed individually, except

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two per cage in Experiment 11, with food and water available ad lib. Rats receiving morphine IV were implanted with jugular catheters of silicone rubber and polyethylene (18) or of silicone rubber and vinyl. The cannulation procedure was essentially that of Weeks (18). Anesthesia was provided by ketamine, 80 mg/kg IM, and xylazine, 6 mg/kg. For IP administration of drugs through catheters (Experiment 9), the catheter was of silicone rubber and polyethylene. All catheters exited the skin at the back of the neck.

Apparatus

Linear boxes. Four identical boxes were used in Experiments 1, 3, 5, and 9–11. The end chambers were 18 cm wide and 27 cm long; one was black, the other white. Floors were tactually distinct, one being of stainless steel rods, the other of either rods of a different diameter or wire mesh. In some experiments, the white chamber had a slight vinegar odor from having a small quantity of 2% acetic acid applied to its end wall. Room light entered both chambers through the lid, with more light provided to the black chamber than to the white one. The middle (neutral) chamber, 18 × 17 cm, had grey sides and a grey floor of smooth plastic 4 cm below the other floors. To facilitate choice behavior, the 11-cm wide walls separating the neutral chamber from the end chambers were of transparent plastic, allowing rats in the neutral chamber to see both end chambers readily. A full-width removable cardboard barrier, white on one side and black on the other, was used as a retrace door for the neutral chamber during training. With the subject in one end chamber, the barrier blocked the rat's view of the other chambers. Electromechanical equipment recorded the number of entries into each end chamber from the neutral chamber along with the total time in seconds spent in each end chamber.

T-shaped boxes (T-boxes). The two identical boxes used in Experiments 2, 4, 6–8, and 12 were shaped like T-mazes without alleys, with the neutral chamber as start box. The black and white end chambers were adjacent and were separated by a common wall. The grey neutral chamber, approximately the size of an end chamber, was centered on the wall separating the other chambers. Thus, as with the CPP box, the only route between end chambers was through the neutral chamber, but, as with a T-maze, the subject faced both end chambers when exiting the neutral chamber. This side-by-side feature, like the transparent end walls of the neutral chamber in the linear box, was designed to promote choice behavior. In the conventional CPP apparatus, a rat leaving the neutral chamber can see only one end chamber at a time.

The end chambers were similar to those of the linear shuttle boxes, and the white chamber had a slight vinegar odor. In Experiment 4 only, the floor of the neutral chamber was elevated 10 cm above those of the other chambers. The openings from the neutral chamber to the end chambers had sliding doors of black or white cardboard. Each time the subject left the neutral chamber, a microcomputer recorded the side entered and, for comparison with the CPP time measure, the duration in seconds of each entry into each end chamber. In some experiments, latencies of entering the end chambers were recorded electrically in tenths of seconds.

General Procedure

In all experiments, subjects were assigned randomly to experimental conditions and to the black or the white side as positive. Some other aspects of the procedures (such as apparatus habituation) varied considerably among experiments. Although CPP effects are held to be quite robust over different laboratories in which procedures also vary (5,6,15), caution is needed here in comparing the results of different experiments. Experiments 5, 6, 8, and 11 started with two 15-min periods of habituation to the entire apparatus, while Experiments 10 and 12 started with one such period. Experiments 2 and 4 gave two 100-s exposures to each of the three chambers, followed by two periods of exposure to the entire apparatus, 15 and 30 min, respectively. Experiments 1, 3, 7, and 9 did not use apparatus habituation. No relationship has been apparent between the use or amount of habituation and performance measures in these experiments or in any other experiments we have done using these methods.

At the start of a trial (except in Experiments 10 and 11, which did not use catheters), the catheter was flushed with saline and connected to a length of small bore plastic tubing leading to a 1-ml syringe, both of which contained either morphine sulfate dissolved in 0.9% sodium chloride with 0.9% benzyl alcohol included or the vehicle. Infusion volume was either 0.5 ml per kg of body weight (Experiment 1) or 1.0 ml/kg (remaining experiments). The solution was infused manually, in about 15 s, beginning as soon as the animal was in the positive chamber with the door closed (or just before placement in Experiment 9). On trials in which neither the drug nor the vehicle was to be given, the catheter was not flushed and a plastic tube was merely connected to the plug in the end of the catheter. In most experiments, saline was not given in the negative chamber because of the possibility that the infusion stimulation could come to elicit conditioned drug responses after being followed repeatedly by the drug effect. The effect of giving saline on the negative side was investigated in Experiments 2, 8, and 12.

To prevent reinforcer satiation or disruption effects from interfering with the process or the measurement of conditioning, morphine was administered no more often than once per day and, in all but one of the experiments that used 10 mg/kg morphine, the drug was given no more often than once every 2 days.

Placement Training

The animal was placed in the positive and negative chambers in alternation, with the doors closed, for the period specified for the experiment. When the side was positive, the drug was infused just before placement (Experiment 9) or just after placement (other experiments). When the side was negative, the rat remained in the chamber without the drug.

Approach Training

Approach training differed from placement training in that the animal entered the chambers rather than being placed there by the experimenter. Two types of approach training procedures were used: nonchoice and choice.

Nonchoice training. The door to one side was closed and the rat was placed in the neutral chamber, facing away from the entrances to the black and white chambers. It was allowed to enter the accessible side, was confined to that side, and received the drug when that side was positive. As in placement training, trials alternated between positive and negative.

Choice training. Both chambers were accessible to the rat on the odd-numbered trials. Even-numbered trials were nonchoice or "forced" trials in which only the chamber not entered on the previous trial was accessible. Hence, the two chambers were entered an equal number of times.

Testing and Data Analysis

Time-and-Entries (T&E) Test. After the conclusion of training, the rat was allowed access to the entire apparatus for 15 or, in Experiment 4, 30 min. Number of entries into each side and times spent in each side were automatically recorded. In Experiments 2, 4, 6, 8, and 12, T&E data were also collected prior to training during apparatus habituation periods. The T&E test was omitted in Experiment 7.

Discrete-Trials Choice Test (DC Test). In all but the first experiment, the T&E test was followed by five or six choice trials, usually given over 3 days (Experiment 10 gave four DC trials on 1 day). On each trial, the rat was placed into the neutral chamber, facing away from the open exits, and was allowed to enter either chamber. In different experiments, it was returned to its living cage after being confined to the chosen side for 1 min, after reentering the neutral chamber, or after either of these occurred. In most experiments, if a rat failed to choose within 1 min it was given up to two additional opportunities, 1 min apart (which were rarely needed), before a failure to choose was recorded. In Experiment 3, there were potentially up to four opportunities, each lasting 2 min. In Experiment 11, the odd and even trials took place under different conditions so there were two DC scores, each based on three trials. Experimenters were blind to conditions during the DC test.

Reinforcement measures during training. In choice training, the subject's choice provided a measure of reinforcement on each odd-numbered training trial. In some of the experiments with nonchoice training, the relative latency of entering the positive and negative chambers constituted a measure of reinforcement (Experiments 6–8 and 12). The procedure for measuring latency is described in Experiment 6.

Treatment of data. For each subject, the entry and choice scores were the percentages of positive side entries from the neutral chamber. In the DC test, trials on which no choice was made were excluded in calculating a subject's score. For choices in training, the first training trial was excluded in computing the percentage. The subject's time score was the percentage of end-chamber time spent in the positive chamber. Individual latency scores for training were the subjects' medians of the final three positive and three negative trials for 8 or 16 training trials and of the final five positive and five negative for 20 training trials. In calculating the medians, latencies for trials on which no entries occurred were arbitrarily scored 180 s, the sum of the three 60-s opportunities in nonchoice training.

Statistical analysis. The statistical analyses were *t*-tests and unweighted-means analysis of variance (ANOVA). The particular forms of these analyses were designed to prevent the error terms from being inflated by any black-white biases, that is, mean score differences between the black and white sides. In ANOVA, this was done by using positive side (black or white) as a variable. For *t*-tests, it was done by comparing the black (or white) entries, times, choices, or latencies of black-positive vs. white-positive subjects. These analyses were used for all significance tests reported here (except when all black- or white-positive rats in a group had scores of 100% on a measure, resulting in zero variance for that cell in the analysis—in which case the variable of black- or white-positive was ignored). In most cases, taking out the effect of black- or white-positive increased the size of the F or t. It also prevented any imbalance in the numbers of black- and white-positive subjects from being able to create a positive or negative bias.

For statistical analysis of latency data, the individual subjects' median latencies were log transformed to reduce heterogeneity of variance. The latencies presented are antilogs of group means of these log median latencies.

In some experiments, we attempted to reduce error variance with the aid of T&E pretests. Given sufficiently high correlation between pre- and posttest, the changes from pre- to posttest could be much less variable than the posttests scores. Most pretest-posttest correlations turned out to be too low to increase statistical power, most were nonsignificant, and several were negative.

PROCEDURES AND RESULTS FOR INDIVIDUAL EXPERIMENTS

EXPERIMENTS USING IV ADMINISTRATION

Experiment 1: Choice Training vs. Placement Training

The purpose of this experiment was to compare choice and placement training with much briefer chamber times than are used in CPP training. The time in the positive chamber was 1 min after the IV infusion of 10 mg/kg morphine or about 75 s. In the negative chamber, it was (inadvertently) 1 min. The linear apparatus was used. Choice training consisted of four choice trials on odd-numbered days, each followed by a forced trial to the opposite side on the next day. For placement training, subjects alternated between positive and negative sides on successive days. The T&E test was given on the ninth day.

In training, the positive side was chosen in 77% of the choice trials after the first, $t(8) = 2.92$, $p = 0.02$ (data not shown). In the T&E test, the groups differed significantly in both time, $F(1,15) = 11.09$, $p = 0.005$, and entries, $F(1,15) = 13.73$, $p = 0.003$. As may be seen in Fig. 1, the approach group spent most of its time in the positive chamber and most

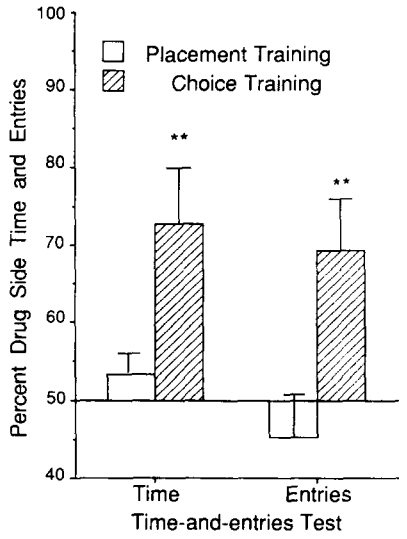

FIG. 1. Placement vs. choice training with brief chamber times (Exp. 1). Data shown are mean percentages for time spent on drug side and for entries into drug side in T&E test; DC test not given. In this and all subsequent figures that depict percentages, the "chance" expectancy is 50%. In all figures, error bars represent standard errors of means and asterisks represent the significance of the results for an individual group when compared to chance: $*p = 0.05$, $**p = 0.01$, $***p = 0.001$. $n = 9$–10.

of its entries were into the positive chamber. Placement training did not produce the greater time in the positive chamber that is usually found with morphine and longer exposures to a compartment. The weakness of placement training was even more striking for the entries measure as the placement subjects entered the positive chamber slightly less than chance.

Experiment 2: Reinforcing or Aversive Effect of IV Saline Tested by Choice and Placement Training

In Experiment 1, saline was not administered on the negative side because of the risk of conditioned reinforcement on that side from the saline. Omitting saline obviously entailed some risk that morphine reinforcement effects could be distorted by any unconditioned reinforcing or aversive effects of saline infusion. The present experiment tested for possible reinforcing or aversive properties of IV saline infusion. Choice and placement training were used with 8 and 16 trials and with 75-s chamber times on both sides. The T-box, which was designed to promote choice behavior, was used. Two 15-min apparatus exploration periods were given, the second one also serving as a T&E pretest. Training was then given, one or two trials daily, in which saline was received in the positive chamber and no infusion was given in the negative chamber. Training was followed by the T&E posttest and the DC test.

None of the four groups showed a statistically significant effect by either T&E measure, although one combination showed a virtually significant effect. This combination was eight trials of choice training for which the entries score was 46% (Table 1) ($p = 0.051$), suggesting that saline might have been slightly aversive. However, no DC test was significant (each $p > 0.4$), nor were the choices of either choice group in training (each $p > 0.14$) (data not shown). No group changed significantly from pre- to posttest in either entries or time; the only F that exceeded 1.0 was for a decrease in entries ($p = 0.20$) (see Table 1). A single nearly significant result in four groups and five or more measures per group is well within chance expectancies.

Experiment 3: Choice vs. Placement Training with Longer Chamber Times

This experiment was essentially a replication of Experiment 1 except that animals spent 15 min in the positive and negative chambers. Chamber times of 15 min or longer are usually employed in CPP training. Results are shown in Fig. 2. With the longer chamber exposures, placement training was approximately as effective as approach training (each $F < 1$). In training, the choice-trained subjects again entered the positive side on 72% of trials after the first, $t(10) = 2.53$, $p = 0.04$ (data not shown).

Experiment 4: Choice vs. Placement Training with Two Morphine Doses

This experiment incorporated several changes intended to promote reliable choice behavior following placement training with brief chamber times; 1) The T-box, which was designed to facilitate choice behavior, replaced the alley-shaped apparatus; 2) the number of training trials was doubled; 3) to increase the number of chamber entries, the T&E test lasted twice as long; 4) group sizes were doubled and pretests were employed to reduce variability from individual differences. A second objective was to test the procedures and the measures with a considerably lower dose of morphine, 1 mg/kg.

The design was a 2 × 2 factorial: placement vs. choice training and 1 vs. 10 mg/kg morphine IV. All subjects received 16 training trials with 75-s periods in the chambers. The choice and placement training procedures were the same as in the first two experiments, again with one trial per day. Prior to training, two daily 30-min apparatus habituation periods were given, the second also serving as a T&E pretest. The posttest also lasted 30 min and was followed by the DC test.

Posttest results are shown in Fig. 3. With choice training, both doses were effective by all three measures. The apparent dose effect in the DC scores of the choice-trained groups was nonsignificant ($p = 0.08$). During training, for the high dose on the last seven of the eight choice trials mean positive choice

TABLE 1

T&E TEST CONDITIONS, PRETEST RESULTS, POSTTEST RESULTS, AND PRE-TO-POST TEST CORRELATIONS FOR GROUPS RECEIVING PRETESTS

				Percent Positive Side Time						Percent Positive Side Entries					
				Change from Pre to Post						Change from Pre to Post					
Experiment	Training	Trial	Dose	Pre	Post	F	p	r	Post F	Pre	Post	F	p	r	Post F
2	Choice	8	0	47	46	0.1		0.45	2.4	47	46	0.3		0.73	4.8
2	Choice	16	0	50	56	0.8		0.30	1.6	54	50	2.0	0.20	0.53	0.0
2	Place	8	0	50	53	0.3		0.20	0.8	49	50	0.1		0.35	0.1
2	Place	16	0	50	53	0.4		0.30	0.8	49	52	0.8		0.37	0.8
4	Choice	16	10	49	69	11.8	0.01	0.23	29.3	51	63	12.1	0.01	0.41	48.0
4	Choice	16	1	42	64	9.2	0.01	−0.55	12.6	50	58	6.3	0.03	0.39	12.5
4	Place	16	10	46	69	58.3	0.001	0.64	46.4	45	58	52.3	0.001	0.68	14.6
4	Place	16	1	55	64	2.9	0.20	−0.21	12.5	54	57	0.5		−0.09	4.1
6	Nonchoice	20	10	48	62	8.8	0.02	0.17	24.0	47	58	23.3	0.001	0.31	20.7
6	Nonchoice	20	1	53	55	0.2		0.47	2.5	52	55	1.9	0.20	0.34	8.3
6	Nonchoice	20	0	53	54	0.1		0.21	1.7	51	52	0.1		−0.07	1.1
8	Nonchoice	16	10*	49	70	22.8	0.001	0.38	119.0	50	60	22.0	0.001	0.41	41.2
8	Nonchoice	16	10†	51	63	6.5	0.04	−0.02	17.0	51	55	1.9	0.25	0.09	4.2
8	Nonchoice	16	0	55	47	3.1	0.11	0.30	0.9	56	48	11.8	0.01	0.24	1.2
12	Nonchoice	8	0‡	57	55	0.1		−0.18	1.2	57	54	0.2		−0.05	1.0

*Saline on negative side; †no infusion on negative side; ‡saline by IP injection.

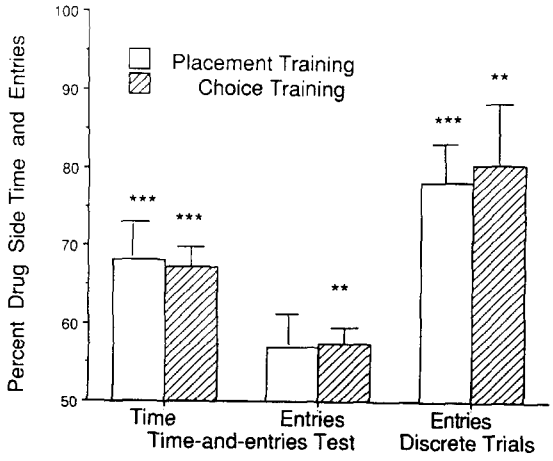

FIG. 2. Placement vs. nonchoice training with 15-min chamber times (Exp. 3). n = 11–12.

was 92%, $t(14) = 11.6, p < 0.001$; for low dose, it was 76%, $t(14) = 6.6, p < 0.001$. This difference between high and low doses was significant, $F(1,28) = 9.07$, $p = 0.005$ (training data not shown). Choices in training also provided some indication of the speed of conditioning. On choice trials 2–4, 85% ($p < 0.001$) and 67% ($p = 0.04$) of the choices were positive for the high- and low-dose groups, respectively, showing that conditioning was rapid.

Unlike Experiment 1, brief-exposure placement training with 10 mg/kg IV morphine was also effective by both T&E measures and not significantly less so than approach training for entries ($p = 0.25$), or for time ($F < 1$). Only by the DC measure was approach training significantly more effective than placement training, $F(1,57) = 5.86, p < 0.025$. Clearly, neither approach training nor prolonged exposure to the positive and negative chambers is required to produce strong choice behavior. By the DC and time measures, placement training was also effective with the 1-mg/kg dose.

Changes from T&E pretest to T&E posttest are shown in Table 1 for all experiments that included pretests. Results were largely similar to those for the T&E posttest, except for time

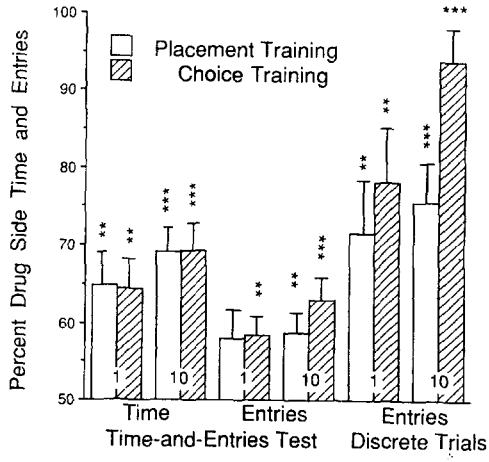

FIG. 3. Placement vs. choice training with brief chamber times, enhanced reinforcement conditions, and two doses of morphine (Exp. 4). n = 14–16. For the one nonsignificant percentage, $p = 0.055$.

in the low-dose placement group, which did not significantly gain in entries or in time. For entries, the high-dose groups gained significantly more than did the low-dose groups, $F(1,53) = 4.16$, $p = 0.05$. Change in time spent showed no significant effects of the training procedure or of the morphine dose ($F < 1$). Correlations between pretests and posttests generally were disappointingly low so the use of change from pretest to posttest did not result in the hoped-for increase in statistical power. In the three cases in which pretest means were 49–51% (i.e., within 1% of the chance expectancy), the F's for pre- to postchange were considerably lower than those for the corresponding posttests (Table 1). Highly significant changes occurred only in association with pretest means that were—by chance—well below chance expectancy.

Addendum to Experiment 4: Regression Effects

A popular CPP design is pretesting, making each subject's nonpreferred side that subject's positive side and measuring reinforcement by the shift in time spent from pre- to posttest. This design reduces both ceiling effects and variability in the data due to some subjects being reinforced toward, and others against, the initial preference. Unfortunately, it also risks confounding treatment effects with 1) sequence effects, for example, a systematic shift toward the white or black side or toward 50% with repeated testing, and 2) statistical regression effects, that is, the tendency for the higher scores to decrease on retesting and lower scores to increase.

The present pseudoexperiment attempted to test for such potential confounding using data from the two pretests of Experiment 4. The data were for the 48 subjects having first pretest entry scores different from 50%. We first tested for imaginary positive reinforcement taking place between the two pretests by making each subject's nominal positive side the one it had initially entered less. The mean of the first pretest was 39.4%, and this increased to 44.6% on the nominal posttest, $F(1,46) = 10.66, p = 0.002$ (data not shown). The same data could instead have been used to test for aversive effects by treating as positive the side initially preferred. The change would have been opposite in direction, but its size, the F, and p would have been the same. To show that these changes were regression effects rather than sequence effects, subjects were then assigned to imaginary treatments on the basis of their second pretest scores and changes from the second pretest back to the first were calculated. For reinforcement, the second pretest mean was 38.9, which increased to 44.8 on the first pretest, $F(1,49) = 17.8, p < 0.001$; alternatively, for imaginary aversive effects, the size of the change, and the F and p, would have been the same (data not shown).

Both sources of confounding could be eliminated by doing the significance tests between changes shown by experimental groups vs. those shown by control groups. Unless the pre- to posttest correlations are quite high, however, such designs may be unnecessarily low in power due to the added variability of the pretest scores and of both scores for the control subjects (9). More importantly, the conclusions would be limited to reinforcement on the nonpreferred side or punishment on the preferred side. Because the initial side bias might reflect neophobia or exploratory reinforcement, either of which could presumably be influenced by the test drug, this limitation would not be a trivial one.

Experiment 5: Nonchoice Approach Training

A useful feature of CPP training is the control it permits over the sequence of positive and negative trials. The present experiment tested an approach training procedure that pro-

vided the same control by the use of forced trials exclusively. Training consisted of two daily forced trials in the linear box, 1.5 h apart, the first to the negative side, the second to the positive. Dose and route of administration were as in the previous experiments: 10 mg/kg IV. As in Experiment 1, time in the positive chamber was 1 min after the 15-s infusion and in the negative chamber 1 min, again by an oversight.

Results are shown in Fig. 4. Discounting any bias from the 15-s time difference, nonchoice training was clearly effective by all three measures. In the DC test, proportion of positive choices was among the highest of any reported here.

Experiment 6: Nonchoice Training with Response Latency Measures and Two Morphine Doses

Discrete-trial studies of nondrug reinforcers like food and electrical brain stimulation in rats often use runways, measuring the time taken to leave the start box and that taken to go from the start box to the goal box. This experiment investigated the ability to detect high and low morphine doses of a similar measure in the OPC apparatus: the time to enter the open chamber. The basic difference between the runway and OPC latency measures is that OPC uses relative latency, that is, the difference between the times taken to enter the positive and negative chambers.

On each training trial, one chamber was open and the time to enter that chamber was measured in tenths of seconds from when the subject was placed into the neutral chamber—facing the rear—until its weight was off the neutral chamber floor and onto the end chamber floor. Three groups of rats received 10, 1, and 0 mg/kg morphine IV with 20 alternating positive and negative forced trials in the T-box, 1 trial per day. Time in the chambers was 75 s. Besides the latency measures taken in training, nine latency test trials were intermixed among the five DC trials that followed the T&E posttest.

For each group, Fig. 5 shows the mean latencies of entering the positive and negative sides during training trials and also for the posttraining latency test. Fig. 6 shows the results of the T&E and DC tests. All five measures classified 10 mg/kg morphine as reinforcing and all but time spent so classified 1 mg/kg. None of the five measures classified saline as reinforcing. All measures but time classified 10 mg/kg as more reinforcing than saline [each $F(1,28) > 7.5$, each $p < 0.015$]. By DC and both latency measures, 1 mg/kg morphine was more

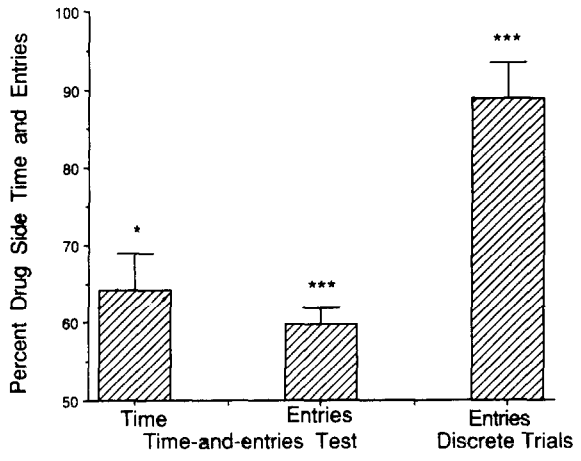

FIG. 4. Nonchoice training (Exp. 5). $n = 12$.

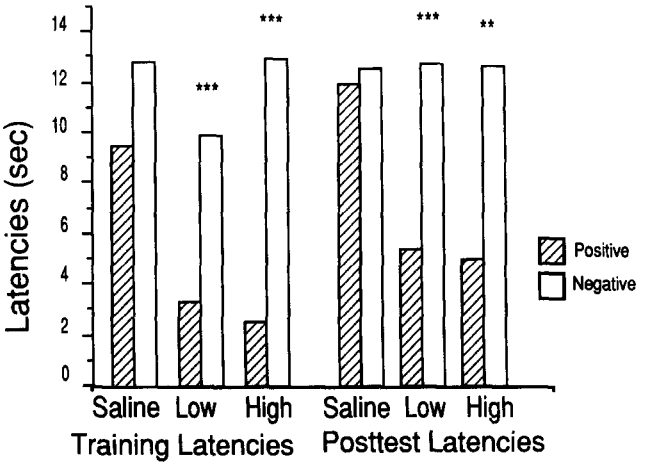

FIG. 5. Nonchoice training with two morphine doses and latency measure (Exp. 6). Data shown are antilogs of mean logs of individual latency scores for entries into the positive and negative chambers during and after training. Because standard errors computed on these logs were deceptively small, error bars are not shown. $n = 15$–17.

reinforcing than saline [each $F(1,28) > 7.8$, each $p < 0.01$]. No measure showed a significant dose effect, although two were borderline: training latencies ($p = 0.07$) and DC ($p = 0.06$).

Changes in time and entries are given in Table 1. The high-dose group showed significant gains in both, whereas the low-dose group showed gains in neither. A dose effect was shown for change in entries, $F(1,26) = 6.94$, $p = 0.02$, but was borderline for change in time, $F(1,26) = 3.74$, $p = 0.07$.

Experiment 7: Omission of the T&E Test

In the previous experiments, T&E scores were consistently lower than either DC scores or measures of reinforcement that were taken during training. The T&E test also requires several times as long as a training trial and so can be inconvenient to schedule in sequence with training. However, because the DC test had always followed a T&E test there was some possibility of it requiring the prior experience of exploring the apparatus following training. The present experiment omitted the T&E test and used only the two reinforcement measures that had shown the largest effects with nonchoice training: relative latencies of entering the positive and negative chambers during training and choices in the DC test. After 20 daily trials of nonchoice training in the T-box, with 5 mg/kg IV morphine given on one side and the same volume of saline on the other, a 6-trial DC test was given. The DC test was then repeated to assess its stability.

Fig. 7 shows the mean latency scores for the last five positive and last five negative trials. As in Experiment 6, the latency difference was marked. Figure 7 also shows the mean percentages for positive and for negative entries in the first and second blocks of DC trials. Although the DC test should entail some degree of extinction of entering the positive side, positive choice decreased hardly at all from the first block of DC trials to the second.

Experiment 8: Nonchoice Training Using Within-Subjects Control for Vehicle Reinforcement or Aversive Effects

The present experiment tested the effect of giving IV saline on the negative side. In addition, like Experiment 2, it tested

the reinforcing and aversive effects of IV saline infusion. One group of rats received 10 mg/kg morphine IV on the positive side and no infusion on the negative as in Experiments 1 and 3-6. A second group received morphine on one side and the same volume of saline, 1 ml/kg, on the other, and a third group received only the saline. After two 15-min exploration periods, the second serving as a pretest, 16 nonchoice training trials were given in the T-box, 1 trial daily, with 75-s chamber times and with latency measured on each trial.

Results for the T&E and DC tests are shown in Fig. 8. Both morphine groups chose the positive side more than four times as often as the negative side in the DC test. In the T&E test, both morphine groups spent more time on the positive than on the negative side. Although only the morphine-saline group entered the positive side significantly more than the negative, both morphine groups entered that side significantly more than did the saline group in both the T&E test and the DC test. For the T&E entry and DC measures with the morphine group, $F(1,20) = 5.26$, $p = 0.04$, and $F(1,22) = 16.00$, $p = 0.001$, respectively. For the same measures with the morphine-saline group, $F(1,22) = 27.6$, $p < 0.001$, and $F(1,22) = 16.44$, $p = 0.001$. Both morphine groups also spent more time on the positive side than did the saline group; for morphine, $F(1,20) = 12.86$, $p = 0.003$; for morphine-saline, $F(1,22) = 42.8$, $p < 0.001$. The saline group was nonsignificantly below 50% in the DC and T&E tests (each $p > 0.3$).

Changes from pretest to posttest in T&E scores are shown in Table 1. The modest decrease in entries in the saline group might reflect either a weak aversive effect or, considering the low pre-post correlation, merely statistical regression. The three groups differed significantly in changes: for entries, $F(2,32) = 12.36$, $p < 0.001$; for time, $F(2,32) = 10.73$, $p < 0.001$. Both morphine groups significantly exceeded the saline group in changes in both time and entries (each $F > 9.0$, $p = 0.01$), but the two did not differ significantly between themselves in gains in either time or entries (each $p > 0.14$).

None of the pre-post correlations was high enough for the pretest to add statistical power. For each of the F's in Table 1 with pretest mean within 1% of chance expectancy, the corresponding F based on the posttest alone was substantially higher, between 1.9 and 5 times as high.

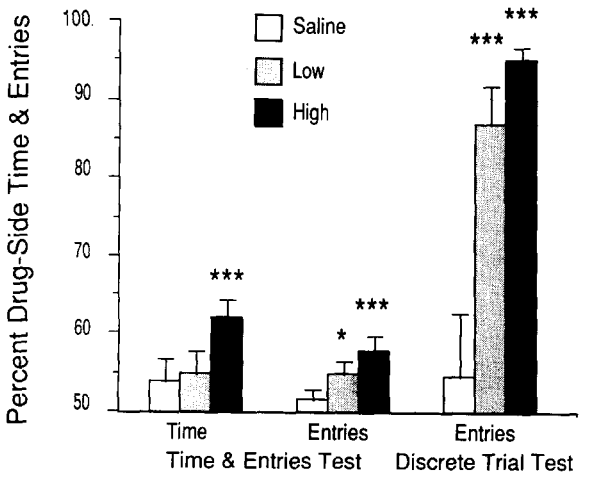

FIG. 6. Nonchoice training with two morphine doses and latency measure (Exp. 6). n = 15-17.

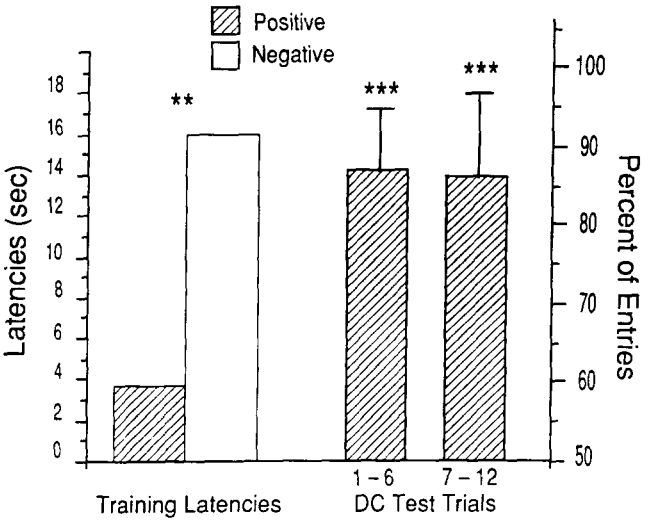

FIG. 7. Nonchoice training with T&E test omitted (Exp. 7). Left portion of graph shows antilogs of mean logs of individual latency scores. Right portion shows mean percentages for entries into positive and negative sides in two blocks of DC trials; T&E test not given. n = 11.

Even if saline has neutral reinforcing properties, the process of preparing the subject for an infusion could markedly affect the latency measure. Apparently, it did not as the saline group entered the positive chamber only slightly faster than the negative one during training ($F < 1$) and the negative side latencies did not differ significantly between the morphine-saline and morphine-only groups, $F(1,22) = 1.36$, $p > 0.25$ (Fig. 9). In contrast, both morphine groups entered the positive chamber faster than the negative chamber. The morphine-saline group showed a significantly greater latency difference than did the saline group, $F(1,22) = 4.67$, $p < 0.05$. For nonchoice training at least, these results suggest that omitting saline on the negative side does not invalidate an experi-

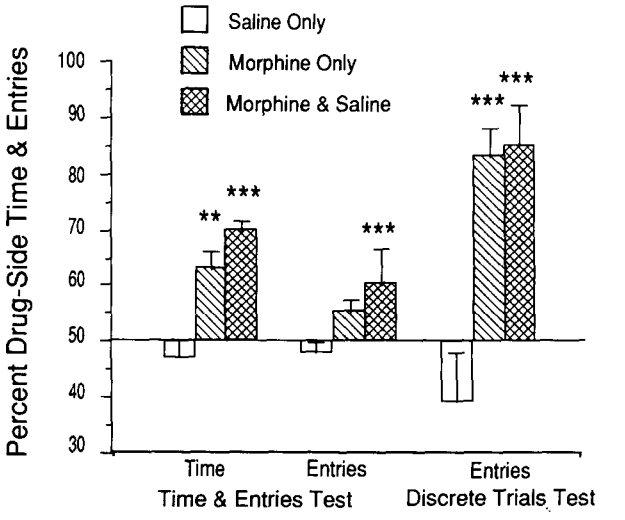

FIG. 8. Control for IV vehicle infusion reinforcement: One group received morphine on one side and saline on the other; other groups received only morphine and only saline (Exp. 8). n = 12-14.

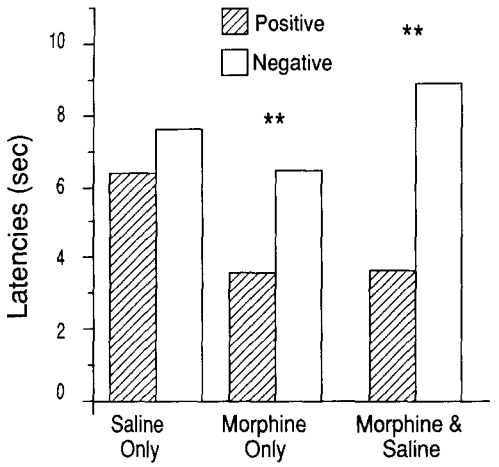

FIG. 9. Control for IV vehicle infusion reinforcement: One group received morphine on one side and saline on the other; other groups received only morphine and only saline (Exp. 8). Data shown are antilogs of mean logs of individual latency scores. n = 12-14.

ment, but also that giving saline on the negative side does not interfere with conditioning and might even increase the power of an experiment.

EXPERIMENTS USING IP MORPHINE ADMINISTRATION

Experiment 9: Choice vs. Placement Training with the Drug Administered Through IP Catheters

This experiment was like Experiment 3 except dose and route of administration were changed from 10 mg/kg IV to 5 mg/kg IP. This route and dose are far more commonly employed than 10 mg/kg IV in CPP experiments using morphine. As in Experiments 1 and 3, choice and placement training were used, one trial per day in the linear box. Choice-trained rats received the drug through IP catheters as soon as they entered the positive compartment. Placement-trained rats received the drug immediately before being placed in the positive chamber to make their experience more like that in CPP experiments on morphine.

In training, the choice-trained group entered the positive side on 81% of the trials after the first, $t(7) = 3.67$, $p = 0.01$. Results of the T&E and DC tests are shown in Fig. 10. By the time measure, both types of training were effective and about equally so, whereas by the T&E entry measure only approach training was effective and the groups differed significantly, $F(1,14) = 8.63$, $p < 0.02$. In the DC test, however, placement training produced clear choice behavior. These results suggest that the T&E test is less sensitive to choice behavior than the DC test. The similar lack of reliable chamber entry differences with placement training in Experiment 1 may also have reflected an insensitivity of the T&E entry measure. Few CPP studies have recorded entries during their time tests. Studies that looked for choice behavior did not find it and concluded that choices in CPP do not measure drug reinforcement (1). The present results suggest that placement training with IP morphine reinforcement can bring about the learning of choice behavior, but that the time test may not promote the occurrence of such choice behavior in the CPP apparatus.

Experiment 10: Approach Training Without Catheters

A major convenience feature of the conventional CPP method is the lack of need for surgically implanted catheters. The rat is merely injected IP or SC and placed in the chamber. The present experiment sought to achieve the same advantage in approach training by using only forced training trials, as was done in the forerunner of the CPP procedure, the 1957 study of Beach (4). Eight forced trials were given in the linear apparatus, one or two per day, at least 5 h apart, with odd-numbered trials negative and even-numbered positive. On positive trials, the rat was injected IP with 5 mg/kg morphine and immediately run to the positive chamber for 15 min. On negative trials, the rat was merely run to the negative chamber for 15 min. The T&E test was followed with another eight training trials, then a second T&E test, and then a DC test.

Results are shown in Fig. 11. The similarity of the entries scores and the DC scores in the present experiment to those of the approach group in Experiment 9 suggests that the effectiveness of approach training can be achieved without cannulation. With the same drug, dosage, and route of administration, placement training yielded negative results for entries during time tests in Experiment 9 and elsewhere (1).

Experiment 11: Nonchoice Training with Test Under Drug Conditions

Several CPP studies have found equivalent time scores whether or not the subjects were drugged during the test (11). As in the previous experiment, rats without catheters were run to the positive chamber following IP injection of 5 mg/kg morphine and run to the negative chamber without injection, with chamber times of 15 min. There were eight training trials in the T-box, two per day, at least 5 h apart, with odd-numbered trials negative and even trials positive. The same dose of morphine was injected 15 min before the T&E test and also 15 min before the first of two daily DC tests, which were given about 45 min apart.

As may be seen in Fig. 12, the results of the T&E test, conducted this time under the influence of the drug, did not differ greatly from those of the previous experiment, although significance was borderline for entries ($p = 0.07$). For the first of the two daily DC tests, given 15 min after a drug

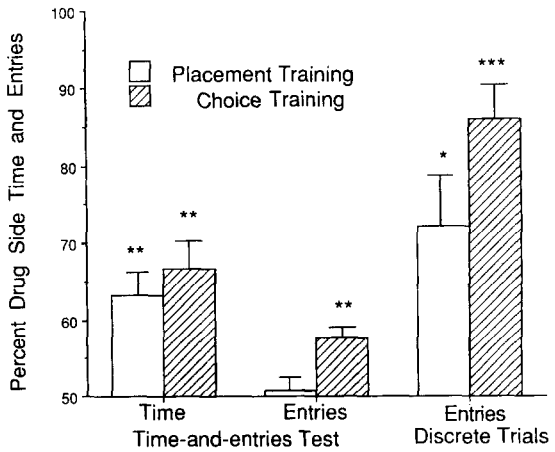

FIG. 10. Placement vs. approach training using IP catheters (Exp. 9). n = 9.

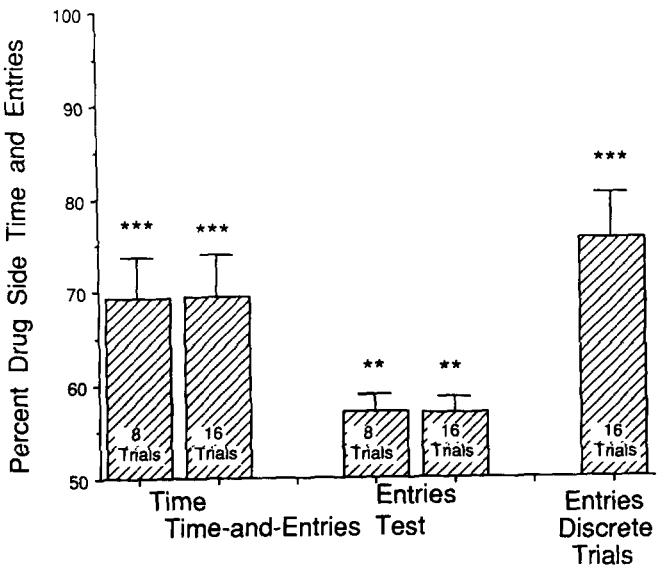

FIG. 11. Nonchoice approach training without catheters, with morphine injected IP just before rat was run to positive side (Exp. 10). Data are for one group of rats tested after 8 training trials (T&E test only) and again after 16 trials (T&E and DC tests). $n = 12$.

injection, the results were quite similar to those of the same test in the previous experiment. These findings partially confirm the reports that drug-reinforced behavior is not diminished by testing under the drug state. On the second of the daily trials, an hour after the drug injection, no preference for the positive side remained. The exact basis for this difference between the two daily tests is unclear; conceivably, it may be related to morphine's biphasic action (17).

Experiment 12: Reinforcing Effects of IP Saline Injection

Experiments 10 and 11 did not include vehicle injection on the negative side under the assumption that IP saline injections given prior to the response of entering a chamber cannot reinforce that response. The present experiment tested this assumption. Nonchoice training with 15-min chamber times was again used. To determine whether injections would cause discrepancies between training latencies and other measures, latencies in training were also recorded. After a T&E pretest, eight forced training trials like those in Experiments 10 and 11 were given in the T-box, then a T&E posttest and a five-trial DC test. To assure adequate statistical power there were 24 subjects, although T&E data were available on only 19 of them.

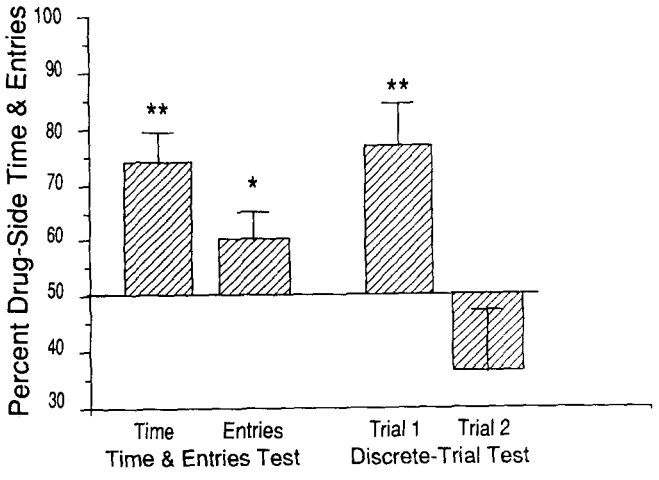

FIG. 12. Nonchoice training with 5 mg/kg IP morphine and testing under drug (Exp. 11). Morphine injected 15 min before T&E test and 15 min before first of two daily DC tests. $n = 10$. Choices on second daily DC trial not significantly below 50%, $p > 0.20$.

None of the T&E and DC scores were significant (each $p > 0.25$) (DC data not shown), nor were the changes in time and entries from pre- to posttest ($F < 1$) (Table 1). Thus, IP injection of saline does not appear to be either reinforcing or aversive in the nonchoice training procedure. The positive side was nevertheless entered faster than the negative one in training, with respective means of 6.0 and 8.3 s ($p = 0.04$) (data not shown). Latency in training would therefore have been an invalid measure of reinforcement in Experiments 10 and 11.

DISCUSSION OF RESULTS

The present findings bear on two issues concerning OPC and CPP methods: whether measures of entry into the positive and negative chambers are better indices of reinforcement overall than the relative time spent in the chambers and whether training that entails active responding is more effective than placement training.

Entry vs. Time Measures of Reinforcement

In the T&E test, time spent was always a stronger measure of reinforcement than entries. Mean time proportion exceeded mean entry proportion in every morphine reinforcement group; also, time was significantly greater on the positive side in all but two groups, while entries was nonsignificant in five groups. On the other hand, the DC measure was considerably more powerful than time spent. It never failed to detect morphine reinforcement with either placement or approach training. Furthermore, in every morphine group in which both measures were employed mean DC proportion exceeded mean time proportion.

Several explanations may be offered for the larger and more reliable entry scores in the DC test than in the T&E test. In the DC test, the number of trials and the intertrial intervals were uniform across subjects and all trials began with the subject in the same location and orientation. In the T&E test, the number of rat-initiated "trials" varied between none and about 30 and the intervals between them ranged from under 3 s to much of the test period (unpublished observations). Because of this combination of deficiencies of the T&E test, we recently stopped using it.

Besides the DC measure, there were three other promising approach measures: choice between chambers during choice training, latency of chamber entry in nonchoice training, and latency in a posttest. Choices in training always showed larger effects than either measure in the T&E test, and latency differences between the positive and negative sides were large. These approach measures may have additional potential utility owing to their number. When there is doubt or controversy over whether or not a particular drug is reinforcing under given conditions, the agreement among multiple independent mea-

sures of reinforcement could add credibility to an experiment's findings.

Approach vs. Placement Training

In all the morphine experiments that used DC tests (i.e., all but the first experiment), placement-trained animals showed that they had learned to go to the positive side. At the same time, when placement and approach training were compared approach training produced DC scores that were always larger, more reliable, or both. Therefore, although both conditioning methods yielded approach behavior, approach conditioning appeared to be somewhat stronger overall. A more fundamental advantage of approach over placement training is in the opportunity it affords to assess reinforcement during conditioning by choice behavior during choice training and latency during nonchoice training. This allows the conditioning process to be charted as it occurs, a feature also possessed by drug self-administration.

Duration of Chamber Exposure

From the results of Experiments 4–8, short exposures are sufficient with IV morphine with placement as well as with approach conditioning. With the CPP method, time efficiency is usually obtained by the use of multiple sets of apparatus, but at increased apparatus cost and laboratory space. With a single OPC apparatus of about 60 × 40 cm, an experimenter can give one training trial or two DC trials to approximately 50–100 subjects per day.

The use of brief chamber exposures presumably requires that the drug be given intravenously, an added cost. But, even with the conventional CPP procedure, IV may be the superior route of administration for studying drug reinforcement, especially when comparability with IV drug self-administration is desirable. Recent evidence suggests that cocaine behaves more like a reinforcer when CPP is based on intravenous infusion rather than IP injection and that morphine appears to produce more rapid CPP conditioning by the IV route (2,11,12).

GENERAL DISCUSSION

In CPP, time spent in a chamber is the sole measure of reinforcement; hence, it seems essential for the time measure to have an unambiguous relationship to the subject's behavior during the test. When more time is spent in the positive than in the negative chamber, does this mean that the subject entered that chamber more than the other or it stayed there longer per entry? The answer, for any given animal or experimental group, could be either or both. Total time in a chamber is based on the number of times a rat enters the chamber combined with the durations of these entries. Therefore, it cannot be used to distinguish between treatments that cause animals to return to places associated with the treatments and those that cause animals to stay longer when they enter those places.

This ambiguity of time spent has led to two different descriptions of the rat's behavior in the CPP test. Reports of some studies describe time-spent data in approach terms as if it showed the animals to have entered the positive chamber more than the negative one. Inasmuch as the actual entering behavior was not recorded in these studies, this interpretation is necessarily unwarranted. Other reports merely state that the rat remains longer ("prefers to remain") in the positive chamber. In all but a very few studies [e.g., (1,2)], even this statement is unwarranted because of the ambiguity of the total time measure just mentioned. More importantly, it raises the question of how time spent in a chamber relates to reinforcement [see (6,10,21)]. Time spent does not correspond to any confirmed measure of reinforcement, that is, rate, probability, or speed of response. Hence, it could only be an indirect measure to be validated by agreement with established measures. However, enough disagreements with drug self-administration findings have been noted (15) to weaken the case for CPP being even an indirect measure of reinforcement.

Rate of lever pressing, spatial choice, and approach latency, on the other hand, are already well-established laboratory measures of reinforcement (10). Self-administration is simply the application to drug reinforcers of free-operant conditioning: A manipulatory response is followed immediately by drug presentation and the rate of that response is found to increase. In OPC, similarly, the response of entering a chamber is immediately followed by a reinforcer, after which the probability of that response increases while its latency decreases. OPC is thus also a special case of a well-established type of laboratory method for measuring reinforcement, discrete-trial place learning, as exemplified by T-maze and runway procedures. The statement that a drug is a positive reinforcer in terms of lever pressing or chamber entry means that it exerts the same kinds of control over behavior that are common to the many reinforcers studied with instrumental conditioning.

Given the weaknesses of time spent as the sole measure of reinforcement, CPP might well be strengthened by either supplementing or replacing time with discrete-trial choices between the positive and negative chambers. The DC test is fully compatible with CPP placement training as several of the experiments reported here showed. It can be given after the CPP time measure or instead of the latter. Besides having shown larger effects than those of time spent, DC is convenient and time efficient.

A possible basic weakness of OPC is that subjects remain in the chambers for a period of time after the drug begins to take effect. Delivery of the drug immediately upon chamber entrance constitutes the operant reinforcement procedure, while leaving the subject in the chamber provides an additional experience, that is, exposure to the positive chamber while under the influence of the drug. The behavioral changes, therefore, cannot be unambiguously attributed to the reinforcement component of this procedure. To do so, it would be necessary to rule out any possible contributions of drug-potentiated chamber exposure effects like habituation and dishabituation of exploratory motivation and of fear, differential attention to cues from the two chambers, or differential activity level while being exposed to these cues. The problem is similar for CPP, in which the (classical) reinforcement procedure consists of presenting the drug effect unconditioned stimulus following the chamber conditioned stimulus.

A second possible deficiency of both OPC and CPP is of a practical nature. OPC was designed specifically for reinforcer detection and there is little evidence that it can also discriminate reinforcer efficacy differences, such as between different drugs or the same drug at different doses or under different conditions. CPP has the same lack of secure evidence that it discriminates reward efficacy, even though significant dose effects have been reported [see (6)]. The problem in CPP is that virtually all significant dose effects reported are based on comparisons that included either the vehicle or doses too low

to be significantly reinforcing [but see (3)]. By the same lenient rules of evidence, Experiment 6 in the present study (the only experiment that included two morphine doses and saline) would have shown significant dose effects for all four OPC measures used.

We are now starting to examine variations in the conditioning and testing procedures in relation to dose effects. Until sensitivity to reward magnitude has been established, OPC will be useful primarily in studies dealing with the presence or absence of reinforcement. Elements of OPC and CPP can also be combined for this purpose, such as by using approach training with IP injections (Experiments 10 and 11) or, as mentioned above, by giving the DC test following CPP training.

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