Pharmacology Biochemistry and Behavior, Vol. 58, No. 1, pp. 133–140, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00010-5

Dosage Choices of Rats for Morphine, for Heroin, and Between Morphine and Heroin

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Received 20 March 1996; Accepted 23 September 1996

HUTTO, C. W., JR. AND W. F. CROWDER. *Dosage choices of rats for morphine, for heroin, and between morphine and heroin.* PHARMACOL BIOCHEM BEHAV **58**(1) 133–140, 1997.—Each of four doses of intravenous morphine and four of intravenous heroin was tested for reinforcing efficacy, and comparisons were made among the four morphine doses, among the four heroin doses, and between morphine and heroin. Rats with venous catheters performed one daily forced run for 75 s to one of the two compartments of a spatial choice apparatus, with compartments and doses or drugs alternating over days. In each trial, the accessible compartment depended on which dose (including zero dose) or drug a rat was to receive. After 18–28 forced trials and 0 or 4 reinforced choice trials, efficacies of the different doses and drugs were measured by subsequent compartment choices. Increasing dose effects on choice were found for morphine (0.32, 1.0, 3.2, and 10.0 mg/kg intravenously) and for heroin (0.032, 0.10, 0.32, and 1.0 mg/kg intravenously). A 32:1 morphine:heroin dose ratio produced nearly equal choice. Dose effects were more evident in within-subjects experiments (each rat received two doses or two drugs) than in between-subjects experiments (each rat received one dose of one drug). © 1997 Elsevier Science Inc.

Opioid reinforcement Dose choice Drug choice Reinforcement efficacy Morphine Heroin Rats

REVIEWS of drug reinforcement have distinguished between two types of research problems: a) presence or absence of reinforcement for any specified combination of drug, dose, and conditions; b) relative magnitude or efficacy of drug reinforcement, i.e., differences in reinforcing efficacy among those combinations that have been shown to be reinforcing. The most appropriate testing procedures have been held to differ for these two types of problems (2,14).

For presence or absence of reinforcement, two general methods are widely used with rats. One is drug self-administration, in which the drugs are typically injected intravenously (IV) in consequence of lever pressing (30,33). It is a direct application of the free-operant method to drug reinforcers. The other is conditioned place preference (CPP), in which the drugs are usually injected intraperitoneally (IP) or subcutaneously (SC), taking effect either before or shortly after the animal is placed into a designated compartment; reinforcement is measured by the proportion of time spent in the drug compartment in a subsequent free-roaming test (7,8,28). CPP was developed specifically to study drug reinforcement (15,21), although it has subsequently been used with a variety of nondrug reinforcers.

For the second type of problem, pertaining to variations in reinforcement efficacy, continuous reinforcement self-administration procedures, which are the self-administration measures used most often with rats, have been held to be unsuitable because they do not ensure long intervals between drug administrations and hence may allow response rates to be distorted by disruption or stimulation and by drug satiation (1,13,32,33). CPP generally provides long interinjection intervals and thus might be expected to be sensitive to drug reinforcement efficacy differences. CPP dose effects for positive reinforcers are usually ascending and are often progressive over wide dose ranges, such as between 0.08 and 15.0 mg/kg of morphine (18). However, although multiple doses are often employed in CPP studies, we have seen only a few studies that showed statistically significant differences among doses that individually yielded significant time-spent scores [e.g., (5,6, 11)]. Reported nonsignificance of apparent dose effects, as well as absence of reported significance, is not unusual. When dose effects are cited (8,24), they are often based on dose sets that include either zero doses or those not producing significant effects. Such comparisons obviate the distinction between the range of reinforcing doses and whether these different reinforcing doses vary in efficacy.

An attractive alternative to self-administration and CPP is

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the straight runway (10,31), which has been used for more than 50 years to study reinforcement and motivational variables with natural reinforcers (12). When used with drug reinforcers, a major drawback is the need for controls, preferably yoked, for any classically conditioned drug effects that can either mimic or mask the effects of drug reinforcement on running speed or starting latency.

We have been using the instrumental conditioning procedure of operant place conditioning (OPC), which is a spatial counterpart of discrete-trial self-administration and is similar to single-unit maze training with drug reinforcement. Receipt of a drug is contingent on entering the positive drug compartment of a spatial choice apparatus, and reinforcement is measured by the subsequent probability of this choice behavior (9,23). OPC and CPP are fundamentally antithetical, both in their conditioning procedures and in their response measures: OPC gives the drug in immediate consequence of the response of entering a place, whereas CPP exposes the subject to the place while the subject is drugged; reinforcement is measured in OPC by increased frequency of the reinforced entry response, and in CPP by increased time spent in the drug compartment.

In OPC, drugs are ordinarily given IV to minimize delay of reinforcement. Because drugs are far more satiating than natural reinforcers like food and water (32), and because we have assumed that even partial drug satiation might attenuate drug reinforcement, drugs are given no more than once a day. As a matter of experimental efficiency, time in drug compartments is only about 1 min.

OPC has been shown to detect the reinforcing effects of morphine, but there is little evidence either for or against a dose effect on reinforcement efficacy (9). This is the subject of the present investigation, which deals with efficacy differences among four reinforcing doses of morphine and among four reinforcing doses of heroin, and also compares various reinforcing doses of morphine and heroin.

METHODS

Subjects and Cannulation

The subjects were male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN, USA), 50–70 days old, housed individually in standard suspended wire cages with food and water available ad lib. Under deep anesthesia by ketamine (80 mg/kg IP) and either xylazine (6 mg/kg IP) or acetopromazine (10 mg/kg IP), they were implanted in the right external jugular vein with Weeks-type catheters of silicone rubber and polyethylene (29). Instead of passing directly through the skin at the back of the neck, the catheter was connected to a loop of 23 ga hypodermic tubing that was enclosed between two disks of knitted polypropylene surgical mesh (Surgipro SPM-149, United States Surgical Corporation, Norwalk, CT, USA). The tubing was bent at a right angle to the plane of the loop at the point where it exited through a puncture wound in the skin. After approximately 1 week, tissue growth had firmly attached the mesh to the skin. On the open end of the tubing was a short vinyl tube plugged with stainless steel wire when not in use.

Apparatus

The apparatus was a T-shaped discrimination box consisting of two drug compartments, each 27 × 18 cm (length × width), and a 27 × 15-cm choice compartment. As is sometimes done (17), the drug compartments were made highly distinctive to expedite training. One was black, with floor of 5-mm stainless steel tubes 10 mm apart; the other was white, with floor of 2.4-mm stainless steel rods 13 mm apart. Room light entered both compartments through the lid, with more light entering the black than the white compartment. The white compartment had a slight vinegar odor from having a small amount of 2% acetic acid applied to one wall. The choice compartment was gray, with a gray plastic floor.

The drug compartments were separated by a common wall, one end of which bisected the open end of the choice compartment. This arrangement was designed to facilitate the control of choice behavior by visual stimuli from the drug compartments by ensuring that the animal faced both drug compartments when leaving the choice compartment. The openings from the choice compartment to the drug compartments had sliding black and white doors. On each trial, the latency of entering a drug compartment was registered automatically in tenths of seconds.

General Procedure

In each experiment, the black and white compartments were assigned equally to the different experimental conditions (drugs, doses), and then subjects were assigned randomly and equally to these combinations of compartment and experimental condition. Prior to the start of training, the rats were briefly handled about 10 times and were placed in each of the three compartments 10 times for approximately 1 min.

Drugs and Drug Administration

The drugs were morphine sulfate (Penick Corporation, Newark, NJ, USA), heroin HCl (diacetylmorphine HCl; NIDA, Rockville, MD, USA), and naltrexone HCl (Sigma Chemical Co., St. Louis, MO, USA). Doses are expressed as the salt. At the start of a trial, the catheter was flushed with saline and connected to a 2-m length of small bore plastic tubing leading to a 1-ml syringe. The syringe and tubing contained either the vehicle (0.9% sodium chloride with 0.9% benzyl alcohol added) or morphine or heroin dissolved in the vehicle. Infusion volume was 1.0 ml/kg, except in some of the choice training trials, as described below. The solution was infused manually in about 15 s, beginning as soon as the animal was in a drug compartment with the door closed.

One minute after the end of the infusion, the animal was removed from the apparatus and, to antagonize the drug in the first seven experiments, was infused with 1.0 ml/kg of either 0.1 mg/ml naltrexone HCl or its vehicle (saline), depending on whether the opioid or the saline had just been given. The catheter was then filled with heparin solution (5 USP units/ml) and the vinyl connecting sleeve was plugged.

The naltrexone was given in the hope of promoting conditioning by isolating the drug effect to the positive compartment or by reducing the development of tolerance to the drug's reinforcing effect. We briefly investigated the effect of naltrexone on the reinforcing efficacies of these two drugs by testing its effects on: a) the reinforcing efficacy of a low dose of heroin, b) within-subject dose choices for morphine and for heroin, and c) within-subject choice between morphine and heroin. We also gave rats the same dose of morphine, or the same dose of heroin, in both compartments, but with one compartment followed by naltrexone. In none of the experiments did postreinforcement naltrexone significantly affect morphine or heroin reinforcement, so these experiments are not reported.

Forced (Nonchoice) Training

This was the only conditioning procedure in several experiments and was used in all but four trials in the others. The door to one drug compartment was closed and the rat was placed in the choice compartment, facing away from the entrances to the compartments. It was allowed to enter the accessible compartment, was immediately infused with morphine, heroin, or saline, and was confined there for an additional 60 s. Trials alternated between opioid and saline, different doses of the same opioid, or different opioids, and therefore also alternated between the two compartments. Experiment 1 used 18 forced trials; the remaining experiments, 24 or 28.

Choice Training

Four choice training trials were given midway through the forced training in most single-opioid experiments. These were like forced training trials except that both doors were open and only a single opioid solution was available to each rat, with dose manipulated by varying the volume in accordance with the rats' choice.

Posttraining Nonreinforced Choice Test

Because of its sensitivity and convenience, this has become the standard OPC measure of reinforcement. It consisted of two trials per day, at least 2 h apart, for 3 days, except in experiment 1, where the 3 days were preceded by a day with one test trial. In each test trial, the rat was placed into the choice compartment, facing away from the open exits, and was allowed to enter either drug compartment. When it did so, the door to the other drug compartment was closed. It was returned to its living cage after remaining in the chosen compartment for 30 s or after reentering the choice compartment, whichever came first. If a rat failed to choose within 1 min, it was given up to two additional opportunities, 1 min apart, before a failure to choose was recorded; such failures were excluded in calculating a subject's score. Experimenters were blind to conditions during this test.

Two additional measures were obtained in most experiments: choices in the four choice training trials, and speed of entering each compartment in forced training trials. These measures turned out to be considerably weaker than the posttraining choice test and are not reported.

Statistical Analysis

The tests employed were *t*-tests and analyses of variance. Their particular forms were designed to prevent the error terms from being inflated by any black or white biases, i.e., mean score differences between the black and white compartments. This was done by comparing the black choices of rats assigned to black positive vs. white positive conditions or by using black positive vs. white positive compartment as a variable of classification in analysis of variance. Taking out the effect of black or white bias in this way has typically increased the size of the F or t somewhat. Many experiments on drug effects include zero-dose control groups, to which each experimental group is compared. No such groups were included here; instead, each subject served as its own control. To test the significance of conditioning in any group, that group's performance was compared with chance expectancy by a *t*-test. If a particular dose was ineffective, the black choices of the black-positive and white-positive subgroups should not have differed except by chance, since assignment of subjects to the two subgroups was random. In the experiments on drug vs. saline (experiments 1 and 4), the *t*-tests were one tailed, because the finding of a dose being chosen significantly less than saline would not have been accepted without replication. In the other experiments, these tests were two tailed.

Criterion For Significant Dose Effects

In most of the reports that we have checked regarding dose effects on drug reinforcement, the means compared were those for all doses, including doses without significant effects, and sometimes even zero doses. This paper uses a more restricted criterion for dose effects: significant differences among significantly reinforcing doses only. This criterion is based on the distinction mentioned above between dose effects on presence or absence of reinforcement and dose effects on the efficacy of those doses that have been shown to be reinforcing. Because the present study is specifically concerned with the latter, such a criterion for significance of differences is essential here.

EXPERIMENTS AND RESULTS

Experiment 1: Effect of Morphine Dose on Reinforcement Efficacy

Separate groups of rats received daily forced training as described above, with morphine doses of 0.32, 1.0, 3.2, and 10 mg/kg in the positive compartment and saline in the negative one. The 18 training trials alternated between positive and negative compartments, with a single nonreinforced choice trial at least 2 h before trials 4, 7, 10, 13, and 16. (These choice trials were included to reveal any large, consistent changes in choice behavior during the course of training. No such changes were seen, so these trials were not included in subsequent experiments.) Beginning on the day after completion of training, the main choice test was given. In experiment 1 only, this consisted of seven trials over 4 days, instead of the usual six trials over 3 days.

Choices are shown in Fig. 1. The first question was whether every dose was reinforcing by the choice measure, because only reinforcing doses were to be compared. The positive compartment was entered on 73% of the trials overall [$F(1, 60) = 55.6$, $p < 0.0001$] and by every dosage group separately (each $p < 0.05$, one-tailed).

The principal question was whether the doses differed significantly, and choice was found to vary significantly among the doses overall [$F(3, 60) = 3.82$, $p = 0.015$]. However, although pairwise comparisons found that the highest dose group significantly exceeded every lower dose group (each $p < 0.02$), no other pair of groups differed significantly (each $F < 1.0$). Thus, the present experiment failed to show a dose effect on reinforcement efficacy among three of the four doses.

Experiment 2: Choices Between Morphine Doses

Experiment 1, in which the different doses were given to different groups of subjects, found no significant differences among the three lowest doses. Experiment 2 attempted to increase dose sensitivity by having each subject receive one of the four doses after entering one compartment and a different dose after entering the other compartment. All six pairs of the doses from experiment 1 were used, with a different group receiving each pair. Because we felt that differential reinforcement might be slower than simple instrumental learning, the previous 18 forced training trials were increased to 24. These trials alternated between the higher dose and lower dose com-

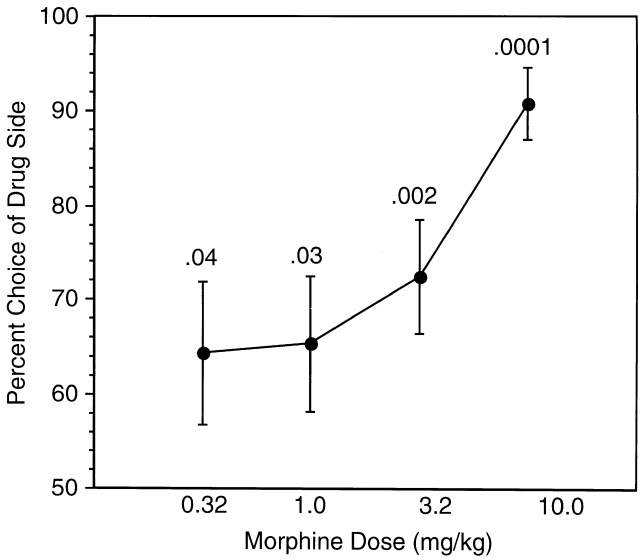

FIG. 1. Experiment 1. Effect of morphine dose on choice of morphine over saline compartments by groups receiving different doses of morphine (n = 16–19 per group). Data shown are mean percentages of morphine compartment choice by four groups receiving different doses of IV morphine. The ordinate value of 50 represents equal choice between morphine and saline. Vertical lines are SEM; above each is the one-tailed probability of the deviation from equality of morphine and saline choice. The choice test is a nonreinforced posttraining discrete-trial test of entry into the drug or nondrug compartment. In experiment 1, there were seven choice test trials over 4 days; in the remaining experiments, there were two trials per day for 3 days.

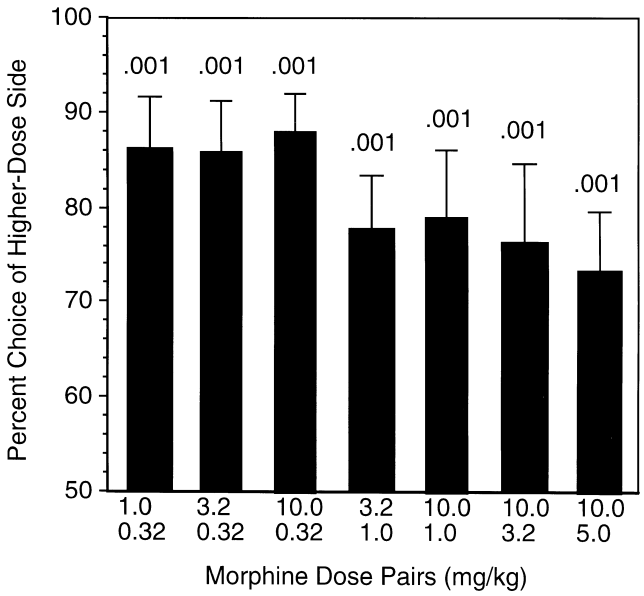

FIG. 2. Experiments 2 and 3. Choices between morphine doses (n = 9–22 per group). Each rat received two doses, one for entering each compartment. Data shown are for all pairs of the four doses that had been used in experiment 1 and for one pair of doses with a closer (2:1) dose ratio. The ordinate value of 50 represents equal choice of the two doses. Vertical lines are SEM; above each is the two-tailed probability of the deviation from equal choice of the two doses.

partments. Four reinforced choice training trials were also given halfway through training. After training was complete, the six-trial choice test was given.

Higher dose choice percentages in the post-test are shown in Fig. 2. The effect of dose in this within-subjects experiment was quite different from the effects found in experiment 1. The higher dose compartment was chosen with an overall mean of 82% [$F(1, 70) = 171.5$, $p < 0.0001$] and by every group individually, from 73% to 88% (each $t > 3.2$, each $p < 0.02$). The extent of higher dose preference did not vary significantly among the six pairs of doses ($F < 1.0$).

Experiment 3: Morphine Dose Choice with a Closer Dose Ratio

The previous experiments used doses at least 0.5 log unit apart, i.e., dose ratios of 3.2:1 and higher. The present experiment compared the highest dose, 10 mg/kg, with half that dose. Rats were trained and tested as in experiment 2.

Choice of the higher dose compartment is shown in Fig. 2. The 10-mg/kg compartment was chosen in 73% of the test trials [$t(20) = 3.80, p < 0.002$].

Experiment 4: Effects of Heroin Dose on Reinforcement Efficacy

As in experiments 2 and 3, rats received 24 forced training trials, alternating between positive and negative compartments, along with 4 choice training trials, with all trials at least 1 day apart. Separate groups received heroin doses of 0.032, 0.1, 0.32, and 1.0 mg/kg in the positive compartment and—except for the four choice training trials—saline in the negative one. Testing was the same as in experiments 2 and 3.

Figure 3 shows the results of the choice test. The heroin compartment was chosen in 84% of the test trials overall [$F(1,$

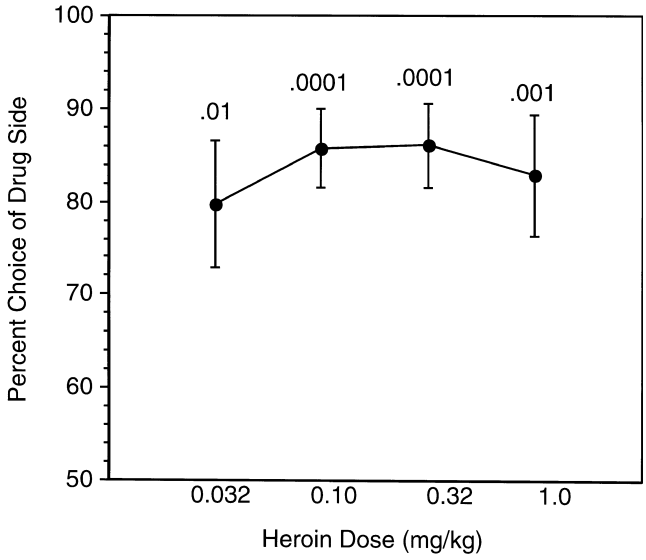

FIG. 3. Experiment 4. Effect of heroin dose on choice of heroin over saline compartments by groups receiving different doses of heroin (n = 9–11 per group). Vertical lines are SEM; above each is the one-tailed probability of the deviation from equality of heroin and saline choice.

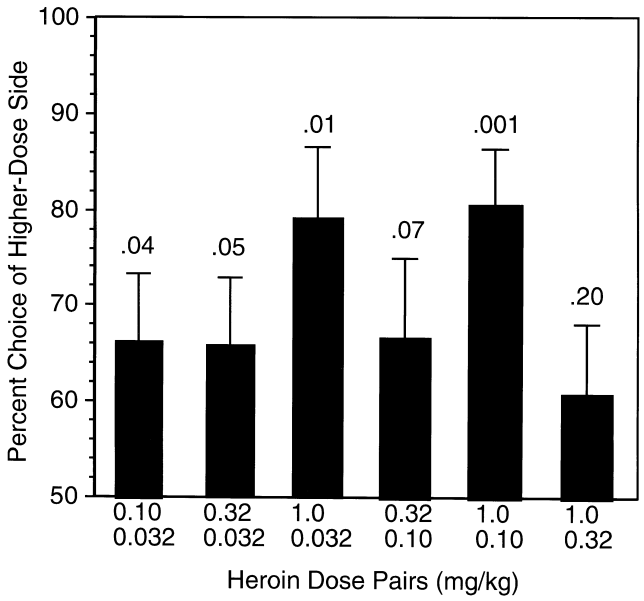

FIG. 4. Experiment 5. Choices between heroin doses (n = 12–14 per group). Each rat received two doses, one for entering each compartment. Data shown are for all pairs of the four doses that were used in the previous experiment. The ordinate value of 50 represents equal choice of the two doses. Vertical lines are SEM; above each is the two-tailed probability of the deviation from equal choice of the two doses.

33) = 149.2, $p < 0.0001$] and by every dosage group separately (each $t > 4.0$, each $p < 0.01$). Dose had no significant effect ($F = 0.3$), with choices of the different doses ranging only from 80% to 86%. Thus, even more clearly than in experiment 1, the between-subjects design failed to show differences in reinforcing potency among the doses tested.

Experiment 5: Choices Between Heroin Doses

This was an attempt to increase dose sensitivity by having each rat receive two doses, one in each compartment, as in experiments 2 and 3. All six pairs of the four doses from experiment 4 were used, with a different group receiving each pair. Training and testing were as in experiments 2 and 3.

Results are shown in Fig. 4. As in experiment 2, the within-subjects dose preference design revealed dose effects that were not shown in its between-subjects counterpart, experiment 4. The higher dose compartment was chosen by each of the six groups, with an overall mean of 70% [$F(1, 63) = 45.6$, $p < 0.0001$], whereas between-subjects experiment 4 found virtually no effect of dose. The graph depicts considerable variation among the group means, with two of the means being highly significant and two others nonsignificant. The most defensible interpretation is that these differences could reflect random sampling variations, inasmuch as the six means did not vary significantly overall [$F(5, 63) = 1.26$, $p = 0.30$].

Experiment 6: Choices Between Morphine and Heroin in Various Doses

Each of six groups of rats received 1.0 or 10.0 mg/kg of morphine in one compartment and 0.1, 0.32, or 1.0 mg/kg of heroin in the other one, in a 2 × 3 factorial design. Each dose of morphine and heroin had been found to be reinforcing in experiments 1 and 4. The four choice training trials were omitted for technical reasons and were replaced by four additional forced training trials. The 28 forced training trials alternated between the morphine and heroin compartments and were followed by the choice test.

Heroin choices are shown in Fig. 5. The effect of morphine dose was significant [$F(1, 77) = 7.09$, $p < 0.01$], whereas that of heroin dose was borderline [$F(2, 77) = 2.78$, $p = 0.07$]. Every heroin dose was chosen significantly over 1.0 mg/kg morphine, 63–75% (each $p < 0.05$). With 10 mg/kg morphine, the heroin and morphine compartments were chosen nearly equally when the morphine:heroin dose ratios were 100:1 and 32:1; when that dose ratio was 10:1, the heroin compartment choice was 69% ($p < 0.01$).

Experiment 7: Choices Between a Low Heroin Dose and Four Morphine Doses

All rats received 0.032 mg/kg of heroin in one compartment (a lower dose than any used in experiment 6), with different groups receiving 0.32, 1.0, 3.2, and 10.0 mg/kg of morphine (the same doses that were used in experiments 1 and 2) in the other compartment. Conditioning and testing were the same as in experiment 6, except that 24 instead of 28 training trials were given.

Morphine choices are shown in Fig. 6. The lowest morphine dose, which was 10 times the heroin dose, was chosen less than half as often as heroin ($p < 0.05$). The next higher morphine dose, which was 32 times the heroin dose, was chosen nearly equally with heroin ($p > 0.80$). For the two highest morphine doses, which were 100 and 320 times the heroin

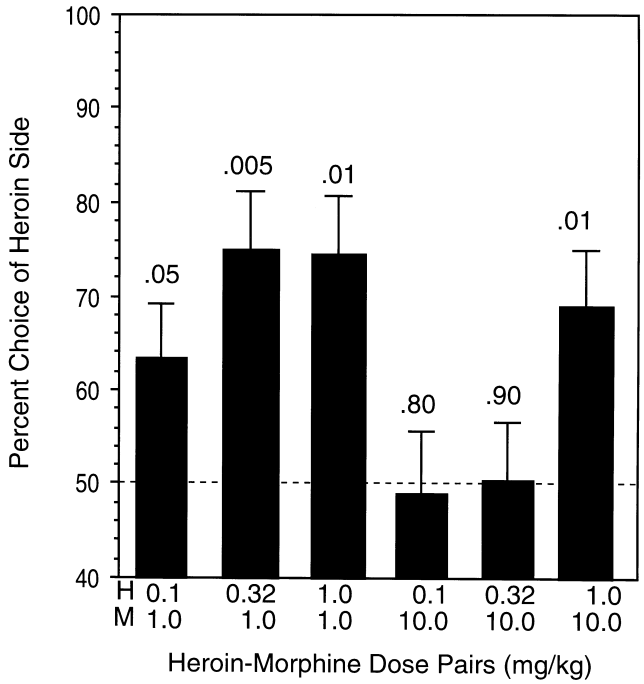

FIG. 5. Experiment 6. Choices between heroin and morphine in various doses (n = 11–16 per group). Each rat received one of two doses of morphine for entering one compartment, and one of three doses of heroin for entering the other compartment, in a 2 × 3 factorial design. The ordinate value of 50 represents equal choice of the two combinations. Vertical lines are SEM; above each is the two-tailed probability of the deviation from equal choice of the two drugs.

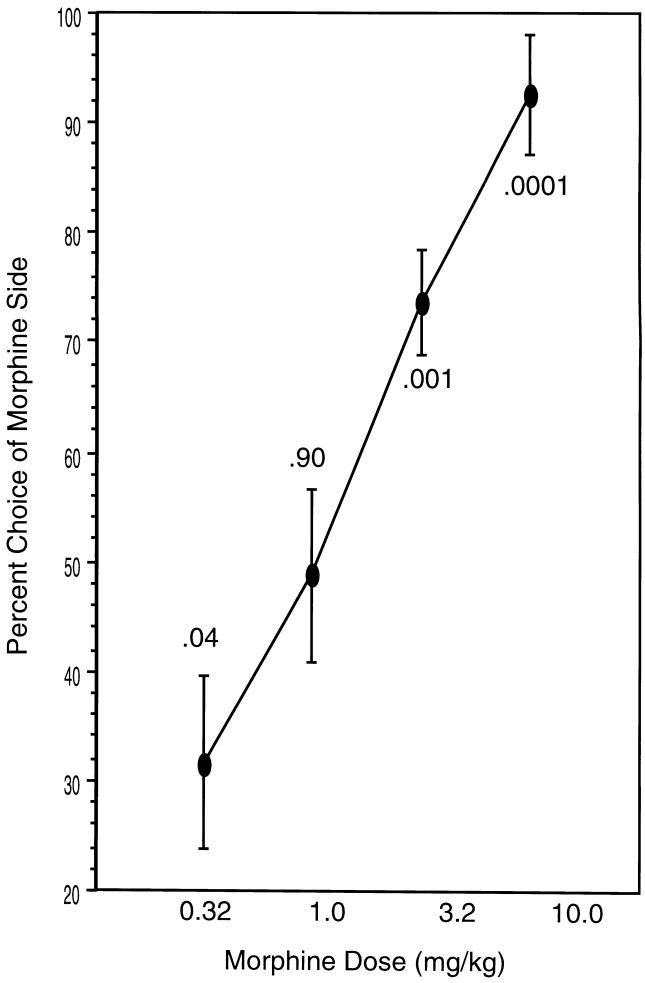

FIG. 6. Experiment 7. Choices between low dose heroin and four doses of morphine (n = 15–17 per group). Each rat received heroin, 0.032 mg/kg (the lowest dose used in this study), for entering one compartment, and one of four doses of morphine (the same morphine doses as in experiments 1 and 2) for entering the other drug compartment. The ordinate value of 50 represents equal choice. Vertical lines are SEM; above each is the two-tailed probability of the deviation from equal choice of the two drugs.

dose, the morphine compartment was heavily favored, 74% and 93% (each $p < 0.001$). A trend analysis for morphine dose found a significant linear component [$F(1, 55) = 43.3, p < 0.001$] and no suggestion of curvilinearity ($F < 0.2$).

The results of experiments 6 and 7 together suggest that, when rats choose between IV morphine and heroin, heroin is about 30 times as potent as morphine on a dosage basis. In experiment 6, the rats chose the 10 mg/kg morphine and 0.32 mg/kg heroin compartments (32:1 ratio) almost equally, and chose the 1.0 mg/kg heroin compartment more than 2:1 over the 10 mg/kg morphine compartment. Similarly, in experiment 7, the rats chose the 0.032 mg/kg heroin compartment and the 1.0 mg/kg morphine compartment (32:1 ratio) almost equally, and chose the 0.032 mg/kg heroin compartment 2:1 over the 0.32 mg/kg morphine compartment. This was a larger potency difference than we had expected when the study was planned. A possible basis for it is discussed below.

DISCUSSION

The posttraining choice test seems to possess adequate sensitivity and dependability for assessing both presence of opioid reinforcement and dose effects on it. As to detection of reinforcement, each of the eight groups in experiments 1 and 4 chose the drug compartment significantly, with an overall mean of 78%. The lowest morphine dose tested, however, was 0.32 mg/kg; by comparison, lever-pressing procedures have obtained morphine reinforcement with 0.032 mg/kg (30), and CPP has been found with morphine at 0.08 mg/kg IV (18) and with heroin at 0.02 mg/kg SC (22).

Our chief concern was sensitivity to dose effects. A major finding was that within-subjects comparisons of doses, in which each subject received both doses, were far more sensitive than between-subjects comparisons. For morphine dose, between-subjects experiment 1 found little difference among groups trained with the three lower doses, whereas within-subjects experiment 2 found each of the six groups choosing the higher dose compartment more than three times as often as the lower dose one. For heroin, between-subjects experiment 4 found virtually no dose effect, with choice varying only between 80% and 86%, while within-subjects experiment 5 found each group choosing the higher dose compartment, four significantly so and one borderline ($p = 0.07$). Similar findings have been reported for natural reinforcers. Between-groups experiments often show little effect of amount of food reinforcement on learning, yet animals that receive different quantities of food in the two goal boxes of a maze readily learn the response that yields the larger quantity (16).

A noteworthy finding of the morphine–heroin choice experiments (experiments 6, 7) was that a morphine dose had to be some 30 times the heroin dose for the two to be chosen equally. (The reinforcement thresholds do not necessarily have a 30:1 ratio, because only reinforcing doses were used.) The explanation may lie in how quickly the two drugs produce reinforcement following injection. Heroin is known to cross the blood–brain barrier much more rapidly than morphine (19), resulting in a much larger proportion of the heroin than the morphine reaching the receptor sites in the first few seconds after the response of drug compartment entry. This interpretation assumes that OPC behaves like instrumental conditioning and not like CPP. If, instead, the CPP interpretation of OPC is correct, the 30:1 finding might hold only when the time in the drug compartment after drug administration is brief, such as the 60 s used here. With longer drug compartment exposure, the equipotent dose ratio might be much lower, because the critical time period would be the time in the drug compartment after the drug reaches the receptors. However, if OPC behaves like instrumental conditioning, the critical time period would be that between the entry response and the drug's arrival at the receptors, so increasing the time in the compartments should not affect the relative potencies of the two drugs.

The monotonically increasing dose effect found in the within-subjects experiments is very different from that usually found in drug self-administration studies, most of which have used fixed ratio 1 or other rich schedules, and which, over most if not all of the reinforcing dose range, have found response rate to decrease as dose increases (3,32–34). It is also at variance with the findings of a recent progressive ratio experiment, which found the breaking point for 0.10 mg/kg heroin to be about half that for 0.05 mg/kg (20). In the present study, 0.10 mg/kg heroin was chosen about 2:1 over 0.032 mg/kg heroin.

The other spatial learning paradigm, CPP, has in recent

years been used approximately as much as self-administration (8). The dose effects it finds with positive reinforcers are almost always positive, and it has a number of significant methodological advantages over drug self-administration. a) It requires very few drug administrations, sometimes as few as one (4), so tolerance, physical dependence, and toxicity are minimized. b) Increases in the response measure never imply decreased reinforcement. c) CPP does not require control groups for responding due to stereotypic behaviors and other direct effects of the drug that could produce spurious signs of reinforcement in drug self-administration (1,3,13). d) By taking its reinforcement measure after the completion of conditioning, it avoids confounding a treatment's effects on drug reinforcement with the treatment's effects on the performance of the conditioned behavior. e) Drug satiation cannot influence CPP's acquisition or its dependent measure, because conditioning trials are well separated in time, and testing is done in the absence of the drug. f) Disruption from motor side effects of a treatment cannot usually interfere with conditioning or testing, because neither requires much gross motor behavior. Thus, CPP is probably the only fully appropriate method for studying reinforcement attenuating effects of receptor blockade and lesions that have severe motor side effects.

OPC possesses the same advantages over self-administration except a and f (above). A practical disadvantage of OPC is the number of conditioning trials needed: up to a dozen or more times as many as CPP has used. In comparison with self-administration, a disadvantage of both OPC and CPP in their typical forms is that they do not track the conditioning process over training trials, because they usually do not test throughout training. However, one OPC procedure we have used consists of alternating forced and choice training trials (unpublished research), and we believe that even 100% choice training would often be appropriate if needed.

A possible advantage of OPC over CPP might be a greater ability to show statistically significant dose effects among significantly reinforcing doses. Whether the two methods actually differ in this respect is unclear at this time, because CPP studies do not usually base their dose effect significance tests on significantly reinforcing doses only.

OPC has one fundamental advantage over CPP: its response measure of reinforcement, proportions of entries into the positive and negative compartments, as opposed to CPP's relative time spent in those compartments. The accepted measures of reinforcement in spatial learning situations have always been based on going to places. This is not an arbitrary convention, because going to a place is the reinforced response in spatial learning. Therefore, times spent in places could be no more than indirect measures of reinforcement, requiring validation through concordance with direct measures of reinforcement, such as measures of going to places or of manipulatory behaviors like lever-pressing. Time spent is also thoroughly ambiguous, because it is based on the number of times a rat enters the compartment, combined with the durations of these entries. Hence, it cannot distinguish between treatments that cause animals to enter specified places more often and those that cause animals to stay longer when they enter those places.

The time-spent measure is not an essential part of CPP, however. We previously reported three experiments in which CPP-type placement training was combined with the post-training choice test, using IP as well as IV drug administration, all with clear positive results [(9), experiments 3, 4, 9]. We have additionally found that the frequent CPP procedure of giving the drug well before the rat is placed into the positive compartment produced significant choice of that compartment (unpublished observations). Choice has also been shown to be considerably more powerful than relative time spent. In each of seven experiments that employed both measures, the choice proportions were higher than the time-spent proportions [(9), experiments 3, 4, 5, 6, 8, 9, 10]. The choice test was included in a recent CPP study of the reinforcing effect of morphine under chronic inflammatory pain (25). It detected the effect of pain on morphine reinforcement, whereas the standard CPP time-spent test failed to do so. Because of this finding, a subsequent study using other analgesics omitted the time-spent measure and used only the choice measure (26,27).

These considerations suggest that CPP could be strengthened by replacing the time-spent measure with a discrete-trial choice measure like the one used here. For examining the effects on reinforcement efficacy of dose and of other variables that can be manipulated between trials, the within-subjects design used here, as well as by Barr et al. (5) to show significant dose effects on CPP, also seems promising.

ACKNOWLEDGEMENTS

This research was supported by NIDA grant DA05498. The technical assistance of J. H. Bolthouse, T. A. Bounds, A. J. Davisson, K. A. Eubanks, K. M. Haraway, J. D. McMillian, J. M. Rappaport, and S. C. Stout is greatly appreciated. A portion of the data presented here was included in a dissertation submitted by C. W. Hutto to the University of Mississippi, 1992, in partial fulfillment of the requirements for the Ph.D. degree.

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