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The Reference-Dose Place Conditioning Procedure Yields a Graded Dose-Effect Function

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A major criticism of the place conditioning procedure for studying conditioned drug reward is that it is relatively insensitive to large quantitative shifts in drug dose (i.e., dose effects are all or none). Experiment 1 demonstrated this lack of sensitivity using a wide range of intravenous (IV) cocaine doses (0.1, 0.3, 0.45, 0.6, 0.9, or 1.2 mg/kg). Rats had cocaine repeatedly paired with one distinct end compartment of a 3 compartment apparatus; vehicle was administered in the other end compartment. In a subsequent drug-free choice test, the 0.45 to 1.2 mg/kg doses of cocaine conditioned a place preference. The magnitude of the effect did not differ. Experiment 2 used a modified version of this standard place conditioning method. In this alternative method termed reference-dose procedure, a fixed dose of cocaine (reference dose) was repeatedly paired with one end compartment (i.e., 0.45 mg/kg); the comparison dose of cocaine was administered in the other end compartment (vehicle, 0.6, or 1.2 mg/kg). Preference for the comparison-dose compartment increased with dose—a graded dose-effect curve. In contrast to the standard procedure, the reference-dose procedure revealed that the conditioned rewarding effect of 1.2 mg/kg of cocaine was greater than that of 0.45 mg/kg. This increase in sensitivity to conditioned reward with the reference-dose procedure will likely increase the utility of the place conditioning method as a preclinical model, as well as a procedure for studying processes mediating associatively-motivated choice behavior.

Drugs of abuse can have a significant impact on the physiology, behavior, and/or cognition of individuals exposed to these compounds. Importantly, research with human and non-human animals indicate that these drug effects enter into learned associations with situational cues such as the environment and drug paraphernalia reliably present when the drug is affecting the nervous system (Childress et al., 1999; O'Dell et al., 1996; Sell et al., 2000; Tzschentke & Schmidt, 1999). Many theorists conceptualize this association as resulting from Pavlovian (classical) conditioning processes (e.g., Kalivas & Nakamura, 1999; Koob, 2004; O'Brien et al., 1998; Pavlov, 1927; Robinson & Berridge, 1993; Self, 1998; Siegel et al., 2000). According to this notion, conditioned associations emerge to those stimuli that occur reliably in close temporal relation with the drug effects. Learned associations might be expressed as drug-like or drug-opposite responses. Although theories of drug abuse that include associative learning processes differ on which factors promote associations and control response type, one factor that they often share is the importance of conditioning to drug abuse etiology.

Of main interest in the present report are learned associations with cocaine. In humans with a history of cocaine abuse, presentation of drug paraphernalia as well as a video enacting the purchase and use of cocaine can elicit decreases in

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skin temperature and resistance, as well as increases in heart rate and questionnaire measures of cocaine cravings (Ehrman et al., 1992; Robbins et al., 1997). In a recent study, these conditioned responses were correlated with alteration in cerebral blood flow to the basal ganglia, anterior cingulate, and amygdala (Childress et al., 1999). These data indicate that discrete environmental cues reliably signaling drug effects can come to evoke behavioral, cognitive, and physiological responses related to the drug. These cue-evoked responses likely contribute to development, maintenance, and relapse of chronic cocaine use.

Various preclinical models have provided an important elaboration to our understanding of cocaine-conditioned drug effects. Cocaine-place conditioning is one such preclinical model that has been widely used for this purpose. In a "typical" place conditioning experiment, the animal (e.g., rat) receives cocaine while confined to one distinct environment (paired environment). At a different time, the same rat receives vehicle while confined to a second environment (unpaired environment) that varies from the cocaine-paired environment along several stimulus dimensions (e.g., flooring, walls, etc.). This two-confinement sequence is considered one conditioning trial. Although single-trial cocaine place conditioning has been attempted (Bevins, 2001; Nomikos & Spyraki, 1988), the typical experiment repeats this sequence several times (e.g., 4 confinements to each environment). Following the last conditioning trial, a drug-free test for conditioning is conducted. The rat is placed in the apparatus and allowed free access to the end compartments (i.e., paired and unpaired environments). More time in the cocaine-paired environment relative to the unpaired environment (or a control value) is taken as evidence for a learned association between environmental cues and cocaine. This effect is described as a conditioned place preference. This expression reflects the assumption that the cocaine-paired environment has been associated with the appetitive and/or rewarding effects of the drug. This association with reward elicits approach and hence increases the amount of time (i.e., preference) in the paired environment (Bardo & Bevins, 2000; Carr et al., 1989).

The majority of research on cocaine place conditioning has focused on neurobiological factors mediating the conditioned rewarding effects of cocaine (for recent examples see Baker et al., 1998; Kimmel et al., 2000; Romieu et al., 2002; Tzschentke & Schmidt, 1999). Much less research has systematically examined the behavioral factors that modulate cocaine place conditioning. Albeit limited, this research has shown that cocaine place conditioning in an all-or-none manner (see following paragraph) is affected by such variables as cocaine dose, administration route, injection to placement interval, number of conditioning trials, and environment duration (e.g., Bardo et al., 1995; Ettenberg et al., 1999; Nomikos & Spyraki, 1988; O'Dell et al., 1996). From our perspective this research is important given its consistency with the assumption that place conditioning indexes a learned association between cues presented in close temporal relation to the drug effects.

Experiment 1

Unfortunately, our understanding of associatively-motivated choice behavior (place preference/conditioning) and its behavioral and biological substrates in this widely used preclinical model of drug reward is limited by the method's relative insensitivity. For example, cocaine place conditioning tends to be all-or-none.

That is, once place conditioning is observed with a particular dose, an increase in dose does not induce a stronger preference (Bardo et al., 1995; Bardo & Bevins, 2000; Mueller & Stewart, 2000; O'Dell et al., 1996). A similar all-or-none outcome is seen in antagonism experiments (see Bardo & Bevins, 2000, for a recent review). This inability to generate dose-effect curves, and hence median effective doses (ED_{50}s), clearly restricts the usefulness of the place conditioning protocol in basic research on potential pharmacotherapies or genetic contributions to conditioned reward. Further, this insensitivity also limits its usefulness in research on behavioral and cognitive processes mediating drug-conditioned choice behavior. For instance, its all-or-none nature does not allow one to readily study variables that might enhance an already existing place preference (e.g., infusion rate, interstimulus interval, context duration, additive effects with qualitatively different rewards, etc.), or choice among qualitatively different rewards (e.g., cocaine vs. copulatory opportunity). Experiment 1 examined the effectiveness of a wide range of cocaine doses using the standard place-conditioning protocol. This replication of past research was required in order to empirically determine a broad dose range that produced the all-or-none place conditioning effect. That range was then used to select the cocaine doses in Experiment 2.

Method

Subjects. The subjects (n = 63) were naive male Sprague-Dawley rats (330 ± 4 g) obtained from Harlan (Indiana, U.S.A.). All rats were housed individually in plastic tubs lined with aspen shavings in a colony on a 12 h light:dark cycle. Experiments were conducted during the light portion of the cycle. Rats had free access to food and water in their home cages and were handled extensively before surgery.

Apparatus. Two similar three-compartment wood chambers were used. One end compartment, with the inside dimensions of 31 x 24 x 45.5 (l x w x h) cm, had white walls and a mesh floor with pine chips lining the litter tray. The other end compartment, with the same inside dimensions, had black walls and a rod floor with newspaper lining the litter tray. A smaller center compartment that had gray walls and an aluminum floor (15 x 24 x 45.5 cm) separated the end compartments. During choice (preference) tests, the solid center walls were lifted 11 cm to allow the rat unrestricted movement between compartments. The experimental room was illuminated with fluorescent lights; a white-noise generator provided 80-dB masking noise. An 8-mm camera mounted above the chambers recorded all sessions.

Surgery. Rats were anesthetized with an IP injection (1 ml/kg) of ketamine hydrochloride (100 mg/ml) followed by an IP injection (0.6 ml/kg) of xylazine hydrochloride (20 mg/ml) purchased from Midwest Veterinary Supply (Iowa, U.S.A.). One end of a silicon catheter was implanted into the external right jugular. The other end was positioned under the skin such that it exited from an incision on the center of the head. Attached to this end was a stainless steel cannula. Dental acrylic and jeweler screws held the cannula in place on the skull (cf. Bevins & Bardo, 1999; Schenk et al., 1993; Weeks, 1972). The catheter was flushed twice a day for the duration of the experiment with 100 to 200 µl of filtered sterile saline mixed with heparin (30 units/ml; Elkin-Sinn, Cherry Hill NJ). Rats were allowed 4 to 5 days of recovery before the start of an experiment. Catheter patency was assessed immediately after the post-conditioning preference test with an approximately 50-µl infusion of xylazine (20 mg/ml). This concentration produced clear motor ataxia within 5 to 10 sec if the catheter was patent. Only rats with patent catheters were included in analyses.

Drug. Cocaine hydrochloride (NIDA, Bethesda MD) was dissolved in heparinized saline and infused intravenously (IV) at a volume of 500 µl/kg. Cocaine was mixed immediately before each daily use.

Procedure. Following recovery from surgery, cocaine place conditioning started. Rats assigned to a cocaine dose (0.1, 0.3, 0.45, 0.6, 0.9, or 1.2 mg/kg) received an IV infusion of cocaine immediately upon placement in one end compartment. Cocaine was cleared from the infusion line and catheter with a 200-µl infusion of heparinized saline. This infusion protocol (cf. Bevins & Bardo, 1999) took approximately 10 sec. Rats were infused with a similar volume of heparinized saline across the same amount of time in the other end compartment. Confinement duration was 10 min. This confinement/infusion protocol was repeated for 4 conditioning trials (i.e., each compartment experienced on 4 separate occasions). All factors (e.g., cocaine-paired compartment, 1st compartment experienced, etc.) were counterbalanced as allowed by the sample size (n = 7 to 10 per group; see Figure 1). A control group included rats whose catheters were deemed clogged during the recovery period or rats that received sham surgery (i.e., an incision on the head and neck but no catheter or head-mount implanted). This control received equal confined exposures without infusions. The post-conditioning preference test was conducted the day after the last conditioning trial. Each rat was placed in the center gray compartment and allowed free access to the end compartments for 10 min.

For this research, we selected the (i.v.) route of administration over the intraperitoneal (i.p.) or subcutaneous (s.c.) route because (i.v.) cocaine appears to produce the most stable place preference effect (Bardo et al., 1995). This stability might reflect decreased variability between individuals' brain and blood levels of cocaine that occurs with (i.v.) administered cocaine (Ma et al., 1999) and/or better temporal contiguity between the paired context and the appetitive effects of cocaine (Bardo & Bevins, 2000; Pavlov, 1927). Regardless, the research by Barr et al. (1985) and Patkina and Zvartau (1998) described later shows the utility of the reference-dose procedure using systemic routes of administration.

The test session was video taped for later observation of time spent in each end compartment during the post-conditioning preference test. A rat was considered to be in a compartment if both front paws were positioned in the compartment. In our laboratory, inter-observer reliability on this behavior by an individual naive to the experimental conditions is high (e.g., $r = 0.86$, $p < 0.0001$, $n = 64$ independent observations, from Besheer et al., 1999).

Time spent in each end compartment on the test day was converted to a preference ratio (score) before statistical analyses. A preference score was calculated using the following formula: (time spent in the cocaine-paired compartment/(time spent in the paired compartment + time spent in the unpaired compartment)). To calculate a preference score for the control group, the time designates as "paired-compartment" was randomly selected such that 4 values were from the white compartment and 5 values were from the black compartment. A preference score of 0.5 indicates similar time spent in each end compartment; a value greater than 0.5 denotes more time spent in cocaine-paired environment and will be taken as evidence of cocaine reward. Omnibus analyses used a one-way between-subject analysis of variance (ANOVA). A significant ANOVA ($p \leq 0.05$, two-tailed) was followed by planned contrasts between the control and each dose of cocaine.

Results and Discussion

For each rat in the control (Ctrl) group, time spent in the two end compartments (black/rod = 219±19 s; white/mesh = 208±16 s) did not differ statistically, $t < 1$. This indicates that there was not a systematic group bias for either end compartment. Figure 1 shows the preference scores for each cocaine dose and the control group. Increasing the dose of cocaine increased the preference for the paired compartment in a stair-step function. The ANOVA revealed a significant effect of cocaine dose, $F(6,56) = 4.69$, $p = 0.0006$, with rats in the 0.45, 0.6, 0.9, and 1.2 mg/kg cocaine conditions showing a significant preference for the paired compartment, ps ≤ 0.002. A striking feature of the results from this standard place conditioning protocol is its insensitivity to large quantitative changes in cocaine dose. The 0.45 mg/kg dose seems to be as rewarding as the 1.2 mg/kg dose in that they control similar preference (choice) for the cocaine-paired environment. As noted earlier, this all-or-none dose effect is very common in the place conditioning literature (e.g., Bardo et al. 1995; Bardo & Bevins, 2000; Mueller & Stewart, 2000; O'Dell et al., 1996).

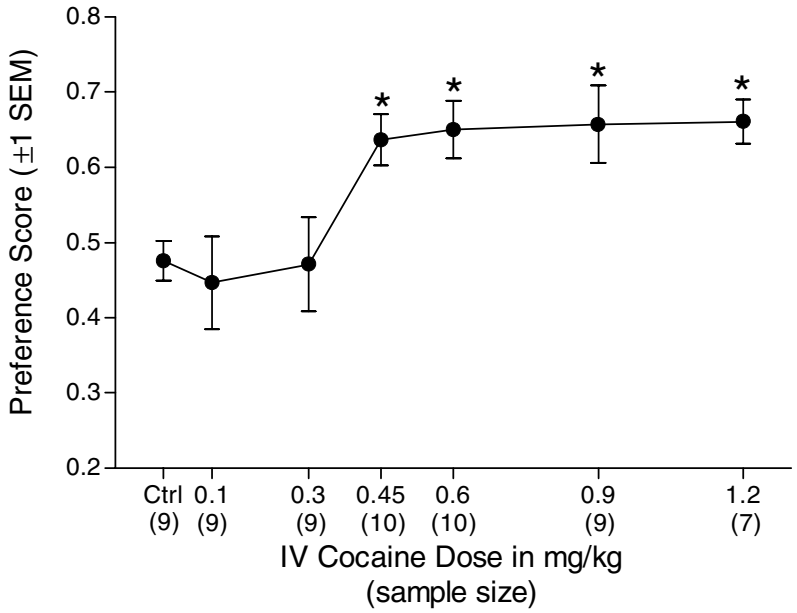

Figure 1. The mean preference score (±1 SEM) for each group in Experiment 1. This experiment used the standard place conditioning procedure in which cocaine was infused intravenously (IV) immediately upon placement in one end compartment; vehicle was administered in the other end compartment. Asterisks (*) denote significant difference ($p \leq 0.05$) from the control (Ctrl) condition. The number in the parentheses below each cocaine dose indicates the number of rats per condition.

Experiment 2

The standard place-conditioning protocol has been an important and widely used preclinical model for understanding the neurobiological mechanisms of the conditioned incentive-motivational properties of cocaine reward. Unfortunately, the lack of sensitivity described earlier, and demonstrated in Experiment 1, makes the standard conditioning protocol a poor candidate for some questions regarding conditioned drug reward. Twenty years ago Barr and colleagues (1985) suggested a variant of the place conditioning procedure that might be better suited for studying choice behavior. Their idea was to extend the effect range by repeatedly pairing a known rewarding dose of morphine (1 mg/kg), termed "reference dose," in one end compartment, while a different dose of morphine (0.1, 0.3, 3.0, or 5.0) was reliably paired with the other end compartment. The portion of time spent in this latter "comparison" compartment is the main measure of interest. Rats spent approximately 25% of their time in the end compartment paired with 0.1 mg/kg of morphine compared with 75% to the compartment paired with the reference dose (1 mg/kg) indicating that the reference dose was more rewarding. Notably, as the "comparison dose" increased so did the proportion of time spent in that compartment suggesting that the conditioned rewarding effects of the comparison dose were competing with the reference dose. From this data pattern, Barr et al. (1985) suggested that 0.3 mg/kg of morphine was statistically equivalent to 1 mg/kg in its ability to condition rewarding value to environmental stimuli; 3 and 5 mg/kg were more effective as indicated by a preference score above 0.5.

Barr et al's. paper has been essentially ignored despite the demonstration of an extended effect range. That is, there is approximately a 45% window (i.e., effect range) in which to observe changes in associatively-motivated choice behavior. This estimate is based on the difference between the highest preference score (ca. 0.70) and the lowest preference score (ca. 0.25). In a standard place-conditioning protocol this window would range from 0.50 (no conditioning) to the highest preference score. Thus, for Experiment 1 the effect range for cocaine using a standard protocol was approximately 19%—less than half the effect range demonstrated by Barr et al. Experiment 2 sought to assess the utility of this reference-dose procedure by extending its use to cocaine place conditioning. We used the 0.45 mg/kg cocaine data (i.e., 0.45 vs. 0 mg/kg) from Experiment 1 as the baseline in which to compare two new groups: 0.45 vs. 0.6 mg/kg and 0.45 vs. 1.2 mg/kg.

Method

Subjects and Apparatus. Fourteen naive male Sprague-Dawley rats (333±9 g) housed and treated similar to Experiment 1 were used in the present study. The apparatus was unchanged.

Procedure. The reference dose of cocaine was 0.45 mg/kg (i.e., the lowest dose that produce maximal preference in Experiment 1). All rats had one end compartment (black or white) paired on 4 separate occasions with an i.v. infusion of 0.45 mg/kg cocaine. Depending on group assignment, the other end compartment was repeatedly paired with an i.v. infusion of 0.0 (see next paragraph), 0.6, or 1.2 mg/kg cocaine (i.e., comparison dose). The remaining procedural details (e.g., infusion, counterbalancing, etc.) were identical to Experiment 1. The post-conditioning preference test was conducted the day after the last conditioning trial. Each rat was placed in the center gray compartment and allowed free access to the end compartments for 10 min.

The 0.45 mg/kg data from Experiment 1 was included in the present study to serve as a control group that received the reference-dose of cocaine (0.45 mg/kg) paired with one compartment and vehicle (0 mg/kg) paired with the other compartment (i.e., comparison dose). Of interest is how much the comparison dose of cocaine competed with the reference dose for choice behavior. Accordingly a preference score was calculated for the comparison compartment: (time in comparison-dose compartment/(time in comparison compartment + time in reference-dose compartment)). Omnibus analyses used a one-way between-subject ANOVA. A significant ANOVA was followed by planned contrasts (i.e., Newman-Keuls Multiple Comparison Test) among the groups.

Results and Discussion

The results from the choice test are presented in Figure 2. As the comparison dose of cocaine increased so did the preference for that environment. The ANOVA revealed a significant effect of comparison dose, $F(2,21) = 11.13$, $p = 0.0005$. Subsequent planned comparisons indicated that the preference score for the 0.6 and 1.2 mg/kg groups were significantly greater than vehicle (0 mg/kg), ps < 0.05. Further, the preference score for the 1.2 mg/kg group was significantly higher than the 0.6 mg/kg group, $p < 0.05$. This latter result is especially important because the cocaine dose-effect curve is not an all-or-none function as typically seen with the standard protocol (cf. Figure 1). Rather, the measure of conditioned reward is graded in which rats' choice behavior indicates that the 1.2 mg/kg dose of cocaine is more rewarding than the 0.6 or 0.45 mg/kg dose.

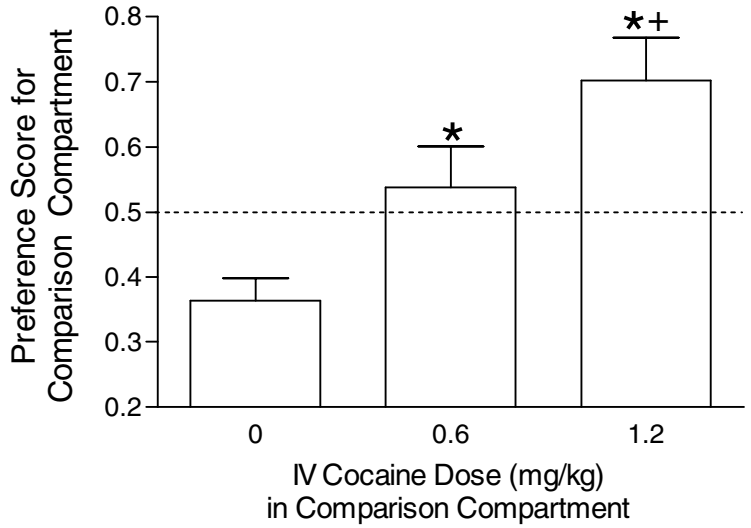

Figure 2. The mean preference score (+1 SEM) for each group in Experiment 2. This experiment used the reference-dose place conditioning procedure in which a fixed dose of cocaine (reference dose) was infused intravenously (IV) immediately upon placement in one end compartment (i.e., 0.45 mg/kg); the comparison dose of cocaine was administered in the other end compartment (see number below each bar). Asterisks (*) denote significant difference ($p \leq 0.05$) from the 0 mg/kg comparison-dose condition. The plus (+) denotes a difference between the 0.6 and 1.2 mg/kg condition.

General Discussion

In Experiment 1, we found using a standard place-conditioning protocol that cocaine (0.45 to 1.2 mg/kg) produced a conditioned preference for the drug-paired environment. Similar to past research we take this preference to reflect a conditioning process in which the environmental cues acquired appetitive and/or incentive-motivational properties by virtue of being paired with cocaine. Appetitive stimuli typically control approach behaviors (e.g., Panksepp et al., 2004). Accordingly, the acquired appetitive properties of the cocaine-paired environment evoke an approach conditioned response that results in an overall increase in time spent in that environment (Bevins & Bardo, 2000). Further, the results of Experiment 1 taken alone would lead to the conclusion that cocaine doses between 0.45 and 1.2 mg/kg were equivalent rewards (see Figure 1). That is, choice for the paired compartment increased at the 0.45 mg/kg dose and remained at that level even as the dose was increased more than 2.5 times (i.e., 1.2 mg/kg). Importantly, the results from the reference-dose procedure in Experiment 2 indicated that this conclusion of reward equivalency was partially misleading. It is the case that the 0.45 and 0.6 mg/kg doses were equivalent as indicated by a preference score near 0.5 (see Figure 2, center bar). Rats distributed their time similarly between the comparison-dose compartment (0.6 mg/kg) and the reference-dose compartment (0.45 mg/kg). However, the 1.2 mg/kg cocaine dose engendered more conditioned rewarding value to the comparison-dose compartment than the 0.45 mg/kg dose conditioned to the reference-dose compartment. This result corroborates the findings of Barr et al. (1985) that the reference-dose procedure can extend the effect range so that an orderly dose-effect function can be obtained in a place conditioning paradigm. Further, the present findings extend this observation from morphine

place conditioning (Barr et al., 1985) to cocaine place conditioning. This is a notable extension because Barr et al. stated in the Discussion that in a preliminary study they found that the reference-dose procedure had not worked for cocaine. The discrepancy between the reference-dose experiment reported here and that noted by Barr et al. cannot be evaluated because his experiment was not described in any detail.

The empirical and theoretical implications of a more sensitive methodology for studying associatively-motivated choice behavior are exciting. To date, calculation of ED_{50}s for the conditioned rewarding effects of a drug, or antagonism of that conditioned reward, have not been possible. The reference-dose procedure allows such experiments to be conducted. This feature could increase the usefulness of the place conditioning procedure as a preclinical model of drug reward. The following narrative describes a hypothetical example of this potential. Say that a laboratory has recently synthesized a ligand that has a range of receptor binding properties. This spectrum of binding properties is sufficiently complex that the drug might serve as a pharmacotherapy for cocaine, but it may also have some characteristics of a stimulant (cf. bupropion hydrochloride, Zyban, as a pharmacotherapy for nicotine use). To test this unknown ligand in the reference-dose procedure the experimenter would use a moderately rewarding dose of cocaine as the reference and comparison dose (e.g., 0.45 mg/kg i.v. cocaine from the present report). Thus, both sides of the place conditioning apparatus would be paired with the moderate dose of cocaine. Pretreatment with the unknown ligand (or vehicle) would occur before placement and infusion of cocaine only in the comparison compartment. Controls that received pretreatment with vehicle would distribute their time equally between the comparison- and reference-dose compartment (i.e., a 0.5 preference score). If the unknown ligand blocks cocaine reward (or has competing aversive properties), then the preference score would be significantly less than 0.5 because the conditioned reward of the unaffected reference dose of cocaine would out compete the attenuated comparison dose for approach behavior. In contrast, if the unknown ligand has a rewarding effect, then preference for the comparison compartment would increase given that the reward is sufficient to combine with the cocaine reward. Notably, this latter effect would be missed by the standard place-conditioning procedure given its all-or-none nature. In brief, using the reference-dose method one can test within the same experiment whether a ligand would increase or decrease conditioned reward.

Most of the discussion to this point has referred to the use of the reference-dose procedure as a method to compare different quantities of the same appetitive stimulus (i.e., doses of drug). However, an important extension of the reference-dose method will be its use to compare qualitatively different rewards. For example, can conditioned reward from access to copulatory opportunity compete with cocaine? If so, at what cocaine dose will choice behavior distribute itself equally between the compartments, or how much mate access is required to out compete a moderate dose of cocaine? Can the conditioned rewarding effects of one abused drug compete with a different one? This latter question has recently been asked. Apparently independent of Barr et al. (1985), the essential features of the reference-dose method reappeared in a paper by Patkina and Zvartau (1998). Briefly, these researchers first determined doses of cocaine, caffeine, and ethanol that would condition a place preference in rats. In a follow-up experiment, they repeat-

edly paired one distinct environment with a purportedly rewarding dose of ethanol (1.2 g/kg, intragastral). A second environment was paired with the rewarding caffeine dose (1.5 mg/kg, i.p.). Rats spent statistically similar time in both compartments suggesting that these drugs are comparably rewarding. In a third experiment, rats preferred an environment associated with cocaine (5 mg/kg, i.p.) over one paired with caffeine (1.5 mg/kg, i.p.).

The reference-dose procedure clearly offers advantages over the standard place-conditioning protocol. However, it still retains some of the drawbacks that are seemingly inherent to place conditioning. For example, generating dose-effect functions demand between-subject designs thus requiring more animals than a comparable self-administration experiment that can use a within-subject design (Bardo & Bevins, 2000). Additionally, the reference-dose procedure requires the investigator to identify a reference dose. Although a drug dose must be selected when using the standard protocol, dose selection with the reference-dose method might require more preliminary research. That is, an investigator using the standard protocol in his or her laboratory needs to determine if the dose selected produces place conditioning. In contrast, similar to the present research, initial use of the reference-dose procedure likely requires the assessment of a range of drug doses before selecting a reference dose. This discussion prompts the important point that selection of different reference doses might lead to different results (e.g., shifted dose-effect curves). This might be perceived as a drawback specific to the reference-dose procedure. However, from our perspective, this perception is incorrect. The ability of an antagonist to block place conditioning in the standard protocol will also vary with dose selection; yet the researcher will not know to what degree and the antagonism-dose effect function will likely be all-or-none. Conversely, in the reference-dose procedure differences in the ability of reference doses to condition a preference can be known and, in principle, the dose-effect function can be graded. To us, the possibility of studying how dose-effect functions vary with the reference doses is an advantage, not a drawback, of the reference-dose method.

The place-conditioning procedure is a very rich learning situation. This learning situation is made even more fascinating by the fact that the test assesses choice between two sets of stimuli that have a different learning history. The reference-dose procedure might encourage learning researchers to critically think about the learning processes involved in associatively-motivated choice behavior. For instance, could alternative theoretical frameworks from the associative learning and behavior system approach (cf. Bevins & Bardo, 2000; Panksepp et al., 2004) be successfully applied to place conditioning research? What new experiments or theoretical advances would be derived if place conditioning was conceptualized as a multiple (acquisition) or concurrent (testing) schedule in which choice might be described by some modified variant of operant choice theory (e.g., Herrnstein, 1961; Mazur, 2000)? Regardless, the present research and that of Barr et al. (1985) and Patkina and Zvartau (1998) suggests that this reference-dose methodology has promise to advance or understanding of behavioral and neurobiological processes mediating choice behavior in this widely used preclinical model of conditioned drug reward.

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