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Effect of Learned Behavior Upon Conditioned Place Preference to Cathinone

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SCHECHTER, M. D. *Effect of learned behavior upon conditioned place preference to cathinone.* PHARMACOL BIOCHEM BEHAV **38**(1) 7–11, 1991.—The purpose of this study was to examine whether first training rats to discriminate the stimulus cues produced by an indirect dopamine agonist, cathinone, would influence a subsequent test of preference. The conditioned place preference (CPP) paradigm was used to evaluate the reinforcing effects of *l*-cathinone in four differently treated groups of rats. Half of the animals were trained to discriminate the interoceptive cues produced by 0.8 mg/kg cathinone in a two-lever, food-motivated operant task. The other animals were equally divided between two groups, one receiving saline and noncontingent reinforcements on the same schedule as those trained to discriminate cathinone; the other group, the "yoked-control" rats, received the same cathinone and saline regimen of administration as the discrimination-trained animals. Results of CPP testing indicate that cathinone produced a statistically significant conditioned place preference only in the group trained to discriminate cathinone and not in the saline or yoked control groups. Furthermore, when half of the cathinone discrimination-trained rats were pretreated with the dopamine release inhibitor CGS 10746B, the conditioned place preference to cathinone was attenuated. The results would indicate that pairing cathinone with a nonpreferred environment tended to make the rat spend more time in that environment and the amount of time spent in the cathinone-associated environment can be increased by prior discrimination training and decreased by diminished dopamine function in the brain.

Conditioned place preference Cathinone Drug discrimination CGS 10746B Rats Dopamine

MANY drugs can serve as unconditioned stimuli and, as such, they may act as reinforcers. The attributes of a reinforcer may be positive in nature and, thus, drugs are capable of increasing the probability of the occurrence of the behavior associated with the drug. Similarly, the frequency of behavior associated with a drug functioning as a negative reinforcer will decrease. In either situation, the presentation of the drug is contingent upon the animal performing the associated behavior. Another assessment of the reinforcing properties of a drug as an unconditioned stimulus is one that does not follow a response with drug administration but, rather, develops an association of the drug effect with environmental stimuli. If the unconditioned effect of the drug is "perceived" by the animal as positive or rewarding, it will spend more time in the environment in which the drug was administered. This behavioral technique is then known as "conditioned place preference" or CPP. In contrast to a positive effect, when the drug that has been associated with specific environmental stimuli produces an aversive effect, or dysphoria, the animals will avoid the environment associated with the drug and this phenomenon is known as "conditioned place aversion." Thus this behavioral paradigm is sensitive to both the aversive and positive reinforcing properties of drugs and it does not require the animal to be in a drugged state when it is tested. The list of drugs that produce conditioned place preference includes (but is not limited to) the following systemically administered drugs, all of which have in common a mechanism of action upon dopaminergic neurons: amphetamine (4–6, 10, 14, 22, 23), methylphenidate and nomifensine (15), apomorphine (23, 25, 27) and cocaine (2, 16, 24).

Cathinone has been shown to produce amphetamine-like effects by a similar action upon the brain, i.e., its ability to release, and block the reuptake, of dopamine (12, 13, 28, 29). Like amphetamine, apomorphine and cocaine, cathinone induces hyperactivity in rodents (8, 11, 30) and has been shown capable of serving as a drug that produces discriminative stimuli (7, 17, 19, 21). In fact, the only differences in the discrimination between cathinone, amphetamine and cocaine have been shown to be temporal (18). The purpose of the present experiment was, therefore, to investigate whether the amphetamine-like, dopaminergically mediated drug cathinone would produce a conditioned place preference at a dose (0.8 mg/kg) previously shown to be effective in controlling differential responding in a drug discrimination paradigm. In addition, experiments were set to investigate whether discrimination training with a drug affects subsequent conditioned place preference, i.e., if animals that have been trained to respond differentially in a two-choice task, based solely upon their recognition of the interoceptive cues produced by a drug, prefer or avoid the environment paired with that drug differently than those animals simply given the drug without being trained to discriminate its central effects. For a drug to produce a conditioned place preference (or, for that matter, aversion), it must produce an effect upon the central nervous system that can be associated with a specific environment. The experiments set in the present study sought to examine if animals that are first trained to "perceive" the central effects of a drug would better associate its actions when it is, subsequently, paired with a distinct environment. Lastly, the involvement of dopamine neurons in the production of conditioned place preference to cathinone was investigated by us-

ing the recently synthesized benzothiadiazepine CGS 10746B as pretreatment prior to cathinone-environmental conditioning. This compound has been shown to decrease dopamine release without either changing dopamine metabolism or occupying dopaminergic receptors (1).

METHOD

Group Selection and Training Procedures

Thirty-two male Sprague-Dawley rats, weighing 270–290 g at the start of the experiments, were purchased from Zivic-Miller Laboratories, Allison Park, PA. The animals were housed singly in hanging wire cages in a room maintained at a relatively constant temperature and humidity and illuminated 12 h per day (lights on at 0600 h). Throughout the study, all rats received free access to water while in their home cages and they were on a restricted diet of standard laboratory rodent chow to maintain their body weight at 85–90% of ad lib weight as determined by the growth chart supplied by the breeder.

The animals were divided randomly into four equal groups (n = 8). Two of these groups, the "discriminant" rats, were trained to discriminate the interoceptive cuing properties of cathinone according to a procedure detailed elsewhere (19). One of these discriminant groups was designated to be tested in the conditioned place preference test after establishment of differential responding based on the cathinone-induced discriminative stimuli. The second of these two groups was, after training to discriminate cathinone from its vehicle, designated to be pretreated with CGS 10746B and then tested with cathinone in the conditioned place preference paradigm. Briefly, the discrimination procedure consisted of training an animal to press one lever 15 min following the intraperitoneal (IP) administration of vehicle (distilled water) at a volume of 1 ml/kg. The initial FR1 reinforcement schedule was gradually increased, over a 5-day period, until an FR10 reinforcement schedule was attained. This procedure was then repeated, with the other lever being reinforced, following a similar volume of water containing 0.8 mg/ml of *l*(−)-cathinone hydrochloride (NIDA) with the weight calculated as the salt. Fifteen min after IP administration, each rat was required to press the opposite lever on an FR1 schedule and the reinforcement requirement was gradually increased, over 3 days, to an FR10. Once lever-pressing behavior was established on both levers, a biweekly repeating injection order was employed: V,D,D,V,V; D,V,V,D,D, where V = vehicle, D = drug, i.e., 0.8 mg/kg cathinone. For each animal, the choice on any given day was considered correct if the first lever to accumulate 10 presses was state-appropriate, i.e., the cathinone lever after cathinone administration and the vehicle lever after vehicle administration. Training was continued until all rats achieved the training criterion of 16 correct lever choices in 20 consecutive sessions.

The remaining 16 rats were randomly assigned to one of two equal groups: one group was a "yoked" control in which cathinone and its vehicle were administered on those days in which the discriminant rats were injected and trained in the discrimination task. Rats in this group were placed into the operant chamber 15 min after injection and 40 reinforcements were delivered on a noncontingent basis. Thus they received the same drug treatment and exposure to the same environment as the discriminant rats but they were not trained to discriminate between cathinone and saline. The last group of eight animals was included as the "vehicle control" and were injected with vehicle on every occasion that the discriminant and yoked control groups received cathinone or vehicle. Thus the number of exposures to injection were the same but these animals never received cathinone until the beginning of conditioned place preference pairings (below). They were, likewise, placed into the operant chamber and provided with 40 reinforcements starting at 15 min postsaline injection.

CPP Apparatus

The apparatus used in the CPP procedure consisted of two modular testing chambers (Model No. 85000, Lafayette Instrument Co., Lafayette, IN) each measuring 30 × 20.5 × 18 cm and connected by a central corridor (the "choice area") measuring 30 × 19.5 × 20 cm. These test modules were covered by a translucent Plexiglas top which allowed light, from either a white or red light bulb, into the chamber. The top of each of the 3 units could be opened to permit entry or removal of the rat. One of the modules consisted of a black, smooth Plexiglas floor and was illuminated by red light. The other module was lighted with a white bulb and had grid floor with wood shavings placed under this floor. These physical differences allowed for distinction by three senses, viz., tactile (floors), visual (lighting) and olfactory (presence vs. absence of "wood" smell). The central corridor was grey and nondistinctive. All testing was carried out between 1000–1600 h in a darkened laboratory with a source of "white noise" in the room.

Conditioning Regimen

The conditioning schedule consisted of three phases. The first phase, the *preconditioning phase*, was 3 days in duration. On days 1–3, the animal was placed into the grey choice area and allowed free access to both test modules for a 15-min period. The alley and modules were thoroughly washed between rats to eliminate olfactory cues. The last day of preconditioning, i.e., day 3, constituted the baseline preference day. The cumulative time that the rat had at least its two front paws in either the "black" (red-lighted) or "white" (lighted) compartment was measured by observations through a one-way glass window on the side of each module.

The next phase was 8 days of *conditioning trials*. On every other day, the rat was administered 0.8 mg/kg *l*-cathinone sulfate IP and returned to its home cage for 10 min prior to being confined to its nonpreferred side for a 30-min period (the nonpreferred side being the chamber in which the individual rat had spent less time when tested on day 3). On alternate days, the animals were administered (IP) an equal volume (1 ml/kg) of the vehicle (distilled water) used to dissolve cathinone and they were confined to the opposite (preferred) side at the same postadministration time and for the same 30-min duration.

After the eight pairings, four with 0.8 mg/kg cathinone and four with its vehicle, the last phase, the *preference test*, was conducted. The same parameters were used as on day 3, i.e., the rat was placed into the choice area and allowed free access to the entire test apparatus for a 15-min period during which the total time spent in each modular environment was recorded.

Effect of Pretreatment With CGS 10746B Upon Preference for Cathinone

Of the sixteen rats that were selected to be trained to discriminate between 0.8 mg/kg *l*-cathinone and its vehicle, one animal's discriminative performance fell below criterion, whereas a second animal died of unrelated causes. Both of these rats were in the group designated to be tested in the CPP-test after conditioning with CGS 10746B and cathinone so that this group had an n = 6. This group of discrimination-trained animals was used to determine the effect of pretreatment with CGS 10746B upon produc-

TABLE 1

EFFECT OF FOUR TREATMENT REGIMENS UPON CONDITIONED PLACE PREFERENCE TO 0.8 mg/kg CATHINONE

Treatment (n)	Discriminant (8)		Yoked-Control (7)		Saline-Control 8		CGS Discriminant (6)	
A. Mean Time (s) Spent in Nonpreferred (NP) Side Before and After Cathinone Conditioning								
	Baseline	Pref. Test	Baseline	Pref. Test	Baseline	Pref. Test	Baseline	Pref. Test
Mean	265.4	434.5*	276.3	428.1	268.5	363.9	283.3	440.0
SD	92.3	94.9	85.4	141.0	47.1	171.2	39.0	130.8
B. Difference Scores: Mean Time (s) in NP Side Minus P Side Before and After Cathinone Conditioning								
	Baseline	Pref. Test	Baseline	Pref. Test	Baseline	Pref. Test	Baseline	Pref. Test
Mean	−219.6	161.3*	−175.3	131.7	−144.1	−4.0	−185.3	75.2
SD	223.3	158.8	149.2	279.4	109.2	395.7	106.6	240.4

*Significant difference from baseline trial; $p<0.01$, paired Student's t-test.

tion of conditioned place preference to cathinone. To this end, a dose of CGS 10746B that had previously been shown to antagonize the discriminative effects of a similar dose of cathinone (19), viz., 20 mg/kg, was administered to these CPP-naive rats after their baseline preference-aversion was determined (day 3 baseline preference). Thus prior to the four cathinone-environment pairings, CGS 10746B was first administered (IP) followed 10 min later by administration of cathinone and 10 min later by placing the animals into their less-preferred side for a 30-min period. On alternate days, this dose of CGS 10746B was administered 10 min prior to the administration of the vehicle and 10 min after the second injection the animals were, likewise, placed into their preferred side for a 30-min period. As previously, the preference test on day 12 was conducted over a 15-min period with the animals being tested without being injected.

Measurements and Statistics

The actual measurements taken were the number of s in which the animals had at least two paws in either the black or white area during the 15-min test periods on days 3 an 12. These two numbers generally did not add up to 900 s (60 s × 15 min) as the time that an animal spent in the grey choice area was not included. After the baseline day (day 3), the side to be referred to as nonpreferred (NP) was determined and it was this side that was paired with all drug treatments, i.e., cathinone in three groups and CGS 10746B + cathinone in a fourth group. After the conditioning trials, the amount of time spent on the nonpreferred side was determined and was compared to baseline. The second measurement used was the difference (scores) between the time spent in the nonpreferred side and the amount of time spent in the preferred side ("NP-P"). This measurement allows for consideration of data on the amount of time spent in the side originally preferred at baseline and, therefore, the side paired during four conditioned sessions with the vehicle. A Student's paired t-test was conducted on both measurements to compare baseline and preference test results in each group (26).

RESULTS

The eight animals trained to discriminate 0.8 mg/kg *l*-cathinone from its vehicle (designated "discriminant") spent a mean of 265.4 s in the side that they least preferred during baseline measurements on day 3 (Table 1A). In addition, they spent a mean of 485.0 s on the preferred side and this is evident in the difference scores (NP-P data), where 265.4 (s spent in NP side) minus 485.0 (s spent in P side) appears as −219.6 s in Table 1B. After four pairings with each of 0.8 mg/kg cathinone and its vehicle, this same group spent an average of 434.5 s on the nonpreferred side, an increase of 64% and a difference that is significant ($t=6.701$, $p<0.01$). Likewise, the pairing of cathinone with the nonpreferred side produced increased mean time spent in that side over baseline in the yoked and saline control groups. These increases were 54.9 and 35.5% and the paired t-values were equal to 2.348 and 1.658, respectively, neither of which reached the level for significance set at $p<0.05$.

When CGS 10746B was administered prior to conditioning with 0.8 mg/kg cathinone on the nonpreferred side, there was a trend towards increased time that the animals spent in that side, i.e., the mean time spent in the nonpreferred side went from 283.3 to 440.0 s, an increase of 55.3%. When a paired t-test was applied to these data, the calculated $t=2.470$ was slightly below the level of significance.

It is of interest to examine the results of Table 1B which indicates the mean time (in s) that the animals spent in the nonpreferred side minus the time spent in the preferred side at baseline and after cathinone conditioning sessions. The baseline will always be a negative number as the preferred side subtracted from the nonpreferred side will be negative, i.e., the preferred side, by definition, is always a greater amount of time. In the discriminant animals, this difference was shown to be significant in that it went from −219.6 at baseline to 161.3 s after cathinone conditioning. This is a difference of 380.9 s. Likewise, the mean difference between NP-P measurements on baseline and preference test days for the yoked control rats was 307.0 s. In contrast, the animals who received vehicle (control) were seen to equally favor the nonpreferred and preferred sides in that their mean NP-P measurement remained negative (−4.0) after the conditioning trials.

DISCUSSION

The results of the present study would indicate that cathinone now joins other psychoactive stimulants, such as cocaine and amphetamine [reviews (3,9)], as a drug that produces conditioned place preference. However, it was necessary to first train the rats to discriminate the interoceptive cues of the dosage used to allow this conditioned place preference to reach significant levels. The most parsimonious explanation for this observation is that the dose used both in discrimination training and in conditioned place

preference (0.8 mg/kg) was just below that needed to produce preference attributes. It was, therefore, required for the animals to be trained to discriminate the subtle interoceptive cueing properties of this dose of cathinone prior to attaining statistically significant conditioned preference. Conditioned place preference using a higher dose of cathinone, without prior discriminative training should, therefore, produce a significant preference. This, indeed, is the case. Subsequent to the analysis of the present results, ten additional rats were administered 1.2 mg/kg cathinone and conditioned with it in their nonpreferred compartment. Whereas mean time (± S.D.) spent in this side during the baseline trial was 215.3 (± 136.1) s, this measurement significantly ($p<0.01$) increased to 366.3 (± 141.1) s after conditioning (Schechter, unpublished results).

In addition to amphetamine and cocaine, other drugs that act upon dopamine receptors have been shown to produce conditioned place preference. These include: bupropion, methylphenidate, nomifensine and apomorphine (9). The common property of these drugs to facilitate dopamine transmission, either by stimulating release, inhibiting the reuptake or directly stimulating dopamine receptors, has led to the suggestion that the rewarding properties measured by the CPP-test are mediated by central dopamine. This hypothesis has been evidenced by the ability of specific dopamine receptor antagonists or neurotoxin lesions to block the stimulant-induced conditioned place preference (9). The present results would indicate that a drug that has selective activity in precluding the release of dopamine from presynaptic stores, viz., CGS 10746B (1), can decrease the preference in animals trained to discriminate the interoceptive cueing properties of 0.8 mg/kg cathinone. The CGS 10746B dose (20 mg/kg) used was previously shown to be capable of attenuating the discriminative properties of both amphetamine (20) and cathinone (19). Although this group of rats was small (n = 6), this observation would suggest a role of dopamine in stimulant-induced place preference.

The conditioned place preference technique appears to be particularly valuable in studying the pharmacology of drug reward. The present results are the first published data to indicate that pairing cathinone with a nonpreferred environment tends to attract the rat to spend more time in that environment. Furthermore, the reinforcing properties of cathinone can be increased by first training the subjects to "perceive" the interoceptive cues produced by it and, in contrast, diminished by decreasing central dopamine function.

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