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Cocaine-Induced Conditioned Place Approach in Rats: The Role of Dose and Route of Administration

TIMOTHY C. DURAZZO,* DAVID V. GAUVIN,†[1] KEVIN L. GOULDEN,† RICHARD J. BRISCOE† AND FRANK A. HOLLOWAY†

*Department of Psychology, Oklahoma State University, Stillwater, OK 74078
†Psychobiology Laboratories, Department of Psychiatry and Behavioral Science, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000*

DURAZZO, T. C., D. V. GAUVIN, K. L. GOULDEN, R. J. BRISCOE AND F. A. HOLLOWAY. *Cocaine-induced conditioned place approach in rats: The role of dose and route of administration.* PHARMACOL BIOCHEM BEHAV **49**(4), 1001-1005, 1994. – The hedonic valence of the interoceptive stimuli associated with a wide range of cocaine doses administered by either SC or intraperitoneal injections was assessed in rats. Ninety-six male Sprague-Dawley rats were randomly assigned to different dose- and route-of-administration dependent groups (n = 8/group) and conditioned in a place learning task. During half of the conditioning trials, rats received either SC or intraperitoneal injections of saline or an individual dose of cocaine from 0.32 to 32 mg/kg (10 groups, 0.5 log common log unit increments), and were immediately placed in the initially nonpreferred compartment of a straight alley-way place-conditioning chamber. Prior to the other conditioning trials, rats received equivalent volumes of saline injections via the same routes of administration and were immediately placed in the initially preferred compartment. Two additional control groups received saline injections on both sides. Each rat received eight conditioning trials (four on each side). Significant conditioned place approach was produced by both SC- and IP-injected cocaine. However, the IP route of cocaine administration required a dose of 10 mg/kg cocaine to elicit a conditioned place approach, whereas a 0.32 mg/kg SC cocaine injection produced a CPP. Saline injections alone did not change the initial preference scores, and conditioned place aversions were not produced by any cocaine dose. The results of the present study demonstrate the relative safety of SC cocaine administration in the rat and a behavioral potency difference between these two routes of administration relative to the hedonic valence of the associated subjective states.

Cocaine Conditioned place preference Rats Place approach

A NUMBER of laboratories previously have demonstrated that the same stimulus condition (including drug conditions) can be both positively reinforcing and aversive, depending on the task conditions under which it is administered (10,13-15). Drugs such as cocaine can be reinforcing and aversive at the same time. In the latter case, cocaine users have described their subjective experiences soon after cocaine administration as anxiety or overly wired (18). Geist and Ettenberg (5) recently have demonstrated that rats self-administering cocaine injections displayed behavioral dissonance, defined as an increase in approach-avoidance behaviors, near the location within the alleyway associated with self-administration.

A number of laboratories have demonstrated a conditioned place approach in rats to unique environmental stimuli associated with cocaine administration [cf., (20)]. However, as reviewed by Schechter and Calcagnetti (20), there is a paucity of data comparing both SC (SC) and intraperitoneal (IP) administration of cocaine across a wide range of doses. Most studies have utilized IP administered cocaine in rats, and conditioned with only a single or limited range of doses. However, in a number of recent studies focusing on the teratogenic effects of prenatal cocaine in rats, in which cocaine cannot be administered intraperitoneally, the drug has been successfully administered via the SC route. For example, a number of studies have demonstrated the SC delivery of cocaine to rats results in plasma and brain cocaine and benzoylecognine levels equivalent to those described in human cocaine abusers (23).

The purpose of the present study was to assess the relative hedonic valence of a wide range of cocaine doses in rats using both SC and IP administered cocaine. The place conditioning

[1] Requests for reprints should be addressed to David V. Gauvin, Ph.D., University of Oklahoma Health Science Center, Research Bldg., 302-R, P.O. Box 26901, Oklahoma City, OK 73190-3000.

task was used because it has been demonstrated, in a number of laboratories and across a wide range of drugs, to be sensitive to hedonically positive, negative, and neutral affective states. Rats are genetically predisposed to forage in their environment (4,12) and show an initial genetic or developmental preference for one of the two conditioning chambers (7,8, 12,21). As innate foragers, rats naturally move about the conditioning environment. As described by White, Messier, and Carr (24) a preference for one of two stimuli implies either that the subjective (affective) state produced by the preferred stimulus is relatively rewarding or that the alternative choice produces relative aversion. The actual observation in a place conditioning task is a tendency for an animal to orient towards or away from a rewarding stimulus. White, Messier, and Carr (24) have concluded that the inferred affective properties or hedonic valences of stimuli are, in fact, based on the observation of approach, withdrawal, or no change in the behaviors those stimuli produce. Bindra (1) and Hearst and Jenkins (6) have suggested that just as rewards elicit approach behaviors and noxious events elicit withdrawal, cues, or signals for such reinforcer events can acquire attributes similar to those reinforcer events. These conditioned attributes contribute to, or enhance, the hedonic qualities of the environmental cues and are, in turn, thought to elicit the conditioned response repertoires [for a more detailed description of the place conditioning procedure see (24)].

METHOD

Subjects

Ninety-six male Sprague–Dawley rats were purchased from Sasco Inc. (Omaha, NE) and housed in standard Plexiglas shoe-box cages (three per box) in a colony room maintained on a 12 L : 12 D cycle (lights on at 0530 h). Food and water was continuously available in the home cage. The colony room and animal care were administered by an AAALAC-accredited team of technicians and veterinarians from the Department of Animal Resources at the University of Oklahoma Health Sciences Center. After a 1-week acclimation period to the new environment, rats were conditioned in a Place Learning task.

Place Conditioning Task

The place conditioning apparatus consisted of two main conditioning compartments (40 × 16 × 24 cm), connected to each other by a third compartment (10 × 16 × 24 cm) in a straight alleyway configuration. The apparatus was constructed of Plexiglas (Cope Plastics, Oklahoma City, OK); the hinged top was clear Plexiglas, and the side panels were double-walled clear Plexiglas with sliding black Plexiglas inserts. The central chamber (compartment 2) had a clear Plexiglas floor, with gray walls. The two conditioning compartments had the following distinguishing stimulus characteristics: in compartment 1, the walls were decorated with white and black vertical stripes with textured indoor/outdoor carpeting on the floor. In compartment 3, walls were decorated with white and black horizontal stripes with smooth indoor/outdoor carpeting on the floor. The apparatus was cleaned between rats by removing feces and wiping the walls, ceiling, and carpeting with warm soapy water. Sets of removable guillotine doors could be inserted between individual compartments at the beginning of each session. Additionally, small partial walls (10 cm high) were inserted between the central compartment and the two conditioning compartments. These partial walls remained in place during the sessions to allow for a clear, detectable, quantitative change in location of the rat from one compartment to the other. The number of compartment entries, time in each compartment, and general activity of the subjects was assessed and recorded by sets of infrared photobeams located near the floors of each compartment and linked by a photobeam controller (DIG-723, Med. Associates, Inc., East Fairfield, VT) to a Commodore 64C microcomputer system. The microcomputer system controlled the experimental contingencies and recorded all measures from four sets of conditioning chambers simultaneously (American Neuroscience Research Foundation, Oklahoma City, OK).

The first 4 days of the procedure consisted of a preexposure period with the guillotine doors removed, to allow for free access to all three compartments. Each rat was placed separately into the apparatus for 40 min. On the fifth day, a 20-min habituation test session was conducted. We have found that the 20-min preference test engendered preference data that is not statistically different from data generated in the longer 40-min preference test sessions. The 20-min test sessions allowed us to run all rats through the test sessions on the same day. The central compartment was sealed off from the two distal compartments by the placement of the guillotine doors. Initially, each rat was sequestered into this small central compartment. Once the computer program was initiated, the guillotine doors were removed, which completed a photobeam sensor circuit and started recording time, activity, and chamber data. The rats had free access to all three compartments. The relative time spent in each of the two conditioning compartments was calculated for each rat (total time spent in compartments 1 and 3). The preference score was calculated (the total time spent in the least preferred compartment ÷ total time spent in compartments 1 and 3) and expressed as a percentage. This initial habituation test session data was used to compare changes in individual preferences/biases for one end compartment over the other. The most nonpreferred compartment was designated as the conditioned stimulus (CS+) side, which would be paired with cocaine during the following conditioning trials. The alternative compartment was paired with saline injections in volumes equivalent to the cocaine injections (dependent upon group assignments; see below).

The cocaine/saline pairing trials began on the day following the habituation test sessions. On a 3-day cycle, each rat was injected with either cocaine or saline and immediately placed into the conditioning apparatus for 40 min. The route of administration, dose, and concentrations of cocaine were group dependent and listed in Table 1. Each cocaine-environment pairing session was followed by a day off to insure that any immediate or residual effects of the cocaine injections did not carryover into the saline-environment pairing sessions. The two groups of rats receiving saline injections on both sides were not conditioned on the day following saline injections paired with placement into the nonpreferred side of the apparatus. After injection each rat was immediately placed into the conditioning apparatus for 40 min. Black Plexiglas partitions were inserted into the guillotine door guides to sequester each rat within one of the two end conditioning compartments. Cocaine was paired with the placement of the rat in the initially nonpreferred compartment [biased strategy, cf., (20)]. Saline injections were paired with the placement of the rat in the initially preferred compartment (CS−). Each rat received a total of eight stimulus/environment pairing sessions (four cocaine + four saline, or eight saline) and two conditioning test sessions (T1 and T2).

Drugs

Cocaine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO), weighed daily (expressed as the salt), and

dissolved in 0.9% saline. Cocaine and saline were injected intraperitoneally or subcutaneously, dependent upon group assignment (see Table 1). We were sensitive to the problems associated with the SC injection of cocaine in rats. We recently reported (2) that a low concentration of cocaine (approx. 1.2 to 1.6 mg/ml) administered in large volumes did not reliably produce dermal necrosis. Each rat received the cocaine and/or saline injection in either a single large bolus or in two injections, administered on alternate sides of the body. The highest concentration of cocaine used in the present study was 1.6 mg/ml. Rats receiving the highest cocaine dose (32 mg/kg, SC) were delivered to the Department of Animal Resources of the University of Oklahoma Health Sciences Center following the completion of the study for necropsy and histopathology analyses of the dermis and SC vault spaces.

Data Analysis

The total time of the 20-min undrugged, free-access, test session spent in the nonpreferred compartment ÷ the total time spent in both conditioning compartments was expressed as a percentage, and is used as a measure of side preference. Although it might seem that this preference score could significantly change from one test session to another by the rat spending more time in the central start chamber, rats demonstrate a genetic predisposition to forage. To date, we have not witnessed a single rat that does not explore and forage the three compartments and spend a significant amount of the test time in one of the two larger conditioning alleyways. Additionally, our studies have failed to show any significant change in the total amount of time each rat spends in the start chamber from test #1 to test #2. Based on these findings within our laboratory, we utilize the described preference score as a measure of hedonic valence. This preference score appears to accurately infer a hedonic valence for the environmental cues of the compartment. A three-way mixed-factor ANOVA (route of administration × (dose-dependent) group × test; repeated measure on one factor (test)) was conducted to make intergroup comparisons and to assess the statistical significance of the change in: a) preference scores and b) the total time spent in the start chamber, between test #1 and test #2. Statistical significance was set at $p < 0.05$.

TABLE 1

GROUP ASSIGNMENTS AND CHARACTERISTICS

Group (n = 8)	Route of Administration	Dose	Concentration
			Equiv. Volumes to
Saline SC	subcutaneous	0.0 mg/kg	Exp. Groups
0.32	subcutaneous	0.32 mg/kg	0.012 mg/ml
1.0	subcutaneous	1.0 mg/kg	0.04 mg/ml
3.2	subcutaneous	3.2 mg/kg	0.12 mg/ml
10	subcutaneous	10.0 mg/kg	0.40 mg/ml
32	subcutaneous	32.0 mg/kg	1.2-1.6 mg/ml
			Equiv. Volumes to
Saline IP	intraperitoneal	0.0 mg/kg	Exp. Groups
0.32	intraperitoneal	0.32 mg/kg	0.012 mg/ml
1.0	intraperitoneal	1.0 mg/kg	0.04 mg/ml
3.2	intraperitoneal	3.2 mg/kg	0.12 mg/ml
10	intraperitoneal	10.0 mg/kg	0.40 mg/ml
32	intraperitoneal	32 mg/kg	1.2-1.6 mg/ml

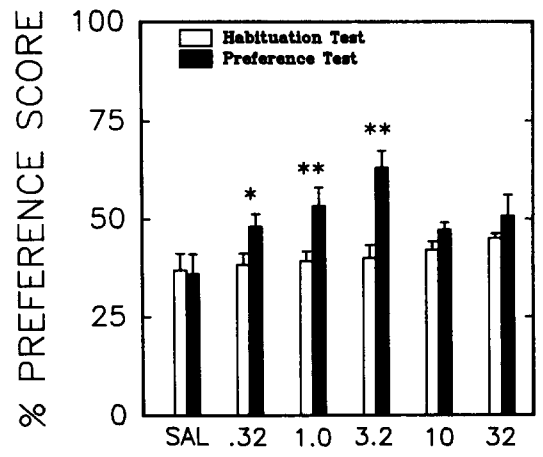

FIG. 1. Conditioned place approach induced by subcutaneously administered cocaine. Mean (SE) preference scores of rats expressed as a function of cocaine conditioning dose. The total time of a 20-min session spent in the nonpreferred compartment ÷ total time spent in both conditioning compartments is expressed as a percentage, and is used as a measure of side preference. Place approach infers a hedonic valence for the unique environmental cues of the compartment. Initial side preference/bias (open bars) was used to determine which of two compartments would be paired with subcutaneously injected cocaine. After double cocaine-saline pairings, rats were retested for side preference (T1 and T2). $*p < 0.05$, $**p < 0.01$.

RESULTS

The differential cocaine concentrations and subsequent SC injection volumes used in the present study did not result in any visible dermal necrosis, as is often reported with SC cocaine injections [cf., (2)]. There were significant dose, $F(5, 84) = 5.93$, $p < 0.001$, test, $F(1, 84) = 52.91$, $p < 0.001$, and dose × test interactions, $F(5, 84) = 2.42$, $p < 0.04$. Additionally, there was a significant three-way (route × dose × test) interaction main effect, $F(5, 84) = 3.84$, $p < 0.003$. Figure 1 presents the change in preference scores for groups of rats place conditioned with SC administered cocaine and/or saline. Significant place approach was conditioned with cocaine doses of 0.32, $F(1, 84) = 4.82$, $p < 0.05$, 1.0, $F(1, 84) = 9.56$, $p < 0.01$, and 3.2 mg/kg, $F(1, 84) = 22.46$, $p < 0.01$. No significant conditioned approach nor avoidance resulted in the groups conditioned with saline nor the higher SC cocaine doses of 10 and 32 mg/kg. In comparison, Fig. 2 presents the change in preference scores for groups of rats conditioned with IP administered cocaine. Significant place approach was conditioned with the higher doses of 10, $F(1, 84) = 10.66$, $p < 0.01$, and 32 mg/kg, $F(1, 84) = 12.98$, $p < 0.01$. Neither approach nor avoidance was conditioned by the lower cocaine doses of 0.32, 1.0, and 3.2 mg/kg. There were no significant group differences in the initial preference scores [simple effects test; groups at time 1 ($F(5, 180) = 0.65$]. The analysis of variance conducted on the total time spent in the start box during test #1 and test #2 revealed no significant change in those times, fully supporting the use of the percent preference scores used for the above analyses (all main effects and interactions: all $F < 1.5$; simple interactive effects: all $F < 1.0$).

Necropsy and histopathology analyses revealed that there was no evidence of chronic inflammation or granuloma formation involving the skin, underlying skeletal muscles or subcutis.

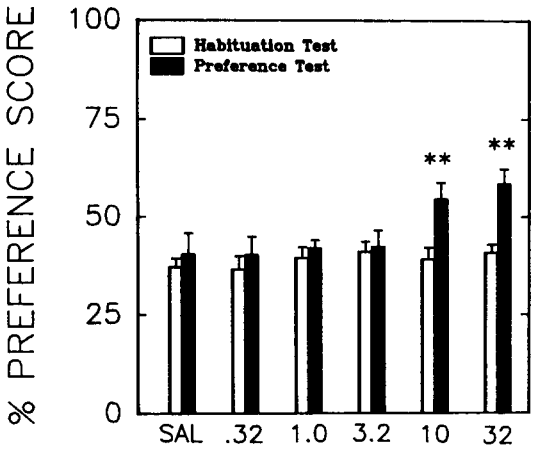

FIG. 2. Conditioned place approach induced by intraperitoneally administered cocaine. Mean (SE) preference scores of rats expressed as a function of cocaine conditioning dose. Details similar to Fig. 1. $^{**}p < 0.01$.

DISCUSSION

Conditioned place approach for unique environmental cues associated with cocaine administration was demonstrated using both SC and IP routes of administration in the rat. Interestingly, when comparing the first dose required to elicit a conditioned place approach, the SC route of administration of cocaine produced a 30-fold leftward shift in the dose-response function compared to IP administration. Conditioned place approach was produced with 0.32 mg/kg SC cocaine compared to 10 mg/kg IP cocaine. Conditioned place avoidance was not produced by any treatment condition, and saline conditioning failed to produce any shifts in the initial preference scores. The data from the present study demonstrate the relative potency difference between SC and IP routes of cocaine administration and the relative safety of SC cocaine administration in the rat. The hedonic valence of cocaine is inferred to be associated with a positive affective state that is both dose and route of administration dependent. The SC route of cocaine has a longer biological half-life compared to the IP route [cf., (9,16)], and we now demonstrate a behavioral potency difference with the SC route producing a more robust hedonically positive interoceptive subjective state in the rat. Interestingly, neither SC or IP injections of cocaine produced conditioned place aversions with any dose of cocaine used in this study. Higher doses of cocaine were not employed due to the electrocorticographic paroxysms produced in rats by doses greater than 32 mg/kg.

One hypothesis to explain the lack of some behaviorally active cocaine doses to effectively change the amount of time spent on the CS+ side after repeated pairings has been previously proposed by White and Hiroi (25) and is based on similar experimental results for other effects of other drugs. As reviewed by White and Hiroi (25), there appears to be an optimally effective dose of posttraining injections of amphetamine on retention of recently learned tasks: both lower and higher doses are ineffective. White and Hiroi have proposed that maximum reward may be produced by an optimum level of activation in the CNS. Higher and lower doses would, thus, produce supra- and suboptimal levels of activation and, correspondingly, less positively hedonic or rewarding effects. Similar biphasic effects were reported by White and Hiroi (25) for a place conditioning task using the nonamphetamine-like psychostimulant, pipradrol.

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REFERENCES

1. Bindra, D. A motivational view of learning, performance, and behavior modification. Psychol. Rev. 81:199-213; 1974.
2. Durrazo, T. C.; Gauvin, D. V.; Holloway, F. A. Technical report: The SC administration of cocaine in the rat. Pharmacol. Biochem. Behav. 49:1007-1010; 1994.
3. El-Maghrabi, E. A.; Calligaro, D. O.; Eldefrawi, M. E. High affinity binding of (^{3}H)cocaine to rat liver microsomes. Life Sci. 42: 1675-1682; 1988.
4. Fantino, E.; Logan, C. A. The experimental analysis of behavior: A biological perspective. San Francisco: W. H. Freeman and Co: 1979:473-499.
5. Geist, T. D.; Ettenberg, A. A simple method for studying intravenous drug reinforcement in a runway. Pharmacol. Biochem. Behav. 36:703-706. 1990.
6. Hearst, E.; Jenkins, H. M. Sign-tracking: The stimulus-reinforcer relation and direction action. Austin, TX: Psychonomic Society: 1974.
7. Hilgard, E. R. Methods and procedures in the study of learning. In: Stevens, S. S., ed. Handbook of experimental psychology. New York: John Wiley and Sons; 1951:517-567.
8. Hinde, R. A.; Stevenson-Hinde, J. Constraints on learning: Limitations and predispositions. New York: Academic Press: 1973.
9. Katz, J. L.; Griffins, J. W.; Sharpe, L. G.; De Souza, E. B.; Witkin, J. M. Cocaine tolerance and cross-tolerance. J. Pharmacol. Exp. Ther. 264:183-192; 1993.
10. Kelleher, R. T.; Morse, W. H. Schedules using noxious stimuli: II Responding maintained with response-produced electric shock. J. Exp. Anal. Behav. 11:819-838; 1968.
11. Kloss, M. W.; Rosen, G. M.; Bauckman, E. J. Cocaine-mediated hepatotoxicity: A critical review. Biochem. Pharmacol. 33:169-173; 1984.
12. Maier, N. R. F.; Schneirla, T. C. Principles of animal psychology. London: Dover; 1964.
13. McKearney, J. W. Maintenance of responding under a fixed-interval schedule of electric shock presentation. Science 160:1249-1251; 1968.
14. Mello, N. K. Behavioral studies of alcoholism. In: Kissin B.; Begleiter, H., eds. The biology of alcoholism. vol. II. Physiology and behavior. New York: Plenum Press; 1972:219-260.
15. Morse, W. H.; Mead, R. N.; Kelleher, R. T. Modulation of elicited behavior by a fixed-interval schedule of electric-shock presentation. Science 157:215-217; 1967.
16. Nayak, P. K.; Misra, A. L.; Mulé, S. J. Physiological disposition and biotransformation of (^{3}H)cocaine in acutely and chronically treated rats. J. Pharmacol. Exp. Ther. 196:556-569; 1977.
17. Pan, H. T.; Manacherry, S.; Justice, J. B., Jr. Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. J. Neurochem. 56:1299-1306; 1991.
18. Petersen, R. C.; Stillman, R. C. Cocaine: 1977. NIDA Research Monograph: 13. Washington, DC: U.S. Government Printing Office.
19. Petit, H. O.; Pan, H. T.; Parson, L. H.; Justice, J. B., Jr. Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J. Neurochem. 55:798-804; 1990.
20. Schechter, M. D.; Calcagnetti, D. J. Trends in place preference

conditioning with a cross-indexed bibliography; 1957-1991. Neurosci. Biobehav. Rev. 17:21-44; 1993.
21. Schneirla, T. C.; An evolutionary and developmental theory of biphasic processes underlying approach and withdrawal. Nebraska Symp. Motivat. 7:1-42; 1959.
22. Shuster, L.; Quimby, F.; Bates, A.; Thomson, M. L. Liver damage from cocaine in mice. Life Sci. 20:1035-1042; 1977.
23. Spear, L. P.; Frambes, N. A.; Kirstein, C. L. Fetal and maternal brain and plasma levels of cocaine and benzoylecognine following chronic SC administration of cocaine during gestation in rats. Psychopharmacology (Berlin) 97:427-431; 1989.
24. White, N. M.; Messier, C.; Carr, G. D. Operationalizing and measuring the organizing influence of drugs on behavior. In: Bozarth, M. A., ed. Methods of assessing the reinforcing properties of abused drugs. New York: Springer Verlag; 1987:591-617.
25. White, N. M.; Hiroi, N. Pipradrol conditioned place preference is blocked by SCH23390. Pharmacol. Biochem. Behav. 43:377-380; 1992.