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Dose History and Occurrence of Conditional Stimuli Determine the Strength of Cocaine-Seeking Behavior of Rhesus Monkeys

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Four adult male rhesus monkeys were trained to lever press for cocaine under a daily two-component MIX PR (progressive ratio) schedule. During the first 10 min of experimental sessions, completion of progressive ratios resulted in 1-s presentations of brief visual stimuli (BS; colored lights) associated with cocaine infusions during the second component. Stimulus lights of different colors were associated with doses of 3, 30, and 300 $\mu\text{g}/\text{kg}$ cocaine as the available self-administered infusate. A 5-min time out period followed the first component, which in turn was followed by a 60-min component during which completion of progressive ratios resulted in cocaine infusions and the associated visual stimuli. Once reinforcer rates had stabilized under each dosing condition in both components, break point tests were conducted separately for BS as the reinforcer and with cocaine + stimuli as the reinforcer. Break points for lever pressing maintained by BS alone increased as they were paired with increasing doses of cocaine. Break points maintained by actual cocaine delivery, however, demonstrated an inverted U-shaped function to cocaine dose. The results of this study suggest that the strength of cocaine-seeking behavior varies monotonically with the self-administered dose of cocaine and that the level of motivation to obtain cocaine may not be directly revealed by levels of actual cocaine self-administration.

The importance of drug-associated stimuli in the initiation, perpetuation, and resumption of drug-seeking behavior has repeatedly been documented within the scientific literature. Drug-seeking behaviors occasioned by environmental stimuli likely develop through classical conditioning processes (O'Brien, Childress, McLellan, & Ehrman, 1992; Stewart, 1983). Presumably, contextual information can be conveyed by these stimuli that have gained motivational significance through repeated pairings with the drug's reinforcing properties. Drug-associated stimuli contribute to drug-seeking behavior, especially during a period of drug abstinence, leading to drug craving and relapse to drug use. Former drug users often report environmental factors as triggers of drug craving (Shulman, 1989) and relapse to cocaine use (Wallace, 1989). In humans with a history of drug abuse, presentation of drug-related stimuli can induce self-reports of drug craving (Dudish-Poulsen & Hatsukami, 2000; Foltin & Haney, 2000) and physiological re-

This research was carried out in an AAALAC-certified facility and in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996) and was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. These studies and the preparation of this report were supported by National Institute on Drug Abuse grant DA-01442. J. L. Newman was supported by a Ruth L. Kirschstein Individual Predoctoral Award by the National Institute on Drug Abuse (F31DA-016845). The authors wish to thank Ellen Soehngen, Elizabeth Goodbody, Mario Dance, and Yohance Goodrich for their expert technical assistance during the execution of these studies. These data were presented in preliminary form at the 2004 Winter Conference on Animal Learning and Behavior, Winter Park, CO. Correspondence concerning this article should be addressed to Patrick M. Beardsley, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Medical College of Virginia Campus, P.O. Box 980613, Richmond, VA 23298-0613, U.S.A. (pbeardsl@hsc.vcu.edu).

sponses (Ehrman, Robbins, Childress, & O'Brien, 1992) long after termination of drug use.

Consequently, investigating the role of drug-associated stimuli in the resumption of drug-seeking behaviors has gained considerable attention. Reinstatement procedures are commonly used in laboratory animals to investigate determinants that may lead to relapse to drug use in man. For example, exposure to stimuli that set the occasion for self-administration, or stimuli paired with drug delivery can precipitate reinstatement of drug-seeking behavior in rats following extinction of lever pressing (Fuchs, See, & Middaugh, 2003; Goeders & Clampitt, 2002; Kruzich, Congleton, & See, 2001; Panlilio, Weiss, & Schindler, 1996; See, Grimm, Kruzich, & Rustay, 1999; Weiss et al., 2001). Gaining a greater understanding of the parameters in which drug-associated stimuli come to control behavior is important for treating drug addiction disorders.

Despite the widely acknowledged significance of drug-associated environmental stimuli in addiction, few studies have been conducted to characterize the relationship between the magnitude of the drug reinforcer (i.e., dose) and the strength of the motivation to seek the drug during a period independent of self-administration and in absence of the drug itself. One study (Schenk & Partridge, 2001) revealed that the influence of drug-associated stimuli on self-administration varies with dose and that the presence of a cocaine-associated stimulus was essential for the maintenance of stable self-administration. Crowder and colleagues (Crowder, Smith, Davis, Noel, & Coussens, 1972) demonstrated that the magnitude of a conditioned reinforcer is dependent upon dose of morphine paired with the stimuli. Further, studies employing second-order schedules have demonstrated that responding in the presence of stimuli during initial drug-free intervals increases dose dependently (Arroyo, Markou, Robbins, & Everitt, 1998; Olmstead, Parkinson, Miles, Everitt, & Dickinson, 2000).

The purposes of the present studies were two-fold. The first goal was to characterize dose response functions relating cocaine dose to drug-seeking behavior in cocaine's absence during a daily, two-component reinforcement schedule under limited access conditions. The second goal was to determine the persistence of cocaine-seeking behavior reinforced by BS presentations associated with different doses of cocaine under progressive ratio (PR) break point test conditions. Under PR schedules, the response requirement (i.e., ratio value) increases systematically following the delivery of each reinforcer until the subject fails to emit responses for the next reinforcer following a predetermined period of time. The value of the ratio at which the subject obtains its final reinforcer is termed the break point and reflects the persistence of a reinforcer to maintain increasing requirements of operant behavior (Griffiths, Brady, & Snell, 1978; Hodos & Kalman, 1963). In drug self-administration procedures, this schedule is commonly used to infer reinforcing efficacy of drug reinforcers relative to other drugs or multiple doses of the same drug (Richardson & Roberts, 1996; Stafford, LeSage, & Glowa, 1998, for reviews). The reinforcing efficacy of many compounds has been characterized using PR schedules; however, there is relatively little information regarding such persistence of drug-seeking behavior maintained by conditioned stimuli under this schedule.

Spear and Katz (1991) demonstrated that cocaine-associated BS presented under a second-order PR schedule enhances response rates for cocaine infusions,

whereas omission of the BS decreases PR break point for cocaine-maintained responding. Ranaldi and Roberts (1996) used a synthesis of second-order and PR schedules to examine the role of conditioned stimuli in the initiation, maintenance, and extinction of cocaine-seeking behavior. These authors demonstrated that the presence of conditioned stimuli engenders higher break points during the maintenance phases of cocaine self-administration, and increases responding during extinction. These studies suggest that the brief presentations of drug-associated stimuli enhance the motivation to obtain drug deliveries.

To examine the role of magnitude of drug reinforcer on the persistence of drug-seeking behavior, break points engendered by dose-specific cocaine associated brief stimulus (BS) presentations were examined in cocaine's absence. For these studies, monkeys were trained to self-administer several doses of cocaine under a two-component MIX PR:BS PR:DRUG schedule in which cocaine-seeking behavior maintained by BS presentations was established in the first component, while cocaine self-administration paired with BS presentations was established in the second component of a daily experimental session. Following stabilization of lever pressing for each dose of cocaine, break point tests were conducted separately for: (1) cocaine infusions paired with dose-related BS presentations, and (2) for dose-related BS presentations without cocaine infusions. The present study revealed that daily cocaine-seeking behavior under limited access conditions, and its persistence under break point test conditions, is influenced by recent self-administered dose of cocaine and associated BS.

Method

Subjects

Four experimentally naïve adult male rhesus monkeys (*Macaca mulatta*; 7.9-12.1 kg) were housed individually in ventilated cubicles (1 x 1 x 1m) and restrained by a stainless-steel harness and tether system. Water was freely available and LabDiet High Protein Monkey Diet (PMI Nutrition International, Missouri, U.S.A.) and fresh fruit were provided to maintain a constant body weight.

Surgery

Monkeys were surgically implanted under sterile conditions with an indwelling silicone catheter (0.08 mm internal diameter; Ronsil Rubber Products, New Jersey, U.S.A.) under phencyclidine (1mg/kg, i.m.) and pentobarbital (i.v. to effect.) anesthesia. The right or left internal and external jugular or femoral veins were catheterized. If a catheter became non-patent it was removed, the monkey was given a minimum of two weeks from testing, after which an alternative vein was catheterized. The catheter ran subcutaneously to the midscapular region and exited through a small incision in the skin and then traveled through a protective stainless-steel harness and restraining arm through which it passed to the rear of the cubicle and connected to a peristaltic pump (Masterflex, Cole-Parmer, Illinois, U.S.A.) that delivered 1-ml infusions over 10 s. The harness and tether were equipped with swivels to allow animals nearly complete freedom of movement within the cubicles.

Apparatus

Two response levers separated by a food hopper were mounted on the clear Plexiglas front door of the experimental cubicles. A modular light emitting diode (LED) lamp (7.5 cm x 12.5 cm; Edwards Triliptical Beacon Light Source, 50/60 Hz) with interchangeable colored LEDs and colored casings (red, green, or blue) was mounted above the drug-reinforced (left-side) lever. Three white or amber stimulus lights were mounted above the left and right levers, respectively. All contingencies for drug or stimulus presentations were arranged for presses of the left-side lever. Scheduling of infusions, stimulus changes, and collection of data were accomplished using Med-PC IV software (Vermont, U.S.A.) and a personal computer- associated interface.

Procedure

Prior to initial catheterization, monkeys were acclimated to the self-administration chambers and trained to press the right-side lever reinforced according to a Fixed Ratio 30 (FR 30) schedule in which every 30 presses produced food pellet delivery (1 g banana-flavored pellets; BioServe, New Jersey, U.S.A.) during daily, 1-hr experimental sessions. The food reinforced sessions were discontinued subsequent to cocaine self-administration training.

Following catheterization, monkeys were trained to self-administer cocaine (30 µg/kg in 10-s, 1-ml infusions) according to a FR 30 schedule of reinforcement in which every 30th press on the left-side lever produced cocaine infusion during 1-hr daily sessions. The beginning of each self-administration session was signaled by illumination of the outer two jeweled stimulus lamps above the drug-reinforced lever. Each 10-s cocaine infusion was paired with a stimulus presentation which consisted of two distinct visual stimulus changes: (1) simultaneous with the beginning of a cocaine infusion, the outer two jeweled stimulus lamps above the left lever were extinguished and the center stimulus lamp was illuminated for 10 s, after which the outer lamps were re-illuminated and the center lamp was extinguished; and (2) the modular LED stimulus became illuminated for 1 s at the beginning of each cocaine infusion and again for 1 s at the end of the 10-s infusion.

Following acquisition of cocaine self-administration, the FR schedule was changed to a PR schedule during the daily 1-h sessions. The progression of ratio requirements was derived from that used by Depoortere and colleagues (1993) and followed the exponential equation:

$$10 * e^{(SR\# * 0.1)} - 3,$$

where SR# = reinforcer number for which the subject was presently working and was initialized at SR # = 15, such that the first ratio requirement was FR 42. The initial response requirement and subsequent progression of ratios was chosen to engender high rates of lever pressing without resulting in rapid cocaine satiation.

Once lever pressing for cocaine became stable under the PR schedule, where stability was defined as no increasing or decreasing trends in cocaine infusions for three consecutive days and daily infusions were $\pm 20\%$ of the three-day mean, a two-component mixed PR:BS PR:DRUG schedule was introduced. During the first 10-min component (BS component), lever pressing according to a PR schedule resulted in BS presentations that were identical to the BS presentations paired with cocaine except that the BS presentation lasted for 1 s (i.e., once a ratio was completed, the red LED stimulus and center jeweled stimulus lamp was illuminated for 1 s, while the outer two jeweled stimulus lamps were extinguished). The BS component was followed by a 5-min time out period during which all stimuli were extinguished and responding was without consequence. The end of the time out period and beginning of the 60-min self-administration component was signaled by the re-illumination of the outside stimulus lamps above the left lever. During the self-administration component, monkeys were allowed to self-administer cocaine accompanied by BS presentation on a PR schedule identical to that in effect during the first component. Progression of ratio values was equal for both drug seeking and self-administration components. Initiation of the self-administration component was independent of responding during the BS component.

Break point tests were scheduled once the number of obtained infusions stabilized, in which there were no upward or downward trends in infusions and the total number of daily infusions was $\pm 20\%$ of the mean number obtained during the most recent three experimental sessions (actual results were typically less variable than this). Break point tests consisted of either single components of cocaine delivery (accompanied by stimulus presentation) or of just BS presentation in the absence of cocaine delivery. During break point tests, cocaine infusions or BS presentations were arranged according to PR schedules identical to those during training sessions with a test session terminating when 10 minutes occurred in the absence of a left lever press. Maximum duration of break point test sessions was set at 5 h, but all monkeys reached break points before 5 h elapsed.

A minimum of two training sessions separated break point tests for cocaine and BS presentation. A BS break point test was scheduled when the numbers of infusions and BS presentations occurring on the intervening two training sessions were 80% or greater than the numbers occurring during the session immediately prior to the infusion break point test day. If, however, three or more sessions intervened between cocaine infusion and BS break point tests, it was additionally required that the numbers of BS presentations and cocaine infusions obtained during the most recent training session were at least 80% of that 3-day mean for each measure.

Subsequent to BS break point tests at a particular dose, a different dose of cocaine was substituted and a different colored LED stimulus was activated during experimental sessions. Blue, red,

and green LEDs were associated with 3, 30 and 300 $\mu\text{g}/\text{kg}$, respectively. When saline was substituted for a dose of cocaine, BS presentations did not occur, however, the white jeweled stimulus lamps above the left lever were illuminated to indicate that the session was occurring. All monkeys received the cocaine doses in the same order. Following saline substitution and break point tests, self-administration was re-established with 30 $\mu\text{g}/\text{kg}$ cocaine and break points at this dose were re-determined. Lever presses on the non-reinforced lever were recorded but were otherwise without scheduled consequence during daily training conditions and break point tests.

For tests under maintenance conditions, numbers of infusions and BS presentations for individual monkeys are presented graphically. For break point tests, numbers of cocaine infusions and BS presentations are presented graphically and break point ratio values for individual monkeys are provided. The break point ratio was defined as the final ratio at which the last reinforcer was obtained. BS presentation values for test and retests with 30 $\mu\text{g}/\text{kg}$ cocaine were averaged for individual monkeys and are presented as mean \pm range; for infusions, data from these tests are presented separately. Break point values for the test and retest condition with 30 $\mu\text{g}/\text{kg}$ were averaged and reflect the nearest scheduled step value.

Cocaine hydrochloride (National Institute on Drug Abuse, Maryland, U.S.A.) was dissolved and diluted in a 0.9% sterile saline and the final solution sterile filtered. All infusions were 10 s in duration and 1 ml in volume.

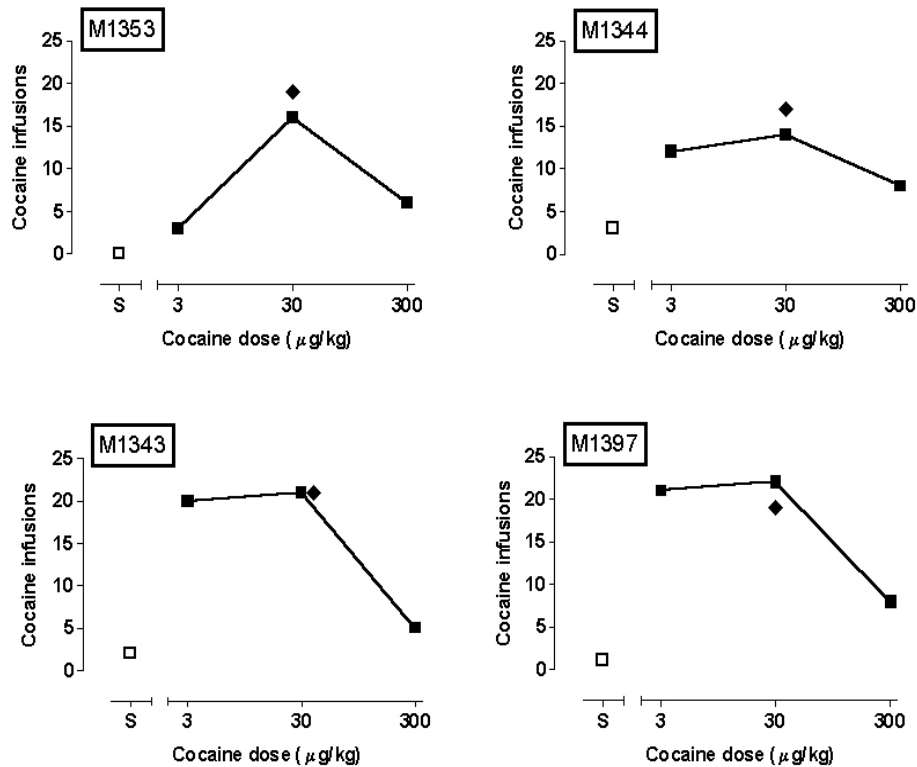


Figure 1. Infusions of cocaine and saline during self-administration under maintenance conditions for individual monkeys. Each data point represents the number of infusions for cocaine (filled squares) and saline (empty squares) as a function of cocaine dose ($\mu\text{g}/\text{kg}/\text{infusion}$) and saline (S). Filled diamonds indicate the re-determination with 30 $\mu\text{g}/\text{kg}$ cocaine.

Results

Performance During Maintenance Conditions

Figure 1 shows the relationship between cocaine dose and infusion numbers obtained during the second component of maintenance conditions for individual monkeys. Substitution of 0 (saline), 3, 30, and 300 $\mu\text{g}/\text{kg}$ cocaine during maintenance conditions revealed that all monkeys dose-dependently self-administered cocaine, and the dose-response function assumed an inverted U-shaped relationship with 3 and 300 $\mu\text{g}/\text{kg}$ maintaining fewer infusions than 30 $\mu\text{g}/\text{kg}$. Monkeys M-1344, M-1343, and M-1397 self-administered nearly as many infusions at 3 $\mu\text{g}/\text{kg}$ as obtained at 30 $\mu\text{g}/\text{kg}$, whereas the number of infusions at 3 $\mu\text{g}/\text{kg}$ for monkey M-1353 was near saline levels. All doses of cocaine maintained greater numbers of infusions than saline in each of the four monkeys. Numbers of infusions obtained upon retest with 30 $\mu\text{g}/\text{kg}$ were similar to the numbers of infusions obtained prior to substitution with other doses of cocaine. Cocaine intake levels increased with dose for all monkeys. Intake for 3, the average of two tests with 30, and 300 $\mu\text{g}/\text{kg}$ were 9, 525, and 1800 $\mu\text{g}/\text{kg}$, respectively, for M-1353; 36, 465, and 2400 $\mu\text{g}/\text{kg}$, respectively, for M-1344; 63, 615, and 2400 $\mu\text{g}/\text{kg}$, respectively for M-1343; and 60, 630, and 1500 $\mu\text{g}/\text{kg}$, respectively for M-1397.

Figure 2 shows numbers of BS presentations occurring during the 10-min drug seeking component as a function of LED stimulus color associated with each dose of cocaine for individual monkeys. BS presentations obtained on the first and last tests with 30 $\mu\text{g}/\text{kg}$ were similar. In contrast to the inverted U-shaped function relating infusions obtained to cocaine dose, BS presentations increased monotonically as the BS color changed with increases in dose. The green BS associated with the highest dose of cocaine, 300 $\mu\text{g}/\text{kg}$, maintained the greatest number of presentations with the exception of monkey M-1397, who obtained a slightly higher number of red BS presentations associated with 30 $\mu\text{g}/\text{kg}$. The number of BS presentations obtained by monkey M-1343 upon re-determination at 30 $\mu\text{g}/\text{kg}$ was slightly higher than the number of BS presentations at 300 $\mu\text{g}/\text{kg}$. BS associated with all doses of cocaine maintained a greater numbers of presentations than the "no stimulus change" condition associated with saline in most monkeys. The blue BS (3 $\mu\text{g}/\text{kg}$ -paired) failed to engender responding in monkey M-1353, and maintained lower levels of presentation relative to other BS in the other monkeys. Green BS presentations associated with 300 $\mu\text{g}/\text{kg}$ cocaine engendered the highest levels of BS presentation in monkeys M-1353 and M-1344. In monkeys M-1343 and M-1397, BS presentations associated with 300 $\mu\text{g}/\text{kg}$ were within the range of the first and second test with the 30 $\mu\text{g}/\text{kg}$ -paired BS. When responding had stabilized under the "no LED" condition associated with saline infusions, none of the four monkeys responded sufficiently to complete the initial ratio requirement (FR 42). This result suggests that behavior extinguished in absence of cocaine and BS presentations.

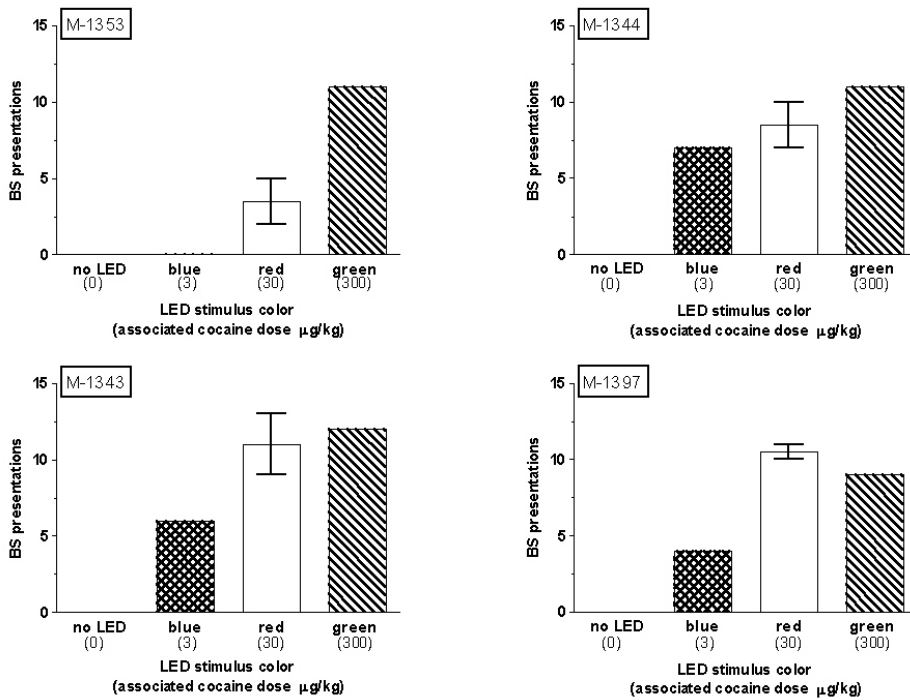


Figure 2. Brief stimulus presentations (BS) obtained as a function of LED stimulus color which had been associated with different cocaine doses under maintenance conditions in individual monkeys. Each bar represents numbers of BS presentations obtained during the 10-min BS components. Bars for the red BS, which were associated with 30 µg/kg/infusion cocaine, represent the means of the test and retest conditions at this dose; brackets through these bars indicate the range of these two determinations. The absence of a bar during the "no LED" (saline) condition indicates that the first FR was not completed under this condition.

Performance During Cocaine Infusion Break Point Tests

Figure 3 shows numbers of cocaine infusions as a function of cocaine dose and for saline obtained under break point test conditions for individual monkeys. Infusions and break points increased with dose to 30 µg/kg, and then decreased at 300 µg/kg, the highest dose tested. Cocaine infusions obtained at 30 µg/kg for both test and retest conditions were similar for individual monkeys. Break points for saline, 3 µg/kg, the scheduled step value nearest the average of the determinations for 30 µg/kg, and 300 µg/kg were FR 0, FR 47, FR 543, and FR 132, respectively for M-1353; FR 0, FR 242, FR 297, and FR 107, respectively for M-1344; FR 0, FR 491, FR 1481, and FR 57, respectively for M-1343; and FR 0, FR 543, FR 734, and FR 79, respectively for M-1397. When saline was the available infusate, none of the monkeys completed the lowest ratio (FR 42) necessary to obtain an infusion. Cocaine intake levels dose-dependently increased with dose. Intake for 3, the average of two tests with 30, and 300 µg/kg was: 6, 765, and 3600 µg/kg, respectively for M-1353; 54, 600, and 3000 µg/kg, respectively for M-1344; 78, 870, and 2100 µg/kg, respectively for M-1343; and 75, 1020, and 1200 µg/kg, respectively for M-1397. Test sessions were terminated when 10 min occurred without a lever press and session durations increased with increases in dose to 30 µg/kg and then de-

creased at 300 $\mu\text{g}/\text{kg}$. Session durations during break point tests with saline, 3, the average of tests at 30, and 300 $\mu\text{g}/\text{kg}$ cocaine were: 643, 1797, 8033, and 5764 s, respectively for M-1353; 2064, 4533, 5794, and 3919 s, respectively for M-1344; 833, 5661, 13268, and 2212 s, respectively for M-1343; and 1365, 4585, 7470, and 3175 s, respectively for M-1397.

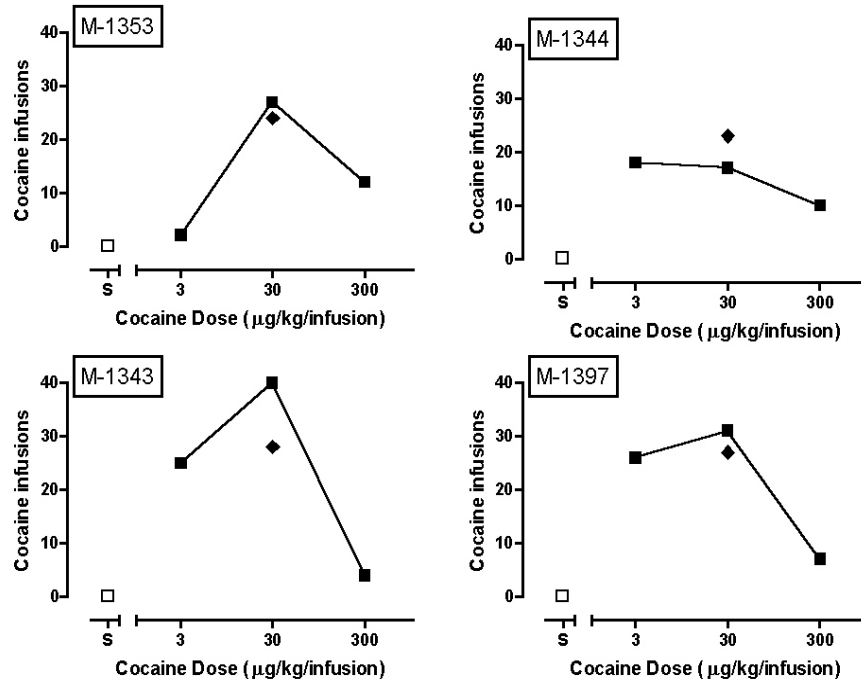


Figure 3. Infusions of cocaine and saline during self-administration break point test conditions for individual monkeys. Each data point represents number of infusions for cocaine (filled squares) or saline (unfilled square) as a function of cocaine dose ($\mu\text{g}/\text{kg}/\text{infusion}$) and saline (S) during the break point tests. Filled diamonds indicate the re-determination with 30 $\mu\text{g}/\text{kg}$.

Performance During BS Break Point Tests

Figure 4 shows BS presentations occurring during break point tests as a function of LED stimulus color associated with each dose of cocaine for individual monkeys. BS presentations and break points increased with dose to 30 $\mu\text{g}/\text{kg}$ cocaine. Numbers of green BS presentations associated with 300 $\mu\text{g}/\text{kg}$ were generally within the range of the BS presentation number observed at 30 $\mu\text{g}/\text{kg}$. Responding was maintained at greater levels in the presence of BS associated with all doses of cocaine compared to the “no LED” condition associated with saline in all monkeys, with the exception monkey M-1344. Monkey M-1344 obtained more BS presentations (8) and had a higher break point value (FR 87) under the “no stimulus” condition than under the blue BS condition which had been associated with 3 $\mu\text{g}/\text{kg}$ cocaine (1 BS presentation at a break point value of FR 42). Monkey M-1353 also obtained one BS presentation associated with 3 $\mu\text{g}/\text{kg}$, whereas monkeys M-1343 and M-1397 obtained 24 and 15 presentations, respectively. Session durations during break point tests for BS presentations associated with saline, 3, the average of two tests at 30, and 300 $\mu\text{g}/\text{kg}$ cocaine were 600, 1336, 3879, and 4265

s, respectively for M-1353; 2125, 905, 3846, and 3197 s, respectively for M-1344; 600, 4278, 6918, and 5466 s, respectively for M-1343; and 600, 3432, 4943, and 4193 s, respectively for M-1397.

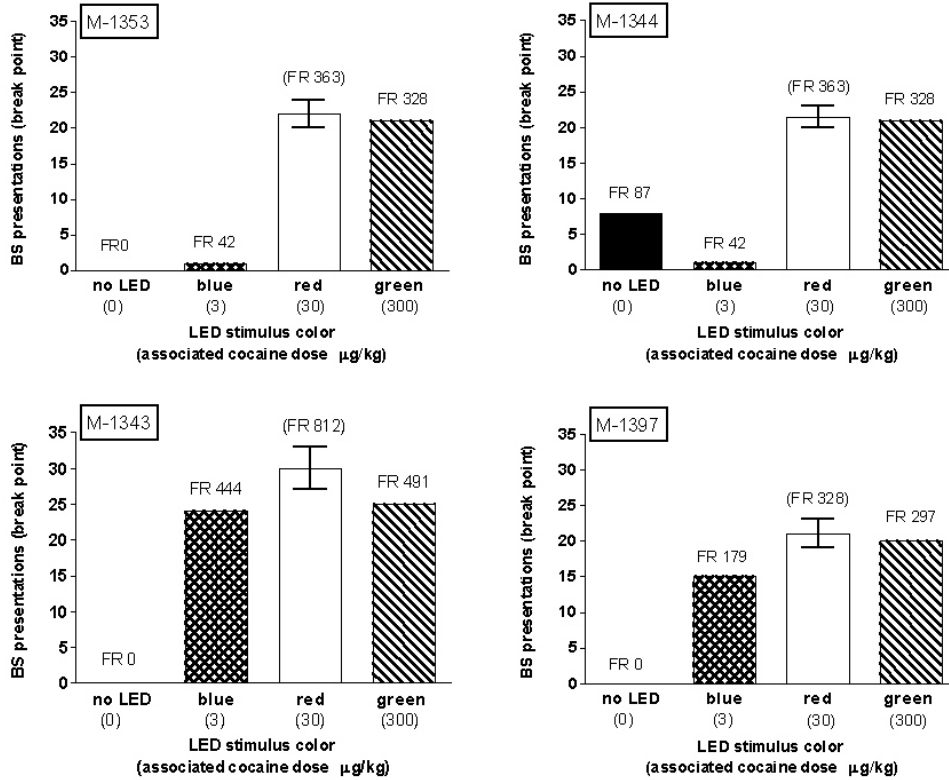


Figure 4. Brief stimulus presentations (BS) obtained as a function of the LED stimulus color associated with different cocaine doses during break point test conditions for individual monkeys. Each bar represents number of BS presentations obtained during the break point test at each dose condition. The bar above the "no LED" condition represents results when saline was tested. Bars for the red BS, which were associated with 30 µg/kg/infusion cocaine, represent the means of the test and retest conditions at this dose; brackets through these bars indicate the range of these two determinations. Numbers above bars represent break point values. The break point value for 30 µg/kg/infusion represents the nearest scheduled step value for the average of the test and retest conditions.

Discussion

This study demonstrated that lever pressing by rhesus monkeys reinforced by infusions of cocaine or their associated stimuli could be maintained under a two component schedule in which a conditional stimulus-reinforced, drug seeking component preceded a cocaine-reinforced, self-administration component. Responding maintained by cocaine infusions was characterized by an inverted U-shaped function relating infusion number to dose, both under maintenance and break point test conditions, however, rates of BS presentations generally progressively increased in the presence of BS associated with increasing cocaine doses.

Cocaine self-administration performance under break point test conditions was characterized by dose-dependent increases in infusions up to 30 µg/kg fol-

lowed by decreases at 300 $\mu\text{g}/\text{kg}$, the highest dose of cocaine tested. The dose-response determination under maintenance conditions, in which the session durations were limited to 60 min, was similar to that observed during break point conditions. Numbers of infusions obtained at 3 and 300 $\mu\text{g}/\text{kg}$ during break point tests were comparable to, and only slightly higher than the numbers of infusions obtained during maintenance conditions. The greatest number of cocaine infusions and highest break point was engendered by 30 $\mu\text{g}/\text{kg}$ cocaine. When saline was the available infusate and no stimulus changes occurred, lever pressing behavior extinguished in most monkeys.

Despite a decrease in overall infusion numbers at the highest dose, levels of cocaine intake continued to increase with dose during both the maintenance and break point test conditions. Compared to intake levels under maintenance conditions, cocaine intake levels were higher under break point conditions. Mean cocaine intake level at 30 $\mu\text{g}/\text{kg}$ was 48% higher under break point conditions than maintenance conditions, whereas intake at 3 and 300 $\mu\text{g}/\text{kg}$ was 26% and 22% higher during break point tests, respectively.

Cocaine intake under fixed ratio conditions also dose-dependently increases with higher doses despite decreases in infusion numbers. Under an FR 30 schedule, cocaine intake at similar doses usually exceeded intake levels observed in the present study (Beardsley, Sokoloff, Balster, & Schwartz, 2001). Therefore, it is likely that cocaine intake at these lower doses are influenced by factors other than behavioral disruption and satiation, and are likely a function of increasing demand arranged by the progressive ratio schedule. The cumulative level of cocaine intake obtained at 300 $\mu\text{g}/\text{kg}$ may have resulted in the decrease in infusion numbers and break points at this dose due to overall behavioral disruption or satiation. It has been demonstrated that increasing levels of cocaine intake can lead to disruptions in the behavior that is maintained by the infusions themselves (Balster & Schuster, 1973). Another potential explanation is that the monkeys became satiated. Previously, it has been suggested that animals will self-administer cocaine to reach and maintain a satiety threshold (Tsibulsky & Norman, 1999). Therefore, it is conceivable that the parameters of the progressive ratio procedure used in the present study rapidly led to satiation at the high dose of cocaine, which is reflected in the shorter break point session durations during testing at this dose. The highest dose of cocaine used in the present study, 300 $\mu\text{g}/\text{kg}$, served as a reinforcer and it is, therefore, unlikely that this dose was aversive. In studies employing choice procedures, in which subjects were allowed to choose one of two doses of cocaine, higher doses of cocaine (often exceeding 300 $\mu\text{g}/\text{kg}$) were almost always chosen over lower doses (Anderson & Woolverton, 2003) despite decreases in response rates (Johanson & Schuster, 1975).

The inverted U-shaped dose response for cocaine break points characterized in this study is consistent with the findings of others (Griffiths, Bradford, & Brady, 1979; Griffiths et al., 1978; Lile et al., 2003; Winger & Woods, 1985). Conversely, other studies using progressive ratio procedures have shown that break points dose-dependently increase monotonically or to asymptotic levels with increasing dose (Depoortere et al., 1993; Griffiths et al., 1978; Rowlett, Massey, Kleven, & Woolverton, 1996; Yanagita, 1973). In the present study, there were no post-reinforcer time out periods that would allow for dissipation of cocaine's effects between infusions. This explanation is supported by the observation that im-

posing inter-infusion intervals of increasing duration can engender higher break points and infusion numbers (Griffiths et al., 1979; Rowlett et al., 1996). However, decreases in break points are observed at very high doses despite incorporating long time out periods (Griffiths et al., 1979). Studies using non-drug reinforcers have shown that break points increase linearly with greater magnitude of the reinforcer (Hodos, 1961; Hodos & Kalman, 1963; Keesey & Goldstein, 1968), suggesting factors other than satiation control break points.

Number of BS presentations was also dose- and stimulus-dependent under break point conditions. However, unlike cocaine infusions, BS presentations obtained at the highest dose did not show marked decreases. The break points and BS presentations reached asymptotic levels in the presence of the green LED stimulus associated with 300 $\mu\text{g}/\text{kg}$. The slight decrease in BS presentations with the 300 $\mu\text{g}/\text{kg}$ cocaine-associated stimulus (i.e., green LED), relative to the 30 $\mu\text{g}/\text{kg}$ cocaine stimulus (i.e., red LED), was interpreted as representing asymptotic levels of performance under these experimental conditions. BS presentations obtained during maintenance conditions increased monotonically with increases in dose. In most monkeys, numbers of BS presentations obtained during break point conditions were at least twice the number of those obtained during maintenance conditions.

These results suggest that the motivation to self-administer cocaine, in absence of the drug, increases monotonically with increases in dose, and assessing the reinforcing efficacy of a self-administered drug could potentially be confounded by effects of the drug not essential for it serving as a reinforcer. This is supported by other studies in which drugs dose-dependently direct behavior during drug-free conditions. Studies using conditioned place preference studies have demonstrated that when tested in a drug-free state, time spent in the cocaine-paired environment increases when the context was previously paired with increasing doses of cocaine (Nomikos & Spyraiki, 1988; O'Dell, Khroyan, & Neisewander, 1996). The findings from these conditioned place preference studies support the findings of the present study in that stimuli previously associated with increasing doses of cocaine reflect higher levels of motivation when examined in absence of the drug itself. The conditioned reinforcing ability of stimuli to maintain lever pressing in rats have been reported to increase with associated morphine dose as well (Crowder et al., 1972).

Second-order schedules provide another method for evaluating daily drug seeking followed by drug self-administration and are frequently used for examining drug-seeking behavior (Arroyo et al., 1998; Di Ciano & Everitt, 2002; Everitt & Robbins, 2000; Olmstead et al., 2000; Semenova & Markou, 2003). Under second-order schedules, subjects are presented with brief stimuli according to one schedule contingency, which is treated as a unitary response; the unitary response itself is then reinforced according to some schedule of primary reinforcement (Kelleher, 1966). Behavior generated during the first unitary schedule that precedes the first drug infusion is often referred to as drug-seeking behavior, as this provides a measure of behavior that is not affected by the rate-altering effects of the drug itself (Everitt & Robbins, 2000). Studies using second-order schedules in rats have demonstrated that cocaine-seeking during the initial drug-free period monotonically increases with increases in self-administered cocaine dose, whereas infusions of cocaine decrease with increases in dose (Arroyo et al., 1998; Olmstead et al.,

2000). The results from studies using second-order schedules to examine drug-seeking behavior thus appear consistent with the results of the present study.

In the present study, transition into the self-administration component was independent of responding during the drug-free component. The independence between behavior during the BS-only component and occurrence of the self-administration component was further enforced by the insertion of the 5-min time out period between these components. As a result, responding during the first component was never directly reinforced by cocaine infusion. This observation suggests that presentation of cocaine-associated brief stimuli obtained conditioned reinforcing properties. Other tests, however, would have more definitively clarified this inference. One such test would have included the re-presentation of cocaine-paired stimuli during extinction to determine if they would reinstate responding. This test was avoided during the present study for fear that it would weaken the control exerted by the conditional stimuli, the retention of which was essential for conducting a long-term study spanning several months.

Drug-associated stimuli can occasion the persistence of drug-seeking behaviors and are often cited as determinants of relapse to cocaine use (Shulman, 1989; Wallace, 1989). Early observations of drug addicted patients have demonstrated that drug conditioned stimuli incur meaningful motivational properties as a result of repeated pairings with the reinforcing effects of a drug. Levine (1974) described two former drug users that had extensive histories of injecting drugs. In both cases, the patients developed a compulsive habit of injecting substances despite the lack of any drug effect. One explanation for this behavior is that the process itself developed conditioned reinforcing properties that manifest in the absence of drug effects. Further evidence of the conditioned reinforcing effects of drug-associated stimuli in humans has been reported by Foltin and Haney (2000) who demonstrated that initially neutral stimuli paired with smoked cocaine can acquire conditioned reinforcing effects. Studies have shown that presenting former drug users with drug-related stimuli (e.g., paraphernalia) results in self-reports of drug craving accompanied by physiological responses such as increased heart rate (Ehrman et al., 1992; Foltin & Haney, 2000), and activation of brain areas important in mediating reward (Bonson et al., 2002; Childress et al., 1999). These observations in humans are supported by reinstatement procedures in laboratory animals demonstrating that drug-associated stimuli can invoke drug-seeking behavior when presented contingently upon lever pressing during extinction (Fuchs et al., 2003; Goeders & Clampitt, 2002; Kruzich et al., 2001; Shelton, Hendrick, & Beardsley, 2004; Weiss et al., 2001). The observations that drug-seeking behavior is a function of stimulus control is important with regard to treating drug addiction and the role that environmental context may play in facilitating relapse to drug taking.

The purpose of the present studies was to characterize dose response functions relating cocaine dose to drug-seeking behavior in cocaine's absence during a daily, two-component reinforcement schedule, and to determine the persistence of cocaine-seeking behavior reinforced by BS presentations associated with different doses of cocaine. The strength of daily cocaine-seeking behavior, and its persistence under break point test conditions, were related to changes in stimuli associated with increases in cocaine dose as a monotonic function. In contrast, the dose-response function for cocaine infusions and break points assumed an inverted U-shaped function. Together, these results suggest that motivation to obtain cocaine

may be more meaningfully measured under conditions in which cocaine is absent than when present and reinforce the importance of the role of drug-associated stimuli in drug-seeking behavior.

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