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Effect of Time-Out Duration on the Reinforcing Strength of Cocaine Assessed Under a Progressive-Ratio Schedule in Rhesus Monkeys

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Abstract

Progressive-ratio (PR) schedules of reinforcement have provided valuable information regarding the reinforcing strength of cocaine and the underlying neurobiological mechanisms. Parametric manipulations, such as altering time-out (TO) values, can affect the shape of the cocaine dose-response curve. Previous studies have used PR schedules with widely varying parameters; thus complicating comparisons across experiments. The present study evaluated the reinforcing strength of cocaine (0.005 – 0.9 mg/kg) as a function of post-reinforcement TO duration (5, 10, 30 or 60 min) under a PR schedule in rhesus monkeys. Daily sessions ended when 2 h elapsed without an injection; the total number of injections was defined as the breakpoint (BP) value. When the TO was 10 min, the relationship between cocaine dose and number of injections received (i.e., BP) was characterized by an inverted U-shaped curve in all monkeys. Increasing the TO to 30-min resulted in a rightward shift of the ascending limb of the dose-response curve, but did not affect self-administration of higher doses. The number of injections received of a low cocaine dose was not further increased when the TO was shortened to 5 min, nor did increasing the TO to 60 min alter self-administration of the highest tested dose. These results suggest that drug accumulation plays a role in determining the reinforcing strength of low and intermediate cocaine doses under PR schedules. However, the reinforcing strength of higher cocaine doses was unaffected by manipulating TO, suggesting that the BP value is a useful measure of reinforcing strength.

Keywords

Self-Administration; Progressive-Ratio; Reinforcing Strength; Rhesus Monkey

Introduction

Whereas simple reinforcement schedules (e.g., fixed-ratio (FR) or fixed interval schedules) indicate whether a drug can have reinforcing effects, progressive-ratio (PR) schedules provide information regarding the strength of a drug as a reinforcer. PR schedules of reinforcement in rodents, nonhuman primates and humans (for review see Richardson and Roberts, 1996; Arnold and Roberts, 1997; Stafford et al.,1998) are characterized by substantial procedural differences which may affect generalization of conclusions across studies. In nonhuman primates, for

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example, the primary dependent variable, breakpoint (BP), is sometimes defined as the value of the last ratio completed before the session ends (e.g., Rowlett et al., 1996) or as the final ratio completed when an injection has not been received for a specific time (e.g., Lile et al., 2002). Moreover, ratio increments are sometimes increased within a session (e.g., Rowlett et al., 1996; Lile et al., 2002) and sometimes across days (e.g., Griffiths et al 1975). The concordance of data obtained with these divergent procedures is not well understood. In order to extend the predictive capability of PR schedules as measures of reinforcing strength, extensive parametric studies are necessary to understand how schedule parameters influence measures of reinforcing strength (cf. Katz, 1990). Ideally, parameters should be used that minimize direct effects of cocaine on operant responding (i.e., behavioral stimulant or disruptive response rate-altering effects) so that alterations in BP represent differences in the strength of reinforcing effects.

Negus and Mello (2003) used a modification of a PR schedule to investigate the role of time-out (TO) duration (i.e., the time following reinforcement during which stimulus lights are off and responding has no scheduled consequences) and found that changes in TO had minimal effects on BP generated by 0.032 mg/kg cocaine or 1-g food pellets. The influence of TO duration on cocaine self-administration under PR schedules has been examined under the procedure used by Woolverton, Rowlett and colleagues (e.g. Woolverton, 1995; Rowlett et al., 1996). In those studies the schedule consisted of five components, each made up of 4 trials for 20 total injections. Each trial had the same response requirement which doubled in the subsequent component. Each trial ended with an injection or expiration of a limited hold (12 or 24 min) followed by a 15– or 30-min TO. In these studies, the 30-min TO duration resulted in a greater number of injections and response rates than the 15-min duration. Additionally, Rowlett et al. (1996) demonstrated that increasing the TO duration produced an asymptotic dose-response curve with no descending limb rather than the biphasic curve typically observed under simple schedules Finally, Woolverton et al. (2002) reported that increasing the TO from 30 to 60 min resulted in decreases in maximum cocaine injections. Thus, it appears that increases in TO produce biphasic effects, with higher values resulting in decreased measures of reinforcing strength.

Our laboratory uses a different procedure (e.g. Lile et al., 2002, Lile et al.,2003; Czoty et al., 2006) in which the response requirement increases with successive injections and the session ends when 2 hours pass without an injection. The primary aim of the present study was to determine whether altering TO duration under this procedure produced similar results to those observed by Woolverton, Rowlett and colleagues. Complete cocaine dose-effect curves (0.005 – 0.9 mg/kg per injection) were generated under conditions in which the post-reinforcer TO duration was 10 or 30 min. We also examined a low dose (0.015 mg/kg) when the TO was 5 min and the highest dose (0.9 mg/kg) when the TO was 60 min. We hypothesized that, at lower cocaine doses, longer TO durations would prevent cocaine accumulation and decrease the number of self-administered injections whereas shorter TO durations would result in increased self-administration due to an increase in the behavioral stimulant effects of cocaine secondary to drug accumulation. At higher cocaine doses, longer TO durations were hypothesized to reduce drug accumulation and the rate-decreasing effects, increasing the number of injections received.

Methods

Subjects and apparatus

Four male rhesus macaques (*Macaca mulatta*), with extensive histories of cocaine self-administration and previous exposure to various monoamine transporter inhibitors, served as subjects (Lile et al., 2002, Lile et al.,2003; Czoty et al., 2006; Martelle et al., 2007). The monkeys' diet consisted of 1-g banana-flavored food pellets which were available under an

FR 50 schedule of reinforcement (see below). Fruit and vegetables were also given several times per week Monkeys were individually housed in sound-attenuating chambers (0.91×0.91×0.91 m; Plas Labs, Lansing, MI). The door was constructed of Plexiglas to allow the monkey visual access to the laboratory. Each cubicle was equipped with two response levers (BRS/LVE, Beltsville, MD) mounted on the chamber door. Four stimulus lights, alternating white and red, were located horizontally above each lever. Each animal was fitted with a stainless-steel harness and spring arm (Restorations Unlimited, Chicago, IL) that attached to the rear of the cubicle. A peristaltic infusion pump (Cole-Parmer Co., Chicago, IL) was located on top of the chamber for delivering injections at approximately 1.5 ml/10 s. Different cocaine doses were studied by changing the available concentration of cocaine.

Catheter Implantation

Under sterile conditions, each monkey was surgically prepared with an indwelling intravenous catheter and vascular access port (Access Technologies, Skokie, IL) as described previously (Martelle et al., 2007). Following surgery the monkey was returned to its home cage where tubing connected to the infusion pump was threaded through the spring arm and attached to the vascular access port via a 20-gauge Huber Point Needle (Access Technologies). To prolong patency, ports and catheters were filled with a solution of heparinized saline (100 U/mL) between experimental sessions.

Experimental Procedure

Monkeys had been previously trained to self-administer (-)cocaine HCl (National Institute on Drug Abuse, Bethesda, MD; dissolved in sterile 0.9% saline) under a PR schedule and 1-g banana-flavored pellets (P.J. Noyes Co., Lancaster, NH) under an FR 50 schedule of reinforcement (Czoty et al., 2006). During the present study, access to food began at approximately 08.00 h and access to cocaine began at approximately 15.00 h. Cocaine was contingent on responding on the right lever, while food pellets were contingent on left lever responding, with availability signaled by the illumination of the white lights above the appropriate lever. Food availability ended once the monkey had received its daily ration of pellets calculated for each monkey to maintain body weight at approximately 95% of free-feeding levels. Importantly, in all cases during the collection of the data in the present report, monkeys earned all pellets and food availability ended prior to the start of drug availability. Usually, this occurred more than three hours before the start of cocaine self-administration session.

Under the PR schedule, completing the response requirement resulted in a 10-s injection, extinguishing of the white lights and illumination of the red lights for 10-s, followed by a 10-min TO period during which all lights were extinguished and responding had no scheduled consequences. The response requirement for subsequent injections was determined by the equation used by Richardson and Roberts (1996): ratio = $[5e^{(\text{injection\#} \times 0.2)}]-5$. For these studies, the first response requirement (50 responses) corresponded with the 12^{th} value given by the equation and was followed by 62, 77, 95, 117, 144, etc (see Fig. 1). Immediately prior to the start of each experimental session (approximately 7 h prior to cocaine availability), the catheter was filled with the drug solution to be administered during that session.

Cocaine dose (saline, 0.005 – 0.9 mg/kg/inj) and TO duration (10 or 30 min) combinations were presented in quasi-random order for at least 5 consecutive sessions and until responding was stable (± 20% of the mean for the previous three sessions with no trend). Once stability was achieved for a given combination, either dose or TO duration was manipulated. When all doses had been examined under the 10- and 30-min TO durations, 0.9 mg/kg cocaine was tested using a 60-min post-reinforcer TO. Subsequently, 0.015 mg/kg cocaine was tested using a 5-min TO.

Data Analysis

The primary dependent variable was the number of injections administered during the PR session. For each monkey, number of injections during the last three sessions at each dose- TO combination was averaged, and data are presented as mean (±SEM) across monkeys. Data were analyzed using one-way and two-way repeated measures analyses of variance (ANOVA) and post-hoc Bonferroni tests. Statistical significance was accepted at the 95% level of confidence ($p<0.05$).

Results

Effect of cocaine dose on breakpoint

Complete cocaine dose-response curves were generated at two TO values. In all subjects, under both 10-min and 30-min TO conditions, the number of injections received increased as a function of cocaine dose and engendered inverted U-shaped dose-response curves, with the peak number of injections occurring at 0.45 mg/kg per injection (Fig. 1). There was a significant main effect of dose for both the 10-min and 30-min TO conditions ($F_{6,3}=24.14$, $p<0.001$ and $F_{6,3}=45.26$, $p<0.001$, respectively). All except the lowest cocaine dose (0.005 mg/kg) resulted in more injections than saline ($p<0.05$).

Effect of TO duration on breakpoint

Increasing the TO duration from 10 min to 30 min resulted in rightward shift in the ascending limb of the cocaine dose-response curve (Fig. 1). A two-way repeated measures ANOVA revealed significant main effects of TO duration ($F_{1,21}=14.10$, $p<0.01$) and dose ($F_{6,21}=28.85$, $p<0.001$) and a significant interaction ($F_{12,21}=3.65$, $p<0.05$). Post-hoc analyses revealed that number of injections differed significantly according to TO duration only during availability of 0.015 and 0.045 mg/kg cocaine ($p<0.05$ and $p<0.01$, respectively). When a low cocaine dose was studied (0.015 mg/kg) with a 5-min TO, a one-way repeated measures ANOVA comparing the self-administration of that dose across the three TO durations showed a significant effect of TO duration ($F_{2,3}=7.18$, $p<0.025$); post-hoc analysis showed that the number of injections administered was significantly higher in sessions with a 5-min versus 30-min TO ($p<0.05$). When 0.9 mg/kg was available with a 60-min TO, a repeated measures one-way ANOVA indicated no significant differences.

Discussion

Based on pharmacokinetic studies in humans (e.g. Foltin and Fischman, 1991) and monkeys (e.g. Mello et al., 2002) and studies manipulating post-reinforcer TO durations under FR and FI schedules (e.g. Balster and Schuster, 1973; Johanson, 1982; Winger, 1993), it was hypothesized that shortening the TO would increase the reinforcing strength of low doses of cocaine by increasing drug accumulation. In the present findings, a shorter TO value (10 min) resulted in a cocaine dose-response curve to the left of that obtained at a longer TO duration (30 min), supporting this hypothesis. However, decreasing the TO duration to 5 min during availability of a low cocaine dose (0.015 mg/kg) did not further increase reinforcing strength, suggesting that maximal effects are observed when the TO is 10 min. These data suggest that the reinforcing strength of doses on the ascending limb of the cocaine dose-response curve are determined by factors in addition to reinforcing effects, perhaps as a result of drug accumulation.

Unlike previous studies, the peak and descending limb of the dose-effect curve were unaffected by manipulating TO duration. Under the PR schedule described above (e.g., Rowlett et al., 1996), increasing the post-injection TO from 15 to 30 min flattened or shifted upward the descending limb of the dose-response curve, resulting in dose-effect curves that were

asymptotic rather than inverted U-shaped. Those authors attributed alterations in the dose-effect curve to reductions in the disruptive or rate-decreasing effects of cocaine at high doses due to reduced drug accumulation at the longer TO duration. In other studies using a PR schedule in which the ratio requirement increased daily until a dose of cocaine failed to maintain responding, the dose-response curve was inverted-U shaped even with a 3-hr TO (Griffiths et al., 1979).

Several procedural differences between those studies and the present study could each have contributed to the differences observed. In the present study the response requirement increased after each injection, the maximum response requirement was determined by the subject, the limited hold was 5 to 10 times longer and a larger range of cocaine doses was tested. The descending limb under the 15-min TO in the previous two studies was situated to the left of the descending limb under the 10-min TO condition in this study, possibly suggesting that the PR schedule used in the present study produced a less flexible descending limb. Overall, the present results indicate that, under PR schedules of drug self-administration, the influence of specific parameters (in this case, post-reinforcer TO duration) on measures of reinforcing strength can differ across procedures. Under the procedure used in the present study, the reinforcing strength of lower cocaine doses was influenced by TO duration, suggesting that drug accumulation may increase self-administration at these doses. Higher cocaine doses, on the other hand, were unaffected by changes in TO duration. Thus, in studies investigating the effects of pharmacological variables on the reinforcing strength of cocaine under this PR procedure, changes in the reinforcing strength of higher unit doses of cocaine might more purely reflect changes in reinforcing versus nonspecific, response rate-increasing or -decreasing effects of cocaine.

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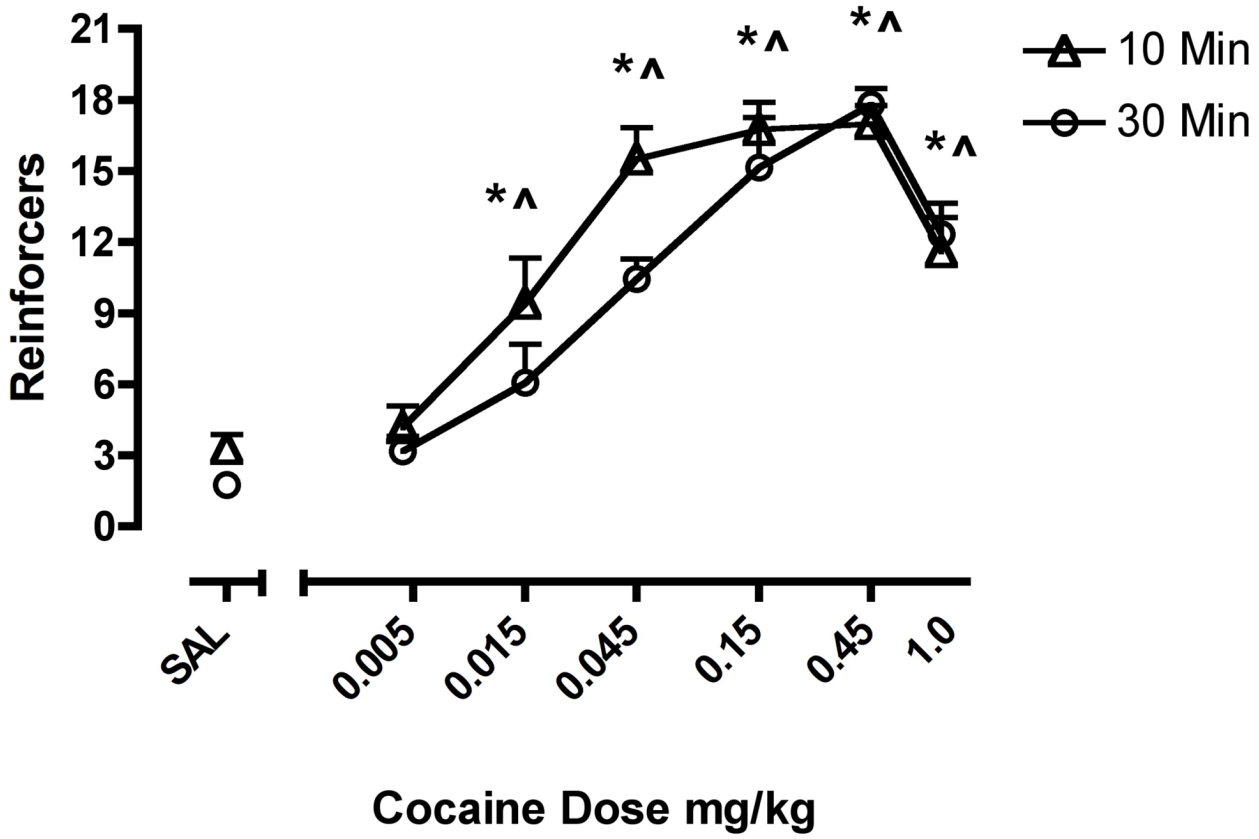

Figure 1.

Mean (+ SEM, n=4) number of injections received under a PR schedule of cocaine self-administration. *Vertical axis*, number of cocaine injections. *Horizontal axis*, available cocaine dose. Symbols represent different TO durations. Asterisks indicate a significant difference between 10- and 30-min TO durations at a given dose (*, $p<0.05$; **, $p<0.01$).