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ORIGINAL INVESTIGATION

J.K. Rowlett · B.W. Massey · M.S. Kleven
W.L. Woolverton

# Parametric analysis of cocaine self-administration under a progressive-ratio schedule in rhesus monkeys

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**Abstract** The present study was designed to investigate parameters and quantitative analysis of cocaine self-administration under a progressive-ratio (PR) schedule of reinforcement, with the goal of enhancing the resolution of PR schedules for measuring reinforcing efficacy. Six rhesus monkeys were prepared with chronic intravenous catheters and trained to self-administer cocaine under a PR schedule. The schedule consisted of five components, each made up of four trials (i.e., 20 trials total). Each trial within a component had the same response requirement. Three initial response requirements were tested: fixed-ratio (FR) 60, FR 120 and FR 240. The response requirements doubled in successive components to a maximum of FR 960, FR 1920 or FR 3840, respectively, in the fifth component. A trial ended with an injection or the expiration of a 12- or 24-min limited hold (LH). The inter-trial interval (ITI) was 15 or 30 min. Four dependent measures were assessed: break point (last FR completed), injections/session, responses/session and response rate (responses/s). For the three initial FRs, the break point, number of injections/session, responses/session and rate increased with dose of cocaine (0.013–0.1 mg/kg per injection) at both ITI/LH values. At the ITI15/LH12, responding decreased at higher doses, i.e., the dose-response functions were biphasic. In contrast, at the ITI30/LH24, responding reached an asymptote at higher doses. In general, cocaine maintained significantly higher break points, injections/session, responses/session and rate at ITI30/LH24 than at ITI15/LH12. However, at both ITI/LHs, as initial FR was increased, injections/session at the higher doses decreased while break point, total responses/session and rate did not change. A ceiling on performance, as assessed by break point, total responses/session and response rate, may have limited the number of cocaine injections an animal could take in a session. The results of this study indicate that optimal conditions for measuring the reinforcing efficacy of cocaine were obtained at the longer ITI/LH and at initial FRs above 60.

**Key words** Self-administration · Reinforcement · Progressive-ratio · Cocaine · Rhesus monkey · Reinforcing efficacy

J.K. Rowlett · W.L. Woolverton (✉)
Department of Psychiatry and Human Behavior,
University of Mississippi Medical Center,
Jackson, MS 39216, USA

B.W. Massey[1] · M.S. Kleven[2] · W.L. Woolverton
Drug Abuse Research Center, Pritzker School of Medicine,
The University of Chicago, Chicago, IL 60637, USA

*Present addresses:*
[1] Astra/Merck Group of Merck & Co., Inc., 6075 Poplar Avenue,
Memphis, TN 38119, USA
[2] Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin,
F-80106 Castrex Cedex, France

## Introduction

Intravenous drug self-administration procedures have been used extensively to characterize and compare the reinforcing effects of drugs (Schuster and Thompson 1969; Johanson and Balster 1978). The concept of reinforcing efficacy, i.e., the maximum reinforcing effect of a drug, has proven useful when comparing among drugs (Griffiths et al. 1975, 1979; Nader and Woolverton 1991; see, however, Katz 1990). Relative reinforcing efficacy has been examined using choice procedures (e.g., Johanson and Schuster 1975; Woolverton and Johanson 1984) and progressive-ratio (PR) procedures (e.g., Griffiths et al. 1975, 1978, 1979; Hoffmeister 1979; Risner and Silcox 1981). Under a PR schedule, the response requirement for obtaining a reinforcer is increased until responding stops. The response requirement at which responding ceases is often termed "break point". PR procedures have been used to examine the reinforcing effects of a variety of stimuli, including sweetened solutions (Hodos 1961), electrical brain stimulation (Hodos 1965), food (Spear and Katz 1991) and drugs (e.g., Griffiths et al. 1975, 1979). In particular, PR procedures have been used to study the reinforcing effects of psychomotor stimulants in rats (e.g., Roberts et al. 1989; Depoortere et

al. 1993; Roberts 1993), dogs (e.g., Risner and Silcox 1981) and non-human primates (e.g., Griffiths et al. 1975, 1978, 1979; Winger and Woods 1985; Spear and Katz 1991). When a psychomotor stimulant (e.g., cocaine) is made available for self-administration under a PR schedule, break points tend to increase with increasing doses and reach an asymptotic level (Bedford et al. 1978; Griffiths et al. 1979; Depoortere et al. 1993) or decline at higher doses (Griffiths et al. 1979; Winger and Woods 1985). Psychomotor stimulants can be rank-ordered according to break points, and these results are generally consistent with knowledge of the relative abuse liability of these drugs in humans (Griffiths et al. 1978). In addition, in a study examing self-administration of cocaine and procaine under PR schedules (Woolverton 1995), the reinforcing efficacy of cocaine relative to procaine was consistent with the intrinsic efficacy of these compounds in blocking dopamine uptake in rat brain (Woodward et al. 1995). Thus, results from drug self-administration studies using PR schedules may provide information useful in understanding in vivo pharmacological action of psychomotor stimulants.

Meaningful rank ordering of drugs in terms of reinforcing efficacy depends upon our ability to quantify reliable differences using relevant dependent and procedural variables. Many issues need to be resolved before definitive conclusions can be made concerning PR procedures and reinforcing efficacy. For example, some investigators have shown that, under certain conditions, break points provide results similar to response rate in fixed-ratio (FR) procedures (Griffiths et al. 1979; Winger and Woods 1985). That is, dose-response functions for many compounds self-administered under PR schedules are biphasic functions, conforming to an inverted U-shape (Griffiths et al. 1979; Winger and Woods 1985). Thus, responding under PR procedures may be determined by effects other than reinforcing effects (e.g., motoric impairment, satiation). This potential confound is similar to that often noted for the more typically used FR procedures (Winger and Woods 1985; Skoldager et al. 1991) and could complicate interpretation of results from PR experiments. In addition, it has been noted that break points have inherent problems in terms of measurement and statistical analyses (Katz 1990; Depoortere et al. 1993).

The present study was designed to investigate parameters and quantitative analysis of cocaine self-administration under a PR schedule of reinforcement, with the goal of enhancing the resolution of PR schedules for measuring reinforcing efficacy. Specifically, this study examined whether and how the initial FR requirement in the progression and the inter-trial interval (ITI) and limited hold (LH) would affect the dose-response function for cocaine. The PR procedure used was designed to improve estimation of break points by including multiple determinations of self-administration at each response requirement in the progression. This procedure has been previously shown to reliably differentiate the maximum reinforcing effects of cocaine and procaine, with cocaine obtaining a greater maximum effect than procaine (Woolverton 1995). The purpose of the ITI/LH manipulation was to assess whether lengthening the time between injections and increasing the amount of time available to complete an FR would alter the cocaine dose-response function. Specifically, it was predicted that cocaine self-administration would increase as the ITI/LH was increased, due to lengthening the time between injections. In addition to manipulating schedule parameters, we compared two different approaches of statistical analyses of the data. The first approach used analyses of variance techniques and the second approach used non-linear regression analysis to examine treatment effects among four dependent measures: break point, injections/session, total responses/session and response rate.

## Materials and methods

### Animals and apparatus

The subjects were six adult male rhesus monkeys (*Macaca mulatta*) with body weights between 5.6 and 11.0 kg. Three of the monkeys (8217, 9126, 9163) were naive, one monkey (9031) had previously been exposed to continuous IV infusions of cocaine while responding under fixed-ratio (FR) schedules of food reinforcement and the other monkeys (9033, 9118) had a history of both food- and cocaine-maintained responding with continuous infusions of various drugs (Kleven and Woolverton, unpublished data). Each monkey was fitted with a stainless-steel restraint harness and spring arm which was attached to the rear of an experimental cubicle (90 cm wide×90 cm deep×90 cm high) in which the monkey lived for the duration of the experiment. Two response levers (BRS/LVE, PRL-001, Beltsville, Md.) were mounted on the inside of the transparent front of each experimental cubicle 10 cm above the floor. Four jeweled stimulus lights, two red and two white, were mounted directly above each lever. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer, Chicago, Ill.) located outside the cubicle. All programming and recording of experimental events was accomplished by a Macintosh II computer and associated interfaces and cumulative recorders located in an adjacent room. Water was available continuously and each monkey was fed, immediately after the experimental session, a sufficient amount of monkey chow (Ralston-Purina, St Louis, Mo.) to maintain a stable body weight. In addition, each monkey was given a chewable vitamin tablet 3 days/week.

Each monkey was adapted to the cubicle and restraint system and had a catheter implanted according to the following protocol. The monkey was injected with a combination of ketamine hydrochloride (1.0 mg/kg, IM) and atropine sulfate (0.04 mg/kg, IM) followed in 20–30 min by inhaled halothane. When anesthesia was adequate, a catheter was surgically implanted into a major vein. For internal and external jugular and femoral veins, a silicone catheter (0.076 cm i.d., 0.26 cm o.d.; Cole-Parmer, Chicago, Ill.) was used. For brachial veins, the catheter was Micro-Renethane (0.1 cm i.d., 0.2 cm o.d.; Braintree Scientific, Braintree, Mass.) drawn to a tapered tip after heating. An antibiotic (Keflin; Eli Lilly, Indianapolis, Ind.), was injected (200 mg) locally to the incision site and was administered (100 mg) IM twice daily for 7 days to prevent infection. Following surgery the monkey was returned to the experimental cubicle and the catheter was threaded through the spring arm, out the back of the cubicle and connected to the infusion pump. If a catheter became non-functional during the experiment, a new catheter was implanted as before following a 1- to 2-week period to allow any infection to clear. Catheters were filled between sessions with a solution of 100 units/ml heparin to prevent clotting at the catheter tip.

### Procedure

Experimental sessions, signaled by the illumination of all white lever lights, were conducted at the same time each day, 7 days a week. During initial sessions, one lever press on either the right or the left lever, individually determined, resulted in an injection of cocaine (0.1 mg/kg per injection). The white lights were turned off and the red lights were illuminated during drug infusion. These initial sessions had no ITI or LH. After stable baseline responding was established (<10% variation in total number of injections/session for at least five consecutive sessions with no consistent trend), the PR schedule was initiated with short ITI/LHs. The response requirements and ITI/LHs were gradually increased to the terminal conditions of the PR schedule. The PR schedule consisted of five components, each made up of four trials (i.e., 20 trials total). Each trial within a component had the same response requirement. Three different initial response requirements were used. Thus, the response requirement in the first component was FR 60/trial, FR 120/trial or FR 240/trial and doubled in successive components to a maximum of FR 960, FR 1920 or FR 3840, respectively, in the fifth component. A trial ended with an injection or the expiration of a limited hold (LH). Trials were separated by an ITI of 15 or 30 min. Following an injection or expiration of the LH (12 or 24 min, respectively), all stimulus lights were extinguished and responding had no consequence for the remainder of the trial. A session ended when the response requirement was not met within the LH for two consecutive trials or when all 20 trials had been completed. Thus, in order to complete a component and progress to the next FR, at least two trials had to be completed within the component.

The ITI/LH manipulation was designed primarily to assess the effects of increasing the time between injections on the cocaine dose-response function, as it varied with initial FR. The proportion of the ITI that consisted of the LH, in the absence of an injection, was held constant. That is, the LH was 80% of the ITI in both cases. Thus, in addition to varying the time between injections, the ITI/LH manipulation also varied the amount of time the monkey had to complete an injection. The purpose for the LH manipulation was to allow more time for a monkey to respond at the higher ITI/LH, in anticipation of higher FRs being completed by the monkey at the higher ITI/LH compared to the lower ITI/LH.

When behavior maintained by cocaine was stable, saline was substituted for cocaine until responding declined to low levels. Cocaine-maintained behavior was then re-established and various doses of cocaine (0.013–0.4 mg/kg per injection) were made available in an irregular order until responding was stable. All conditions (cocaine dose, ITI, initial FR) were presented to each monkey in an irregular order.

### Data analysis

Four dependent measures were analyzed: break point (last FR completed), total number of injections/session, total responses/session and the mean response rate (responses/s). Response rate was calculated by dividing the total responses by the total time between initiation of a trial and injection, plus the limited holds on trials that were not completed. Each dose×initial FR×ITI/LH condition was available to the monkeys until responding achieved the 5-day stability criteria. Each dependent measure was obtained for an individual monkey by calculating the mean over the 5 days of stability for each condition. Individual monkey data and mean values were calculated for each dependent measure and presented in Figs 1–4. Incomplete trials were defined as any trial in which responding occurred but no injection was delivered (i.e., the monkey initiated but did not complete the FR). The total number of incomplete trials was obtained across the 5 days of stable performance for each monkey in each condition.

The data presented in Figs 1–4 were analyzed initially for violations of homogeneity of variance, since violation of homogeneity of variance has been noted previously in data from progressive-ratio experiments (Depoortere et al. 1993). Violation of homogeneity of variance was assessed in two ways. First, the data were analyzed using Cochran's test (see Kirk 1982). Second, Huyn-Feldt epsilon values were calculated for the data (Huyn and Feldt 1976). Degrees of freedom were corrected if the epsilon value was <0.75. To further evaluate the nature of the distribution of variance to mean values, SEM values were plotted as a function of the means independent of various factors in the analysis and a Pearson product moment correlation coefficient ($r^2$) calculated. All animals were used in the tests for violation of homogeneity of variance, except for Huyn-Feldt epsilon values, which were calculated using a subset of the data ($n$=4 monkeys) used in the analysis of variance computations (see below).

In cases where data violated the assumption of homogeneity of variance, the dependent measure to be analyzed was transformed according to the method of Kirk (1982, p. 83). Briefly, four transformations ($\log_{10}$, square root, reciprocal, inverse sine) were applied to the largest and smallest values of each treatment condition and a range computed. The ratio of largest to smallest range was computed for each transformed data set, and the transformation with the smallest ratio was used where appropriate.

The data presented in Figs 1–4 were analyzed using two approaches. First, each dependent measure (non-transformed or transformed) was analyzed using a repeated measures analysis of variance (ANOVA). Because not all cells in the analysis had equal sample sizes, the ANOVA was performed on the conditions in which the largest sample size could be obtained (four doses, using monkeys 8217, 9033, 9118, 9163). Thus, the analysis was a 4 (dose levels: 0.025, 0.05, 0.1, 0.2)×2 (ITI/LH: 15/12, 30/24 min)×3 (initial FR: FR 60, FR 120, FR 240) repeated measures ANOVA, performed on a subset of the sample ($n$=4). Multiple comparisons were made using the Bonferroni $t$ procedure. Because these ANOVAs were performed on a subset of the data, the data from these four monkeys are presented as mean and standard error of the mean (SEM) values using insets in Figs 1–4.

The second approach to analyzing the data used GraphPad Prism software to calculate non-linear regression analyses for the dose-response functions for each treatment level (non-transformed only). The non-linear regression analysis used was an iterative curve-fitting technique using equations for sigmoidal dose-response functions with variable slopes. The equation used for the analyses was the four parameter logistic equation:

$$Y=\text{minimum}+(\text{maximum}-\text{minimum})/(1+10^{((\log ED_{50}-X)*\text{slope})}).$$

Non-linear regression analyses were performed separately on means for each dependent measure (the 0.4 mg/kg per injection condition was omitted, because only one monkey was tested in this condition). The minimum value of each dependent measure was constrained to zero (i.e., either no injections taken or responses made), all other parameters were free to vary. For each treatment and dependent measure, $ED_{50}$ (dose at which 50% of the maximum effect was obtained) and $E_{max}$ (maximum effect obtained) estimates were obtained. Confidence intervals (95%) were obtained for both parameters and used to test differences between treatment means. Goodness-of-fit coefficients also were obtained for each curve ($r^2$).

The number of incomplete trials was analyzed using a similar ANOVA procedure used to analyze the data in Figs 1–4. Thus, the analysis was a 4 (dose levels: 0.025, 0.05, 0.1, 0.2)×2 (ITI/LH: 15/12, 30/24 min)×3 (initial FR: FR 60, FR 120, FR 240) repeated measures ANOVA, performed on a subset of the sample ($n$=4).

## Results

### General

For all ANOVAs, the general pattern of results for all six monkeys was similar to that observed for the four monkeys used in the analyses. Cochran's tests revealed that break point [$C(3, 24)=0.40$, $P<0.05$] and responses/ses-

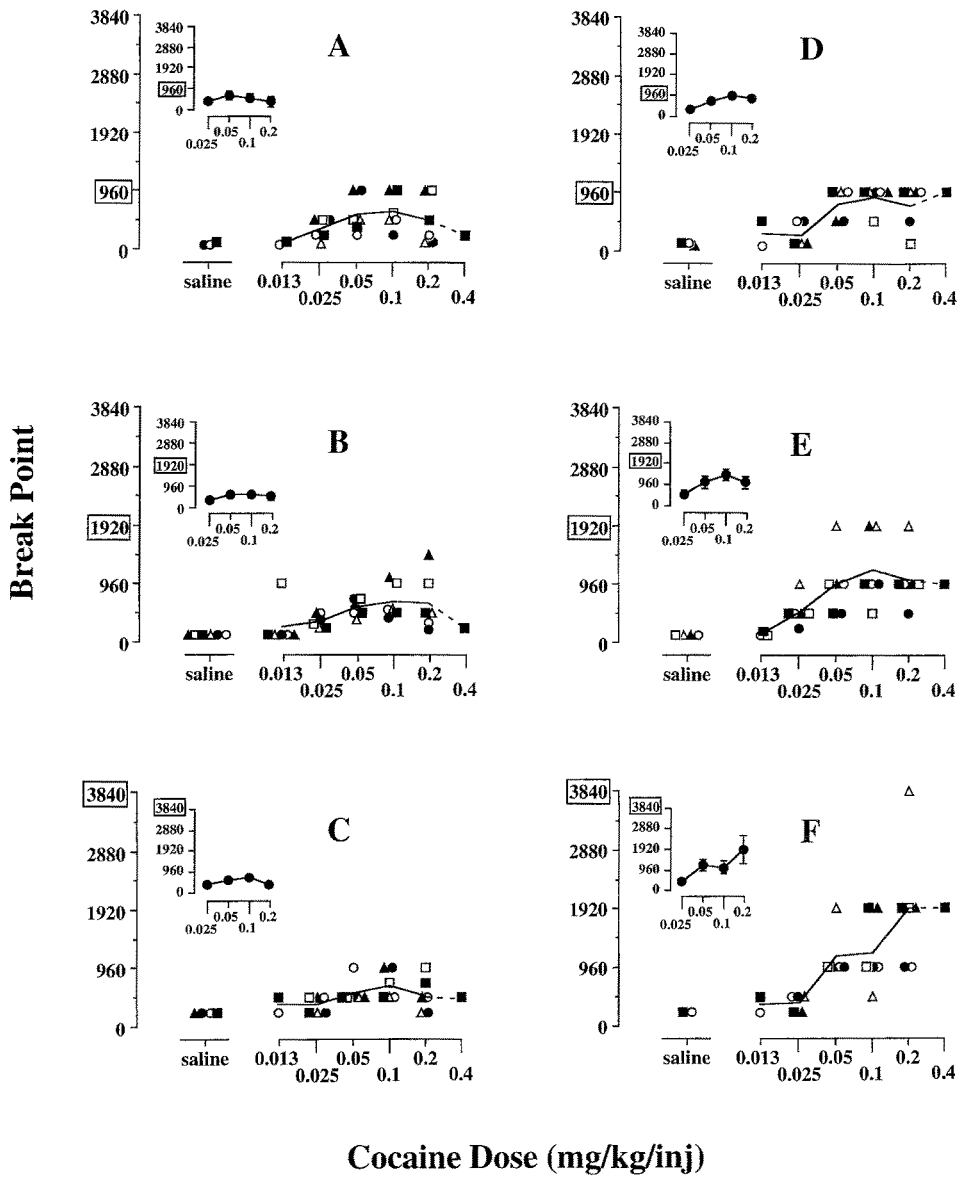


**Fig. 1A–F** Cocaine self-administration under a progressive-ratio schedule, expressed as break point (maximum ratio completed). The three panels on the *left* represent the following initial fixed-ratios (FRs) under the *inter-trial interval (ITI) 15 min/limited hold (LH) 12 min* condition: **A** initial FR 60, **B** initial FR 120, **C** initial FR 240. The three panels on the *right* represent the following initial FRs under the *ITI30 /LH24* condition: **D** initial FR 60, **E** initial FR 120, **F** initial FR 240. *Symbols* represent individual monkeys and lines without symbols represent means (*broken lines* indicate only one monkey in that condition). Insets are means ±SEM (represented by error bars) for monkeys 8217, 9033, 9118 and 9163, used in the ANOVAs (see text). Symbols without error bars indicate that the bars were smaller than the symbols. Boxes around y-axis numbers represent the maximum obtainable break point under an initial FR condition. Symbols for individual monkeys: ○ (*open circle*) 8217; ● (*filled circle*) 9033; △ (*open triangle*) 9118; ▲ (*filled triangle*) 9163; □ (*open square*) 9126; ■ (*filled square*) 9031

sion [$C(3, 24)=0.27$, $P<0.05$] violated the assumption of homogeneity of variance. Therefore, transformations were used in the ANOVAs for break point and responses/session. The ratio of the largest and smallest ranges for break point data was smallest for the inverse sine transformation and, for responses/session, smallest for the square root tranformation. Correlational analyses comparing means and standard errors for each dependent measure revealed a positive relationship for break point ($r^2=0.67$, $P<0.001$) and responses/session ($r^2=0.68$, $P<0.001$). The correlations for injections/session and rate were not reliably different from zero ($r^2=0.01$ and 0.25, respectively).

Of the 24 functions analyzed in the non-linear regression analyses, no fit failed to reach convergence and all curves had goodness-of-fit coefficients that ranged from $r^2=0.960$–$0.999$. Thus, even with some dose-response functions tending toward an inverted U-shape, the functions in general conformed well to a sigmoidal dose-response equation.

## Break point

Mean break point (Fig. 1, insets) increased as a function of dose [main effect of dose, $F(3, 9)=9.556$, epsilon=1.003, $P<0.05$]. The main effects of initial FR and ITI/LH were not significant. There was a significant interaction between ITI/LH and cocaine dose [$F(3, 9)=8.473$, epsilon=2.24, $P<0.01$]. This interaction likely reflects that, collapsed across initial FRs, the dose-response function was an inverted U-shaped function at ITI15/LH12 (Fig. 1, left panels) while generally sigmoidal in shape at ITI30/LH24 (Fig. 1, right panels). Bonferroni *t*-tests performed on the ITI/LH×cocaine dose interaction revealed that break point (collapsed

**Table 1** Estimates for $ED_{50}$ (dose producing 50% of maximum effect) and $E_{max}$ (maximum effect obtained) for cocaine self-administration under a progressive-ratio schedule in rhesus monkeys ($n$=6). Abbreviations: *ITI* inter-trial interval, *FR* fixed-ratio, *CI* 95% confidence intervals. Estimated parameters were derived from non-linear regression analysis of sigmoidal dose-response functions (see text)

| Measure | Initial FR | ITI/LH | $ED_{50}$ (CI) | $E_{max}$ (CI) |
|---|---|---|---|---|
| Break point | 60 | 15/12 | 0.020 (0.012–0.033) | 563 (367–757) |
| | | 30/24 | 0.030 (0.023–0.038) | 888 (760–1020)*** |
| | 120 | 15/12 | 0.023 (0.013–0.042) | 614 (419–809) |
| | | 30/24 | 0.026 (0.016–0.042) | 1130 (825–1430)* |
| | 240 | 15/12 | 0.014 (0.005–0.041) | 572 (223–920) |
| | | 30/24 | 0.059 (0.003–1.3) | 2210 (1140–5560)* |
| Injections/session | 60 | 15/12 | 0.017 (0.011–0.027) | 14 (10–20) |
| | | 30/24 | 0.022 (0.019–0.026) | 18 (17–19)** |
| | 120 | 15/12 | 0.020 (0.017–0.023) | 11 (10–12) |
| | | 30/24 | 0.019 (0.016–0.022) | 15 (13–17)* |
| | 240 | 15/12 | 0.014 (0.006–0.033) | 7 (3–11) |
| | | 30/24 | 0.028 (0.016–0.050) | 12 (8–16) |
| Responses/session | 60 | 15/12 | 0.022 (0.015–0.033) | 3288 (2196–4381) |
| | | 30/24 | 0.028 (0.020–0.040) | 5534 (4287–6781) |
| | 120 | 15/12 | 0.024 (0.018–0.032) | 3747 (3078–4415) |
| | | 30/24 | 0.024 (0.018–0.033) | 6850 (5513–8186)* |
| | 240 | 15/12 | 0.038 (0.011–0.13) | 3140 (336–5945) |
| | | 30/24 | 0.019 (0.003–0.12) | 8894 (1733–16060) |
| Rate (responses/s) | 60 | 15/12 | 0.020 (0.016–0.024) | 1.5 (1.3–1.7) |
| | | 30/24 | 0.031 (0.014–0.065) | 2.2 (1.2–3.2) |
| | 120 | 15/12 | 0.022 (0.014–0.033) | 1.5 (1.1–1.9) |
| | | 30/24 | 0.025 (0.017–0.037) | 2.0 (1.6–2.4) |
| | 240 | 15/12 | 0.020 (0.005–0.087) | 1.2 (0.3–2.1) |
| | | 30/24 | 0.034 (0.011–0.099) | 2.0 (0.7–3.3) |

Note that $P<0.05$ by comparing confidence intervals for the following: * compared to 15-min ITI, ** compared to initial FR 240 in the same ITI, *** compared to 15-min ITI and initial FR 240 in the same ITI

across initial FR) for 0.1 and 0.2 mg/kg per injection were reliably greater at ITI30/LH24 compared to ITI15/LH12.

$ED_{50}$ estimates for the break point data ranged from 0.014 to 0.059 mg/kg per injection (Table 1). However, the mean $ED_{50}$ values tended to fall within the CIs across treatment conditions and all CIs overlapped with one another. Thus, the $ED_{50}$ estimates were not reliably different as a function of ITI/LH or initial FR, suggesting that the potency of cocaine remained the same across conditions. $E_{max}$ estimates (Table 1) at ITI30/LH24 were reliably greater than estimates at ITI15/LH12 for all initial FRs. Within the ITI30/LH24 condition, $E_{max}$ estimates were significantly greater at initial FR 240 compared to initial FR 60.

## Injections/session

Mean injections/session (Fig. 2, insets) increased as a function of dose [main effect of dose, $F(3, 9)=7.667$, epsilon=0.89, $P<0.01$] and decreased as a function of initial FR [main effect of initial FR, $F(2, 6)=42.252$, epsilon=0.649, $P<0.01$]. The main effect of ITI/LH was not significant. There was a significant interaction between ITI/LH and cocaine dose [$F(3, 9)=10.591$, epsilon=1.47, $P<0.01$]. This interaction likely reflects that, collapsed across initial FRs, the dose-response function was an inverted U-shaped function at ITI15/LH12 (Fig. 2, left panels) while generally sigmoidal in shape at ITI30/LH24 (Fig. 2, right panels). Bonferroni $t$-tests performed on the ITI/LH×cocaine dose interaction revealed that injections/session (collapsed across initial FR) for 0.2 mg/kg per injection were reliably greater at ITI30/LH24 compared to ITI15/LH12.

$ED_{50}$ estimates for injections/session ranged from 0.014 to 0.028 (Table 1). The $ED_{50}$ estimates were not reliably different as a function of ITI/LH or initial FR, suggesting that the potency of cocaine remained the same across conditions. $E_{max}$ estimates (Table 1) for initial FR 120 at ITI30/LH24 were reliably higher than this estimate at the ITI15/LH12. Also, compared within the ITI30/LH24 condition, the $E_{max}$ estimate at FR 60 was reliably higher than that of FR 240.

## Responses/session

Total responses/session (Fig. 3, insets) increased as a function of cocaine dose [main effect of dose, $F(3, 9)=6.330$, epsilon=0.852, $P<0.05$]. The main effects of initial FR and ITI/LH were not significant. There was a significant interaction between ITI/LH and cocaine dose [$F(3, 9)=12.664$, epsilon=2.228, $P<0.01$]. For the ITI/LH×dose interaction, responses/session were reliably higher for 0.05, 0.1 and 0.2 mg/kg per injection (collapsed across initial FR) at ITI30/LH24 compared to ITI15/LH12 (Bonferroni $t$-tests, $Ps<0.05$).

$ED_{50}$ estimates for responses/session ranged from 0.019 to 0.038 mg/kg per injection (Table 1). The $ED_{50}$

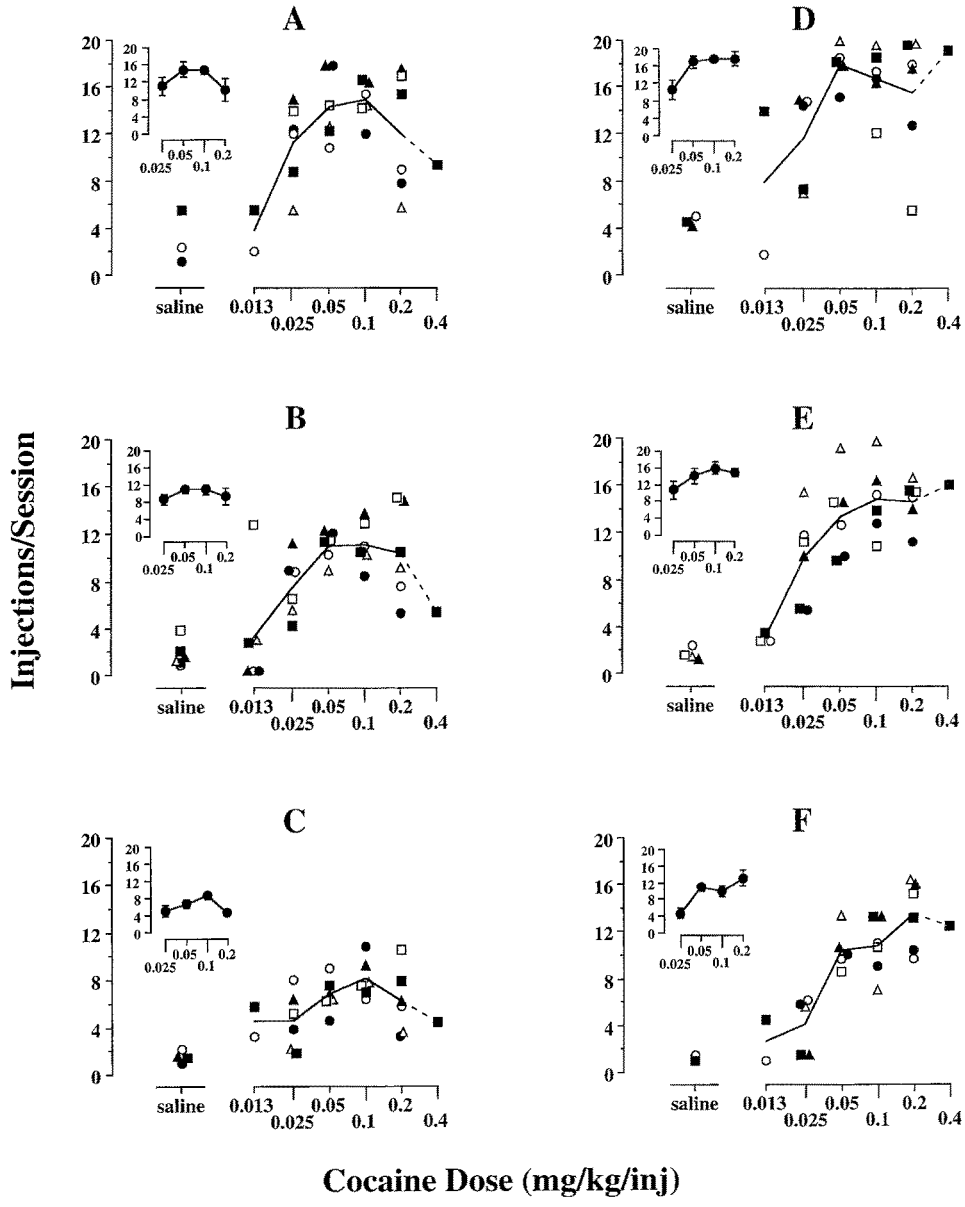


**Fig. 2A–F** Cocaine self-administration under a progressive-ratio schedule, expressed as injections/session. See Fig. 1 for details

estimates were not reliably different as a function of ITI/LH or initial FR, suggesting that the potency of cocaine remained the same across conditions. $E_{max}$ estimates (Table 1) for initial FR 120 were significantly greater at ITI30/LH24 compared to ITI15/LH12.

Response rate

Response rate (Fig. 4, insets) increased as a function of cocaine dose [main effect of dose, $F(3, 9)=5.790$, epsilon=0.749, $P<0.05$]. The main effects of initial FR and ITI/LH were not significant. There was a significant interaction between ITI/LH and cocaine dose [$F(3, 9)=8.316$, epsilon=0.66, $P<0.05$]. At the dose of 0.2 mg/kg per injection, rate was higher at ITI30/LH24 compared to ITI15/LH12 (Bonferroni $t$-test, $P<0.05$). No other multiple comparisons were significant.

$ED_{50}$ estimates for response rates ranged from 0.020 to 0.034 mg/kg per injection (Table 1). The $ED_{50}$ estimates were not reliably different as a function of ITI/LH or initial FR, suggesting that the potency of cocaine remained the same across conditions. No effects were observed for $E_{max}$ estimates (Table 1).

Incomplete trials

The overall mean was three incomplete trials per 5 days of stability. The range was one to seven incomplete trials per 5 days of stability. There was no evidence that incomplete trials varied as a function of dose, initial FR or ITI/LH (No main effects or interactions were significant, data not shown).

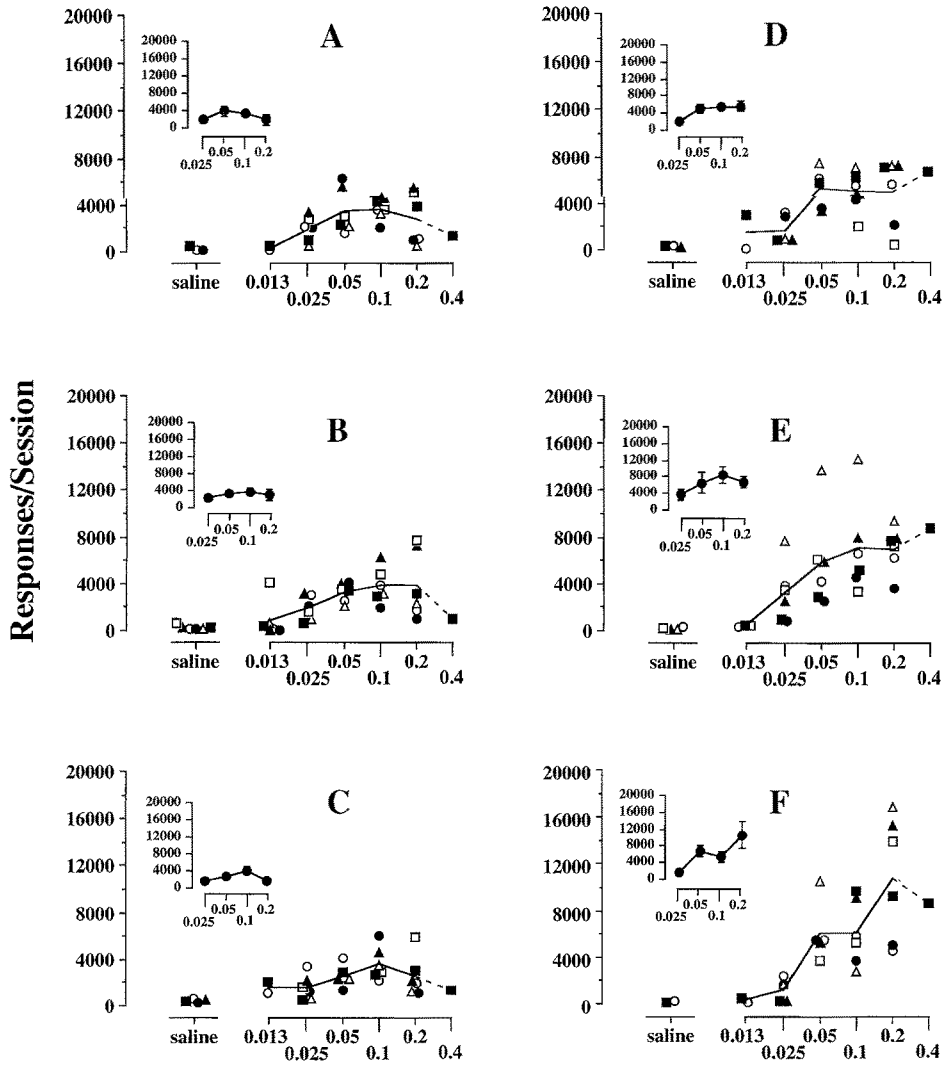


**Fig. 3A–F** Cocaine self-administration under a progressive-ratio schedule, expressed as responses/session. See Fig. 1 for details

## Discussion

Consistent with previous studies examining cocaine self-administration under PR schedules (Griffiths et al. 1975, 1978, 1979; Bedford et al. 1978; Winger and Woods 1985; Spear and Katz 1991; Depoortere et al. 1993), cocaine-maintained behavior increased as a function of dose for low to moderate doses. Under the ITI15/LH12 condition, the cocaine dose-response functions tended to first increase, then decrease at higher doses for all dependent measures while under the ITI30/LH24 condition, the dose-response functions for all dependent measures tended to increase to a maximum, at which performance was asymptotic. This general finding is consistent with previous results from our laboratory (Woolverton 1995). Inverted U-shaped functions, such as those found at ITI15/LH12, have been interpreted as reflecting rate disruptions due to accumulation of drug at high doses. Consistent with this interpretation, Jones et al. (1995) found that non-contingent cocaine administration can disrupt responding maintained by food under a PR schedule. In the present study, all dependent measures tended to be higher at ITI30/LH24 compared to ITI15/LH12, a result also consistent with disrupted responding due to drug accumulation at ITI15/LH12. Taken together, these results indicate that the cocaine dose-response function was optimal (i.e., a monophasic, sigmoidal curve) under the ITI30/LH24 condition compared to the ITI15/LH12 condition. Indeed, these findings generally suggest that the use of longer ITIs, which presumably would decrease drug accumulation, results in a less contaminated estimate of reinforcing efficacy in the PR procedure.

While drug accumulation may have accounted for inverted U-shaped dose-response functions in the present study, Griffiths et al. (1979) found inverted U-shaped dose-response functions in baboons responding for cocaine under a PR schedule with 3-h time-outs separating injections. Thus, drug accumulation may not have accounted for the inverted U-shaped functions found by Griffiths et al. (1979). The peak of the inverted U-shaped

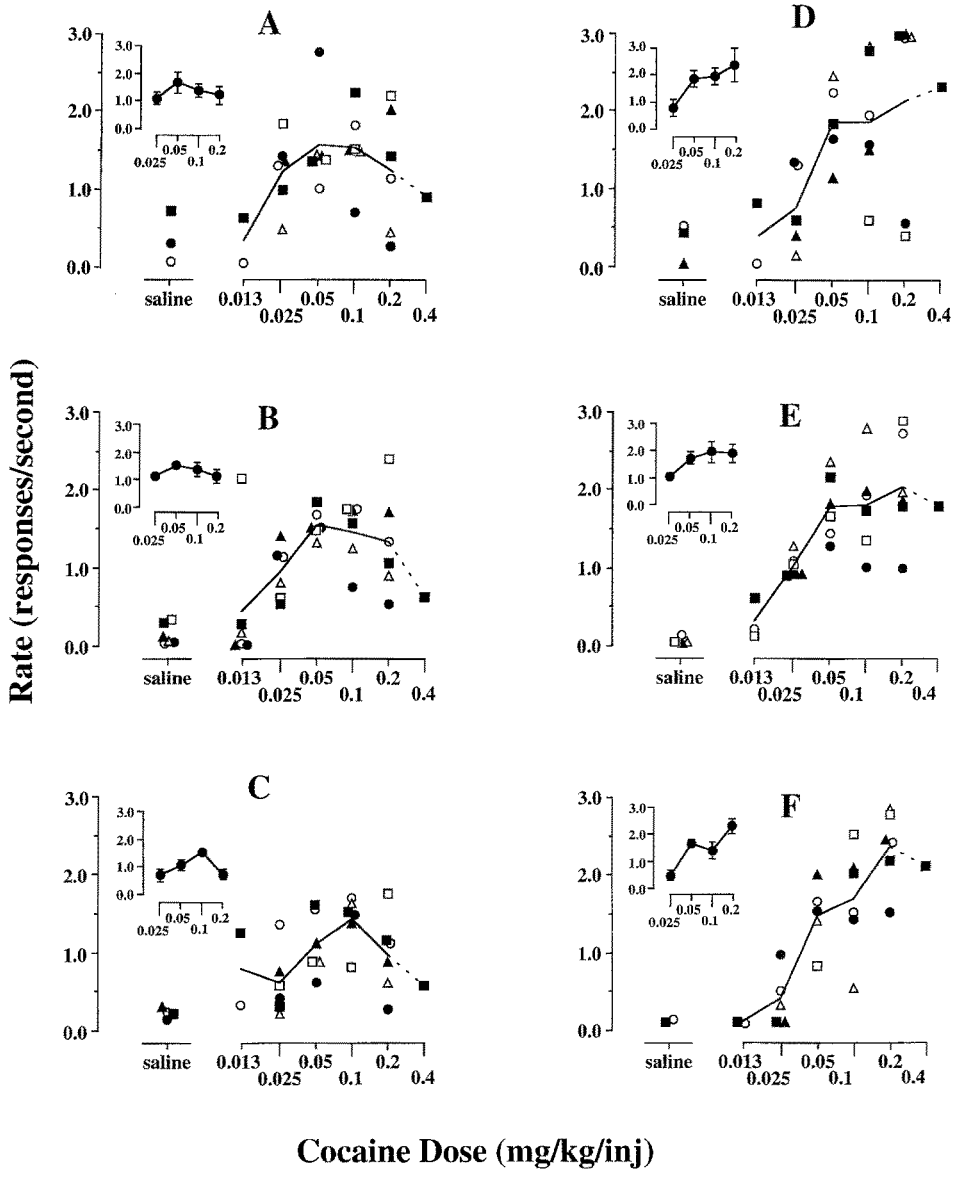


**Fig. 4A–F** Cocaine self-administration under a progressive-ratio schedule, expressed as response rate (responses/s). See Fig. 1 for details

functions in the Griffiths et al. (1979) study occurred in the range of 0.1–1.0 mg/kg per injection across baboons, while the highest dose tested (3.0 mg/kg per injection) resulted in lower break points than doses in the range of 0.1–1.0 mg/kg per injection. Thus, the descending limb of the dose-response functions in the Griffiths et al. (1979) study occurred at higher doses than those tested in the present study. It is possible that in the present study, inverted U-shaped dose-response functions would have been observed even under the ITI30/LH24 condition, if higher doses had been tested. However, while the cocaine dose-response functions over similar dose ranges were comparable between the two studies, it is possible that differences in sensitivity to cocaine between rhesus monkeys and baboons may also contribute to the absence or presence of inverted U-shaped dose-response functions.

For break point, total responses/session and rate, changes in the initial FR had little effect on the dose-response functions for cocaine. Although there was evidence for an increase in break point at the initial FR 240 in the ITI30/LH24 condition when analyzed using nonlinear regression, concordant results were not obtained with ANOVA. Since responding resulted in fewer injections as the initial FR was increased as an independent measure, injections/session decreased as initial FR increased. Although under the present conditions initial FR had little effect on responding maintained by cocaine, it seems likely that a point would be reached at which an increase in this variable would decrease responding maintained by cocaine. At initial FR 60, injections/session at the higher doses was at or near 20 for most monkeys, suggesting that the initial FR 60 resulted in maximal performance that was determined by the number of injections available (i.e., a ceiling effect). Thus, optimal conditions for measuring the reinforcing efficacy of cocaine were obtained at the initial FRs above 60.

The present results suggest that all four dependent variables provided asymptotic measures of the reinforc-

ing efficacy of cocaine under the ITI30/LH24 condition. One obvious implication of this finding is that reinforcing efficacy increases with dose to a maximum and is asymptotic after that point. However, other interpretations of the data should be considered. It is possible that there was a ceiling on responding maintained by cocaine. Some procedural limitation, e.g., the LH, could have limited maximal responding such that most animals peaked at approximately 3500 total responses at ITI15/LH12 or approximately 8000 total responses at ITI30/LH24. To assess this possibility, the number of incomplete trials (i.e., trials in which the FR was initiated but not completed) was assessed. If the 12-min LH was too short, then the number of incomplete trials should have significantly increased as the initial FR was increased and should have been lower at LH24 than at LH12. This was not the case, as no significant effects of initial FR or ITI/LH were observed when incomplete trials were analyzed. Indeed, the average number of incomplete trials across all conditions was three per 5 days of stability (which in some cases included up to 95 trials total), indicating that incomplete trials were relatively rare. Moreover, Woolverton (1995) found that increasing the LH under similar conditions had no effect on performance. Another possibility for a ceiling on responding was a physical limitation, e.g., fatigue, which may have limited maximum responding. Fatigue may have been less of a factor with the ITI30/LH24 condition, with responding increasing as a result. Finally, the interaction of accumulation of cocaine levels and increasing the FR over the course of the session may have inhibited responding. That is, as cocaine levels and response requirement were concomitantly increased over the course of a session, responding may have been disrupted to a point that limited further performance, just enough to result in asymptotic levels of performance. Support for this latter notion comes from the finding that measures of responding were lower at ITI15/LH12 compared to ITI30/LH24. However, if drug accumulation were playing a major role in limiting responding, one would expect responding to decrease again at higher unit doses where drug consumption would be higher.

Several issues with regard to data analysis should be considered as well. Conclusions based upon mean data assume that means were representative of effects in individual monkeys. As can be seen in the figures, for some monkeys, responding reached an asymptote at high doses of cocaine while for others, responding continued to increase or decreased again with dose. Although it seems likely that these latter two outcomes would have been asymptotic at longer ITI/LHs, this remains an assumption. A second consideration is the fact that, for responses/session and break point, variance increased as injections increased. The most significant ramification of this scaling problem is that at maximum responding, reliable differences between doses may be more difficult to detect (see also Depoortere et al. 1993; Woolverton 1995). Conclusions based upon non-linear regression analyses were similar to those based upon ANOVA; however, the regression approach does offer some advantages. For example, non-linear regression analysis allowed the use of all data, thus allowing unequal *n*s (which often occur in self-administration procedures), and calculation of pharmacologically relevant variables ($ED_{50}$, $E_{max}$). However, many of the dose-response functions tended toward a biphasic, inverted U-shaped function. The equation used in the nonlinear regression analysis assumes a sigmoidal dose-response function, i.e., this equation assumes an asymptote at the top of the function, and would not provide the most accurate fit of an inverted U-shaped dose-response function. Thus, the $ED_{50}$ and $E_{max}$ values obtained in the present study may have been contaminated estimates of reinforcing potency and reinforcing efficacy, at least at the ITI15/LH12 condition.

Based on the results of the present study, several general comments are possible concerning dependent measures in PR studies. First, as noted in other studies (Depoortere et al. 1993; Woolverton 1995), injections/session and rate provided estimates of reinforcing efficacy that were more amenable to statistical analysis than the typically used break point. Injections/session and response rate data were free of violations of assumptions common to standard statistical tests. Break point and responses/session provide direct measures of how much behavior the animal is emitting (Hodos 1961), which is not reflected directly in a measure of injections/session or rate. Responses/session provided number of responses on trials that were not complete, a contribution that may be especially critical at higher response requirements, and was not reflected in break point. Indeed, in the present study, responses/session, break point and rate gave somewhat different information than injections/session, although it should be noted that all of these dependent measures were highly correlated. In addition, because asymptotic performance measured by responses/session, breakpoint and rate data was relatively unaffected by initial FR, these three dependent measures may potentially allow comparisons across studies using different PR procedures. In a previous study comparing cocaine and procaine self-administration using one of the PR schedules described in the present study (ITI15/LH12 or ITI30/LH24, initial FR 120), similar dissociations between injections/session, total responses/session and rate were observed (Woolverton 1995). However, in comparing cocaine to procaine, injections/session and responses/session led to similar conclusions, while the dose-response functions for response rate were flat with few effects of drug dose. Taken together, the findings of the present study and the previous one (Woolverton 1995) suggest that the dependent measures may be dissociated, especially when comparing between drugs. Thus, when comparing drugs, multiple comparisons using injections/session, a measure of responses (either break point or responses/session) and rate may be warranted.

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## References


Bedford JA, Bailey LP, Wilson MC (1978) Cocaine-reinforced progressive-ratio performance in the rhesus monkey. Pharmacol Biochem Behav 9: 631–638

Depoortere RY, Li DH, Lane JD, Emmett-Oglesby MW (1993) Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacol Biochem Behav 45: 539–548

Griffiths RR, Findley JD, Brady JV, Dolan-Gutcher K, Robinson WW (1975) Comparison of progressive-ratio performance maintained by cocaine, methylphenidate and secobarbital. Psychopharmacologia 43: 81–83

Griffiths RR, Brady JV, Snell JD (1978) Progressive-ratio performance maintained by drug infusions: Comparison of cocaine, diethylpropion, chlorphentermine, and fenfluramine. Psychopharmacology 56: 5–13

Griffiths RR, Bradford LD, Brady JV (1979) Progressive-ratio and fixed-ratio schedules of cocaine-maintained responding in baboons. Psychopharmacology 65: 125–136

Hodos W (1961) Progressive-ratio as a measure of reward strength. Science 134: 943–944

Hodos W (1965) Motivational properties of long durations of rewarding brain stimulation. J Comp Physiol Psychol 59: 219–224

Hoffmeister F (1979) Progressive-ratio performance in the rhesus monkey maintained by opiate infusions. Psychopharmacology 62: 181–186

Huyn H, Feldt LS (1976) Estimation of the Box correction for degrees of freedom for sample data in randomized block and split-plot designs. J Edu Stat 1: 69–82

Johanson CE, Balster RL (1978) A summary of results of a drug self-administration study using substitution procedures in rhesus monkeys. Bull Narc 30: 43–54

Johanson CE, Schuster CR (1975) A choice procedure for drug reinforcers: cocaine and methylphenidate in the rhesus monkey. J Pharmacol Exp Ther 193: 676–688

Jones CA, LeSage M, Sundby S, Poling A (1995) Effects of cocaine in pigeons responding under a progressive-ratio schedule of food delivery. Pharmacol Biochem Behav 50: 527–531

Katz JL (1990) Models of relative reinforcing efficacy and their predictive utility. Behav Pharmacol 1: 283–301

Kirk, RE (1982) Experimental design: procedures for the behavioral sciences, 2nd edn. Brooks/Cole, Belmont, Calif.

Nader MA ,Woolverton WL (1991) Effects of increasing the magnitude of an alternative reinforcer on drug choice in a discrete-trials choice procedure. Psychopharmacology 105: 169–174

Risner ME, Silcox DL (1981) Psychostimulant self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology 75: 25–30

Roberts DCS (1993) Self-administration of GBR 12909 on a fixed ratio and progressive ratio schedule in rats. Psychopharmacology 111: 202–206

Roberts DCS, Loh EA , Vickers G (1989) Self-administration of cocaine on a progressive-ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. Psychopharmacology 97: 535–538

Schuster CR, Thompson T (1969) Self-administration of and behavioral dependence on drugs. Annu Rev Pharmacol 9: 483–502

Skjoldager P, Winger G, Woods JH (1991) Analysis of fixed-ratio behavior maintained by drug reinforcers. J Exp Anal Behav 56: 331–343

Spear DJ, Katz JL (1991) Cocaine and food as reinforcers: effects of reinforcer magnitude and response requirement under second-order fixed-ratio and progressive-ratio schedules. J Exp Anal Behav 56: 261–275

Winger G, Woods JH (1985) Comparison of fixed-ratio and progressive-ratio schedules of maintenance of stimulant drug-reinforced responding. Drug Alcohol Depend 15: 123–30

Woodward JJ, Compton DM, Balster RL, Martin, BR (1995) In vitro and in vivo effects of cocaine and selected local anesthetics on the dopamine transporter. Eur J Pharmacol 277: 7–13

Woolverton WL (1995) Comparison of the reinforcing effects of cocaine and procaine in rhesus monkeys responding under a progressive-ratio schedule. Psychopharmacology 120: 296–302

Woolverton WL, Johanson CE (1984) Preference in rhesus monkeys given a choice between cocaine and *d,l*-cathinone. J Exp Anal Behav 41: 35–43