Monoamine Releasers with Varying Selectivity for Dopamine/Norepinephrine vs. Serotonin Release as Candidate "Agonist" Medications for Cocaine Dependence: Studies in Assays of Cocaine Discrimination and Cocaine Self-Administration in Rhesus Monkeys

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Abbreviations: FR (fixed ratio), FI (fixed interval), PAL-353 (m-fluoroamphetamine), PAL-314 (m-methylamphetamine), PAL-287 (1-naphyl-2-aminopropane)

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ABSTRACT
Monoamine releasers constitute one class of drugs under investigation as candidate medications for the treatment of cocaine abuse. Promising preclinical and clinical results have been obtained with amphetamine, which has high selectivity for releasing dopamine/norepinephrine vs. serotonin. However, use of amphetamine as a pharmacotherapy is complicated by its high abuse potential. Recent preclinical studies suggest that non-selective monoamine releasers or serotonin-selective releasers have lower abuse liability and may warrant evaluation as alternatives to amphetamine. To address this issue, the present study evaluated the effects of five monoamine releasers in assays of cocaine discrimination and cocaine self-administration in rhesus monkeys. The releasers varied along a continuum from dopamine/norepinephrine selective to serotonin selective (PAL-353, methamphetamine, PAL-314, PAL-287, fenfluramine). In drug discrimination studies, rhesus monkeys were trained to discriminate saline from cocaine (0.4 mg/kg, SC) in a two-key, food-reinforced drug discrimination procedure. Substitution for cocaine was positively associated with selectivity for dopamine/norepinephrine vs. serotonin release. In drug self-administration studies, rhesus monkeys responded for cocaine (0.01 and 0.032 mg/kg/inj) and food (1 gm pellets) under a second-order FR2(VR16:S) schedule. In general, monoamine releasers produced dose-dependent and sustained decreases in cocaine self-administration. However, the dopamine/norepinephrine-selective releasers decreased cocaine self-administration with minimal effects on food-maintained responding, whereas the more serotonin-selective releasers produced non-selective reductions in both cocaine and food-maintained responding. These results are consistent with the conclusion that dopamine/norepinephrine-selective releasers retain cocaine-like abuse-related effects but may also be capable of producing relatively selective reductions in the reinforcing effects of cocaine.
INTRODUCTION

Cocaine abuse and dependence constitute a significant public health problem, and reliable pharmacotherapies are not yet available (Mendelson and Mello, 2004; Vocci et al., 2005). One potential approach to the treatment of cocaine dependence has been suggested by the effective treatment of opioid dependence with opioid agonists like methadone and of tobacco dependence with formulations of nicotine. These medications share pharmacological mechanisms of action with the abused drug and produce some effects in common with the abused drug, and they have been referred to as “agonist” medications (Rothman and Glowa, 1995). Cocaine blocks the reuptake of dopamine, serotonin and norepinephrine, and the abuse-related effects of cocaine are thought to be mediated primarily by its dopaminergic effects (Woolverton and Johnson, 1992). Consequently, both indirect and direct dopamine agonists have been evaluated as candidate agonist medications (Mello and Negus, 1996; Carroll et al., 1999; Newman and Kulkarni, 2002; Platt et al., 2002; Rothman et al., 2002; Grabowski et al., 2004). As one example, chronic treatment with the monoamine releaser d-amphetamine, which selectively releases dopamine and norepinephrine, produced a dose-dependent and sustained decrease in cocaine self-administration under second-order, progressive-ratio and choice schedules in rhesus monkeys (Negus, 2003; Negus and Mello, 2003a; Negus and Mello, 2003b). Moreover, amphetamine-induced decreases in cocaine self-administration were relatively selective, in that cocaine self-administration was reduced by amphetamine doses that had lesser effects on responding maintained by a non-drug reinforcer (food pellets) and that produced little other evidence of toxicity. Amphetamine maintenance also decreased cocaine use with low toxicity in clinical trials (Grabowski et al., 2001; Grabowski et al., 2004), and effective doses in humans were almost identical to doses that reduced cocaine self-administration in non-human primates.
These results suggest that amphetamine may be a promising lead compound in the development of agonist medications for cocaine abuse and dependence. However, amphetamine is itself a drug of abuse, and its high abuse potential limits its utility as a candidate medication for addiction treatment. One strategy for building on these results with amphetamine is to identify other monoamine releasers that have lower abuse potential than amphetamine but that retain amphetamine’s ability to produce sustained and selective decreases in cocaine self-administration. A growing body of literature suggests that a reduction in the selectivity of monoamine releasers to release dopamine/norepinephrine vs. serotonin is associated with a reduction in reinforcing effects (Locke et al., 1996; Rothman et al., 2005; Wee et al., 2005). Serotonergic activity also appears to limit the reinforcing effects of monoamine reuptake inhibitors (Czoty et al., 2002). Less is known about the degree to which selectivity for release of different monoamines influences the effects of monoamine releasers on cocaine self-administration. In one study, acute administration of an appropriate dose combination of phentermine (which selectively releases dopamine/norepinephrine >> serotonin) and fenfluramine (which selectively releases serotonin >> dopamine/norepinephrine) decreased cocaine self-administration more than food-maintained responding in rhesus monkeys; however, phentermine alone reduced cocaine self-administration with even lesser effects on food-maintained responding (Glowa et al., 1997). Similarly, chronic administration of the non-selective releaser PAL-287 produced a marginally selective decrease in cocaine self-administration vs. food-maintained responding in rhesus monkeys, but more selective reductions in cocaine self-administration were achieved with chronic amphetamine treatment (Negus and Mello, 2003b; Rothman et al., 2005). Taken together, these results suggest that reducing the dopamine/norepinephrine vs. serotonin selectivity of releasers may reduce their abuse potential, but this may also reduce their ability to produce behaviorally selective reductions in cocaine- vs. food-maintained responding.

The purpose of the present study was to further investigate the degree to which dopamine/norepinephrine vs. serotonin selectivity would influence the cocaine-like abuse-related
effects of monoamine releasers and the ability of these compounds to produce selective reductions in cocaine self-administration. Five compounds were selected for study, ranging from PAL-353 (m-fluroamphetamine, selectively releases DA/NE>>5HT) to the serotonin-selective releaser fenfluramine. Figure 1 shows the chemical structures of these five drugs, and Table 1 shows potency and selectivity data for the drugs to serve as substrate-type releasers at dopamine, norepinephrine and serotonin transporters. As is commonly true for existing monoamine releasers, the potency of these compounds to release norepinephrine was similar to or higher than potency to release dopamine, and compounds with exclusive selectivity for dopamine or norepinephrine release are not yet available (Rothman et al., 2001). All compounds were evaluated first in an assay of cocaine discrimination in rhesus monkeys to assess the relative potency and efficacy of these compounds to produce cocaine-like discriminative stimulus effects. These studies provide one measure of the ability of these compounds to produce cocaine-like abuse-related effects. Second, all compounds were evaluated for their effects on cocaine- and food-maintained responding under a second-order schedule that has been used previously to assess the effects of amphetamine (Negus and Mello, 2003b) and other candidate medications (Negus et al., 1997; Mello and Negus, 1998; Negus et al., 1999; Negus and Mello, 2002). Preclinical studies with this procedure have shown good concordance with clinical trials (Mello, 2005). We hypothesized that decreases in selectivity for dopamine/norepinephrine vs. serotonin release would be associated with decreases in cocaine-like discriminative stimulus effects and decreases in the selectivity of reductions in cocaine self-administration.
METHODS

Subjects

Studies were conducted in 11 adult male rhesus monkeys (Macaca mulatta) that weighed 7.3-9.6 kg. Six monkeys were studied in the drug discrimination experiments, and five monkeys were studied in the drug self-administration experiments. All monkeys had an experimental history involving the evaluation of dopaminergic and/or opioid compounds in assays of drug discrimination or drug self-administration. Monkeys were maintained on a diet of multiple vitamins, fresh fruit and Lab Diet Jumbo Monkey biscuits (PMI Feeds, Inc., St. Louis, MO). In addition, monkeys could receive 1 gm banana flavored pellets (Precision Primate Pellets Formula L/I Banana Flavor, P. J. Noyes Co., Lancaster, NH) during daily operant sessions as described below. Water was continuously available. A 12 hr light-dark cycle was in effect (lights on from 7 a.m. to 7 p.m.).

Animal maintenance and research were conducted in accordance with the guidelines provided by the NIH Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was periodically monitored by consulting veterinarians. Monkeys had visual, auditory and olfactory contact with other monkeys throughout the study. Operant procedures and foraging toys provide an opportunity for environmental manipulation and enrichment.

Drug Discrimination Procedures

Apparatus: Each monkey was housed individually in a well-ventilated, stainless steel chamber (56 x 71 x 69 cm). The home cages of all monkeys were modified to include an operant panel (28 x 28 cm) mounted on the front wall. Three round translucent response keys (5.1 cm in diameter) were arranged 3.5 cm apart in a horizontal row 9 cm from the top of the operant panel. Each key could be transilluminated by red or green stimulus lights (Superbright LED's, Fairchild Semiconductor, San Jose, CA). In addition, three circular translucent panels (1.9 cm in
diameter) were located in a vertical column below the center response key and could be transilluminated by red or green stimulus lights. The operant panel also supported an externally-mounted pellet dispenser (Gerbrands, Model G5210, Arlington, MA) that delivered 1 gm food pellets to a food receptacle mounted on the cage beneath the operant response panel. Operation of the operant panel and pellet dispenser and data collection were accomplished with custom-written software operating on microprocessors and software purchased from Med Associates Inc. (Georgia, VT) and located in a separate room.

**Discrimination Training:** Subjects were trained to discriminate cocaine (0.4 mg/kg IM) from saline using procedures identical to those used for our previous studies of the effects of indirect and direct dopamine agonists (Lamas et al., 1996; Negus et al., 1999). Discrimination sessions consisted of multiple, 20-min cycles and were conducted five days per week. Each cycle consisted of a 15-min time-out period followed by a five-min response period. During the time-out, all stimulus lights were off and responding had no scheduled consequences. During the response period, the right and left response keys were transilluminated red or green, and monkeys could receive up to 10 food pellets by responding under a fixed-ratio (FR) 30 schedule of food presentation. For three of the six monkeys, the left key was illuminated green and the right key was illuminated red. For the other three monkeys, the colors of the response keys were reversed. The center key was not illuminated at any time, and responding on the center key had no scheduled consequences. If all available food pellets were delivered before the end of the five-min response period, the stimulus lights transilluminating the response keys were turned off, and responding had no scheduled consequences for the remainder of that response period.

On training days, monkeys were given an i.m. injection of either saline or 0.40 mg/kg cocaine five-min after the beginning of each time-out period (i.e., 10 min before the response period). Following administration of saline, responding on only the green key (the saline-appropriate key) produced food, whereas following administration of 0.40 mg/kg cocaine, only responding on the red key (the drug-appropriate key) produced food. Responses on the
inappropriate key reset the FR requirement. Daily sessions consisted of one to five cycles, and if the training dose of cocaine was administered, it was administered only during the last cycle.

During the response period of each cycle, three dependent variables were determined: (1) Percent injection-appropriate responding prior to delivery of the first reinforcer \(\frac{(\text{Injection-Appropriate Responses Emitted Prior to 1st Reinforcer})}{\text{Total Responses Emitted Prior to 1st Reinforcer}} \times 100\); (2) Percent injection-appropriate responding for the entire response period \(\frac{(\text{Injection-Appropriate Responses Emitted During Response Period})}{\text{Total Responses Emitted During Response Period}} \times 100\); and (3) Response Rate \(\frac{(\text{Total Responses Emitted During Response Period})}{\text{Total Time Stimulus Lights Were Illuminated}}\).

Monkeys were considered to have acquired cocaine discrimination when the following three criteria were met for seven of eight consecutive training sessions: (1) the percent injection-appropriate responding prior to delivery of the first reinforcer was greater than or equal to 80% for all cycles; (2) the percent injection-appropriate responding for the entire cycle was greater than or equal to 90% for all cycles; (3) at least one pellet was earned during all training cycles.

**Discrimination Testing:** Once monkeys met criterion levels of cocaine discrimination, testing began. Test sessions were identical to training sessions except that responding on either key produced food, and test compounds were administered using a cumulative dosing procedure. In this procedure, increasing doses of the test compound were administered at the beginning of each successive 20-min cycle, instead of saline or the cocaine training dose, and each successive dose increased the total cumulative dose by 0.5 log units. Each drug was tested in a group of five monkeys, and testing in each monkey was conducted up to a dose that produced >90% cocaine-appropriate responding (i.e. full substitution) or that eliminated responding. (Note that an original group of five monkeys was used to test cocaine, PAL-353, PAL-314, PAL-287 and fenfluramine. One monkey died of causes unrelated to the study before methamphetamine could be tested. Accordingly, a sixth monkey was used to test cocaine and methamphetamine.)
Test sessions were usually conducted on Tuesdays and Fridays, and training sessions were usually conducted on Monday, Wednesday and Thursday. Test sessions were conducted only if the three criteria listed above were met during the training day immediately preceding the test day. If responding did not meet criterion levels of discrimination performance, then training was continued until criterion levels of performance were obtained for at least two consecutive days.

Data Analysis: Where possible, an ED50 value for each drug in each monkey was defined as the dose of a test compound that produced 50% cocaine-appropriate responding. Log ED50 values were calculated by log-linear interpolation from individual subject dose-effect curves. Log ED50 values were averaged to yield mean values, and these log values were converted to linear values for presentation. In addition, the percent cocaine-appropriate responding and the percent control response rate were determined for the highest dose of each compound tested in each monkey. These data were averaged and are reported in tabular form. For determination of the percent control response rate, the control response rate was determined for each monkey as the mean response rate during saline training cycles immediately preceding test sessions throughout the study. For graphical display, the cumulative percentage of monkeys in which full substitution (>90% cocaine-appropriate responding) was observed is shown as a function of drug dose.

Drug Self-Administration

Apparatus: Each monkey was housed individually in a well-ventilated stainless steel chamber (64 x 64 x 79 cm). The home cages of all monkeys were modified to include an operant panel and pellet dispenser identical to those described above for drug discrimination studies. In addition, a double-lumen catheter was surgically implanted into each monkey under aseptic conditions as described previously (Negus et al., 1999; Negus and Mello, 2003b).
intravenous catheter was protected by a tether system consisting of a custom-fitted nylon vest connected to a flexible stainless steel cable and fluid swivel (Lomir Biomedical, Malone, NY). Two syringe pumps (Model B5P-IE, Braintree Scientific, Braintree, MA; or Model 980210, Harvard Apparatus, South Natick, MA) were mounted above each cage for delivery of saline or drug solutions through the two lumen of the intravenous catheters. One syringe pump (the self-administration pump) was used to deliver self-administered cocaine injections. The second syringe pump (the treatment pump) was used for non-contingent delivery of saline or test drugs. The treatment pump delivered injections every 20 min from 10:30 a.m. each day until 9:30 a.m. the next morning for a total of 3 injections/hr and 69 injections/day. No treatment injections were delivered between 9:30 a.m. and 10:30 a.m. During this period, monkeys received their morning ration of food, and their health status was evaluated by the technical staff. Catheter patency was periodically evaluated by i.v. administration of ketamine (5 mg/kg) or the short-acting barbiturate methohexital (3 mg/kg) through the catheter lumen. The catheter was considered to be patent if i.v. administration of ketamine or methohexital produced a loss of muscle tone within 10 sec. Operation of the operant panel, pellet dispenser and drug pumps and data collection were accomplished with custom-written software operating on microprocessors and software purchased from Med Associates Inc. (Georgia, VT) and located in a separate room.

**Training Procedure:** Procedures for the evaluation of cocaine- and food-maintained responding were identical to those used in our previous studies of indatraline and amphetamine (Negus et al., 1999; Negus and Mello, 2003b). Alternating daily sessions of food and cocaine availability were associated with different colored stimulus lights projected on the center response key of the operant response panel. Red stimulus lights signaled food availability, and green stimulus lights signaled the availability of cocaine injections (delivered in a volume of 0.1 ml in 1 sec). Under the terminal schedule, the completion of a variable ratio of 16 responses on the center response key resulted in the illumination for 1 sec of an appropriately colored stimulus light (red for food, green for drug) underneath the center key (VR16:S). In addition, completion
of this VR response requirement a fixed ratio of two times (FR2) resulted in delivery of the available reinforcer and the initiation of a 10 sec time-out period, during which the stimulus light illuminating the center response key was turned off and responding had no scheduled consequences. This terminal second-order schedule is designated as FR2(VR16:S). The two side keys were not transilluminated during sessions of food and cocaine availability, and responding on these keys had no scheduled consequences. Four food sessions and four drug sessions were conducted during each experimental day. Food sessions began at 11 a.m., 3 p.m. 7 p.m. and 6 a.m. the next morning, and drug sessions began at 12 noon, 4 p.m., 8 p.m. and 7 a.m. the next morning. At all other times, responding had no scheduled consequences. Each food and drug session lasted one hour or until 25 food pellets or 20 injections had been delivered, whichever occurred first. Thus, monkeys could earn a maximum of 100 food pellets per day and 80 injections per day. Studies were conducted seven days a week.

During initial training, responding was maintained by delivery of 1gm food pellets during food sessions and by 0.032 mg/kg/inj cocaine injections during drug sessions. Training continued until monkeys met the following criteria for stable food and cocaine self-administration under the terminal schedule: (1) three consecutive days during which the number of drug injections/day differed by no more than 20% from the mean number of drug injections/day during those three days and there was no upward or downward trend; and (2) during the same three consecutive days, the mean number of both drug injections per day and food pellets per day was greater than 50.

**Testing Procedures:** Once cocaine- and food-maintained responding stabilized, testing began. Each dose of each drug was tested for a period of 7 consecutive days. During the 7-day test period, the unit dose of cocaine was changed to 0.01 mg/kg/inj, and saline or a dose of a test drug was administered by the treatment pump through one lumen of the double lumen catheter as described above (one injection every 20 min from 10:30 am each day until 9:30 a.m. the next day unless ). A unit dose of 0.01 mg/kg/injection cocaine was used for these studies, because
previous studies have demonstrated that this is the lowest dose to reliably maintain high rates of cocaine self-administration in all monkeys, and because behavior maintained by this unit dose of cocaine is sensitive to the effects of pretreatment compounds (e.g. Negus et al., 1999; Negus and Mello, 2003b). The infusion rate for test drugs was identical to that used in our previous studies with amphetamine and opioids, and a similar infusion rate was employed in the present study to permit direct comparison with those previous studies (Negus et al., 1997; Mello and Negus, 1998; Negus and Mello, 2002; Negus, 2003; Negus and Mello, 2003a; Negus and Mello, 2003b; Pereira Do Carmo et al., in press). The dose ranges for each test drug were as follows: PAL-353 (0.032-0.32 mg/kg/hr), methamphetamine (0.01-0.056 mg/kg/hr), PAL-314 (0.32-1.0 mg/kg/hr), PAL-287 (0.1-1.0 mg/kg/hr) and fenfluramine (0.1-1.0 mg/kg/hr). For all drugs except PAL-314, these dose ranges were empirically determined to cover a range from doses that produced little or no effect to doses that decreased rates of cocaine self-administration to less than 20% of control. The dose range for PAL-314 was limited by the solubility of the drug. In particular, delivery of 1.0 mg/kg/hr PAL-314 required an increase in infusion rate from 0.3 ml/hr (0.1 ml every 20 min) to 1.2 ml/hr (0.1 ml every 5 min). Doses higher than 1.0 mg/kg could not be reliably tested, because sustained use of higher infusion rates placed undue strain on the catheter. At the conclusion of each test period, the maintenance dose of cocaine (0.032 mg/kg/inj) and saline control treatment were reinstated for a period of at least four days and until the number of reinforcers per day maintained by cocaine and food returned to baseline levels. This interval between successive treatments was designed to reduce the possibility of carry-over effects from one treatment condition to the next.

Both PAL-353 and methamphetamine produced relatively selective decreases in responding maintained by 0.01 mg/kg/inj cocaine vs. food. To evaluate the effects of these two drugs on self-administration maintained by a higher unit dose of cocaine, a follow-up study was conducted. Specifically, the highest dose of each of these drugs (0.32 mg/kg/hr PAL-353 and 0.056 mg/kg/hr methamphetamine) was tested during availability of 0.032 mg/kg/inj cocaine.
These experiments were identical to those described above, except for the higher cocaine unit dose.

The effects of each test drug on cocaine- and food-maintained responding were evaluated in groups of three to four monkeys. In general, all doses of one drug were tested in a given monkey before initiation of studies with another drug. Both the sequence of drug doses and the sequence of drugs were mixed across monkeys. It should also be noted that results with PAL-287 were described previously (Rothman et al., 2005).

Data Analysis: The primary dependent variables were the total injections per day and total pellets per day delivered during the last three days of treatment with saline or each dose of each test drug. For statistical analysis, values for cocaine- and food-maintained responding during drug treatments were expressed as a percentage of control values for cocaine- and food-maintained responding obtained during saline treatment. Test drug effects were then analyzed by two-factor ANOVA, with test drug dose as one factor, and reinforcer type (cocaine or food) as the other factor. A significant analysis of variance was followed by individual means comparison using simple effects or the Duncan post hoc test. ED50 values for each drug to reduce cocaine- and/or food-maintained responding were also determined. The ED50 was defined as the dose of test drug that reduced levels of cocaine- or food-maintained responding to 50% of control levels. ED50 values to reduce cocaine- and food-maintained responding were determined in each monkey using log-linear interpolation, and these ED50 values were compared by t-test. In the event that a drug reduced cocaine self-administration but not food-maintained responding to levels below 50% of control, a conservative estimate of the ED50 value to reduce food-maintained responding was determined by assuming that the next incremental dose would have eliminated food-maintained responding. For all analyses, the criterion for significance was set at p<0.05, and all analyses were conducted using commercially-available software (CLRANOVA, Clear Lakes Research, Houston, TX). In addition to these
statistical analyses, levels of cocaine- and food-maintained responding are also displayed for all seven days of treatment with saline and the highest dose of each drug.

Drugs

Cocaine HCl was obtained from the National Institute on Drug Abuse (NIH, Bethesda, MD) and was dissolved in sterile saline. PAL-287 (1-naphthyl-2-propylamine hydrochloride), PAL-314 [m-methylamphetamine; 1-(3-toluyl)-2-propylamine fumarate] and PAL-353 [m-fluoroamphetamine; 1-(3-fluorophenyl)-2-propylamine fumarate] were provided by B. Blough of Research Triangle Institute (Research Triangle Park, NC). d-Methamphetamine HCl and dl-fenfluramine HCl were purchased from Sigma Chemical Co. (St. Louis, Mo). All drug solutions were dissolved in water and were filter-sterilized using a 0.22 micron Millipore filter. Doses were calculated using the salt forms of the drugs given above.
RESULTS

Effects of monoamine releasers in monkeys trained to discriminate cocaine from saline.

During the training days preceding test days, monkeys responded primarily on the saline key during saline cycles (mean percent saline-appropriate responding ± SEM=99.92±0.08) and primarily on the cocaine key during cocaine training (mean percent cocaine-appropriate responding ± SEM= 99.69±0.28). Mean response rates ±SEM were 2.27 ± 0.27 and 1.96 ± 0.37 responses/sec during saline and drug training cycles, respectively. Response rates during saline training cycles were used to calculate Percent Control Response rates shown in Table 2.

Figure 2 and Table 2 show that cocaine produced a dose-dependent and complete substitution for the training dose of cocaine in all six monkeys. Doses of cocaine that produced complete substitution (i.e. >90% cocaine-appropriate responding) either did not affect response rates or increased response rates. Figure 2 and Table 2 also show data for each of the test drugs. Both PAL-353 and methamphetamine produced dose-dependent and complete substitution for cocaine in all monkeys tested. However, response rates at doses that produced complete substitution were more variable than with cocaine. Increases in response rates were seen in some monkeys, but decreases in response rates were seen in other monkeys. PAL-314 and PAL-287 produced a dose-dependent and complete substitution for cocaine in four of five monkeys (the same four monkeys for both drugs); in the fifth monkey, PAL-314 and PAL-287 produced less than 2% cocaine-appropriate responding up to doses that eliminated responding. Fenfluramine produced a dose-dependent and complete substitution for cocaine in one monkey, but substitution was observed only at a dose that reduced response rates to less than 15% of control. In the other four monkeys, fenfluramine produced only saline-appropriate responding up to doses that eliminated responding. Overall, the rank order of relative potencies of these drugs to
produce cocaine-appropriate responding was methamphetamine $\geq$ cocaine $\geq$ PAL-353 $\geq$ PAL-314 $\geq$ PAL-287 $\geq$ fenfluramine. The rank order of maximal cocaine-like discriminative stimulus effects (defined as maximal percent monkeys in which complete substitution was observed; Fig. 2) was cocaine=PAL-353=methamphetamine>PAL-314=PAL-287>fenfluramine.

**Effects of monoamine releasers on cocaine- and food-maintained responding**

We have shown previously that, under the current second-order schedule, unit doses of 0.01 and 0.032 mg/kg/inj cocaine lie at the peak of the cocaine self-administration dose-effect curve (Negus et al., 1999; Negus and Mello, 2003b). During control saline treatment in the present study, monkeys usually self-administered the maximum numbers of 80 cocaine injections per day and 100 food pellets per day during availability of both 0.01 and 0.032 mg/kg/inj cocaine. Specifically, during the last three days of 7-day control treatments with saline, monkeys responded for 79.2±0.8 cocaine injections per day and 95.3±2.5 food pellets per day during availability of 0.01 mg/kg/injection cocaine, and for 78.6±1.0 cocaine injections per day and 97.2±2.8 food pellets per day during availability of 0.032 mg/kg/inj cocaine.

Figure 3 shows percent control levels of responding for 0.01 mg/kg/inj cocaine and food during treatment with each of the monoamine releasers (results with PAL-287 reported previously; Rothman et al., 2005). Results of statistical analysis are reported in Table 3, and ED50 values for each drug to reduce cocaine- and food-maintained responding are shown in Table 4. PAL-353, methamphetamine, PAL-287 and fenfluramine produced dose-dependent decreases in cocaine self-administration. However, the relative effects on food maintained responding varied. PAL-353 and methamphetamine significantly reduced cocaine self-administration at doses that either did not affect food-maintained responding, or that decreased
food-maintained responding significantly less than cocaine self-administration. ED50 analysis indicated that both drugs were at least 3-fold more potent in decreasing cocaine self-administration than in decreasing food-maintained responding. PAL-287 at a dose of 1.0 mg/kg/hr was the lowest dose to significantly decrease both cocaine- and food-maintained responding, but cocaine self-administration was decreased more than food-maintained responding. ED50 analysis indicated that PAL-287 was less than two-fold more potent in decreasing cocaine self-administration than food-maintained responding, and this small difference in ED50 values was not statistically significant. Finally, fenfluramine produced virtually identical effects on cocaine- and food-maintained responding. A dose of 1.0 mg/kg/hr was the lowest dose to significantly decrease responding maintained by either reinforcer, and the magnitude of this decrease was similar for both reinforcers. ED50 analysis also indicated similar potencies of fenfluramine to decrease cocaine- and food-maintained responding. PAL-314 did not significantly alter cocaine or food-maintained responding relative to baseline across the dose range tested (Table 3), and higher doses could not be tested due to the limited solubility of the drug. However, at the highest dose of 1.0 mg/kg/hr PAL-314, cocaine- and food-maintained responding were 57.1±6.6 and 98.8±2.7% of control, respectively, indicating a trend for this compound to produce at least a marginally selective decrease in cocaine- vs. food-maintained responding.

Figure 4 shows levels of cocaine self-administration and food-maintained responding during all seven days of treatment with saline or the highest dose of each monoamine releaser. In general, monkeys responded at high, stable levels for both cocaine and food reinforcement during saline treatment. During treatment with monoamine releasers, maximal decreases in cocaine self-administration were achieved within 2-5 days, and decreases in cocaine self-
administration were generally sustained for the duration of the treatment. The only exception was PAL-314, and levels of cocaine self-administration appeared to recover toward baseline on Day 7 of treatment with 1.0 mg/kg/hr PAL-314. As noted above, effects on food-maintained responding varied across monoamine releasers. The smallest effects were observed with PAL-353 and methamphetamine, and the greatest effects were observed fenfluramine.

Figure 5 shows the effects of the highest doses of PAL-353 (0.32 mg/kg/hr) and methamphetamine (0.056 mg/kg/hr) on responding maintained by food and a higher unit dose of 0.032 mg/kg/inj cocaine. Results of statistical analysis are reported in Table 3. Results were similar to those reported during availability of the lower unit dose of 0.01 mg/kg/inj cocaine. Specifically, 0.32 mg/kg/hr PAL-373 significantly decreased both 0.032 mg/kg/inj cocaine self-administration and food-maintained responding, but cocaine self-administration was decreased more than food-maintained responding. A dose of 0.056 mg/kg/hr methamphetamine significantly decreased 0.032 mg/kg/inj cocaine self-administration, but did not significantly alter food-maintained responding.
DISCUSSION

The present study evaluated the effects of monoamine releasers in assays of cocaine discrimination and cocaine self-administration in rhesus monkeys. Five different monoamine releasers were examined, and these compounds varied along a continuum of selectivity for dopamine/norepinephrine vs. serotonin release from selective dopamine/norepinephrine releasers through non-selective compounds to selective serotonin releasers. In drug discrimination studies, selectivity for dopamine/norepinephrine vs. serotonin release was positively correlated with the degree of substitution for the discriminative stimulus effects of cocaine. In drug self-administration studies, all monoamine releasers decreased cocaine self-administration; however, pharmacologic selectivity for dopamine/norepinephrine vs. serotonin release was associated with behavioral selectivity to reduce cocaine self-administration with lesser effects on food-maintained responding. These results support the hypothesis that decreases in the pharmacologic selectivity of monoamine releasers to release dopamine/norepinephrine vs. serotonin produce decreases in both cocaine-like abuse-related effects and in behavioral selectivity to reduce cocaine- vs. food-maintained responding.

Effects of monoamine releasers in the assay of cocaine discrimination. The results of the present study agree with previous findings that dopamine/norepinephrine-selective releasers substitute more effectively than serotonin-selective releasers for a cocaine training stimulus in drug discrimination studies. For example, amphetamine substituted for cocaine in rats and rhesus monkeys trained to discriminate cocaine from saline, whereas fenfluramine did not (D'Mello and Stolerman, 1977; Schuster and Johanson, 1985; Wood and Emmett-Oglesby, 1988). Similarly, cocaine and amphetamine produced relatively similar profiles of
discriminative and subjective effects in humans, whereas fenfluramine did not produce stimulant-like subjective effects (Chait et al., 1986; Oliveto et al., 1998). The present results also provide some insight into the degree of dopamine/norepinephrine vs. serotonin selectivity that is required to produce reliable cocaine-like discriminative stimulus effects. Specifically, methamphetamine (which is approximately 31-fold selective for dopamine vs. serotonin release in assays of monoamine release, see Table 1) produced full substitution for cocaine in all monkeys, whereas PAL-314 and PAL-287 (which have 6.1-fold selectivity and 0.27-fold selectivity, respectively) did not substitute for cocaine in all monkeys. The present results also agree with the finding that reductions in selectivity for dopamine/norepinephrine vs. serotonin release are associated with reductions in the reinforcing efficacy of monoamine releasers in rhesus monkeys (Locke et al., 1996; Rothman et al., 2005; Wee et al., 2005). Interestingly, PAL-314 and PAL-287 produced similar degrees of substitution for cocaine in the present study (i.e. full substitution in 4 of 5 monkeys). However, these compounds displayed very different abilities to maintain self-administration, with PAL-314 maintaining self-administration more reliably than PAL-287 (Rothman et al., 2005; Wee et al., 2005). Thus, despite the general concordance between these drug discrimination and drug self-administration results, there remain potentially important differences in the pharmacological mechanisms responsible for production of cocaine-like discriminative stimulus vs. reinforcing effects. Specifically, reinforcing effects of monoamine releasers may be more vulnerable than cocaine-like discriminative stimulus effects to increases in serotonergic activity.

Effects of monoamine releasers on cocaine- vs. food-maintained responding. With regard to the preclinical evaluation of candidate pharmacotherapies for drug dependence, we
have argued previously that optimal medications might be those that produce sustained decreases in self-administration of the target drug of abuse across a wide range of drug unit doses while producing lesser effects on responding for a non-drug reinforcer (e.g. food) and minimal evidence of undesirable effects (Mello and Negus, 1996; Mello, 2005). By these criteria, dopamine/norepinephrine-selective monoamine releasers produce a more promising profile of effects than less-selective or serotonin-selective releasers as candidate medications for cocaine dependence. In the present study, the dopamine/norepinephrine-selective releasers PAL-353 and methamphetamine produced sustained decreases in cocaine self-administration across a one-half log unit range of cocaine unit doses while having significantly lesser effects on food-maintained responding. Amphetamine, which also has high selectivity for dopamine/norepinephrine vs. serotonin release, also produced decreases in cocaine self-administration that were sustained (for up to 28 days), apparent across a 30-fold range of cocaine doses, and selective for cocaine- vs. food-maintained responding under three different schedules of reinforcement (Negus, 2003; Negus and Mello, 2003a; Negus and Mello, 2003b). Possible mechanisms underlying these effects were discussed previously (Negus and Mello, 2003b).

These findings are concordant with earlier preclinical studies that chronic amphetamine treatment produced rightward/downward shifts in cocaine discrimination and self-administration dose-effect curves in rodents (Peltier et al., 1996), and with clinical findings that amphetamine may serve as a relatively safe and effective maintenance medication for the treatment of cocaine dependence in humans (Grabowski et al., 2001; Grabowski et al., 2004). By contrast, monoamine releasers with lower pharmacologic selectivity for dopamine/norepinephrine vs. serotonin release displayed less behavioral selectivity in reducing cocaine- vs. food-maintained responding. These results suggest that non-selective or serotonin-selective releasers may have
decreased cocaine self-administration by producing a non-selective reduction in the reinforcing effects of both cocaine and food (e.g. Higgins and Fletcher, 2003), a reduction in stimulus control, or non-selective motoric effects that impaired the ability of the subjects to respond. This conclusion based on these chronic treatment studies is consistent with results of a previous study that examined the acute effects of phentermine and fenfluramine administered either alone or in combination in monkeys responding for cocaine and food (Glowa et al., 1997). In that study, the most selective decreases in cocaine self-administration were accomplished with phentermine alone.

**Prospects for the Use of Monoamine Releasers as Treatments for Cocaine Dependence.** We have interpreted the results of the present study to suggest that dopamine/norepinephrine-selective monoamine releasers may reduce cocaine use with fewer undesirable effects than non-selective or serotonin-selective releasers. However, several caveats to this conclusion warrant mention. First, it is well-established that monoamine releasers with relatively high selectivity for dopamine/norepinephrine vs. serotonin function as strong reinforcers in drug self-administration studies, and drugs such as amphetamine and methamphetamine have acknowledged abuse liability. Although agonist medications for other forms of drug dependence also have high abuse liability (e.g. methadone for the treatment of opioid abuse), the abuse liability of dopamine/norepinephrine-selective monoamine releasers certainly poses an obstacle to their use in the treatment of stimulant dependence. Second, the present study documented optimal effects with releasers selective for dopamine/norepinephrine vs. serotonin release; however, the degree to which the dopaminergic and/or noradrenergic effects of these drugs contributes to their profiles of behavioral effects remains to be determined. Releasers with selectivity for dopamine vs. both norepinephrine and serotonin would help
address this issue. Lastly, the present study used food-maintained responding to provide a 
measure of potentially undesirable, non-selective effects of candidate medications, and 
dopamine/norepinephrine-selective releasers produced more selective reductions in cocaine- vs. 
food-maintained responding than non-selective or serotonin-selective releasers. However, as 
discussed previously (Mello and Negus, 1996), a reduction in food-maintained responding may 
be acceptable if a medication produces few other undesirable effects. Further assessment of the 
concordance between preclinical and clinical results awaits results from clinical studies with 
monoamine releasers.

One final caveat to the present study is that differences in the pharmacokinetics and time 
courses of the drugs may have influenced measures of relative potency. In drug discrimination 
studies, for example, test drug effects were examined from 15-20 min after their administration 
in cumulative dosing experiments. If a drug did not produce peak effects at this time, then its 
relative potency may have been underestimated relative to other drugs that were at peak effect. 
Similarly, in the drug self-administration studies, all drugs were administered using the same 
chronic infusion regimen. Again, this may have influenced relative potency measures, because 
long-acting drugs may have accumulated to higher degrees and appeared more potent than drugs 
with shorter durations of action. However, the primary dependent measures in the present study 
were not relative potencies, but rather (a) maximal degree of cocaine-like discriminative stimulus 
effects, and (b) selectivity to reduce cocaine- vs. food-maintained responding. To address this 
focus on efficacy, drugs were studied in both drug discrimination and drug self-administration 
procedures from ineffective to maximally effective doses (except PAL-314 in drug self-
administration studies, and in this case, PAL-314 was studied up to the highest dose possible 
given its limited solubility). Thus, all drugs displayed sufficiently rapid onsets and durations of
action to produce robust behavioral effects that permitted an assessment of their cocaine-like
discriminative stimulus effects and their selectivity to reduce cocaine- vs. food-maintained
responding. By this measure, dopamine/norepinephrine-selective releasers produced optimally
selective reductions in cocaine- vs. food-maintained responding.
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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Structures of the monoamine releasers examined in this study. The structure of amphetamine is included for comparison. Note that PAL-353, PAL-314 and fenfluramine are ring-substituted amphetamine analogs, whereas PAL-287 is a non-amphetamine naphthylene analog. In vitro potency and selectivity for these compounds to release dopamine, norepinephrine and serotonin are shown in Table 1.

Figure 2. Cocaine-like discriminative stimulus effects of monoamine releasers. Abscissa: Dose in mg/kg (log scale). Ordinate: Cumulative percentage of monkeys in which complete substitution for cocaine was observed (≥90% cocaine-appropriate responding). Cocaine was tested in a group of 6 monkeys, and all other drugs were tested in groups of 5 monkeys.

Figure 3. Effects of chronic treatment with monoamine releasers on responding maintained by cocaine (0.01 mg/kg/inj) or food pellets. Abscissae: Dose of test compound in mg/kg/hr (log scale). Ordinates: Percent control levels of cocaine- and food-maintained responding. Each point shows mean±SEM data obtained during the last three days of a seven-day treatment in three or four monkeys. Asterisks indicate that the test compound produced a significant reduction in responding for a given reinforcer as compared to control levels of responding for that reinforcer (*p<0.05, **p<0.01). Crosses indicate a significant difference between percent control levels of cocaine- and food-maintained responding at a given dose of the test compound († p≤0.05, †† p<0.01).

Figure 4. Time course of effects of monoamine releasers on responding maintained by cocaine (0.01 mg/kg/inj) or food pellets. Abscissae: Consecutive days of treatment. Left ordinates: Number of food pellets per day (maximum = 100). Right ordinates: Number of
cocaine injections per day (maximum = 80). Each point shows mean±SEM data for 3-4 monkeys.

Figure 5. Effects of chronic treatment with PAL-353 and methamphetamine on responding maintained by cocaine (0.032 mg/kg/inj) or food pellets. Abscissae: Treatment. Ordinate: Percent control levels of cocaine- and food-maintained responding. Each bar shows mean±SEM data obtained during the last three days of a seven-day treatment in four monkeys. Asterisks indicate that the test compound produced a significant reduction in responding for a given reinforcer as compared to control levels of responding for that reinforcer (*p<0.05, **p<0.01). Crosses indicate a significant difference between percent control levels of cocaine- and food-maintained responding († p≤0.05, †† p<0.01).
Table 1. IC50 values (nM) for monoamine releasers to release dopamine, norepinephrine and serotonin. The ratio of 5HT IC50/DA IC50 is also shown. Higher ratios imply greater selectivity for releasing dopamine. For all compounds, potency to release NE was similar to or higher than potency to release DA. All values were published previously, and references are provided. Data for amphetamine are included for comparison with the compounds tested in the present study.

<table>
<thead>
<tr>
<th>Drug</th>
<th>DA</th>
<th>NE</th>
<th>5HT</th>
<th>5HT/DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24.8±3.5</td>
<td>7.2±0.44</td>
<td>1765 ± 94</td>
<td>71</td>
</tr>
<tr>
<td>PAL-353&lt;sup&gt;3&lt;/sup&gt;</td>
<td>24.2 ± 1.1</td>
<td>16.1±1.7</td>
<td>1937 ± 202</td>
<td>80</td>
</tr>
<tr>
<td>Methamphetamine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24.5 ± 2.1</td>
<td>12.3±0.7</td>
<td>736 ± 45</td>
<td>31</td>
</tr>
<tr>
<td>PAL-314&lt;sup&gt;3&lt;/sup&gt;</td>
<td>33.3 ± 1.3</td>
<td>18.3±1.4</td>
<td>218 ± 22</td>
<td>6.5</td>
</tr>
<tr>
<td>PAL-287&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12.6 ± 0.4</td>
<td>11.1±0.9</td>
<td>3.4 ± 0.2</td>
<td>0.27</td>
</tr>
<tr>
<td>Fenfluramine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&gt;10,000</td>
<td>739±57</td>
<td>79.3 ± 11.5</td>
<td>&lt;0.0079</td>
</tr>
</tbody>
</table>

<sup>1</sup>Rothman et al., 2001
<sup>2</sup>Rothman et al., 2005
<sup>3</sup>Wee et al., 2005
Table 2. Mean (range) ED50 values and mean (range) percent cocaine-appropriate responding and percent control response rate at highest dose for cocaine and monoamine releasers in rhesus monkeys trained to discriminate 0.4 mg/kg cocaine from saline. The fraction of monkeys in which an ED50 value could be determined is also shown under “#.” When the fraction is less than 1, the remaining monkeys showed ≤2% cocaine-appropriate responding up to doses that eliminated responding. Note that mean (range) ED50 values show results only in those monkeys in which an ED50 value could be calculated. The Percent Cocaine Responding and Percent Control Response Rate show data obtained with the highest dose tested in each monkey. Control response rates in individual monkeys ranged from 1.47 to 3.43 responses/sec.

<table>
<thead>
<tr>
<th>Drug</th>
<th>#</th>
<th>ED50 (mg/kg)</th>
<th>Percent Cocaine Responding</th>
<th>Percent Control Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>6/6</td>
<td>0.13 (0.07-0.23)</td>
<td>99.6 (97.7-100)</td>
<td>151.3 (93.5-257)</td>
</tr>
<tr>
<td>PAL-353</td>
<td>5/5</td>
<td>0.23 (0.18-0.56)</td>
<td>100 (100-100)</td>
<td>147.9 (44.8-283)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>5/5</td>
<td>0.10 (0.05-0.18)</td>
<td>99.5 (96.7-100)</td>
<td>95.5 (9.4-320)</td>
</tr>
<tr>
<td>PAL-314</td>
<td>4/5</td>
<td>0.32 (0.18-0.56)</td>
<td>80.3 (1.3-100)</td>
<td>80.3 (0-210)</td>
</tr>
<tr>
<td>PAL-287</td>
<td>4/5</td>
<td>0.49 (0.18-1.8)</td>
<td>80.0 (0-100)</td>
<td>84.0 (0-204)</td>
</tr>
<tr>
<td>Fenfluramine</td>
<td>1/5</td>
<td>0.94</td>
<td>20 (0-100)</td>
<td>2.8 (0-14.1)</td>
</tr>
</tbody>
</table>
Table 3. P values for the main effect of monoamine releaser dose and reinforcer type (cocaine or food) and for the dose-reinforcer interaction from two-factor ANOVA of monoamine releaser effects on cocaine self-administration and food-maintained responding. Analyses were conducted on data shown in Figures 2 and 4. Data for PAL-287 were reported in Rothman et al., 2005. All significant effects (p<0.05) are shown in **bold**.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Main Effect of Dose</th>
<th>Main Effect of Reinforcer</th>
<th>Dose-Reinforcer Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL-353</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01 Coc</td>
<td>&lt;0.001</td>
<td>0.008</td>
<td>0.002</td>
</tr>
<tr>
<td>0.032 Coc</td>
<td>0.076</td>
<td>0.030</td>
<td>0.076</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01 Coc</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.032 Coc</td>
<td><strong>0.013</strong></td>
<td><strong>0.008</strong></td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>PAL-314</td>
<td>0.32</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>PAL-287</td>
<td>&lt;0.001</td>
<td>0.114</td>
<td>0.116</td>
</tr>
<tr>
<td>Fenfluramine</td>
<td><strong>0.001</strong></td>
<td>0.40</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Table 4. ED50 values (95% confidence limits) for monoamine releasers to decrease responding maintained by 0.01 mg/kg/inj cocaine and food. For PAL-353 and methamphetamine, ED50 values could not be determined for suppression of food-maintained responding, because no doses decreased rates of food-maintained responding by ≥50% in all animals. In these cases, a conservative estimate of the ED50 value was determined by assuming that the next higher dose would eliminate food-maintained responding. For PAL-314, ED50s could not be determined for either cocaine- or food-maintained responding across the dose-range tested, and higher doses could not be tested due to limited solubility. The ratio of Food ED50 ÷ Cocaine ED50 is also shown to provide an approximate measure of selectivity in reducing cocaine self-administration vs. food-maintained responding. Higher ratio values indicate greater selectivity for reducing cocaine- vs. food-maintained responding. ** Indicates significantly lower ED50 to decrease cocaine self-administration vs. food-maintained responding as determined by t-test, p<0.01.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cocaine ED50</th>
<th>Food ED50</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL-353</td>
<td>0.14 (0.09-0.23)**</td>
<td>&gt;0.42</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>0.024 (0.019-0.030)** &gt;0.073</td>
<td>&gt;3.0</td>
<td></td>
</tr>
<tr>
<td>PAL-314</td>
<td>&gt;0.74</td>
<td>&gt;1.75</td>
<td>≈2.4</td>
</tr>
<tr>
<td>PAL-287</td>
<td>0.48 (0.42-0.56)</td>
<td>0.82 (0.57-1.17)</td>
<td>1.7</td>
</tr>
<tr>
<td>Fenfluramine</td>
<td>0.36 (0.16-0.77)</td>
<td>0.41 (0.25-0.66)</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Figure 1

- PAL-353
- Amphetamine
- Methamphetamine
- PAL-314
- PAL-287
- Fenfluramine
Figure 2

The figure presents a graph showing the percentage of monkeys substituting in response to different doses of various substances. The x-axis represents the dose (mg/kg) ranging from 0.01 to 10, and the y-axis represents the percentage of monkeys substituting, ranging from 0 to 100.

Key:  
- □ Cocaine  
- ▲ PAL-353  
- ▼ Methamphetamine  
- ◻ PAL-314  
- ● PAL-287  
- △ Fenfluramine
Figure 3

Graphs showing the effects of different drugs on % Control against Dose (mg/kg/hr). The graph for PAL-353 shows a decline in % Control with increasing dose, with statistical significance indicated by ** and ††. For PAL-314 and PAL-287, similar trends are observed with significant decreases at certain doses. The graph for Methamphetamine and Fenfluramine also demonstrates a decrease in % Control with dose, with significant decreases at higher doses, indicated by **.

Legend:
- O: Food-Maintained Responding
- △: Cocaine-Maintained Responding
Figure 4

[Graph showing the number of food pellets per day and cocaine injections per day across consecutive days of testing for different conditions and treatments.]