1521-0103/359/1/1–10$25.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

http://dx.doi.org/10.1124/jpet.116.234252
J Pharmacol Exp Ther 359:1–10, October 2016

Discriminative Stimulus Effects of Binary Drug Mixtures: Studies with Cocaine, MDPV, and Caffeine

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Received April 7, 2016; accepted August 1, 2016

ABSTRACT

Illicit drug preparations often include more than one pharmacologically active compound. For example, cocaine and synthetic cathinones [e.g., 3,4-methylenedioxypyrovalerone (MDPV)] are often mixed with caffeine before sale. Caffeine is likely added to these preparations because it is inexpensive and legal; however, caffeine might also mimic or enhance some of the effects of cocaine or MDPV. In these studies, male Sprague-Dawley rats were trained to discriminate 10 mg/kg cocaine from saline, and the discriminative stimulus effects of cocaine, caffeine, and MDPV were evaluated alone and as binary mixtures (cocaine and caffeine, MDPV and caffeine, and cocaine and MDPV) at fixed-dose ratios of 3:1, 1:1, and 1:3 relative to the dose of each drug that produced 50% cocaine-appropriate responding. Dose-addition analyses were used to determine the nature of the drug-drug interactions for each mixture (e.g., additive, supra-additive, or subadditive). Although additive interactions were observed for most mixtures, supra-additive interactions were observed at the 50% effect level for the 1:1 mixture of cocaine and caffeine and at the 80% effect level for all three mixtures of cocaine and caffeine, as well as for the 3:1 and 1:3 mixtures of cocaine and MDPV. These results demonstrate that with respect to cocaine-like discriminative stimulus effects, caffeine can function as a substitute in drug preparations containing either cocaine or MDPV, with enhancements of cocaine-like effects possible under certain conditions. Further research is needed to determine whether similar interactions exist for other abuse-related or toxic effects of drug preparations, including cocaine, synthetic cathinones, and caffeine.

Introduction

Many illicit drug preparations include more than one pharmacologically active compound (Cole et al., 2011). For example, seized cocaine often includes caffeine, lidocaine, phenacetin, or levamisole. Several recent reports suggest that up to 50% of cocaine shipments are mixed with caffeine before being exported from Brazil (Bernardo et al., 2003; Magalhães et al., 2013; Lapachinske et al., 2015). Although the amount of caffeine can vary from sample to sample, several reports suggest that as much as 40% of the weight of cocaine exports can be accounted for by caffeine (Fukushima et al., 2014; Zacca et al., 2014). Similarly, analyses of purchased or seized "designer drugs" [e.g., synthetic cannabinoids (K2/spice) and synthetic cathinones (bath salts)] suggest that they also comprise more than one pharmacologically active compound (Shanks et al., 2012; Seely et al., 2013). Although most bath salt preparations include at least one synthetic cathinone [e.g., 3,4-methylenedioxypyrovalerone (MDPV), 4-methylmethcathinone (mephedrone), 3,4-methylenedioxy-N-methylcathinone (methylone)], preparations containing two or more synthetic cathinones and mixtures of a synthetic cathinone and caffeine have been reported at different times and in different geographic locations (Brandt et al., 2010; Davies et al., 2010; Spiller et al., 2011; Żukiewicz-Sobczak et al., 2012; Caudevilla-Gálligo et al., 2013; Zuba and Byrska, 2013). For instance, analysis of drugs seized in Arkansas between 2010 and 2012 suggests that up to 80% of bath salts preparations contain both a synthetic cathinone and caffeine (Seely et al., 2013); however, the composition of bath salts preparations changes over time with regard to both the identity of the synthetic cathinone (e.g., MDPV, mephedrone, methylone) as well as the caffeine content (e.g., from 100% MDPV to 100% caffeine) (Davies et al., 2010; Shanks et al., 2012), making it difficult, if not impossible, to predict what people are using at any given time. Nevertheless, despite clear evidence that many recreational drugs, including cocaine and bath salt preparations, are often mixtures of multiple drugs, most drug abuse research evaluates the effects of drugs in isolation.

Similar to other drugs of abuse (e.g., cocaine, methamphetamine), synthetic cathinones interact with monoamine

This work was supported, in part, by a National Institutes of Health (NIH) research grant (R01 DA039146) from the National Institute on Drug Abuse (NIDA), as well as the NIH Intramural Research Programs of NIDA and the National Institute of Alcohol Abuse and Alcoholism (NIAAA). C.P.F. is supported by a Senior Scientist Award [K05 DA017918] from NIDA as well as a grant from the Welch Foundation [Grant AQ-0039].
dx.doi.org/10.1124/jpet.116.234252.

ABBREVIATIONS: ANOVA, analysis of variance; DAT, dopamine transporter; E_{max}, maximal effect level; FR, fixed ratio; MDPV, 3,4-methylenedioxypyrovalerone; NIDA, National Institute on Drug Abuse; SERT, serotonin transporter.

transporters [dopamine transporter (DAT), norepinephrine transporter, and serotonin transporter (SERT)], where they function as either inhibitors (inhibit uptake only) or substrates (inhibit uptake and stimulate release) (e.g., Baumann et al., 2013; Simmler et al., 2013). Similarities also exist with regard to the behavioral and physiologic effects of synthetic cathinones and cocaine, with both drugs or family of drugs capable of increasing locomotor and cardiovascular activity, maintaining self-administration, and producing discriminative stimulus effects that overlap with other drugs of abuse with similar mechanisms of action (e.g., methamphetamine and MDMA) (Aarde et al., 2013; Baumann et al., 2013; Fantegrossi et al., 2013; Gatch et al., 2013; Marusich et al., 2014; Watterson et al., 2014; Schindler et al., 2016). Despite differences in their mechanisms of actions, caffeine (adenosine A_1 and A_{2A} receptor antagonist) and cocaine (monoamine transporter inhibitor) are also known to produce similar behavioral effects (e.g., Garrett and Holtzman, 1996). This partially overlapping profile of activity results from interactions between adenosine and dopamine systems, through which adenosine receptor antagonists, such as caffeine, stimulate or enhance the activity of dopamine systems (Mumford and Holtzman, 1991; Garrett and Holtzman, 1994, 1996; Powell et al., 1999; Solinas et al., 2002). In addition to interactions at the presynaptic level that can affect dopamine release, modulation of dopamine neurotransmission can also occur through heteromeric adenosine-dopamine receptor complexes (Ferré, 1997).

The primary goal of the current study was to test the hypothesis that caffeine enhances the effects of drugs with which it is mixed (e.g., cocaine and MDPV). Dose-addition analyses provide a powerful approach to characterizing drug-drug interactions (e.g., additive, supra-additive, or subadditive) when both drugs produce the same pharmacologic effect (Tallarida, 2000). Because MDPV and caffeine both produce cocaine-like discriminative stimulus effects (Harland et al., 1989; Justinova et al., 2009; Gatch et al., 2013), and because drug-discrimination procedures provide a stable baseline from which to quantitatively assess changes in the potency and effectiveness of a drug (or drug mixture), the current studies evaluated the effects of cocaine, MDPV, and caffeine administered alone and as binary mixtures in rats trained to discriminate 10 mg/kg cocaine from saline. Each pair of drugs was mixed at three fixed-dose ratios (3:1, 1:1, and 1:3; relative to their ED_{50} values), with dose-addition analyses used to determine whether combining drugs with similar (cocaine and MDPV) or dissimilar (cocaine and caffeine; MDPV and caffeine) mechanisms of action resulted in cocaine-like discriminative stimulus effects that differed from those predicted for a strictly additive interaction.

Materials and Methods

Subjects. Seven adult male Sprague-Dawley rats (275–300 g) were obtained from Harlan (Indianapolis, IN) and individually housed for the duration of the study in a temperature- and light-controlled vivarium (24°C; 14/10 hour light/dark cycle). Rats were maintained with free access to tap water and were fed 15 g of Purina rat chow per day. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and the 8th edition of the Guide for Care and Use of Laboratory Animals (National Research Council, 2010).

Apparatus. All experiments were conducted in standard operant conditioning chambers (ENV-008CT; Med Associates, Inc., St. Albans, VT) located within ventilated, sound-attenuating enclosures (ENV-022M; Med Associates, Inc.). The right wall was equipped with two response levers (fixed, nonretractable) and two stimulus lights (2.5 cm in diameter) that could be illuminated with a 100-mA white light. A pellet trough was located between the two levers and allowed for the delivery of 45-mg food pellets (PJAI-0045; Noyes Precision Pellets, Research Diets Inc., New Brunswick, NJ). Experiments were controlled by an IBM computer, and data were collected using MED-PC IV software and a PC-compatible interface (Med Associates, Inc.).

Single-Cycle Discrimination Training. Rats were trained to discriminate cocaine (10 mg/kg; i.p.) from saline using a two-lever discrimination procedure, with the position of the cocaine-appropriate lever (i.e., left or right) counterbalanced across rats. Cocaine and saline training sessions were conducted in a pseudorandom order, with the restriction that a particular type of training session was not conducted on more than 3 consecutive days. Immediately after cocaine or saline administration, rats were placed in a dark operant chamber for a 10-minute pretreatment period. The start of the session was signaled by the illumination of a white light above each lever, and completion of ratio requirements on the injection-appropriate lever resulted in the delivery of a food pellet. Initially, food was delivered after a single response on the injection-appropriate lever, with the response requirement gradually increased to a fixed ratio (FR) of 10, based on performance. Responding on the alternate lever reset the response requirement on the injection-appropriate lever. Sessions were terminated after 20 minutes or after 50 food pellets were delivered, whichever occurred first. Testing began once rats satisfied the acquisition criteria, defined as five consecutive sessions (or six of seven) in which ≥80% of responses occurred on the injection-appropriate lever both before delivery of the first reinforcer and for the entire session. During test sessions, rats received an injection of either saline or one of four doses of cocaine (1, 3.2, 10, or 17.8 mg/kg, i.p.), and completion of 10 consecutive responses on either lever resulted in the delivery of a food pellet. Test sessions were terminated after 20 minutes or 50 food pellets were delivered, whichever occurred first.

Multiple-Cycle Discrimination Training. Once satisfactory dose-response curves were generated for cocaine under single-cycle conditions (i.e., at least one dose of cocaine that resulted in ≥80% of responding on the cocaine-appropriate lever and at least one dose of cocaine that resulted in ≤20% of responding on the cocaine-appropriate lever), the experimental conditions were changed to a multiple-cycle procedure, which consisted of two to six cycles per session. Each cycle began with a 10-minute pretreatment period, followed by a 10-minute response period during which a maximum of five food pellets could be received for responding on the injection-appropriate lever. The stimulus conditions were identical to those for single-cycle conditions (i.e., pretreatments were spent in a dark chamber, and illumination of the white lights above the levers signaled the availability of food under an FR10 schedule of reinforcement); however, lever lights were extinguished after five pellets had been delivered, with the remainder of the 10-minute response period spent in the dark. At the end of each 10-minute response period, rats were briefly removed from the chamber for the next training or testing injection.

Seven distinct training conditions were used to allow for roughly comparable frequencies of reinforcement on the cocaine- and saline-appropriate levers across each of the six possible cycles. Briefly, 10 mg/kg cocaine could be administered before any cycle or not at all (i.e., six saline injections); however, if cocaine was administered before cycles 1–5, it was always followed by a sham cycle in which rats were removed from the chamber and poked in the abdomen with a capped syringe. During sham cycles, food was delivered for responding on the cocaine-appropriate lever. Sham cycles were included to normalize the frequencies of reinforcement on the saline- and drug-appropriate levers across training sessions.

Multiple-Cycle Discrimination Testing. Test sessions were performed every third session as long as testing criteria were satisfied during two consecutive training sessions (at least three reinforcers earned in each cycle, with ≥80% of responding occurring on the injection-appropriate lever, both before the first reinforcer and for the duration of the cycle). Test sessions always began with saline administered before the first cycle, followed by cumulative doses of the test drug (or drug mixture) administered before cycles 2–6. The following drugs were administered i.p. alone: cocaine (1.78, 3.2, 5.6, 10, and 17.8 mg/kg), caffeine (3.2, 5.6, 10, 17.8, and 32 mg/kg), methamphetamine (0.178, 0.32, 0.56, 1.0, and 1.78 mg/kg), MDPV (0.1, 0.178, 0.32, 0.56, and 1.0 mg/kg), and midazolam (0.178, 0.32, 0.56, 1.0, and 1.78 mg/kg), which served as a negative control. Rats were also tested in sessions comprising six consecutive saline injections. The following drugs were administered as binary mixtures: cocaine and caffeine, MDPV and caffeine, and cocaine and MDPV. Drug mixtures were based on the concept of dose equivalence (i.e., doses of each constituent drug that produce the same effect level) (Tallarida and Raffa, 2010) and prepared at fixed dose ratios of 3:1, 1:1, 1:3, relative to the mean ED_{50} for each drug administered alone (i.e., dose estimated to produce 50% cocaine-appropriate responding) (Table 1). To allow for the observation of leftward or rightward departures from the predicted line of additivity, the fixed-dose pair expected to produce ∼50% cocaine-appropriate responding was always tested during the fourth cycle. The dose for each constituent of the mixture was decreased by 1/4 log unit for cycles 2 and 3 and increased by 1/4 log unit for cycles 5 and 6. The details of each drug mixture are shown in Table 2. Dose-response curves for cocaine and caffeine were determined in triplicate before testing cocaine:caffeine mixtures and again (in triplicate) after testing cocaine:caffeine mixtures, methamphetamine, midazolam, MDPV, and saline. Thus, mixtures of cocaine and caffeine were designed using the maximal effect level (E_{max}), ED_{50}, and slope parameters from the first determinations, whereas mixtures of MDPV and caffeine, and cocaine and MDPV used the mean E_{max}, ED_{50}, and slope parameters from all determinations.

The order of testing was as follows: cocaine, caffeine, cocaine: caffeine mixtures, methamphetamine, midazolam, saline, cocaine, caffeine, MDPV, MDPV:caffeine mixtures, and cocaine:MDPV mixtures. For drug mixtures, the 1:3, 1:1, and 3:1 fixed-dose ratios were evaluated according to a pseudo-Latin square design.

Drugs. (-)-Cocaine hydrochloride was provided by the NIDA Drug Supply Program. Caffeine and (+)-methamphetamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Midazolam hydrochloride was purchased from Hospira Inc. (Lake Forest, IL) as a 5 mg/ml solution. (+/−)-MDPV was synthesized in the Laboratory of Medicinal Chemistry at NIDA by Dr. Kenner Rice. Drug mixtures were prepared in a single solution according to the concentrations listed in Table 2. All drugs were dissolved in physiologic saline and administered i.p. Most drugs were administered in a volume of 1 ml/kg; however, because of the solubility limits of caffeine (∼14 mg/ml), the maximum concentration of caffeine used was 10 mg/ml, with injection volumes increased to achieve the appropriate unit dose (e.g., 1.78 ml/kg of 10 mg/ml caffeine to achieve 17.8 mg/kg caffeine).

Statistical Analyses. Dose-response curves for each drug (alone) were evaluated in triplicate for each rat. The mean values obtained for each dose were then used to determine the E_{max} for each drug in each rat. Linear regression of the portion of log dose-response curve spanning the 20% and 80% effect levels was used to determine ED_{50} values for % cocaine-appropriate responding as well as slope parameters for each drug alone. No more than one dose with >80% effect and no more than one dose with <20% effect were included in these analyses. A one-way analysis of variance (ANOVA) with repeated measures and post hoc Dunnett's tests was used to determine statistically significant differences between the E_{max} and slope values obtained for each drug alone. Data for percent cocaine-appropriate responding are reported as the mean (± 1 S.E.M.) percentage of responding on the cocaine-appropriate lever and were included in analyses only for cycles in which rats received at least one reinforcer. When reported, response-rate data represent the mean (± 1 S.E.M.) rate of responding in responses per second for all test sessions, regardless of whether a reinforcer was delivered.

Analysis of Drug Mixtures. Predicted effect levels were calculated for all drug mixtures based on the concept of dose equivalence using the E_{max}, ED_{50}, and slope parameters obtained for each drug in individual rats. For all drugs, the ED_{50} refers to the dose that produced 50% cocaine-appropriate responding, regardless of whether the E_{max} was ∼100% (i.e., cocaine and MDPV) or less than 100% (i.e., caffeine). To determine the predicted effect level for a pair of drugs (e.g., drug A and drug B), the dose of drug B in each fixed dose pair was converted to an equivalent dose of drug A [$b\mathrm{eq}(a)$] using the following function (eq. 1) described by Grabovsky and Tallarida (2004):

$$b\mathrm{eq}(a) = \mathrm{ED_{50}A}\Big/\left[(\mathrm{E_{max}A}/\mathrm{E_{max}B})(1 + \mathrm{ED_{50}B}^{q}/b^{q}) - 1\right]^{1/p} \quad (1)$$

where ED_{50} A and ED_{50} B are the doses of drugs A and B estimated to produce 50% cocaine-appropriate responding, E_{max} A and E_{max} B are the maximal effect levels obtained for drugs A and B, a is a dose of drug A, and q and p are slope parameters derived from the linear portion of the dose-response curves for drugs A and B, respectively. The total dose equivalents of drug A (eqA) present in each fixed dose pair was then determined by adding $b\mathrm{eq}(a)$ to a. Parameters derived for individual rats were then used to calculate the predicted effect level for individual rats using the following function (eq. 2) (Grabovsky and Tallarida, 2004):

$$\text{Predicted Effect Level} = [\mathrm{E_{max}A}(\mathrm{eqA}^{p})]/[(\mathrm{E_{max}A}(\mathrm{eqA}^{p})) + (\mathrm{ED_{50}A}^{p})] \quad (2)$$

Because rats were trained to discriminate cocaine from saline, all drug mixtures were converted to cocaine equivalents before calculating the predicted effect levels for each fixed dose pair, including mixtures that did not include cocaine (i.e., MDPV and caffeine). For the MDPV:caffeine mixtures, each component was first converted to

TABLE 1
Discriminative stimulus effects of saline and test drugs administered alone

Drug	E_{max} (% DAR)		ED_{50} (mg/kg)		Slope	
	Mean	(S.E.M.)	Mean	(95% CI)	Mean	(S.E.M.)
Cocaine[a]	100	(0.0)	4.9	(4.2–5.9)	1.7	(0.08)
Saline	1.8	(1.5)*	n.a.	n.a.	n.a.	n.a.
Caffeine[a]	77.5	(6.3)*	14.4	(9.5–21.9)	1.2	(0.12)
MDPV	98.3	(1.1)	0.39	(0.25–0.58)	2.1	(0.33)
Methamphetamine	99.8	(0.2)	0.37	(0.34–0.41)	2.3	(0.32)
Midazolam	7.6	(5.0)*	n.a.	n.a.	n.a.	n.a.

n.a., not applicable.
[a]E_{max}, ED_{50}, and slope parameters were derived from two sets of dose-response curves determined in triplicate (i.e., six dose-response curves).
*Significant difference from cocaine as determined by one-way analysis of variance with repeated measures and post-hoc Dunnett's test for multiple comparisons.

TABLE 2
Composition of binary mixtures of cocaine and caffeine, MDPV and caffeine, and cocaine and MDPV

Cycle	Cocaine (mg/kg):Caffeine(mg/kg)	MDPV (mg/kg): Caffeine (mg/kg)	Cocaine (mg/kg):MDPV (mg/kg)
	3:1	3:1	3:1
1	saline:saline	saline:saline	saline:saline
2	1.4:1.5	0.10:0.98	1.1:0.03
3	2.5:2.6	0.19:1.7	2.0:0.06
4	4.5:4.6	0.33:3.1	3.6:0.11
5	8.0:8.2	0.59:5.5	6.4:0.20
6	14.3:14.7	1.0:9.8	11.4:0.35
	1:1	1:1	1:1
1	saline:saline	saline:saline	saline:saline
2	0.9:2.9	0.07:2.0	0.75:0.07
3	1.7:5.2	0.12:3.5	1.3:0.12
4	3.0:9.3	0.22:6.2	2.4:0.22
5	5.3:16.5	0.39:11.0	4.3:0.39
6	9.5:29.3	0.70:19.3	7.6:0.70
	1:3	1:3	1:3
1	saline:saline	saline:saline	saline:saline
2	0.5:4.4	0.03:2.9	0.38:0.03
3	0.8:7.8	0.06:5.2	0.67:0.06
4	1.5:13.9	0.11:9.3	1.2:0.11
5	2.7:24.7	0.20:16.6	2.1:0.20
6	4.8:44.0	0.35:29.5	3.8:0.35

cocaine equivalents, and then the cocaine equivalents of MDPV and cocaine equivalents of caffeine were summed to provide the total cocaine equivalents for each fixed dose pair of MDPV and caffeine. Because dose-response curves for cocaine and caffeine were redetermined throughout the experiment, slightly different E_{max}, ED_{50}, and slope parameters were used to calculate the predicted effect levels. For cocaine:caffeine mixtures, the predicted effect levels for the cocaine:caffeine mixtures were determined using the mean values from both sets of dose-response curves (i.e., those determined before and after testing); however, because evaluations of the MDPV:caffeine and cocaine:MDPV mixtures were more closely matched temporally to the second set of dose-response curves for cocaine and caffeine, only parameters derived from these functions were used to calculate the predicted effect levels for mixtures of MDPV and caffeine and cocaine and MDPV.

Predicted dose-response curves for each drug mixture represent the mean (± 1 S.E.M.) of the total cocaine equivalents (mg/kg) and the mean (± 1 S.E.M.) predicted effect level (percent cocaine-appropriate responding) for each fixed dose pair of each drug mixture. Predicted dose-response curves were compared with the observed dose-response curves for each fixed dose ratio using two-factor (dose and mixture) ANOVA with repeated measures and post-hoc Holm-Sidak's multiple comparisons tests. In addition, E_{max} values and slope parameters obtained from the predicted and observed dose-response curves were compared by paired, two-tailed t tests. Predicted and observed effects were compared at three effect levels: 20%, 50%, and 80%. Accordingly, ED_{20}, ED_{50}, and ED_{80} values for predicted and observed dose-response curves were used to calculate potency ratios (e.g., $ED_{50}^{observed} / ED_{50}^{predicted}$) for individual rats, with departures from additivity deemed significant if the 95% confidence interval for the group did not include 1.

Correlation analyses were also performed to determine whether ED_{50}, slope, or maximal effect levels for constituent drugs were related to the potency ratios at the 20%, 50%, and 80% effect levels for each fixed-dose ratio of each binary mixture. Pearson's correlation coefficients are reported to indicate factors for which a significant interaction was observed.

Results

Discriminative Stimulus Effects of Constituent Drugs: Cocaine, Caffeine, and MDPV. Rates of responding during training sessions (defined as the two sessions before each test) were comparable across the three conditions (0.82 ± 0.05, 1.0 ± 0.05, and 0.94 ± 0.04 responses/s, for saline, 10 mg/kg cocaine and sham, respectively). Accuracy of responding during training sessions was also comparable, with greater than 99% of responding occurring on the injection-appropriate lever both before the first reinforcer and for the cycle as a whole. As shown in Fig. 1, dose-dependent increases in cocaine-appropriate responding were observed after cumulative doses of cocaine, MDPV, caffeine, and methamphetamine, whereas responding occurred on the saline-appropriate lever after cumulative doses of midazolam and when rats received saline before all six cycles. Although cocaine, MDPV, and methamphetamine all produced high levels of drug-appropriate responding at doses smaller than those that decreased responding (Table 1), rate-decreasing effects limited the doses of caffeine that could be tested, resulting in a maximum effect significantly smaller than that produced by cocaine. The rank-order potency for increasing cocaine-appropriate responding was methamphetamine = MDPV > cocaine > caffeine (Table 1). The cocaine and caffeine dose-response functions were determined (in triplicate) at two different times during the experiment, once before testing cocaine:caffeine mixtures, and again after testing of cocaine:caffeine mixtures was complete. Because the E_{max}, ED_{50}, and slope parameters for these two determinations were not significantly different, the data in Table 1 represent the mean of the two determinations for each drug. Dose-response curves for all other drugs were determined, in triplicate, only once.

Discriminative Stimulus Effects of Binary Mixtures of Cocaine and Caffeine. As shown in Fig. 2 (top row), when combined at fixed dose ratios of 3:1, 1:1, or 1:3, mixtures of cocaine and caffeine dose dependently increased cocaine-appropriate responding, with >90% cocaine-appropriate responding observed for at least one fixed-dose pair of each mixture. Analysis of predicted and observed dose-response curves by two-way ANOVA with repeated measures revealed a main effect of dose for all three mixtures [3:1 – $F(4,24) = 81.8$; $P < 0.0001$; 1:1 – $F(4,24) = 67.4$; $P < 0.0001$; 1:3 – $F(4,24) = 65.6$; $P < 0.0001$]; however, a main effect of mixture was observed only when cocaine and caffeine were combined

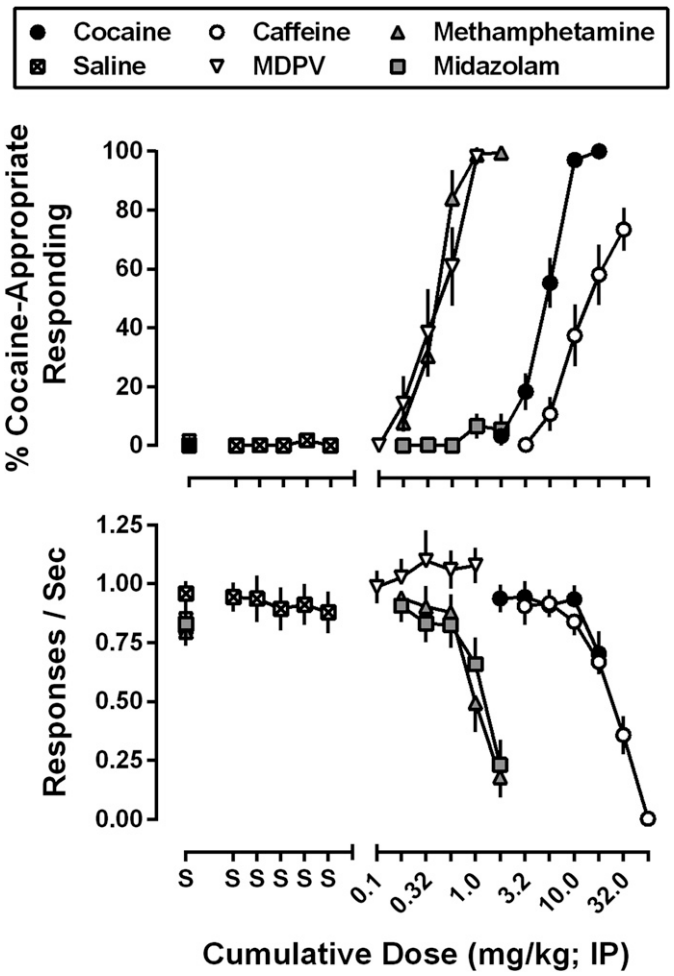

Fig. 1. Dose-response curves for the percent of responding that occurred on the cocaine-appropriate lever (top panel) during test sessions in which cumulative doses of cocaine, caffeine, MDPV, methamphetamine, or midazolam were administered, as well as when saline (S) was administered before each of six cycles. Data represent the mean (± 1 S.E.M.) for seven rats, with each dose-response curve determined in triplicate for MDPV, methamphetamine, midazolam, and saline, or doubly determined in triplicate for cocaine and caffeine. Bottom panel shows the mean (± 1 S.E.M.) rate of responding observed during test sessions. Response-rate data reflect data from all cycles of each test session; however, data for the percent cocaine-appropriate responding were included only for doses/cycles in which a rat earned at least one reinforcer.

at the 1:1 ratio [$F(1,6) = 9.0$; $P < 0.05$]. A significant dose X mixture interaction [$F(4,24) = 7.1$; $P < 0.001$] was also observed for the 1:1 fixed-dose ratio. Post-hoc Holm-Sidak's tests for multiple comparisons revealed significant differences between the observed and predicted dose-response curves for the third and fourth dose pairs of the 1:1 cocaine:caffeine mixture, which corresponds to total cocaine equivalent doses of 6.5 (±0.6) and 10.5 (±1.1) mg/kg, respectively. Although ED_{50} values for each of the cocaine:caffeine mixtures were smaller than predicted, as indicated by a potency ratio ($ED_{50}^{observed}/ED_{50}^{predicted}$) less than 1, a significant departure from additivity at the 50% effect level was observed only for the 1:1 mixture of cocaine and caffeine (Table 3). As expected, based on the significant dose X mixture interaction, differential effects were observed when comparisons were extended to smaller (20%) and larger (80%) effect levels, with mean ED_{20} values greater than 1 for all fixed dose mixtures and mean ED_{80} values significantly less than 1 for all fixed-dose mixtures of cocaine and caffeine, suggesting additive interactions at small effect levels, and supra-additive interactions at larger effect levels.

To illustrate individual differences in the nature and magnitude of the interactions between cocaine and caffeine, potency ratios for three different effect levels (20%, 50%, and 80%) are shown for individual subjects in Fig. 3 (top panels). A potency ratio of 1 indicates no difference between the predicted and observed dose required to produce 20%, 50%, or 80% cocaine-appropriate responding, whereas a potency ratio less than 1 indicates that the dose required to produce the effect at a designated level was smaller than predicted for an additive interaction, and a potency ratio greater than 1 indicates that dose required to produce the effect at a designated level was larger than predicted for an additive interaction. There were no systematic differences in the rank ordering of individual potency ratios across the 3:1, 1:1, and 1:3 fixed-dose ratios of cocaine and caffeine; however, potency ratios for some rats (1, 3, and 6) were consistently less than 1 for each effect level, suggesting a supra-additive interaction, whereas another rat (7) showed potency ratios consistently greater than 1 at the 20% and 50% effect levels, suggesting a subadditive interaction between cocaine and caffeine. Correlation analyses failed to identify any significant interactions among ED_{50}, E_{max}, or slope for cocaine and caffeine and the observed potency ratios at any effect level for 3:1 and 1:1 mixtures of cocaine and caffeine; however, ED_{50} values for caffeine were negatively correlated with the potency ratio for the 80% effect level of the 1:3 mixture of cocaine and caffeine ($R^2 = 0.72$; $P < 0.05$).

Discriminative Stimulus Effects of Binary Mixtures of MDPV and Caffeine. Predicted and observed dose-response curves for mixtures of MDPV and caffeine (fixed-dose ratios of 3:1, 1:1, and 1:3) are shown in Fig. 2 (middle row). Similar to the effects observed when caffeine was mixed with cocaine, dose-dependent increases in cocaine-appropriate responding, with maximal effect levels exceeding 90%, were observed for all fixed-dose ratios of MDPV and caffeine. Analysis of predicted and observed dose-response curves by two-way ANOVA with repeated measures revealed a main effect of dose for all three mixtures [3:1 – $F(4,24) = 97.9$; $P < 0.0001$; 1:1 – $F(4,24) = 66.8$; $P < 0.0001$; 1:3 – $F(4,24) = 81.5$; $P < 0.0001$]. Although there was no main effect of mixture (i.e., effects of mixtures did not depart from additivity), there was a significant dose X mixture interaction for all three fixed-dose mixtures [3:1 – $F(4,24) = 6.1$; $P < 0.01$; 1:1 – $F(4,24) = 3.1$; $P < 0.05$; 1:3 – $F(4,24) = 8.1$; $P < 0.001$]. Post-hoc Holm-Sidak's tests for multiple comparisons revealed significant differences between the observed and predicted dose-response curves for the first and second dose pairs of the 3:1 and 1:3 MDPV:caffeine mixtures, indicative of a subadditive interaction at small dose pairs.

Unlike for mixtures of cocaine and caffeine, grouped potency ratios for the 3:1, 1:1, and 1:3 fixed-dose mixtures of MDPV and caffeine were not different from 1 at either the 50% or 80% effect level (Table 3); however, the potency ratio for the 1:3 mixture of MDPV and caffeine was significantly greater than 1 at the 20% effect level, again suggesting a subadditive interaction at small dose pairs. As observed with mixtures of cocaine and caffeine, individual differences were seen in the absolute nature of the interactions across the 3:1, 1:1, and 1:3

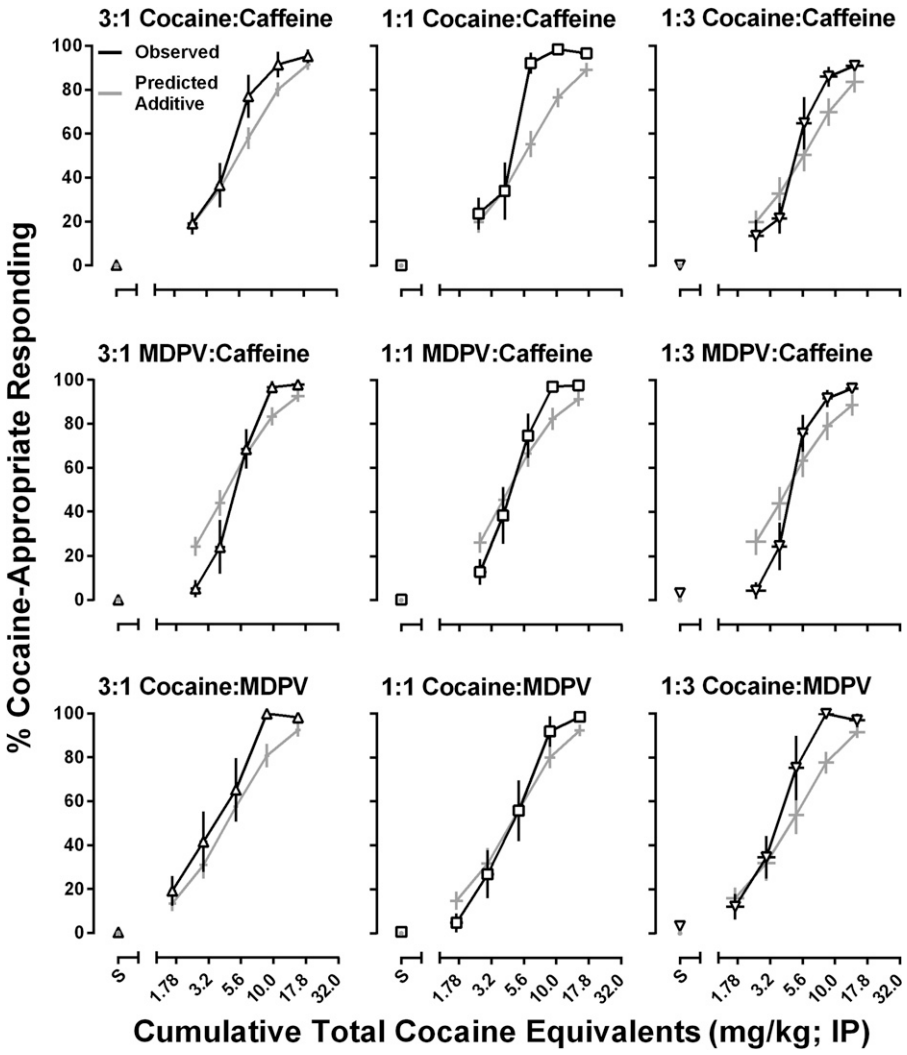

Fig. 2. Dose-response curves for the percent of responding that occurred on the cocaine-appropriate lever during test sessions in which cumulative doses of binary mixtures of cocaine and caffeine (top row), MDPV and caffeine (middle row), or cocaine and MDPV (bottom row) were administered. Each mixture was tested at three fixed-dose ratios [3:1 (left column), 1:1 (center column), and 1:3 (right column)] relative to the ED_{50} for each constituent drug. Experimentally determined dose-response curves (black lines, open symbols) represent the mean (± 1 S.E.M.) for seven rats, with all dose-response curves determined in triplicate for each rat. Predicted, additive dose-response curves represent the mean (± 1 S.E.M.) for seven rats and are depicted as gray lines (no symbols). Because total cocaine equivalents were calculated for individual rats, cumulative total cocaine equivalents (mg/kg) represent the mean (± 1 S.E.M.) for seven rats.

mixtures of MDPV and caffeine (Fig. 3; middle panel). Most notably, the potency ratios for rat 5 were consistently greater than 1 and tended to become larger as the proportion of MDPV to caffeine increased. Conversely, rat 7 showed the opposite trend, with a potency ratio less than 1 for the 3:1 mixture of MDPV and caffeine and progressively greater than 1 as the proportion of MDPV to caffeine was decreased (i.e., 1:3 > 1:1 > 3:1). As with mixtures of cocaine and caffeine, potency ratios at the 80% effect level were less than 1 for the vast majority of rats, however, when grouped potency ratios were not different from 1, suggesting that the interactions between MDPV and caffeine were largely additive. Correlation analyses failed to identify any significant interactions among ED_{50}, E_{max}, or slope for MDPV and caffeine and the observed potency ratios at any effect level for 3:1, 1:1, or 1:3 mixtures of MDPV and caffeine.

Discriminative Stimulus Effects of Binary Mixtures of Cocaine and MDPV. Dose-response curves for the predicted and observed effects of mixtures of cocaine and MDPV are shown in Fig. 2 (bottom row). As observed for the other binary mixtures, dose-dependent increases in cocaine-appropriate responding were observed when cocaine and MDPV were mixed at fixed dose ratios of 3:1, 1:1, or 1:3 (cocaine:MDPV), with near exclusive responding on the cocaine-appropriate lever observed at larger dose pairs. Analysis of predicted and observed dose-response curves by two-way ANOVA with repeated measures revealed a main effect of dose for all three mixtures [3:1 – $F(4,24) = 64.0$; $P < 0.0001$; 1:1 – $F(4,24) = 84.6$; $P < 0.0001$; 1:3 – $F(4,24) = 52.4$; $P < 0.0001$], and a main effect of mixture for the 1:3 fixed dose ratio of cocaine and MDPV [$F(1,6) = 10.3$; $P < 0.05$]. Post-hoc Holm-Sidak's tests for multiple comparisons revealed significant differences between the observed and predicted dose-response curves at the third and fourth dose pairs of the 1:3 cocaine:MDPV mixture, corresponding to total cocaine equivalent doses of 5.3 (±0.7) and 9.1 (±1.3) mg/kg, respectively. A significant dose X mixture interaction was observed when cocaine and MDPV were combined at both the 1:1 and 1:3 fixed dose ratios of their ED_{50}s [1:1 – $F(4,24) = 3.4$; $P < 0.05$; 1:3 – $F(4,24) = 5.2$; $P < 0.01$].

Despite a significant main effect of mixture for the 1:3 cocaine:MDPV mixture, analysis of the potency ratios for the 3:1, 1:1, and 1:3 mixtures suggests that the interactions between cocaine and MDPV were strictly additive at both the 20% and 50% effect levels, with supra-additive interactions apparent at the 80% effect level for both 3:1 and 1:3 mixtures of cocaine and MDPV (Table 3). With regard to individual differences in the nature of the interaction between cocaine

TABLE 3
Observed and predicted discriminative stimulus effects of binary mixtures of cocaine and caffeine, MDPV and caffeine, and cocaine and MDPV

Mixture	ED_{50} (mg/kg; Cocaine Equivalents)		Potency Ratio	Potency Ratio	Potency Ratio
	Predicted Mean (95% CI)	Observed Mean (95% CI)	ED_{20}OBS / ED_{20}PRED Mean (95% CI)	ED_{50}OBS / ED_{50}PRED Mean (95% CI)	ED_{80}OBS / ED_{80}PRED Mean (95% CI)
Cocaine:Caffeine					
3:1	5.4 (4.0–7.4)	4.7 (3.4–6.7)	1.31 (0.77–1.85)	0.92 (0.67–1.14)	0.67 (0.52–0.82)+
1:1	5.1 (3.7–7.0)	3.8 (2.7–5.5)	1.23 (0.82–1.64)	0.78 (0.59–0.97)+	0.52 (0.36–0.68)+
1:3	4.9 (3.7–6.5)	4.3 (3.3–5.7)	1.60 (0.42–2.79)	0.95 (0.61–1.28)	0.67 (0.34–0.99)+
MDPV:Caffeine					
3:1	4.3 (3.2–5.9)	4.9 (3.7–6.5)	1.69 (1.00–2.38)	1.20 (0.76–1.64)	0.87 (0.60–1.15)
1:1	4.4 (3.2–5.9)	4.4 (3.3–5.8)	1.52 (0.86–2.17)	1.07 (0.69–1.44)	0.76 (0.51–1.02)
1:3	4.3 (3.1–5.8)	4.4 (3.5–5.6)	1.62 (1.03–2.21)+	1.10 (0.73–1.46)	0.76 (0.50–1.02)
Cocaine:MDPV					
3:1	4.3 (3.2–5.8)	3.3 (2.3–4.9)	1.12 (0.48–1.76)	0.84 (0.46–1.23)	0.67 (0.37–0.97)+
1:1	4.3 (3.2–5.8)	4.3 (3.1–5.9)	1.37 (0.87–1.87)	1.06 (0.63–1.49)	0.82 (0.47–1.17)
1:3	4.4 (3.3–5.9)	3.5 (2.4– 4.9)	1.25 (0.70–1.80)	0.84 (0.54–1.13)	0.60 (0.44–0.77)+

+Significant difference between observed and predicted ED_{20}, ED_{50}, or ED_{80} values for an additive interaction as defined by 95% confidence interval (CI) that does not include 1.

and MDPV, four rats (1, 2, 3, and 4) had potency ratios that were consistently less than 1 at both the 50% and 80% effect levels, with rat 3 generally showing the largest departures from additivity across the three fixed dose ratios. As with mixtures of MDPV and caffeine, rat 5 exhibited consistent and large rightward departures from additivity, with potency ratios ranging from 1.5 to 2.5 over the 20% and 50% effect levels and, notably, was the only rat for which potency ratios were greater than 1 at the 80% effect level. Correlation analyses failed to identify any significant interactions among ED_{50}, E_{max}, or slope for cocaine and MDPV and the observed potency ratios at any effect level for any fixed-dose mixture of cocaine and MDPV.

Upon completion of testing binary mixtures, dose-response curves for cocaine alone, caffeine alone, and MDPV alone were redetermined, and the 95% confidence interval for these single determinations overlapped those derived from the previous dose-response curves for each drug alone (data not shown).

Discussion

Illicit drug preparations often include multiple pharmacologically active compounds, yet relatively little is known about the nature of the interaction(s) among constituents of these drug mixtures or between drugs used concurrently (i.e., polydrug abuse). Using the concept of dose equivalence, the current study characterized the nature of interactions between drugs with similar (cocaine and MDPV) and dissimilar (cocaine and caffeine or MDPV and caffeine) mechanisms of action in rats trained to discriminate cocaine from saline. Despite differences in their mechanisms of action, cocaine (a nonselective monoamine transporter inhibitor), MDPV (a dopamine- and norepinephrine-selective transporter inhibitor), and caffeine (an adenosine A_1 and A_{2A} receptor antagonist) each dose dependently increased cocaine-appropriate responding, indicating at least partially overlapping discriminative stimulus effects. For mixtures containing cocaine, the interactions tended toward additivity at smaller effect levels (i.e., 20% and 50%) and toward supra-additivity at larger effect levels (i.e., 80%). When the synthetic cathinone MDPV was mixed with caffeine, strictly additive interactions were observed for all fixed dose mixtures. Although previous studies have pointed toward additive interactions between cocaine and caffeine (Harland et al., 1989; Kleven and Koek, 1998; Justinova et al., 2009), the current study is the first to use dose-addition analyses to describe the nature of the interaction between binary mixtures of cocaine, MDPV, and caffeine. Given that caffeine is often identified in drug preparations containing cocaine or MDPV, these findings provide an important first step toward a more complete understanding of the behavioral and abuse-related effects of both cocaine and bath salts preparations.

Caffeine is the most widely used drug worldwide. Similarities between the behavioral and neuropharmacologic effects of caffeine and drugs such as cocaine and methamphetamine have led others to investigate possible interactions among these drugs (e.g., Garrett and Holtzman, 1994; Solinas et al., 2002). Although numerous studies have demonstrated that caffeine can increase the locomotor, discriminative stimulus, or reinforcing effects of cocaine when administered before or coadministered with cocaine (e.g., Gauvin et al., 1990; Schenk et al., 1990, 1994; Horger et al., 1991; Kuribara, 1994; Comer and Carroll, 1996; Garrett and Griffiths, 1997; Kleven and Koek, 1998; Gasior et al., 2000; Poleszak and Malec, 2002; Justinova et al., 2009; Prieto et al., 2015), few studies have attempted to characterize the nature of the interaction(s) between these two drugs (e.g., Harland et al., 1989). Harland and colleagues combined fixed doses of caffeine with a range of cocaine doses (and vice versa), and concluded that these drugs had strictly additive interactions; however, these analyses failed to incorporate parameters (i.e., maximal effect and slope) now known to impact statistical analyses regarding departure from additivity (Grabovsky and Tallarida, 2004); both these parameters were incorporated into the current analyses.

Indeed, and as reported by Harland et al. (1989) and Justinova et al. (2009), although caffeine increases cocaine-appropriate responding, the slope of this function is shallower and the maximum effect is smaller than that obtained with cocaine. Although the additive interactions observed at smaller effect levels of cocaine:caffeine mixtures are in general agreement with reports by Harland and colleagues (1989), the current studies found consistent supra-additive interactions at larger effect levels (i.e., 80%) for each of the cocaine and

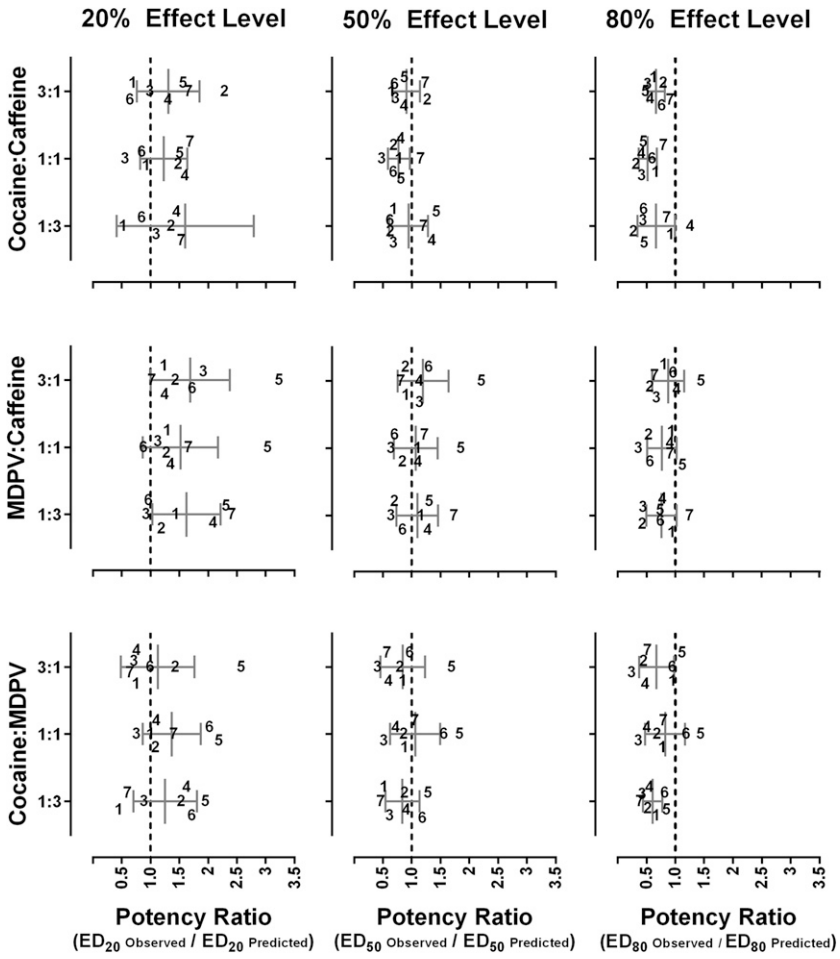

Fig. 3. Potency ratios (observed/predicted additive) for binary mixtures of cocaine and caffeine (top row), MDPV and caffeine (middle row), and cocaine and MDPV (bottom row) at three different effect levels: 20% (left column), 50% (center column), and 80% (right column). Potency ratios for individual subjects are depicted by their ID no. The group mean and 95% CI are depicted by the vertical gray line and error bars, respectively.

caffeine mixtures. One possible explanation for this apparent discrepancy is that the current study designed mixtures to include fixed-dose pairs that spanned the linear portion of the constituent drug's dose-response curves, whereas Harland et al. (1989) combined relatively ineffective doses of caffeine (i.e., less than 40% cocaine-appropriate responding) with relatively large doses of cocaine (i.e., greater than 50% cocaine-appropriate responding). As a result, high levels of cocaine-appropriate responding (> 40%–50%) were observed with the first dose pair for most of their cocaine and caffeine mixtures that reduced the accuracy of ED_{50} values and subsequent isobolographic analyses. Nevertheless, with the exception of the supra-additive interactions observed at larger effect levels, the current findings are in general agreement with previous reports (Harland et al., 1989).

To determine whether similar interactions occur between caffeine and other monoamine transporter inhibitors, the current studies also characterized the cocaine-like discriminative stimulus effects of mixtures of caffeine and MDPV. Consistent with previous reports, MDPV produced cocaine-appropriate responding with near exclusive levels of cocaine-appropriate responding observed at a dose of 1 mg/kg (Gatch et al., 2013). Regardless of the proportion at which they were mixed, MDPV and caffeine exhibited strictly additive interactions with respect to their cocaine-like discriminative stimulus effects. As observed for mixtures of cocaine and caffeine, a dose *X* mixture interaction was observed for all three mixtures of MDPV and caffeine, suggesting that the nature of the interaction varied as a function of dose. Unlike with mixtures of cocaine and caffeine, the tendency toward supra-additivity at larger effect levels failed to reach significance. Although it is unclear why MDPV and caffeine exhibited seemingly opposing interactions at small and large effect levels, this could be due to the quantal nature of responding in drug-discrimination assays (i.e., rats are trained to respond exclusively on the saline-appropriate or cocaine-appropriate lever, thereby reducing the likelihood of observing graded responses) and/or due to MDPV and/or caffeine having discriminative stimulus effects that only partially overlap with those of cocaine (which was a component of both cocaine:caffeine and cocaine:MDPV mixtures).

The ability of monoamine transporter inhibitors to enhance discriminative stimulus effects of cocaine has been consistently demonstrated in rats (Cunningham and Callahan, 1991; Kleven and Koek, 1998; Li et al., 2006; Soto et al., 2009), although with some exceptions (e.g., modafinil; Loland et al., 2012). Unlike previous studies, which have largely investigated the effectiveness of pretreatments with fixed doses of DAT, SERT, and norepinephrine transporter inhibitors to shift the cocaine dose-response curve to the left (enhancement) or right (inhibition), the current studies used dose-addition analyses to characterize the nature the interaction(s) between cocaine and MDPV across a range of fixed-dose pairs and at three different ratios relative to their

ED_{50} values. Using this approach, it is clear that the nature of the interaction between cocaine and MDPV was generally additive, as would be predicted for two drugs with similar mechanisms of action; however, supra-additive interactions were observed at large effect levels (i.e., ED_{80} values for 3:1 and 1:3 mixtures of cocaine and MDPV). Such an outcome is consistent with the leftward shifts in the cocaine dose-response curves reported in the previous pretreatment studies, as well as the results of isobolographic analyses which characterized the effects of pretreatments with drugs that interact with monoamine transporters on the discriminative stimulus effects of cocaine (Li et al., 2006). Additivity has also been observed with regard to the reinforcing effects of binary mixtures of cocaine and another monoamine transporter inhibitor, RTI-117 (Woolverton et al., 2008). Although cocaine and MDPV have comparable mechanisms of action, MDPV is much more selective for DAT relative to SERT (300- to 800-fold selective) than cocaine (1.5- to 3-fold selective) (Baumann et al., 2013; Simmler et al., 2013). Together with work by Kleven and Koek (1998), these functional profiles suggest that the supra-additive interactions observed between cocaine and MDPV at the larger effect levels likely result from drug actions or interactions at DAT.

With regard to individual differences in the drug-drug interactions, some rats (i.e., rats 1, 2, and 3) tended toward supra-additivity, whereas others (i.e., 5 and 7) tended toward subadditivity. Because the nature of the interaction is known to be dependent on the ratio at which the drugs are mixed, it is possible that these differences resulted from the use of a single mixture based on the group mean ED_{50} value rather than customized mixtures for individual subjects. Although this approach introduced some intersubject variability with regard to the precise ratio at which drugs were mixed [e.g., the mean ($\pm$S.E.M.) ratio for the 3:1 mixture of cocaine and caffeine was 3.1 ($\pm$0.3):1.2 ($\pm$0.2) when individual differences were taken into account], these differences were not statistically significant and did not impact the magnitude or nature of the interaction across subjects. Moreover, individual differences in the ED_{50}, E_{max}, or slope parameters of constituent drugs were not correlated with the magnitude or nature of the interactions for the vast majority of the binary mixtures, suggesting that the use of group ED_{50} values did not interfere with characterizations of the drug-drug interactions.

In summary, the present study used dose-addition analyses to characterize the nature of the interactions for the cocaine-like discriminative stimulus effects of binary mixtures of cocaine, caffeine, and MDPV. There were four main findings: (1) when tested alone, cocaine, MDPV, caffeine, and methamphetamine dose dependently increased cocaine-appropriate responding, midazolam and saline did not; (2) when tested as binary mixtures, the cocaine-like discriminative stimulus effects of fixed-dose mixtures of cocaine and caffeine, MDPV and caffeine, and cocaine and MDPV generally did not depart from additivity, although supra-additive interactions were observed at large effect levels when cocaine was mixed with either caffeine or MDPV; (3) across conditions, interactions tended toward additivity (or subadditivity for MDPV and caffeine) for smaller dose pairs and toward supra-additivity for larger dose pairs, an effect that might be related to the quantal nature of responding in drug-discrimination assays; and (4) the nature of the interaction differed across subjects, with some rats consistently showing supra-additive interactions and others consistently showing subadditive interactions. Together, these results provide clear evidence for additive interactions with respect to the cocaine-like discriminative stimulus effects of binary mixtures of cocaine, caffeine, and MDPV and suggest that supra-additive interactions can occur between drugs with different mechanisms of action (cocaine and caffeine) or different selectivity values for DAT over SERT (cocaine and MDPV). Although these findings demonstrate that caffeine can function as a substitute in drug preparations containing either cocaine or MDPV, further research is required to determine whether similar interactions exist for the reinforcing or toxic effects of cocaine and synthetic cathinones when they are mixed with caffeine.

Authorship Contributions

Participated in research design: Collins, France.
Conducted experiments: Collins, Abbott, Galindo, Rush.
Contributed new reagents or analytic tools: Rice.
Performed data analysis: Collins.
Wrote or contributed to the writing of the manuscript: Collins, Rice, France.

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