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Self-administration of drug mixtures by monkeys: combining drugs with comparable mechanisms of action

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Abstract

Rationale—Abuse of drug mixtures is common. Drug interactions that are super-additive in terms of reinforcing effects may contribute to this phenomenon. Although quantitative methods for assessing drug interactions have been developed, they have not been widely applied to the analysis of reinforcing effects.

Objectives—The present experiment was designed to study self-administration of mixtures of drugs with comparable pharmacological mechanisms of action. Our hypothesis was that the drugs would be dose-additive.

Materials and methods Rhesus—monkeys prepared with i.v. catheters were allowed to self-administer cocaine or saline under a progressive-ratio schedule in baseline sessions. When responding was stable, two mu opioid agonists, alfentanil and remifentanil, were tested alone in one group ($n=5$). Two dopamine (DA) uptake blockers, cocaine and RTI-117 were tested in the other group ($n=6$). Next, mixtures of doses of the two opioids or the two DA uptake blockers were

tested in approximate 1:1, 1:2, and 2:1 ratios of their ED₅₀s. Results were analyzed using isobolographic techniques.

Results—All drugs alone and drug mixtures functioned as positive reinforcers in a dose-related manner. There was no difference between experimentally determined ED₅₀ values and predicted additive ED₅₀ values for any mixture. Maximum responding maintained by mixtures, a measure of reinforcing strength, did not differ from that for single drugs.

Conclusions—Mixtures of various proportions of two drugs with comparable mechanisms of action were additive, i.e., they did not interact. This result will serve as the basis for comparison to studies of mixtures of drugs with various mechanisms of action.

Keywords

Drug abuse; Self-administration; Rhesus monkey; Opioid; Dopamine uptake blocker; Drug mixture; Isobologram; Additivity

Abuse of drug mixtures is common. Mechanisms that have been advanced to account for polydrug abuse include interactions that enhance positive effects or diminish negative effects of one or more of the combined drugs (Ellinwood et al. 1976; Kosten et al. 1986; 1987; Kreek 1987). Several drug combinations have been studied in the laboratory in humans and non-humans under a variety of conditions, including ethanol/barbiturate (DeNoble et al. 1985; Meisch and Lemaire 1990), cocaine/marijuana (Foltin and Fischman 1990), benzodiazepine/caffeine (Rush et al. 1994), ethanol/cocaine (Ikegami et al. 2002), ethanol/marijuana (Liguori et al. 2002), and dextromethorphan/diphenhydramine (Jun et al. 2003). It is not always clear, however, whether and how results can be accounted for by these broad mechanisms. Even for the stimulant/opioid mixture, the mixture that has been most frequently studied, the nature of any interaction between drugs is equivocal. Some studies have found little evidence of an interaction between stimulants and opioids (Hemby et al. 1996; Mattox et al. 1997; Mello et al. 1995). Other studies provided evidence for a significant interaction between stimulants and opioids in drug self-administration. Some have reported an increase in potency of the combination relative to either drug alone (Duvauchelle et al. 1998; Rowlett and Woolverton 1997; Rowlett et al. 1998; Winger et al. 2006), while other results support the conclusion that the reinforcing effects of a stimulant/opioid combination are enhanced relative to either drugs alone (Ranaldi and Munn 1998; Wang et al. 2001). One conclusion that is possible from these experiments and that would be consistent with the drug self-administration literature is that the effects of a drug combination depend upon the conditions of availability (Ward et al. 2005). Although certainly true, in the absence of a quantitative definition of a drug interaction, it is difficult to conclude whether the interaction between drugs depends upon the conditions of availability.

Traditionally, interactions between drugs have been quantified in reference to dose-additivity (see Loewe 1953; Tallarida 2000; Wessinger 1986; Woolverton 1987). Additivity serves as a reference point because it is the outcome that defines no interaction. Thus, experimental approaches that allow the distinction between a combination that is additive and one that is super-additive or sub-additive should enhance our understanding of interactions between abused drugs. The quantitative analysis of drug interactions has been substantially refined over recent years (see Tallarida 2000), although these approaches have not been systematically applied to studies of drug self-administration and the reinforcing effects of drugs. One hypothesis with regard to abuse of drug mixtures is that drugs that are abused in combination are super-additive with regard to their reinforcing effects. Negus (2005) tested this hypothesis using the dose-addition model with combinations of cocaine and heroin in rhesus monkeys allowed to choose between i.v. drug injections and food.

Interestingly, the combination of cocaine and heroin was additive in some combinations and sub-additive in others. Super-additivity was not observed. Smith et al (2006) also reported additivity between cocaine and heroin in rats. Although these experiments suggest that this is a viable approach to studying the self-administration of drug combinations, it has not been systematically investigated with regard to reinforcing effects.

The objective of the present experiment, then, was to study the self-administration of mixtures of drugs with comparable mechanisms of action and to quantify the interaction between them, if any, in terms of reinforcing effects. Rhesus monkeys were prepared with chronic i.v. catheters and allowed to self-administer drugs under a progressive-ratio (PR) schedule of reinforcement with an inter-trial interval (ITI) between injections. A PR schedule was used because it allows measurement of both potency and strength (maximum reinforcing effect) as a reinforcer (e.g., Griffiths et al. 1975, 1978; Hoffmeister 1979; Rowlett et al. 1996). The ITI allows time for the non-specific rate-altering effects of the drugs to dissipate, so that responding is more clearly determined by reinforcing effects (e.g., Griffiths et al. 1978; Winger 1993; Rowlett et al. 1996). In one group of monkeys, two mu opioids, alfentanil and remifentanil, were studied alone and in combination. In a second group, two dopamine transporter (DAT) ligands, cocaine and RTI-117 (Carroll et al. 1995). Reinforcing effects were measured for each drug alone and for combinations of fixed ratios of doses. With the “fixed-ratio” design, drug doses are combined in a particular ratio, for example, the ratio of the ED₅₀s. The fixed-ratio approach is preferred to the fixed-dose approach for a number of theoretical and statistical reasons, detailed by Tallarida (2000). The quantitative analysis of the effects of combining drugs with the same mechanisms of action will serve as the basis of comparison for combinations of drugs with different mechanisms of action. The hypothesis was that drugs with comparable mechanisms of action would be additive in terms of reinforcing effects.

Materials and methods

All animal use procedures were approved by the University of Mississippi Medical Center's Animal Care and Use Committee and were in accordance with the National Research Council's Guide for Care and Use of Laboratory Animals (1996).

Animals and Apparatus

The subjects were 11 male rhesus monkeys (*Macaca mulatta*) weighing between 7.0 and 10 kg at the beginning of the study. Monkeys 96R0679, 96R0661, and 97R0111 had a history of self-administration of the phenyltropane RTI-31 under a fixed-ratio 1 (FR1) schedule of reinforcement (Wee et al. 2006). Monkeys M1388, M1389, and RiK2 had a history of self-administration of a variety of compounds under PR schedules similar to the one used in this study (Wang and Woolverton 2007a; Wee and Woolverton 2006; Wee et al. 2006). Monkey R0463 had a brief history of cocaine self-administration under a choice schedule (unpublished). The other monkeys (97R0697, M1338, CK47, and R0209) were experimentally naive. All monkeys were provided with sufficient food to maintain stable initial body weights (140–200 g/day, Teklad 25% Monkey Diet, Harlan/Teklad, Madison, WI) and had unlimited access to water. Fresh fruit was provided daily, and a vitamin supplement was given three times a week. Lighting was cycled to maintain 16 h of light and 8 h of dark, with light on at 0600 hours.

Each monkey was fitted with a stainless-steel harness (E&H Engineering, Chicago, IL) or a jacket (Lomir Biomedical, Malone, NY) that attached by a tether to the rear wall of the experimental cubicle (1.0 m³, Plaslabs, Lansing, MI). The front door of the cubicle was made of transparent plastic, and the remaining walls were opaque. Two response levers (PRL-001, BRS/LVE, Beltsville, MD) were mounted on the inside of the door. Four jeweled

stimulus lights, two red and two white, were mounted above each lever. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer, Chicago, IL). A Macintosh computer with custom interface and software controlled all events in an experimental session and recorded data.

Procedure

Monkeys were implanted with a silastic catheter (0.26 cm o.d. × 0.076 cm i.d.; Cole-Parmer, Chicago, IL) into the jugular (internal or external) or femoral vein under isoflurane anesthesia. Brachial veins were implanted with a microrenethane catheter (0.2 cm o.d. × 0.1 cm i.d.; Braintree Scientific, Braintree, MA) heated and drawn to approximately half size at the proximal end. The proximal end of the catheter was inserted into the vein and terminated in the vena cava near the right atrium. The distal end was threaded subcutaneously to exit the back of the monkey, threaded through the tether, out the rear of the cubicle and connected to the peristaltic pump. In the event of catheter failure, surgery was repeated using another vein, after the veterinarian confirmed the health of the monkey.

Experimental sessions began at 11:00 each day and were conducted 7 days per week. Thirty minutes before each session started, catheters were filled with drugs for the sessions without infusing the drugs into monkeys. At the start of a session, the white lights were illuminated above both levers and pressing the right lever resulted in the delivery of a drug injection for 10 s. During the injection, the white lights were extinguished and the red lights were illuminated. Responding was maintained under a PR schedule of reinforcement comparable to that described by Wilcox et al. (2000). A session consisted of 20 trials, with 1 injection available per trial. The response requirement started at 50 responses/injection (opioids) or 100 responses/injection (cocaine, RTI-117) and doubled after every fourth trial. There was an ITI after each injection of 5 min (opioids) or 10 min (cocaine, RTI-117) during which lights were extinguished and levers were inactive. A subject had 30 min to complete a trial (limited hold 30 min, LH 30'). A trial ended with a 10-s drug injection or the expiration of the LH. If the response requirement was not completed for two consecutive trials (i.e., the LH expired) or the animal self-administered all 20 injections, the session ended. After the session, catheters were filled with 0.9% saline containing heparin (40 units/ml).

In baseline sessions, injections of cocaine or saline were available. The baseline dose of cocaine was the lowest dose that maintained the maximum injections in individual monkeys, between 0.1 and 0.4 mg/kg/injection. The baseline dose of cocaine or saline was initially available under a double-alternation schedule, i.e., two consecutive daily cocaine sessions were followed by two consecutive daily saline sessions. This sequence was sometimes modified to allow extra sessions for responding to be maintained by cocaine or to extinguish with saline injection, according to the behavior of the individual monkey. Responding was considered stable in baseline sessions when injections/session varied by no more than two for both cocaine and saline for at least two consecutive double-alternation sequences. At this point, test sessions were inserted to the daily sequence between two saline and two cocaine sessions. To prevent monkeys from learning this session sequence, a randomly determined saline or cocaine baseline session was inserted after every other test session. Thus, the final daily sequence of sessions was C, S, T, S, C, T, R, C, S, T, S, C, T, R, where "C," "S," "R," and "T," respectively, represent a cocaine baseline, a saline, a randomly determined cocaine/saline, and a test session.

In one group on monkeys, various doses of alfentanil (0.2–1.6 µg/kg per injection) and remifentanil (0.025–0.8 µg/kg per injection) were made available to a monkey in test sessions that were otherwise identical to baseline sessions. Alfentanil was tested first in two of the monkeys and remifentanil first in the other three. In the other group, one of various doses of cocaine (0.012–0.2 mg/kg per injection) or RTI-117 (0.006–0.2 mg/kg per

injection) was made available to a monkey in test sessions that were otherwise identical to baseline sessions. Cocaine was tested first in half the monkeys and RTI-117 first in the other half. All doses of one compound were tested before moving on to the next compound. For the first monkey tested with a given drug, doses were available in an ascending order. For the other monkeys, doses were tested in an irregular order. After a test session, a monkey was returned to baseline conditions until responding again met stability criteria or a new stable baseline was established. At least five doses of each drug were tested. All doses were tested at least twice in each monkey, once with a saline session the day before and once with a cocaine session the day before. When the two test sessions of a dose showed high variability (each of the two determinations $\geq \text{mean} \pm 3$ injections), the dose was re-tested twice, once after a saline and once after a cocaine baseline session.

After testing individual drugs, ED_{50} s were calculated for individual monkeys. Monkeys were then tested with combinations of doses of alfentanil and remifentanil or cocaine and RTI-117, in fixed ratios of their ED_{50} s. This was done in the same manner as with individual drugs on a milligram/kilogram basis, with total doses of mixtures calculated by adding together the doses of the individual drugs in the mixture (see Tallarida 2000). As the nature of the interaction between two drugs can change with the dose ratios, combinations were tested in approximate 1:1, 2:1, and 1:2 ratios of their ED_{50} s. Order of testing of the ratios was counterbalanced across monkeys.

Data analysis

The mean number of injections per session was calculated individually from the two test sessions at a dose and mean values were calculated for the group. The mean effect at each dose was used in a nonlinear regression analysis (Tallarida 2000, pp. 173–179) to yield curve fits and ED_{50} values (with SEM) for the individual drugs. The individual ED_{50} values allow the calculation of the additive total ED_{50} of a combination as given in Tallarida (2000). Briefly stated, for two drugs, A (the lower potency drug) and B (higher potency drug) having a constant potency ratio, the total additive ED_{50} , denoted Z_{add} , is calculated from the individual ED_{50} values of drugs A and B as

$$Z_{\text{add}} = ED_{50A} / [p + R(1 - p)] \quad (1)$$

where R is the potency ratio (ED_{50A}/ED_{50B}) and p is the proportion of the total dose that is drug A. An equivalent expression, based on the conversion of p and $(1-p)$ to fractions, f of ED_{50A} and $(1-f)$ of ED_{50B} is given by

$$Z_{\text{add}} = f(ED_{50A}) + (1 - f)(ED_{50B}) \quad (2)$$

which allows an estimation of the variance $V(Z_{\text{add}})$, the square of the standard error, as

$$V(Z_{\text{add}}) = f^2 V(ED_{50A}) + (1 - f)^2 V(ED_{50B}) \quad (3)$$

Experimental values of the total ED_{50} were obtained from linear regression of effect on $\log(\text{dose})$ for each subject, and these values were averaged to give the mean and standard error of the mean for subsequent statistical comparison with Z_{add} (Tallarida 2000, pp. 60–62). For graphical display, isobolograms were constructed (Tallarida 2006). For constant R ,

this yields a straight line of additivity in Cartesian coordinates that is obtained by connecting the intercepts that are the individual ED₅₀ values. Departures from simple additivity occur when it can be shown that $Z_{\text{mix}} < Z_{\text{add}}$, indicative of synergism, or when $Z_{\text{mix}} > Z_{\text{add}}$, which indicates sub-additivity. Equality, of course, means that the drug interaction is simply additive. On the isobologram experimentally derived dose combination pairs (for the 50% effect) that do not lie significantly off this line are said to be additive, whereas points above and below are indicative of sub-additivity and super-additivity (synergism), respectively. All calculations were assisted by the program PharmTools Pro (The McCary Group, Emmaus, PA).

Additionally, the maximum number of injections, regardless of dose, was used as a measure of reinforcing strength in an individual subject, and mean group maximums were calculated for each drug and mixture. Statistical significance of differences was analyzed using paired *t* test for drugs alone or one-way analysis of variance for repeated measures for the five subjects tested in all conditions for each drug pairing.

Drugs

Cocaine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD). RTI-117 was synthesized as described previously (Carroll et al. 1995). Alfentanil hydrochloride and remifentanil hydrochloride were purchased commercially. Final solutions were prepared using 0.9% saline. Doses were expressed as the salt forms of the drugs.

Results

Alfentanil and remifentanil maintained responding in all monkeys (Fig. 1), both with asymptotic dose–response functions whose maximums were not statistically different. Slopes of the dose–response functions for alfentanil and remifentanil did not differ. Together, these indicate a constant potency ratio (i.e., parallelism), a requirement for a linear isobole of additivity. The ED₅₀ values were 0.46 (±0.082, SEM) for alfentanil and 0.090 (±0.01, SEM) for remifentanil, i.e., remifentanil was approximately fivefold more potent than alfentanil. The observed mean maximum responding maintained by remifentanil was 14.5 (±0.8, SEM) and occurred at doses of 0.2, 0.4, or 0.8 µg/kg/injection in different monkeys. The observed maximum maintained by alfentanil was 13.4 (±0.6, SEM) at doses between 0.4 and 1.7 µg/kg per injection.

Combinations of the two drugs in fixed dose ratios were tested (Fig. 2: I, II, and III). The proportions (*p*) of the total dose combination were I, *p*(alfentanil)=0.89; *p*(remifentanil)=0.11 (approximate 2:1 based on ED₅₀s); II, *p*(alfentanil)=0.80; *p*(remifentanil)=0.20 (approximate 1:1 based on ED₅₀s); and III, *p*(alfentanil)=0.66; *p*(remifentanil)=0.34 (approximate 1:2 based on ED₅₀s). Mixtures maintained a dose-related increase in responding in all monkeys that was comparable to that maintained by the component drugs. The experimental points (Z_{mix}) were slightly below the predictions of additivity (Z_{add}), but differences were not statistically significant (Fig. 2, Table 1). Mean maximum responding did not differ across the individual drugs and the combinations (alfentanil/remifentanil 1:1, 14.5±1.3; 2:1, 14.7±1.3; 1:2, 13.7±1.3, SEM).

Similarly, both cocaine and RTI-117 maintained responding in all monkeys (Fig. 3). Maximums and slopes of the dose–response functions did not differ. The curve fits for cocaine and RTI-117 also indicated a constant potency ratio, and a linear isobole was again constructed. The ED₅₀ values were 0.034 mg/kg per injection (±0.007, SEM) for cocaine and 0.023 mg/kg per injection (±0.007, SEM) for RTI-117. The observed mean maximum responding maintained by cocaine was 16.5 (±1.0, SEM) and occurred at doses between

0.05 and 0.4 mg/kg per injection in different monkeys. The observed maximum maintained by RTI was $14.3 (\pm 1.0, \text{SEM})$ at doses between 0.025 and 0.05 mg/kg injection.

Combinations of the two drugs in fixed dose ratios were tested (Fig. 4: I, II, and III). The proportions (p) of the total dose combination were I, $p(\text{cocaine})=0.48$; $p(\text{RTI-117})=0.52$ (approximate 2:1 based upon ED_{50} s); II, $p(\text{cocaine})=0.63$; $p(\text{RTI-117})=0.37$ (approximate 1:1 based upon ED_{50} s); and III, $p(\text{cocaine})=0.81$; $p(\text{RTI-117})=0.19$ (approximate 1:2 based upon ED_{50} s). Mixtures maintained a dose-related increase in responding in all monkeys that was comparable to that maintained by the component drugs. The experimental points (Z_{mix}) were slightly above or below the predictions of additivity (Z_{add}), but differences were not statistically significant (Fig. 4; Table 2). Mean maximum responding did not differ across the individual drugs and the combinations (1:1, 17.7 ± 0.9 ; 2:1, 16.4 ± 0.6 ; 1:2, 15.2 ± 1.4).

Discussion

Both alfentanil and remifentanil functioned as positive reinforcers under the present PR schedule in monkeys. This result is consistent with a previous report that both of these drugs served as positive reinforcers when the response requirement for an injection was increased across sessions (Ko et al. 2002). Absolute and relative potencies of these compounds were comparable to those reported by Ko et al. (2002). In the present study, these two mu opioid agonists were not different in terms of their relative strength as reinforcers, i.e., the maximum responding maintained under a PR schedule did not differ between the two drugs. This result is also consistent with the findings of Ko et al. (2002) who used demand curve analysis and concluded equal strength as reinforcers. The main difference between the drugs, i.e., the somewhat shorter duration of action of remifentanil, did not appear to influence the results of either study. Taken together, the two studies suggest a convergence of measures of relative reinforcing strength across PR schedules and economic analysis. This convergence is not particularly surprising given that both approaches are based upon measurement of the effects of response requirement on drug consumption.

Similarly, both cocaine and RTI-117 functioned as positive reinforcers under the present PR schedule in monkeys. Reinforcing effects of cocaine in monkeys have been amply demonstrated using a wide variety of schedules of reinforcement, including the present one (e.g., Wilcox et al. 2000). This is the first demonstration of the reinforcing effects of RTI-117. Because RTI-117 is a cocaine analog, and relatively selective DA transporter ligand (Carroll et al. 1995), reinforcing effects were not particularly surprising. In vitro, RTI-117 showed approximately 13-fold higher affinity for the dopamine transporter than cocaine. Cocaine and RTI-117 have been compared behaviorally in terms of the effects of chronic administration of locomotor activity in rodents (Izenwasser et al. 1999). In that study, RTI-117 was approximately sevenfold more potent than cocaine. The potency difference found in the present study, although not as large as in other studies, is at least consistent with a DA mechanism involved in the reinforcing effect of RTI-117. In the Izenwasser et al. (1999) study, there were some qualitative differences in the effects of cocaine and RTI-117. Mechanisms responsible for those differences were not clear, although both pharmacokinetics and pharmacodynamic differences may have contributed. RTI-117 has both a slower onset and longer time course than cocaine and is more selective for DA relative to other monoamine systems (Carroll et al. 1995; Stathis et al. 1995). Qualitative differences between the two drugs in terms of reinforcing effects were not pronounced. The RTI-117 dose-response function was more biphasic in shape than was the case with cocaine under the present conditions. It seems likely that this can be attributed to the longer duration of action of RTI-117, resulting in drug accumulation over the session that reduced lever pressing. At any rate, the drugs differed slightly in potency and were not significantly

different in terms of maximum responding maintained, a measure of their relative strength as reinforcers.

When two μ opioids or two DAT ligands were combined, self-administration was well maintained by the mixtures and comparable to that maintained by the drugs alone. Given the pharmacodynamic similarities of the combined drugs, it was hypothesized that one would function as a substitute for the other in combination, i.e., that their combinations would be dose-additive. Experimental results support this hypothesis. Moreover, any pharmacokinetic differences between combined drugs apparently did not contribute to an interaction between them. The use of the i.v. route of administration, which avoids differences in absorption seen with peripheral administration, and an ITI to minimize drug accumulation over the session may have minimized any pharmacokinetic influences. Additionally, there was no evidence for an increase in the reinforcing strength of the mixtures relative to the individual drugs alone. Although these results may not seem surprising, it has been reported previously using isobolographic analysis with other endpoints that interactions between drugs can vary with the proportions of the compounds (see Tallarida 2000; Wessinger 1986; Woolverton 1987).

A primary rationale for conducting the present study was to establish in self-administration the effects of combining drugs with comparable mechanisms of action, to serve as a reference point for further studies of mixtures of drugs with different mechanisms. We have recently reported that the mixture of cocaine with the antihistamine diphenhydramine can be super-additive in combinations of 1:1, 1:2, and 2:1 ED₅₀ mixture ratios (Wang and Woolverton 2007b). It may be that super-additivity as reinforcers is confined to drugs with different mechanisms of action. On the other hand, this is not always the case, as both additivity and sub-additive interactions have been reported for self-administration of mixtures of cocaine and drugs with different mechanisms of action (Negus 2005; Rowlett et al. 2007; Smith et al. 2006). For the combination of heroin and cocaine, increases in maximum responding under a PR schedule, a measure of increased reinforcing strength, have been reported in some cases (Duvauchelle et al. 1998; Ranaldi and Munn 1998) but not in others (Rowlett and Woolverton 1997; Ward et al. 2005). Clearly, additional research will be required to examine the generality of this speculation, both with drugs of similar and different mechanisms of action.

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Fig. 1. Self-administration of alfentanil and remifentanil by monkeys responding under a PR schedule of reinforcement. Each data point represents the mean injections/session of each dose for two to five monkeys. *Vertical lines* represent SEM

Fig. 2.

Isobologram representing the self-administration of mixtures of alfentanil (*abscissa*) and remifentanil (*ordinate*). The *solid diagonal line* connects the ED₅₀s of the drugs alone. This *line* represents combinations of doses that would be predicted to have a 50% effect were the drugs additive, i.e., the additive isobole. *Dashed radial lines* (I, II, and III) represent the three different dose ratios that were tested. *Symbols* on the *radial lines* represent the experimentally determined ED₅₀s of those mixtures, and the *horizontal and vertical lines* through these points represent the SEM

Fig. 3. Self-administration of cocaine and RTI-117 by monkeys responding under a PR schedule of reinforcement. Details are as in Fig. 1

Fig. 4. Isobologram representing the self-administration of mixtures of cocaine (*abscissa*) and RTI-117 (*ordinate*). Details are as in Fig. 2

Table 1

ED₅₀ values (total dose+SEM) for three fixed ratio dose combinations as determined experimentally (Z_{mix}) and as predicted by additivity (Z_{add}) for combinations of alfentanil and remifentanil

Group	Z_{mix} : ED ₅₀ (experimental)	Z_{add} : ED ₅₀ (additive)
I	0.30±0.08	0.31±0.05
II	0.21±0.03	0.25±0.04
III	0.16±0.04	0.19±0.025

Table 2

ED₅₀ values (total dose $\pm$ SEM) for three fixed ratio dose combinations as determined experimentally (Z_{mix}) and as predicted by additivity for combinations of cocaine and RTI-117

Group	Z_{mix} : ED ₅₀ (experimental)	ED ₅₀ (additive)
I	0.018 $\pm$ 0.004	0.027 $\pm$ 0.005
II	0.034 $\pm$ 0.005	0.029 $\pm$ 0.005
III	0.041 $\pm$ 0.011	0.031 $\pm$ 0.006