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Reinforcing Effects of Caffeine, Ephedrine, and Their Binary Combination in Rats

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BRISCOE, R. J., S. A. VANECEK, M. VALLETT, T. J. BAIRD, F. A. HOLLOWAY AND D. V. GAUVIN. *Reinforcing effects of caffeine, ephedrine, and their binary combination in rats.* PHARMACOL BIOCHEM BEHAV **60**(3) 685–693, 1998.—The reinforcing effects of caffeine, ephedrine, and caffeine + ephedrine combinations were tested in rats maintained to self-administer 0.5 mg/kg/injection of cocaine in daily 4 h limited access periods. The dose–response relationship for cocaine demonstrated a a typical inverted U-shaped function. The dose-dependent administration of cocaine was stable over the 3-day substitution epochs. Similar to earlier reports, neither caffeine nor ephedrine engendered stable patterns of self-injections. Combinations of caffeine + ephedrine produced biphasic patterns of administration only on the first day of substitution. Days 2 and 3 of the caffeine–ephedrine substitution periods engendered variable and inconsistent reinforcer deliveries that did not significantly differ from saline substitution tests. These reduced patterns of self-administered caffeine–ephedrine combinations were not attributed to behavioral toxicity. Progressive-ratio tests demonstrated rank ordered break points of: food > cocaine > caffeine–ephedrine combination = caffeine = ephedrine = saline. Caffeine–ephedrine pretreatments failed to show any significant change in the administration of the maintenance dose of cocaine except at the highest combination dose tested. Although previous data from this laboratory demonstrated symmetrical crossgeneralization between the discriminative effects of caffeine–ephedrine combinations and cocaine (5,6), the present data suggest limited reinforcing effects of these combinations in rats. © 1998 Elsevier Science Inc.

Caffeine Ephedrine Cocaine Self-administration Abuse liability Progressive ratio Fixed ratio Drug combinations

CAFFEINE, ephedrine, and caffeine–ephedrine combinations have been packaged and sold over the counter for the treatment of the symptoms associated with the common cold and as dietary aids for weight control. These drugs have enjoyed multimillion dollar sales per annum for over 25 years, with limited reports of abuse or requirements for medical intervention upon their abrupt withdrawal or discontinuance (28). In more recent controlled clinical trials, the relative safety and effectiveness of caffeine–ephedrine combinations have been established as an adjunct in the treatment of obesity. These clinical trials have demonstrated a low incidence of misuse and minimal potential for abuse among the participants (4,19).

Recently, "herbal ecstasy" (Ecstacy®) has been sold over the counter in a variety of specialty stores. Ecstacy® and many other health food supplements principally contain *ma huang,* the natural source of ephedrine, and cola nut, a natural source of caffeine. The popularity of these new designer-type herbal products has exploded over the past few years, particularly because they are centrally active, considered dietary aids, and do not fall under the general jurisdiction of the Drug Enforcement Agency. Most of these herbal compounds are packaged without safety or administration instructions and, as a result, there have been some recent isolated case reports in the popular media of ephedrine-associated toxicity or overdose. The Food and Drug Administration has recently acted on these reports and has issued a warning to consumers regarding problems associated with the possible abuse of the ephedrine-containing herbal products (32).

This laboratory has conducted a series of preclinical studies investigating the abuse liability of another class of caffeine–ephedrine products commonly referred to as "look-

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alike" stimulants or "turkey drugs." These drug combinations were sold to the public through magazine mail orders and to many interstate transportation workers through illicit "truck stop" markets (1). Gauvin et al. (5) have demonstrated the qualitative and quantitative similarities between a 10 mg/kg cocaine discriminative stimulus and the over-the-counter stimulants, caffeine and ephedrine, when administered singly or in combination. In these reports, caffeine alone engendered partial generalization to the cocaine cue, while a drug mixture of caffeine, ephedrine, and phenylpropanolamine engendered full generalization. These compounds have also been shown to crossgeneralize to an amphetamine cue (15). In later studies, Gauvin et al. (6) utilized the two-choice drug discrimination procedure with saline and either caffeine, ephedrine, or a caffeine–ephedrine combination as training stimuli. Cocaine and amphetamine demonstrated only partial generalization to the caffeine cue in these studies, while both fully generalized to the ephedrine cue. Cocaine and amphetamine did produce full generalization to the caffeine–ephedrine drug mixture cue. Together, these studies suggest that caffeine–ephedrine combinations demonstrate symmetrical crossgeneralization to cocaine. Overton (21) has suggested those compounds with the most similar subjective effects demonstrate symmetrical discriminative crossgeneralization. Inasmuch as the discriminative stimulus effects of drugs as measured in these preclinical assays can accurately predict their subjective profile in humans (26), these data may suggest a potential for abuse of the caffeine–ephedrine combinations. Individually, the subjective profiles of ephedrine and IV caffeine administered alone in humans have been reported to produce a similar constellation of euphoric or positive effects to cocaine as well as other abused stimulants in experienced drug abusers (20,25).

Although the discriminative or subjective profile of the caffeine–ephedrine aggregate seems similar to the subjective effects associated with the controlled stimulants, cocaine and amphetamine, there is not a strong correspondence between the subjective effects produced by drugs and the corresponding drug effects that will initiate or maintain self-administration (21). The reinforcing effects of both caffeine and ephedrine have been examined singly in self-administration procedures (2,7,11), with little evidence that either drug will maintain stable rates of self-administration, but there are no published reports on the reinforcing effects of the caffeine–ephedrine combinations.

The purpose of the present study was to test the reinforcing effects of caffeine, ephedrine, and their combination in rats maintained to self-administer cocaine. This enabled a replication of the assessment of the reinforcing effects of single drug availability and, for the first time, the assessment of the reinforcing effects of the joint administration of a number of different caffeine–ephedrine doses. The measurement of the reinforcing effects of single and binary doses of compounds were conducted during cocaine substitution test sessions. The relative work or effort that each rat would expend to administer the test compounds was also quantified by use of a progressive-ratio schedule. The relative break points [cf. (13)] or maximum number of lever-press responses engendered prior to the delivery of the last reinforcer during a single test session for caffeine–ephedrine combinations, caffeine, ephedrine, food, and cocaine provided a second measure of the rank-ordered subjective value or potential for abuse. And finally, pretreatments with the caffeine–ephedrine combination prior to cocaine self-administration sessions were conducted to determine the behavioral disruption induced by coadministration of caffeine–ephedrine and cocaine.

METHOD

Subjects

Sixty-five male Sprague–Dawley rats (Sasco Laboratory, Inc., Omaha, NE) weighing between 250 and 300 g at the onset of the study were used. The rats were individually housed in suspended stainless steel cages in an AAALAC- accredited colony room. The colony room was maintained at 20°C on a 12 L: 12 D cycle, with lights on at 0500 h. The rats underwent a 7-day acclimation period in the colony room prior to the start of the study, and were given ad lib access to food and water. Subsequently, food access was regulated to allow for an approximate 10% increase in weight each month to allow for normal growth. All experimentation was conducted in accordance with the NIH guidelines as described in The Handbook for the Use of Animals in Neuroscience Research (29).

Apparatus

Each of 12 operant chambers (Lafayette Instruments, Lafayette, IN) was equipped with a retractable lever, a food cup and dispenser, and a stimulus lamp. The start of the self-administration session was signaled by extension of the lever and the illumination of the stimulus lamp located directly above the lever. White noise (Model 15800, Lafayette Instruments, Lafayette, IN) was presented throughout the session through a centrally placed external speaker. Drug delivery was initiated by depression of the lever that was interfaced to an IBM-compatible computer using input/output controller cards (MED-Associates, Inc., Georgia, VT). A custom Clarion-based software package (American Neuroscience Research Foundation, Yukon, OK) controlled all behavioral contingencies and data collection. Each drug's bolus were delivered in approximately 1 s using a pneumatic syringe system (Ledger Technical Services, Kalamazoo, MI). The rat was connected to the syringe system by a catheter fitted through a single channel stainless steel swivel (Harvard Bioscience, South Natick, MA).

Behavioral Training

Following the acclimation period, the animals body weights were reduced and maintained at 85% of their free feeding weight. Each animal was trained to the location and operation of the pellet dispenser, and trained to lever press to receive food pellets (one 45 mg pellet, P. J. Noyes Inc., Lancaster, NH) by the method of successive approximations. The requirements for food delivery was gradually raised over sessions until a fixed-ratio (FR) 10 was achieved. Upon reaching the criterion level of food-maintained responding (FR10) for 3 consecutive days, surgery was performed (see below). Following surgery, the animals were initially retrained in the daily 4-h session to a maintenance dose of cocaine (0.5 mg/kg/injection) and food on day 1 only. On all subsequent days, the food delivery system was inactivated and lever press responding produced a single injection of the cocaine training dose available at FR10. Each self-administration session began with two experimenter administered priming injections. After stable cocaine-maintained responding (3 days with $\pm 10\%$ or less variability of responding between days) was reached, testing began.

Surgery

Each rat was pretreated with atropine (0.1 ml of 0.54 mg/ml, SC) 20 min prior to administration of the anesthetics. An-

esthesia was achieved by a single injection of sodium pentobarbital (45 mg/kg, IP) with supplemental injections of ketamine (10 mg/kg, IP), as needed to maintain anesthesia. A chronic indwelling catheter was implanted into the right jugular vein using the procedure first described by Weeks (31). The distal end of the catheter was passed subcutaneously to the point of exit in the back at the midscapular level, and was anchored there with either a plastic (Kent Scientific, Litchfield, CT) or a stainless steel anchoring skin button (Harvard Bioscience, South Natick, MA). The animals were given a 4-day recovery period, each day of which the catheters were flushed with a 0.1-ml saline solution containing heparin (10 units/ml) and penicillin G sodium (250,000 units/ml). On self-administration days, the catheters were flushed just prior to the operant session with 0.1 ml of saline to ensure patency, and immediately following the drug session with heparinized (10 units/ml) saline solution. Proper placement of the catheters was confirmed weekly by a single bolus of sodium methohexital (0.20 ml of 5 mg/ml) injected into the catheter which, if properly placed, would produce an immediate loss of the righting reflex (30). Proper catheter placement was also verified visually during necropsy at the completion of testing.

Experiment 1—Substitution tests

Test sessions were conducted in which a novel dose of cocaine or a dose from a preselected dose range of caffeine, ephedrine, or a caffeine + ephedrine combination would be delivered instead of the training or maintenance dose of cocaine. Prior to these tests a dose range was selected for each test compound based on previous published reports from our laboratory using drug discrimination assays or from other laboratories using self-administration assays. Each dose was selected to produce a complete dose–response curve throughout a safe behaviorally active dose range that would minimize, to the best of our knowledge, any long-term toxic effects or lethality. Each of these substitution tests was preceded by 3 days of self-administration of the cocaine maintenance dose. A specific dose of either cocaine, caffeine, ephedrine, or a caffeine + ephedrine combination was tested for 3 consecutive days in the same rat.

At the start of the project each rat and the specific dose to be administered in substitution tests was randomly selected from the entire group of 65 rats by random selection of rat identification numbers drawn from a hat. We estimated 45 separate test conditions would be needed to complete the study, and planned on eight rats per test condition. Once eight different numbers were randomly drawn from the hat for each dose of the dose–effect function all numbers were replaced and another random selection occurred for the next planned dose–effect function or comparison. An individual rat was tested only once per each dose–effect function for a true "between subjects (dose)" design. Each dose of a selected dose range was tested in eight different rats for all planned dose–response functions. Not all 65 rats were instrumented at one time; however, for each rat identified with a unique number the exact number and dose tests needed to be completed for each rat were known prior to catheterization. Once all the tests designated for a given rat were completed the rat was transferred to other pilot projects being conducted in the lab. In a final review of these selection procedures, rats were tested in a range from three to eight conditions.

For the cocaine dose–effect function the dose range tested was 0.01 to 1.0 mg/kg/inj. Due to the differential efficacy and potency of caffeine and ephedrine in this assay that has been demonstrated in previous studies (2,7,11), a 0.25 to 1.0 mg/kg/inj dose range was tested. The first set of drug combination tests assessed the drugs in a combination dose ratio similar to the actual caffeine:ephedrine dose ratio found in bogus or look-alike cocaine street drug samples (1,28). Two additional sets of substitution tests were completed with a fixed dose of either caffeine (0.7 mg/kg/inj) or ephedrine (0.7 mg/kg/inj) with increasing doses of the alternate drug. All self-administration sessions were 4 h in duration. The drug volumes were varied between 0.03 and 0.05 ml, depending on the individual animal's weight, thus maintaining a fixed drug concentration at each dose tested. This allowed for very precise control of the dose administered with each injection.

Experiment 2—Progressive-Ratio Tests

The progressive-ratio (PR) schedule of reinforcement has been used extensively to specifically compare the reinforcing effects of cocaine to other drugs [cf. (8–10,22,23)]. Although there is some controversy as to the level of measurement that this specific assay is able to quantify, most authors agree with Katz (16) that, at least, ordinal comparisons between reinforcers seem the least problematic level of measurement with respect to the PR schedule. At the ordinal level of measurement a relative value can be quantified for each test compound and can be used to give a rank order to the tests. Stimulants in particular have been rank ordered in terms of their break points and have been shown to be generally consistent with their known abuse liability in humans (9). The PR tests were conducted in 30 randomly selected rats to assess the relative break points between cocaine, food pellets, caffeine, ephedrine, and caffeine-ephedrine combinations. The PR test consisted of the same training criteria as discussed above with a 4-h access. Rats were randomly selected from the entire group for each dose test by simply drawing numbers from a hat. The "break points" (13,14), total number of responses, and total number of reinforcer deliveries were determined for the cocaine maintenance dose and then compared to those engendered by delivery of saline, food pellets, caffeine, ephedrine, and caffeine + ephedrine combinations. The food-reinforced PR test was conducted when the animals were at 85% of their free-feeding weights. Roberts et al. (24) described the logarithmic series that was utilized in this study, and its use has been reported to engender robust responding in the first 1 to 2 h of the drug session with complete response-rate suppression occurring prior to the end of the 4-h session. To reduce toxicity of multiple injections in a short period of time, the response requirements for the first six injections were modified as suggested by Depoortere et al. (3). The response requirements to earn an injection increased according to the following series: 3, 6, 10, 15, 18, 23, 28, 33, 41, 49, 57, 70, 83, 96, 117, 138, 156, 200, 225, 275, 300, 325, 350, 375, and 425. Each progressive-ratio test was preceded by 2 days of stable baseline self-administration of the cocaine maintenance dose.

Experiment 3—Pretreatment Tests

Following completion of the substitution tests, pretreatment tests of IP administered cocaine or caffeine + ephedrine combinations were conducted. The pretreatments were conducted to assess possible adverse behavioral consequences resulting from the coadministration of these drugs on the maintenance dose of cocaine. The eight animals per condition used in this test were randomly selected from the main group of 65 rats. All of these animals had varied histories of self-administration of cocaine, caffeine, ephedrine, or caffeine-ephedrine

combinations. Following 2 days of stable self-administration of the cocaine maintenance dose, each rat was given a pretreatment injection prior to the next daily session. The animals received IP injections of either 3.2, 10, or 32 mg/kg cocaine (15 min) or one of three caffeine + ephedrine combinations (3.2 + 1.8, 10 + 5.6, or 32 + 17.8 mg/kg; 30 min) prior to the self-administration session. The specific pretreatment was randomly selected prior to each test until each dose of the preselected dose range was tested for each compound ($n = 8$ animals per dose).

Drugs

Atropine sulfate (0.54 mg/ml; Vedco, Inc., St. Joseph, MO), sodium pentobarbital (Nembutal, 50 mg/ml; Abbott Laboratories, North Chicago, IL), sodium methohexital (Brevital, 500 mg; Eli Lilly, Indianapolis, IN), and Penicillin G (for IV injection; Marsam Pharmaceuticals, Cherry Hill, NJ) were purchased from University Hospital pharmacy (University of Oklahoma Health Sciences Center, Oklahoma City, OK). Anhydrous caffeine (expressed as the base), ephedrine hydrochloride, and ketamine hydrochloride were purchased from Sigma Chemical Company, St. Louis, MO. The cocaine hydrochloride, expressed as the salt, was supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). All drugs were mixed daily in sterile 0.9% saline and placed in sterile amber, light-attenuating, serum bottles.

Data Analysis

All dose–effect functions for the substitution tests were assessed using a between-subjects repeated-measures mixed-design ANOVA. Specific drug doses were loaded as between subjects factors and the specific days of substitution were loaded as the within-subjects factor. If the main ANOVA was significant, individual simple-effect tests (group × day ANOVAs) were conducted to compare individual dose groups across the three day presentation (33). The PR tests were analyzed using an one-way subject × treatment ANOVA; because of the somewhat controversial nature of the progressive-ratio tests (8–10,16) the progressive-ratio data were expressed and analyzed in three different ways: 1) as the mean break points, 2) as the mean total number of responses emitted on the lever during the test period, and 3) as the mean total number of food pellets delivered during the test period. Pretreatment tests were compared using a between-subjects, repeated-measures, mixed-factor ANOVA. All post hoc comparisons were done using the Tukey (A) multiple comparison procedure (33). Data were analyzed using the Complete Statistical System (CSS: Statistica, Tulsa, OK) personal computer software program. Statistical significance was set at $p < 0.05$ for all comparisons.

RESULTS

Experiment 1: Substitution Tests

The cocaine dose–response function (Fig. 1) clearly demonstrated a dose-dependent relationship between the injection dose and the total number of injections earned during each of three consecutive daily sessions. For the 3 consecutive days of tests, a typical inverted U-shaped dose–response function was generated. Upon analysis, there was a main dose effect, $F(6, 49) = 5.12$, $p = 0.0003$, but there were no significant main day or main dose × day interactive effects [main day effect: $F(2, 98) = 0.2$, $p = 0.7$; main dose × day interaction: $F(12, 98) = 0.73$, $p = 0.7$. The 0.06 mg/kg/injection dose of cocaine demonstrated a high degree of variability. This may suggest that this dose was the threshold for cocaine self-administration. Simple-effect tests on the main dose (group) effects were conducted. The group mean number of injections self-administered during saline test sessions were significantly lower than the four highest cocaine test doses (0.25, 0.5, 0.75, and 1.0 mg/kg/inj; all simple-effect (dose) tests $ps < 0.001$; all Tukey post hoc (day) comparisons $ps < 0.01$). Additionally, the differences between the number of self-injections demonstrated during saline tests and the number of self-injections demonstrated during tests with the two lowest cocaine test doses (0.01 and 0.06 mg/kg/inj) were not significantly different. Individual simple-effect ANOVA comparisons (33) between the number of injections self-administered during tests relative to the training or maintenance dose of 0.5 mg/kg/inj of cocaine demonstrated that saline, and the lowest cocaine test doses (0.01 mg/kg/inj), engendered significantly lower numbers of self-injections during each day of their respective tests (all simple-effect test $ps < .01$, all Tukey individual post hoc comparisons $ps < 0.01$). Due to the low variability in responding occasioned during the substitution tests conducted with the two highest cocaine doses (0.7 and 1.0 mg/kg/inj), the number of reinforcer deliveries were significantly lower during these tests than that engendered by the maintenance dose of 0.5 mg/kg/inj of cocaine (simpe-effects tests: $ps < 0.01$)

Caffeine substitution tests (Fig. 2) demonstrated essentially flat dose–response functions over the 3-day substitution test period; there was no main dose effect, $F(4, 35) = 1.4$, $p = 0.2$, for caffeine. By visual analysis, on day 1 of the caffeine substitution tests there was a slight inverted U-shape to the distribution that was significantly reduced or flattened on the 2 subsequent test days. There was a main day effect, $F(2, 70) = 12.23$, $p = 0.00002$, and main dose × day interactive effects, $F(8, 70) = 1.0$, $p = 0.3$, for the caffeine substitution data. Ephedrine substitution tests (Fig. 3) did not maintain significant rates of self-administration over the 3-day substitution period [main dose effect: $F(4, 35) = 1.8$, $p = 0.1$]. With respect to the ephedrine dose–response function, there were significant main day effects, $F(2, 70) = 12.72$, $p = 0.00002$, and significant main dose × day interactive effects, $F(8, 70) = 0.6$, $p = 0.7$).

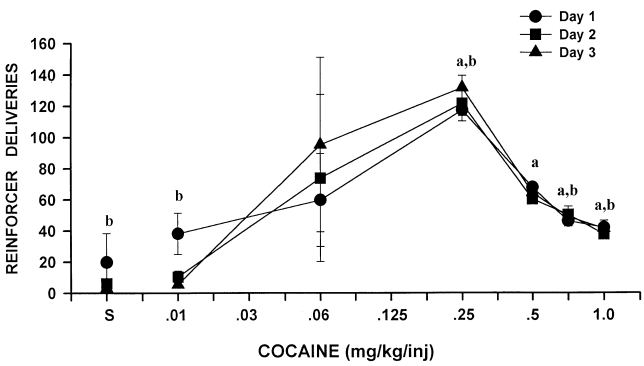

FIG. 1. Cocaine substitution dose–effect curve. Group means (±SE) of cocaine injections are plotted as a function of cocaine test dose over the 4-h substitution test. Cocaine substitution tests were conducted on 3 consecutive days. Each point represents the mean of eight rats. Simple-effect test comparisons: (a) significantly different from saline substitution tests: $p < 0.001$; (b) significantly different from maintenance dose of cocaine (0.5 mg/kg/inj), $p < 0.001$.

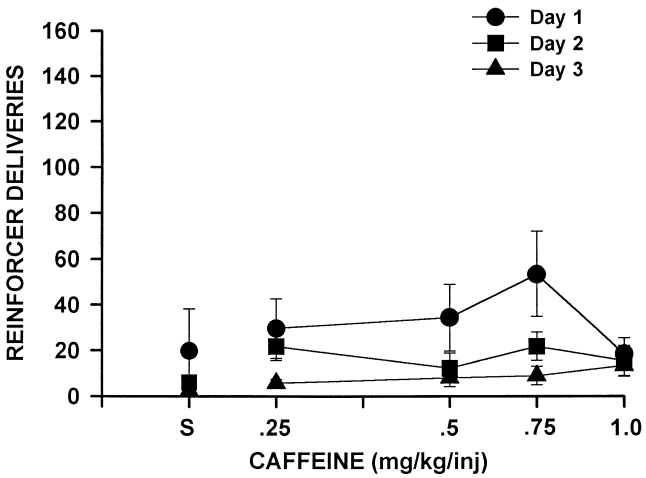

FIG. 2. Caffeine substitution dose–effect curve. Group means (±SE) of caffeine injections are plotted as a function of caffeine test dose over the 4-h substitution test. Caffeine substitution tests were conducted on 3 consecutive days. No caffeine dose was significant from saline. Each point represents the mean of eight rats.

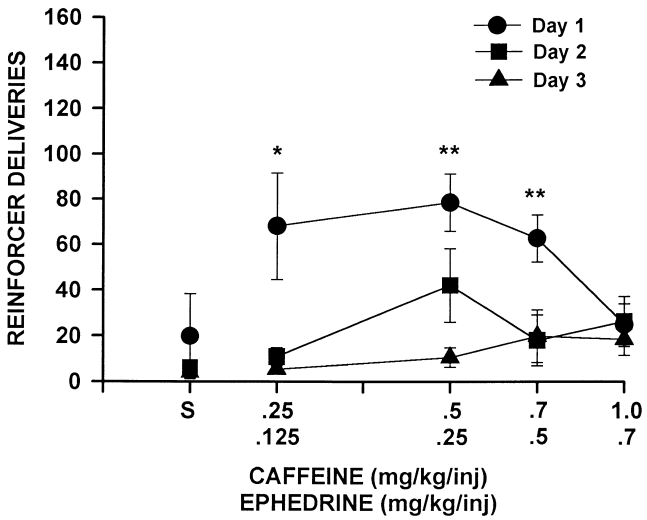

FIG. 4. Caffeine–ephedrine combination dose–effect curve. Group means (±SE) of caffeine–ephedrine injections are plotted as a function of the combination test dose over the 4-h substitution test. This figure demonstrates a fixed caffeine:ephedrine ratio, expressed in one-third common log unit increments. Caffeine–ephedrine combination substitution tests were conducted on 3 consecutive days. Each point represents the mean of eight rats. *$p < 0.05$, **$p < 0.01$ vs. saline.

The first of the series of caffeine + ephedrine combinations tested for substitution for the cocaine maintenance dose was a fixed caffeine:ephedrine dose ratio (Fig. 4). Similar to the initial cocaine dose–effect function upon visual inspection of the dose–response relationship there was a dose-dependent biphasic pattern to the number of injections administered in the 4-h session on day 1 only. On the 2 subsequent days of substitution there was a significant reduction in the total number of injections administered that resulted in more flattened functions. Upon analysis, the dose combination tests resulted in significant main dose effects, $F(4, 34) = 3.82$, $p = 0.01$, main day effects, $F(2, 68) = 23.63$, $p = 10^{-6}$) and main dose × day interactive effects, $F(8, 68) = 2.76$, $p = 0.01$. On day 1 of the substitution tests the three lowest dose combinations engendered higher numbers of self-injections than was demonstrated with saline tests. Tukey post hoc analyses demonstrated that the specific dose combination of 0.25 caffeine + 0.125 ephedrine mg/kg/inj on day 1 was significant when compared to day 2 ($p = 0.005$) and day 3 ($p = 0.001$). Additionally, post hoc tests revealed that day 1 of the 0.5 caffeine + 0.25 ephedrine and 0.7 caffeine + 0.5 ephedrine mg/kg/inj combinations significantly differed from day 3 ($p < .05$) but not day 2.

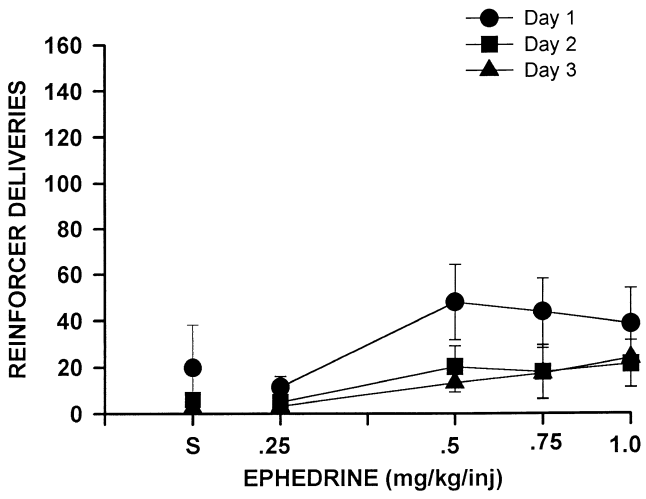

FIG. 3. Ephedrine substitution dose–effect curve. Group means (±SE) of ephedrine injections are plotted as a function of ephedrine test dose over the 4-h substitution test. Ephedrine substitution tests were conducted on 3 consecutive days. No ephedrine dose was significant from saline. Each point represents the mean of eight rats.

The second series of caffeine + ephedrine substitution tests (Fig. 5) were conducted with a fixed dose of ephedrine (0.7 mg/kg/inj) and varying the concomitantly administered dose of caffeine. This dose–response function provided similar conclusions to that of the first series of combination tests described above. There was a significant biphasic, dose-dependent change in the total number of injections administered in the 4-h sessions. This biphasic pattern of injections was evident for the first day of substitution, only. Overall, the analyses of these combination tests revealed main dose effects, $F(4, 34) = 5.69$, $p = 0.001$, main day effects, $F(2, 68) = 8.8$, $p = 0.0003$, and significant main dose × day interactive effects, $F(8, 68) = 1.9$, $p = 0.06$. Individual dose (group) × day post hoc comparisons demonstrated that the number of reinforcer deliveries on day 1 of both the 0.7 ephedrine + 0.5 caffeine mg/kg/inj and 0.7 ephedrine + 0.7 caffeine mg/kg/inj tests were significantly different from mean number of deliveries engendered during saline tests ($p < 0.05$ and $p < 0.01$ respectively).

The third, and last, series of caffeine + ephedrine combination substitution tests (Fig. 6) was conducted with a fixed dose of caffeine (0.7 mg/kg/inj) with various doses of concomitantly administered ephedrine. Similar to all of the previous combination tests, visual inspection of this dose–response function suggested a significant dose-dependent biphasic pattern of injections on day 1 only. These combination tests pro-

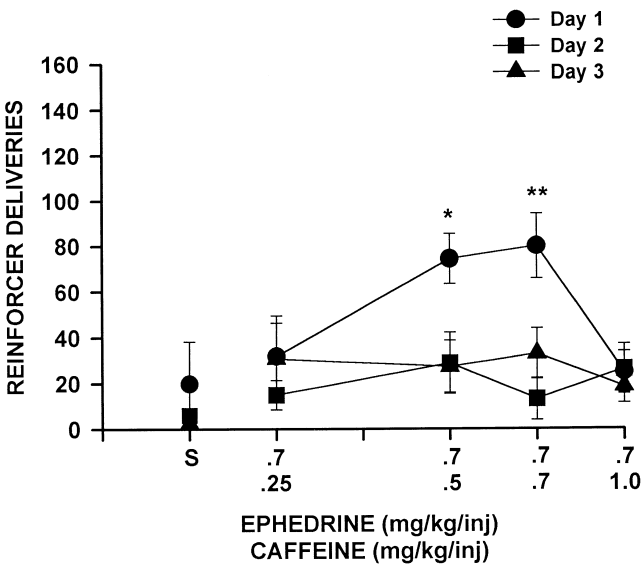

FIG. 5. Ephedrine–caffeine fixed-dose combination dose–effect curve. Group means (±SE) of ephedrine–caffeine injections are plotted as a function of the combination test dose over the 4-h substitution test. This figure demonstrates a fixed dose of ephedrine with increasing doses of caffeine. Ephedrine–caffeine combination substitution tests were conducted on 3 consecutive days. Each point represents the mean of eight rats. $*p < 0.05$, $**p < 0.01$ vs. saline.

duced significant main dose effects, $F(4, 35) = 5.5$, $p = 0.001$. The number of injections significantly decreased on the two subsequent substitution days demonstrated by a main day effect, $F(2, 70) = 24.3$, $p = 10^{-6}$, and a significant main dose × day interaction, $F(8, 70) = 1.9$, $p = 0.06$. Post hoc comparisons

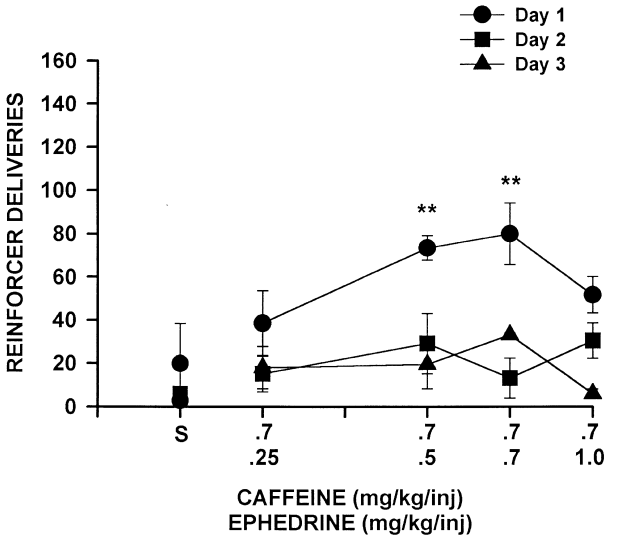

FIG. 6. Caffeine–ephedrine fixed-dose combination dose–effect curve. Group means (±SE) of caffeine–ephedrine injections are plotted as a function of the combination test dose over the 4-h substitution test. This figure demonstrates a fixed dose of caffeine with increasing doses of ephedrine. Caffeine–ephedrine combination substitution tests were conducted on 3 consecutive days. Each point represents the mean of eight rats. $**p < 0.01$ vs. saline.

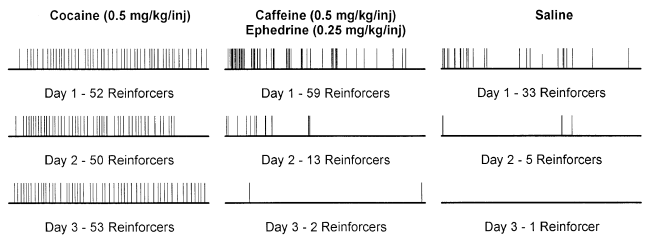

FIG. 7. Representative rat event records. The left panel demonstrates the event records for the pattern of reinforcer deliveries from a representative rat for the 3 days of the cocaine maintenance dose self-administration. The center panel demonstrates the event records for the pattern of reinforcer deliveries from a representative rat over the 3-day cocaine substitution period for caffeine (0.5 mg/kg/inj) + ephedrine (0.25 mg/kg/inj). The right panel demonstrates the event records for the pattern of reinforcer deliveries from a representative rat over the 3-day cocaine substitution period for saline. Each vertical blip indicates one reinforcer delivery; all event records represent 4 h.

revealed that day 1 of the 0.7 caffeine + 0.5 ephedrine mg/kg/inj and 0.7 caffeine + 0.7 ephedrine mg/kg/inj was significantly different from saline ($p < 0.01$).

The injection event records depicted in Fig. 7 show the pattern of injection for the 0.5 mg/kg/inj cocaine, 0.5 caffeine + 0.25 ephedrine mg/kg/inj combination and saline across the 4-h substitution test sessions for one representative rat. The cocaine event records (left panel) demonstrated a regular pattern of responding across the 4-h session for all 3 days. When these data are compared to the caffeine–ephedrine combination event records (center panel) a somewhat different pattern of self-injections on day 1 of the substitution was demonstrated, but the caffeine–ephedrine combination was self-administered throughout the duration of the 4-h session. Days 2 and 3 of the caffeine–ephedrine combination substitution demonstrated steadily declining numbers of reinforcers earned. The saline substitution event records (right panel) demonstrated that most of the reinforcer deliveries occurred in the first hour of day 1, with almost no reinforcers earned on days 2 and 3 of the saline tests. The caffeine (0.7 mg/kg/inj) event records over the 3-day substitution period for two representative rats are depicted in Fig. 8. The ephedrine (0.7 mg/kg/inj) event records over the 3-day substitution period for two representa-

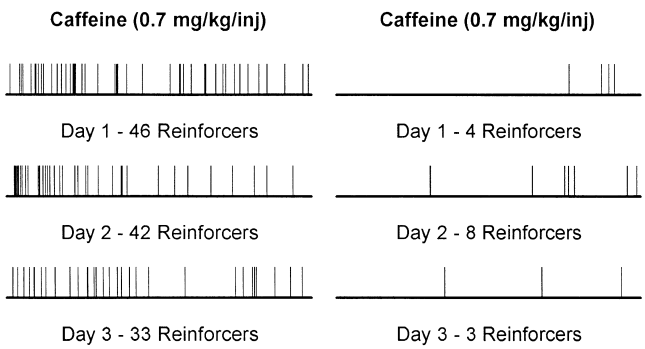

FIG. 8. Caffeine event records. Event records for caffeine (0.7 mg/kg/inj) are demonstrated in two animals for the 3-day substitution tests. These event records demonstrate the high degree of variability between animals. Each vertical blip indicates one reinforcer delivery, all event records represent 4 h.

tive rats are depicted in Fig. 9. To illustrate the high degree of variability between animals, two different event records are demonstrated, as a single representative animal was not available.

Experiment 2: Progressive-Ratio Tests

Progressive-ratio break point tests for the cocaine maintenance dose, saline, food (45 mg pellets), caffeine 0.7 mg/kg/inj, ephedrine 0.7 mg/kg/inj, and several caffeine + ephedrine combinations were conducted to rank order the relative break points (Fig. 10).

Relative to the left panel of Fig. 10 only, a significant main treatment effect was demonstrated between the reinforcers tested regardless of the data being expressed as the group mean break points, $F(8, 69) = 17.2$, $p = 10^{-6}$, group mean total number of responses emitted during the progressive-ratio test session, $F(8, 69) = 16.5$, $p = 10^{-6}$, or as the group mean number of reinforcer deliveries during the progressive-ratio tests, $F(8, 69) = 16.17$, $p = 10^{-6}$. At 85% of their free-feeding weight, rats engendered higher break points, response rates, and reinforcer deliveries for food than any other test condition. Similarly, regardless of the specific dependent measure utilized for comparisons, Tukey post hoc comparison tests demonstrated significant differences between food and the maintenance dose of cocaine. Additionally, all three expressions of performance in the progressive-ratio tests for the maintenance dose of cocaine was significantly different from caffeine ($p < 0.001$), ephedrine ($p < 0.001$), 0.25 caffeine + 0.125 ephedrine mg/kg/inj ($p < 0.001$), 0.7 caffeine + 0.5 ephedrine mg/kg/inj ($p < 0.01$), and 1.0 caffeine + 0.7 ephedrine mg/kg/inj ($p < 0.01$). In summarizing the left panel of Fig. 10, the break points, total number of responses emitted, and total number of reinforcer deliveries for cocaine ($p < 0.001$) and food ($p < 0.001$) were shown to be the only tests conditions that were significantly different from saline.

With respect to the right panel of Fig. 10, progressive-ratio tests conducted with a number of caffeine:ephedrine combinations demonstrated that only one test combination engendered self-injection rates significantly different from saline (0.5 caffeine + 0.25 ephedrine; $p < 0.05$, but the group mean break point engendered by this combination was significantly lower than that engendered by the maintenance dose of cocaine ($p < 0.01$).

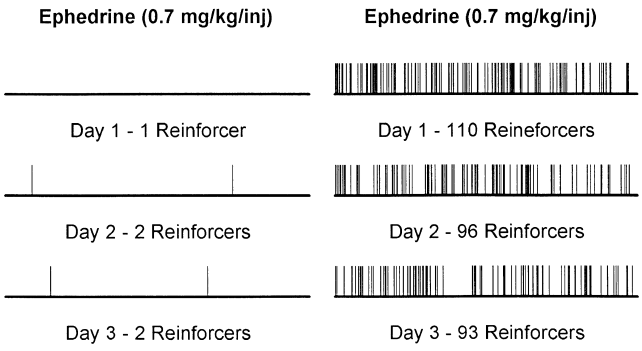

FIG. 9. Ephedrine event records. Event records for ephedrine (0.7 mg/kg/inj) are demonstrated in two animals for the 3-day substitution tests. These event records demonstrate the high degree of variability between animals. Each vertical blip indicates one reinforcer delivery; all event records represent 4 h.

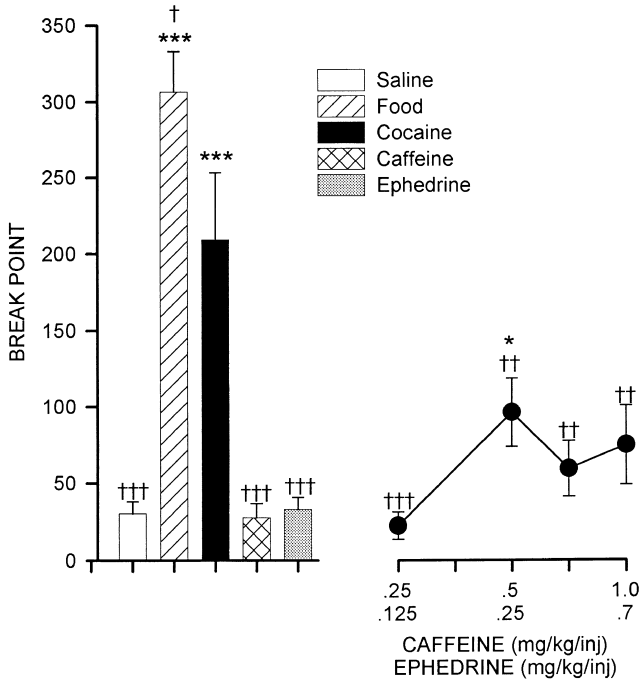

FIG. 10. Relative break points. Group means (±SE) of the progressive-ratio break points for saline, food, cocaine (0.5 mg/kg/inj), and several caffeine + ephedrine combinations. Each point represents the mean of eight rats. *$p < 0.05$, **$p < 0.001$ vs. saline, $p < 0.01$, $p < 0.001$ vs. cocaine.

Experiment 3: Pretreatment Tests

All dose and drug tests were analyzed for total reinforcers earned over the entire 4-h self-administration session and in the first hour of the session to assess the differential time course of the test compounds. Figure 11 (right panel) depicts cocaine pretreatment tests conducted across a full log unit range of

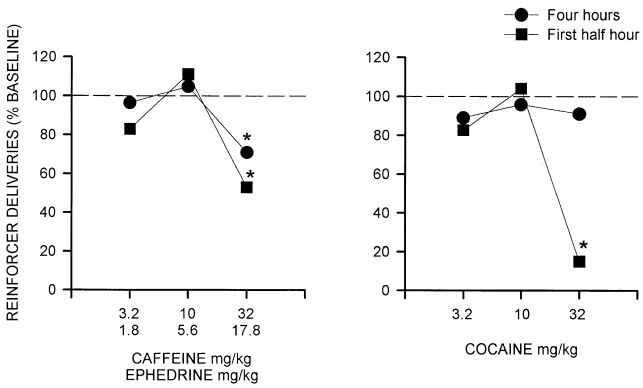

FIG. 11. Preload dose– and time–effect functions. Total number of reinforcer deliveries expressed as a percentage of baseline, plotted as a function of the preload test dose. Baseline responding is the number of reinforcers earned during the preceding maintenance training day with 0.5 mg/kg/inj. Preload injection of either cocaine (right panel) or caffeine + ephedrine (left panel) are shown for the first half hour of the session (squares) or for the full 4-h session (circles). Each preload test was conducted in eight rats with the cocaine maintenance dose (0.5 mg/kg/inj). *$p < 0.05$ (significant change from baseline).

doses. Only the 32 mg/kg cocaine pretreatment dose significantly reduced the total number of injections self-administered during the first half hour of the 4-h access period [main dose effect: first half hour: $F(1, 7) = 8.8, p = 0.02$], but not the full 4 h when compared to baseline. Figure 11 (left panel) depicts the caffeine–ephedrine pretreatment tests. The highest dose of the caffeine–ephedrine combination (32 mg/kg caffeine + 17.8 mg/kg ephedrine) demonstrated a significant reduction in the total number of reinforcers earned during the 4-h access period, $F(1, 7) = 8.7, p = 0.02$. The first, $F(1, 7) = 12.1, p = 0.01$, and second, $F(1, 7) = 9.9, p = 0.01$, half hours of the test session were significantly lower than baseline as well.

DISCUSSION

The present results replicate, in part, the unstable or erratic patterns of self-administration of caffeine and ephedrine by animals when substituted for cocaine (7,10). We have extended those findings to include caffeine + ephedrine combinations. Neither caffeine nor ephedrine was self-administered above saline levels or in any dose-dependent manner, and the pattern of self-injection was erratic between animals. The substitution of the caffeine + ephedrine combinations was somewhat different from the single drugs in terms of both the pattern in a single session and the day-to-day performance. The combination was administered in a biphasic, dose-dependent manner on day 1 of substitution. The total number of reinforcers earned was significantly reduced during the next 2 days. On the second and third days of caffeine–ephedrine substitution, the number of injection administrations was not statistically different from those engendered by saline. This pattern of self-injection was characteristic of all animals tested on the caffeine + ephedrine combinations with relatively little deviation from this pattern across the 3-day substitution test. These data suggest that the combination of caffeine and ephedrine may possess some of the qualitative interoceptive cues to those engendered by cocaine initially, but the combination will not easily maintain self-administration over the course of the 3 days of substitution. These data, taken along with previous reports by this laboratory (5,6) might imply that this combination of over-the-counter compounds possesses very limited abuse potential, and would not support long-term abuse. The low abuse potential is also supported by the fact that these compounds have been available over the counter for more than 20 years, with few reports of abuse.

Tests conducted under the progressive-ratio schedule of reinforcement demonstrated that the break points for the caffeine + ephedrine combinations were, for the most part, not significantly different from saline. Sekita et al. (27) have also demonstrated rhesus monkey self-administration of a pseudoephedrine–caffeine combination with break point values similar to saline levels, but with dose-dependent substitution for cocaine.

The pretreatment tests were conducted to assess the potential behavioral disruptive effects of coadministration of caffeine + ephedrine combinations with cocaine. This could be a critical factor in predicting if this drug combination would be safe to administer to individuals who are prone to cocaine use or relapse. Only the highest doses of the caffeine–ephedrine combination and cocaine pretreatments were shown to have any potentially behaviorally disruptive effects. This was demonstrated by the marked decrease in reinforcer deliveries as well as the experimenter's observations of marked increases in stereotypy during those test sessions. Although a potential for behavioral disruption was demonstrated here, the effects were apparently not long lasting or fatal. No animal was lost during these pretreatment tests, and each rat regained steady baseline responding on subsequent days of cocaine access. The high doses of cocaine and caffeine + ephedrine shown here to reduce cocaine self-administration, have also been shown to significantly reduce rates of responding in a simple food reinforced task (6). This may suggest that these drugs are simply disrupting behavior and not altering the reinforcing strength of the cocaine. This may be important to consider because King et al. (17) have suggested that reductions in responding due to pretreatments could actually reflect increases in the unit dose of the self-administered drug, thus contributing to an entrenchment of the cocaine abuse itself. The pretreatment tests also demonstrated that the animals were not primed to increase lever-press responding for the cocaine maintenance dose at any of the pretreatments tested. The behaviorally disruptive effects of concomitant administration of caffeine and cocaine have been previously reported by this laboratory for rats maintained on a fixed-ratio 10 schedule of food delivery in a two-choice discrimination task (12) and a fixed-interval 5-min schedule in a single lever operant task (18). Neither study demonstrated a significant deleterious effect produced by any caffeine:cocaine test combination.

In conclusion, Overton (21) has suggested that not all sensory consequences of drug actions are conducive to drug abuse. There appears to be at least three categories of subjective drug effects: 1) effects such as euphorigenic actions, which promote drug abuse; 2) aversive effects, which deter drug abuse; and 3) neutral subjective effects, which can provide a basis for stimulus control but that neither increase nor decrease a drug's abuse liability (21). There is only a modest correlation between the degree of discriminability and abuse liability (21). The robust discriminative stimulus properties of caffeine–ephedrine combinations previously demonstrated in this laboratory (6) and the lack of reinforcing effects of these combinations demonstrated in the present study may suggest that the subjective effects of categories 2 and 3 (above) form the basis of these drug discriminations. Inasmuch as the animal preclinical assays of the discriminative and reinforcing effects of drugs accurately predict the abuse liability in humans, the data from the present self-administration study and those of Gauvin et al. (6) using a drug discrimination assay, may suggest that the combination of caffeine and ephedrine is centrally active, relatively safe, and has limited abuse potential.

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