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The Effects of Cocaine in a Gustatory Avoidance Paradigm: A Procedural Analysis[1]

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FOLTIN, R. W. AND C. R. SCHUSTER. *The effects of cocaine in a gustatory avoidance paradigm: A procedural analysis*. PHARMAC. BIOCHEM. BEHAV. **16**(2) 347–352, 1982.—When the presentation of a novel food to a rat is followed by the injection of certain compounds, the animal consumes less of that food on subsequent presentations. Conflicting results have been obtained when cocaine is used in this gustatory avoidance paradigm. In the present study, fluid intake of rats was limited to a single presentation, seven days a week. Following the determination of baseline water intake, sweetened fluid was given during the session following the determination of baseline water intake, sweetened fluid was given during the session followed by an injection of cocaine. In Experiment 1, 24.0 mg/kg cocaine-fluid pairings occurred on alternate days with two-bottle preference tests occurring between pairings. Animals treated with cocaine had lower preference ratios than saline controls although both groups consumed more novel fluid than tap water. In Experiment 2, preexposure to the fluid prior to pairing it with cocaine eliminated differences between cocaine and saline treated rats. In Experiment 3 no effect of cocaine on novel fluid consumption was seen in male or female rats when 24.0 mg/kg cocaine-fluid pairings were given for five consecutive days. In addition, 36 mg/kg cocaine when paired with novel fluid every other day failed to induce an avoidance response in Experiment 4. These results indicate that cocaine is a weak avoidance-producing agent in the gustatory avoidance paradigm, and suggests the need for a standardized procedure for studying drugs in this paradigm.

Gustatory avoidance CTA Cocaine CS-preexposure effect Rats

IT HAS been demonstrated in rats that the simultaneous occurrence of the ingestion of a novel substance (i.e. saccharin solution) and the presentation of ionizing radiation, which produces an acute illness, results in the development of an avoidance of the saccharin solution on subsequent presentations [9]. Psychomotor stimulants, opiate analgesics, and antianxiety agents also induce similar avoidance responses [3]. In evaluating the efficacy of cocaine in inducing avoidance responses there have been conflicting results. For instance, one study [3] failed to obtain an avoidance response with cocaine in male albino rats, while another study [10] using a similar procedure, but with female rats, report dose-dependent cocaine-induced avoidance responses. In addition avoidance responses have been reported in male hooded rats using a more complicated procedure involving the development of a discrimination of two distinct flavors, one paired with saline injections and the other with cocaine injections [1,4]. The present report describes the results of four experiments designed to further assess the efficacy of cocaine in the gustatory avoidance paradigm.

EXPERIMENT 1

Novel fluid presentation was followed by the injection of 24 mg/kg cocaine every other day. On the alternate days, a two-bottle preference testing procedure was given to assess changes in fluid preference consequent to cocaine-fluid pairings. It was predicted that cocaine would significantly decrease the preference for the novel solution. Although saline treated rats had larger preference ratios for the novel fluid than the cocaine treated rats, both groups drank more novel sweetened fluid than tap water.

Method

Animals and apparatus. Thirty-two adult male Sprague-Dawley rats (Holtzman, Madison, WI) weighing between 250

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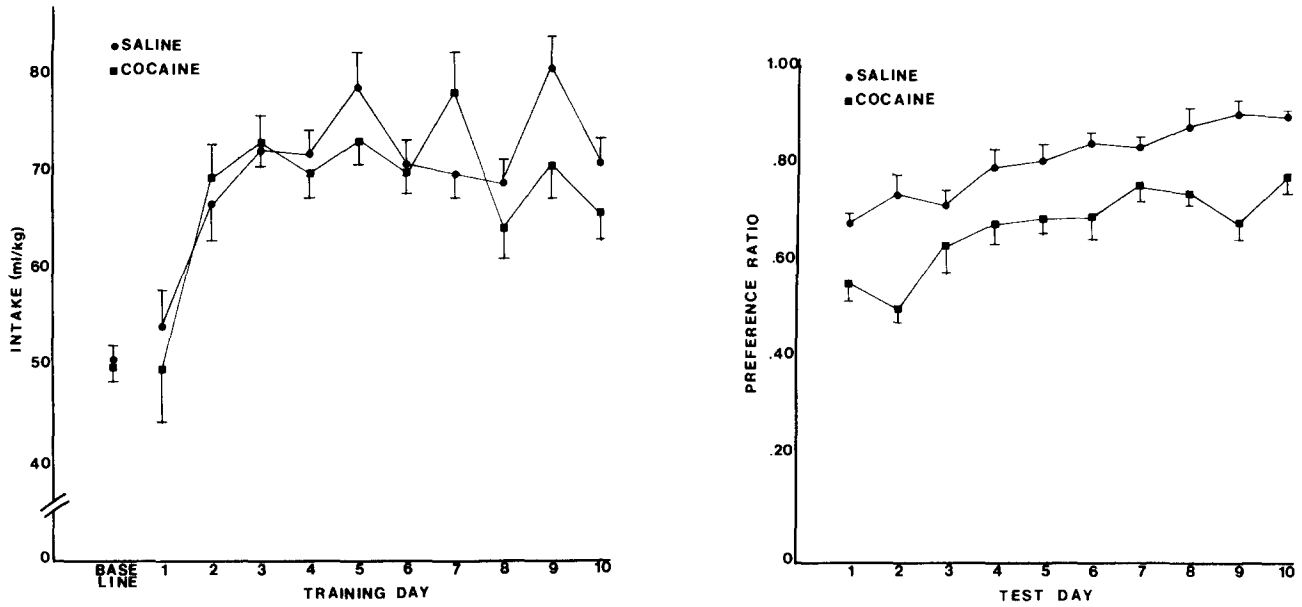

FIG. 1. Mean fluid intake and SEMs as a function of training day (left panel) and mean preference ratios and SEMs as a function of test day (right panel) for cocaine and saline treated rats in Experiment 1.

and 300 gm at the start of the experiment were individually housed in stainless steel ceiling suspended cages with food (Teklad, Winfield, OH) available ad lib and a light dark cycle from 8:00 a.m. to 8:00 p.m. All animals were handled daily and maintained at 23.67 hr water deprivation by limiting access to water to a single 20 min presentation occurring at the same time (6:00-6:20 p.m.) each day, seven days a week. All fluids were presented in Wahman (Baltimore, MD) 100 ml calibrated drinking bottles attached to the front of each cage.

Procedure. Animals were randomly assigned to four groups, with all groups containing eight animals. Animals were acclimated to drinking all of their daily fluid during the 20 min presentation over a five day period. On the following day half of the animals (Group 1 and 2) received sweetened condensed milk (Borden's Columbus, OH, 2:1 tap water to milk) and half (Group 3 and 4) received saccharin solution (0.1% w/v sodium saccharin in tap water) during the 20 min session. Ten minutes after the session, half of the rats (Group 1 and 3) received an injection of cocaine hydrochloride (24 mg/kg) and the remaining rats (Group 2 and 4) received an injection of physiological saline. The 20 min session on the next day consisted of the presentation of two bottles, one containing tap water and the other the milk or saccharin solution with position of the tap water bottle (right,left) randomized between animals. A preference ratio was calculated by dividing intake of the milk or saccharin solution by total fluid intake for that session. A ratio of 0.00 indicated that only tap water was consumed. This 2-day sequence of a single bottle cocaine or saline pairing session followed by a two-bottle test session continued for twenty days for a total of ten pairings and ten test sessions.

Drug. Cocaine hydrochloride was dissolved in physiological saline and injected (IP) in a volume of 1 ml/kg. Doses are expressed as total weight of the salt.

Results

The left panel of Fig. 1 shows the mean baseline water intake and the mean milk or saccharin solution intake for both groups for the ten training (pairing) days; the right panel shows the mean preference ratios for the ten test days and indicates that the values for the saline animals were higher than for the cocaine animals. These data were analyzed using two repeated measures analysis of variance, one for the first five days, and one for the second five days. For the first five days there was a significant effect of day, $F(4,60)=2.50$, $p<0.05$, with preference ratios increasing over that period, and as previously noted a significant effect of treatment, $F(1,60)=17.73$, $p<0.001$. There was also a significant difference between the saline and cocaine treated animals for the last five days, $F(1,60)=37.57$, $p<0.0001$.

EXPERIMENT 2

In classical conditioning experiments, preexposure to the conditioned stimulus prior to conditioning decreases the effectiveness of that stimulus when later paired with the unconditioned stimulus during conditioning [13,16]. The CS-preexposure effect is also seen in gustatory avoidance conditioning. That is, if an animal has prior experience with the solution subsequently paired with a drug, the avoidance response is attenuated [17,18]. The larger the number of preexposure sessions the greater the attenuation [8]. The animals in Experiment 1 were exposed to the flavored solutions during the two-bottle test session, which were not followed by a cocaine injection, therefore over the course of the ten testing sessions the avoidance-inducing properties of cocaine may have been attenuated. In Experiment 2, the role of previous exposure to the novel solution in attenuating the avoidance induced by cocaine was investigated. The procedure used in

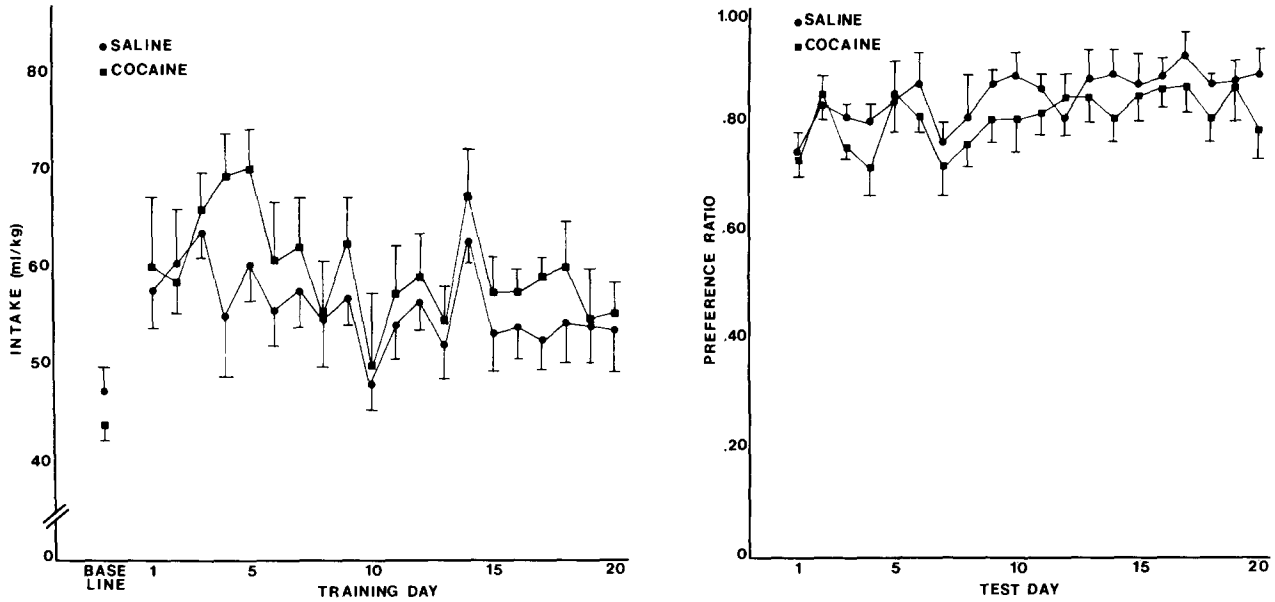

FIG. 2. Mean fluid intake and SEMs as a function of training day (left panel) and mean preference ratios and SEMs as a function of test day (right panel) for cocaine and saline treated rats in Experiment 2.

Experiment 1 was repeated with the addition of fluid preexposure sessions given prior to the cocaine-fluid pairing sessions. The fluid preexposure completely eliminated all differences between saline and cocaine treated rats.

Method

Animals and procedure. Twenty-four albino rats weighing 250 to 325 gm were housed and water deprived as in Experiment 1 with fluid intake limited to a single 20 min session occuring at the same time each day (4:00–4:20 p.m.), seven days a week. Following a seven day baseline water intake period, all animals were assigned randomly to one of two groups; twelve rats received 21 consecutive days of saccharin solution presentation and 12 received 21 days of sweetened condensed milk presentation. After the preexposure period, training and testing were done in the same manner as in Experiment 1 but with a total of 20 pairings, and 20 tests rather than 10 each. Half of the saccharin exposed rats received cocaine (24 mg/kg) and half received saline during these pairings. The milk exposed animals were divided into the same two groups.

Results

The preference ratios and training day fluid intake for all animals were almost identical (Fig. 2) indicating that solution preexposure eliminated the differences in solution preferences seen in Experiment 1.

EXPERIMENT 3

Since the effects of cocaine in decreasing preference ratios was attenuated by preexposure to the flavored solution, Experiment 3 was designed to increase the avoidance response induced by cocaine by always pairing the drug with the novel solution and eliminating preference testing on alternate days. Since the previous experiments showed no differences between saccharin and sweetened milk, only sweetened milk was used as the novel stimulus. In addition, the role of sex of the rats was investigated as a study [3] which did not obtain an avoidance response with cocaine had used male rats and another study [11] which did report cocaine-induced avoidance responses had used female rats. However, no significant differences between cocaine and saline treated male or female rats were seen following five consecutive days of cocaine-fluid pairings.

Method

Animals and procedure. Twenty-four male albino rats weighing 300 to 350 gm and 24 female albino rats weighing 250 to 300 gm were housed and water deprived as in Experiment 1, with water access limited to one 20 min session (2:00–2:20 p.m.) daily, seven days a week. Following five days of baseline water intake, sweetened condensed milk was presented for five consecutive days with 12 animals of each sex receiving cocaine (24 mg/kg) and 12 of each sex physiological saline ten min after each session. Twenty-four hours after the last cocaine-milk pairing session an additional milk presentation occurred which was not followed by injections.

Results

Milk intake (Fig. 3) increased for both groups over the five days, with no significant differences between groups in intake.

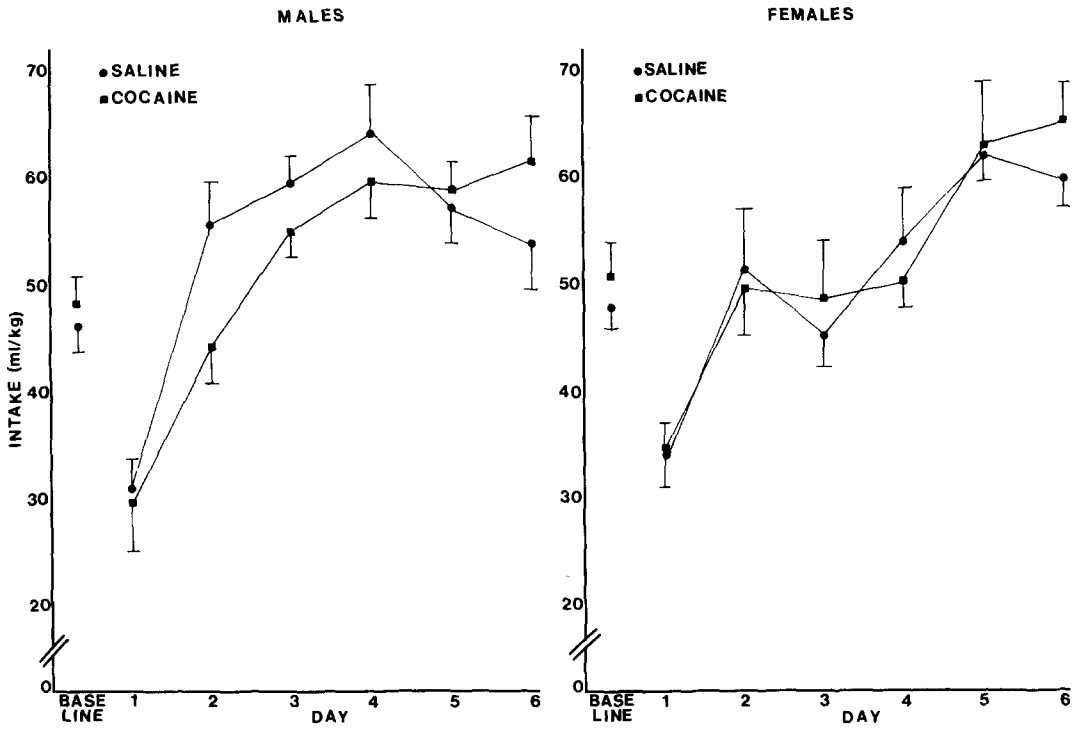

FIG. 3. Mean milk intake and SEMs for male and female rats as a function of training day in Experiment 3.

EXPERIMENT 4

Sex of the rats was not a factor mediating the difference between the present study and an earlier study [11]. There was a transient difference between cocaine and saline treated male rats, with the saline group consuming more milk on the first test day. It is possible that by pairing cocaine with the novel fluid for five consecutive days any aversive effects of cocaine were attenuated by an increase in deprivation state. In Experiment 4, drug-flavor pairing occurred every other day with water available on alternate days during the test session. By giving water on alternate days each test session follows an equivalent deprivation period (24 hr), eliminating potential differences in deprivation between different test sessions. This is similar to other studies in which the drug-fluid pairings occurred once every three days with water presentations on intervening days [3,11]. In addition the dose of cocaine was increased to 36 mg/kg given five minutes after the session with each drug-fluid pairing day separated by a day of tap water presentation. In spite of this increase in dose, decrease in potential deprivation and decrease in latency to injection no significant differences were seen between cocaine and saline treated groups of rats.

Method

Animals and procedure. Twenty male albino rats weighing 275 and 325 gm were housed and water deprived as in Experiment 1. Sessions were from 9:30 to 9:50 a.m. daily. Following five days of baseline water intake, animals were exposed to sweetened milk during the twenty min session followed five minutes later by an injection of cocaine (36 mg/kg, n=10) or saline (n=10). The next day all animals were given tap water as their only fluid. This sequence of one drug training day followed by one day of tap water without injections continued for 14 days for a total of seven drug days and seven no-drug days.

Results

Even though a larger dose of cocaine was used there was still no difference between saline and cocaine treated rats in intake for the seven days of testing and both groups drank more milk on the last day than their baseline water intake (Fig. 4). In addition, deprivation state was the same for each test session, arguing against the possibility that deprivation increased with each test session obfuscating any aversive stimulus properties of cocaine in Experiment 3. Although it might be argued that a larger dose of cocaine would be effective, any significant increase in dose above 36 mg/kg greatly increases the probability of inducing convulsions.

DISCUSSION

The effects of cocaine when given following the presentation of a novel fluid were analyzed using several different procedural manipulations. In Experiment 1 rats received cocaine-fluid pairings and two-bottle preference tests on alternate days. Animals treated with saline following fluid presentation had significantly larger preference ratios for the novel fluid than the animals treated with cocaine. However, repeated pairings did not further increase the size of the

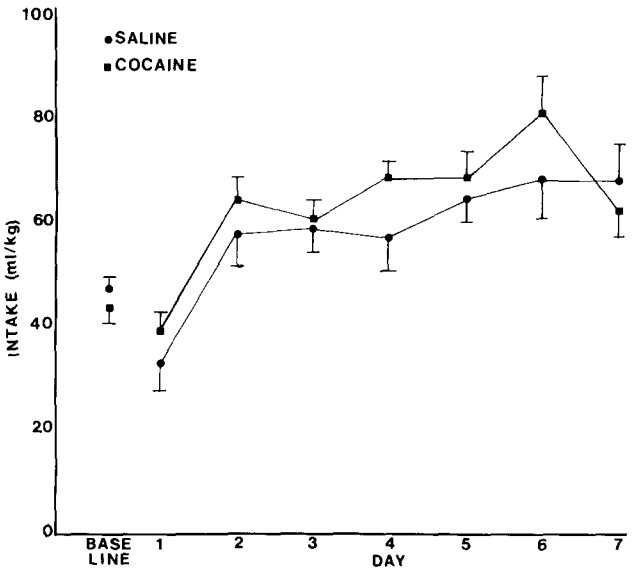

FIG. 4. Mean milk intake and SEMs as a function of training day for cocaine and saline treated rats in Experiment 4.

avoidance response as the preference ratios for both groups increased during the repeated pairings, with the saline group always have a stronger preference than the cocaine treated animals. This indicates the increase in sensitivity obtained with two-bottle tests as no differences in total intake were seen between the cocaine and saline treated groups using the one-bottle measure.

It has been reported that preexposure to the novel fluid before fluid-drug pairings results in the attenuation of the subsequent avoidance response when methyl-atropine [18], lithium chloride (e.g. [15,17]) or cyclophosphamide [8] is the drug used in conditioning. In addition, the greater the number of fluid preexposures, the greater the attenuation until significant avoidance responses are eliminated [8]. This phenomonon known as latent inhibition, or the CS-preexposure effect, has been reported to occur during classical conditioning for stimuli associated with shock presentation [14]. In the first experiment the novel fluid was presented without cocaine or saline injections during the preference testing sessions which may have attenuated the avoidance inducing properties of cocaine due to fluid preexposure without cocaine on test days. This was investigated using two approaches. If fluid preexposure did decrease the effects of cocaine in Experiment 1 then it should be possible to eliminate the differences between cocaine and saline treated animals by increasing the number of fluid preexposures and to increase the effect of cocaine by eliminating preference testing.

In Experiment 2, fluid preexposure sessions completely eliminated the differences between saline and cocaine treated groups of rats as seen in Experiment 1. This indicates a similarity between cocaine and other agents which induce larger avoidance responses. In order to decrease the fluid preexposure the two-bottle preference tests were removed and consecutive one-bottle fluid-drug pairing sessions were given in Experiment 3. Two bottle-tests are more sensitive in measuring avoidance responses [7,12] but are not used during repeated trials to avoid the CS preexposure effect. A difference between saline and cocaine treated male rats was seen only on the first of five test days, with cocaine treated rats consuming less of the novel fluid. This inability to condition a long lasting cocaine-induced avoidance response replicated one previous study [3] but is different from another [11] which reported long lasting cocaine-induced avoidance responses using female rats. Since no significant differences were seen between saline and cocaine treated female rats it is unlikely that a sex difference mediates the discrepancy between the present results and the latter study [11].

The remaining differences between the two studies of dose of cocaine and drug-fluid pairing separated by a day of water presentation were analyzed in Experiment 4. In Experiment 3 conditioning of an avoidance response may have been attenuated by an increased fluid deprivation state by presenting only the fluid associated with cocaine for five consecutive days. In order to decrease the possible influence of increased deprivation during testing, cocaine-fluid pairings were given every other day in Experiment 4. A higher dose of cocaine was used and following seven days of cocaine-fluid pairings there was no significant differences between saline and cocaine treated groups. This procedure differed from the latter study [11] only in the number of days between each fluid-drug pairing. In that study the pairings occurred once every three days rather than every other day.

In no experiment did cocaine induce an avoidance response as large as those reported by others [1, 4, 10, 11]. The earlier studies, which differ from the present one used male hooded rats and a more complicated procedure involving the training of a discrimination between two flavors, one paired with cocaine given after the session and the other with saline [1,4]. It has been reported, [2] using male Wistar rats that four pairings of 1.0 mg/kg amphetamine reduced novel fluid intake to less than one ml, while another study [5] reported equivalent results using the same dose of amphetamine, male hooded rats and the two-flavor discrimination procedure indicating that at least for amphetamine differences in procedure have a minimal effect on the resulting avoidance responses. However, cocaine induces only small avoidance responses and is clearly more susceptible to the effects of slight differences in procedure. The two-choice discrimination procedure is more sensitive in analyzing aversive stimulus properties of drugs. The single-bottle procedure is commonly used when repeated pairings are to be used and clearly, with cocaine the single-bottle is a poor measure while the two-bottle procedure which is a cross between the single-bottle tests and the two flavor discrimination procedure used in the first study was more sensitive. It was more sensitive than the single-bottle procedure but due to the CS-preexposure effect is less sensitive than the two flavor discrimination procedure. A final variable which may further account for differences in results between this study and the earlier one is the fact that Long-Evans hooded rats have been reported to acquire large avoidance responses with greater resistance to extinction that Sprague-Dawley Albino rats [6]. Although the difference in procedure and strain between the present study and several others [1,4] may explain the discrepancy in results between these studies, there was only a minor procedural difference between the present report and another earlier study [11]. The reasons for the discrepancy between these two studies remains unclear.

In conclusion the present study in combination with other reports on the effects of cocaine in the gustatory avoidance

paradigm indicates that cocaine is a weak avoidance producing agent [1, 4, 10, 11]. This weak efficacy appears to be a property of cocaine which is susceptible to the differences in procedure and rat strain shown between the published reports. In addition, research aimed at determining what property(s) of cocaine are responsible for this effect should all use the more sensitive two-bottle and two-flavor discrimination procedures to facilitate the acquisition of this knowledge.

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