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The Aversive Stimulus Properties of Repeated Infusions of Cocaine[1]

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FOLTIN, R. W., K. L. PRESTON, G. C. WAGNER AND C. R. SCHUSTER. *The aversive stimulus properties of repeated infusions of cocaine.* PHARMAC. BIOCHEM. BEHAV. **15**(1) 71–74, 1981.—When the presentation of a novel food to a rat is followed by the administration of certain compounds, including psychomotor stimulants, the animal consumes less of the food on subsequent presentations relative to an animal administered saline. This phenomenon has been termed gustatory avoidance conditioning. Conflicting results have been obtained when cocaine is used in this procedure. Therefore, the possibility that the weak efficacy of cocaine in this paradigm is due to its relatively short duration of action was investigated. Fluid intake was limited to a single 15 min presentation, seven days a week. Following the determination of baseline water intake, sweetened milk was given during the session followed by a series of infusions of cocaine through chronic indwelling peritoneal catheters. Four infusions, spaced 15 min apart, of 9.0 mg/kg cocaine induced an avoidance response, while one infusion of 36 mg/kg cocaine followed by three saline infusions did not. This supports the hypothesis that the low efficacy of cocaine in this paradigm is due, at least in part, to its short duration of action.

Gustatory avoidance conditioning Conditioned taste aversion Cocaine Rats

IF certain consequences (e.g., lithium chloride-induced illness) occur following the presentation of a novel fluid or food (e.g., saccharin solution) to a rat, the animal on subsequent presentations consumes less of that substance. This response has been termed conditioned taste aversion or gustatory avoidance conditioning. Initially, it was believed that only agents that induce illness could produce a gustatory avoidance response. However, subsequent studies have demonstrated that the administration of psychoactive substances can also induce this type of avoidance response [3]. The effects of cocaine in this paradigm have been inconsistent. Some investigators report a weak efficacy [1, 4, 8] while others report that cocaine produces no avoidance responses [3,6]. Although the reasons for the discrepancy are unclear, most investigators would agree that cocaine is a weak agent, at best, in inducing a gustatory avoidance response.

It has been reported that the potency of other stimulants in this procedure was positively correlated with their duration of action [9]. In an attempt to determine whether duration was a factor in cocaine's effects, a previous study [3] held total dose constant and gave multiple and successive injections of cocaine to rats following a novel solution presentation. Both rats who received a single injection of 36 mg/kg cocaine, which alone was ineffective, followed by three saline injections and rats who received 9 mg/kg cocaine at each of the four injections drank significantly less saccharin solution than a group of animals given four similarly spaced saline injections. This indicates that the combination

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of the handling and injection procedure plus cocaine administration was necessary to induce a gustatory avoidance response.

The present investigation is a replication of that study [3] using repeated injections, but the handling of the animals was eliminated by the use of chronic indwelling intraperitoneal catheters. The group receiving four cocaine infusions of 9 mg/kg each drank significantly less novel fluid than the groups receiving either one cocaine infusion (36 mg/kg) with three saline infusions or four infusions of only saline.

EXPERIMENT 1

METHOD

Animals

Eighteen male, Sprague-Dawley albino rats (Holtzman Co., Madison, WI) weighing between 400 and 550 g at the start of the experiment were individually housed in stainless steel ceiling-suspended cages with food (Teklad, Winfield, IA) available ad lib. Access to water was limited to a single 15 min presentation occurring from 9:30 to 9:45 a.m. daily, seven days a week. Fluid was presented in Wahman (Baltimore, MD) 100 ml calibrated bottles attached centrally to the front of each cage.

Surgery

Silicone intraperitoneal catheters (1 mm i.d., 3 mm o.d.) were implanted into each rat. Following ether anesthetization, a small slit was made in the skin about 1.5 cm below the left side of the rib cage. The peritoneal cavity was punctured and 5 cm of catheter tubing inserted. The cavity was tied off with purse string stitches using nonabsorbable surgical suture which had been cemented to the catheter. The catheter was passed subcutaneously exiting through a dorsal puncture wound centered about 2.5 cm below the neck. The catheter was held in place by a jacket made of adhesive tape wrapped securely around the animal which allowed unrestricted movement. Protection of the catheter was provided by flat silver coated copper braid cable (0.64 cm wide, No. 14 AWG, Newark Electronics, Chicago, IL). The catheter and cable were passed through a hole in the roof of the cage and attached to a swivel [2] which allowed the subject to move without twisting the catheter.

Procedure

The animals were randomly assigned to one of three groups, with each group containing six subjects. Following four days of measuring baseline water intake during the 15 min session, the animals were prepared with catheters as described above. After another day of exposure to the 15 min session, sweetened condensed milk (Borden's, Columbus, OH, 2:1 tap water to milk) was substituted for the water. One group of rats received one infusion of cocaine (36 mg/kg) 15 min after milk presentation followed by three infusions of physiological saline spaced at 15 min intervals (C:S group). A second group received four cocaine infusions (9 mg/kg) spaced at 15 min interval (C:C group) and the last group received four infusions of saline also at 15 min intervals (S:S group). During the next session, milk was again available followed by the appropriate infusions for each group. Twenty-four hours later all subjects were given a two-bottle choice test during the 15 min session. One bottle contained tap water and the other milk with their position (left, right) randomized between animals. Preference was determined by the use of a ratio of milk intake divided by total fluid intake. A ratio of 0.00 indicated that tap water was consumed and a ratio of 1.00 indicated the consumption of only milk.

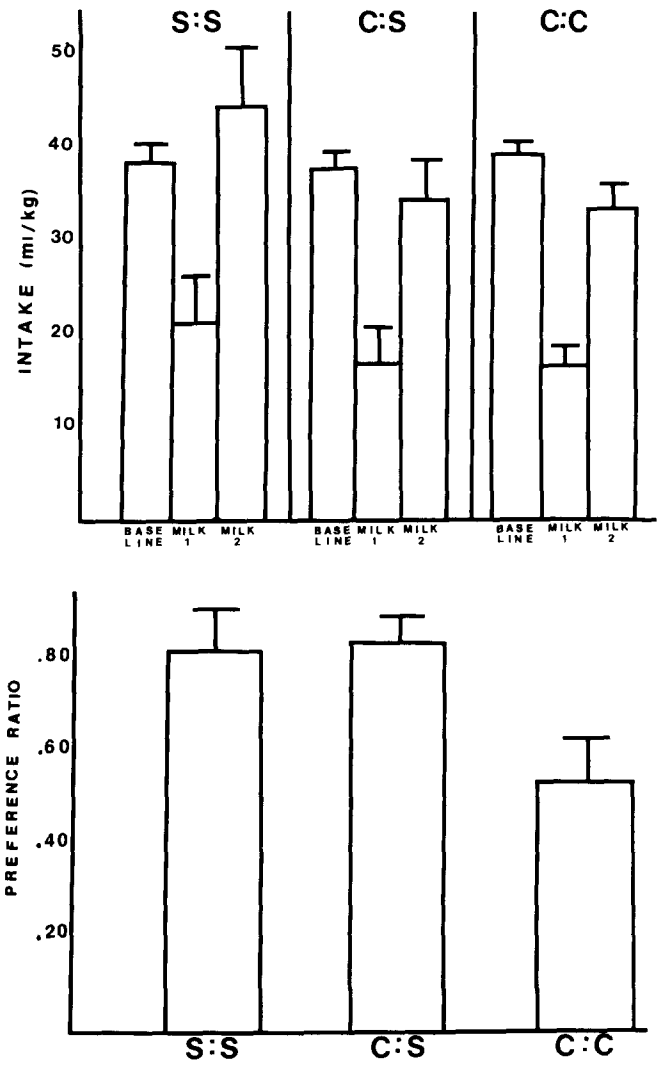

FIG. 1. Upper Panel. Mean baseline water intake and intake of milk on the first and second milk presentations with SEMs as a function of experimental condition. Lower Panel. Mean preference ratios for milk with SEMs as a function of experimental condition.

Drug

Cocaine hydrochloride was dissolved in physiological saline with the volume of all infusions being 1 ml/kg. Doses are expressed as the salt.

RESULTS

There were no significant differences between groups for baseline water intake and milk intake on the first and second days of the single bottle presentations (Fig. 1, upper panel). The preference ratios are shown in the bottom panel of Fig.

1. There were no significant differences between groups using an analysis of variance test, $F(2,17)=2.22, p>0.25$, and a planned comparison of the S:S and C:S groups with the C:C group was significant at a $p<0.1$ level, $F(1,15)=4.37$, with the C:C group having a lower preference ratio than the other two groups.

EXPERIMENT 2

The results of Experiment 1 suggest that repeated infusions of cocaine were more effective than a single infusion of cocaine in inducing a gustatory avoidance response. Experiment 2 was a replication of Experiment 1 with an increase in the number of rats in each group, an improvement in the leash apparatus and a shortening of the time period between removal of the milk and the first infusion.

METHOD

Animals and Surgery

Twenty-four male Sprague-Dawley albino rats weighing from 250 to 325 g at the start of the experiment were housed, fed, water deprived and prepared with catheters as in Experiment 1. The cable for shielding the catheters was replaced by a 25.4 cm length of 0.32 cm (ID) brass tubing (K and S Engineering, Chicago, IL) epoxied to a 2.54 cm length of spring which was riveted to a 2.54 cm wide washer with a 0.32 cm centerhole. The catheter was threaded through the spring and tubing with the washer being held in place by the adhesive tape jacket. The tubing pased through the hole at the top of the cage and the catheter opening was capped.

Procedure

Animals were randomly assigned to three groups as described in Experiment 1 (C:S, C:C, S:S) with all groups containing eight rats. Surgery was performed after the fourth water intake baseline session. Following the fifth baseline session, sweetened milk was presented followed by the appropriate series of infusions. Infusions occurred 5, 20, 35 and 50 min after the removal of the milk bottle. Twenty-four hours later water was presented for the daily session. The sequence of milk presentation followed by a day of water presentation occurred two more times for a total of three milk-cocaine pairing sessions. A fourth presentation of milk without infusions occurred after the third water presentation in the series. Milk intake for each presentation was determined and used as a measure of avoidance conditioning.

RESULTS

One rat did not drink during baseline and was removed from the C:S group and one rat in the S:S group died during surgery. There were no differences between groups in baseline water intake or first day of milk intake (Fig. 2). The intake for the second and third training day and the last milk presentation were analyzed using a repeated measures analysis of variance. There were significant differences between the three groups of rats with the C:C group consuming less milk than the other groups, $F(2,19)=7.05, p<0.01$. When intake for all three groups are combined for each test day there is a significant effect of test day with mean intake increasing on successive test days, $F(2,38)=5.01$, $p<0.025$. There was also a significant interaction between milk intake for each test day and each group, $F(4,38)=3.91, p<0.01$. The C:S and the S:S group consumed similar amounts of milk on all three test days, while the intake for the C:C group increased over the three test days. In fact, on the third test day all three groups consumed nearly the same amount of milk (Fig. 2).

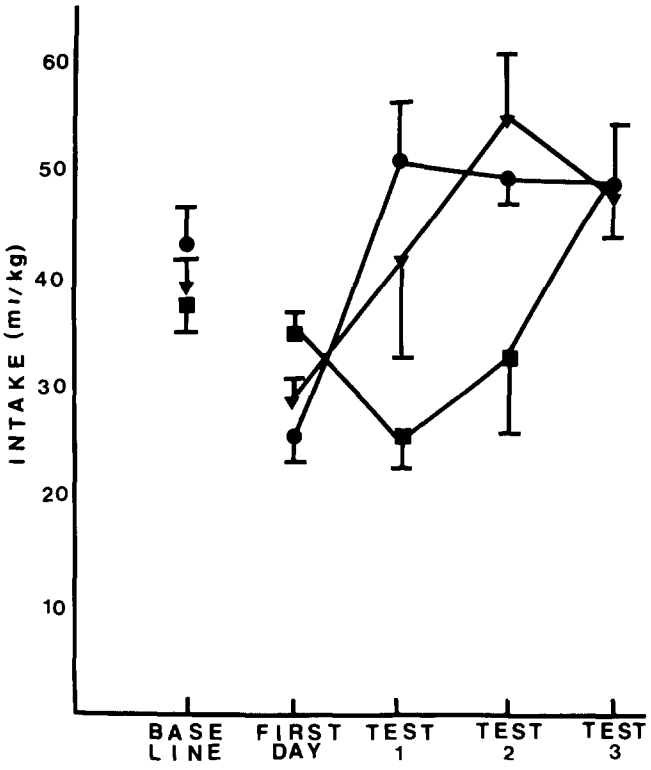

FIG. 2. Mean baseline water intake and intake of milk on test sessions with SEMs as a function of test day and experimental condition in Experiment 2: ● S:S, ▲ C:S, ■ C:C.

DISCUSSION

Conflicting results have been obtained in the gustatory avoidance paradigm with several reports indicating that post-session cocaine can produce avoidance responses [1, 4, 8] while other reports indicate the ineffectiveness of cocaine in this procedure [3,6]. It has been postulated that the relatively short duration of action of cocaine is in part responsible for its ineffectiveness [3]. In a previous study [3], the duration of action of cocaine was prolonged by giving repeated injections following novel fluid presentation, with total dose held constant between groups. Rats receiving four spaced injections of cocaine, as well as animals receiving one cocaine followed by three saline injections, developed avoidance responses. Both experiments in this study replicated the procedure of that earlier report [3], but in addition used chronic indwelling catheters to avoid the influence of handling. Animals receiving four spaced infusions of cocaine did show a significant avoidance response when compared to the animals receiving four infusions of saline or one cocaine followed by three saline infusions. However, neither experiment demonstrated a long-lasting cocaine-induced avoidance response.

It has been reported that WIN 35,428, a long-acting co-

caine analog, is more potent than cocaine in inducing avoidance responses [4], although the intensity of the maximum avoidance response obtained does not differ between the two compounds. This increase in potency without a change in efficacy indicated to the authors that the low efficacy of cocaine is not related to its relatively short duration of action [4]. On the other hand, increasing the duration of action of lithium chloride [5] or nitrous oxide [7] does increase the magnitude of the avoidance response induced by a specific dose. In combination, these studies [4, 5, 7] and the present one indicate that duration of action is a variable influencing the effects of drugs in the gustatory avoidance paradigm. Although the nature of this influence requires further investigation.

The results also indicate that the cocaine-induced avoidance response is a transient one. This differs from other reports [1, 4, 8] in which the cocaine-induced avoidance response remained at a constant level or increased during the duration of the repeated pairings in the experiment. In two of these studies a complicated procedure was used involving the training of a discrimination between a flavor paired with cocaine injections following the session and another flavor paired with saline [1,4] which may have enhanced the effects of cocaine in inducing avoidance responses while the third study [8] used a procedure similar to that of Experiment 2 (i.e., cocaine-flavor pairings separated by sessions of tap water presentation) and did not find the cocaine response to be short lasting.

In summary, four spaced infusions of cocaine were found to produce a greater avoidance response than a single cocaine infusion followed by three saline infusions. This supports the hypothesis that the relatively short duration of action of cocaine is in part responsible for its weak efficacy in this paradigm. However, it appears that the duration of action is not the only factor affecting cocaine's efficacy as WIN 35,428, a longer acting cocaine analog, is more potent than cocaine, but equally efficacious [4]. The relationship between duration of action and efficacy and potency requires further investigation.

REFERENCES

1. Booth, D. A., C. W. T. Pilcher, G. D. D'Mello and I. P. Stolerman. Comparative potencies of amphetamine, fenfluramine and related compounds in taste aversion experiments in rats. *Br. J. Pharmac.* **61:** 669–677, 1977.
2. Brown, Z. W., Z. Amit and J. R. Weeks. Simple flo-thru swivel for infusions into unrestrained animals. *Pharmac. Biochem. Behav.* **5:** 363–365, 1976.
3. Cappell, H. and A. E. LeBlanc. Gustatory avoidance conditioning by drugs of abuse: Relationships to general issues in research on drug dependence. In: *Food Aversion Learning*, edited by N. W. Milgram, K. Krane and T. M. Alloway. New York: Plenum Press, 1978, pp. 133–167.
4. D'Mello, G. D., D. M. Goldberg, S. R. Goldberg and I. P. Stolerman. Conditioned taste aversion and operant behavior in rats: Effects of cocaine and a cocaine analogue (WIN 35,428). *Neuropharmacology* **18:** 1009–1010, 1979.
5. Domjan, M., K. Foster and D. J. Gillan. Effects of distribution of the drug unconditioned stimulus on taste-aversion learning. *Physiol. Behav.* **23:** 931–938, 1979.
6. Foltin, R. W. and C. R. Schuster. The failure of cocaine to function as an aversive stimulus in a gustatory avoidance paradigm. *Pharmac. Biochem. Behav.*, submitted.
7. Goudie, A. J. and D. W. Dickens. Nitrous oxide-induced conditioned taste aversion in rats: The role of duration of drug exposure and its relation to the taste aversion-self-administration "paradox". *Pharmac. Biochem. Behav.* **9:** 587–592, 1978.
8. Goudie, A. J., D. W. Dickins and E. W. Thornton. Cocaine-induced conditioned taste aversion in rats. *Pharmac. Biochem. Behav.* **8:** 757–761, 1978.
9. Stolerman, I. P. and G. D. D'Mello. Amphetamine-induced hypodipsia and its implications for conditioned taste aversion in rats. *Pharmac. Biochem. Behav.* **8:** 333–338, 1978.