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Cocaine-Induced Conditioned Taste Aversions in Male and Female Wistar Rats

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VAN HAAREN, F. AND C. E. HUGHES. *Cocaine-induced conditioned taste aversions in male and female Wistar rats*. PHARMACOL BIOCHEM BEHAV **37**(4) 693–696, 1990.—After an initial period of adaptation to 20 min per day of limited water availability, male and female Wistar rats were allowed access to water or a 0.1% sodium-saccharin solution. Saccharin exposures were followed by the subcutaneous administration of 0, 5, 10 or 20 mg/kg cocaine for different groups of rats. Four pairings of the saccharin solution with cocaine administration resulted in a consistent decrease in saccharin consumption only in female subjects injected with the largest dose of cocaine (20 mg/kg). Choice testing in which subjects could choose between two drinking tubes, one containing water, the other one containing the saccharin solution, was then conducted during extinction. During four of such experimental sessions, subjects which had previously been injected with vehicle mostly consumed the saccharin solution or showed a position bias. Conditioned taste aversions were not only observed in the group of female subjects injected with 20 mg/kg cocaine, but also in males previously treated with 20 mg/kg cocaine. In addition, compared to vehicle control groups, males and females injected with 5 and 10 mg/kg cocaine tended to avoid the saccharin solution in favor of regular water. It is suggested that previous failures to obtain consistent cocaine-mediated taste aversions may have been a function of the experimental procedures used to assess cocaine's efficacy in inducing conditioned taste aversions.

Conditioned taste aversion Cocaine Single tube procedure Two-tube choice procedure Male and female rats

WHETHER or not the administration of cocaine immediately after the ingestion of a novel substance results in the avoidance of this substance on future presentations remains a matter of controversy. Even though it has been shown that many drugs of abuse, including opiate analgesics, antianxiety agents as well as other psychomotor stimulants may produce a conditioned taste aversion (CTA) in rats (4), the evidence with respect to the effects of cocaine is contradictory. Many investigators have failed, or only partially succeeded, in inducing cocaine-mediated taste aversions in male rats at doses as large as 36 mg/kg, approximately half the lethal dose (2–4, 11, 12). If anything, the efficacy of cocaine in inducing CTA is relatively weak as compared to other psychomotor stimulants, e.g., amphetamine (4).

Even though some investigators have argued that the short duration of action of cocaine might be responsible for its weak potency in inducing CTA (11), others have disputed this claim (10). Foltin et al. (11) showed that temporally spaced repeated infusions of cocaine (4×9 mg/kg, at 15-min intervals) produced CTA in male rats, whereas one infusion of the accumulated dose (36 mg/kg) did not. However, D'Mello et al. (10) have presented evidence to suggest that male rats exhibit CTA after administration of a large dose of cocaine (18 mg/kg) which is comparable to that observed after administration of this dose followed by additional smaller doses at 30 min intervals ($18+9+9$ mg/kg). These investigators also observed that the effects of a long-acting cocaine analogue (WIN 35,428) were not different from those of cocaine itself.

Of course, many of the contradictory effects of cocaine in taste aversion experiments may also have been caused by differences in experimental procedures, the strain of rats used in the experiments, as well as the gender of the experimental subjects. Foltin and Schuster (12) have argued that single drinking tube procedures are clearly inferior to two-drinking tube discrimination procedures when assessing the effects of cocaine in CTA procedures. The gender of the experimental subjects is another variable which may have confounded the assessment of cocaine's efficacy in establishing CTA, although the evidence with respect to this variable is contradictory as well. Goudie et al. (15) have shown that female rats consumed less of a saccharin-flavored fluid after ingestion had been paired with the intraperitoneal administration of cocaine. Evidence also indicated that the amount of saccharin consumed decreased in a dose-dependent manner, the largest decrease being observed after the administration of 36 mg/kg cocaine. However, Foltin and Schuster in the only available experiment directly comparing cocaine-induced CTA in male and female rats [(12), Experiment 3] showed that male as well as female rats did not avoid sweetened condensed milk in a single drinking tube procedure after this solution had been paired with a large dose of cocaine (24 mg/kg). That gender of the experimental subjects may be an important factor determining the behavioral effects of cocaine is suggested by experiments that have shown that gonadal hormones may modify the behavioral effects of agents which, like cocaine, interact with the dopaminergic system (13, 14, 21–23). In addition, it has recently been shown that cocaine self-administration is strongly influenced by the presence or absence of different gonadal hormones at the time of testing (1, 8, 9, 16).

The present experiment was designed to assess explicitly the efficacy of cocaine in establishing CTA in male and female Wistar rats. Groups of water-deprived rats were subcutaneously injected

with different doses of cocaine hydrochloride (0, 5, 10 or 20 mg/kg) after the ingestion of a 0.1% sodium-saccharin solution. The development of CTA was not only monitored during subsequent presentations of the saccharin solution, but was also assessed during extinction in a two-drinking tube choice procedure at the conclusion of the experiment.

METHOD

Subjects

Thirty-two male (mean weight 456 g) and 32 female (mean weight 325 g) Wistar rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) when they were 60 days old. They were housed in stainless-steel suspended cages (4 same-sex subjects to a cage) upon arrival in the laboratory. The light-dark cycle was reversed for the duration of the experiment (lights on 7:00 p.m.–7:00 a.m.). Subjects were allowed free access to food and water in their home cages until the start of the experiment when only food was freely available, while access to water was limited to the time periods to be described below. All subjects were repeatedly handled during the 6 weeks preceding the start of the experiment.

Procedure

Twenty-four hours preceding the first experimental session, all water bottles were removed from the home cages and subjects were randomly assigned to four different treatment groups. Starting with the first session, subjects were removed from their home cages, weighed and transferred to an individual cage fitted with a calibrated drinking tube on the right-hand side of the cage. They were then allowed access to water for 20 min and immediately returned to their home cage. This procedure was repeated during 5 sessions. Preceding session 6, subjects were removed from their home cages, weighed and transferred to the same individual cage as before. During this session, however, the calibrated drinking tube did not contain water, but a 0.1% sodium-saccharin solution. Subjects were removed from the individual cage after 20 min, put in a holding cage and subcutaneously (nape of neck) injected with one of four different doses of cocaine hydrochloride (Sigma, St. Louis, MO) dissolved in physiological saline (0, 5, 10 or 20 mg/kg body weight, dependent upon group membership). Subjects were then immediately transferred to their home cages. Over the next nine sessions, two sessions in which subjects were allowed to drink water and were not injected were followed by one session in which they were exposed to saccharin and subsequently injected with the appropriate dose of cocaine. A total of 4, 3-session blocks was thus completed.

During sessions 16 through 19 subjects were transferred from their home cages to the individual cage, which this time was fitted with two calibrated drinking tubes, one containing water and the other one containing the 0.1% saccharin solution. The position of the water tube and saccharin tube was alternated across the four sessions. Subjects remained in the individual cage for 20 min and were then immediately returned to their home cages. All experimental sessions were conducted between 9:00 a.m. and 1:00 p.m.

RESULTS

One male and one female subject, each belonging to the group which received 20 mg/kg cocaine, developed ulcers not obviously related to the experimental treatment. Their data were excluded from the present analysis.

Figure 1 shows group-averaged solution intake during water presentation (left bar), the first saccharin presentation prior to drug injection (middle bar) and saccharin consumption averaged over the three days after saccharin consumption had been paired with postsession cocaine administration (right bar).

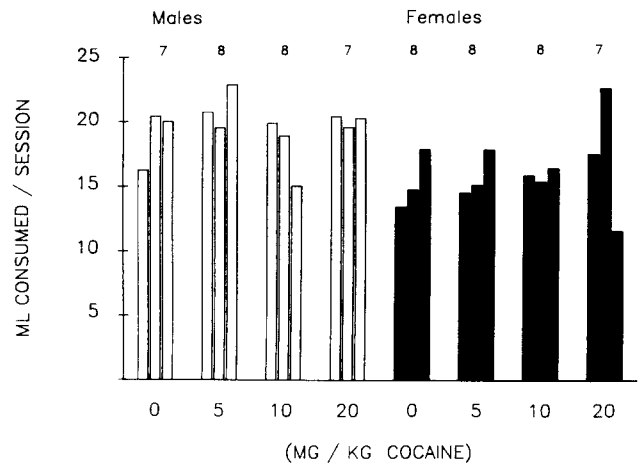

FIG. 1. Group-averaged solution intake during water presentation (left bar), the first saccharin presentation prior to drug injection (middle bar) and saccharin consumption averaged over the three days after saccharin consumption had been paired with postsession cocaine administration (right bar). Males are shown on the left, females on the right. The number of subjects in each group is shown at the top.

Analysis of Variance, including the factors sex, dose (0, 5, 10 or 20 mg/kg cocaine) and type of drinking fluid (water or saccharin) confirmed the observations in Fig. 1 that females, in general, consumed less fluid than males [sex, $F(1,53)=9.10$, $p<0.01$], but that group assignment [dose, $F(3,53)=1.60$, n.s.] and type of fluid [fluid, $F(1,53)=1.44$, n.s.] were irrelevant. Then, averaged saccharin intake during sessions following the first pairing of saccharin intake with the administration of different doses of cocaine was subjected to ANOVA involving the factors sex and dose. Neither sex, $F(1,53)=3.79$, nor dose, $F(3,53)=2.53$, proved significant, but a sex by dose interaction, $F(3,53)=3.10$, $p<0.03$, suggested that saccharin intake of the females, but not that of the males, was differentially affected by the dose of cocaine. Sex differences were observed as a function of the administration of 20 mg/kg cocaine, $F(1,12)=5.97$, $p<0.03$, but not as a function of any of the other doses. Females injected with 20 mg/kg cocaine consumed significantly less saccharin solution than vehicle-treated controls (Duncan's new multiple range test, 0 vs. $20=7.54$, $p<0.01$).

Figure 2 shows the intake of saccharin solution as a percentage of total session fluid intake during the four choice sessions of the present experiment.

The data presented in Fig. 2 strongly suggest that the intake of saccharin-flavored fluid in males varied greatly as a function of bottle position, especially at the lower doses of cocaine. ANOVA thus was limited to final choice session when the saccharin solution was presented on the preferred side of the cage. Differences between males and females were not observed in this analysis [sex, $F(1,54)=1.02$, n.s.], but the dose of previous cocaine administration functionally decreased the percentage of saccharin intake relative to total session fluid consumption [dose, $F(3,54)=18.60$, $p<0.001$]. Duncan's new multiple range test showed that males previously injected with 5, 10 and 20 mg/kg cocaine consumed significantly less saccharin solution than vehicle-treated controls (0 vs. $5=29.63$, $p<0.05$, 0 vs. $10=47.25$, $p<0.01$ and 0 vs. $20=74.49$, $p<0.01$). Similar observations were made between the different groups of females (0 vs. $5=34.13$, 0 vs. $10=$

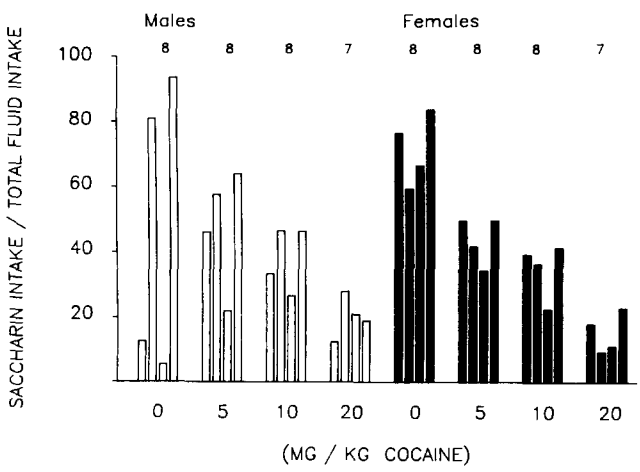

FIG. 2. Group-averaged intake of saccharin solution as a percentage of total session fluid intake during four sessions in which subjects could choose between water and a saccharin-flavored solution. Males are shown on the left, females on the right. The number of subjects in each group is shown at the top.

42.38, 0 vs. 20 = 62.59, all $p<0.01$). Thus, whereas vehicle-treated control subjects mostly consumed saccharin solution during choice testing, cocaine-treated groups either were indifferent (5 and 10 mg/kg) or showed a strong preference for water during choice testing (20 mg/kg).

DISCUSSION

The results of the present experiment show that the administration of cocaine following the ingestion of a novel substance may result in avoidance of this substance on future occasions. The data suggest that the procedure used to measure the presence of a conditioned taste aversion may be crucial to its observation. When the development of CTA was measured during consecutive exposures to the drinking tube containing saccharin, CTA was only observed in females injected with 20 mg/kg cocaine, confirming results also obtained by others (15). As such these data suggest that cocaine administration in the single drinking tube conditioned taste aversion paradigm affected saccharin consumption in a gender-specific manner. In this procedure, the behavior of the females seemed to be more sensitive to the effects of cocaine than that of males, especially in view of the fact that males, because of their body weights, received an absolutely larger dose of cocaine than females. Apparently, some confounding aspects of the single drinking tube procedure (effects of fluid deprivation vs. the aversive properties of cocaine administration) acted to mask the observation of CTA mostly in males.

The choice procedure employed in the second part of the present experiment proved to be more sensitive. When male and female rats were allowed to choose between one drinking tube containing water and one containing saccharin, the majority of male and female subjects which had had saccharin paired with 20 mg/kg cocaine showed evidence of CTA by consistently consuming larger amounts of water than saccharin-flavored fluid. Males and females which had been injected with 5 and 10 mg/kg cocaine consumed smaller quantities of saccharin-flavored solutions than vehicle-treated control groups, but were relatively indifferent between the two solutions. However, given the fact that the control groups consumed mostly saccharin solution during choice testing, it seems reasonable to suggest that a mild CTA was observed in these groups also. In addition, it has to be taken into account that CTA during choice sessions may have been attenuated by the fact that saccharin consumption during choice sessions was no longer followed by cocaine administration.

The present experiment has shown that male rats may show evidence of a CTA after the administration of cocaine at doses which were considerably smaller than those used to induce CTA in other experiments. In this context it might be worthwhile to suggest that the present experiment differed from other experiments in one other, possibly important aspect. Whereas male rats had been housed individually in previous experiments, they were housed in groups of 4 during the present experiment. Evidence is available to suggest that the behavioral effects of psychomotor stimulants in male rats may vary dependent upon housing conditions (17). For instance, Schenk et al. (19) have shown that male rats housed in groups failed to self-administer cocaine reliably, while male rats which were housed in isolation readily engaged in operant behavior which produced cocaine infusions varying between 0.1 and 1.0 mg/kg/infusion. However, the effects of housing conditions on the behavioral effects of cocaine are not necessarily straightforward. In another experiment, Schenk et al. (18) have shown that isolated male rats, trained in a place preference paradigm, failed to develop a place preference, whereas male rats housed in groups showed large place preference effects at a dose as low as 0.31 mg/kg cocaine. Interestingly, however, housing conditions did not seem to affect the behavioral effects of amphetamine, another psychomotor stimulant (20). Evidence is also available to suggest that housing conditions may affect the extinction of CTA in male rats through variations in the level of endogenous testosterone (5–7). Additional experiments will have to be conducted to elucidate the contribution of gonadal hormones to the behavioral effects of cocaine not only in conditioned taste aversion experiments, but also in other experimental procedures designed to evaluate the behavioral effects of acute and chronic cocaine administration.

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REFERENCES

1. Bennett, S. A. L.; Robert, D. C. S.; Vickers, G. J. Gender and estrous cycle affect cocaine self-administration on a progressive ratio schedule. Soc. Neurosci. Abstr. 268.7; 1988.
2. Booth, D. A.; Pilcher, C. W. T.; D'Mello, G. D.; Stolerman, I. P. Comparative potencies of amphetamine, fenfluramine and related compounds in taste aversion experiments in rats. Br. J. Pharmacol. 61:669–677; 1977.
3. Cappell, H.; Le Blanc, A. E. Conditioned aversion by psychoactive drugs: does it have significance for an understanding of drug dependence. Addict. Behav. 1:55–64; 1975.
4. Cappell, H.; Le Blanc, A. E. Gustatory avoidance conditioning by drugs of abuse: relationship to general issues in research on drug dependence. In: Milgram, N. W.; Krames, L.; Alloway, T. M., eds. Food aversion learning. New York: Plenum Press; 1977.
5. Chambers, K. C. Hormonal influences on sexual dimorphism in rate of extinction of a conditioned taste aversion in rats. J. Comp. Physiol. Psychol. 90:851–856; 1976.
6. Chambers, K. C.; Sengstake, C. B. Pseudo-castration effects of social isolation on extinction of a taste aversion. Physiol. Behav. 21: 29–32; 1978.

7. Chambers, K. C.; Sengstake, C. B.; Yoder, R. L.; Thornton, J. E. Sexually dimorphic acquisition of a conditioned taste aversion in rats: Effects of gonadectomy, testosterone replacement and water deprivation. Physiol. Behav. 27:83–88; 1981.
8. Dalton, J. C. H.; Vickers, G. J.; Roberts, D. C. S. Cocaine self-administration and hormonal status: the estrous cycle and the antiestrogen tamoxifen. Soc. Neurosci. Abstr. 349.3; 1985.
9. Dalton, J. C. H.; Vickers, G. J.; Roberts, D. C. S. Increased self-administration of cocaine following haloperidol: Sex-dependent effects of the antiestrogen tamoxifen. Pharmacol. Biochem. Behav. 25: 497–501; 1986.
10. D'Mello, G. D.; Goldberg, D. M.; Goldberg, S. R.; Stolerman, I. P. Conditioned taste aversion and operant behavior in rats: effects of cocaine, apomorphine and some long-acting derivatives. J. Pharmacol. Exp. Ther. 219:60–68; 1981.
11. Foltin, R. W.; Preston, K. L.; Wagner, G. C.; Schuster, C. R. The aversive stimulus properties of repeated infusions of cocaine. Pharmacol. Biochem. Behav. 15:71–74; 1981.
12. Foltin, R. W.; Schuster, C. R. The effects of cocaine in a gustatory avoidance paradigm: A procedural analysis. Pharmacol. Biochem. Behav. 16:347–352; 1982.
13. Glick, S. D.; Badalamenti, J. I. Sex difference in reward asymmetry and effects of cocaine. Neuropharmacology 25:633–637; 1986.
14. Glick, S. D.; Hinds, P. A. Sex differences to cocaine-induced rotation. Eur. J. Pharmacol. 99:119–121; 1984.
15. Goudie, A. J.; Dickens, D. W.; Thornton, E. W. Cocaine-induced conditioned taste aversions in rats. Pharmacol. Biochem. Behav. 8: 757–761; 1978.
16. Roberts, D. C. S.; Vickers, G.; Dalton, J. Increased self-administration of cocaine following haloperidol is attenuated by ovariectomy. Soc. Neurosci. Abstr. 245.11; 1984.
17. Sahakian, B. J.; Robbins, T. W.; Morgan, M. J.; Iversen, S. D. The effects of psychomotor stimulants on stereotypy and locomotor activity in socially-deprived and control rats. Brain Res. 84:195–205; 1975.
18. Schenk, S.; Hunt, T.; Malovechko, R.; Robertson, A.; Klukowski, G.; Amit, Z. Differential effects of isolation housing on the conditioned place preference produced by cocaine and amphetamine. Pharmacol. Biochem. Behav. 24:1793–1796; 1986.
19. Schenk, S.; Lacelle, G.; Gorman, K.; Amit, Z. Cocaine self-administration in rats influenced by environmental conditions: implications for the etiology of drug abuse. Neurosci. Lett. 81:227–231; 1987.
20. Schenk, S.; Robinson, B.; Amit, Z. Housing conditions fail to affect the intravenous self-administration of amphetamine. Pharmacol. Biochem. Behav. 31:59–62; 1988.
21. van Haaren, F.; Heinsbroek, R. P. W.; Louwerse, A.; van de Poll, N. E. *d*-Amphetamine differentially affects low, but not high response rates of male and female Wistar rats. Psychopharmacology (Berlin) 89:73–76; 1986.
22. van Haaren, F.; Heinsbroek, R. P. W.; van Hest, A. Behavioral differences between male and female rats: Effects of gonadal hormones on learning and memory. Neurosci. Biobehav. Rev. 14:23–33; 1990.
23. van Hest, A.; van Haaren, F.; van de Poll, N. E. Haloperidol, but not apomorphine differentially affects low response rates of male and female Wistar rats. Pharmacol. Biochem. Behav. 29:529–532; 1988.