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Cocaine discrimination and time-course effects in male and female Wistar rats

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Abstract

Previously, sex differences have been observed in the behavioral effects of acute and chronic cocaine administration. In the present experiment, male and female rats were trained to discriminate intraperitoneal injections of 10.0 mg/kg cocaine from its vehicle. It was hypothesized that the subjective effects of cocaine might differ between male and female rats. It was further hypothesized that generalization gradients between male and female rats might differ as a function of the time since cocaine administration. In addition, we were interested to see whether multiple generalization gradients could be determined within the same experimental session. For that purpose, two different types of generalization tests were conducted in extinction, one in which subjects were tested both 10 min and 30 min following cocaine administration (vehicle, 1.0, 3.0, 5.6, 10 or 17 mg/kg) and one in which subjects were only tested 30 min after cocaine administration. The generalization gradients obtained 30 min following drug administration were shifted to the right of the gradient obtained 10 min following drug administration. The two 30-min gradients were not different from one another, showing that multiple generalization gradients can be obtained within the same experimental session.

Keywords: Cocaine discrimination; Generalization gradient; Time course; Lever press; (Rat, male); (Rat, female)

1. Introduction

It has been well established that gonadal hormones affect functional adaptation to changes in the (experimental) environment (for review, see Beatty, 1979; van Haaren et al., 1990). Gonadal hormones also alter the physiological and behavioral sequelae of pharmaceutical challenge. With respect to cocaine, it has been reported that its systemic toxicity is greater in male rats than in intact or ovariectomized female rats (Morishima et al., 1993). Others have demonstrated that the behavioral effects of acute or chronic cocaine administration differ as a function of the subject's sex. In early reports, Glick et al. (1983) reported that female rats were more sensitive than male rats to cocaine-induced rotation. Roberts et al., (1989) demonstrated that female rats reached higher breaking points than male rats on a progressive ratio (PR) schedule of cocaine self-administration. Since then, others have shown that female rats were more sensitive than male rats to the effects of acute cocaine administration on locomotor activity (van Haaren and Meyer, 1991; van Luijtelaar et al., 1996) and that female rats required a smaller dose of cocaine than male rats to show evidence of a taste aversion (van Haaren and Hughes, 1990). It has also been shown that intact male rats were less sensitive than castrated male rats and intact and ovariectomized female rats to the effects of cocaine on schedule-controlled behavior (van Haaren, 1994; van Haaren and Anderson, 1994a). Gender differences in cocaine metabolism have previously been shown in rats after acute and chronic cocaine administration (van Haaren et al., 1997; see also Vernotica and Morrell, 1998). Others have shown that the LD_{50} for cocaethylene was much lower in male rats than in female rats (Sobel et al., 1998). These observations have human relevance as it has been shown that males reach highest cocaine peak plasma levels faster than females, detected cocaine effects faster than females and reported more episodes of good and bad effects than females (Lukas et al., 1996).

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The present experiment was designed to evaluate the subjective effects of cocaine administration at different time points following cocaine administration in male and female rats. Craft and Stratmann (1996) have previously presented evidence to show that intact male and female rats required the same number of sessions to discriminate between vehicle administration and the administration of 5.6 or 10 mg/kg cocaine, despite the fact that the higher dose of cocaine produced more locomotor activation in female rats than in male rats. Craft and Stratmann, however, did not attempt to assess sex differences in cocaine discrimination as a function of time since cocaine administration. It was hypothesized that such could be an important variable as van Haaren et al. (1997) have previously shown that sex differences in cocaine metabolism may be observed after repeated cocaine administration. The present experiment addressed this issue. In addition, the experiment was designed to further test the hypothesis that a drug's time-course effects may be evaluated within subjects within the same experimental session. Data in support of such an approach have previously been presented by Jarbe (1993) and Gauvin et al. (1990) who reported that in pigeons and male rats the proportion of responses emitted on the previously established cocaine-appropriate manipulandum decreased as time since injection increased. Time-course effects are typically investigated by injecting the same dose of cocaine (or any drug for that matter) at different times prior to the start of an experimental session. In the present experiment, time-course effects were investigated by conducting two consecutive tests within the same experimental session, one 10 min after drug administration and one 30 min after drug administration. To assess the reliability of the data obtained during the second test, other sessions were conducted in which subjects were tested only 30 min after drug administration. Within-session determination of a drug's timecourse effects (if shown feasible and reliable) would greatly reduce the amount of time needed to establish such drug-effect relationships.

2. Materials and methods

2.1. Subjects

Six experimentally naive, male and female Wistar rats served as subjects. All subjects were housed in groups (three same-sex subjects to a cage) under constant temperature and humidity conditions and a reversed 12-h light–dark cycle (lights on at 7 p.m.). Water was always available in the home cage. The subjects were fed approximately 16 g of Purina Rat Chow immediately following each experimental session. This feeding schedule resulted in each rat being food deprived for approximately 22 h prior to each experimental session. At the start of experimentation, female rats weighed 396 g (range: 381–419 g) and male rats weighed 470 g (range: 436–491 g).

2.2. Apparatus

Experiments were conducted in six identical Coulbourn Instruments modular, rodent operant-conditioning chambers that were 25 cm wide, 30 cm long, and 29 cm high. The side walls of the chamber, except for the front panel, were made of translucent Plexiglas. The floor consisted of 16 rods, spaced 2 cm apart (center to center). Two non-retractable rodent levers were located symmetrically on either side of the pellet tray, 7 cm from the floor of the chamber. The levers protruded 2 cm from the front panel and required a force in excess of 0.20 N to be operated. Three stimulus lights were located directly above each lever. A Sonalert was mounted above each lever, approximately 6 cm from the ceiling of the chamber. A houselight was located 2 cm from the ceiling in the middle of the front panel. The pellet tray could be illuminated by a white light. Each experimental chamber was contained in a sound-attenuating, ventilated cabinet. The chambers were connected to a PDP 11-23 microcomputer (Digital Equipment Corporation, Maynard, MA) located in the experimental room itself. Experimental contingencies and data acquisition procedures were programmed using SKED-11 (Snapper and Inglis, 1985), obtained from State Systems (Kalamazoo, MI).

2.3. Procedure

2.3.1. Lever press acquisition

All subjects were trained to press the levers using a free-operant acquisition procedure (van Haaren, 1992). In this arrangement, food pellets were delivered on an average of once per minute or when a lever press was emitted. After five sessions of this initial training procedure, five sessions were conducted in which subjects were required to complete a Fixed-Ratio 1 (FR 1) schedule of reinforcement that alternated between the two levers after the presentation of every fifth reinforcer. During the next three sessions, subjects completed a Random-Interval 15-s (RI 15-s) schedule. Completion of an RI 30-s was required during the final two sessions of lever press acquisition training. Experimental sessions were conducted Monday through Friday of each week.

2.3.2. Discrimination training

Injection of the drug (D) or the vehicle (V) prior to any particular session was determined according to the following schedule: VDVDD DVDVV. Immediately after the intraperitoneal (I.P.) administration of either 10.0 mg/kg cocaine or the vehicle (saline), the subject was placed in the darkened operant chamber. Ten minutes post-injection the houselight and both lever lights were illuminated. Responses on one lever (cocaine- or saline-appropriate) were followed by pellet presentation according to a Tandem Random-Time 30-s Fixed-Ratio 10 (TAND (RT 30-s,

FR 10)) schedule of reinforcement. Presses on the other lever were never followed by pellet presentation (extinction). To avoid the possibility of discriminative olfactory cues left behind from a previous subject on the lever associated with pellet presentation, the position of the cocaine- or saline-appropriate lever was switched after each subject. When a response was made and recorded, an auditory feedback stimulus was provided. A 2-s change-over delay (COD) was initiated following a response on the lever associated with extinction to prevent adventitious reinforcement of switching between levers.

All sessions were terminated after 40 pellets had been presented or after 30 min, whichever came first.

2.3.3. Generalization testing

Generalization testing began after at least 30 discrimination training sessions and when the subjects made at least 80% correct lever presses during five consecutive sessions. Generalization tests were conducted in extinction. In phase A, subjects were placed in the darkened experimental chamber immediately after drug administration. Ten minuntes later the houselight and lever lights were illuminated for either 5 min or until the response requirement necessary for pellet presentation established during training sessions (TAND RT 30-s, FR 10) had been met (Test 1). Following the completion of the response requirement, instead of pellet delivery, all stimulus lights were extinguished and responding had no scheduled consequences. If the response requirement was not completed within these five min, all lights were extinguished until the start of the second test. Thirty minutes following the pre-session drug injection, a second generalization test identical to the first was conducted (Test 2). Different doses of cocaine (saline, 1.0, 1.7, 3.0, 4.2, 5.6, 10.0 and 17.0 mg/kg) were administered in a semi-random order (range: 2–7) on Tuesdays and Fridays of each week if the criterion of 80% correct responding had been met on the preceding day.

During phase B, only one test (Test 2 Only) was conducted as subjects were injected, placed in the darkened experimental chamber and tested 30 min after the administration of saline or 1.0, 3.0, 5.6, 10.0 or 17.0 mg/kg of cocaine.

2.4. Determination of stage of estrous cycle

When an intact female rat received cocaine or saline during generalization testing, a vaginal smear was obtained to determine the stage of the estrous cycle.

2.5. Data analysis

The percentage of correct lever presses was determined by dividing the number of responses emitted on the stimulus-appropriate lever by the total number of responses emitted on both levers prior to the delivery of the first food pellet. Calculations were identical during generalization tests, which were conducted in extinction, except the recording was terminated at the time when the food pellet was programmed to be delivered. ED_{50}s were calculated by log–linear interpolation of the descending portion of the dose–effect curve and includes one point above 80% and one point below 20%.

3. Results

Fig. 1 shows the percentage of total responses on the cocaine-appropriate lever for each of the test doses of cocaine and saline for female rats (top panel) and male rats (bottom panel) during generalization tests conducted 10 min (left hand panels) and 30 min (middle panels) after cocaine administration in the same session and when the generalization test was conducted 30 min following cocaine administration only (right hand panels). Two female rats and two male rats did not complete the experiment for various reasons and their data were excluded from this analysis.

When injected with the 10.0 mg/kg training dose of cocaine, all subjects emitted 95% to 100% of all responses on the cocaine-appropriate lever during the generalization test conducted 10 min post-injection (Test 1). These results did not differ from those obtained in training sessions. When injected with saline and tested 10 min post-injection (Test 1), average responding was greater than 80% on the saline-appropriate lever. This is also representative of responding during the training sessions. The percentage of total responses emitted on the cocaine-appropriate lever was functionally related to the dose of cocaine administered during generalization tests conducted 10 min post-injection, such that, the smaller the dose of cocaine, the lower the percentage of total responses emitted on the cocaine-appropriate lever (see left panel in Fig. 1). When the test dose of cocaine exceeded the training dose, i.e., when 17.0 mg/kg was administered, some of the subjects failed to emit any responses on either lever. The generalization gradients of male and female rats and the ED_{50}s with 95% confidence limits (as presented in the first column of Table 1) did not differ 10-min following cocaine administration in the test sessions.

Response rates on injection-appropriate levers were also evaluated during these test sessions 10 min after administration of the cocaine training dose (10 mg/kg) and 10 min after vehicle administration. During test sessions, response rates on the cocaine-appropriate lever averaged 28.38 responses per minute (range 11.23–55.18) for female rats, while those on the saline-appropriate lever averaged 38.31 (range 27.55–48.26). For male rats, response rates averaged 48.97 responses per minute (range 47.55–50.25) after the administration of cocaine, while those after saline administration averaged 34.03 (10.56–52.57).

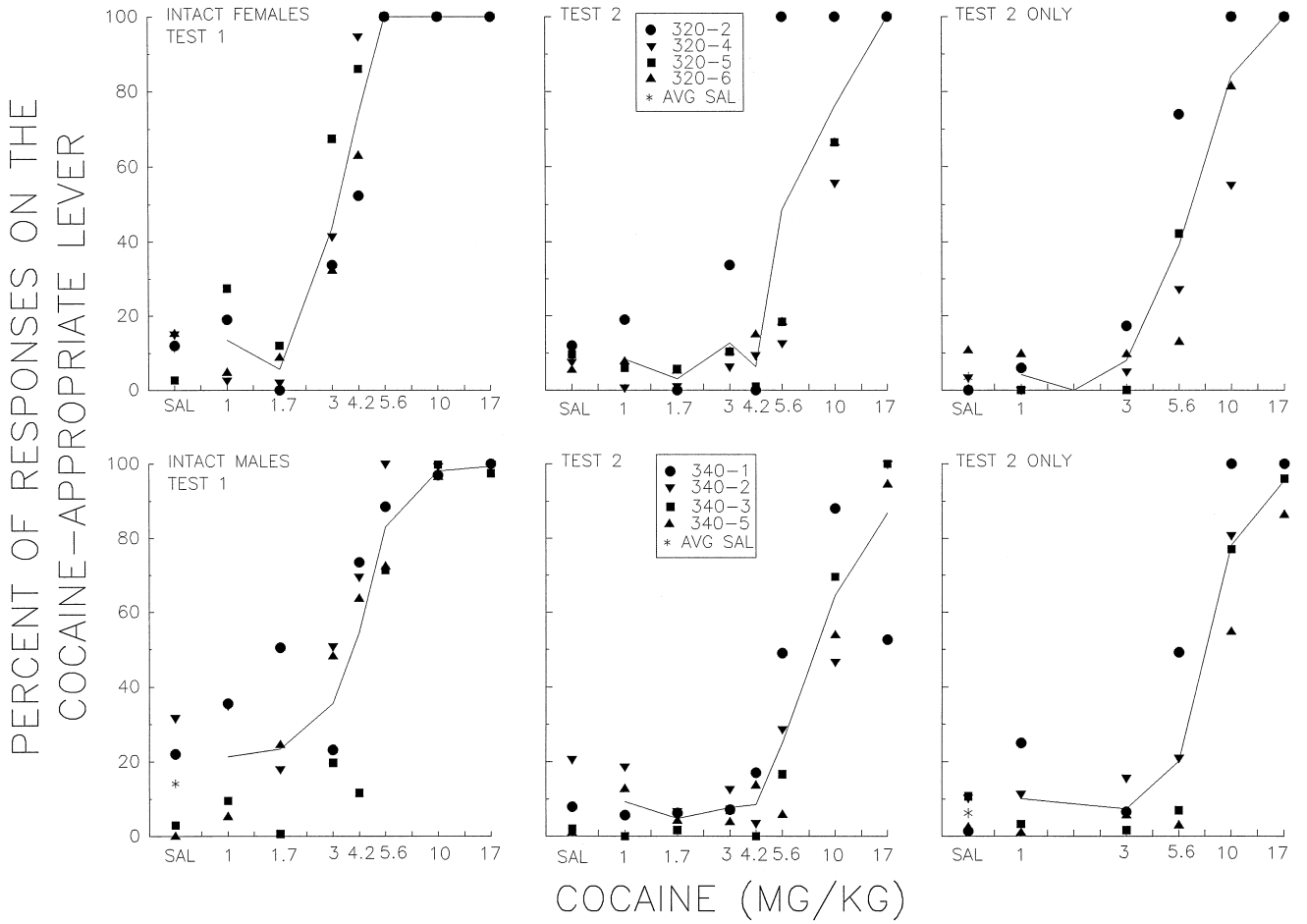

Fig. 1. Percentage of the total responses emitted on the cocaine-appropriate lever during Test 1 (left panels), Test 2 (center panels), and Test 2 Only (right panels) for female (upper panels) and male subjects (lower panels). Single unconnected filled symbols represent the mean data for each individual female and male subject whose responding was maintained by a TAND (RT 30-s, FR 10) schedule of reinforcement. The solid lines (cocaine) and the asterisks (saline) represent group averages for female subjects responding on the TAND (RT 30-s, FR 10) schedule.

When subjects were tested again within the same experimental session 30 min following cocaine administration, the generalization gradients were shifted to the right for male, as well as female rats and the ED_{50}s and 95% confidence limits (middle column of Table 2) were larger relative to those obtained in Test 1. Sex differences were not observed.

A comparison of the data presented in the right panel of Fig. 1 (generalization test 30 min post-injection not preceded by another generalization test) with those presented in the middle panel (a generalization test 30 min post-injection which was preceded by another generalization test 10 min post-injection) shows no differences between the two generalization gradients. In addition, the ED_{50}s for Test 2 were similar to and within the 95% confidence limits for those obtained during Test 2 Only.

3.1. Stage of estrous cycle

Examination of vaginal smears did not yield any obvious correlation between the stage of the estrous cycle and the behavioral effects or the discrimination of cocaine.

Table 1
ED_{50}s and 95% confidence limits

Test 1	Test 2	Test 2 Only
Female rats		
3.07 mg/kg (2.75–3.43 mg/kg)	6.07 mg/kg (5.37–6.86 mg/kg)	6.09 mg/kg (2.71–13.70 mg/kg)
Male rats		
3.16 mg/kg (1.76–5.68 mg/kg)	8.23 mg/kg (7.63–8.89 mg/kg)	5.37 mg/kg (0.86–33.63 mg/kg)

4. Discussion

These results confirm and extend those previously reported by others. First of all, they show that cocaine's generalization gradient did not differ between male and female rats when assessed 10 min post-injection. As such, these findings confirm those obtained by Craft and Stratmann (1996) who also showed that there were no differences in cocaine generalization gradients between intact male and female rats after the administration of 5.6 and 10 mg/kg cocaine as the training dose. On the basis of previously observed differences in cocaine metabolism between male and female rats (van Haaren et al., 1997) we had hypothesized that generalization gradients might differ if assessed 30 min, instead of 10 min after cocaine administration. As expected the generalization gradients obtained 30 min after cocaine administration were shifted to the right of those obtained 10 min after cocaine administration. This was evidenced by the fact that cocaine's ED_{50}s obtained during the 30-min post-injection tests were at least twofold greater than those obtained during the 10-min post-injection tests. However, sex differences were not observed, lending credence to the statement that there might exist a dissociation between cocaine's physiological fate and its behavioral effect (at least under the present experimental conditions).

The results of the present experiment also indicate that there were no differences between generalization gradients obtained 30 min after cocaine administration whether or not such gradients were obtained following a generalization test 10 min after cocaine administration. This finding supports the notion that, at least under the present experimental parameters, consecutive cocaine generalization tests may be conducted within the same experimental session. The present findings support those of Jarbe (1993) who showed that separate and repeated determinations of the (cocaine) time course yielded similar estimates in pigeons and further extends the findings of Hiltunen and Jarbe (1986) and Jarbe et al. (1981), who showed the feasibility of repeated testing using delta 9-tetrahydrocannabinol as the discriminative stimulus in pigeons and rats. In the present experiment, comparison of the generalization gradients and ED_{50}s obtained during Test 2 and Test 2 Only, both obtained 30 min following cocaine administration, revealed enough similarity to conclude that the results are a function of the time-course effects of cocaine. If exposure to extinction in the preceding test (Test 1) had influenced responding in Test 2 by setting the occasion to respond on the alternate lever, an inverse of the obtained function from Test 2 Only would have been expected. These results show that repeated generalization tests within one experimental session may be useful in conducting a functional analysis of the discriminative stimulus properties of cocaine. Experiments should be conducted to assess the feasibility of this procedure to determine full time-course functions within one experimental session.

Cocaine has been shown to produce different behavioral effects in male and female rats (van Haaren, 1994; van Haaren and Hughes, 1990; van Haaren and Meyer, 1991; van Haaren and Anderson, 1994a). Although no significant differences in the discrimination of the various doses of cocaine were observed between male and female rats in the present experiment, discrimination of the subjective effects of cocaine remains an important issue (at least as it relates to the drug's abuse potential). Research involving human subjects has shown that the reported subjective effects of intranasally administered cocaine vary between male and female volunteers (Lukas et al., 1996). In that context, it would be important to assess the extent to which hormonal variables may affect a subject's proclivity to self-administer cocaine (or any other drug, for that matter). Such research efforts, unfortunately, have been sorely lacking with only a few exceptions (Roberts et al., 1989; van Haaren and Anderson, 1994b,c; van Haaren et al., 1998).

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