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The Effects of Chlordiazepoxide on Low-Rate Behavior Are Gender Dependent

FRANS VAN HAAREN, ERIC KATON AND KAREN G. ANDERSON

University of Florida, Gainesville, FL

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VAN HAAREN, F., E. KATON AND K. G. ANDERSON. *The effects of chlordiazepoxide on low-rate behavior are gender dependent.* PHARMACOL BIOCHEM BEHAV **58**(4) 1037–1043, 1997.—Gender differences in anxiety have long been assumed to exist, but the experimental evidence is contradictory. It has also been suggested that antianxiety agents may have gender-dependent behavioral effects. The present experiment was designed to establish whether or not intact male and female rats behave differently when exposed to a Differential-Reinforcement of Low-Rate 72-s schedule of reinforcement (assumed to assess some of the inhibitory behavioral tendencies associated with anxiety), and whether or not the behavioral effects of acute chlordiazepoxide administration would differ between the sexes. There were no differences between male and female rats in the total number of responses, the total number of obtained reinforcers, or response efficiency in the absence of drug administration. Male and female Wistar rats were then challenged with different doses of chlordiazepoxide (vehicle, 1, 3, 10, 17, and 30 mg/kg). Low doses of chlordiazepoxide (1 and 3 mg/kg) decreased response efficiency, and medium doses (10 and 17 mg/kg) increased response efficiency in male and female rats. The highest dose of CDP (30 mg/kg) further increased response efficiency in male rats, but decreased response efficiency in female rats. These results suggest that the behavioral effects of chlordiazepoxide are dose dependent and that the effects of a large dose of chlordiazepoxide differ between male and female rats. Whether or not gender differences in drug metabolism or whether schedule contingencies played an important role in these observations remains to be determined in future experiments. © 1997 Elsevier Science Inc.

Differential-Reinforcement of Low-Rate schedule Chlordiazepoxide Number of responses Number of obtained reinforcers Response efficiency Lever press Male and female rats

BENZODIAZEPINE agonists, such as chlordiazepoxide (CDP) and diazepam (DZ), are widely prescribed drugs known for their anxiolytic, anticonvulsant, sedative/hypnotic, and muscle relaxant properties (9). Some 10% of the population in the United States and Europe uses tranquilizers and hypnotics (1). Elderly women are most likely to use benzodiazepines for a prolonged period of time (17).

Gender differences in anxiety were initially postulated on the basis of observations in open-field tests in which male rats tend to ambulate less and defecate more than female rats (2,7). In other experiments it has been shown that punished licking rates of female hooded Lister rats were lower than those of male rats in the Vogel conflict task, that female rats spent more time than male rats in the open arms of an elevated plus-maze, and that female rats were less likely than male rats to engage in social interactions during familiarization to the apparatus (12,32). These observations were interpreted to show that female rats were less anxious than male rats in the elevated plus-maze, but more anxious than male rats in the Vogel conflict test, while the data from the social interaction test were considered ambiguous. Blanchard et al. (3,4) have recently presented evidence to support the contention that female rats are more anxious than male rats in response to the potential dangers (cat and cat odor) presented in an anxiety/defense test battery. In summary, the evidence to support the existence of gender differences in experimental paradigms used to study anxiety is inconclusive at best, indicating the need for further experimentation.

The Differential-Reinforcement of Low-Rate (DRL) schedule generates low response rates because reinforcement presentation only occurs when responses are separated by a minimum time interval (e.g., DRL 15 s or DRL 72 s). Low to intermediate doses of CDP increase the total number of responses and decrease the number of earned reinforcers when male rats respond on a DRL 15-s schedule. High doses generally eliminate responding altogether. Low to intermediate doses of CDP also increase the propensity for burst responding and disrupt the interresponse time (IRT) distribution (18,22,23). It has also been reported, however, that CDP (or DZ) administration does not increase response rates or de-

Requests for reprints should be addressed to Frans van Haaren, Department of Psychology, University of Florida, Gainesville, FL 32611-2250.

crease reinforcement frequency when subjects respond on a DRL 72-s schedule of reinforcement [(19,30), but see (11)] for a report in which CDP decreased response rates and increased reinforcement frequency on that schedule of reinforcement). Nonbenzodiazepine anxiolytics such as buspirone and gepirone increase reinforcement rate, but disperse the IRT distribution on DRL 72-s schedules of reinforcement without an increase in burst responding (19,24). It has been suggested that benzodiazepine agonists reduce the behavioral inhibition associated with signals for nonreward inherent to DRL schedules of reinforcement in view of their response rate-increasing and reinforcement-frequency decreasing behavioral effects (11,27). The present experiment was designed to further evaluate the behavioral effects of CDP administration on behavior maintained by a DRL 72-s schedule of reinforcement and to establish whether or not CDP's behavioral effects would be gender dependent.

METHOD

Subjects

Six male and six female Wistar rats were obtained from a commercial breeder when they were approximately 60 days old. They were housed in same-sex groups of three under a re-

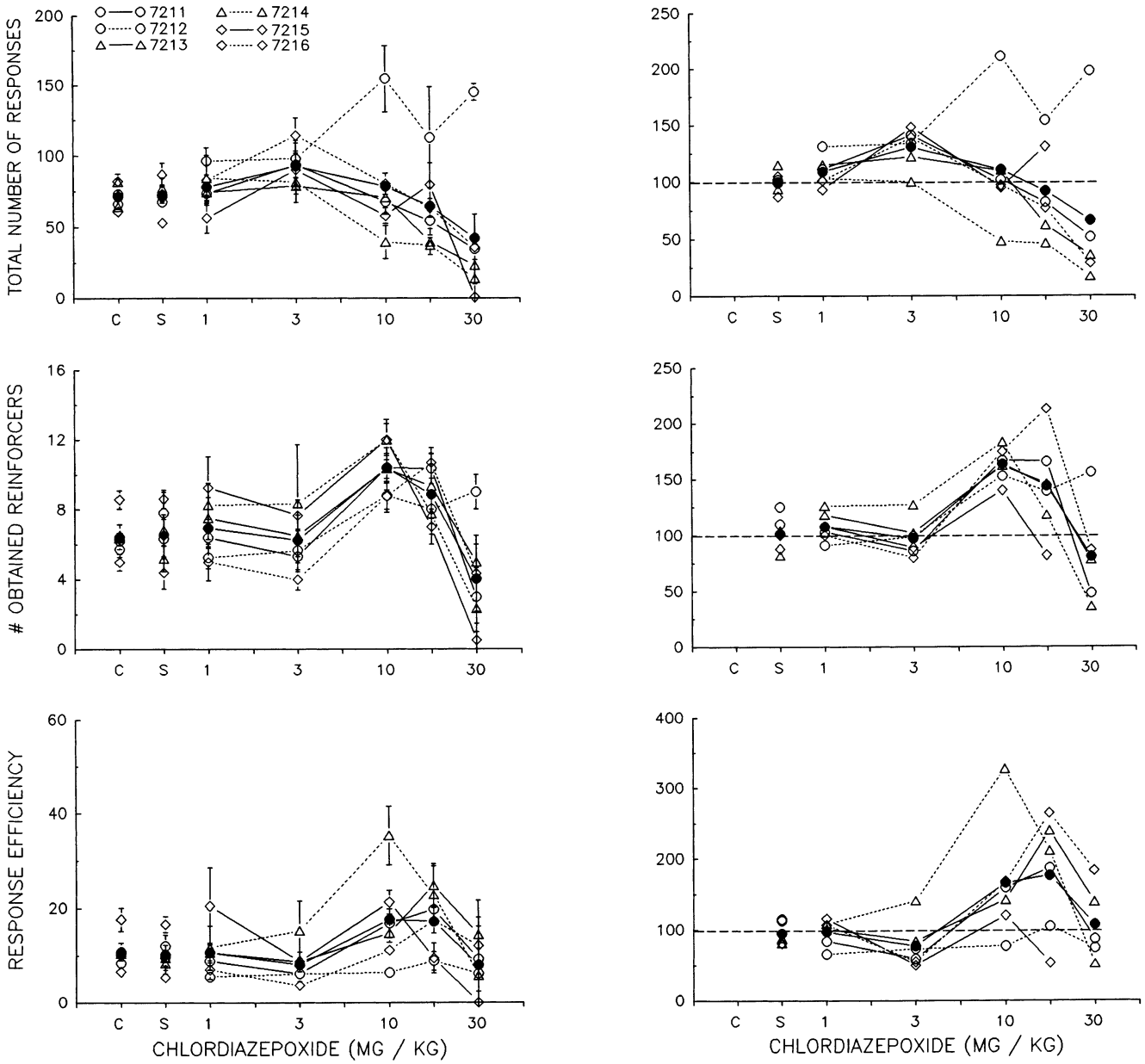

FIG. 1. The total number of responses (top left-hand panel), the number of obtained reinforcers (middle left-hand panel), and response efficiency (bottom left-hand panel) during control sessions (C), after vehicle administration (S) and following the different doses of chlordiazepoxide for individual female rats responding on a DRL 72-s schedule of reinforcement. The right-hand panels show the same data expressed as a percentage of control values. Filled symbols represent the average of the individual dose–effect curves.

versed 12 L:12 D cycle (lights on 0700 h) and constant temperature and humidity conditions. All subjects had limited access to food resulting in approximately 22 h of food deprivation before each experimental session (10), but tap water was always available in the home cage.

Apparatus

Experiments were conducted in six identical, two-lever, Coulbourn Instruments modular rodent operant-conditioning chambers. Only the right lever was active and each press on this lever resulted in feedback from a clicker. A houselight was located 2 cm from the ceiling in the middle of the intelligence panel. The pellet trough was in between the two levers and could be illuminated by a white light bulb. Each experimental chamber was enclosed in an individual sound-attenuating, ventilated cabinet. The chambers were connected to a PDP 11-23 microcomputer (Digital Equipment Corporation) located in the experimental room itself. The experimental contingencies and data acquisition procedures were programmed in SKED-11 (26).

Procedure

All subjects were trained to press the right lever using an automated training procedure [for details, (28)]. Once sub-

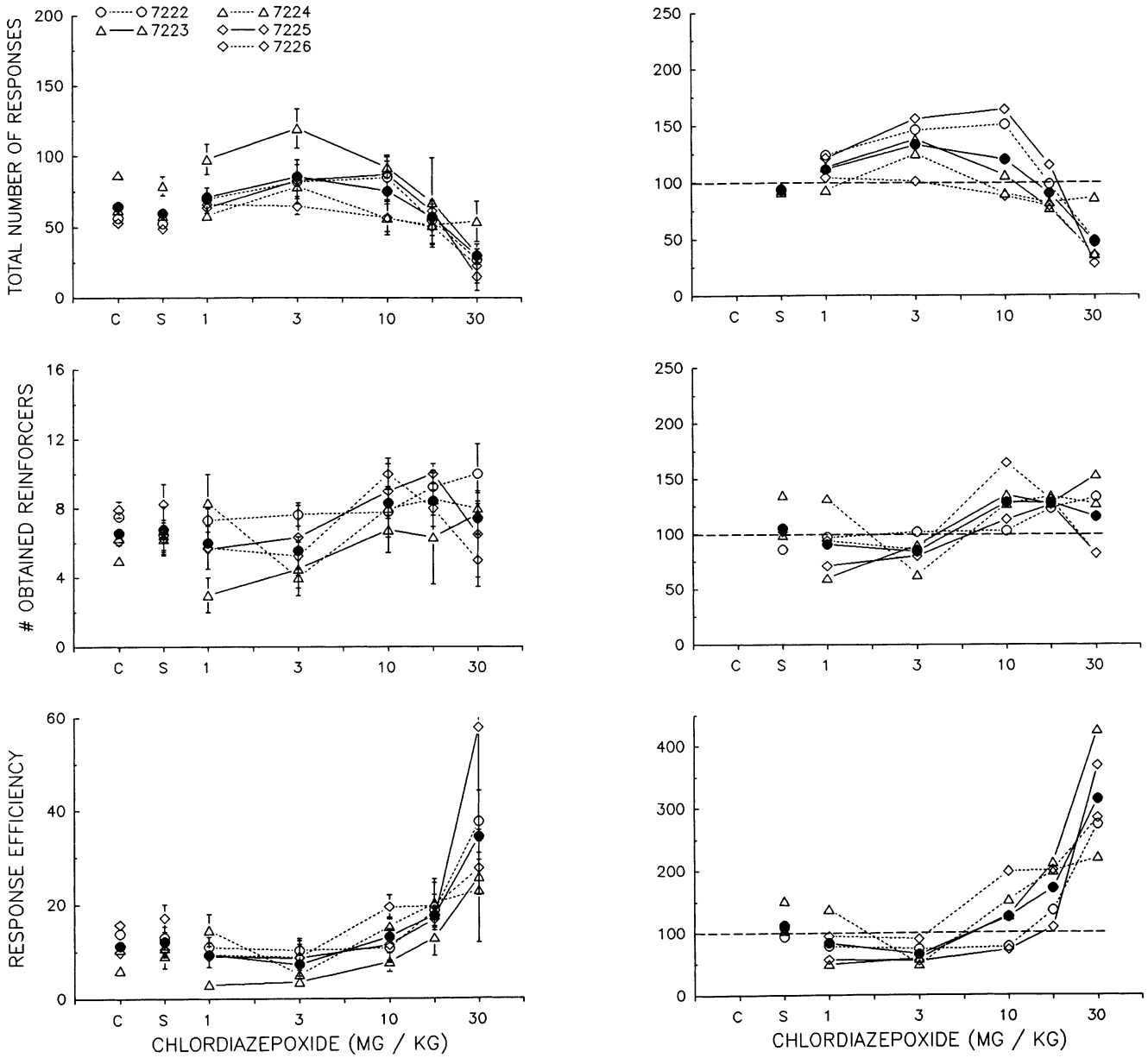

FIG. 2. The total number of responses (top left-hand panel), the number of obtained reinforcers (middle left-hand panel), and response efficiency (bottom left-hand panel) during control sessions (C), after vehicle administration (S) and following the different doses of chlordiazepoxide for individual male rats responding on a DRL 72-s schedule of reinforcement. The right-hand panels show the same data expressed as a percentage of control values. Filled symbols represent the average of the individual dose–effect curves.

jects reliably pressed the lever they were immediately exposed to the DRL 72-s procedure, during which the houselight and the stimulus lights above the right lever were illuminated at the start of the session. A 45-mg BioServe food pellet was presented when consecutive lever presses occured at least 72 s apart. The pellet tray was illuminated for 2 s during pellet presentation. The session duration was 60 min. Subjects participated in multiple, nightly training sessions to accelerate the acquisition of steady-state DRL behavior. They were exposed to five sessions per night during 6 consecutive weeks. Each session was separated from the next by 30 min, during which the subjects remained in the darkened chamber and lever presses did not have any scheduled consequences. Thereafter, experimental sessions were conducted once a day, Monday through Friday. After baseline response rates had stabilized (as evidenced by visual inspection of day-to-day data plots) subjects were intraperitoneally (IP) injected with different doses of CDP (vehicle, 1, 3, 10, 17, and 30 mg/kg), 10 min prior to the start of an experimental session. CDP was dissolved in an isotonic sodium-chloride solution, which was stored for a maximum of 7 days at 5°C. The solution was allowed to attain room temperature prior to injection. All doses were tested at least twice in irregular order on Tuesdays and Fridays of each week. Doses were tested more often when the behavioral effects of the initial two determinations were inconsistent. This occured mostly when subjects had been injected with the intermediate doses of CDP (3, 10, and 17 mg/kg). The mean of all determinations was taken to represent the behavioral effect of a particular dose of CDP for further analysis.

RESULTS

The left-hand panels of Figs. 1 and 2 show the total number of responses, the number of obtained reinforcers, and the response efficiency of six female (Fig. 1) and five male rats (Fig. 2, one of the male rats expired before the dose–effect curve was completed). Response efficiency was calculated as the number of obtained reinforcers divided by the total number of responses observed during the session. The right-hand panels of these figures show the same data expressed as a percentage of control values. The filled symbols in each graph represent the average of the individual data plots. The total number of responses emitted during the session, the number of obtained reinforcers, and response efficiency were averaged across all sessions, which immediately preceded those during which CDP or vehicle was administered to assess schedule control in the absence of drug administration. There were no

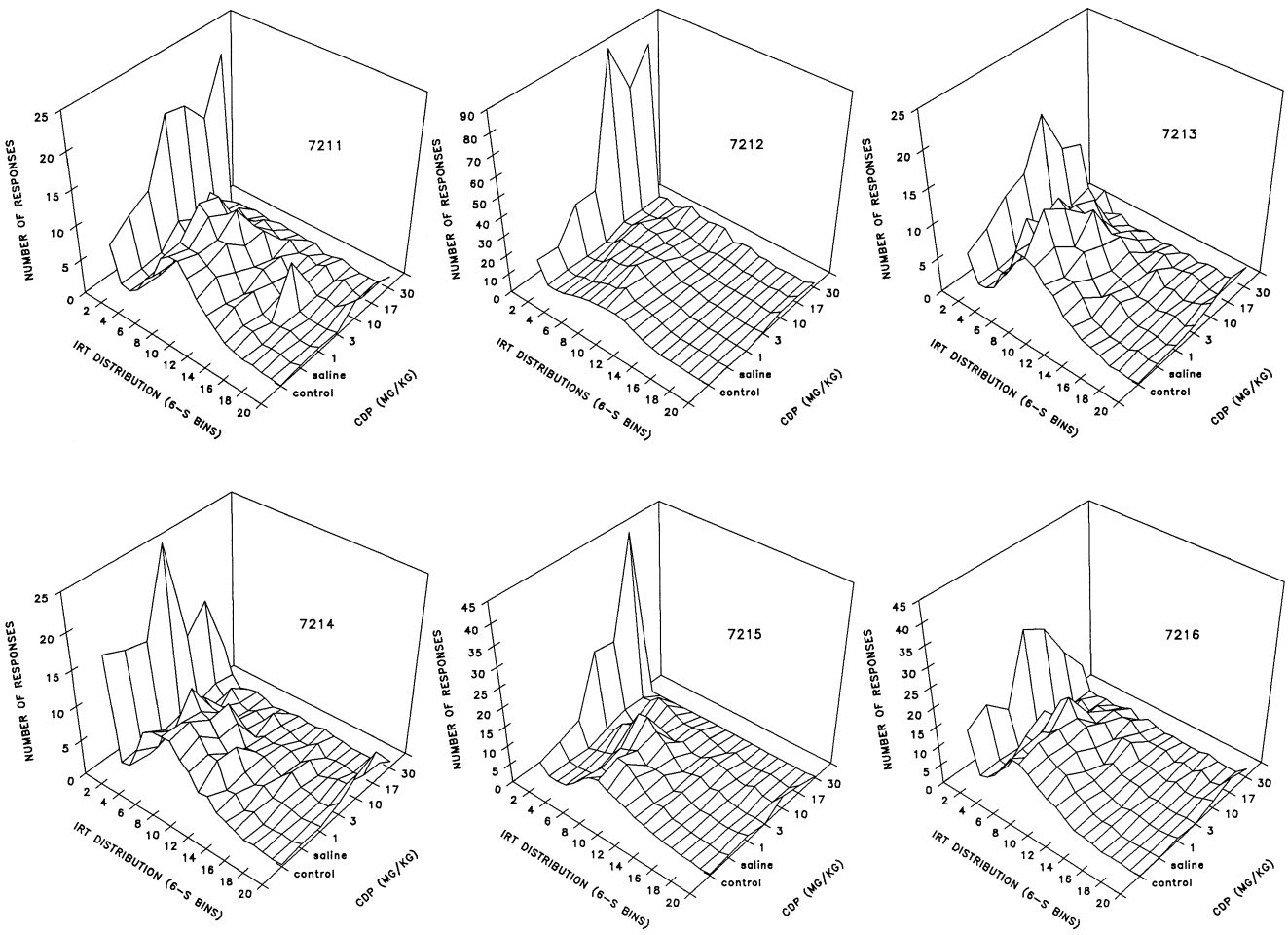

FIG. 3. Interresponse time distributions for individual female rats. The total number of responses (y-axis) in 6-s bins (x-axis) as observed during control sessions (C), following vehicle administration (S) and as a function of the different doses of chlordiazepoxide (z-axis).

differences between female and male Wistar rats in the total number of responses emitted during the session, $t(9) = 1.014$, NS, the number of obtained reinforcers, $t(9) = -0.233$, NS, or response efficiency, $t(9) = -0.202$, NS.

The lowest dose of CDP (1 mg/kg) slightly increased the total number of responses in four of six female rats and four of five male rats, while 3 mg/kg CDP increased the total number of responses in all but one female (7214) and one male (7226) rat. The tendency of 3 mg/kg CDP to increase the total number of responses is reflected in a decrease in the number of obtained food pellets, which also translates into a lowered response efficiency for five of six female rats and five of five male rats. The behavioral effects of 10 mg/kg CDP were mixed because of individual differences, but the total number of responses was less than observed after 3 mg/kg CDP with few exceptions. The total number of responses decreased after 17 mg/kg CDP, and the decrease was most pronounced after 30 mg/kg CDP with only one exception (7212). Medium doses of CDP (10 and 17 mg/kg) increased response efficiency in male and female rats. The highest dose of CDP (30 mg/kg) further increased response efficiency in male rats, but decreased response efficiency in female rats. Response efficiency measures were analyzed with an analysis of variance involving the factors gender and dose of CDP. CDP significantly changed response efficiency in male and female rats [dose: $F(6, 48) = 10.44$, $p < 0.001$]. A significant gender by dose interaction, $F(6, 48) = 8.69$, $p < 0.01$, confirmed the observations described in more detail above. Duncan's Multiple Range Test indicated a significant difference in response efficiency between male and female rats following the administration of 30 mg/kg CDP (MSE = 108.58, $df = 8$, $p < 0.05$).

Figures 3 and 4 show the interresponse time (IRT) distributions for individual subjects as a function of CDP administration. The total number of responses (y-axis) in 6-s bins of the IRT distribution (x-axis) is shown during control sessions (C), as a function of the administration of physiological saline (S) and following the different doses of CDP (z-axis). The analysis of IRT distributions during control sessions revealed two distinct modes, one during the shortest IRT bin, and one that most often occurred at about half the duration of the reinforced IRT. These bimodal IRT distributions changed very little following vehicle administration or after the administration of 1 mg/kg CDP in female and male Wistar rats. When male and female rats were injected with 3 mg/kg CDP or more, the number of responses in the shortest IRT bin (between 0 and 6 s) increased consistently. This increase was accompanied by a decrease in the number of responses shorter than the reinforced IRT and a small increase in the number of responses

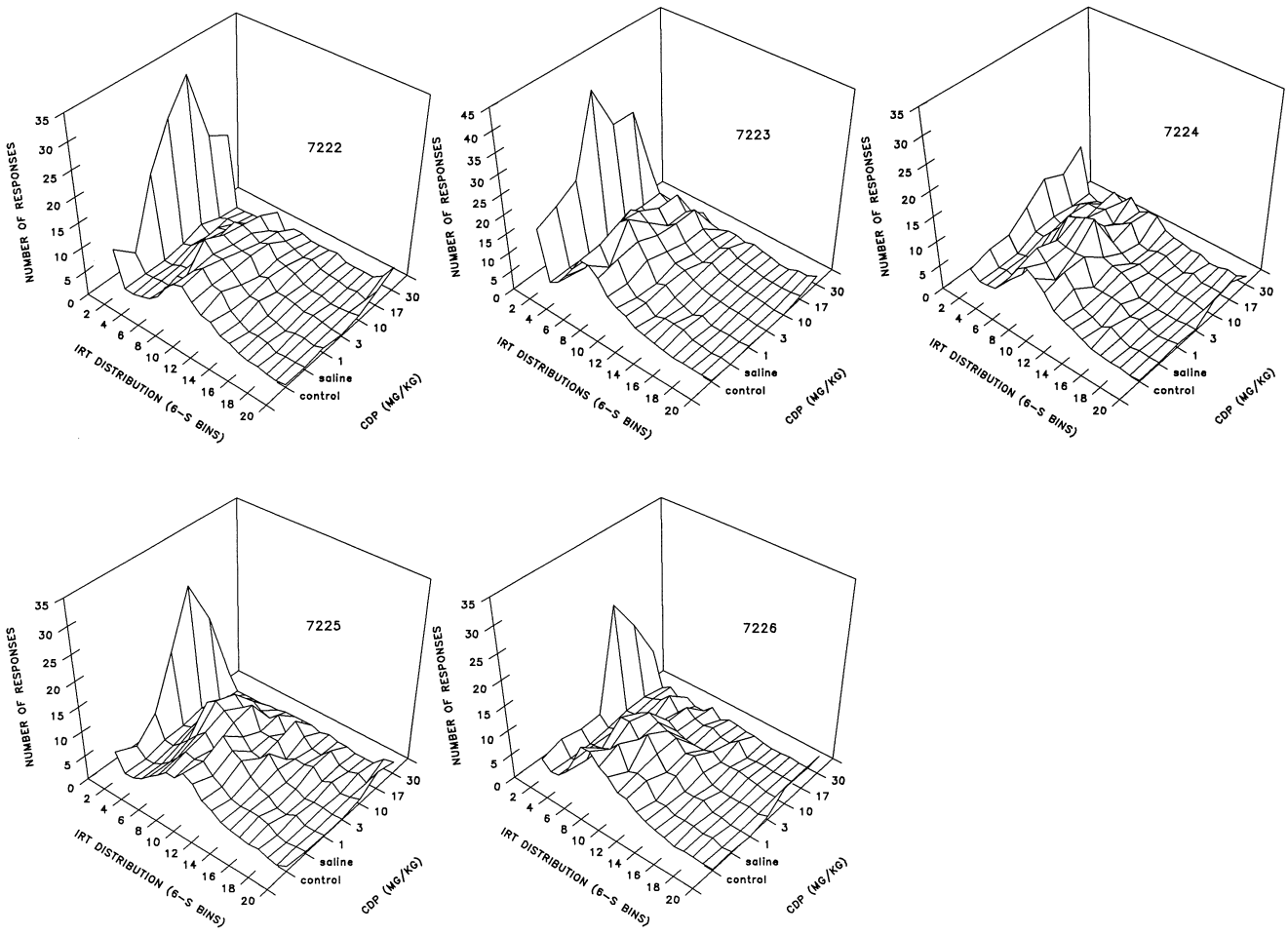

FIG. 4. Interresponse time distributions for individual male rats. The total number of responses (y-axis) in 6-s bins (x-axis) as observed during control sessions (C), following vehicle administration (S) and as a function of the different doses of chlordiazepoxide (z-axis).

longer than the reinforced IRT, mostly in the final bin of the distribution (120 s or more), especially in male subjects.

DISCUSSION

There were no differences in DRL schedule performance between male and female rats in the absence of CDP administration, contrary to what has previously been reported when male and female rats were exposed to DRL schedules of different durations (DRL 15 s, 30 s) (29,31). This suggests that gender differences in anxiety may only be observed under experimental conditions that have not yet been fully identified, but that may be different from those generally assumed to be indicative of such subjective experience. Interestingly, baseline IRT distributions did not peak around the expected 72-s bin, but observed peaks ranged from the 24-s to the 54-s bin. The absence of a peak at the reinforced IRT may have been a function of the massed training sessions with immediate exposure to the DRL 72-s schedule. The present experiment showed that low doses of CDP (1 and 3 mg/kg) generally increased the number of responses and decreased the number of obtained reinforcers in both male and female rats. Higher doses of CDP (10 and 17 mg/kg) decreased the number of responses and increased the number of obtained reinforcers, while the highest dose of CDP (30 mg/kg) further decreased the number of responses for both males and females, while the number of obtained reinforcers decreased for female rats and was not affected for male rats. These observations imply that 1 and 3 mg/kg CDP decreased response efficiency in most male and female rats and that 10 and 17 mg/kg CDP increased response efficiency in male and female rats. The highest dose of CDP (30 mg/kg) further increased response efficiency in male rats, but decreased response efficiency in female rats. As had been previously reported, CDP administration facilitated burst responding and disrupted the IRT distribution in male and female rats (20). These results show that the behavioral effects of CDP were dose dependent and that the contradictory results observed in previous experiments may well have been a function of drug dose and schedule parameters (11,18–20,22–24). The present results also show that the behavioral effects of large doses of CDP differed between male and female rats. Whether or not gender differences in drug metabolism played a role in these observations remains to be determined (25), but such seems unlikely in view of the fact that gender differences were not observed following administration of the smaller doses of CDP. It could also be argued, of course, that the present experimental procedure may not have been sensitive enough to detect such differences.

Gonadal steroid hormones or their metabolites affect the $GABA_A$/benzodiazepine/chloride ionophore receptor complex through which benzodiazepine agonists exert their behavioral effects (16). Gender differences have been demonstrated in $GABA_A$-receptor binding in the preoptic area and mediobasal hypothalamus of male and female Sprague–Dawley derived rats (13). Others have shown that $GABA_A$ receptor levels do not vary across the estrus cycle in rats, but that they are increased in late gestational periods and immediately postpartum (8,15). GABAergic neurotransmission is altered by estrogen and progesterone treatment, as these hormones have been reported to affect levels of binding sites within the GABA receptor complex (5,14). It seems likely that gender differences in the effects of CDP on GABAergic neurotransmission underly the behavioral differences observed in the present experiment.

Gender differences in the behavioral and physiological effects of benzodiazepines have also been observed in humans. Ellinwood et al. (6) studied the effects of oral contraceptives on DZ-induced psychomotor impairment. Young men, and women taking oral contraceptives, received three different doses of DZ (0.07, 0.14, and 0.28 mg/kg). The women took the drug on day 10 and day 28 of their cycle. Performance was significantly impaired after the highest dose. Peak impairment occured after 20 min for men and for women on day 28 of their cycle, but on day 10 women reached peak impairment after 60 min. Other studies have shown that women who take oral contraceptives have higher volumes of CDP distribution than men, or women who do not take contraceptives (21). Taken together, animal and human data suggest that the behavioral effects of benzodiazepine agonists may be gender dependent. To what extent the differences are affected by behavioral, pharmacological, and/or neurochemical variables should be subject of further study.

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