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Effects of Chlordiazepoxide on Food Anticipation, Drinking and Other Behaviors in Food-Deprived and Satiated Rats

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NIETO, J. AND A. POSADAS-ANDREWS. *Effects of chlordiazepoxide on food anticipation, drinking and other behaviors in food-deprived and satiated rats.* PHARMACOL BIOCHEM BEHAV 20(1) 39–44, 1984.—Two groups of rats, Deprived and Satiated, were presented with food according to a fixed time 60-sec schedule. They were then injected with saline, 5, 10, and 15 mg/kg of chlordiazepoxide hydrochloride according to a Latin square design. During saline administration time spent visiting the food tray, time spent drinking, number of tray entries and the amount of water ingested were always greater in the Deprived than in the Satiated group; whereas the opposite was true for grooming. As chlordiazepoxide dose increased time spent visiting the food tray increased in both groups, but the effect was bigger in the Satiated than in the Deprived group. Drinking was not affected by the drug. Grooming and sniffing-rearing were reduced as the dose increased.

Chlordiazepoxide Food anticipation Drinking Grooming Deprivation level Fixed-time schedule Rat

WHEN rats are presented periodically with small amounts of food irrespective of their behavior, as in fixed time (FT) schedules, they develop a pattern of anticipatory visits to the food tray; that is, rats are more likely to orient towards and approach the tray as time to food presentation decreases (e.g., [26]). In addition, the rats develop the habit of drinking, grooming, rearing, etc., at times when anticipatory visits to the tray are occuring at low strength, i.e., soon after food delivery (e.g., [9,27]).

There have been few studies assessing the effects of drugs on behavioral patterns engendered by FT schedules despite the fact that they offer advantages over other procedures: changes of response rate and distribution do not alter the rate and pattern of food delivery, and these schedules encourage the analysis of interactions between behaviors occurring in the situation. Nieto, Makhlouf and Rodriguez [16] using a FT schedule reported that d-amphetamine facilitated anticipatory visits to the tray while concurrently suppressed activities that were incompatible with visiting the tray. Thus, d-amphetamine appears to increase responding, but within a progressively reducing diversity of responses (see also [19,20]). The purpose of the present experiment was to extend such an analysis to the effects of a benzodiazepine, chlordiazepoxide hydrochloride (CDP), on behavioral patterns engendered by a FT schedule.

Several studies have analyzed the effects of benzodiazepines on behavioral performance under fixed interval (FI) schedules, where food also occurs at regular intervals but depends on the emission of a particular response. Typically, CDP administration has been found to increase overall rate of lever pressing and key pecking over a range of doses, and to alter temporal pattern of behavior by increasing response rate during the early portions of the interval (see [19] and [25] for reviews). Thus, the effects of CDP on schedule-controlled responding are often described as dependent on rate of occurrence of target response (e.g., [3]). Other studies have investigated the effects of CDP on drinking induced by FI and FT schedules (see [24] for a review). Most of those studies have reported increments in the amount of water ingested (e.g., [2,23]), although some have failed to observe such an effect (e.g., [13]; see [15] for a review). However, several detailed questions about the effects of CDP are not well documented.

First, are the effects of CDP on food anticipation in FT schedules similar to those observed on lever pressing in FI schedules? Food anticipation is known to be affected by factors such as the interpellet interval [21], deprivation [22], and d-amphetamine [16] in a way similar to lever pressing.

Second, are the increments of food anticipation and drinking matched by corresponding reductions in other behaviors such as general activity and grooming? Grooming is known to be reduced by increasing deprivation levels [22], and benzodiazepines are assumed to elicit ingestion through mechanisms that mimick hunger (see [5,6] for reviews). Alternatively, CDP may only disinhibit all activities occurring in

such a situation, thus grooming and general activity could also be enhanced.

Third, how do deprivation levels and CDP interact? Posadas-Andrews, Burton and Cooper (unpublished data) found that CDP and deprivation interact additively, but that facilitation is greater in satiated than in deprived rats.

The present study attempted to answer these questions by exposing groups of satiated and deprived rats to periodic food presentations, and by recording their behavioral patterns.

METHOD

Subjects

Eight male albino rats weighing 227 g in average, range 215 to 240 g, were housed individually with water continuously available.

Apparatus

Four identical chambers manufactured by Campden Instruments Ltd. and measuring 23×25×20 cm were used. All walls in the chamber were aluminium except for the clear Plexiglas entrance door and the translucent ceiling. A recessed food tray (5.3×6.0×3.5 cm) was mounted on the front wall at floor level. In order to obtain food from the tray the rats had to push open a hinged Plexiglas flap (5.3×6.0 cm) connected to a microswitch. Food pellets (Campden Instruments Ltd., 45 mg, complete diet) were delivered to the tray by an automatic dispenser. Every pellet was signaled by briefly turning on a light located behind the flap. A stainless-steel ball-valve drinking spout connected to a calibrated reservoir was mounted 8 cm to the left of the tray, 4 cm above the floor. Contact with the drinking spout could be recorded by a contact sensor. Mounted above the translucent ceiling was 40 W 240 VAC houselight. The floor of the chamber consisted of stainless steel rods.

Each chamber was enclosed in a sound attenuating shell provided with an exhaust fan and a one-way observation window. The controling and recording equipment was located in an adjacent room.

Procedure

Weight determination and pretraining. For ten days rats were allowed continuous access to food and water in their home cages. Their ad lib weight was the median weight over the last five days. The rats were then reduced to 80% of their ad lib weights by feeding them limited amounts of chow. The weights were held at this level for a further 15 days when the rats were magazine trained, and then exposed daily to a fixed-time 60-sec (FT 6) schedule of food delivery. That is, a single pellet was delivered every 60 sec irrespective of the rat's behavior. Throughout the experiment sessions lasted 50 min.

The rats were then allocated to two groups. Four rats (Satiated group) were randomly selected, fed until their initial weights were recuperated and kept at their ad lib weights for the remainder of the experiment. The rats in the Deprived group were kept at 80% of their ad lib weights throughout the experiment. Both groups were tested daily under the FT 60-sec schedule for a further 15 sessions prior to any pharmacological manipulation.

Drug Administration

Chlordiazepoxide hydrochloride (Roche) was administered in doses of 5, 10, and 15 mg/kg. Each dose was dissolved in 0.9% saline solution to provide an injection volume of 1 ml/kg and was injected intraperitoneally 35 min before the start of the session. CDP doses and saline were administered to both groups according to a Latin square design and at least four days were allowed to elapse between each injection.

Recording of Behavior

Time for which tray flap was open, number of tray flap openings, time spent in contact with water spout, and the amount of water ingested were recorded every session. In addition the rats' behavior was videotaped during each CDP dose; the behavior of each rat was then recorded by an observer using a manually operated keyboard connected to a multichannel event recorder. The observer did not know the rat's identity or the dose administered until observations were complete.

The following categories of behavior were recorded: (a) *Tray orientation* defined as pushing the tray flap, or any movement ostensibly directed towards the tray in the absence of the pellet; (b) Drinking defined as contact with any part of the spout or movements directed towards the spout; (c) *Grooming* defined as rubbing body with forepaws, or scratching and licking any part of the body; (d) *Rearing-sniffing* defined as lifting forelegs or moving about in the chamber in an undirected manner; and (e) *Chewing* defined as jaw movements or mouthing. This last category was included since preliminary evidence indicated that a dose of 15 mg/kg of CDP can induce chewing even in the absence of food.

RESULTS

Behavior of the two groups over the last three preinjection sessions was typical of periodic food deliveries: In both groups rats always went to the tray during pellet delivery; they then engaged in some activities such as drinking, rearing-sniffing, and grooming; finally, as time since pellet delivery increased rats were more likely to approach the tray. However, the total time spent in each of the activities monitored automatically differed between groups in these sessions. Comparing the Deprived group with the Satiated group, time spent entering the tray (308 and 128 sec, respectively), time spent drinking (655 and 309 sec, respectively), number of tray entries (297 and 97, respectively), and the amount drunk (27 and 11 ml, respectively) were always greater in the Deprived group; the smallest Student's t test value was 2.54, $p<0.02$.

Effects on Overall Measures

The effects of CDP on the total time opening the tray and drinking, the number of tray entries and the amount drunk were weak. Analysis of Variance with Groups (Deprived and Satiated) and Doses (Saline, 5, 10 and 15 mg/kg) as factors for each of those measures revealed significant Groups effects on time pushing the tray flap open, $F(1,24)=7.07$, $p<0.01$; number of tray entries, $F(1,24)=10.61$, $p<0.001$; time drinking, $F(1,24)=6.34$, $p<0.02$; and amount of water drunk, $F(1,24)=32.19$, $p<0.001$. On the other hand, the Dose effect was never significant; $Fs(3,24)=1.49$, 1.09, 1.12 and 1.79 respectively, $p>0.05$; nor were the Groups × Doses interaction significant, all $Fs(3,21)<1$.

Figure 1 shows results from observation data. The histo-

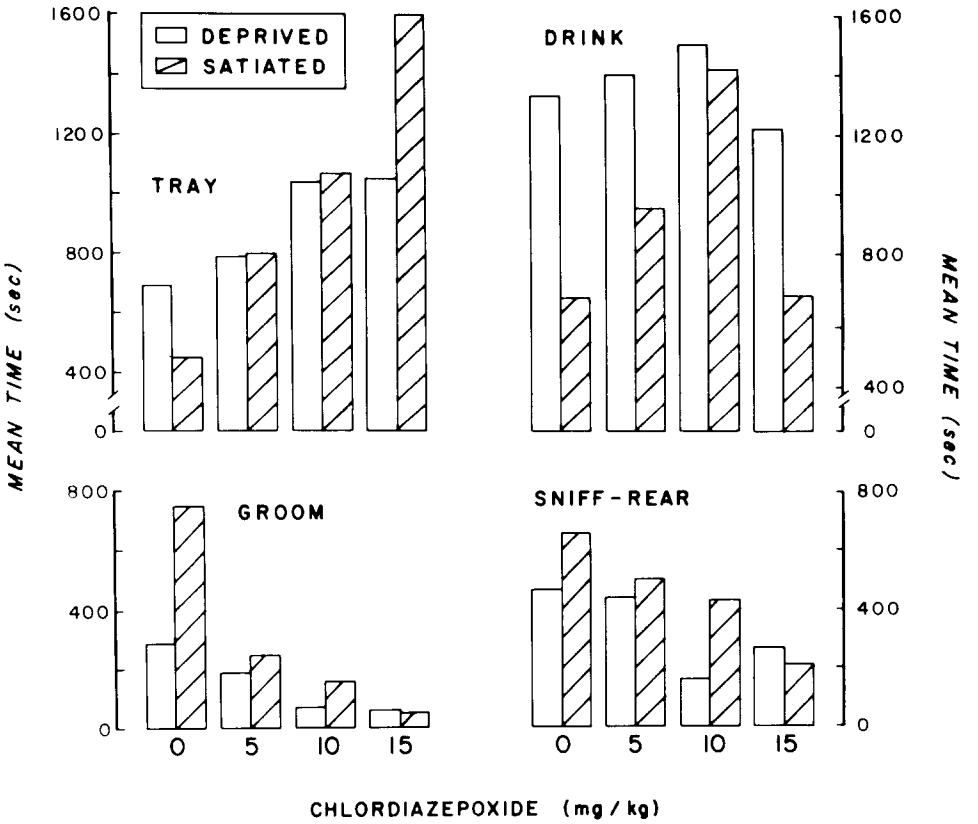

FIG. 1. Mean total time engaged in different activities for each group under the effects of saline and of three doses of CDP.

grams show mean total time spent in each activity under each dose of CDP for the two groups. As seen in Fig. 1, tray orientation increased as a function of the dose in both groups, grooming and sniffing-rearing were reduced, whereas drinking appeared to increase in the Satiated group.

Analysis of each activity with Groups and Doses as factors indicated that the groups did not differ significantly in the amount of tray orientation, $F(1,24)<1$; but that tray orientation increased as the dose increased, $F(3,24)=5.33$, $p<0.005$. On the other hand, groups differed significantly in time spent drinking, $F(1,24)=4.84$, $p<0.03$, although time drinking was not significantly altered by CDP administration, $F(3,24)=1.12$, $p>0.05$.

The groups also differed significantly in time spent grooming, $F(1,24)=3.98$, $p<0.05$; grooming was decreased by increasing doses of CDP, $F(3,24)=7.15$, $p<0.001$.

Sniffing-rearing did not differ significantly between groups, $F(1,24)=2.03$, $p>0.05$, but this behavior was also reduced by the drug, $F(3,24)=4.56$, $p<0.05$. In none of the above analyses was the interaction term significant, all $Fs(3,24)<1$. Finally, time spent gnawing (not shown) seemed to be facilitated at high doses of CDP in the Satiated group, but neither a significant Groups effect nor a Doses effect was detected, $F(1,24)<1$, and $F(3,24)=1.27$ respectively, $p>0.05$.

Effects on Patterns of Behavior

Figure 2 shows the effects of saline and each CDP dose on distributions of each activity throughout the 60-sec interpellet interval in each group. The figure shows that under Saline administration tray orientation increased as time to pellet delivery decreased; drinking occurred soon after pellet ingestion; and sniffing-rearing and grooming occurred late in the interval but before tray orientation appeared with strength. It is clear that tray orientation and drinking occurred with more vigour in group Deprived than in group Satiated, but the opposite was true for grooming.

Increasing doses of CDP altered the pattern of tray orientation by increasing the amount of time spent by the tray early in the interval in both groups. However, the groups were affected to different extents: in group Satiated tray orientation started to increase earlier in the interval and remained at asymptotic levels with low doses. At high doses rats in group Satiated spent most of their time facing the tray entrance. The alterations of patterns of tray orientation were associated with reductions in the spread and frequency of grooming and sniff-rearing in both groups. CDP administration did not cause marked changes in the peak and spread of the patterns of drinking and gnawing.

In order to determine the quantitative properties of the relation between control levels and drug effects on tray orientation shown in Fig. 2, the log of the effects of each dose of CDP on tray orientation in successive 6-sec bins of the interpellet interval were plotted as a function of the log control tray orientation of the corresponding bins (i.e., log(drug/control) $\times 100$). As this analysis would be complicated by the inclusion of the relatively high tray orientation

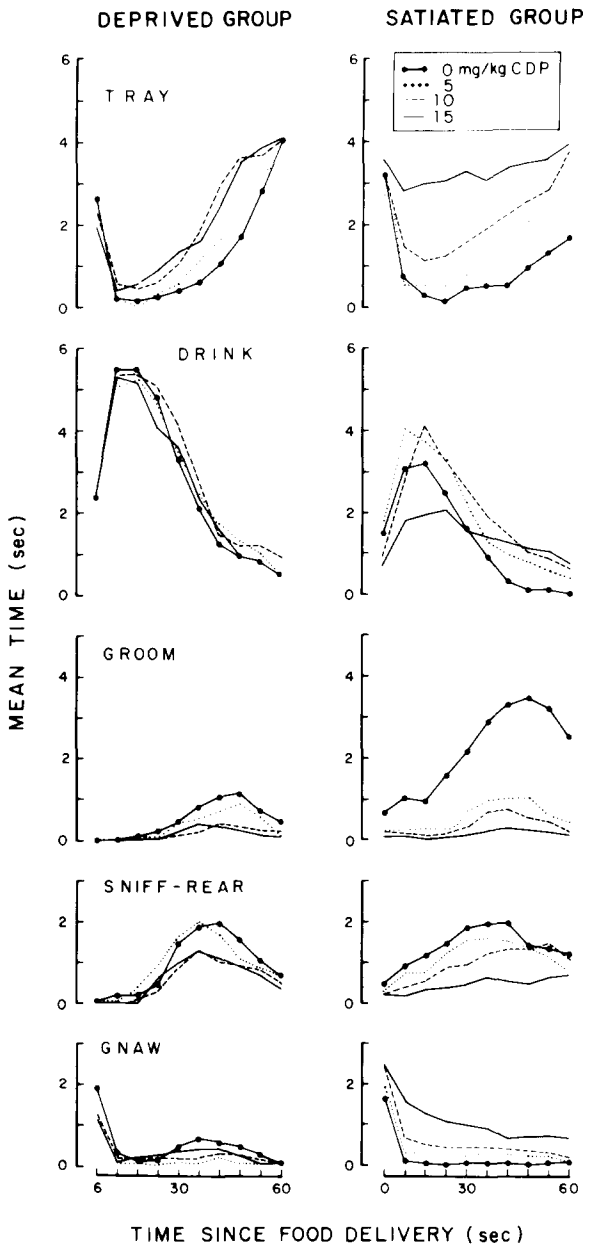

FIG. 2. Mean temporal distributions of different activities within the interpellet interval for each group under the effects of saline and of three doses of CDP. Each activity is plotted at successive 6-sec intervals from pellet delivery.

early in the interval, the data from the first bin were excluded from the analysis. The equations describing the best-fitting regression lines fitted by the method of the least squares are shown for each subject in Table 1. It is clear that for the group Deprived the effects of CDP were weakly linearly and inversely related to control levels as seen by the slopes of the fitted lines. On the other hand, it is clear that the slopes of the lines fitted to the data of group Satiated were always negative indicating that CDP effects were inversely related to control levels, and that the slopes of the lines increased to values close to, but never greater than, −1 as the dose increased. A further difference was that the equations accounted fairly well for the data of the group Satiated but this was not the case for the group Deprived.

DISCUSSION

The present data demonstrate that CDP does not act by disinhibiting all activities occurring between pellet deliveries. Instead, tray activities were increased, drinking was relatively unaffected, while grooming and sniff-rearing were reduced. The effects of CDP on tray activities resemble the effects reported on lever pressing and keypecking in FI schedules (e.g., [1, 3, 12]). That is CDP increased the total time visiting the tray in a dose-related manner, and this effect can be attributed to a marked facilitation of tray orientation during the early portions of the interval when it normally occurred at low levels. Consequently, the response-reinforcer contingency required by FI schedules and the nature of the anticipatory response do not appear to be important determinants of CDP effects on behavior maintained by periodic schedules.

Although CDP increased tray orientation in both groups, it was clear that they were greater in the group Satiated than in the group Deprived. This effect is consistent with the results of a study by Posadas-Andrews *et al.* (unpublished), who found that CDP increased eating in ad lib and 24-hr food deprived rats. The causes of this additive effect are unclear at the present; it may be due to differences in the rates of absorption and excretion of the drug by chronically deprived and satiated rats (see [10]), or as discussed below, it may be determined by the differences in control levels of responding. ing.

The fact that the effects on tray orientation were greatest at times when control levels were lowest, and that they were greatest in the group Satiated which showed lower levels of tray orientation than the group Deprived suggests that the present data may be accounted for in terms of the rate-dependent effects of drugs [8]. Indeed, since the regression analysis for the data of the group Satiated showed the negative slopes typical of the rate dependent effects of amphetamines [11, 13, 24]. However, it is also clear that the rate-dependent analysis cannot account for the entire pattern of results. First, the lines fitted to the data of the group Deprived did not show a consistent inverse relationship that would be expected from the rate-dependent analysis. Second, the drug did not reduce drinking which is a high rate activity [4], nor did it increase drinking (and the other activities) late in the interval when they occurred with low probability. Finally, the drug did not increase grooming and sniff-rearing which occurred at low levels. The case of grooming is particularly interesting since the groups differed in the overall levels of this activity, but CDP reduced it in both groups. Thus, although the effects of CDP on tray activities resemble the rate-dependent effects of amphetamines, such an analysis fails to predict why other activities such as those measured here are not altered in a rate-dependent fashion (see [19] for a discussion of other exceptions).

One interpretation of the present data is that since benzodiazepines increase food and water ingestion [7, 17, 18, 20] and this effect has been attributed to a direct effect on systems controlling hunger and thirst [5,6], appetitive responses such as orienting and approaching the food and water sites would be expected to increase after CDP administration, while responses incompatible with them would be expected

TABLE 1
BEST FITTING REGRESSION LINES

	5 mg/kg			10 mg/kg			15 mg/kg		
	A	B	r	A	B	r	A	B	r
Group Deprived									
Rat 2	1.76X	1.50	0.81	1.72X	1.71	0.74	1.44X	2.00	0.81
Rat 4	2.14X	1.32	0.64	0.28X	2.43	0.29	0.31X	2.42	0.44
Rat 7	2.02X	1.25	0.76	0.12X	2.21	0.20	−0.26X	2.32	0.56
Rat 9	0.28X	2.38	0.15	0.18X	2.36	0.10	0.18X	2.25	0.10
Group Satiated									
Rat 3	−0.58X	2.28	0.69	−0.88X	2.52	0.99	−1.00X	2.74	0.99
Rat 5	−0.57X	2.19	0.84	−0.55X	2.42	0.78	−0.88X	2.42	0.99
Rat 6	−0.25X	2.00	0.48	−0.50X	2.07	0.84	−0.69X	2.21	0.91
Rat 8	−1.56X	1.53	0.63	−0.89X	2.00	0.57	−0.99X	2.52	0.98

Regression lines fitted by the method of the least squares. (A) represents the slope, (B) represents the intercept, and (r) stands for the correlation coefficient.

to decrease. It is surprising that CDP did not increase drinking as much as tray orientation (see also [13,23]), but the restrictions imposed by the FT schedule on the nature of the anticipatory response [21], and the fact that both groups were food deprived may have determined predominance of food related behaviors over drinking.

Although the above view suggests a direct motivational effect on appetitive responses, other factors could also contribute in the facilitation of tray activities. Nieto *et al.* [16] using a similar procedure to that used in the present experiment found that d-amphetamine also increases tray orientation and reduces all the other activities; other studies have found that several other drugs increase responding and reduce drinking in FI schedules (see reviews by [5, 18, 23, 24]). The similarity of the effects across a wide variety of drugs suggests that the present pattern of results may not be caused by changes in hunger and thirst alone, but that some nonspecific factors (e.g., [11, 15, 28]) could contribute as well. In any event, further reserarch is needed to understand the mechanisms underlying drugs effects on behavioral patterns generated by FT and FI schedules.

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