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Chlordiazepoxide and food-deprivation compared using a food-preference test in the rat

Comparación del clorodiacepóxido y la privación de alimento mediante una prueba de preferencia en la rata

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ABSTRACT

The effects of chlordiazepoxide (CDP 0,2.5, 5 and 10 mg/kg) on eating, contact with food, latency to begin-eating and other behavioral categories of male hooded rats were investigated using a food-preference test under 2 conditions of food deprivation (2 and 22 hr). Both CDP and food deprivation inceased eating time. The statistical analysis showed that the effects of both variables were essentially additive. But that CDP does not induced a state identical to that produced by hunger,

DESCRIPTORS: Chlordiazepoxide, Food-deprivation, Eating

RESUMEN

El efecto del clorodiazepóxido (CPD 0, 2,5, 5 y 10 mg/kg) sobre ingestión de alimento, contacto con el alimento, latencia para iniciar la ingesta y otras categorías conductuales se investigaron usando una pueba de preferencia con ratas machos bajo dos condiciones de privación de alimento (2 y 22hr). El CDP y la privación de alimento, aumentan la ingesta. Sin embargo, el efecto observado después de la administración de CDP no es idéntico al inducido por el hambre.

DESCRIPTORES: Clorodiazepóxido, Privación de alimento, Ingesta

There is increasing theoretical interest that benzodiazepine mechanisms are involved in appetite processes (Baile and McLaughin, 1979; Cooper, 1980a; Cooper, 1981; Morley, 1980). Intrahypothalamic injection of benzodiazepines can elicit eating in satiated animals (Anderson-Baker *et al,* 1979; Kelly and Grossman, 1979), whilst systemic benzodiazepine treatment has been shown to facilitate stress-induced feeding (Morley and Levine, 1980; Robbins *et al,* 1977), feeding elicited by electrical stimulation of hypothalamic sites

(Soper and Wise, 1971; Watson *et al,* 1980), and to increase food intake in hungry (Iwahara and Iwasaki, 1969; McLaughlin and Baile, 1979; Niki, 1965; Randall *et al,* 1960) or satiated animals (Cooper and Posadas-Andrews, 1979; Mereu *et al,* 1976; Wise and Dawson, 1974).

A form of food-preference test has been introduced to examine the loss of food neophobia following bilateral lesions of the basoleteral amygdala in the rat (Box and Mogenson, 1975; Rolls and Roll, 1973). Whilst control animals initially favored familiar food, the lesioned rats showed a marked shift in preference towards novel, palatable foods. It is thought that benzodiazepines can reduce food neophobia (Poschel, 1971; Robbins *et al,* 1977; Soubrié *et al,* 1975) and/or container neophobia (Tye *et al,*1975). Benzodiazepines should therefore mimic the effect of the basolateral amygdala lesions, and similarly induce a shift in preference away from familiar food towards novel palatable foods. This prediction has been upheld in several recent studies (Cooper, 1980b; Cooper *et al,* 1981; Cooper and McClelland, 1980; Hodges and Green, 1981).

At doses lower than those that produce the antineophobic effect following acute benzodiazepine administration (Cooper, 1980b; Cooper and Crummy, 1978; Fletcher *et al,* 1980), or after chronic administration (Cooper *et al,* 1981), the contrary effect has been observed. In these instances, benzodiazepine treatment enhanced the choice of the familiar food, without producing any evidence of an antinoephobic effect. It could be argued that promoting the intake of familiar food in the food-preference test is a sign of the benzodiazepine's appetite-enhancing action (Cooper, 1980a; Soubrié *et al,* 1975; Wise and Dawson, 1974). The aim of ths study was to determine whether or not, chloridiazepoxide treatment acts analogously to hunger. This question may be addressed by examining the effects of chlordiazepoxide in hungry and relatively non-hungry animals.

METHOD

Animals

The subjects were 48 male hooded rats obtained from the Laboratory colony, weighing 250-300 g. The rats were housed 4 per cage, and were given free access to tap water and Purina Laboratory chow. Room temperature was maintained at 22-23° C, and room lighting operated on a 12 h light; 12 h dark cycle (light on at 7 a.m.).

Apparatus

The food preference test was run in a hardboard box (44 x 41 x 33 cm) in which 6 aluminium food containers (5 cm square; 1.2 cm) were equally spaced on the grill floor. Before each test, 6 types of food were freshly prepared and placed in the containers. All foods were prepared in comparably sized pieces, and equivalent volumes were placed in each dish. The test box was housed in a sound-attenauting room, and each test was observed over closed-circuit TV. Illumination was provided by a single 60 w bulb placed 50 cm above the centre of the box. Recordings were made using a Sony TV camera fitted with a Cosmicar 8.5 mm 1:1.5 lens. Each test was recorded using a Sony VTR 1360 time-lapse record at 2 frames persecond.

Procedure

The rats were handled daily for two weeks before testing as a taming procedure. Half the subjects were deprived of food 22 hours before the food preference test, and half were deprived 2 hours before the test. Each rat was placed in the box for 10 minutes, and its behavior was recorded. The 6 foods available to the rat were the familiar chow pellets, and 5 novel foods: carrot, apple, cheddar cheese, sugar-Puffs (manufactured by Kellogg's Ltd.) and chocolate-coated cookies (MacVitie's).

Videotapes of the experimental sessions were played back a single frame at a time, and the animal's behavior in each frame was identified according to 8 exclusive categories: contact with the familiar food (head directly over the food container, or head/paws touching food without eating); eating the familiar food; contact with the novel foods; eating the novel foods; sniffing (animal is stationary, displays pronunced head and vibrissae movements and sniffing); walking; rearing; grooming. A BASIC program (written by Dr. R. Fitzgerald) was then used to generate total duration scores for each behavioral category, and also the latency to begin eating. In subsequent data analysis, the sniffing, walking and rearing scores were combined to yield a single exploration measure.

Drug Administration

Within each food deprivation group, subjets were randomly assigned to one of 4 injection conditions: a control 0.9% NaCl solution and 2.5, 5.0 and 10.0 mg/kg CDP HCl conditions. All injections were administered i.p. 30 minutes before start of the test.

Statistical Analysis

A Multivariate Analysis of Variance (MANOVA) was applied to the data. A description of the method is available in Bock (1975). The MANOVA was used to avoid overestimating levels of significance by inference errors which can occur if an Univariate Analysis of Variance is applied to correlated dependent variables.

RESULTS

The results of the MANOVA carried out on the data of the experiment are shown in Table 1. There were significant effects for both food-deprivation level and drug condition. There was a highly significant linear drug effect. However, the interaction between food-deprivation and drug treatment was not significant. Univariate F test were next carried out with respect to individual dependent variables (Table 1).

Figure 1 shows the results for the latency to begin eating for each deprivation condition and drug treatment. Univariate F tests revealed a significant effect of food-deprivation level F (1.40) = 6.82, p = 0.13, the hungry rats were faster to start eating, but there was not a significant effect due to drug dose F (3,40) = 2.22, n.s.

Figure 2 shows the results for the total time (sec) devoted to feeding in the 10 minute test as a function of CDP dose and food deprivation level. Total eating time increased with increasing drug dose for both levels of deprivation, giving a significant linear drug effect, F (1,40) = 12.76, p = 0.001. Eating time was also enhanced at the higher level of food deprivation, F (1,40) = 8.73, p = 0.005). Hence, over the dose range employed in the present experiment, the effect of CDP was additive, not interactive, with that of food-deprivation in extending the time devoted to feeding.

A food-preference index was calculated in terms of the ratio of time spent eating familiar chow to the total time devoted to feeding. In general, all

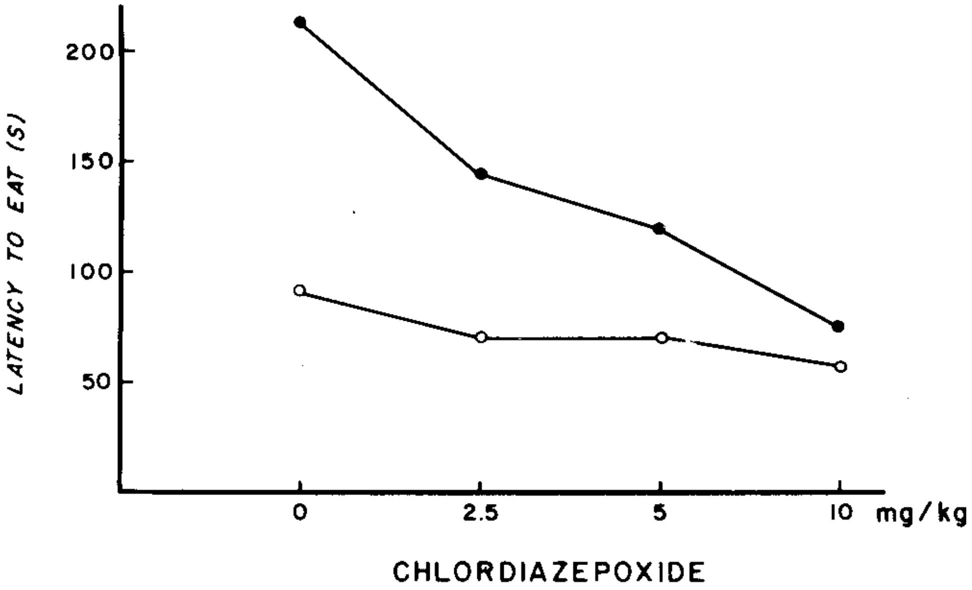

Figure 1. Latency to begin eating in seconds. Effects of chlordiazepoxide (2.5, 5 and 10 mg/kg) and, 2 h (O—O) and 22 h (O—O) food-deprived rats. Both variables affected significantly the latency to begin eating.

SOURCE	WILKS' lambda	APPROX. F	df	SIGNIFICANCE OF F	df FOR UNIVARIATE F tests	SIGNIFICANT UNIVARIATE F TESTS
Food-dep by drug	.54036	.9876	27	.71	3,40	
Food-deprivation	.57000	2.682	9	.019	1,40	eating (.0052); contact (.814); grooming (.2517); exploration (.0110) latency (.01236)
Drug	.20598	2.501	27	.0006	3,40	eating (.004); contact (.39); grooming (.371); exploration (o.0621) latency (.100)
Linear	.34036	.35036	9	00003	1,40	eating (.0009); contact (.391); grooming (.371); exploration (.0621); latency (.0192)

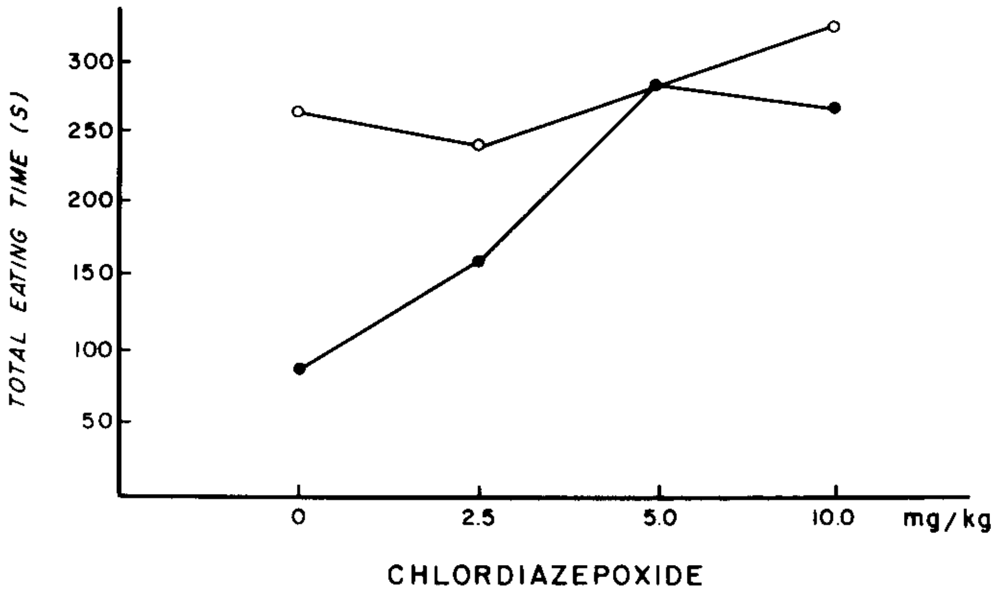

Figure 2. Total eating time (sec) following chlordiazepoxide administration (2.5, 5 and 10 mg/kg) in 2 h (0—0) and 22 h)0—0) food-deprived rats. There was a significant linear drug effect and a singnificant food-deprivation effect.

groups spent more than 50% of their eating time on the familiar food (Fig. 3). The single exception to this was the 22 h food-deprived group that were injected with 10 mg/kg CDP. These animals displayed a marker shift in favor of novel foods. Otherwise, neither food-deprivation nor CDP treatment affected the food-preference measure.

Increasing the level of food-deprivation had a significant effect on the time devoted to exploratory behavior (sniffing, walking, rearing). The hungruier rats showed less non-food directed activity than did the 2 h food-deprived group, F (1, 40) = 7.11, $p = 0.01$ (Fig. 4). In contrast, there was not a significant effect of CDP treatment on the exploratory responses.

DISCUSSION

The food-preference test was sensitive to the effects of both the CDP treatment and the level of food-deprivation. Making rats hungry by 22 h food-deprivation significantly reduced the latency to begin eating, increased the overall duration devoted to feeding, whilst leaving the relative preference for familiar food unchanged. At the same time, the hungrier animals engaged in less exploratory activity. These results can be used to assess the extento to which CDP can be said to mimic hunger.

In 2 h deprived rats, CDP treatment did tend to reduce the latency to feed (Fig. 1), and to increase the duration of feeding (Fig. 2), without affecting the relative preference for the familiar food (Fig. 3). With these comparisons, there are conspicuous similarities between administering CDP to minimally-deprived animals and increasing the level of food-deprivation. However, the statistical analysis of the data for all the groups indicated that the effects of CDP and those of food-deprivation were essentially additive. This result leaves little ground for suggesting that CDP acts to produce a state akin to hunger. Instead, CDP appeared to act independently of food-deprivation level, but produced an additive effect in relation to hunger.

The single instance where an interaction did appear to take place is therefore interesting. Injection of CDP (10 mg/kg) in 22 h food-deprived animals exerted an anti-neophobic effect, with a pronounced shift in preference from the familiar to the novel foods taking place (Fig. 3). This effect has been reported previously (Cooper, 1980b; Cooper and McClelland, 1980, File, 1980; Hodges and Green, 1981). The present results indicate that the effect was not elicited in minimally-deprived animals at the same dose level.

Several previous reports have examined possible relationships between benzodiazepine treatments and food-deprivation conditions. Iwahara and Iwasaki (1969) reported that CDP increased food intake in rats over a 2 h test period, showing similar dose-response relationship in both 2 h and 22 h food-deprived animals. Hence, the effectiveness of CDP was not modified by the level of hunger. Using a fixed-ratio satiation test, Wedeking (1973) showed that CDP administered to 22 h food-deprived rats decreased the latency to the first food reinforcement. A similar decrease was achieved by increasing the period of deprivation to 46 h. CDP administration to 22 h deprived

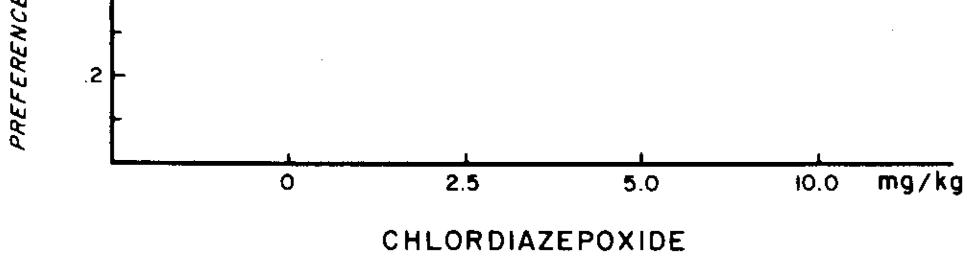

Figure 3. Food-preference index (calculated in terms of the ratio of time spent eating familiar chow to the total time devoted to feeding)). (0–0) 2 h and (0–0) 22 h food-deprived rats. Neither the deprivation nor CDP treatment affected the food-preference exhibited by the rats.

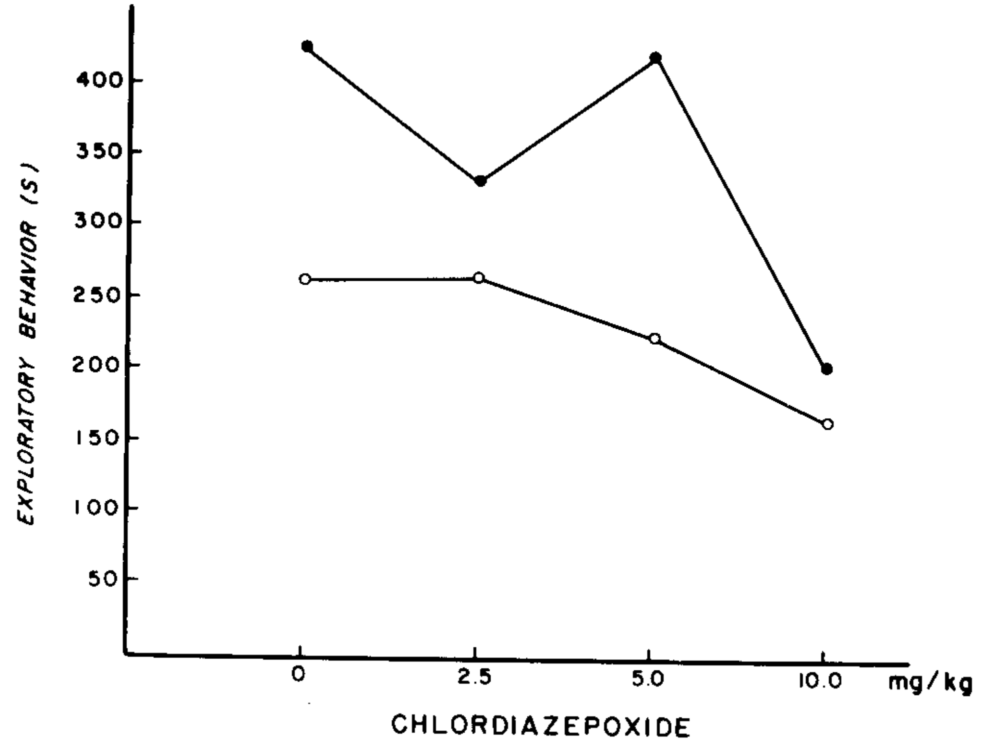

Figure 4. Time devoted to exploratory behavior (sniffing, walking, rearing) in seconds. (0–0) 2 h and (0–0) 22 h food-deprived rats significantly affected exploratory behavior. There was no significant effect of CDP treatment.

animals increased the total food reinforcements taken in the satiation test, whilst a reduction in the period of deprivation led to an opposite effect (Wedeking, 1973). Wise and Dawson (1974) demostrated that hungry rats trained to lever press for food, showed a transfer of training to a diazepam condition. Taken together therefore, the data from feeding experiments (Iwahara and Iwasaki, 1969; Wedeking, 1973; Wise and Dawson, 1974; present study) indicate that therefore is an overlap between the effects of benzodiazepine treatment and food-deprivation. However, that should not be taken to imply that benzodiazepines induce a state which is identical to that of hunger. Instead, benzodiazepines appear to be able to duplicate some of the effects of food-deprivation, by some mechanism which is analogous to, but also independent of, the state of hunger. In the present experiment, CDP and food-deprivation exhibited essentially additive effects on feeding responses in a food-preference test.

In contrast, the anti-punishment action of benzodiazepines is not duplicated by increasing the level of hunger (cook and Davidson, 1973; Dantzer, 1978; Margules and Stein, 1967). Here, there appears to be a marked dissociation between the two manipulations.

Benzodiazepines are perhaps not appropriate drug models for the hunger produced by food deprivation. Firstly, their effects upon feeding behavior are best described as additive to those of food-deprivation. Secondly, their anti-punishment action (which may contribute under some circumstances to alterations in feeding), are not duplicated by food-deprivation.

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