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Effects of Chlordiazepoxide, Food Familiarization, and Prior Shock Experience on Food Choice in Rats

STEVEN J. COOPER[1] AND AUDREY MCCLELLAND

Department of Psychology, Queen's University of Belfast
Belfast BT7 1NN, Northern Ireland

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COOPER, S. J. AND A. McCLELLAND. *Effects of chlordiazepoxide, food familiarization, and prior shock experience on food choice in rats.* PHARMAC. BIOCHEM. BEHAV. **12**(1) 23–28, 1980.—Chlordiazepoxide (5, 10 mg/kg) increased the time devoted to eating familiar laboratory chow without altering the response to a range of novel, palatable foods which were also available to the food-deprived rats. Prior experience with the same range of alternative foods (food familiarization) radically changed the effect of the drug. After familiarization with these foods, chow was virtually ignored as a food choice, indicating its low relative palatability; chlordiazepoxide then prolonged the time eating the familiarized foods without significantly increasing the response to chow. These results are not consistent with an anti-food neophobia action of chlordiazepoxide. They suggest instead that chlordiazepoxide enhances feeding responses related to food saliency. Footshock, delivered two days before the food choice test affected performance within the test. Its effects were opposite those of chlordiazepoxide, but they competed additively with the drug's effects. These results indicate that chlordiazepoxide's action was not simply to remove any inhibitory effect on feeding produced by fear; instead the drug promoted approach to food antagonizing any deficit in approach associated with fear. These findings are viewed as consistent with an action of chlordiazepoxide to augment the level of feeding motivation. Chlordiazepoxide (15 mg/kg) may act to overcome food neophobia.

Chlordiazepoxide Fear Food familiarization Food novelty Food preference Foot-shock Neophobia

RATS with bilateral lesions of the basolateral amygdala are deficient in food neophobia responses [15]. As such, they show a marked increase in preference for foods which are palatable but novel, when given a choice amongst a variety of novel and familiar foods [2,17]. Some authors have suggested that benzodiazepines, like bilateral amygdala lesions, facilitate feeding behaviour by acting to attenuate food neophobia responses [12,16]. In previous studies, we therefore expected to find that benzodiazepines would similarly increase the relative preference for novel, palatable foods in a food-choice situation. However, chlordiazepoxide (CDP) and diazepam (DIAZ), contrary to prediction, produced a clear increase in preference for the familiar food [3,4]. The action of benzodiazepines, at least over certain dose ranges, is therefore opposite that of bilateral amygdala lesions in the food-preference test, and is not consistent with a reduction in food neophobia [3].

The present experiment continues to test the suggestions that benzodiazepines, in this instance CDP, mainly facilitate feeding responses either by a reduction in food neophobia [16], or by removing the inhibitory effects of aversive states, like fear [14]. Both mechanisms primarily ascribe to benzodiazepines a *disinhibitory* action, which secondarily can produce increased feeding. If CDP mainly acts to reduce food neophobia, then any facilitatory drug effect observed in the food-preference test should be eliminated when novel foods are made familiar before the test is run. If CDP mainly acts by alleviating a fear or anxiety condition, then its effects on feeding behavior should be more pronounced in more fearful animals. The present experiment examined these two predictions.

We employed a food-preference procedure which has been used previously in lesion [1,17] and drug [3, 5, 7] studies. In general the results of the experiment are consistent with a relatively direct action of CDP to facilitate approach to food, and to affect food choice. They do not support behavioral disinhibition as a primary action of the drug. However, different actions of the drug emerge at lower and high dose level respectively. Chlordiazepoxide, at lower dose levels, appears to act in a way that is more consistent

[1]Address correspondence to S.J.C., Department of Psychology, University of Birmingham, Birmingham B15 2TT, U.K.

with a relatively direct increase in feeding motivation [3, 18, 19]. At a higher dose, CDP may act to overcome food neophobia.

METHOD

Animals

Subjects were 96 male Sprague-Dawley rats, approximately 70 days old on receipt, and supplied by Charles Rivers U.K. Ltd. On arrival, the rats were randomly allocated four per cage and given individual identification marks. Room temperature was maintained between 20–23°C, and humidity >50%. Room lighting operated on a 12 hr dark–12 hr light cycle (lights on 0800 hr).

Food Familiarization

The 24 home cages were divided into 2 equal groups. In half, standard laboratory food pellets (Diet 41B, Robert Morton Ltd.) and tap water were freely available. When run in the food-preference test, these animals would encounter novel foods. In the other half, however, rats were given a fresh daily supply of carrot, cheddar cheese, currants and chocolate-coated cookies, in addition to regular maintenance chow. For these animals, therefore, all foods in the food-preference test were highly familiar. The rats were maintained under these feeding conditions for 14 days before food-preference tests were run. Each animal received 60 sec gentle handling throughout the pre-test period as a taming procedure.

Shock Treatment

Two days before the food-preference tests were run, half the rats in each feeding treatment group (described above) were randomly selected to receive shock treatment. Each rat received 6 shocks (1.3 mA constant current; 1 sec duration; 15 sec inter-shock interval) in a Grason-Stadler operant chamber. Non-shocked animals were similarly treated, except that the chamber was disconnected from the shock generator. Pilot work had shown that such shock treatment does modify subsequent behavior in the food-preference test; in particular, the latency to begin eating can be considerably enhanced, and this we take to be a sign of conditioned fear in otherwise tamed animals.

Food-preference Test

Each rat was deprived of food from 1230 hr on the day prior to the test day. The food-preference test, run during the following morning, was conducted in a Bowman's MRC-type rat cage. The cage floor was made of a wire grid (apertures 0.4 cm^2). Six round plastic trays (diameter 5.5 cm; rim height 1.1 cm) were fixed to the grid floor. Before each test, six types of food were freshly prepared and placed in the containers. These were Diet 41B food pellets, carrot, cheddar cheese, currants, and chocolate-coated cookies. All foods were prepared in pieces of comparable size, and equivalent volumes were placed in a shallow pile in each dish. For half the animals, the five foods other than chow pellets were completely novel. But for the other half, all the available foods were very familiar.

In the test, each animal was tested individually and was placed for 10 min in the test cage. The first measure to be recorded by the observer was the latency to begin eating; subsequently, the time spent eating each type of food was separately recorded for each occasion that eating occurred. Eating time was recorded only when food was taken into the mouth and chewed; any time spent in contact with food without eating (e.g. touching food with front paws, carrying food about the cage held in the mouth) was not recorded. After each trial, if necessary, each food container was replaced in position, and refilled. Any spillage was removed, and a paper sheet placed beneath the cage was replaced by a clean one.

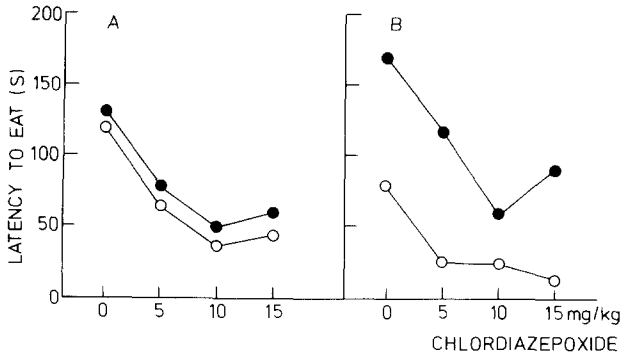

FIG. 1. Latency to eat (sec) in a 10-min food-preference test. A. Effects of CDP in rats pre-exposed to all available food (food-familiarization group) (○–○), and in rats offered familiar chow and five novel foods (●–●). B. Effects of CDP in rats receiving prior shock treatment (●–●) and in rats with no prior shock treatment (○–○). Each point indicates the mean for 12 rats.

Injection Conditions

Within each of the four major groups (food familiarization–shock vs no-shock; food novelty–shock vs no-shock), the rats were further randomly assigned to four injection conditions (n=6 per group for 16 groups). These conditions were (1) 5.0 mg/kg chlordiazepoxide HCl (CDP), (2) 10.0 mg/kg CDP, (3) 15.0 mg/kg CDP, (4) isotonic saline vehicle. Doses are expressed in terms of the salt. Solutions were made up fresh daily in isotonic saline, and were injected IP in a volume of 1 ml/kg. All injections were given 30 min before the beginning of the food-preference test.

Design and Analysis

The experiment used a 3-factor design. The first factor was food exposure prior to the food-preference test (2 levels). The second factor was the experience of shock or no-shock (2 levels). The third factor consisted of 4 levels of CDP treatment. All measures taken in the test were analysed using a 3-factor analysis of variance (ANOVA) [21], using raw data. The design of the experiment permits a comparison amongst the main treatment effects, and also an assessment of the types of interaction which may occur between them. Individual comparisons between groups were made using a *t*-test.

RESULTS

Latency to Eat

Chlordiazepoxide treatment invariably reduced the latency to begin feeding (sec) in the 10 min food-preference test, $F(3,80)=6.66$, $p<0.001$ (Fig. 1). In contrast, exposure to foot-shock two days before the food-preference test signifi-

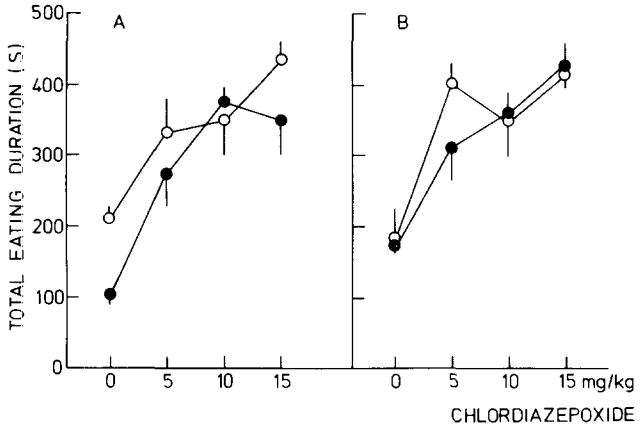

FIG. 2. Total time (sec) devoted to eating in a 10-min food-preference test. A. Effects of CDP and prior shock exposure in rats presented with familiar chow and five additional novel foods. (○–○) Rats which had not received prior shock treatment; (●–●) rats which had received prior shock treatment. B. Effects of CDP and shock exposure in rats pre-exposed to all available foods (food-familiarization group). (○–○) Rats which had not received prior shock treatment; (●–●) rats which had received prior shock treatment. Each point indicates the mean for 6 animals; vertical line indicates SE mean (half-range).

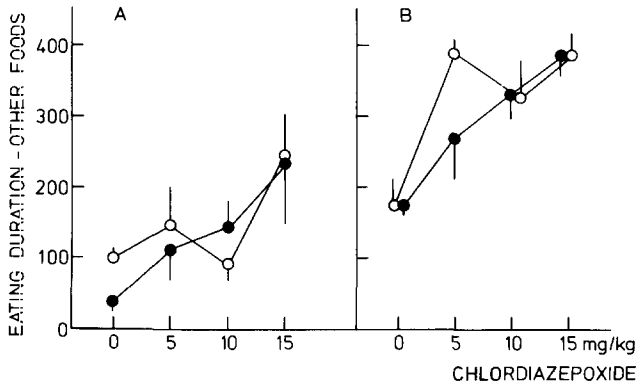

FIG. 4. Total time (sec) devoted to eating five alternative foods to laboratory chow. A. Effects of CDP and prior shock exposure in rats presented with chow and five additional novel foods. (○–○) Rats which had not received prior shock treatment; (●–●) rats which had received prior shock treatment. B. Effects of CDP and shock exposure in rats pre-exposed to all available foods (food-familiarization group). (○–○) Rats which had not received prior shock treatment; (●–●) rats which had received prior shock treatment. Each point indicates the mean for 6 animals; vertical line indicates SE mean.

cantly prolonged the latency to feed, $F(1,80)=25.23$, $p<0.001$ (Fig. 1, panel B). There was no interaction between the effects of CDP treatment and prior shock experience, $F(3,80)=0.87$. Thus, CDP and foot-shock were strictly additive in their effects on the latency measure (Fig. 1, panel B). Food familiarization before the food-preference did not exert a significant main effect on latency, $F(1,80)=0.66$, and did not interact significantly with CDP treatment, $F(1,80)=0.66$, (Fig. 1, panel A). Chlordiazepoxide significantly reduced the

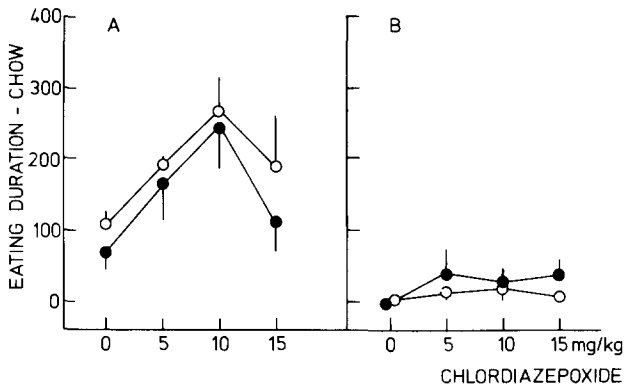

FIG. 3. Total time (sec) devoted to eating familiar laboratory chow in a 10-min food-preference test. A. Effects of CDP and prior shock exposure in rats presented with chow and five additional novel foods. (○–○) Rats which had not received prior shock treatment; (●–●) rats which had received prior shock treatment. B. Effects of CDP and shock exposure in rats pre-exposed to all available foods (food-familiarization group). (○–○) Rats which had not received prior shock treatment; (●–●) rats which had received prior shock treatment. Each point indicates the mean for 6 animals; vertical line indicates SE mean.

latency to feed at each of the three dose levels ($p<0.01$) (Fig. 1).

Total Eating Time

Chlordiazepoxide significantly extended the total time (sec) devoted to feeding in the food-preference test, $F(3,80)=32.11$, $p<0.001$, in a dose-related manner (Fig. 2). Prior experience of foot-shock had the opposite effect of acting to reduce the total feeding duration, $F(1,80)=4.29$, $p<0.04$ (Fig. 2). There was no significant interaction between the effects of CDP treatment and prior shock exposure, $F(3,80)=1.16$. Food familiarization had no effect on the total feeding duration, $F(1,80)=1.70$, and there were no significant interactions between it and the other two main factors. The effect of CDP to prolong the total time devoted to feeding was not affected therefore by prior shock treatment or by food familiarization.

Total Time Eating Chow

The total eating time can be divided between the time spent eating the familiar laboratory chow and the time devoted to eating the five alternative foods. These are now considered separately. In the food-preference test as conventionally used, e.g. [17], chow is presented together with five novel foods. Under this condition, CDP significantly increased the chow eating time, $F(3,80)=3.23$, $p<0.03$. The effect is, however, non-monotonic with respect to dose; the peak effect occurred at 10 mg/kg. At 15 mg/kg, the time spent eating chow did not differ significantly from the control value (Fig. 3, panel A). After the food familiarization procedure, half the animals were presented with chow as a choice amongst five other palatable and familiar foods. Under this condition chow was ignored by virtually all of them (Fig. 3, panel B). Chlordiazepoxide treatment did not then produce any significant increase in the time devoted to the chow. Hence, CDP only significantly affected chow eating, when

other, more palatable foods were novel. Once they had been made familiar, CDP no longer increased chow eating.

Food familiarization obviously exerted a decisive effect on the time allocated to chow eating, $F(1,80)=50.58$, $p<0.001$; chow was a preferred food provided the alternative foods were novel. When they were familiar, chow was put into the position of being a non-preferred food (Fig. 3). Prior shock treatment did not exert a significant effect on chow eating times, $F(1.80)=0.91$.

Total Time Eating Alternative Foods

The effects of CDP depended on whether the five alternative foods to chow were novel or familiar. When they were novel, CDP (5 or 10 mg/kg) did not significantly affect the time spent eating them (Fig. 4, panel A). At 15 mg/kg, however, CDP did produce a significant increase ($p<0.05$); it was at this dose that CDP showed a marked fall in its effect on chow eating (Fig. 3, panel A). At 15 mg/kg, therefore, CDP did produce a shift in choice towards the unfamiliar foods. When the alternative foods were familiar, CDP significantly increased the time spent eating them, in a dose-related manner (Fig. 4, panel B). In this second condition, CDP did not increase chow eating (Fig. 3, panel B).

Clearly, familiarization was an important determinant in the choice of the five foods offered as alternatives to chow, $F(1,80)=41.76$, $p<0.001$. In contrast, previous shock experience did not produce a significant main effect on eating these foods, $F(1,80)=0.63$, or produce significant interactions with either CDP treatment or food familiarization.

DISCUSSION

The results of the present experiment confirm our previous report [3] in showing that CDP (5 or 10 mg/kg) enhanced the choice of familiar laboratory chow, without significantly affecting the response to several novel foods, in a food-preference test. The present results go further in distinguishing between high- and lower-dose related effects of CDP on food choice. When CDP was given at 15 mg/kg, a switch occurred in the rats' relative preference towards the novel foods (Figs. 3 and 4). This high-dose effect of CDP is quite comparable to that produced by bilateral amygdala lesions [1,17]. The change in drug effect as the dose is increased, is not restricted to CDP, since DIAZ also enhances novel food eating at a dose level above that which promotes familiar food eating [4].

Providing that a relatively high dose is used, therefore, these results may indicate that benzodiazepine-induced reduction in food neophobia is possible [16]. If that is the case, then a different mechanism of action must be brought into play at the lower dose levels. We agree with other authors [3, 18, 19] in arguing that the effect of CDP, and other benzodiazepines, at relatively low dose levels is specifically involved in a strengthening of feeding motivation. Thus, we argue, that CDP enhanced the choice of familiar laboratory chow, leaving the response to the novel, palatable foods unchanged because CDP acted like an increase in hunger.

One of the strongest effects observed in the present experiment was the radical change in food choice brought about by the food familiarization procedure. When familiar chow was presented with five novel foods, then the chow was the single most preferred food (c.f. [17]. When, however, the five alternative foods were available before the food-preference test to ensure their familiarity, then chow almost dropped out as a food-choice (present experiment). The same switch from chow to other, palatable foods can be brought about by repeatedly testing the animals in the food-preference test. On the first trial, chow is likely to be the most preferred food; little time is devoted to alternative novel foods. However, with repeated testing, animals progressively spend less time eating chow, and more time eating the alternative foods ([17] Cooper and Posadas-Andrews, unpublished data). When all the available foods are familiar, therefore, a good estimate of relative preference of individual foods can be obtained. It is clear that in the present experiment rats preferred eating the foods other than chow once they had become familiar.

Chlordiazepoxide essentially acts to enhance the response to the preferred foods. This implies a specific mode of action, and is to be distinguished from an indiscriminate increase in feeding behavior. When chow was available with novel, alternative foods, then CDP (5 or 10 mg/kg) increased the time spent eating the familiar chow, since in that situation it formed the more preferred or salient food. In exactly the same way, increasing the level of food deprivation to make rats more hungry, increases their response to familiar chow without altering their response to novel foods [2]. Administering CDP therefore is like an increase in feeding motivation; both act specifically to increase the feeding response to highly familiar (salient) food. But the alternative foods are intrinsically more palatable than chow. Once familiar, they are preferred to chow. In that case, administering CDP (present experiment) or increasing the level of food deprivation (Cooper and Posadas-Andrews, unpublished results) specifically enhances the response to the preferred alternatives to chow, without significantly altering the response to chow. In summary, CDP appears to act like an increase in the level of feeding motivation, in that both manipulations enhance the rat's response to salient foods in a food choice situation.

The results of the present experiment run counter to any general disinhibition hypothesis concerning the action of benzodiazepines [14]. For example, one effect of familiarizing rats with palatable foods was to inhibit strongly their response to their accustomed laboratory chow in a choice test. Yet, there is no evidence that CDP acted preferentially to disinhibit chow-feeding (this experiment; Cooper and Posadas-Andrews, unpublished results). Instead, CDP acted to confirm the dominant feeding response.

Clearly, the major effects of CDP observed in the present experiment (reduction in latency to feed; increase in total eating time; enhanced choice of salient food) were not a function of food novelty. Hence, a reduction in food neophobia as a major explanation of CDP's actions in the food-preference test cannot be sustained.

It has been suggested that benzodiazepines might facilitate feeding responses by suppressing the inhibitory effects of fear or other aversive motivational states [14]. We used prior to shock exposure to instill a mild emotional reactivity in otherwise tamed animals. The effects of the shock experience were to increase the latency to feed, and to reduce the total eating duration. These effects were expected, and are similar to the differences which distinguish non-handled (more emotional) animals from animals accustomed to handling (less emotional) [3]. The effects of the shock experience on the two parameters of latency and total eating time were opposite those produced by CDP. However, CDP's effects were statistically independent of those produced by the shock experience, and it cannot be concluded

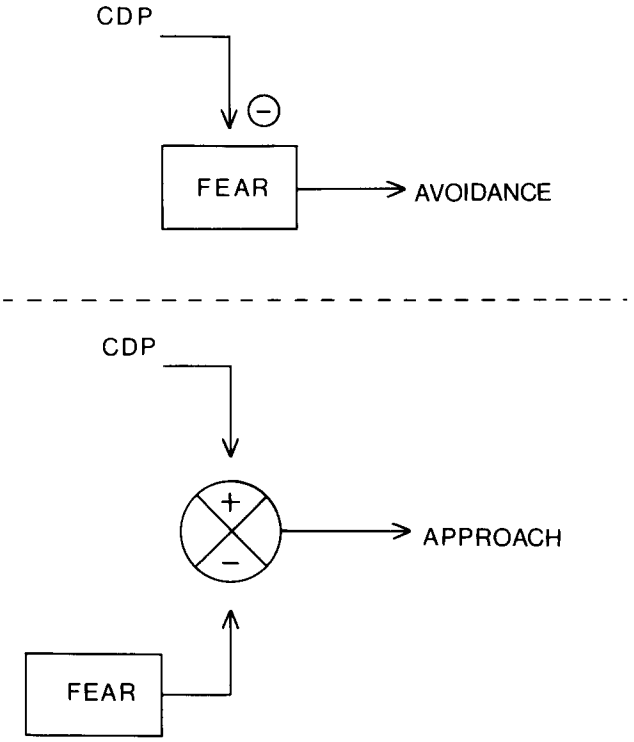

FIG. 5. Two behavioral models of chlordiazepoxide (CDP action. The conventional model [14] incorporates a *disinhibitory* action of CDP on aversive states which motivate avoidance behavior. CDP therefore antagonizes avoidance, although the presence of the aversive state is a necessary condition for the drug action. In contrast, we propose that CDP can more directly facilitate approach behavior. The action of CDP summates (⊗) with the opposite action of an aversive state, if it is present. However, the aversive state is not a necessary condition for the drug action. This second model provides a better prediction of the data of the present experiment.

that elevated emotionality is a necessary condition for the drug's effects to emerge (Fig. 5).

Benzodiazepines are now known to increase the efficiency of GABAergic transmission, when GABA synapses are activated [8, 10, 11]. Preliminary data suggest that GABAergic transmission may have an important function in determining food choice behavior (H. Hodges, University of London, personal communication). Picrotoxin, a potent antagonist of the action of GABA synapses [8,13], at 0.5 and 1.0 mg/kg increases the latency to feed, decreases total feeding duration, and induces a change in preference from familiar chow towards novel foods in a food-preference test. These behavioral effects are opposite those of lower doses of CDP; it is established that picrotoxin can block the facilitatory action of benzodiazepines on GABA synaptic transmission [8]. Further work is necessary to establish whether the benzodiazepine effect on GABAergic transmission is responsible for CDP's specific action in changing food choice behavior.

In summary, the actions of CDP (at relatively low doses) in the food-preference test can be explained in terms of an increase in feeding motivation. The results are not strongly consistent with either a general disinhibitory effect or a specific reduction in food neophobia. Instead, CDP, like increasing the level of food deprivation [2] enhanced the feeding response to the more salient food available. This mechanism accounts for the findings that CDP and other benzodiazepines can promote feeding in free-feeding and food-sated animals [6, 9, 19].

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