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Food-Choice in a Food-Preference Test: Comparison of Two Mouse Strains and the Effects of Chlordiazepoxide Treatment

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Abstract. In male mice of the C57 strain, chlordiazepoxide (CDP) (2.5, 5.0 and 10.0 mg/kg) reduced the latency to begin eating and prolonged the total time devoted to eating in a food-preference test. The increase in feeding duration arose from an increase in the mean duration of individual eating episodes and not from a change in the number of episodes that were initiated. In contrast, in male mice of the A2G strain CDP (at the same dose levels) did not reduce the latency to begin eating and only prolonged the total time devoted to eating at a single dose level (5.0 mg/kg). The two strains differed in their choice between novel and familiar foods that were concurrently available in the test. The A2G mice virtually ignored the palatable novel foods and devoted all their feeding to the familiar food. The C57 strain, however, spent more time eating the novel foods than the familiar food. CDP at all doses increased the duration of feeding devoted to familiar food in the C57 animals, but did not increase feeding duration in the A2G mice. However, CDP (10.0 mg/kg) increased the time spent eating novel foods in both strains to the same degree. Possible mechanisms underlying the effect of CDP on food-preference behaviour in mice and accounting for the strain difference in response to CDP are considered.

Key words: Chlordiazepoxide – Familiar and novel foods – Feeding motivation – Food preference – Mouse strains

Benzodiazepines reliably facilitate feeding responses in a variety of mammalian species (Cooper, 1978; Dantzer, 1977), although there remains some uncertainty concerning the mechanisms underlying the facilitation of feeding behaviour. Poschel (1971), for example, suggests that benzodiazepines suppress the avoidance of novel foods (neophobia), and so act to enhance feeding behaviour by secondary means. In contrast, some authors maintain that benzodiazepines can exert a direct facilitatory effect on feeding motivation (Soubrié et al., 1975; Wise and Dawson, 1974), presumably by affecting hunger or by raising the incentive value of the food. Recently, Cooper and Crummy (1978) introduced a form of food-preference test in order to help determine the mechanisms responsible for the effects of CDP in a food choice situation. They showed that CDP administered to a food-deprived rat increased the choice of a familiar food whilst leaving the response to palatable, novel foods unchanged. Their results suggest that CDP facilitated eating responses directed to the familiar food by a direct increase in feeding motivation.

In mice Stephens (1973) demonstrated that benzodiazepines (CDP, diazepam, nitrazepam) increased food consumption in a 30-min test. A similar effect was reported by Soubrié et al. (1975), and in the case of CDP they reported significant percentage increases in food intake over a dose-range of 4–8 mg/kg. The first aim of the present experiment was to examine the effects of CDP treatment on the feeding behaviour of mice in the food-preference test used previously with rats (Cooper and Crummy, 1978). The effects of CDP and other benzodiazepines on feeding have not been described in terms of possible strain differences. A second aim of the present experiment was to compare two mouse strains in terms of their behaviour in the food-preference test. We found that the general effect of CDP treatment was to reduce the behavioural differences between the two strains.

Materials and Methods

Animals. The subjects were 24 adult male C57 mice (blackcoated) and 24 adult male A2G mice (white-coated) bred in our laboratory from

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stock originally supplied by the Medical Research Council. The mice were 5 weeks old at testing and weighed approximately 30 g (C57) and 35 g (A2G) respectively. Before the experiment they were maintained in group cages with food (Diet 41B laboratory chow pellets) and water available ad lib. Room temperature was maintained at 21 – 23° C; room illumination operated on a 12 h dark-12 h light cycle (light on at 8 a.m.).

Apparatus. The food-preference test was conducted in a clear perspex mouse box (26.4 × 19.2 × 9.6 cm). Six round plastic trays (dia 25 mm; rim height 10 mm) were placed at equal intervals about the floor of the box. Before each trial of the test six types of food were freshly prepared and placed in the containers. The six types of food consisted of, firstly, the familiar Diet 41B pellets, and secondly, five palatable novel foods: apple, carrot, Cheddar cheese, currants and plain chocolate-coated wheatmeal biscuits (Cooper and Crummy, 1978). All foods were prepared in pieces of comparable size, and equivalent volumes were placed in each dish. There was one type of food per dish. The floor of the box was marked with two intersecting black lines which bisected the long and the short sides of the box to divide the floor area into four equal parts. Locomotor activity was scored in terms of entries into these areas.

Procedure. Before the food-preference test (Cooper and Crummy, 1978; Cooper et al., 1979; Rolls and Rolls, 1973) each animal was deprived of food from 5 p.m. on the day prior to the test day. In the test each animal was placed individually in the test box for 10 min between (10 – 11 a.m.). The recorded measures for the feeding responses were the latency to begin eating (s) and, subsequently, the duration of each episode of feeding, in s according to the type of food consumed. Eating time was scored by direct observation only when food was taken into the mouth and chewed; any time spent sniffing or holding food or otherwise in contact with food was not scored. After each trial, if necessary, the floor of the box was cleared and each food container was replaced in position and refilled. Locomotor activity was scored in terms of the total number of floor-areas (marked on the floor as indicated above) entered with all four limbs during the 10-min test period. Animals of both strains were treated equivalently.

Injections were given 30 min before the start of the test session. Within each strain the mice were randomly assigned to four groups (n = six/group); isotonic saline vehicle and 2.5, 5.0 and 10.0 mg/kg CDP HCl. Doses are expressed in terms of the salt. Solutions were made with isotonic saline and injected IP.

Statistical Analysis. Comparisons between experimental treatments and control groups were made using a Student's t-test for independent groups. The effects of strain and drug dosage factors were analyzed using a two-factor analysis of variance (Anova), with two levels for strain and four levels for drug dosage. Statistical analyses were performed on untransformed data, except in the case of individual episode duration scores which were first log transformed to correct a skewed distribution of scores.

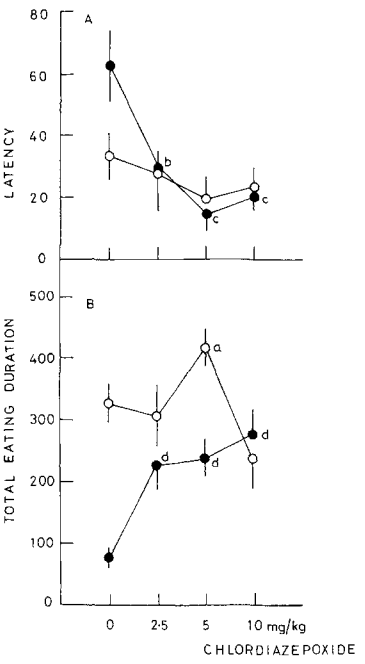

Fig. 1. A Latency to begin eating (s) in two strains of mice in a 10-min food-preference test. The two strains were: (●——●) C57 mice (black-coated); (○——○) A2G mice (white-coated). Dose levels of CDP are 2.5, 5.0 and 10.0 mg/kg. Isotonic saline solution served as a control injection. Each point represents the mean of six animals. *Vertical lines* indicate SEM. Significant differences between group scores and control scores were calculated using a t-test for independent groups. The significance levels are: $^{a}P < 0.05$; $^{b}P < 0.025$; $^{c}P < 0.01$; $^{d}P < 0.005$. **B** Effect of CDP on the total time devoted to eating (s) for two mouse strains (C57, A2G) in the food-preference test. Conventions are as described for Fig. 1 A

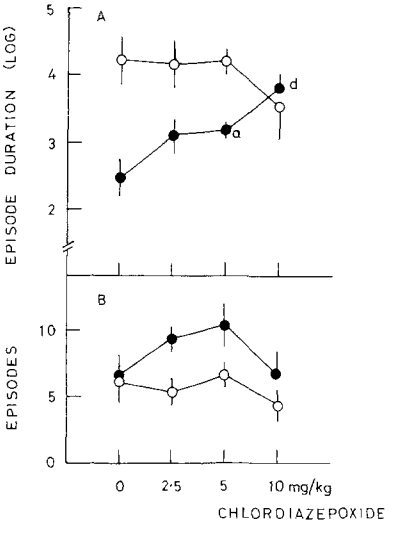

Fig. 2. A Effect of CDP on the mean duration (s) of individual eating episodes (log-transformation of raw data) for two mouse strains in the food preference test. **B** Effect of CDP on the number of individual eating episodes for two mouse strains in the food-preference test. Conventions are as described for Fig. 1 A

Results

Before analyzing the effects of CDP on the feeding responses of the mice, it is necessary first to compare the behaviour of the two strains in a controlled undrugged condition. Differences in the underlying behaviour of the two strains may be related to the responses of the strains to CDP treatment.

Mouse Strain Comparisons — Controls. The A2G strain had shorter latencies to begin feeding than the C57 mice (t = 1.85, 10 df, $P < 0.05$), (Fig. 1 a) and spent a much larger proportion of the test duration in feeding

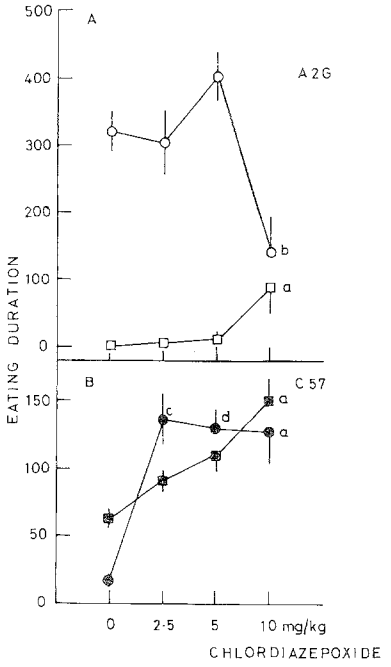

Fig. 3. A Effect of CDP on the time spent eating familiar laboratory chow (O——O) and on the time spent novel foods (apple, carrot, chocolate-coated biscuit, cheese, currants) (□——□) for the A2G strain of mice in the food-preference test. **B** Effect of CDP on the time spent eating familiar laboratory chow (●——●) and novel foods (■——■) for the C57 strain of mice in the food-preference test. Other conventions are as described in Fig. 1A. All durations in s

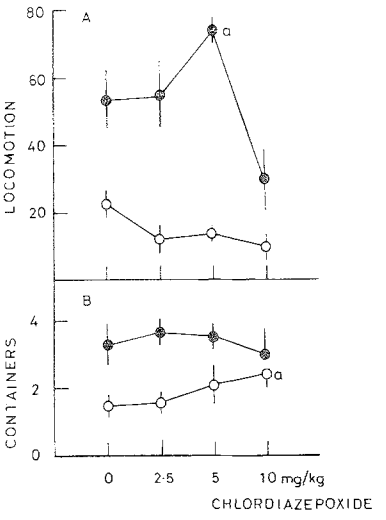

Fig. 4. A Effect of CDP on locomotor activity (as described in Procedure) in two strains of mice in the food-preference test. **B** Effect of CDP on the number of food containers from which food was sampled by two strains of mice in the food-preference test. Conventions are as described in Fig. 1

compared with the C57 mice (t = 6.61, 10 df, P < 0.001) (Fig. 1 b). The difference in the total duration of feeding between the two strains was not due to a difference in the number of individual eating episodes (Fig. 2 b); instead, the A2G strain exhibited significantly longer durations of individual eating episodes compared with the C57 mice (t = 3.35, 10 df, P < 0.005) (Fig. 2a). Hence, once an eating episode had begun, A2G mice had a tendency to remain engaged in feeding for longer periods than the C57 mice. The two strains did not differ, however, in the number of occasions upon which feeding behaviour was initiated.

In addition to the difference in the duration of feeding episodes, the two mouse strains displayed distinctively different choices amongst the familiar and novel varieties of food. The A2G mice, despite spending a longer time engaged in feeding than the C57 mice, showed an almost complete avoidance of the novel foods. Virtually all their feeding, therefore, was devoted to eating the familiar chow (Fig. 3a). In contrast, the C57 mice spent the greater proportion of their feeding time in sampling the novel food choices (Fig. 3b). In keeping with this effect, the C57 mice sampled food from more containers than did the A2G animals (t = 2.45, 10 df, P < 0.025) (Fig. 4b). In addition to the strain differences in feeding behaviour, the two strains differed in their levels of locomotor activity. The C57 mice were considerably more active over the course of the test session than the A2G mice (t = 2.71, 10 df, P < 0.025).

Effects of Chlordiazepoxide Treatment: Latency to Eat. CDP significantly reduced the latency to begin eating at each dose level in the C57 mice (Fig. 1a), but had no effect on the latencies of the A2G mice. After the drug treatment there were no differences between the two strains in their latencies to eat (Fig. 1a), so that CDP reduced the longer latency of the C57 mice to a level equal with that of the A2G mice.

Total Eating Duration. CDP produced a significant increase in the total time devoted to feeding in the C57 strain at each dose level (Fig. 1b), but exerted a stimulant effect on feeding in the A2G mice at only the 5.0 mg/kg dose level. Despite a pronounced difference between the strains under control conditions, the total feeding durations of the two strains were not significantly different after CDP treatment at either 2.5 or 10.0 mg/kg. Hence, at these doses CDP increased the duration of feeding of C57 mice towards the level of feeding shown by the A2G mice. At 5.0 mg/kg CDP, however, the total feeding duration of the A2G mice remained significantly greater than that of the C57 strain (t = 3.73, 10 df, P < 0.005).

Eating Episodes and Episode Durations. The total duration of feeding can first be subdivided into the number of individual episodes of continuous feeding

and the mean duration of the individual eating episodes. An increase in the total eating duration could be due to an increase in the number of eating episodes, an increase in the individual episode duration, or possibly to both.

The results for the mean duration of eating episodes were first submitted to a log transformation since the distribution of scores for the A2G animals, in particular, was skewed. CDP significantly increased the individual eating episode duration at the two higher dose levels in the C57 strain (Fig. 2a), but exerted no significant effects on the episode duration for A2G mice. At the highest dose level (10.0 mg/kg) there was no difference between the values of the eating episode duration for the two mouse strains. Hence, administration of CDP at this dose changed the performance of the C57 mice to a value comparable with that shown by the A2G mice. Once the C57 mice began a feeding episode, they therefore continued to eat for a longer duration, on the average, after CDP treatment.

Within each strain CDP appeared to have no reliable effects on the number of eating episodes begun in the test session (Fig. 2b). However, if a comparison is made across strains, then, despite no difference between the two strains under control conditions, significant differences did emerge after administration of CDP. Thus, C57 mice showed significantly more eating episodes than A2G mice, when treated with CDP at 2.5 mg/kg ($t = 2.72$, 10 *df*, $P < 0.025$) or 5.0 mg/kg ($t = 1.88$, 10 *df*, $P < 0.05$).

Type of Food Chosen. The total duration of feeding can be divided into the time spent eating the familiar chow pellets and the time spent eating the novel foods. A comparison of the durations gives an indication of the choice between the familiar or novel foods.

Figure 3 shows the total eating duration separately for the familiar food (chow) and the five novel food items (carrot, cheese, apple, currants and chocolate-coated biscuit) for the two strains of mice. First consider the effect of CDP on the time devoted to eating the novel foods. The effect of CDP at 10.0 mg/kg was to increase the time spent eating novel foods by 88.0 s in the A2G mice and by 87.2 s in the C57 mice (Fig. 3). That is, despite a greater acceptance by the C57 strain of novel foods under non-drugged conditions, treatment with CDP at 10.0 mg/kg increased the time spent eating novel foods in both strains to the same degree. An analysis of variance confirms this comparison. There was a highly significant effect of mouse strain on the time spent eating novel foods [$F(1,40) = 37.9$, $P < 0.001$], reflecting the greater acceptance of novel foods by C57 mice than A2G mice. There was also a significant drug effect [$F(3,40) = 3.1$, $P < 0.04$], indicating an increase in eating novel foods after CDP treatment. However, there was a non-significant interaction term ($P > 0.05$) for these two factors, which is consistent with there being a comparable effect of the drug on both mouse strains. Hence, there is no substantial indication that the two strains differ in response to CDP treatment so far as the time devoted to novel foods is concerned. CDP at 10.0 mg/kg significantly increased the time spent eating the novel foods in both strains (Fig. 3).

With feeding directed to familiar food, however, there were significant differences in the response of the two mouse strains to CDP treatment. In the C57 strain CDP at each dose level significantly increased the time devoted to eating familiar food. Hence, at the two lower dose levels in this strain CDP increased the response to familiar food without significantly affecting the time spent eating the novel foods (Fig. 3b). In contrast, in the A2G strain administration of CDP at the two lower doses did not affect their response to the familiar food (Fig. 3a). In effect, CDP raised the duration of feeding directed towards familiar food in the C57 mice to a level which was closer to that of the A2G mice. At the highest CDP dose (10.0 mg/kg) the A2G showed a decline.

Discussion

In C57 mice, CDP reduced the latency to begin eating and prolonged the time spent feeding in a food-preference test. The increase in feeding duration was due mainly to an increase in the duration of individual eating episodes, and not to significant change in the number of episodes. CDP treatment greatly increased the time spent eating familiar chow; at 2.5 and 5 mg/kg doses this effect occurred without an increase in the time spent eating the novel foods. These effects of CDP in C57 mice have also been reported for the rat (Cooper and Crummy, 1978; Cooper et al., 1977). They are consistent with an action of CDP to increase the level of feeding motivation. In recent experiments in the rat (Cooper and Posadas-Andrews, unpublished results) increasing the level of food deprivation also resulted in a reduction in latency to eat and a substantial increase in the time spent eating familiar chow without change in the time spent eating novel foods.

In contrast, the A2G mouse strain did not show a reduction in eating latency or an increase in the time spent eating after CDP treatment (except for an increase at 5 mg/kg). CDP did not increase the time spent eating familiar food in this strain. The difference between the two strains is most likely that the A2G animals are more hungry as a result of overnight food deprivation than the C57 mice. This explains why A2G mice were quicker to begin feeding and, once they had started, continued to eat longer than the C57 mice.

Under the non-drugged condition A2G mice behaved in a more food-directed manner than C57 mice; their behaviour finds a parallel in that of well-deprived rats (Cooper and Posadas-Andrews, unpublished results). In effect, CDP made C57 mice more hungry, so that they tended to respond to food like the A2G mice.

At the highest dose level CDP increased the time spent eating novel foods in both strains to the same degree. Hence, the two strains did not differ in this respect, suggesting that the effect was not directly linked to the animals' level of hunger. Instead, the effect is consistent with the proposal that benzodiazepines can overcome food neophobia (Poschel, 1971), provided, as the present experiment indicates, that a sufficiently high dose is used.

Our results agree with others which have demonstrated that CDP facilitates feeding responses in mice (Soubrié et al., 1975; Stephens, 1973). However, they emphasize that the facilitation will depend on the mouse strain, and importantly, the effect of CDP on choice of food will depend on the mouse strain.

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