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Social Learning of a Food Preference in Male and Female Mongolian Gerbils is Facilitated by the Anxiolytic, Chlordiazepoxide

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CHOLERIS, E., P. VALSECCHI, Y. WANG, P. FERRARI, M. KAVALIERS AND M. MAINARDI. *Social learning of a food preference in male and female Mongolian gerbils is facilitated by the anxiolytic, chlordiazepoxide.* PHARMACOL BIOCHEM BEHAV **60**(2) 575–584, 1998.—Social transmission of a food preference in Mongolian gerbils *(Meriones unguiculatus)* depends on the presence of a social bond between the interacting animals. An "observer" gerbil can acquire a preference for a novel food item from a familiar and, or related "demonstrator" animal. However, exposure to an unfamiliar and unrelated demonstrator gerbil does not lead to acquisition of a food preference, even though the extent of social interaction and likelihood of transmission of food information is unaffected. Likewise, individual preexposure to a novel food does not affect diet preference in individual animals. Here we show that oral, nongavage, administration of the benzodiazepine anxiolytic, chlordiazepoxide (CDP, 2.5, 5, and 10 mg/kg) has significant dose-associated differential facilitatory effects on social learning in male and female gerbils, while having no significant effects on either individual learning or total food consumption. These results suggest that the CDP mediated reduction of the anxiety associated with the interactions between unfamiliar/unrelated gerbils facilitates social learning. These findings also rise the possibility of sex differences in socially related anxiety and the effects of CDP on social learning in gerbils. © 1998 Elsevier Science Inc.

Anxiety Behavioral interactions Benzodiazepines Chlordiazepoxide Feeding Food intake Gerbils Neophobia Sex differences Social learning

SMALL rodents are continuously faced with the problem of selecting an appropriate diet. They can, through "trial-and-error" individual learning, develop a preference for metabolically appropriate and an aversion to inappropriate foods (19). Social learning has also been shown to be involved in determining dietary and food preferences. Results of a number of studies have shown that diet-related information can be obtained during brief interactions with a recently fed conspecific. In initial studies Galef and co-workers showed that after a naive rat (*Rattus norvegicus*), an "observer," interacted with a recently fed conspecific, a "demonstrator," the observer exhibited a clear preference for the food of the demonstrator [(24,25); for recent reviews, see (22,23)]. This capability of extracting dietary preferences through social learning has been subsequently demonstrated to occur in several species of social rodents, including the Belding's ground squirrel, *Spermophilus beldingi* (46), the spiny mouse, *Acomys cahirinus* (39), the house mouse, *Mus musculus domesticus* (58), and most recently, the Mongolian gerbil, *Meriones unguiculatus* (61).

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In rats and mice social learning of food preferences takes place between both familiar and unfamiliar individuals (25,59). In contrast, in Mongolian gerbils the social transmission of a food preference apparently depends on the presence of a social bond between the interacting animals. Either genetic relatedness or prior familiarity seems to be necessary for the transfer of food information and social learning. Exposure to an unfamiliar and unrelated demonstrator gerbil does not lead to the acquisition of a subsequent food preference (61). As well, gerbils display a high neophobic response to novel food items and flavors (65) and in brief laboratory exposure show no individual acquisition of food preferences (61).

In the wild, gerbils form stable, monogamous pairs that live with related juveniles through the reproductive season. Families occupy nonoverlapping territories with the reproductive pair aggressively excluding nonresidents (2,4,55). Likewise, in the laboratory unfamiliar same-sex gerbils are highly aggressive to one another (3,45).

Aggression between demonstrator and observer may contribute to the lack of social learning of a food preference between unfamiliar/unrelated gerbils (61). It is possible that the aggression-associated behaviors reduce the amount of time the observers spend investigating the demonstrator's muzzle. Consequently, the exposure to the odor of the food carried by the demonstrator might not be of a sufficient duration for the observer to acquire the diet-related information.

Alternatively, socially induced anxiety may interfere with the acquisition of a food preference from an unfamiliar/unrelated conspecific. Results of investigations with laboratory mice and rats have suggested that aggressive interactions are associated with heightened anxiety (25). Anxiety has been associated with a variety of behavioral responses including the increased avoidance of novel environmental factors (10,11, 27). Anxiety has also been shown to affect performance of a number of laboratory spatial learning tasks, including the Morris water maze and radial maze (47,52). These effects have been further suggested to be sexually dimorphic with females being more susceptible than males to the adverse effects of anxiety on learning [e.g., (47)]. This raises the possibility that anxiety may also influence social learning and contributes to the lack of social transmission of food preference between unfamiliar gerbils.

Anxiolytics such as the benzodiazepine agonist, chlordiazepoxide (CDP), have been shown to reduce various anxiety related behavioral responses associated with aggressive interactions and other anxiogenic situations (25,49). Anxiolytics, including CDP, are also reported to reduce neophobic responses and avoidance to novel environmental conditions (27). However, whether anxiolytics have any effects on, either social learning or individual learning of food preferences, in gerbils and other species of rodents, is not known.

In the present study we considered the effects of CDP on social learning and related behaviors. We examined the effects of CDP on the social acquisition of a food preference by an observer gerbil interacting with an unfamiliar demonstrator. In view of the evidence suggesting sex differences in anxiety and learning (47,48), the effects of CDP were examined in both male and female observers. In addition, we examined whether CDP affected individual learning and the expression of the neophobic responses of gerbils to novel food.

METHOD

Animals

Male and female Mongolian gerbils approximately 3 months of age, weighing 50–70 g and with no significant sex difference in body weights, were used as subjects. Animals were housed in same-sex sibling groups (two to three animals per group) in Plexiglas cages (58 × 38 × 20 cm) at 20 + 1°C, under a 12 L:12 D cycle (lights 0600–1800 h), with food (Mil Morini Rodent Chow, Morini, Reggio Emilia, Italy) and water available ad lib. Gerbils were from an outbred stock (Tumblebrook Farm, MA) reared in our laboratory in Parma. Outbreeding was carefully maintained by pairing only totally unrelated animals. At 30 days of age gerbils were removed from their parents and placed in same-sex groups. All studies with the gerbils were performed in accordance with EEC/Italian requirements.

Flavored Diets

Standard powdered mouse food (Mil Morini Rodent Chow, Morini, Reggio, Italy) was made up to contain either 2% cocoa powder (COC diet) or 1% cinnamon powder (CIN diet). Results of prior unpublished studies had established that the two diets, which had equivalent metabolic and physical features, were of equal palatability to this population of gerbils.

Oral Drug Administration

In contrast to laboratory mice and rats, gerbils can be easily stressed by experimental handling. Subcutaneous and intraperitoneal injections can elicit a range of aversive responses including seizures [(56) for review]. These adverse effects of injections are not, however, limited just to gerbils. Sham-injected laboratory mice have also been reported to display some of the behavioral characteristics of stressed or anxious animals (38). Therefore, to avoid any possible confounding anxiogenic effects of injection an oral, nongavage, drug administration procedure was developed and used in the present study. The oral administration of benzodiazepines, although not common in laboratory animal studies, is extensively used in the clinical treatment of anxiety (15). Results of those investigations have revealed a rapid central uptake of benzodiazepines, such as chlordiazepoxide, comparable to that evident after peripheral injections.

In the week prior to the treatment with the experimental drug gerbils were trained to spontaneously drink from a syringe. During this training same-sex groups of two to three gerbils were kept under a light water deprivation: on days 1 and 2, twice a day (0900 and 1500 h), they were given a 1-h access to a water bottle containing a 0.5 M sucrose solution (in 0.9% saline). On days 3–6 of training, gerbils were, twice a day, provided access to a syringe (1 ml, without needle) containing the 0. 5 M sucrose–saline solution. The gerbils spontaneously inspected the syringe and drank the contents, which was gently "squeezed" into their mouths by the experimenter. Each gerbil was given as many syringes of solution as it would consume. After drinking from the syringes gerbils were given a 1-h access to a sucrose–saline bottle. On day 7 the trained animals were given ad lib access to the sucrose–saline solution during the light period, with no fluid available during the dark period. At the end of the training period the gerbils quickly drank the content of a syringe without any spillage.

Procedures

Experiment 1—social learning. Unfamiliar and unrelated male and female gerbils, arbitrarily designated as "demonstrators" and "observers," were individually housed for a total of 24 h. During this period demonstrator gerbils were food de-

prived for the first 16 h, while the observer animals were water deprived with free access to food. At the end of this 16-h period a weighed feeder [3 × 4.5 × 4.5 cm; for details of feeders, see (60)] containing the COC or CIN diet was presented for 6.5 h to each demonstrator held in a 25 × 38 × 15 cm Plexiglas cage. Individual food intakes were determined by weighing the feeder on a balance accurate to 0.01 g. Only gerbils that ate at least 0.30 g of the flavored food (CIN or COC) over the 6.5-h measurement period were used as demonstrators.

While the demonstrators were consuming this novel diet the standard food of the observers was removed. To limit possible water deprivation-induced stress, observers were orally administered 1 ml of the 0.5 M sucrose–saline solution. Six hours later each food-deprived observer was orally administered either chlordiazepoxide hydrochloride (CDP, Roche Spa, Italy), or the sucrose–saline vehicle (10 ml/kg). Doses and sample sizes used were: CDP 2.5 mg/kg: males = 16, females = 20; CDP 5 mg/kg: males = 18, females = 17; CDP 10 mg/kg: males = 18, females = 15; vehicle: males = 13, females = 14. In view of the lack of any previous studies with gerbils, these doses of CDP were established on the basis of the results of studies of anxiety (10), behavioral interactions (16,20), and learning (42,64) in rats and mice.

Thirty minutes after drug treatment observers and demonstrators were allowed to interact. Demonstrators just fed on either a COC or CIN diet and the unfamiliar/unrelated observer (male–male and female–female pairings only) were placed on opposite sides in a Plexiglas cage (27 × 14 × 14 cm) that was divided into half by a wire mesh partition. The demonstrators and observers were allowed to interact across the mesh for 20 min. Results of prior investigations had established that the wire mesh did not impair social acquisition of food preference in familiar and related gerbils (61). After the social interactions, the observer gerbils were individually placed in a cage provided with two feeders, one containing the COC-flavored diet and the other the CIN-flavored diet. Total intakes of the two diets were determined after 2, 16, and 24 h. The feeders had been previously shown to be reliable for the determination of food intakes of as little as 0.10 g. Observers that failed to eat at least 0.10 g of one of the two diets were considered to have "zero" intake.

All of the social interactions were videotaped and the behaviors that the observer displayed towards the demonstrator through the wire mesh were scored. Ten videotapes from each group were randomly chosen (approximately one-third of the total animals recorded) and scored with ethological software (Eva, developed in this Department by Dr. Marco Lugli) by a trained researcher who was blind to the group designation. The amount of time the observer gerbil spent investigating the muzzle of the demonstrator was scored. This behavior was chosen because the contextual cues responsible for social enhancement of animal's food preferences are indicated to be contained in the demonstrator's breath (26,58). In addition, the time spent by the observer at the wire mesh when the demonstrator was not present at the mesh was scored. This behavior was considered to be indicative of the degree of interest expressed by the observer in the demonstrator.

Experiment 2—individual learning. Individually housed male and female gerbils, which were naive to COC and CIN flavored food, were trained to consume fluid from a syringe according to the previously described procedure. The animals were water deprived overnight, but had food freely available. To limit any possible water deprivation-induced stress, 6 h before drug treatment, when the food was removed, the gerbils were provided 1 ml of the 0.5 M sucrose–saline solution. After the 6 h of food deprivation the gerbils were orally administered chlordiazepoxide (2.5, 5.0, or 10 mg/kg) 10 ml/kg or the sucrose vehicle (10 ml/kg). 30 min after drug or vehicle administration (CDP 2.5 mg/kg: males = 19, females = 13; CDP 5 mg/kg: males = 13, females = 15; CDP 10 mg/kg: males = 16, females = 13; vehicle: males = 14, females = 15) gerbils were individually placed in a clean cage with a cylindrical Plexiglas feeder (3.5 cm diam, 6.5 cm height) filled with either a COC- or a CIN-flavored diet. The flavored diets were covered with wire mesh that allowed the gerbils to smell a food for 20 min but prevented them from eating it. During this 20-min period, all gerbils were videotaped to subsequently score the amount of time spent sniffing the novel food through the wire mesh. A researcher who was blind to the experimental treatments scored 10 randomly chosen tapes from each treatment (approximately one-third of the total animals recorded). The gerbils were then individually placed in a cage provided with two weighed feeders, one containing the COC-flavored diet and the other the CIN-flavored diet. Total intake of the two diets was determined after 2, 16, and 24 h. Subjects that failed to eat at least 0.10 g of one of the two diets were considered to have "zero" intake.

Data Analysis

Food preference of the observers from Experiments 1 and 2 were expressed as the percentage of CIN diet eaten out of the total food consumed. The arcsine transformed percentages were analyzed by a mixed-design repeated-measures analysis of variance (ANOVA) consisting of three between-group factors: demonstrator sex (two levels; males and females), demonstrator/familiar food (two levels; COC and CIN), and treatment (four levels; CDP 2.5, 5.0, and 10 mg/kg and vehicle) and one within-group factor: percentage of CIN (three levels; 2, 16, and 24 h). Food preferences of male and female observer gerbils of the four treatment groups at each time interval were analyzed with a three-way multivariate analysis of variance (MANOVA). Least mean squares comparisons were carried out to determine the source of differences found in the MANOVA model.

Total food intake of the observers in Experiments 1 and 2 was analyzed with a two-way ANOVA with sex (two levels; male and female) and treatment (four levels; CDP 2.5, 5.0, and 10 mg/kg and vehicle) as between-subject factors and total food intake as a repeated-measure within subjects factor (three levels; 2, 16, and 24 h). Because food intake displayed a Poissonian distribution the data were square-root transformed before analysis (66). As there were some zero intakes, 0.50 was added to all values before transformation.

The total duration of the behaviors collected from the observers during the social interactions in Experiment 1 (demonstrators muzzle's investigation and time spent at the mesh) and Experiment 2 (cup investigation) were analyzed with a two-way ANOVA with treatment (four levels; CDP 2.5, 5.0, and 10 mg/kg and vehicle) and sex (two levels; male and female) as dependent variables and total duration of the behavior as independent variable. Mean comparisons were planned a priori in the ANOVA model. Because behavioral data displayed an asymmetrical distribution, durations were natural log-transformed (ln) before statistical analysis. As there were some zero values, 1.0 was added to all data before transformation (66).

A further analysis was run to compare the time gerbils were exposed to the food related-information in the two experiments. With a three-way ANOVA (dependent variables: sex, treatment, and experiment) the time the observers spent investigating the

demonstrator muzzle (Experiment 1) was compared to the time subjects of Experiment 2 spent sniffing the food cup. Mean comparisons were planned a priori in the ANOVA model.

All analysis were performed with SuperANOVA computer package (1) with $\alpha = 0.05$ used as the criterion for significance.

RESULTS

Experiment 1—Social Learning

Observers' food preference. The effects of CDP on the social transmission of a food preference in unrelated/unfamiliar

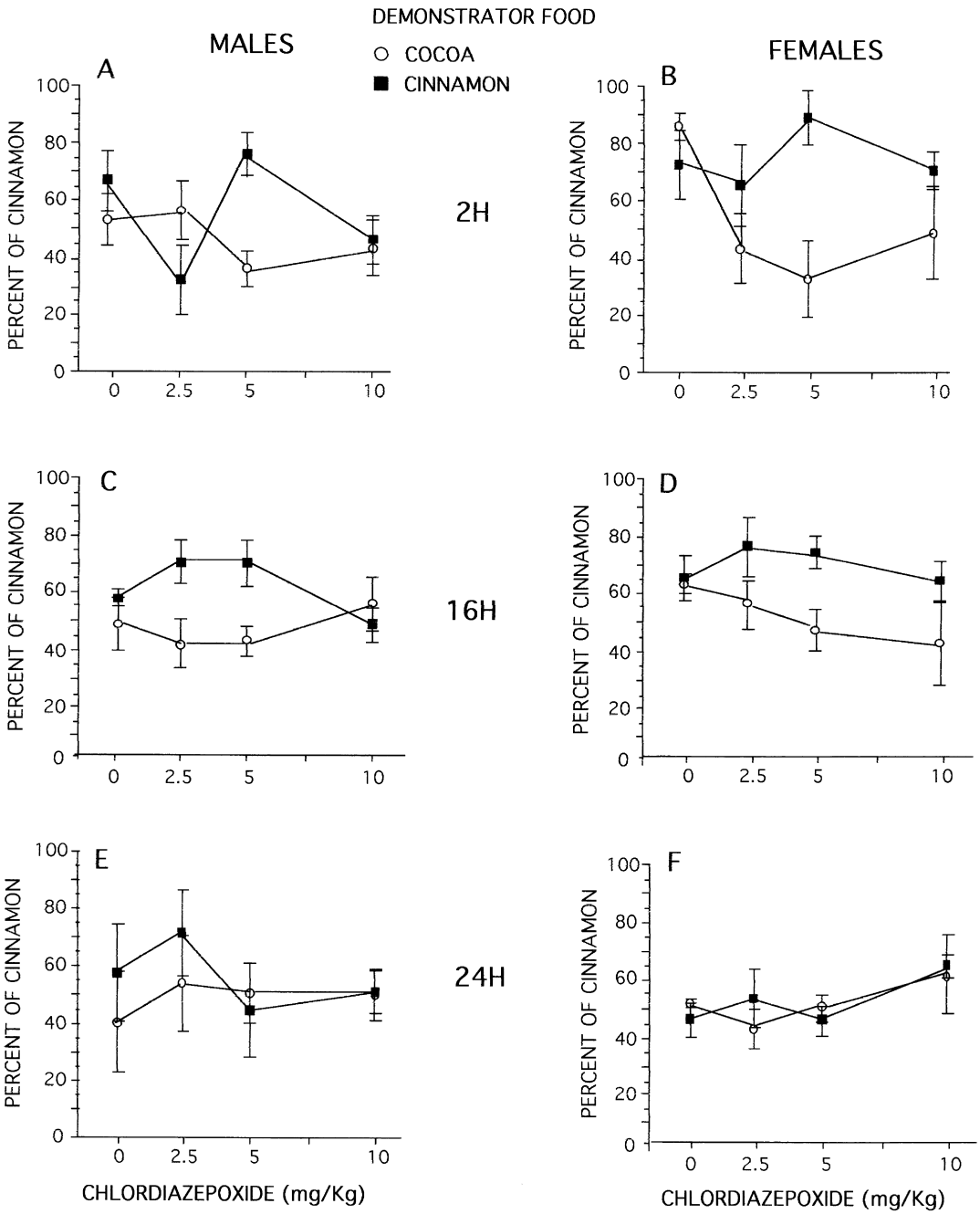

FIG. 1. (A–F) Effects of chlordiazepoxide on the mean amount of cinnamon diet ingested, as a percentage of total amount eaten by male (A, C, E) and female (B, D, F) observers gerbils 2, 16, and 24 h after the social interaction with an unfamiliar and unrelated same-sex demonstrator. Bars indicate SE. (COC = cocoa diet; CIN = cinnamon diet). In observers that were exposed to a cinnamon demonstrator, an increase in the percent of cinnamon diet consumed indicates an enhanced preference for the demonstrator diet (CIN), whereas in observers that were exposed to a cocoa demonstrator, a decrease in cinnamon percent indicates an enhanced preference for the demonstrator diet (COC).

male and female gerbils are shown in Fig. 1A–F. Data is presented as the percent of cinnamon diet consumed by an observer gerbil that was exposed to an unfamiliar/unrelated demonstrator that had previously ingested either cocoa (COC)- or cinnamon (CIN)-flavored diet. In observers that were exposed to a cinnamon demonstrator, an increase in the percent of cinnamon diet consumed indicates an enhanced preference for the demonstrator diet (CIN), whereas in observers that were exposed to a cocoa demonstrator, a decrease in cinnamon percent indicates an enhanced preference for the demonstrator diet (COC).

The overall ANOVA revealed a significant interaction of percent of cinnamon diet consumed at different times (2, 16, 24 h) × treatment (CDP) × demonstrator food (COC, CIN), $F(6, 168) = 2.71$, $p = 0.02$. This indicates that the acquisition of a food preference by the observers was affected by the demonstrator's food, CDP treatment, and time after treatment.

Two hours postsocial interaction (Fig. 1A–B) there was a highly significant effect of the interaction of sex × treatment × demonstrator food, $F(3, 84) = 4.41$, $p = 0.006$. Mean comparisons (COC demonstrator vs. CIN demonstrator) showed that the CDP at 2.5 mg/kg affected the observers diet selection in males, but not in females (males, $t = 2.84$, $p = 0.006$; females, $t = 1.53$, NS). At this dose, male observers preferred food that was opposite to that eaten by their demonstrator. This was primarily due to a decrease in the percent of cinnamon eaten by male gerbils exposed to cinnamon demonstrators (CDP at 2.5 mg/kg-treated males vs. vehicle-treated males: $t = 2.00$, $p = 0.049$). CDP at 5 mg/kg significantly enhanced the preference displayed by the observers for the demonstrator's food in both sexes (COC demonstrator vs. CIN demonstrator: males, $t = 2.98$, $p = 0.004$; females, $t = 2.34$, $p = 0.02$).

Sixteen hours postsocial interaction (Fig. 1C–D) the CDP at 2.5 mg/kg significantly enhanced the male observer's pref-

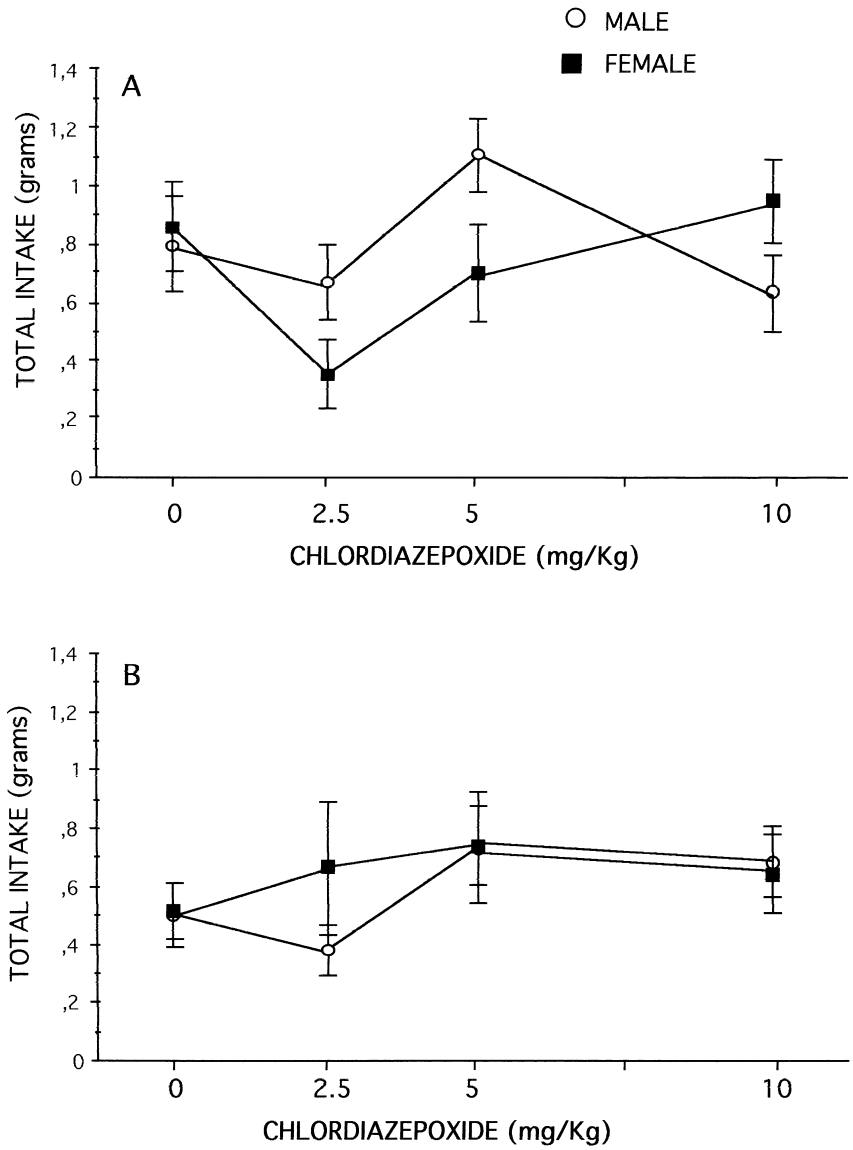

FIG. 2. (A–B) Effects of chlordiazepoxide on the mean amount of total food eaten by gerbils 2 h after a social interaction with an unfamiliar/unrelated conspecifics (A) or after individual exposure to a novel food (B). Bars indicate SE.

erence for their demonstrator's food, while in females this enhancement approached significance (COC demonstrator vs. CIN demonstrator: males, $t = 2.58$, $p = 0.01$; females, $t = 1.88$, $p = 0.06$). CDP at 5 mg/kg enhanced the observer's preference for their demonstrator's food in both sexes (males, $t = 2.10$, $p = 0.004$; females, $t = 2.76$, $p = 0.007$).

In the last 8 h (from 16 to 24 h, Fig. 1E–F) observers of all groups and treatments did not display any enhanced preference for their demonstrator's food.

Vehicle and 10 mg/kg CDP-treated male and female observers showed no significant preference for their demonstrator's food at any time after treatment (Fig. 1A–F). All of the animals in these groups, apart from the vehicle-treated females, equally preferred the COC and CIN diets. The vehicle-treated females showed an initial preference for the CIN diet that was, however, not lowered by interaction with a COC-fed demonstrator. These results from the control group (vehicle, Fig. 1A–F) are consistent with prior findings showing that untreated male and female observer gerbils do not acquire diet-related information from an unfamiliar and unrelated demonstrator (61).

Total food intake. As the total food intake at 16 and 24 h after treatment revealed no significant treatment or sex effects, a second analysis (two-way ANOVA) was run only on the 2-h food intakes. The effect of CDP on the total amount of food ingested by male and female gerbils 2 h after social interactions is shown in Fig. 2A. CDP did not cause any significant dose-dependent pattern of increase in food intake. There was a main effect of treatment, $F(3, 120) = 2.89$, $p = 0.04$, as well as an interaction between sex and treatment, $F(3, 120) = 2.93$, $p = 0.04$.

CDP at 2.5 mg/kg significantly decreased the food intake of the females but not of the males (CDP at 2.5 mg/kg-treated vs. vehicle-treated: males, $t = 0.22$, NS; females, $t = 2.49$, $p = 0.01$. The intakes of males and females tended to be different at this dose ($t = 1.91$, $p = 0.058$). The dose of 5 mg/kg did not increase food intakes in either sexes. However, intakes of the two sexes were significantly different at this dose, with males consuming significantly more food than females ($t = 2.25$, $p = 0.033$). CDP at 10 mg/kg and vehicle had no significant effect on food intake.

Behavioral interactions. The ANOVA run on the time observer spent investigating the demonstrator muzzle through the screen revealed neither significant main effects (treatment and sex), nor an interaction of sex × treatment [treatment: $F(3, 34) = 0.46$, NS; sex: $F(1, 35) = 0.28$; treatment × sex: $F(3, 35) = 1.196$] (not shown). Male and female observer gerbils in the four treatment groups investigated the demonstrator's muzzle to the same extent.

The analysis run on the time observers spent at the mesh in the absence of a direct contact with the demonstrator revealed a significant effect of treatment, $F(3, 34) = 107.243$, $p = 0.0001$. Mean comparisons showed that male and female observers treated with CDP at 5 mg/kg spent significantly more time at the mesh and interest in the demonstrator than vehicle-treated gerbils [males: $F(1, 17) = 106.965$, $p = 0.0001$; females: $F(1, 16) = 107.137$, $p = 0.0001$] (not shown).

Experiment 2—Individual Learning

Food preference. Figure 3A–F shows the overall percentage of CIN diet eaten by the animals of the four groups in the 24 testing hours. Male and female gerbils did not show any significant preference for the food they had previously encountered [treatment: $F(3, 144) = 1.73$, NS; familiar food: $F(1, 144) = 0.38$, NS; sex: $F(1, 144) = 6.12$, NS).

Total food intake. As the total food intake at 16 and 24 h after treatment revealed no significant treatment or sex effects, a second analysis (two-way ANOVA) was run only on the 2 h food intakes. The analysis revealed no significant main effects (treatment and sex), or interaction of sex × treatment [treatment: $F(3, 114) = 1.25$, NS; sex: $F(1, 114) = 0.53$] (Fig. 2B).

Novel food investigation. The total time spent investigating the wire mesh placed on top of the feeder containing the novel food was not affected by CDP treatment, $F(3, 34) = 1.59$, NS in either sexes, $F(3, 34) = 1.42$, NS (not shown). The time gerbils in Experiment 2 spent sniffing the novel food through the wire mesh was not significantly different from the time observers in Experiment 1 spent in sniffing the novel food from the muzzle of the demonstrator through the mesh partition, $F(1, 69) = 0.22$, NS (not shown).

DISCUSSION

The results of the present study with male and female Mongolian gerbils showed that the putative anxiolytic, chlordiazepoxide (CDP), had facilitatory effects on the learning of a food preference from a strange conspecifics but not through individual preexposure to the food in male and female gerbils. In rats and mice social learning of a food preference takes place between both familiar and unfamiliar animals (25,59). In contrast, the social transmission of a food preference in gerbils appears to depend on the presence of a social bond between the interacting animal, with either genetic relatedness or prior familiarity necessary for the transmission of information and social learning. As shown here with vehicle-treated, and previously (61) in untreated observer gerbils, exposure to an unfamiliar and unrelated gerbil does not lead to the acquisition of a subsequent food preference. However, oral administration of CDP elicited social learning and the acquisition of a food preference by "observer" gerbils that were unfamiliar/unrelated to the "demonstrator" animal. Moreover, the temporal duration and the degree of the preference for the demonstrator food expressed by observer's treated with CDP (dose 5 mg/kg) was not different from the preference expressed by untreated observers that spontaneously acquired a diet preference from a demonstrator [familiar and/or related, (61)]. In both cases the acquired dietary preference was no longer evident after 24 h. The experimental procedures (e.g., individual housing) used with the familiar and unfamiliar gerbils were identical and, thus, unlikely to contribute to the differences in social learning. This induction of social learning was also independent of any alterations in individual learning or total food intake, CDP having no consistent, significant effects on the latter two.

These facilitatory effects of CDP on the acquisition of the novel diet preference by the observer were not related to an enhanced exposure to the food stimulus (odor) carried by the demonstrator (20). Vehicle-treated and CDP-treated observer gerbils spent equivalent amounts of time investigating the muzzle of their demonstrator. The observers were, therefore, exposed to similar amounts of transfer of information ["odor" on breath: (26)] about the diet. It may be that CDP affected the nature of the social interactions and reduced the levels of agonistic behavior-related stress and anxiety. A reduction in anxiety would be supported by the heightened interest expressed by the CDP (5 mg/kg)-treated observer towards the demonstrator as measured by time spent at the wire mesh. This may reflect a heightened social interest [e.g. (20)]. Social interactions between unfamiliar and unrelated gerbils are characterized by high levels of aggression and social stress

(3,28,45). Results of investigations with various other species of rodents have shown that agonistic interactions are associated with social stress and an accompanying enhancement of anxiety and anxiety-related behavior (5,49).

Stress has been shown to influence learning in animals, mainly through an inhibitory effect. Acute nonsocial stress has been shown to have inhibitory effects on learning in several species of rodents (33,52,63), with the extent of the impairment dependent on the nature of the learning task and the species/strain of animal examined (21). In contrast, relatively little is known about the effect of socially induced stress/anxiety on learning. Chronic social stress has been shown to impair spatial working memory in rats tested in a 14 radial-arm maze (17). However, in that study the stress manipulations of rats involved not only social exposure, but also a conditioning procedure and a forced cold water swimming.

The facilitatory effects of chlordiazepoxide (5.0 mg/kg) on social learning observed here could be related to a reduction in stress and anxiety. However, this enhancement of social learning is at apparent variance with the negative effects of

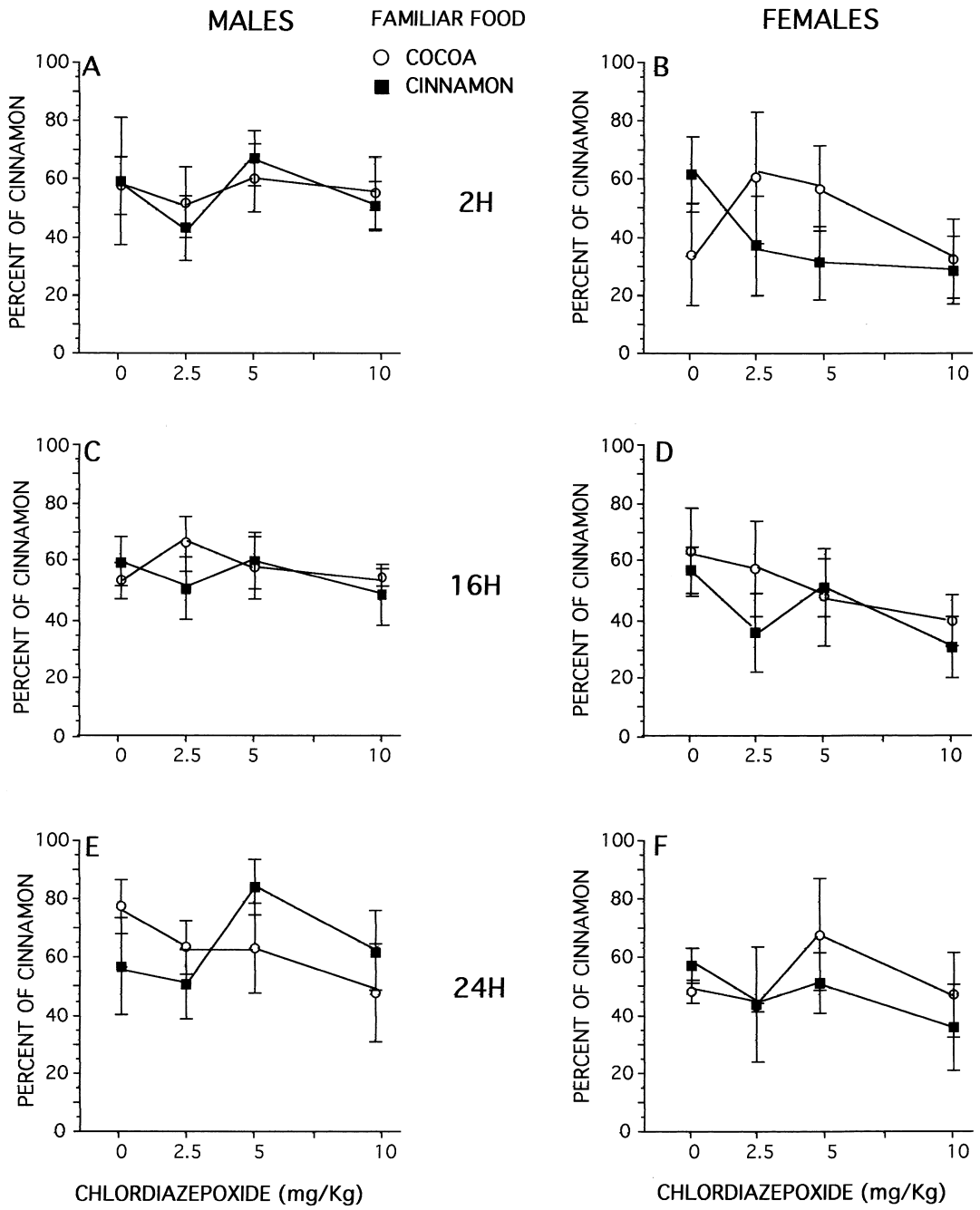

FIG. 3. (A–F) Effects of chlordiazepoxide on the mean amount of cinnamon diet ingested, as a percentage of total amount eaten, by male (A, C, E) and female (B, D, F) gerbils 2, 16, and 24 h after they were individually preexposed to the odor of a novel diet. Bars indicate SE. (COC = cocoa diet; CIN = cinnamon diet).

benzodiazepines reported for other learning tasks [see reviews: (36,42)]. For example, Venault et al. (62) found that the benzodiazepine, diazepam, impaired avoidance learning in mice. Similarly, CDP and diazepam were indicated to reduce learning in the water maze task (40,41,43). In two conditioned avoidance learnings (discrete lever-press avoidance and discrete shuttle avoidance) male gerbils were shown to be more sensitive to the diazepam-induced impairment of learning than rats and mice (37,57). These learning tasks are largely aversively motivated, and performance on them is proposed to be dependent on the activation of hypothalamic–pituitary–axis, whose activity is reduced by benzodiazepines such as CDP [see review (36)]. The effects of benzodiazepines on performance in the radial maze, a less aversively motivated task, are more ambiguous, with either no effects or slightly reduced performance reported (29,30,64). In the present study the social learning paradigm was substantially different with the acquisition of a food preference being a positively motivated learning task that requires the presence of a conspecifics. In addition, assessment of performance (feeding) was carried out immediately after the social interaction (1 h from CDP treatment), whereas in other tasks learning was assessed after a substantial delay (36). Thus, in the present study, the amnesic effects of benzodiazepines that are typically reported in studies on learning and memory (i.e., (36)] were unlikely to be observed. These methodological and motivational differences may account for the positive effects of CDP on social learning observed here.

The absence of social learning in gerbils treated with CDP at 10 mg/kg may be due to a reduced or altered efficacy of this higher dose. Anxiolytics such as CDP display a classic U-shaped dose–response curve, with lower effectiveness at higher doses in their effects on nonsocial and social behaviors (16,18). In addition, benzodiazepines at higher doses may display sedative effects as well as possible nonspecific interactions with other neurotransmitter systems that could interfere with social learning [e.g., (53)]. It should be noted that the lack of effect of CDP (10 mg/kg) on social learning cannot be attributed to lack of information transfer, with the observer and demonstrator displaying equivalent amounts of muzzle interactions after treatment with the various doses of CDP. Rather, it may reflect a lowered efficacy in promoting social interest of the observer in the demonstrator, as indicated by the low level of time spent at the wire mesh.

The finding that CDP at any dose did not elicit the development of a food preference in gerbils individually exposed to the odor of a novel diet indicates that CDP only influenced social learning. In both cases individual gerbils were exposed to the odor of the same novel diet in the same experimental setting and spent the same amount of time investigating the novel flavor. The only difference was that in the social learning experiment the information about the novel food was carried by a conspecific.

Mice exposed to the odor of a novel diet have been shown to develop a preference for that diet (59). In comparison to mice, gerbils appear to possess a greater food neophobia (61,65), which could not be countered here by CDP administration. This is in agreement with the finding that novel odor-induced analgesia is insensitive to benzodiazepine manipulations in deer mice, *Peromyscus maniculatus* (35). However, in balb/c mice novel place avoidance could be countered by the benzodiazepines CDP and diazepam (27). It may be that flavor/odor neophobia is benzodiazepine insensitive, whereas novel experimental setting is sensitive to benzodiazepines. This remains to be confirmed with other possibly more palatable flavors, odors, and diets.

The facilitatory effects of CDP on the acquisition of a food preference were not due to, or associated with, a simple increase in total food consumption. Moreover the present results on learning are presented as a percentage of total food intake and not an absolute intake. As such, they are independent of any possible nonspecific effects of CDP on food intake. In the present study there was no consistent pattern in, or effect of, CDP on total food consumption. Results of studies with a variety of species of mammals have reported that benzodiazepine cause dose-dependent increases in food consumption [see review (13)]. However, those increases in food intake are related to the characteristics of the food rather than any anxiolytic effects. In rats, benzodiazepines preferentially enhance the consumption of highly palatable familiar foods (e.g., chocolate chip cookies), with relatively little effect on other food items [(7,12,14,31,50); for a recent review, see (6)]. In the present study the food used was standard laboratory powdered food, which is likely not of high palatability. As such, it is not surprising that CDP did not significantly enhance the total food intake of gerbils.

Sex differences have been reported in anxiety and stress-related behavioral responses in various other species of rodents [e.g., see (9,32,34,47,54,67)] and the effects of benzodiazepines and other anxiolytics (8,51). The patterns of male–female responses are, however, dependent on the nature of the behavioral measures used, as well as species and strain of rodent examined (21). The results of the present study suggested that there may also be sex differences in social-related anxiety and the effects of CDP on social learning in gerbils. Administration of the lowest dose of CDP (2.5 mg/kg), while having no significant effect on food acquisition by female observers, caused male observers to display initially a preference for the food opposite to that of their demonstrator. Results of studies with male rats have shown that diazepam and CDP reduce aggression at moderate to high doses, while at low doses they enhance aggression (44). Thus, the low dose of CDP through this enhancement of aggressiveness could have led to a negative association with, and aversion to, the demonstrator's food. Whether this also incorporates, or reflects, male–female differences in social learning and anxiety and sensitivity to CDP remains to be determined. The present study does, however, indicate that social learning of food preferences provides a useful and novel method for examining the impact of anxiety and anxiolytics on learning.

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