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Effects of Chlordiazepoxide on Maternal Aggression in Mice Depend on Experience of Resident and Sex of Intruder

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PALANZA, P., R. J. RODGERS, P. F. FERRARI AND S. PARMIGIANI. *Effects of chlordiazepoxide on maternal aggression in mice depend upon experience of resident and sex of intruder.* PHARMACOL BIOCHEM BEHAV **54**(1) 175–182, 1996.—Lactating mice respond differentially to intruders of differing sex, displaying defensive attack against the male and offensive attack against the female. Such a phenotypic dichotomy appears to have adaptive value in that unfamiliar males pose a much greater threat to the offspring than do females. The present study examined the effects of the benzodiazepine anxiolytic chlordiazepoxide (CDP) (2.5–10.0 mg/kg) on this differential response pattern in aggression-naive (nonscreened) (NS) and aggression-experienced (screened) (S) lactating female mice (*Mus musculus domesticus*) confronting intruders of either sex in a 10-min test. This procedure was used to evaluate the influence of both the type of opponent and previous aggressive experience on basal behavioural profiles and drug action. Results showed that both intruder sex and prior screening for attack modulated the behaviour of lactating females toward intruders. In turn, both variables strongly influenced CDP effects on maternal aggression. In particular, in S dams, CDP dose-dependently increased maternal attack against males but decreased attack against female intruders. Conversely, in NS dams, CDP decreased attack (and fear) against males but did not affect it against females. In both S and NS conditions, CDP modified the attack strategy of lactating females against the male, switching it from a defensive to an offensive pattern. Exploration, social investigation, eating, and immobility were differentially affected by the drug treatment, depending on screening and/or intruder sex condition. These differential effects of CDP between S and NS conditions, toward either male or female intruders, cannot be fully explained by differences in the baseline levels of these behaviours. Alternative hypotheses are discussed. These findings demonstrate that the effects of CDP on maternal attack behaviour depend on not only the drug but also the object of attack, and hence the function of attack and the prior experience of the attacker.

Maternal aggression Intruder sex Prior screening Offence Defence Fear Chlordiazepoxide Mice

FEMALE mice exhibit intense aggressive behaviour during lactation; this behaviour, referred to as maternal aggression, is a complex and heterogeneous phenomenon ranging in form from offensive to defensive attack and subserving a variety of functions according the context and characteristics of conspecific intruders (38,40). Specifically, analysis of bite target patterns has revealed that different patterns of attack are generated when lactating females confront sexually naive males and virgin female intruders. Sexually naive males are severely attacked by lactating females with bites primarily directed toward the head, ventral surface, and inguinal area (a defensive pattern), whereas virgin females are comparatively rarely bitten on such vulnerable regions of the body (an offensive pattern). Furthermore, female intruders elicit more social investigation than do males, whereas males evoke more responses indicative of fearfulness in lactating females than do intruder females (39).

The clear differentiation in attack patterns shown toward intruders of differing sex suggests a nonunitary nature of the underlying motivational substrates that, in turn, may reflect

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diverse functions of maternal attack. The defensive type of attack directed against the male (i.e., the sex which is more likely to kill pups) has been interpreted as a counterstrategy to infanticide (35,38,43); the offensive attack toward females may serve to establish a social hierarchy or space rivals of the same sex (36,39,40).

An analysis of motivational substrates in maternal aggression has been conducted using ethopharmacological techniques. Thus, the opioid antagonist naloxone reduced attack on females to a much greater extent than attack on males and induced more fear-related behaviour in response to male vs. female intrusion (42). The serotonergic agonist fluprazine selectively inhibited lactating female aggression toward female but not male intruders, without potentiating fear responses (41). The selective "antioffence" action of these drugs, as observed in other behavioural paradigms [e.g., (9,12,21,23,34)], suggests underlying differences in the neurochemical substrates for offensive and defensive attack. Importantly, our data also demonstrate the crucial importance of the object of attack in assessing the effects of pharmacological manipulations.

These studies indicated a remarkable difference in drug sensitivity between offensive and defensive attack patterns. In particular, our experiments showed that the offensive pattern of maternal attack is much more sensitive to the inhibitory effects of both naloxone and fluprazine than is the defensive pattern. In fact, only by increasing the doses of naloxone (by over five times) and fluprazine (by over 10 times) was attack on males decreased to a level comparable to that produced by the lower doses against female intruders.

In this context, it would be predicted that an opposite profile should be apparent when animals are treated with drugs that act primarily to reduce fear and anxiety. Specifically, these agents should affect the defensive attack pattern more profoundly. Benzodiazepines are a traditional class of drugs with antianxiety properties that have been documented to reduce aggressive behaviour in a wide variety of species [see (44) for a review]. This property seems to reflect a relatively greater benzodiazepine sensitivity of defensive behaviour rather than a nonspecific inhibitory action on attack. A wealth of literature supports the view that benzodiazepines inhibit defensive responding at doses much lower than those required to inhibit offensive behaviour [for a review, see (44,45)]. For example, Blanchard and co-workers (4) observed that at nonsedative doses chlordiazepoxide, diazepam and midazolam consistently reduce defensive threat and attack reactions in wild rats confronting predator-related stimuli. Dixon and Kaesermann (18) found that benzodiazepines consistently reduce defensive postures in intruder mice. Krsiak and colleagues (22) showed that drugs with anxiolytic properties, including diazepam and chlordiazepoxide, selectively inhibit defence behaviour in timid mice.

As maternal aggression toward male intruders is thought to be primarily defensively motivated (fear is a major element), benzodiazepine treatment should have the most pronounced effects on maternal attack against male intruders relative to female intruders (i.e., the opposite profile to that obtained with naloxone and fluprazine). In comparison to the more traditional male intrusion test, the characteristics of attack by lactating mice on male (defensive form) and female (offensive form) conspecific intruders provide a unique model for investigating the involvement of the GABA-benzodiazepine system in offensive and defensive forms of aggression.

Although the antiaggressive properties of benzodiazepines have been substantiated in many studies, it should be noted that low doses of some benzodiazepines can actually enhance aggression (18,28,32,46). Such proaggressive effects appear to depend on the basal level of aggression and test conditions as well as the experience and status of the animals used, and are thought to reflect reduced anxiety (4,29,44). Although prior social experience can strongly affect the propensity for individuals to show aggressive responses (6,7,11,48), the impact of previous experience on the effects of benzodiazepines on aggression in lactating females has not been systematically studied [e.g., (31–33,46,47)].

Against this background, we aimed to investigate the effects of chlordiazepoxide on offensive and defensive attack patterns in lactating mice. Moreover, we considered as an independent variable the aggressive characteristics and experience of animals, by using an independent experimental design with aggression-inexperienced dams and dams screened for attack toward either female or male intruders.

METHOD

Animals and Husbandry

Subjects were outbred Swiss albino mice (CD1), derived from a stock originally purchased from Charles River Italia (Calco, Lecco). At weaning (28–30 days), animals were housed with same-sex peers in groups of eight to 10 in transparent Plexiglas cages (55 × 33 × 20 cm). They were maintained in a temperature-controlled environment (22–24°C) on a 12 L : 12 D cycle (lights on at 0700 h).

Lactating females (n = 246) were obtained by mating virgin females aged 80–90 days with sexually naive males of the same age in breeding cages (27 × 13 × 12 cm). Males were removed when their partners were evidently pregnant, approximately 1 week before parturition.

Two days after delivery, females were randomly assigned to one of two main experimental groups: One group was screened for aggression against conspecifics intruders of both sexes (screened) (S); the other was left undisturbed until testing (nonscreened) (NS). Group-housed (eight to 10 per cage) sexually naive male and female mice served as stimulus animals in all intruder tests, and these animals were used once only.

Drugs

Chlordiazepoxide hydrochloride (CDP; Sigma, St. Louis, MO) was dissolved in physiological saline (0.9%), which alone served for control injections. All injections were administered intraperitoneally (10-ml/kg injection volume) 20 min before testing.

Screening Test for Aggression

At 48 h after parturition, dams in the S condition (n = 130) were confronted in their home cages with a male or female intruder. Those residents that attacked and delivered at least one bite to the opponent in a 3-min test were considered aggressive, and the test was immediately terminated. To avoid the possibility of attacks on pups by male intruders (which might influence dams' responses) (36), the experimenter discouraged males from entering the nest by gently pushing them away with a short wooden dowel. As a result of this screening test, 56 animals were obtained that would readily attack male (n = 28 of 39 tested dams, 71%) or female (n = 28 of 90 tested dams, 32%) intruders. These aggressive dams remained in their home cages for a further 24 h and were then transferred to the experimental apparatus.

Apparatus and Procedure

Each apparatus consisted of two chambers (each 40 × 20 × 20 cm), linked by a tunnel (50 cm long) that could be closed at either end by removable barriers. The floor of the apparatus was covered by sawdust, and food and water were freely available. The external walls of the chambers and the tunnel were made of transparent Plexiglas to allow observation and videotape recording (39). The VHS videocamera and videocassette recorder were situated 3 m distant from and 0.5 m above the apparatus.

Testing was conducted when pups were 4 days old. Twenty-four h before testing, dams (S and NS) along with their litters and nest material were introduced into individual test apparatus. On test days, the dams were further randomly allocated to four treatment conditions: saline [n = 33 (NS) and n = 14 (S)], 2.5 [n = 28 (NS) and n = 14 (S)], 5.0 [n = 28 (NS) and n = 14 (S)], and 10.0 [n = 27 (NS) and n = 14 (S)] mg/kg CDP. Within each of these conditions, NS animals were further randomly allocated to receive a sexually naive male or female intruder. S animals were confronted with an intruder of the same sex as that encountered in the screening test.

Testing commenced by closing the ends of the tunnel, thereby confining the resident dam to the nest chamber. An intruder was then placed into the opposite chamber, and 3 min later, tunnel barriers were removed, allowing free interaction between the animals. Each resident was tested once only in a 10-min videotaped encounter, and the test duration was timed from the initial social contact.

Behavioural Analysis

Behaviour was scored off videotape by a trained observer who remained blind to treatment conditions until data analysis was complete. Data were logged by a series of electronic counters and timers. With the exception of attack latency and bite frequency, all measures were scored as duration (s). Because attempted infanticide resulted in the premature termination of behavioural testing, duration data were recalculated as a proportion of total session duration (i.e., percent of time measures).

The following categories and elements of behaviour were recorded: a) proportion of intruders attacked; b) latency to attack (i.e., time from initial contact to first biting attack); c) accumulated attacking time [i.e., total duration of biting attack (AAT)]; d) number of bites; e) percentage of bites to vulnerable (head/ventrum) body regions; f) fear-related behaviours (summed duration of freezing evoked by intruder proximity, flight in response to intruder approach, startle, upright and/or vocalisation reactions in response to approach from or contact with intruder); g) self-grooming; h) nest-oriented behaviour (on the nest, crouching over pups, suckling, rearranging nest material); i) social investigation; j) exploratory behaviour; k) eating and drinking; and l) immobility (except freezing).

Ethical Considerations

Throughout this study, and in accordance with recent recommendations (19), care was taken to minimise the distress caused to the animals, both adult and infant. As the presence of the whole litter is necessary in tests of maternal aggression (19), the use of a single stimulus pup was not feasible. However, the amount of stress imposed on dams and pups was reduced by intervening and removing intruders as soon as they attacked any pup.

Statistics

Data on the proportion of attacking dams were compared using the Fisher exact probability test. Data on attack latency were analysed by nonparametric tests (Kruskall-Wallis and Mann-Whitney tests). All duration (percent time) data were initially arcsin-transformed to give normal distributions. These results, along with those for percent vulnerable bites, were then analysed by three-way analyses of variance (ANOVA) (independent factors of screening, intruder sex, and drug treatment; 2 × 2 × 4). Unplanned comparisons were used for binary contrasts.

RESULTS

Table 1 summarizes data on the proportion of attack and latency to attack.

Proportion of Attack

In the control group, the proportion of attacking dams was affected by screening for female ($p < 0.04$) but not male intruders. Only in the NS condition was a tendency for a higher proportion of dams attacking male relative to female intruders found ($p < 0.09$). CDP had no effect on S dams. However, in NS dams, the high drug dose significantly reduced the proportion of male intruders that were attacked (10 mg/kg, $p < 0.02$, Fisher test).

Latency to Attack

In the control condition, NS dams showed higher attack latencies toward female than male intruders ($p < 0.003$, $z = 2.97$). Screening reduced latencies of attack toward female ($p < 0.001$, $z = 3.61$) but not male intruders. Only in NS dams did CDP increase latencies to attack male intruders at a 10-mg/kg dose ($p < 0.02$, $z = 2.42$).

Figure 1 shows data on accumulated attacking time, bite frequency, and attack pattern.

Accumulated Attacking Time (AAT)

ANOVA revealed a highly significant three-way interaction of Screening × Intruder sex × Drug [$F(3, 156) = 5.33$, $p < 0.002$]. In the control group, levels of attack on male intruders were similar in both S and NS dams. In NS dams, AAT was higher against male intruders [$F = 6.90$, $p < 0.01$]. However, screening increased AAT toward female intruders [$F = 45.07$, $p < 0.0001$], thereby reversing this pattern. In S dams, CDP dose-dependently increased AAT on males (10 mg/kg, $F = 5.35$, $p < 0.03$), whereas against females, the highest drug dose reduced AAT ($F = 7.50$, $p < 0.007$). In NS dams, CDP dose-dependently reduced AAT on male intruders (10 mg/kg, $F = 4.94$, $p < 0.03$) but had no effect on AAT toward female intruders.

Bite Frequency and Attack Pattern

ANOVA on bite frequency revealed a significant Screening × Intruder sex × Drug interaction [$F(3, 156) = 4.84$, $p = 0.003$]. In the control condition, screening markedly increased the number of bites directed against female (but not male) intruders ($F = 42.00$, $p < 0.0001$). In S dams, 10 mg/kg CDP reduced biting directed against female intruders ($F = 12.88$, $p < 0.0004$), whereas against male intruders, this drug dose dose-dependently increased biting (10 mg/kg, $F = 3.59$, $p < 0.06$).

An analysis of the percentage of vulnerable bites (calcu-

TABLE 1

EFFECTS OF CHLORDIAZEPOXIDE ON ATTACK BY LACTATING MICE CONFRONTING MALE AND FEMALE CONSPECIFICS INTRUDERS IN A 10-min TEST

Treatment Condition	Proportion of Attacked Intruders (%)				Attack Latency (s)*			
	Nonscreened		Screened		Nonscreened		Screened	
	M	F	M	F	M	F	M	F
SAL	13/16 (81)	8/17 (47)†	7/7 (100)	7/7 (100)	3 (1-600)	600 (1-600)§	1 (1-484)	1 (1-8)
2.5 mg/kg	13/14 (93)	5/14 (35.7)	5/7 (71.4)	6/7 (85.7)	19 (1-600)	600 (1-600)	1 (1-600)	1 (1-600)
5.0 mg/kg	11/15 (73)	3/13 (23)	7/7 (100)	7/7 (100)	61 (1-600)	600 (224-600)	1 (1-1)	1 (1-48)
10.0 mg/kg	5/13 (38)‡	3/13 (21)	7/7 (100)	7/7 (100)	600 (1-600)¶	600 (1-600)	1 (1-13)	2 (1-600)
Statistics for drug effect		NS	NS	NS	$H = 8.01$, $p < 0.05$	$H = 3.34$, NS	$H = 2.59$, NS	$H = 3.55$, NS

*Median and ranges (in parentheses) are given.
†Differ from S dams toward female intruder, $p < 0.04$ (Fisher exact probability test).
‡Differ from SAL, $p < 0.02$ (Fisher exact probability test).
§Differ from male intruder, $p < 0.003$; from S dams toward female intruder, $p < 0.001$ (Mann-Whitney test).
¶Differ from SAL, $p < 0.02$ (Mann-Whitney test).

lated only on attacking dams) revealed a significant main effect for intruder sex [$F(1, 97) = 27.90$, $p = 0.0001$] and for drug [$F(3, 97) = 3.68$, $p < 0.02$]; the Screening × Drug interaction approached statistical significance [$F(3, 97) = 2.33$, $p = 0.078$]. In the control condition, male intruders received a higher percentage of bites to vulnerable regions than did female intruders (NS dams: $F = 5.80$, $p < 0.02$; S dams: $F = 3.96$, $p < 0.05$). However, screening per se did not affect patterns of attack. In NS dams, 5 mg/kg CDP decreased the percentage of vulnerable bites against males ($F = 5.35$, $p < 0.03$). Drug effects on attack patterns against female intruders could not be evaluated, because of the low number of attacking dams (Table 1). In S dams, the high dose of CDP reduced the percentage of attacks on the vulnerable regions of male ($F = 6.93$, $p < 0.01$) and, marginally, female ($F = 3.01$, $p < 0.09$) intruders.

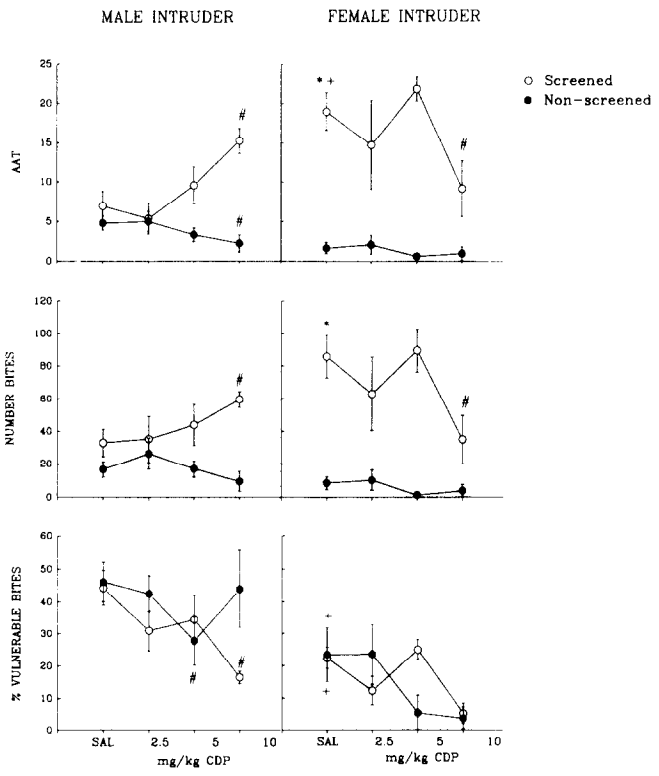

FIG. 1. Effects of CDP on accumulated attacking time (AAT), number, and pattern of bites toward intruders of either sex by aggression-nonscreened and aggression-screened lactating females (means ± SE). *Significant screening effect. +Significant intruder sex effect. #Significant CDP dose effect (vs. SAL).

Figure 2 shows data on fear-related behaviour, self-grooming, and nest-oriented behaviour.

Fear

ANOVA revealed significant Screening × Intruder sex [$F(1, 156) = 4.44$, $p < 0.03$] and Intruder sex × Drug [$F(3, 156) = 3.73$, $p < 0.01$] interactions. In the presence of female intruders, control levels of fear were very low for both S and NS dams. In the presence of male intruders, S dams showed lower control levels of fear than NS dams ($p < 0.05$). Dams showed a higher level of fear behaviour toward male intruders ($F = 10.93$, $p < 0.002$); as screening reduced fear responses, this intruder sex effect was observed only in the NS condition ($p < 0.0001$). In VS dams, 10 mg/kg CDP reduced the level of fear expressed toward male, but not female, intruders ($F = 8.51$, $p < 0.004$). In NS dams, although it did not reach significance ($F = 2.77$, $p < 0.1$), this CDP dose virtually eliminated fearlike behaviour in presence of male intruders.

Self-Grooming

ANOVA indicated main effects for screening [$F(1, 156) = 8.96$, $p < 0.003$] and drug [$F(3, 156) = 4.10$, $p < 0.008$], but no significant interactions. Control levels of self-grooming were not affected by either screening or intruder sex. A tendency for the low dose of CDP to increase self-grooming in S dams ($p = 0.09$) and for the high dose of CDP to decrease it in NS dams ($p = 0.064$) was found.

Nest-Oriented Behaviour

ANOVA indicated significant main effects of screening [$F(1, 156) = 8.67$, $p < 0.004$] and intruder sex [$F(1, 156) =$

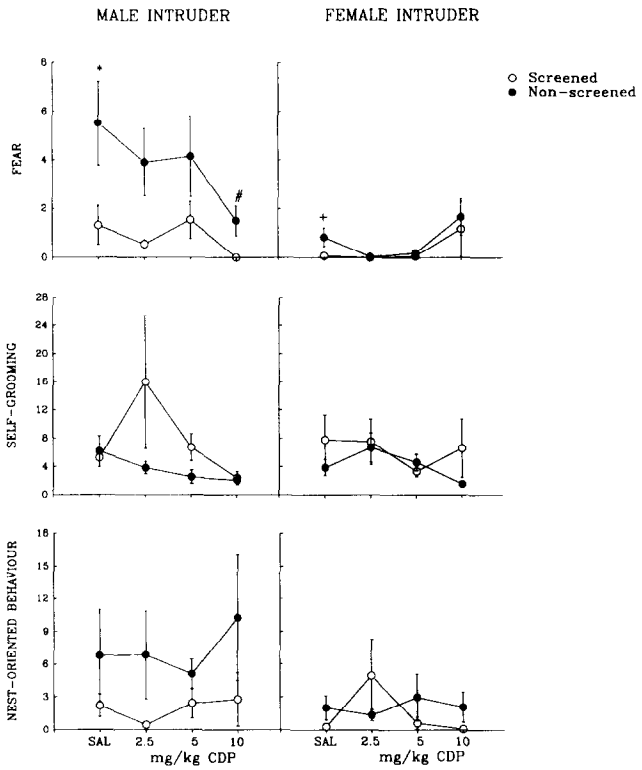

FIG. 2. Effects of CDP on fear, self-grooming, and nest-oriented behaviour shown by aggression-nonscreened and aggression-screened lactating females in response to male or female intruders (mean ± SE). *Significant screening effect. +Significant intruder sex effect. #Significant CDP dose effect (vs. SAL).

5.89, $p < 0.02$]. Screening generally reduced nest-oriented behaviour, whereas such behaviour tended to be higher in the presence of male intruders. No other effects or interactions were significant.

Data on social investigation, exploration, eating and drinking, and immobility are summarized in Fig. 3.

Social Investigation

ANOVA revealed a significant Screening × Intruder sex × Drug interaction [$F(3, 156) = 3.16, p = 0.03$]. In the control condition, screening tended to reduce the social investigation of female ($p < 0.06$) but not male intruders. NS dams displayed more social investigation toward female than toward male intruders ($F = 7.62$, $p < 0.007$), whereas in S dams, male and female intruders received similar amounts of social investigation. Drug treatment increased the social investigation of female intruders in both S (10 mg/kg CDP, $F = 19.4, p < 0.0001$) and NS (5 mg/kg CDP, $F = 7.39, p < 0.008$) dams. CDP did not alter the social investigation of male intruders.

Exploratory Behaviour

ANOVA failed to reveal any significant main effects or interactions for exploratory behaviour. However, the three-way interaction (Screening × Intruder sex × Drug) approached statistical significance [$F(3, 156) = 2.47$, $p = 0.06$). Binary contrasts indicated that in S dams, 10 mg/kg of CDP significantly reduced exploratory behaviour when confronting female ($p = 0.01$), but not male, intruders. Conversely, in NS dams, this dose tended to reduce exploratory behaviour in the presence of male ($P = 0.084$), but not female, intruders.

Eating and Drinking

ANOVA revealed a significant Screening × Intruder sex × Drug interaction [$F(3, 156) = 5.74$, $p < 0.001$]. In S dams, control levels of eating and drinking were virtually nonexistent, and this pattern was unaffected by the sex of the intruder. However, although the control level of ingestion in NS dams was also very low in the presence of males, such behaviour did occur in the presence of female intruders (vs. male: $F = 28.54$, $p < 0.0001$; vs. screened counterparts: $F = 17.00$, $p < 0.0001$). CDP dose-dependently enhanced eating and drinking in NS dams, but only in the presence of male intruders (5 mg/kg CDP, $F = 6.30$, $p < 0.02$; 10 mg/kg, $F = 15.96$, $p < 0.0001$). In contrast, an enhancement of ingestive behaviour was seen with 10 mg/kg CDP ($F = 9.50$, $p < 0.03$) in S dams confronted with female, but not male, intruders.

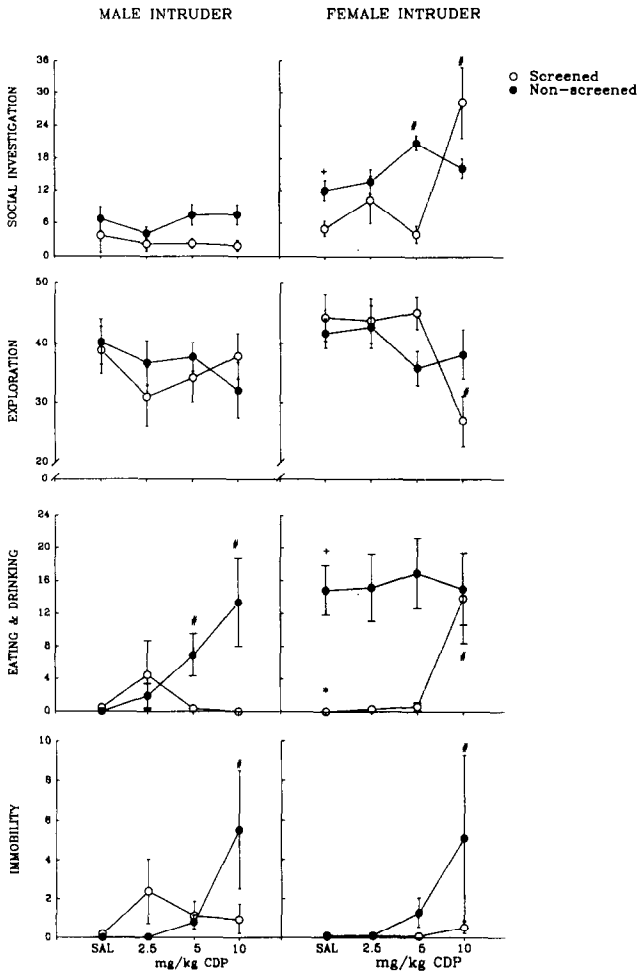

FIG. 3. Effects of CDP on social investigation of intruders, exploration, eating and drinking, and immobility by aggression-nonscreened and aggression-screened lactating females in response to male or female intruders (means ± SE). *Significant screening effect. +Significant intruder sex effect. #Significant CDP dose effect (vs. SAL).

Immobility

ANOVA indicated a significant Screening × Drug interaction [$F(3, 156) = 3.93$, $p < 0.01$]. In the control dams, immobility was virtually nonexistent. CDP dose-dependently enhanced immobility in the presence of both types of intruders (5 mg/kg, $p < 0.06$; 10 mg/kg, $p < 0.0003$). Although screening per se had no effect on immobility, this drug effect was observed only in NS dams.

DISCUSSION

The present data confirm previous findings that intruder sex is an important variable in modulating maternal aggressive responses in mice. They also show that prior screening for attack on intruders affects the behaviour of lactating females in subsequent encounters. In turn, both of these variables strongly influence drug effects on maternal aggression.

The profile of the nonscreened control groups basically confirms our previous findings on maternal aggression; male intruders received more attack behaviour and a higher percentage of bites on vulnerable body regions, and evoked more fearlike responses and less social investigation and eating and drinking than did female intruders. This is consistent with the proposed dichotomy between the offensive (female intruder) and defensive (male intruder) nature of maternal aggression in Swiss mice (38,41,42).

Screening altered some of these behavioural responses, and this effect was very much more pronounced against female intruders. In fact, in the presence of male intruders, the only significant variation in lactating female behaviour produced as a result of screening was a reduction of fearlike responses. Despite this variation in baseline fear levels between S and NS dams, male intruders were attacked with a similar defensive pattern in both conditions; this suggests that the occurrence of defensive-type aggression may be not strictly related to fear or anxiety levels. In direct contrast, against female intruders, screening increased attack behaviour and reduced social investigation and eating and drinking activities. As a result, S dams directed more attacks toward female than male intruders, thereby reversing the pattern observed in NS animals. However, screening did not alter the difference in the bite-attack patterns (i.e., males still received significantly more attacks to vulnerable body regions).

It is important to point out that although baseline fear levels of S dams in the presence of male and female intruders did not differ, the patterns of attack did (defensive and offensive, respectively). Again, this suggests that at least in experienced animals, the motivational substrates underlying the two types of attack may be relatively fear-independent.

Several studies have shown that CDP influences the behavioural responses of lactating females confronting male intruder. In particular, nonsedating high doses of CDP reduce aggressive behaviour, whereas low doses either have no effect or increase aggression (29,30). The differential effects of CDP on aggressive behaviour have been tentatively explained as based on rate-dependency factors (28,30). Current results, however, show a complex profile of CDP activity on maternal aggression in mice. In fact, CDP had differential effects on the responses of lactating mice to intrusion, depending on both prior screening for attack and intruder sex, effects that cannot be ascribed, at least not fully, to differences in the baseline levels of these behaviours. On the whole, none of the behavioural effects observed with CDP treatment can readily be attributed to nonspecific effects. Although immobility was increased in the high-dose group of NS dams confronting both male and female intruders, the same dose did not impair other active behaviours (such as exploration and social investigation).

Against male intruders, CDP showed an opposite profile of effects on attack-related measures (AATs, number of bites) in S vs. NS lactating females. Specifically, in S dams, CDP dose-dependently increased attacks, thus confirming the proaggressive effect reported in the studies mentioned earlier. In contrast, in NS dams, CDP dose-dependently decreased attack and fear-related behaviour while increasing immobility and eating. Despite the opposite effects on attack in S and NS dams, CDP reduced the percentage of bites directed on vulnerable regions of the opponent's body in both conditions. The differential effects of CDP on attack, eating and drinking, and immobility between S and NS dams cannot be explained on the basis of different baseline levels of these behaviours, as control levels did not differ in the two conditions. However, a baseline effect could account for the differential effect of CDP on fear.

Against female intruders, CDP decreased maternal attack and exploration and increased social investigation and eating and drinking in S dams. In NS dams, CDP increased social investigation and immobility. The relative lack of drug effect on aggressive responses in NS dams is likely due to rate dependency factors (i.e., baseline differences in levels of aggression). However, the differential effect of CDP on exploration and immobility between S and NS dams cannot be explained by baseline effects.

Miczek (25) first showed a biphasic effect of CDP on intermale aggression in rats. Further studies have shown that CDP can also exert a biphasic effect on maternal aggression toward male intruders; low doses of CDP (i.e., 5–10 mg/kg) increased attacks, whereas higher doses (15–20 mg/kg) either decreased (in mice) (46) or returned it to control levels (in rats) (31,32). It has been proposed that proaggressive effects of CDP occur more easily when animals are naive, are confronted with a more difficult situation, or have a low baseline of aggression (29,30). Such proaggressive activity has been interpreted to be a rate-dependent phenomenon reflecting the disinhibitory effects of these compounds as a consequence of reduced anxiety (28–30). According to this hypothesis, an anxiety or fear element may have resulted in an inhibition of aggression that can be removed by the anxiolytic effects of benzodiazepines.

Our findings do not confirm this hypothesis, but suggest greater complexity. In our study, NS dams confronting male intruders showed higher control levels of fear that were reduced by drug treatment; this suggests an anxiolytic effect of CDP. According to the hypothesis mentioned earlier, we could have expected a parallel increase of maternal aggression following drug treatment. On the contrary, we observed a decrease in aggression. Furthermore, a proaggressive effect of CDP was recorded in those animals in which the baseline fear was low and not altered by the drug (i.e., in S dams). By comparison, against female intruders (fear virtually absent), CDP treatment either decreased (S dams) or did not affect (NS dams) attack. As a matter of fact, these findings do not contradict data in the literature, as in previous studies a proaggressive effect was observed in benzodiazepine-treated lactating females that confronted only male intruders and had been subjected to prior screening for aggression (i.e., only aggressive dams were used in drug tests), although this factor was generally not fully considered in discussing the results [e.g., (30–32,46)].

Our data suggest that the proaggressive effects of CDP cannot be attributed to direct antifear effects. This could be

because in S dams, baseline levels of fear were very low, thus preventing a further observable reduction (floor effect). On the other hand, a working hypothesis could be that the prior fighting experience against the male intruder (i.e., the screening test) might have altered the GABA functioning in the brain, thus lowering the future fear and anxiety responses and affecting the action of CDP. It is known that experience with intruders can decrease the resident's fear of strange conspecifics, thereby substantially altering future reactions (5,13). In this connection, it has been reported that rats exposed to potentially stressful stimuli (such as handling and foot-shock), show an increase of $GABA_A$ receptors in the brain (1-3,8,14). Corda and Biggio (17) tentatively explained this as an enhancement of GABA functionality, probably related to a decrease in the emotional state of animals. The benzodiazepines' ability to potentiate GABA responses thus should be altered by enhanced functionality of the GABA system. Clearly, further studies are needed to clarify whether the proaggressive effect of CDP on maternal aggression (in S dams confronting a male intruders) involve an anxiolytic action mediated by the benzodiazepine-GABA receptor complex, as also suggested by Mos and co-workers (30).

Although producing different profiles in S and NS conditions, it is clear that CDP's effects on the behaviour of lactating females are heavily influenced by the sex of the intruder. This differential action of CDP on intra- and intersexual aggression in lactating females would be consistent with our original hypothesis that these behaviours (offence and defence, respectively) involve different motivational and neurochemical substrates. Such motivational dichotomy appears to have adaptive value in that strange males pose a much greater threat to the offspring than do females (38,40).

In this context, an interesting feature of our data is that CDP appears to modify the attack strategy of lactating females, an effect that has been also reported by Mos and coworkers (30). It is worth noting that despite having an opposite effect on the intensity of attack in S and NS dams, CDP appeared to induce a shift from a defensive to an offensive pattern of attack in both groups. This finding is consistent with a number of studies showing that defensive responses are much more sensitive to benzodiazepines than are offensive patterns [e.g., (4,22,27,45)]. As a consequence, in both S and NS conditions, the aggression against males under CDP treatment became—phenotypically—like that shown against female intruders in the control group [i.e., high or low level of aggression (in S or NS conditions, respectively), offensive attack-pattern, and low fear levels]. Thus, the earlier hypothesis (41) of a continuum between offence and defence is supported. According to this hypothesis, based on the findings of Blanchard and co-workers (6) and Brain (10), there is a direct relation between fear and the pattern of attack employed, in that higher levels of fear are associated with defensive pattern of attack. However, current results show that, whereas in the NS condition the shift from a defensive to an offensive attack pattern caused by CDP was actually associated with reduced fear, this does not account for the S condition, where fear levels were not affected by drug treatment. Thus, at least in experienced animals, CDP appears to affect the attack strategy of lactating females on conspecific intruders relatively directly rather than indirectly via anxiety reduction.

Prior screening for attack and the sex of intruders influenced the effects of CDP on eating and drinking behaviour in the present study. Benzodiazepines are known generally to stimulate food and water consumption, an effect that appears to be relatively independent from anxiolytic effects (15,16). Current results, however, show that the effects of CDP on eating and drinking cannot be attributed solely to a general stimulation of ingestive behaviour. CDP increased eating and drinking in NS dams confronting male intruders and in S dams confronting female intruders. Although in the former condition this effect may be related to an anxiolytic action (fear was reduced by CDP), such an explanation cannot account for findings in the latter condition.

In summary, our results show that the effects of a benzodiazepine anxiolytic on maternal attack behaviour depend not only upon the drug, but also the object of attack, and hence, the function of attack, and the prior experience of the attacker. The issue of the possible effects of context, functions, previous experience, and genetic and/or interindividual variability in aggressive responses has not received much attention in psychopharmacological analyses of aggression (21,24,26). Indeed, several studies on benzodiazepine effects on maternal aggression located nonaggressive lactating females by means of a screening test and discarded them from subsequent testing [e.g., 32,46,47)]. Our findings suggest that an understanding of drug effects on behaviour demands consideration of the biological variability in phenotype (e.g., animals with varying backgrounds and varying baseline levels of aggression) in an evolutionary perspective (37). The ultimate causation of any phenotype (including behaviour) is the result of selective pressures operating on proximal mechanisms (e.g., neurochemical substrates). It follows that is vitally important to consider the context and function of behaviour when studying its control processes. An evolutionary approach to behavioural pharmacology, which distinguishes ethopharmacology from psychopharmacology, may allow for the development of a better understanding of drug action and help to clarify proximal mechanisms of a given behaviour.

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