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Maternal Aggression Towards Different Sized Male Opponents: Effect of Chlordiazepoxide Treatment of the Mothers and d-Amphetamine Treatment of the Intruders

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MOS, J , B OLIVIER AND R VAN OORSCHOT *Maternal aggression towards different sized male opponents Effect of chlordiazepoxide treatment of the mothers and d-amphetamine treatment of the intruders* PHARMACOL BIOCHEM BEHAV **26**(3) 577–584, 1987 —Lactating female rats vigorously attack equally sized conspecific males introduced into their home cage Under conditions of such high aggression, the previously reported pro-aggressive action of a low (5 mg/kg) dosage of chlordiazepoxide (CDP) is hardly detectable When opponents are large, the intensity of the aggression is less than what is seen with small ones In this situation treatment of the females with CDP increases aggression levels substantially The importance of intruders evoking aggression was further investigated by treating different sized opponents with d-amphetamine d-Amphetamine treatment did not lead to major changes in the defensive capacities of either types of intruder The data demonstrate that drug effects, such as pro-aggressive actions, may be observed using larger sized opponents that are not so easily defeated and show more adequate defense than small ones The subtleness of the dyadic interactions in maternal aggression indicates that drug effects should be considered carefully before extrapolation to other conditions

Lactation Aggression Rat Opponent size Attack topology Defense strategy
Chlordiazepoxide d-Amphetamine Female

IN general, some type of 'standard' opponent is used in laboratory tests for aggression in rodents Such an opponent should evoke a reliable level of aggression but should not win the contest, since losing affects the subsequent behaviour of the experimental animal, as is also the case with the experience of winning [15,16] Under natural conditions, experience is also of vital importance and largely determines whether an animal fights or flees [2]. Laboratory aggression tests in rodents usually offer little opportunity for the animals to flee Adequate defense mechanisms in the intruder, however, are of paramount importance Moreover, the behaviour of opponents in itself is of great interest because it influences the behaviour of the resident and, especially after drug administration, the dyadic interaction appears to be an important variable in which the behaviour in the intruders counts [6]

We have studied aggression in lactating female rats and found that maternal aggression during the first two weeks postpartum is characterized by a short latency to the first attack and by high levels of bite attacks during the first five minutes of a test session [14]. These high levels of aggression in females, especially of the S3 strain, renders slightly smaller male intruders virtually defenseless. Although the predictable high levels of aggression are advantageous in the testing of anti-aggressive drugs, they may be less appropriate in other studies We have recently found that low doses of chlordiazepoxide (5 mg/kg PO) had pro-aggressive effects in lactating females, but only when the basal level of aggression was not too high [12] Apparently the facilitatory effect of CDP depends on the basal level of aggression.

From these findings, the idea emerged to use both large and small intruders in maternal aggression tests and to record the behaviour of the females towards these different sized intruders. In analogy to experiments on prey of different size [1], it was hypothesized that CDP might exert its facilitatory effect on the aggressive behaviour of females

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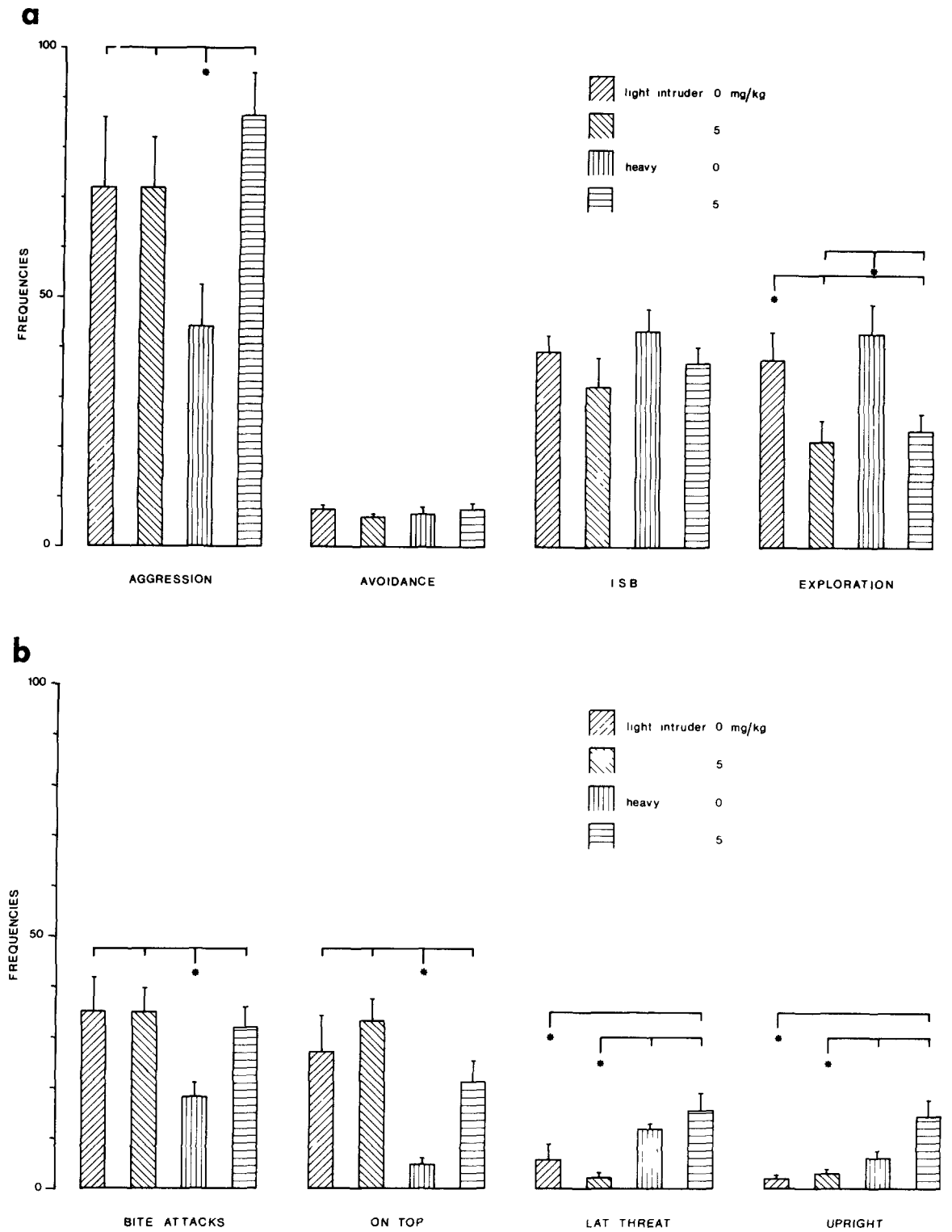

FIG 1 (a) Mean frequencies (±SEM) of the major behavioural categories Exploration, Avoidance, Introductory Social Behaviour (ISB) and Aggression of the lactating females under the different CDP/intruders test conditions Groups that are significantly different ($p<0$ 05) from a particular intruder/drug condition are connected and indicated by an asterisk For detailed description of the behavioural elements of the categories, see the Method section (b) Mean frequencies (±SEM) of Bite Attacks, Lateral Threat, Upright Posture and On Top by the lactating females under the different treatment regimes Groups that are significantly different ($p<0$ 05) from a particular intruder/drug condition are connected and indicated by an asterisk

confronted with heavy male intruders, but not at all or to a lesser degree towards the usual light partners. On the basis of previous experiments [12,13] we selected a dose of 5 mg/kg of CDP which was shown to reliably increase aggression.

To extend these observations, we treated light and heavy intruders with a single dose of amphetamine in a second experiment. Amphetamine treatment of opponents induces increased aggression by the resident [6]. Whether or not this is caused by increased locomotor activity of the opponent (i e , less prolonged submissive postures, less freezing) is not clear In any case, it is intriguing to study whether amphetamine differentially affects the defensive capabilities of opponents of varying size and consequently affects the aggressive behaviour of the females One single dose (4 mg/kg) was chosen on the basis of some preliminary studies (Olivier and Mos [14]) In both experiments we recorded in detail the behaviour of the maternal females

METHOD

Maternal aggression of primiparous S3 female rats was studied under the same conditions as described previously [12,14] Briefly, twelve (CDP-treated) and sixteen (d-amphetamine-treated) females (250–350 grams, 4–9 months old) were placed together with a breeding male in their polycarbonate cage (30×20×15 cm) A gauze wire cloth was placed on the bottom of this cage which enabled the collection of ejaculation plugs

After ejaculation plugs were detected, the male was left for a further week with the female, after which she was placed in the observation cage (40×30×30 cm) where she remained for the rest of the experiment. This cage was provided with nesting material and food and water were freely available These cages were housed in an observation room with a reversed day-night rhythm (12L/12D) with night starting at 7 00 a m The date of parturition was regarded as postpartum day 0

Tests were performed early in the dark period (between 8 30 a m and 12 30 p m.) under red light conditions Tests for the CDP experiments were performed on days 4, 6, 8 and 10 postpartum and for the amphetamine experiment on days 11 to 13, during which time aggression had not yet diminished

Intruder males were group housed and weighed 176.7±7.3/422.8±6 3 (mean±SEM light and heavy group respectively, CDP experiment) and 169 7±3 2/377.2±11.5 (mean±SEM light and heavy group respectively, amphetamine experiment) A male intruder was placed in the female's home cage for 5 min The ongoing behaviour was videotaped and later analysed Each intruder was used once and sacrificed immediately after the morning sessions with an IP overdose of pentobarbital. In the second experiment (amphetamine), this was followed by shaving and describing the localization of the lesions on wound charts [10].

As an ethogram, we used 32 elements divided over 7 behavioural categories The behaviour of the female was scored using the methods described by Olivier and Olivier *et al* [11,12], where a more extensive description of most elements can be found Briefly, *Exploration* includes attention, sniffing, rearing, locomotion, marking, digging, carrying and picking up of food particles *Introductory Social Behaviour* (ISB) comprises moving towards the partner, sniffing the intruder, social grooming and "crawling under "

The following elements belong to *Aggressive Behaviour* bite attack on head, bite attack on body, lateral threat, upright posture, teeth chattering, nipping, pulling, clinch, kicking, lunge and "on top." *Avoidance Behaviour* includes moving away and "keeping off."

Drugs

Clordiazepoxide-HCl (Hoffman-La Roche, Basle) was suspended in tragacanth 1% and given orally in a volume of 5 ml/kg body weight one hour before testing (5 mg/kg) d-Amphetamine (4 mg/kg) was dissolved in water and given intraperitoneally in a volume of 2 ml/kg bodyweight thirty minutes before testing

Statistical Analysis

All statistical analyses were performed using non-parametric statistics since the data were distributed asymmetrically or the structure of the data did not always warrant a symmetrical or known distribution.

Balanced randomised block designs were employed for the experiments which were analysed by Friedman analysis of variance followed by multiple comparison tests (as described by Conover [4]), to compare the effects of body size or drug treatment The females served as their own controls and intruders were used for only one test. The wounds on the different parts of the body were expressed as a percentage of the total number of wounds. However, if a female caused no wounds, no percentage could be calculated and this value had to be omitted. A Kruskal-Wallis analysis of variance was applied to the wound pattern data which were considered as unrelated samples. All other "single" group comparisons were made with the Mann-Whitney U-test

RESULTS

CDP Treatment of the Resident Females

Figure 1 summarizes the results of CDP or vehicle treatment of the female in combination with light or heavy opponents Only frequencies are given though duration data generally follow the same pattern

Exploration was decreased under CDP conditions, independent of the size of the opponent, $\chi^2(3)=14.7$, 15 1, $p=0$ 002 and 0 0017, frequencies and durations, respectively Avoidance ($p<0.3$), as well as Introductory Social Behaviour (ISB), were unaffected by either CDP or the type of intruder, $\chi^2(3)=4.4$, 5.2, $p=0$ 22, 0 16, frequencies and durations, respectively. Aggression was lowest in frequency and duration when the opponent was heavy and the female under vehicle conditions, $\chi^2(3)=10$ 9, 19.8, $p=0$ 012, 0 0002, frequencies and durations, respectively. However latency to first attack did not differ, $\chi^2(3)=3$ 2, $p=0.36$ CDP was able to "restore" the aggression level to that displayed towards the light intruders As in earlier experiments, we did not observe an increase in the frequency of bite attacks and other aggressive elements after CDP if the opponent was a weaker, light-weight male (Fig 1b). However, the duration of total time spent on aggression was significantly enhanced, a feature not detectable when only bite attacks are recorded (data not shown). The differentiation between the various aggressive acts (Fig. 1b) demonstrated that the topology of aggression differed according to the size of the intruder. Under vehicle conditions and when using large intruders, bite attacks and "on top" were less, while lateral threat and upright posture were increased These data are based on the absolute frequencies of occurrence, which is not the most

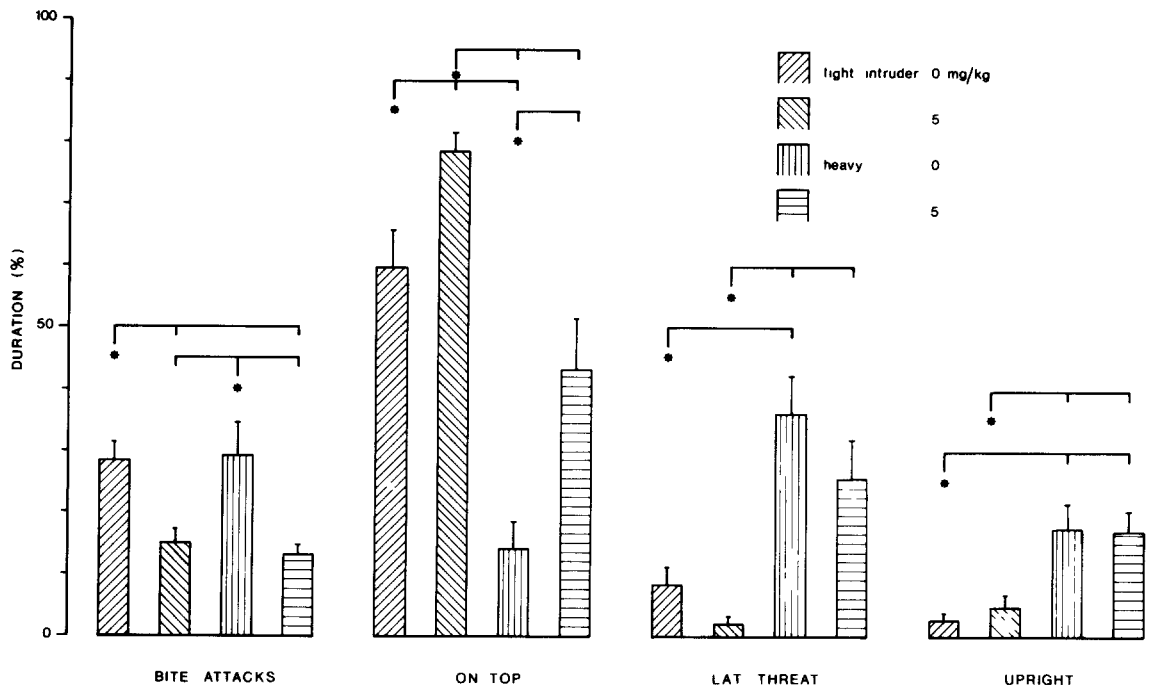

FIG 2 Relative importance of the duration of the various aggressive elements expressed as percentage of total time spent on aggression under the various CDP/intruder conditions Most time is spent during On Top position under the light intruder condition, followed by Bite Attacks When confronted with heavy opponents the female increased the relative time spent on lateral threat and Upright Posture CDP treated females spent relatively less time on Bite Attacks when compared to vehicle conditions, although the frequency of attacks is not changed (Fig 1b) The size of the opponent does not influence the proportion of time spent on bite attacks, in contrast to the frequency Friedman analysis revealed an overall significant effect for On Top, $\chi^2(3)=19\ 5$, $p=0\ 0002$, Upright Posture, $\chi^2(3)=15\ 6$, $p=0\ 0014$, Lateral Threat, $\chi^2(3)=19\ 3$, $p=0\ 0002$, and Bite Attacks, $\chi^2(3)=13\ 0$, $p=0\ 0005$ Groups that are significantly different from another condition are connected and indicated by an asterisk

appropriate measure to compare the importance of individual behavioural elements. Because the different testing conditions do not result in equal levels of aggression it is better to compare the relative duration of behavioural elements.

Therefore, data were also expressed as percentage of the total time spent on aggression (Fig. 2) Introduction of heavy opponents (under vehicle conditions) resulted in a relative increase of the duration of lateral threat and upright posture. Heavy intruders, however, were less held down on the back when compared to light weight intruders and bite attacks were also decreased CDP effects were again most marked when heavy intruders were used The duration of "on top" increased, though was still well below what was seen with light intruders. The relative duration of bite attacks was not increased by CDP treatment.

d-Amphetamine Treatment of the Male Intruders

Figure 3a summarizes the overall results for the maternal females expressed in the behavioural categories: Exploration, Avoidance, ISB and Aggression. Since Inactivity and Pup care hardly occurred during the test period, they were omitted from the figure.

Exploration remained at the same level, both in frequency and duration, under the different intruder conditions and treatments, $\chi^2(3)=7.1$, 6.4, $p=0.07$ and 0.09, frequency and duration, respectively. Introductory Social Behaviour was not significantly different in frequency and duration, $\chi^2(3)=4.2$, 6.2, $p=0.24$ and 0 10, respectively. To our surprise this also held for the total frequency and duration of aggression. The variance was so large that Friedman analysis failed to reveal significant overall effects, $\chi^2(3)=3\ 4$, $2\ 2$, $p=0.34$ and 0.54, frequency and duration. However, aggression tended to be somewhat lower when the females were confronted with a large opponent Latency to first attack did not differ, $\chi^2(3)=2.9$, $p=0\ 41$

Figure 3b summarizes the differences in the composition and topology of the aggression by the females when faced with the different intruder males Under heavy intruder conditions, bite attacks and "on top" decreased greatly, both in frequency and duration.

Concomitantly, lateral threat and upright posture increased when heavy intruders were used. d-Amphetamine treatment had no effect which suggests that amphetamine treated intruders do not use a different defense strategy Moreover, bite targets and number of wounds did not vary significantly with type of intruder or with amphetamine treatment (Table 1). Only a tendency to an increased number of wounds on small, amphetamine treated intruders was found Analysis of the target areas for biting failed to reveal any differences, illustrating that the defensive capacities remain intact after amphetamine treatment A similar analysis on the relative contribution of individual aggressive acts as performed for the CDP experiment is given in Fig 4 It

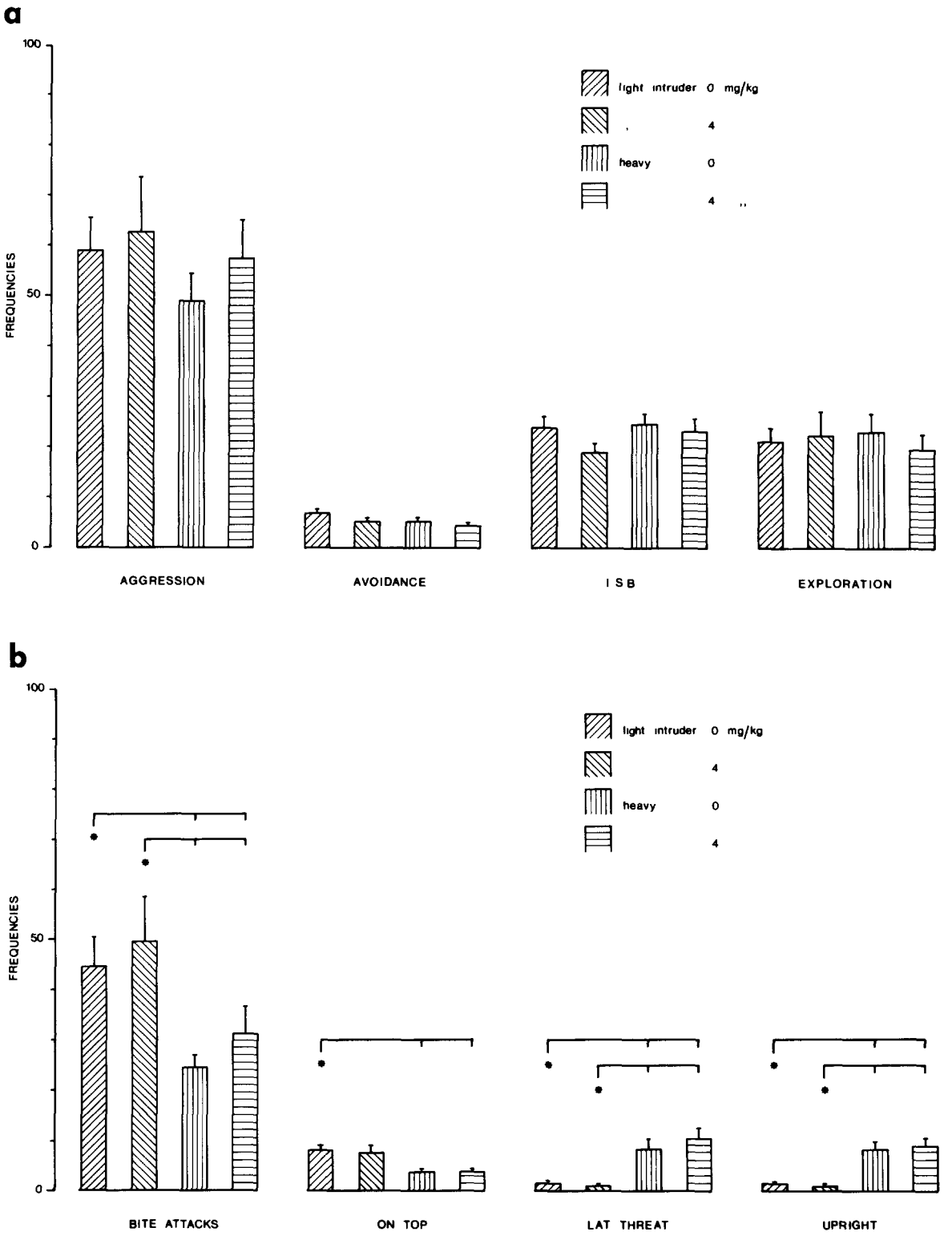

FIG 3 (a) Mean frequencies±SEM of the behavioural categories Exploration, Avoidance, Introductory Social Behaviour (ISB) and Aggression displayed by the lactating females versus light and heavy, amphetamine or vehicle treated intruders (b) Mean frequencies±SEM of Bite Attacks, Lateral Threat, Upright Posture and On Top by the lactating females under the various treatment conditions Groups that are significantly different ($p<0$ 05) from a particular intruder/drug condition are connected and indicated by an asterisk

TABLE 1

PERCENTAGE OF TOTAL NUMBER OF WOUNDS ON THE INTRUDERS IN THE AMPHETAMINE EXPERIMENT

	0/Light	4/Light	0/Heavy	4/Heavy	Kruskal-Wallis H	Kruskal-Wallis p
No of wounds	21 0 ± 5 9	34 7 ± 9 5	13 4 ± 3 0	15 9 ± 4 3	χ^2=1 3	p=0 72*
head	45 9 ± 6 5	35 3 ± 5 5	43 1 ± 5 9	37 2 ± 5 8	1 18	0 76
upper back	37 1 ± 5 7	50 2 ± 6 4	40 5 ± 5 9	45 5 ± 6 1	1 45	0 69
lower back	1 9 ± 0 8	1 2 ± 0 4	1 4 ± 0 5	3 7 ± 1 2	1 39	0 71
upper belly	1 7 ± 0 6	2 2 ± 0 6	3 6 ± 1 5	0 8 ± 0 5	4 24	0 24
lower belly	0 9 ± 0 5	0 ± 0	0 2 ± 0 2	0 ± 0	6 38	0 09
forepaws	9 9 ± 2 5	10 1 ± 2 7	8 1 ± 2 1	11 5 ± 7 2	3 61	0 31
hindpaws	2 3 ± 0 9	1 1 ± 0 6	3 1 ± 2 4	0 7 ± 0 4	3 01	0 39
g region/tail	0 ± 0	0 ± 0	0 ± 0	0 ± 0	not done	

*Friedman analysis of variance Kruskal-Wallis analysis of variance was applied to the percentage of wounds made on the different body areas No overall significance was observed between the different sized or drug treated opponents confronted with the maternal females

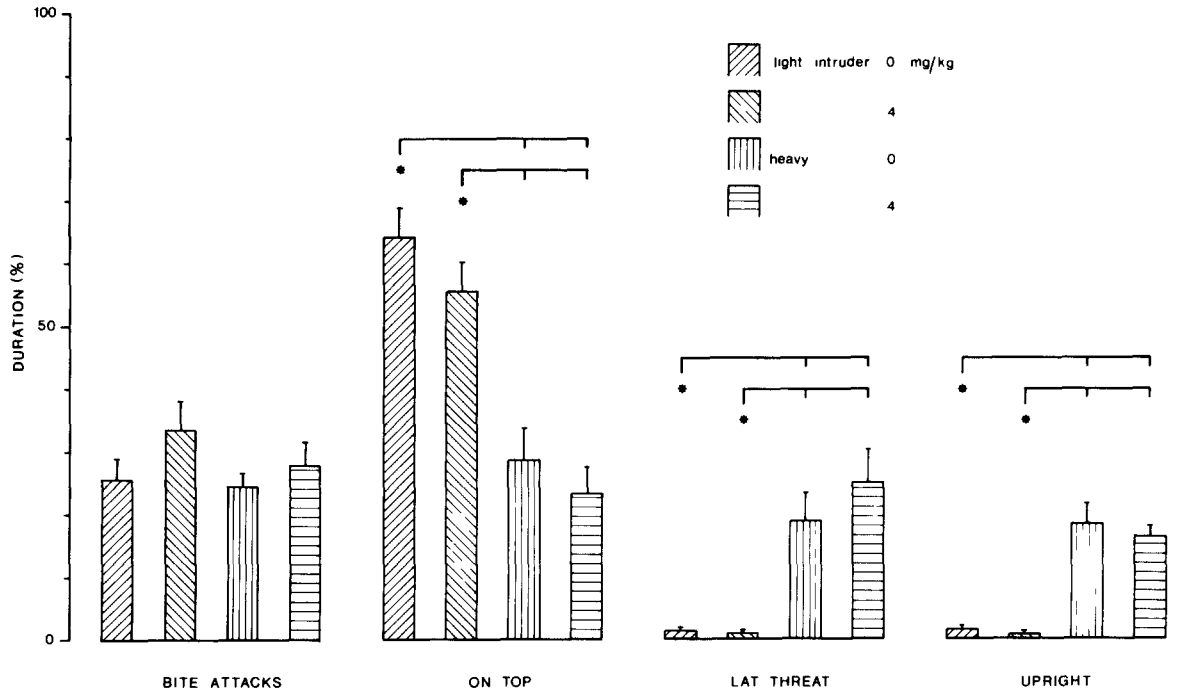

FIG 4 Relative importance of the duration of the various aggressive acts, expressed as percentage of the total time spent on aggression under various d-amphetamine/intruder conditions With light opponents, On Top takes most time, followed by Bite Attacks. Large intruders evoke more Lateral Threat and Upright Posture, while On Top and Bite Attacks decrease somewhat in their relative importance Notice the discrepancy between the data of On Top expressed as duration or frequency (Fig 3b) Friedman analysis revealed an overall significant effect in the percentages of time spent On Top, $\chi^2(3)=25.3$, $p<10^{-4}$, Upright Posture, $\chi^2(3)=32\ 1$, $p<10^{-4}$ and Lateral Threat, $\chi^2(3)=21\ 1$, $p=0\ 0001$ Subsequent multiple comparison tests demonstrated significant effects of opponent size, but not of amphetamine treatment Groups that are significantly different ($p<0\ 05$) from a particular intruder/drug condition are connected and indicated by an asterisk

seems to be a matter of physical strength; heavy intruders are better equipped to maintain an upright posture and avoid the full submissive posture. This was successful in the sense that fewer bite attacks were sustained. The females used an alternative strategy of attack, i.e., lateral threat and sideways approach to be able to bite the back and push over the opponent. Upright posture by the female may serve the purpose of bringing about the on the back posture in the intruder This upright posture of the female appeared to be more often followed by jump attack, a feature never seen in the intruders. In summary, a similar attack/defense strategy is used in the CDP and amphetamine experiment, although minor differences exist. Amphetamine treated intruders showed no marked change in defensive capabilities compared to similar sized control intruders

DISCUSSION

The present paradigm for the study of psychoactive drugs on maternal aggression reveals two important aspects. First, the disinhibitory effects of low doses of chlordiazepoxide on aggression can be made more readily manifest when the females are confronted with a more difficult task, i.e., a larger intruder Second, the changed attack patterns of the females indicate that heavy intruders use a different strategy to withstand the fiercely attacking females. The differential behavioural strategy of lactating females against different sized male intruders is at variance with the notions of Rodgers *et al* [17] who suggested that lactating females do not respond to social postures of the intruders nor on restrictions to bite targets.

Customary practice in aggression research is to use smaller and weaker animals as opponents. Brain and coworkers [3] studied various types of intruders in mice and found that they induced different patterns of fighting depending upon their size and previous experience Moreover, they reacted with other patterns of defense and were differentially successful in avoiding bites on certain areas of the body. They concluded that it may be difficult to extrapolate between tests using intruders of different experience. Testing drugs in randomized block designs requires a consistent experimental situation, or more specifically, the same motivational state of the tested animals In that respect, there are many arguments in favour of using weaker animals, since defeat (an ultimate consequence of more matched intruders) has a pronounced, negative effect on aggressive behaviour in subsequent tests [15,16] However, subtle drug effects are sometimes hard to detect since the intruders are defeated too easily and do not show active defensive behaviour.

In the present experiments it transpired that heavy intruders are also defeated, although the total time spent on aggression is somewhat reduced. What mechanism causes this reduction remains unclear The latency to first attack is not changed, indicating that a general reduction in the propensity to attack does not seem likely After the first attack, the females learn that it is harder to bite the intruder and consequently have to adopt other strategies, which they do quite well. Bite attacks and on top may become suppressed, perhaps by increased fear. Flannelly and Flannelly [5] suggest that larger males evoke fear and that fear reduces offense. In their experiments, most females no longer attacked the larger intruders. Our females were more experienced fighters and did attack, but increased fear may well be present. Miczek and O'Donnell, and Apfelbach suggest that CDP treatment increases aggression and predatory behaviour that were suppressed [1,9]. In our experiments, the suppression may result from the difficulties that the lactating females meet when confronted with males that are hard to bring to a full submissive posture.

In view of previous results of Miczek [6,7] and Miczek and O'Donnell [8], we expected that d-amphetamine treatment of the intruders would have influenced their defensive capabilities Miczek observed that d-amphetamine-treated animals are more readily and consistently attacked, and that d-amphetamine-treated intruders escape more than vehicle-treated intruders, although Miczek [7] suggests this to be a direct effect of the attacks, rather than a direct effect of d-amphetamine on escape behaviour. Although we found little effect of d-amphetamine on the level of aggression, there was a slight indication that amphetamine-treated intruders sustained more bite attacks, resulting in significantly more wounds in light, amphetamine-treated intruders. However, the distribution of these wounds was similar under all conditions, a finding which is at variance with data of Brain *et al* [3] with different types of intruder mice.

Similar to the CDP experiments, the defending strategy of the heavy intruders was markedly different from light intruders Recording only bite attacks as the main parameter of aggression would have led to erroneous conclusions as in the CDP experiment.

The finding that in both experiments light and heavy intruders adopt different strategies in agonistic interactions stresses the need for a complete ethological analysis in describing drug effects. Extrapolations between experiments with different types of intruders should be done very cautiously. However, the manipulations of the defensive capabilities of intruders also opens new perspectives to describe drug effects, most notably pro-aggressive actions and adaptive defensive strategies.

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