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Role of Anxiety in Subordinate Male Mice Sexual Behavior

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D'AMATO, F. R. AND F. PAVONE. *Role of anxiety in subordinate male mice sexual behavior.* PHARMACOL BIOCHEM BEHAV **43**(1) 181–185, 1992.—Dominant and subordinate male mice behave differently when exposed to a female, with subordinates showing impairment of their sexual performance in the presence of the male antagonist. In the present study, we investigate whether these rank-related behavioral differences can be modified by an anxiolytic treatment. In a first experiment, diazepam (0.25 mg/kg) improves the performance of subordinate mice toward the female, as shown by the increase of proxemic behavior, anogenital sniffing, and social grooming of the female. Social grooming of the female is the only behavior modified by a higher dose of the anxiolytic drug (0.5 mg/kg). A second experiment, in which dominant and isolated mice are subjected to the same experimental procedure, demonstrates that social behavior of these two classes of males is not affected by the pharmacological treatment. The results are discussed in terms of the advantages of using subordinate males in such a sexual context as a model for the study of anxiolytic drugs.

Mice Social rank Sexual behavior Anxiety Diazepam

DOMINANT and subordinate male mice differ in their behavior when exposed to a female (7–9,13). These rank-related behavioral differences persist even in the absence of direct physical interaction with the male antagonist. In fact, behavioral differences linked to social rank are detectable when the male is temporarily introduced into the female home cage, in the absence of the male antagonist (7,9), and when the male exposed to the female is separated from its male antagonist by a transparent Plexiglas partition that allows only visual, olfactory, and auditory communication between mice (8). Subordinate males show less interest in the female than their dominant partners and higher levels of displacement activities (2,3,7). The presence of the female acts as a catalyst leading rank-related behavioral differences to appear (8). These results suggest that the lower behavioral performance of subordinate males could be interpreted as a result of long-lasting physiological modification decreasing sexual motivation (5,6,15), but also as the outcome of conflicting tendencies between sexual motivation and the condition of subordination. In fact, subordinates males in the presence of a female are exposed to potential aggression by the dominant male (9,17), which represents the aversive stimulus, and this might inhibit their sexual behavior. If the impairment exhibited by these males in interacting with a female is due only to an anxiety condition, an anxiolytic treatment should be able to cancel such an emotional status.

Several rodent models have been proposed for the study of antianxiety agents [for a review, see (19)]. For the majority of them, based upon conflict or conditioned fear, only the pharmacological validation following administration of the anxiolytic drug has been performed. File (10) elaborated a test, validated behaviorally, physiologically, and also pharmacologically, in which the measure of anxiety was given by changes in the amount of social interaction following manipulation of environmental factors.

The aim of this study was to investigate whether the poor performance of subordinate males in the presence of the female could be improved by an anxiolytic treatment and if subordinate males in such a context could be used as a simple model of anxiety in mice.

EXPERIMENT 1

The aim of the first experiment was to evaluate the effect of an anxiolytic treatment on subordinate males' performance in the presence of a female with the dominant male being confined by a transparent Plexiglas partition.

METHOD

Subjects

Outbred, sexually naive mice of the Swiss-Webster strain were used. Animals were purchased from Plaisant (Rome, Italy) when about 80 days old and housed in groups of 10–15 subjects in 40 × 23 × 15 cm Plexiglas cages with wood chips on the floor and food and water available ad lib. They were maintained under a 12L : 12D cycle, with lights on at 0700 h. Room temperature ranged from 21–24°C.

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Procedure

Five to 7 days after their arrival in the laboratory, male mice were individually marked and housed in pairs in 30 × 13 × 13 cm cages with wood chips and food and water ad lib (day 1). Each mouse of the pair came from a different group cage. On day 8, the two males of the pair were transferred into a bigger cage (40 × 23 × 15 cm) divided in two equal compartments by a transparent Plexiglas partition. The partition was provided with 16–18 holes (0.3-cm diameter) that allowed visual, auditory, and olfactory communication between the members of the pair. A bigger hole in the partition (3.0-cm diameter) could be opened to allow the two mice to interact physically. On day 8, the big hole was left open all day; starting from late evening, it was closed, leaving each mouse in its own compartment.

To determine the social status of the members of the pair, from day 9 the door was opened twice a day and behavioral observations were conducted until agonistic interactions occurred. If no overt aggression was observed, animals were separated again after a 30-min period. Each animal was rehoused in its compartment. On the basis of the presence of wounds and agonistic postures displayed (20), subordinate roles were assigned. A member of a pair was classified as subordinate if it was wounded (scuffed fur or abrasions) and observed in a crouching, upright, and side posture or had fled. When one animal of the pair was more severely wounded, the pair was omitted from the sample. The roles were considered stable if the same animal was classified with a subordinate role more than once a day, for at least 2 consecutive days, without any reversal. This long cohousing period with daily physical contact sessions and constant olfactory, auditory, and visual interactions was chosen to differentiate the effects of occasional defeats from those characterizing submission. If this criterion for assessing roles had not been satisfied by day 13, the pair were not included in the sample.

Behavioral Test

From days 11–13, mice were subjected to the behavioral test. The test was conducted between 1500–1700 h in a soundproof cabin. The home cages were transferred to the cabin 12–14 min before starting the test.

The test consisted of introducing one female into the home cage of the subordinate male of the pair, the dominant male being confined behind the partition. During the behavioral test, physical interaction between animals in different sections of the cage was prevented by closing the large hole. Each male participated in the experiment only once. The estrus condition of females was not ascertained as it is reported in the literature (11) that sexually naive males behave similarly and seem unable to discriminate between estrus and nonestrus females.

Data Collection

The data collection method used was instantaneous sampling (1): The behavior of each male was recorded once every 30 s for a 30-min period, totalling 60 sampling points per subject. Behavioral data were collected on the basis of van Oortmerssen's (20) ethogram. Data analysis was based upon the following behavioral categories: a) proxemic behavior—approaching and following the female; b) leavings; c) investigation—sniffing any part of the body of the female, with the exception of the anogenital area; d) anogenital sniff; e) social groom—wiping, licking, and nibbling female's fur; f) mounts—all attempts to mount the partner that fall short of intromission and mounts with intromission; g) genital groom; h) self-groom—wiping, licking, and nibbling its own fur and scratching movements; i) cage exploration—sniffing the substratum, sniffing with the nose up in the air, rearing; j) partition—sniffing, rearing, and digging against the Plexiglas partition separating the mice; k) climb—climbing on the roof of the cage; l) attention posture—holding attention posture with eyes open and ears erect; m) dig—digging and push-digging movements apparently aimed at removing substrate material; n) feed—eating and drinking; o) rest.

The behavior of females was not recorded.

Drug Treatment

Thirty minutes before the behavioral test, subordinate males were injected with vehicle or 0.25 or 0.5 mg/kg diazepam. All injections were made IP in a volume of 10 ml/kg body weight. For diazepam injections, the content of Valium ampoules (Roche, Milan, Italy) was diluted with distilled water. Vehicle injections consisted in diazepam solvent diluted in distilled water.

Data Analysis

Only animals that did not show more than 50 over the total of 60 sampling points in resting activity were considered for statistical analysis. For each behavioral category, a one-way analysis of variance (ANOVA) was conducted to investigate the effect of the two doses of diazepam in subordinates, followed by Fisher's posthoc tests in the case of significance of the former.

RESULTS

The effects of diazepam treatments on subordinate males' behavior are shown in Fig. 1. The statistical analysis demonstrated a significant drug effect on proxemic behavior, $F(2, 47) = 4.04$, $p < 0.05$, anogenital sniff, $F(2, 47) = 5.13$, $p < 0.01$, social groom, $F(2, 47) = 3.21$, $p < 0.05$, and self-groom, $F(2, 47) = 12.39$, $p < 0.001$. Posthoc comparisons indicated that, in comparison with vehicle-injected subordinate males, the lower dose of diazepam (0.25 mg/kg) increased proxemic behavior, anogenital sniff, and social groom of the female. By contrast, self-groom decreased following diazepam treatment. The higher dose of the drug (0.50 mg/kg) only modified social and self-grooming behaviors, significantly increasing the former and reducing the latter. The above results indicate that a low dose of diazepam is able to increase subordinate males' behavior toward the female, suggesting that at least part of the impairment of their sexual performance was due to a state of anxiety in relation to their social status.

EXPERIMENT 2

A second experiment was conducted to verify if the modifications induced by diazepam on the male's behavior toward the female were specific of subordinate males. For this purpose, the most effective dose of diazepam was tested in two other male classes: dominant and isolated males.

Dominant males can further clarify the role of social rank in this experimental procedure. In fact, it could be hypothesized that the behavioral performance of subordinate males was not only affected by social status per se but also by the interfering presence of a second male behind the partition. If this was the case, we should expect dominant males to respond

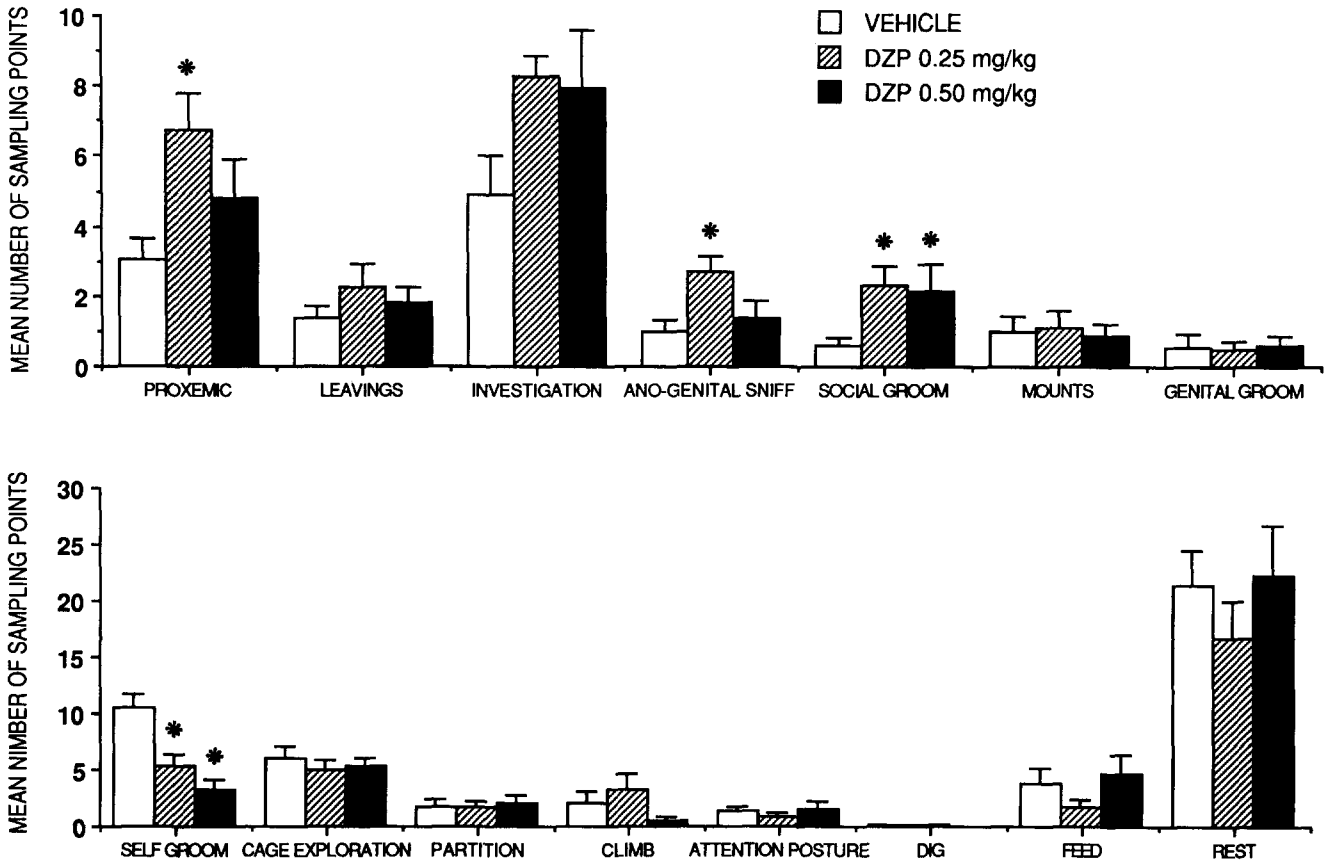

FIG. 1. Effects of diazepam on behavioral profiles of subordinate males in the presence of a female (mean ± SE). *$p < 0.05$, in comparison with vehicle-injected mice.

to drug treatment as subordinate males, as they shared the same experimental procedure and housing.

Isolated mice were chosen because they shared repeated stressful experiences with subordinate animals, that is, social deprivation housing the former, cohabitation with the dominant aggressive partner the latter. However, even if these two classes of males both experienced chronic stress from a physiological and behavioral point of view subordinates are different from isolated subjects (2,4,12,14) and anxiety could perhaps play a role in subordinates but not in isolated animals.

METHOD

Subjects

Subjects of this experiment had the same origin and were housed as mice of Experiment 1 before the experiment started. About 7 or 15 days after their arrival, mice were housed in pairs or singly, respectively, 30 × 13 × 13 cm cages with wood chips and food and water ad lib.

Behavioral Test and Data Collection

The experimental procedure of this experiment follow the same rules of the previous one. Dominant males were those animals in the pairs that were observed to attack, bite, or chase the partner. From days 11–13 of cohousing, dominant males were tested with a female in the partitioned cage.

Isolated males were behaviorally tested in the presence of a female in their home cage after 3–4 days of experimental housing. The length of isolation was brief to avoid large behavioral alterations but long enough to represent a stressful procedure. In addition, the duration of the isolation was comparable with the time the males of the dyad spent in the partitioned cage.

The data collection method and behaviors recorded were the same as in the previous experiment with the exception of the behavioral category partition, which was absent in isolated males.

Drug Treatment and Data Analysis

Dominant and isolated males were injected IP with vehicle or diazepam 0.25 mg/kg, the most effective dose in the previous experiment. The pharmacological treatment preceded the behavioral test by 30 min.

The effects of diazepam on dominant and isolated mice were compared with the effects of the drug on subordinates (data reported in Experiment 1).

General one-way ANOVAs were conducted for each behavioral category to compare the effect of diazepam in subordinate, dominant, and isolated males. Posthoc comparisons were carried out by Fisher's test.

RESULTS

Table 1 reports data for the three classes of males treated with the anxiolytic agent and injected with vehicle solution.

TABLE 1

EFFECTS OF DIAZEPAM (DZP) (0.25 mg/kg) TREATMENTS ON SUBORDINATE, DOMINANT, AND ISOLATED MALES EXPOSED TO A FEMALE

	Subordinate Males		Dominant Males		Isolated Males			
	Vehicle (n = 18)	DZP 0.25 (n = 15)	Vehicle (n = 15)	DZP 0.25 (n = 15)	Vehicle (n = 17)	DZP 0.25 (n = 16)	$F(5, 95)$	p
Proxemic	3.06	6.73*	6.20†	5.93	5.88†	7.50	2.67	<0.05
Leavings	1.44	2.27	2.93	3.53	2.47	2.19	1.75	ns
Investigation	4.94	8.27*	8.20†	8.47	12.59†‡	10.31	6.66	<0.0001
Anogenital sniff	1.00	2.73*	2.60†	2.47	1.29‡	2.00	3.44	0.01
Social groom	0.61	2.33	2.33	2.27	1.23	2.44	1.88	ns
Mounts	1.00	1.13	2.60†	2.00	0.82‡	1.06	2.65	<0.05
Genital groom	0.56	0.47	0.93	0.67	0.65	0.12	0.86	ns
Self-groom	10.50	5.40*	9.13	6.73	5.12†‡	4.69	5.62	<0.001
Cage exploration	6.06	5.00	6.73	7.07	14.35†‡	9.81*	9.69	<0.001
Partition	1.78	1.73	2.13	2.33			0.22§	ns
Climb	2.00	3.20	0.87	0.87	0.12	0.69	1.80	ns
Attention posture	1.33	0.93	1.47	0.73	1.35	1.94	1.68	ns
Dig	0.11	0.13	0.80	0.33	0.59	0.56	2.04	ns
Feed	3.72	1.80	1.47†	1.20	0.41†	0.50	2.74	<0.05
Rest	21.33	16.67	11.20	14.87	11.59	13.75	1.46	ns

Behaviors are expressed as mean number of sampling points.
*$p < 0.05$ in comparison with their vehicle group.
†$p < 0.05$ in comparison with subordinate vehicle group.
‡$p < 0.05$ in comparison with dominant vehicle group.
§F (3, 62).

Posthoc comparisons allowed to evidence not only the effect of the drug in each class of males but also the difference between the three males' categories in baseline condition. In fact, as shown in the table, diazepam affected almost only subordinate males' behavior and acted, as already reported in the first experiment, only on behaviors directed toward the female. Dominant males treated with diazepam modified neither behaviors directed toward the female nor those directed toward the physical environment. Isolated males showed only a decrease of the exploration of the physical environment when injected with diazepam.

Dominant differed from subordinate males for several behavioral categories and, in particular, for those behaviors that are modifiable by the anxiolytic treatment, with the exception of mount behavior, which was higher in dominant males and cannot be increased in subordinates by diazepam treatment. By contrast, the difference between isolates and subordinates referred also to nonsocial behaviors, such as self-grooming, exploration, and feed. In addition, they also show a different behavioral profile in comparison with dominant males to which they have been commonly associated (2,14).

GENERAL DISCUSSION

The results of this study indicate that a low dose of diazepam modifies subordinate males' behavioral performance in the presence of a female but does not affect that of isolated and dominant males. In particular, subordinate males showed an increased interest in the female, as shown by their scores in proxemic behavior, olfactory exploration, and social grooming of the female. By contrast, all other activities directed toward the physical environment were not significantly affected.

Compared to dominant and isolated males, subordinate males seem to be characterized by a different emotional background since they respond to the anxiolytic treatment.

The importance of this study is twofold. First, the results confirm our hypothesis that subordinate males exposed to a female in the presence of the dominant partner suffer from an anxiety condition that contributes to impair their sexual performance. However, anxiolytic treatment, even if able to increase subordinate males' interest in the female, has no effect on such specific sexual behavior as mounting. In fact, subordinate males treated with diazepam did not show any modification in mounting activity and genital grooming, a behavior usually temporarily linked to copulatory activity (16). Thus, we can speculate that the difference in copulatory activity observed between dominant and subordinate males does not simply reflect a condition of anxiety but is probably linked to complex physiological modifications that impair subordinate males' sexual performance (5,6).

The above statement is supported also by data on mice injected with the higher dose of diazepam (0.5 mg/kg): This dose was not able to further increase the improvement of subordinate males' behavior toward the female already obtained with the lower dose so no increase in mounting activity was observed. This suggests that the behavioral differences in sexual activity that subordinate mice injected with 0.25 of diazepam continue to show are not only linked to anxiety.

It is interesting to note that the three different grooming behaviors recorded in subordinates, that is, self-, genital, and social grooming, respond differently to the anxiolytic treatment. Social grooming is increased, self-grooming is lowered, and genital grooming is unaffected by the treatment. This suggests that grooming behavior should be looked into carefully as it reflects different motivational backgrounds, and only self-grooming seem to express conflictual motivations (18) and is sensible to an anticonflict agent such as diazepam.

The second point of relevance of this study is based upon the suggestion to use subordinate males in a sexual context as a model for anxiolytic drugs. In fact, these males express not only a decrease in conflict behavior but, what is more, they show an improvement in their performance. In the experimental procedure used in this study, this improvement was already evident with the low dose of the drug and it appeared only in subordinate males, which are expected to suffer from an anxiety condition.

The advantages of such a model for the study of anxiolytic agents in comparison with classical antianxiety tests based upon conflict or conditioned fear [Geller-Seifter and Vogel tests, (19)] consist in the absence of a procedure of learning that prolongs and complicates the experimental procedure. Unlike the social interaction test (10), in which the anxiolytic effect of benzodiazepines was observed only after chronic administration, in this test the behavioral response to the drug appears just after acute treatment. In addition, in the social interaction test the effect of the drug is evaluated by summing the time spent in active social behaviors independently of their affiliative or disruptive nature; in the present test, aggressive behaviors, usually absent during sexual interactions, are also analyzed separately. Furthermore, the separate analysis of different behaviors conducted in this test presents a more detailed picture of behavioral modifications induced by the drug treatment, evidencing the improvement that subordinate males showed in sexual interactions.

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