Effect of Diazepam on Anxiety, Sexual Motivation, and Blood Testosterone in Anxious Male Mice

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The effects of diazepam on anxious behavior, sexual motivation, and blood level of testosterone in the presence of a female were studied in male mice with elevated anxiety. Diazepam produced an anxiolytic effect in novel environment, but was ineffective during social contacts. The drug potentiated the primary sexual interest, but failed to correct exhaustion of sexual motivation. The drug produced no effect on blood testosterone.

Key Words: *diazepam; $GABA_A$-receptors; social stress; anxiety; sexual motivation*

Diazepam is a synthetic benzodiazepine used for the treatment of anxiety and insomnia. It is a positive modulator of $GABA_A$-receptors and facilitates neurotransmission of GABA, the major inhibitory neurotransmitter in CNS [14]. GABA is widely involved in the regulation of sexual behavior in mammals ranging from motivation and excitation to refractoriness [5,7]. In addition, it affects production and plasma level of testosterone [1,11]. Few data are available on benzodiazepine regulation of behavioral and hormonal components involved in male sexual function [9,13], although some papers report about anxiolytic potency of testosterone [6]. It seems important to study the relationships between the level of anxiety and sexual motivation in animals and the principles of their neurochemical and hormonal regulation.

Our aim was to study behavioral effects of diazepam on anxiety, communicativeness, and sexual motivation. In addition, we examined possible influence of diazepam on plasma testosterone in stressed and intact animals during interactive test with a female.

MATERIALS AND METHODS

The experiments were carried out on male C57B1/6J mice aging 2.5-3 months and weighing 24-28 g. We used behavioral model of chronic social stress: experience of daily (20 days) social defeats in intermale confrontations led to the development of antious status [3]. The controls were grouped intact males without experience of chronic defeats. Each group comprised 10-12 animals.

Acute stress was induced using the restriction model (limited mobility). To this end, the males were placed into plastic cylinders with a diameter of 3 cm for 30 min. Simultaneously with the restriction stress, a receptive female was placed behind the perforated transparent partition. After 30 min, the males were decapitated and blood testosterone was measured.

The following tests were used: elevated plus-maze (EPM) for evaluation of animal anxiety in a novel environment [12]; "partition" test for evaluation of communicativeness as the index of anxiety [10]; behavioral activity of the males was examined by the reaction to known and unknown partner (5+5 min). In addition, test for sexual motivation was carried out to assess the intensity and stability of the behavioral reaction to estrous female of the same genotype behind the perforated transparent partition [2]. The behavioral reactions of males were examined in a 30-min test consisting of 4 successive 5-min sessions (2 sessions in the beginning and 2 sessions before the end of the test). After the test, the males were decapitated, the blood was drawn for testosterone assay. All be-

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havioral parameters were recorded using "Mouse" software.

Diazepam (seduxen) was administered in doses of 0.1, 0.5, and 1.0 mg/kg. The doses were chosen in such a way as to exclude pronounced sedative effect. The drug was injected in a single dose 40 min before testing.

The level of plasma testosterone was determined by solid phase enzyme immunoassay with a SteroidIFA-testosterone-01 standard kit.

The data were processed statistically using nonparametrical Mann—Whitney *U* test and Wilcoxon *T* test.

RESULTS

In EPM test, experimental males subjected to the long-term social conflicts demonstrated lower number of entries into open areas and open arms of the maze, lower total number of entries/exits, and higher number of entries into closed arms compared to intact controls; these animals also spent less time in the center, open arms and open areas, but more time in closed arms (Table 1). In the "partition" test (social contact) with both familiar or unknown partner, the experimental males made significantly lower number of approaches to the partition and spent less time near it (Fig. 1). Our data corroborated the reports on enhanced anxiety and diminished communicativeness of mouse males subjected to chronic social conflicts [3].

Diazepam produced no behavioral effect on intact males tested in EPM. By contrast, high doses of this drug restored almost all behavioral parameters in experimental males (losers in intermale confrontations) to the control values (Table 1). Thus, these experiments reproduced the classical anxiolytic effect of diazepam.

Diazepam produced no significant effect on the behavior of control males during social contacts (Fig. 1). On the contrary, administration of diazepam in low doses shortened social contacts with familiar and unfamiliar males and decreased the number of approaches to the partition in both tests. None dose of diazepam restored parameters of social interaction in anxious mice to the control level. The drug was inefficient in correcting diminished communicativeness of anxious males during social contacts, although there are data on its re-socializing effect [8]. Probably, the drug produced no effect under conditions, which were associated with traumatic chronic social stress. Injection of anxiolytic dehydroepiandrosterone sulfate under similar experimental conditions activated the behavior of anxious males near the partition [4]. Probably, the

TABLE 1. Effect of Diazepam (1 mg/kg) on Male Behavior in EPM Test

Behavioral parameter	Intact		Anxious	
	physiological saline	diazepam	physiological saline	diazepam
Closed arm, L	32.8±8.6	25.6±11.1	11.4±3.0	47.4±19.9
Closed arm, N	3.0±0.6	3.6±0.6	2.2±0.4	3.1±0.6
Closed arm, N, %	39.5±3.0	43.2±2.6	48.6±1.4^{+}	36.8±4.0*
Closed arm, T	244.0±13.1	252.0±14.4	283.0±4.1^{+}	234±20*
Closed arm, T, %	82.6±4.1	64.5±4.5	94.2±1.4^{+}	79.1±6.7*
Center, N	3.8±0.6	4.0±0.6	2.2±0.4^{+}	4.1±0.9*
Center, N, %	50.1±1.8	46.2±2.1	48.6±1.4	49.0±1.9
Center, T	29.8±4.7	18.5±3.7	17.2±4.2^{+}	31.7±10.1
Center, T, %	10.2±1.6	6.3±1.3	5.7±1.4^{+}	10.8±3.4
Open arm, L	186.0±40.3	184.0±40.6	275.0±24.7	142.0±45.8*
Open arm, N	1.0±0.4	1.4±0.6	0.1±0.1^{+}	1.3±0.4*
Open arm, N, %	10.3±3.4	10.6±3.7	2.8±2.8	14.3±4.2*
Open arm, T	20.5±9.4	26.8±12.0	0.4±0.4^{+}	29.8±15.0*
Open arm, T, %	7.2±3.4	9.3±4.1	0.1±0.1^{+}	10.1±5.2*
Number of entries, %	60.5±3.0	96.8±2.6	51.4±1.4^{+}	63.2±4.0
Duration of entries, %	17.4±4.1	15.5±4.5	5.6±1.4^{+}	21.0±6.7
Number of entries/exits	4.0±0.8	5.0±1.1	2.3±0.4^{+}	4.4±1.0

Note. $p<0.05$ compared to *injection of physiological saline in the respective group; $^{+}$intact (physiological saline). *L* — latency, *N* — number, *T* — time.

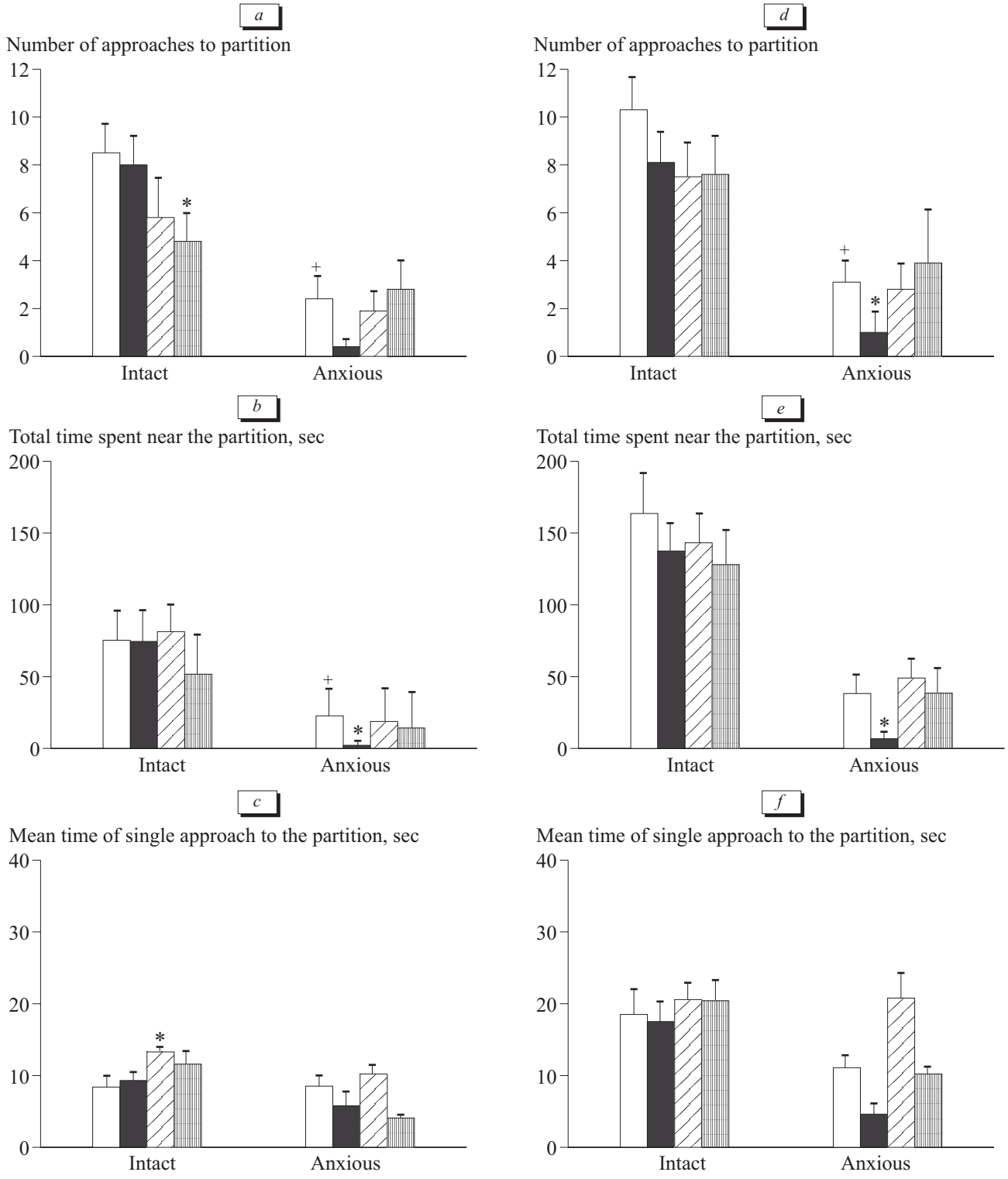

Fig. 1. Effect of diazepam on behavior of mouse males in the partition test with familiar (*a-c*) and unfamiliar (*d-f*) partners. Here and in Fig. 2: light and dark bars correspond to physiological saline and diazepam (0.1 mg/kg). The oblique and vertical shadings correspond to diazepam in doses of 0.5 and 1 mg/kg, respectively. $p<0.05$ compared to: *corresponding group injected with physiological saline; +intact group.

anxiolytic potency of diazepam can be realized in novel anxiogenic environment, but not under conditions previously provoking the development of anxious status in animals.

In the test for sexual motivation, the primary behavioral reaction to the female during the first 5 min of communication in anxious males was shorter than in intact controls (Fig. 2, *a-c*). In

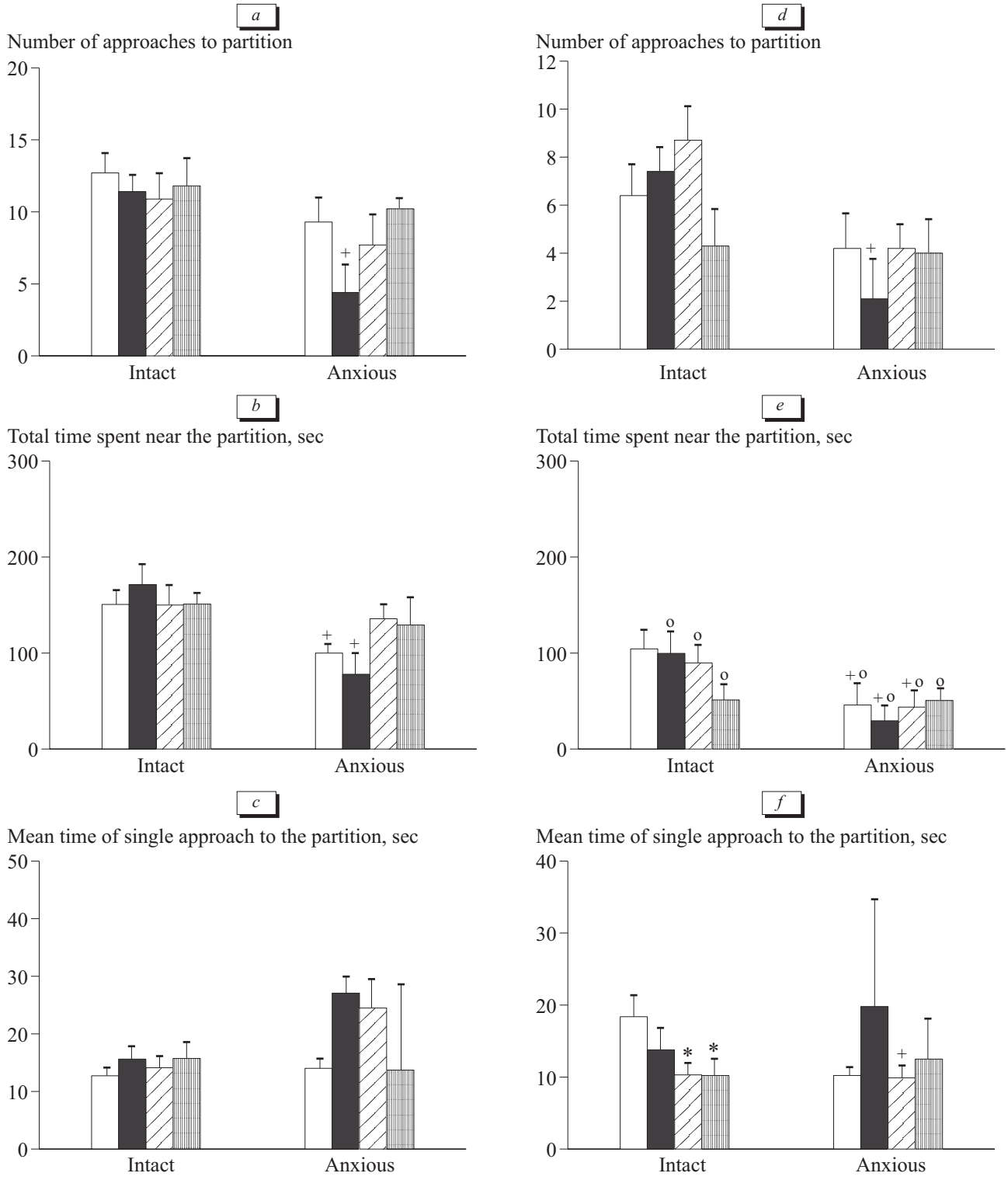

Fig. 2. Effect of diazepam on parameters of sexual motivation in the first (*a-c*) and last (*d-f*) sessions of the test. °$p<0.05$ compared to the total duration of the stay at the partition in the first session of the test in the corresponding group.

control males, the total duration of the reaction to female in the last session of the test did not differ from the value measured during the first 5 min of the communication. By contrast, in anxious males the time spent near the partition in the last session was shorter than that during the first session, which attests to exhaustion of sexual motivation [2].

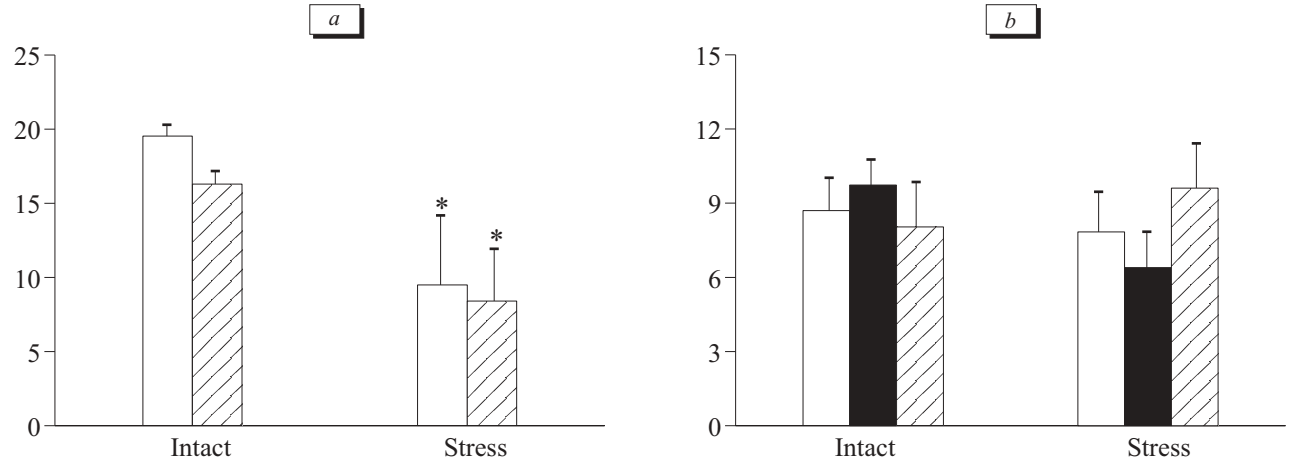

Fig. 3. Effect of stress and diazepam on plasma testosterone level (nmol/liter) in mouse males in the presence of a receptive female. *a*) acute stress; *b*) chronic stress. Light bars correspond to physiological saline. Dark and dashed bars correspond to diazepam administered in doses of 0.1 and 0.5 mg/kg, respectively. *$p<0.05$ compared to the corresponding group without restriction.

Similar to communication with males, diazepam produced no effect on behavioral indices in intact mice during the first 5 min of the partition test. By contrast, in anxious mice moderate doses of the drug slightly increased the total (U=43, p=0.094) and mean (U=38, p=0.085) time spent near the partition (the test on female) in comparison with anxious males injected with physiological saline. The maximum dose of the drug restored the primary sexual interest in submissive males to a level measured in intact animals. Thus, diazepam cannot restore communicativeness of anxious males, but activates suppressed primary behavioral response to the female.

In control males, diazepam produced no effect on the number of approaches to the partition in the last session (Fig. 2, *d-f*). When injected in a dose of 0.5 mg/kg, diazepam decreased the mean duration of the response to female in comparison with that of intact males injected with physiological saline in the same session of the test. The higher dose (1 mg/kg) decreased the total and mean duration of the response under the same experimental conditions. In anxious animals, none doses of diazepam affected sexual motivation in the last session of the test in comparison with intact males injected with physiological saline. In all groups diazepam led to exhaustion of provoked motivation during 30-min test. Probably, GABAergic cerebral mechanisms (specifically, $GABA_A$-benzodiazepine receptor site) are involved in the fundamental neurochemical mechanisms underlying instability of sexual interest in animals.

Therefore, diazepam produces different effects on primary sexual interest in mouse males and its maintenance. The drug produces no effect on primary sexual interest in intact animals, but can potentiate it in anxious males. Diazepam does not prevent exhaustion of sexual motivation in anxious males. Moreover, it provokes the development of similar exhaustion in intact animals.

Acute restriction test 2-fold decreased plasma level of testosterone in mouse males, which was previously elevated due to perception of a female (Fig. 3). Diazepam injected in a dose of 0.5 mg/kg produced no effect on plasma testosterone level during interaction with the female in both tests (with and without immobilization). Chronic stress of long-term social conflicts produced no effect on blood testosterone in the presence of receptive female: in anxious males, the concentration of this hormone did not differ from the control value. In control and anxious mouse males, diazepam (0.1 and 0.5 mg/kg) produced no effect on testosterone level stimulated by the presence of a female. Therefore, diazepam does not affect blood testosterone during olfactory contact with the female, which excludes the testosterone-mediated mechanism of action of this drug on the appetent phase of sexual behavior in mouse males.

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