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GABAergic Drugs and Socio-Sexual Behavior

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PAREDES, R. G., P. KARAM, L. HIGHLAND AND A. AGMO. *GABAergic drugs and sociosexual behavior.* PHARMACOL BIOCHEM BEHAV **58**(2) 291–298, 1997.—To elucidate the role of GABA in the control of sexual behavior, the effects of the $GABA_A$ agonist 4,5,6,7-tetrahydroisoxazolo[5,4*c*]-pyridin-3-ol (THIP), the GABA transaminase inhibitors sodium valproate and γ-acetylen-GABA (GAG), and the GABA synthesis inhibitors isoniazide and deoxypyridoxine were evaluated in sexual behavior, exploration, and sociosexual interactions with a receptive female or a castrated male. Furthermore, to discriminate possible general inhibitory effects from those specific to sexual behavior, the doses of the drugs that produced a significant inhibition of copulation were tested in a free drinking procedure. THIP (16 mg/kg), sodium valproate (400 mg/kg), GAG (100 mg/kg), and deoxypyridoxine (400 mg/kg) produced a strong inhibition of sexual behavior. The percentage of animals displaying mounts and intromissions as well as the mean number of mounts and intromissions were significantly reduced. Sociosexual interactions with a receptive female or a castrated male and exploratory behaviors were also reduced. The most consistent effects observed were reductions in sniffing, self-grooming, and rearing. Drinking behavior was significantly reduced in doses that inhibited sexual behavior. These results further support the hypothesis that altered GABAergic neurotransmission produces reduced sensitivity to environmental stimuli and thereby inhibits sexual and drinking behavior in a nonspecific way. © 1997 Elsevier Science Inc.

GABA Sex behavior Exploratory behavior Sociosexual interactions Drinking behavior

MALE sexual behavior is among the many behaviors modified by GABA [reviewed in (30)]. The observation that the concentration of GABA in the cerebrospinal fluid of male rats significantly increases after ejaculation (36) may suggest that GABA is related to the postejaculatory behavioral inhibition. In support of this hypothesis, it was reported that infusion of the $GABA_A$ agonist muscimol and the GABA transaminase inhibitor ethanolamine-*O*-sulphate into the medial preoptic area (MPOA) produced strong inhibition of most aspects of sexual behavior (16). In contrast, infusion of $GABA_A$ antagonists into the MPOA facilitated sexual behavior in castrated testosterone-treated rats (17), reduced the duration of the ultrasonic vocalizations that are normally emitted by the male rat after ejaculation (18), and shortened the postejaculatory interval (15). However, direct infusion of GABA had little effect on copulation, producing only a slight increase in intromission latency (16). Moreover, the facilitatory effects on pre-ejaculatory parameters, i.e., interintromission interval and ejaculation latency, observed in the same study (15) do not agree with a role for GABA in only postejaculatory events. In fact, a facilitation of sexual behavior similar to that produced by infusion of $GABA_A$ antagonists has been observed shortly after an electrolytic lesion of the MPOA, giving no clear evidence for a role of the $GABA_A$ receptor in the control of male sexual behavior (31). Rather, it seems that any enhancement of activity within the MPOA stimulates male sexual behavior [for a discussion, see (31)].

The role of GABA receptor subtypes in sexual behavior has been analyzed in previous pharmacological studies. The systemic administration of the $GABA_A$ agonist 4,5,6,7-tetrahydroisoxazolo[5,4*c*]-pyridin-3-ol (THIP) inhibits sexual behavior (6). However, the inhibitory effects of THIP were not blocked by concurrent administration of bicuculline; another $GABA_A$ agonist, 3-aminopropanesulfonic acid (APSA), had no effect on sexual behavior. These data further support the hypothesis that the $GABA_A$ receptor is not involved in the control of male sexual behavior (6).

The enhancement of GABAergic neurotransmission produced by GABA transaminase inhibitors (GTIs) also inhibits sexual behavior in the male rat. However, a detailed behavioral analysis has shown that the inhibitory effects produced by GTIs are associated with strong motor deficiencies, i.e.,

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sexual behavior is inhibited only at doses at which motor execution is much impaired (7). Furthermore, this class of drugs reduces the intromission ratio, thereby suggesting difficulties in achieving penile insertion. However, GTIs did not disturb the copulatory thrusting pattern that allows the penis to find the vaginal orifice (1). Rather, GTIs appear to reduce the duration of contraction of the ischiocavernosus muscles in copula (34). The contraction of these muscles is necessary for penile insertion (27).

The stimulation of $GABA_B$ receptors by baclofen inhibits penile reflexes ex copula (10,23) and sexual behavior in doses not associated with an impairment of motor execution (29). The inhibitory effects on sexual behavior produced by baclofen are blocked by concurrent administration of the $GABA_B$ antagonist CGP35348 (32). It has also been reported that baclofen inhibits precopulatory and copulatory behaviors in male rats without affecting nonsexual social interactions or exploratory behaviors (29). It appears, then, that stimulation of the $GABA_B$ receptors has a specific inhibitory effect on behaviors associated with the initiation of copulatory activity.

To further understand the complex role of GABA in the control of sexual behavior, a detailed analysis is required. In the present experiment, copulatory parameters as well as exploratory behaviors and sociosexual interactions with a castrated male and a receptive female were evaluated in male rats after: a) specific stimulation of $GABA_A$ receptors, b) enhancement of GABA levels by administration of GTIs, and c) reduction of GABA concentrations by inhibition of the synthesis of this neurotransmitter. If drug effects were similar in social interactions with a castrated male and sexual interactions with a receptive female, it could be argued that a generalized behavioral impairment is produced. If the effects were observed only when the animals are tested with a receptive female, they could be specific to sexual behavior. The behavioral specificity of the drug actions was further analyzed in a free drinking procedure. In that way, it could be determined if the drugs that inhibited sexual behavior also reduced another biologically relevant behavior.

METHODS

Subjects

Adult, sexually naive male Wistar rats (300–450 g) from a local colony were used. They were maintained under a reversed light/dark cycle (12 D:12 L, lights off 0900 h) and were given free access to commercial rat pellets and tap water. Stimulus females were injected with estradiol benzoate (Sigma, St. Louis, MO, USA), 25 μg/rat, 52–56 h before mating and with progesterone (Aldrich, Milwaukee, WI, USA), 1 mg/rat, 4–6 h before. Stimulus males used in tests of social interactions were castrated at least 2 months before being used.

Drugs

The following drugs were used: the $GABA_A$ agonist 4,5,6,7-tetrahydroisoxazolo[5,4*c*]-pyridin-3-ol (THIP) HCl (Research Biochemicals, Natick, MA, USA); the GABA transaminase inhibitors sodium valproate (VAL; Ciba-Geigy Mexicana, Mexico City, Mexico) and γ-acetylen-GABA (GAG; Merrell International, Strasbourg, France); and the GABA synthesis inhibitors isonicotinic acid hydrazide (isoniazid, ISO; Aldrich, Milwaukee, WI, USA) and 4-deoxypyridoxine (DEOXY) HCl (Sigma). THIP was dissolved in physiological saline and injected intraperitoneally (IP) in a volume of 1 ml/kg. All other drugs were dissolved in distilled water and injected IP in a volume of 5 ml/kg. The doses of the drugs were chosen based on previous studies from this laboratory (6,9). The doses of THIP, GAG, and VAL used were the lowest that have been consistently shown to reduce sexual behavior and have also been evaluated with regard to motor execution. In the case of ISO and DEOXY, the doses used have been shown to efficiently reduce cerebral GABA concentrations without being convulsive (24) and to have motor actions similar to other GABAergic compounds (9)

The intervals between drug administration and behavioral observation were as follows: THIP, 30 min; VAL, 15 min; GAG, 3 h; ISO, 30 min; and DEOXY, 1 h. All drugs were administered according to a Latin-square design. That is, the execution of each animal was compared over the different doses of a given drug plus saline. To minimize any possible effect being carried over from previous treatment, the interval between drug injections was 7 days. No animal received more than one drug.

Procedure

Males were tested three times in 30-min tests with receptive females. Only those males that ejaculated in each of the screening tests were used in the experiment. Because GABA appears to modulate endocrine functions [reviewed in (30)], the subjects were castrated to reduce the possibility of an endocrine effect of the GABAergic drugs. Castration was performed under ether anesthesia, and a testosterone (Sigma) filled silastic capsule (i.d. 0.062 inch, o.d. 0.125 inch; Dow Corning Corporation, Midland, MI, USA) 20 mm long was implanted subcutaneously. This implant maintained normal sexual activity in the animals for several weeks (13). Testing began 2 weeks after castration and capsule implantation.

Sexual behavior. A detailed description of the testing procedure can be found elsewhere (29). Briefly, the subjects were placed into an observation cage where a receptive female had already been placed. The following parameters were registered in a 20-min test: mount latency (time from the introduction of the male until the first mount with pelvic thrusting), intromission latency (time from the introduction of the male until the first mount with vaginal penetration), ejaculation latency (time from the first intromission until ejaculation), number of mounts, and number of intromissions during the test period or until ejaculation. In addition, the intromission ratio was calculated by adding the number of mounts and intromissions and dividing the sum by the number of intromissions.

Exploratory behaviors and sociosexual interactions. These behaviors were registered when the subject was placed with either a receptive female or a castrated male. The interactions with a receptive female were recorded for the first 10 min of the sexual behavior test, and those with a castrated male were registered for the same amount of time in a separate test done at least 1 week apart. In that way, the interactions with a receptive female and a castrated male were observed in the same subject. Exploratory behaviors included the frequency and duration of rearing (the subject standing on the hind legs anywhere in the observation cage) and sniffing (rapid movements of the head or whiskers while the animal is exploring). Sociosexual interactions included the frequency and duration of self-grooming (licking or gently biting different areas of the fur, the limbs, or the genital area), grooming partner (licking or gently biting different areas of the partner's fur or limbs), genital exploration (sniffing or licking the partner's anogenital region), pursuit (the experimental animal following the stimulus animal and keeping close contact), and resting (lying

or standing still, without any particular overt activity), which is characteristic of postejaculatory behavior and might be an indicator of sedation.

Lickometer. Intact animals were water deprived for 24 h and placed for 10 min in a lickometer apparatus for a habituation session. Licks were recorded with an optical lickometer (Coulbourn) in a standard animal chamber (Lafayette). Only those animals that licked more than 200 times were used in the experiment. They were given water for 10 min and deprived again for 24 h before being tested under drug treatment. Only the doses that produced a significant inhibition of sexual behavior were used in this part of the experiment. Drugs were administered according to a parallel-groups design. No animal received more than one drug.

Statistical Analysis

In tests of sexual behavior, percentages of mounts, intromissions, and ejaculation were analyzed by Cochran's *Q*-test and/or McNemar's test for the significance of changes or the binomial test when appropriate. Numbers of mounts and intromissions were evaluated with Friedman's analysis of variance (ANOVA) and/or the Wilcoxon matched-pairs signed-ranks test. In these analyses, all animals were included. Latencies were analyzed by Kruskal–Wallis one-way ANOVA and/or the Mann–Whitney *U*-test. Here, only subjects from which data were actually obtained were included. This was considered more appropriate than assessing values for noncopulating males.

All sociosexual and exploratory behaviors were analyzed for each drug by a single two-factor multiple ANOVA (MANOVA) for repeated measures on one factor (doses of a given drug). The between-groups factor was stimulus animal (receptive female vs. castrated male). Pillai's trace statistic was used because it has been shown to be more robust than other MANOVA statistics (11). In case of a significant omnibus test, univariate ANOVA was performed for each variable. The Bonferroni correction was then applied to determine significant differences. In case of significant interactions in the omnibus test, multivariate tests for simple main effects were performed, followed by univariate ANOVAs with Bonferroni corrections for significance levels. When the factor dose had more than two levels, pairwise multivariate contrasts were performed using Hotelling's T2. When a significant result was obtained, pairwise univariate comparisons were made with Tukey's honestly significant difference (HSD) procedure.

For the lickometer, a one-way ANOVA Kruskal–Wallis test was used. To determine the differences between control treatment and each dose, Tukey's HSD procedure or the Mann–Whitney *U*-test was used following significant ANOVA.

RESULTS

A total of 54 male rats were used in tests of sexual behavior and sociosexual interactions. They were randomly assigned to one of the drugs tested: THIP ($n = 12$), VAL ($n = 10$), GAG ($n = 12$), DEOXY ($n = 10$), and ISO ($n = 10$).

THIP

Sexual behavior. Although most parameters of sexual behavior were reduced after THIP 8 mg/kg, no significant effect was found. However, a dose of 16 mg/kg produced a complete inhibition of sexual behavior (Table 1).

Sociosexual interactions. When the frequency of sociosexual interactions was analyzed with the MANOVA, significant main effects of stimulus animal and dose as well as a significant interaction between stimulus animal and dose were found. Therefore, simple main effects were analyzed. MANOVA statistics are summarized in Table 2 and are not mentioned in the text. The duration of sociosexual interactions differed between stimulus animals and drug doses. However, no interaction between stimulus animal and dose was found with respect to duration. Therefore, these data were not further analyzed.

Analysis of the simple main effects of stimulus animal within each dose showed reduced frequency of pursuit and increased frequency of rearing when the animals where ob-

TABLE 1

SEXUAL BEHAVIOR PARAMETERS IN MALE RATS AFTER ADMINISTRATION OF THIP

		THIP	
Behavior	Vehicle	8	16
Mount percentage	92	50	0**
Intromission percentage	92	50	0**
Ejaculation percentage	75	41	0**
Number of mounts	7.0 ± 1.7	3.2 ± 1.5	0**
Number of intromissions	9.8 ± 1.4	4.6 ± 1.6	0**
Intromission ratio	0.6 ± 0.7	0.7 ± 0.9	0

Drug doses are expressed in mg/kg; $n = 12$ for each dose. Number of mounts, number of intromissions, and intromission ratio are mean ± SE.

**$p < 0.01$ vs. vehicle.

TABLE 2

MANOVA STATISTICS ON SOCIOSEXUAL INTERACTIONS AFTER TREATMENT WITH THIP

	Pillai's *V*	df	*F*	*p*
Main effects				
Frequency				
Stimulus animal	0.659	7, 15	4.139	0.010
Dose	1.018	14, 74	5.485	<0.001
Stimulus animal × dose	0.735	14, 74	3.072	0.001
Duration				
Stimulus animal	0.670	6, 16	5.421	0.003
Dose	0.776	12, 76	4.015	<0.001
Stimulus animal × dose	0.444	12, 76	1.808	0.062
Simple main effects				
Frequency				
Stimulus animal				
Vehicle	0.796	7, 15	8.382	<0.001
THIP 8 mg/kg	0.452	7, 15	1.768	0.168
Dose				
Castrated male	0.939	14, 74	32.988	<0.001
Receptive female	0.931	14, 74	28.859	<0.001

For THIP 16 mg/kg, it was impossible to perform multivariate tests because of zero error variance in some variables; therefore, the Mann–Whitney *U*-test was performed for each variable. No significance was obtained (all $ps > 0.3$). The simple main effects of the duration of sociosexual behaviors were not analyzed because there was no significant interaction between stimulus animal and dose.

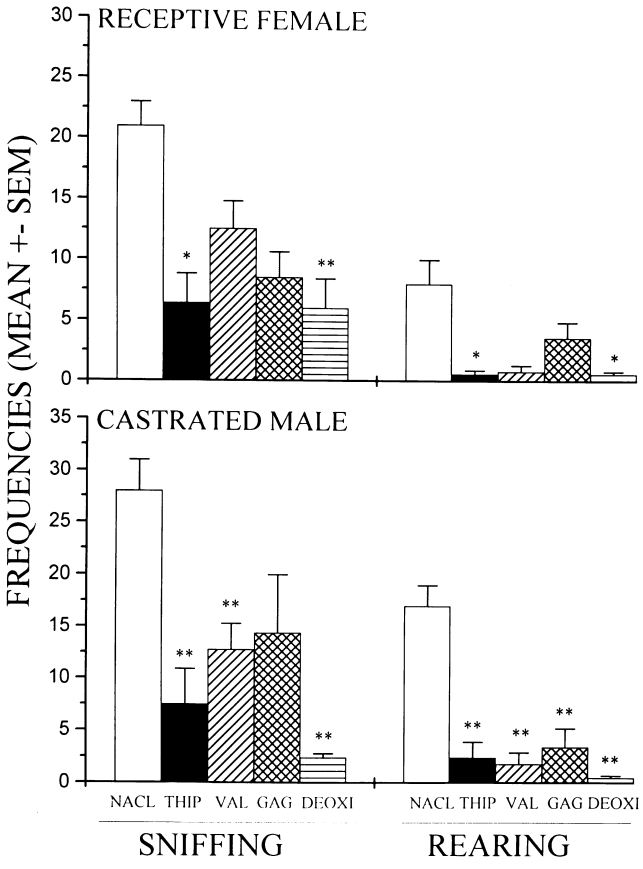

FIG. 1. Frequency of sniffing and rearing in male rats treated with NaCl, THIP (16 mg/kg), VAL (200 mg/kg), GAG (100 mg/kg), and DEOXY (400 mg/kg) while tested with a receptive female or a castrated male. Data are mean ± SEM. $*p < 0.05$ and $*p < 0.01$ vs. NaCl treatment.

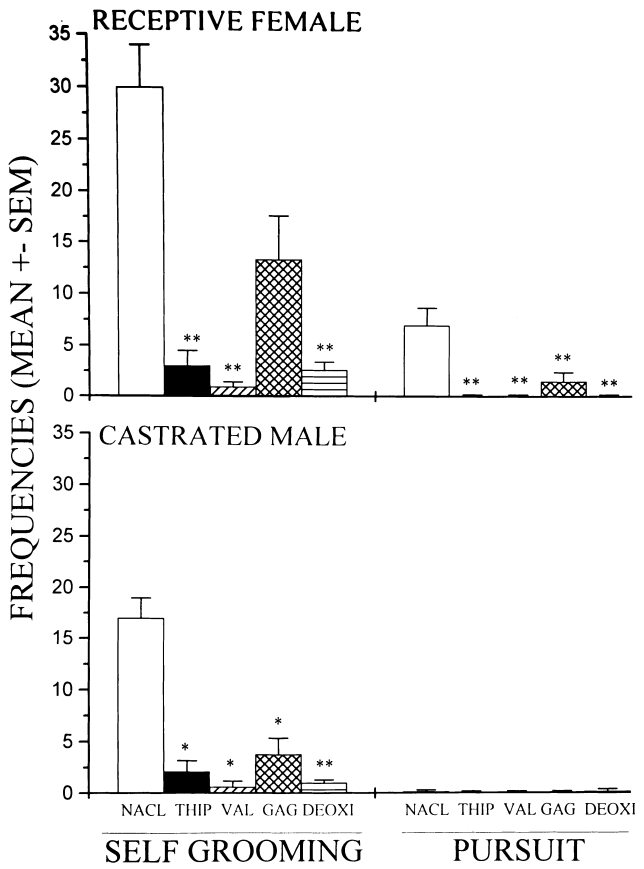

FIG. 2. Frequency of self-grooming and pursuit in male rats treated with NaCl, THIP (16 mg/kg), VAL (200 mg/kg), GAG (100 mg/kg), and DEOXY (400 mg/kg) while tested with a receptive female or a castrated male. Data are mean ± SEM. $*p < 0.05$ and $*p < 0.01$ vs. NaCl treatment.

served with a castrated male in comparison to when they were observed with a receptive female after saline but not after THIP 8 or 16 mg/kg. When the simple main effects of dose were analyzed within each stimulus animal, it was found that a dose of 16 mg/kg produced inhibition of sniffing, rearing (Fig. 1), and grooming (Fig. 2) when the animals were tested with both a receptive female and a castrated male. Pursuit of the receptive female and grooming of the castrated male were also significantly reduced. These results demonstrate that THIP produces a generalized inhibition of behavior. That is, the drug inhibits sexual and social interactions as well as exploratory behavior.

TABLE 3

SEXUAL BEHAVIOR PARAMETERS IN MALE RATS AFTER ADMINISTRATION OF VAL OR GAG

	VAL			GAG		
Behavior	Vehicle	200	400	Vehicle	50	100
Mount percentage	100	80	0**	100	75	25**
Intromission percentage	90	60	0**	100	58	25**
Ejaculation percentage	30	10	0	83	41	17*
Number of mounts	5.7 ± 1.4	5.6 ± 1.6	0**	7.6 ± 1.4	4.6 ± 1.3	1.9 ± 1.4*
Number of intromissions	8.2 ± 1.3	2.3 ± 0.1*	0**	10.3 ± 1.3	4.9 ± 1.6*	1.5 ± 0.1**
Intromission ratio	0.6 ± 0.1	0.3 ± 0.1*	—	0.6 ± 0.6	0.4 ± 0.3	0.5 ± 0.5

Drug doses are expressed in mg/kg; $n = 10$ for VAL and $n = 12$ for GAG. Values for number of mounts, number of intromissions, and intromission ratio are mean ± SE.

$*p < 0.05$ and $**p < 0.01$ vs. vehicle for each drug.

TABLE 4
MANOVA STATISTICS FOR SOCIOSEXUAL INTERACTIONS AFTER TREATMENT WITH VAL OR GAG

	Pillai's *V*	df	*F*	*p*
VAL: Main effects				
Frequency				
Stimulus animal	0.786	7, 12	6.338	0.003
Dose	1.126	14, 62	5.707	<0.001
Stimulus animal × dose	0.852	14, 62	3.290	0.001
Duration				
Stimulus animal	0.769	6, 13	7.218	0.001
Dose	1.056	12, 64	5.964	<0.001
Stimulus animal × dose	0.523	12, 64	1.889	0.052
VAL: Simple main effects				
Frequency				
Stimulus animal				
Vehicle	0.874	7, 12	11.880	<0.001
VAL 200 mg/kg	0.961	7, 12	42.714	<0.001
Dose				
Castrated male	0.679	14, 62	2.276	0.014
Receptive female	1.218	14, 62	6.906	<0.001
GAG: Main effects				
Frequency				
Stimulus animal	0.828	7, 13	8.929	<0.001
Dose	0.743	14, 66	2.790	0.003
Stimulus animal × dose	0.750	14, 66	2.827	0.002
Duration				
Stimulus animal	0.540	6, 14	2.739	0.056
Dose	0.761	12, 68	3.479	0.001
Stimulus animal × dose	0.485	12, 68	1.817	0.063
GAG: Simple main effects				
Frequency				
Stimulus animal				
Vehicle	0.816	7, 13	8.250	0.001
GAG 50 mg/kg	0.671	7, 13	3.787	0.018
GAG 100 mg/kg	0.469	7, 13	1.638	0.210
Dose				
Castrated male	0.979	14, 66	87.779	<0.001
Receptive female	0.970	14, 66	60.626	<0.001

For VAL 400 mg/kg, it was impossible to perform multivariate tests because of zero error variance in some variables; therefore, the Mann–Whitney *U*-test was performed for each variable. The simple main effects of the duration of sociosexual behaviors were not analyzed because there was no significant interaction between stimulus animal and dose.

GABA Transaminase Inhibitors

Sexual behavior. The intermediate doses of VAL (200 mg/kg) and GAG (50 mg/kg) significantly reduced the number of intromissions when compared with controls. Higher doses of VAL (400 mg/kg) and GAG (100 mg/kg) produced a strong inhibition of sexual behavior (Table 3). GAG 100 mg/kg reduced the percentage of animals displaying mounts, intromissions, and ejaculations. A similar reduction was observed in the number of mounts and intromissions (Table 3). No effects were observed on mount, intromission, and ejaculation latencies (data not shown).

Sociosexual interactions. Significant main effects of stimulus animal and dose as well as a significant interaction between them were found in the frequencies of sociosexual interactions for both VAL and GAG. Therefore, the simple main effects were analyzed. MANOVA statistics are summarized in Table 4 and are not mentioned in the text. For VAL, significant main effects of stimulus animal and dose were found in the duration of sociosexual interactions. A significant main effect of dose was also found for GAG. However, no significant interaction was found when the duration of sociosexual behaviors was analyzed with MANOVA for both GTIs. Therefore, no further analysis of the durations was done.

When the simple main effects of frequencies of sociosexual behaviors were evaluated in the VAL-treated group, significant differences were observed between animals placed with a castrated male or a receptive female under the saline condition. The frequency of pursuit and self-grooming was lower, whereas the frequency of rearing was higher when the animals were tested with a castrated male. This difference is also observed after VAL 200 mg/kg but was absent at 400 mg/kg. Tests for simple main effects of dose within each stimulus animal were then performed. No differences were observed between the animals treated with VAL 200 mg/kg and the saline-treated subjects. When the behaviors were analyzed after a VAL dose that completely inhibited sexual behavior (400 mg/kg), a variety of sociosexual interactions were reduced in both the receptive female and castrated male conditions when compared with vehicle. Sniffing, rearing, and self-grooming were reduced when the animals were tested with a castrated male. Self-grooming and pursuit were reduced, whereas resting increased when the animals were tested with a receptive female (see Figs. 1, 2). These results indicate that VAL also produced a generalized inhibition of behavior affecting both social and sexual interactions.

When the simple main effects of frequencies of sociosexual behaviors were evaluated in the GAG-treated group, significant differences were observed between animals placed with a castrated male or a receptive female under the saline condition. The frequency of pursuit was lower and that of rearing higher in the animals tested with a castrated male in comparison with those tested with a receptive female. The same difference was observed in the animals treated with GAG 50 mg/kg but was absent after 100 mg/kg. Tests for simple main effects of dose showed no difference between the animals treated with GAG 50 mg/kg and those treated with saline. The dose of GAG that produced a drastic inhibition of sex behavior, 100 mg/kg, also inhibited social and sexual interactions. The frequency of self-grooming and rearing was reduced when the animals were tested with a castrated male, whereas the frequency of pursuit was reduced in animals tested with a receptive female (see Figs. 1, 2). Animals observed with a receptive female after GAG 100 mg/kg spent more time resting than they did after vehicle administration. GAG inhibited behaviors related to sociosexual interactions as well as those related to exploratory behavior. These results indicate that GAG produces a generalized inhibition of behavior.

GABA Synthesis Inhibitors

Sexual behavior. A dose of 200 mg/kg of ISO had no effect on any of the copulatory parameters (data not shown). A higher dose of ISO was not used because after pilot injections of 400 mg/kg, a high percentage of animals developed convulsions. A dose of 200 mg/kg of DEOXY reduced the number of mounts (Table 5). A higher dose, 400 mg/kg, drastically inhibited copulation. This inhibition was reflected in a reduced

TABLE 5

SEXUAL BEHAVIOR PARAMETERS IN MALE RATS AFTER ADMINISTRATION OF DEOXY

Behavior	DEOXY		
	Vehicle	200	400
Mount percentage	100	70	10**
Intromission percentage	80	70	10**
Ejaculation percentage	40	10	0
Number of mounts	7.8 ± 1.8	2.9 ± 1.1*	0.1 ± 0.1**
Number of intromissions	9.3 ± 2.1	5.5 ± 1.3	0.1 ± 0.1*
Intromission ratio	0.5 ± 0.9	0.7 ± 0.7	1.0 ± 0.0

Drug doses are expressed in mg/kg; $n = 10$ for each dose. Values for number of mounts, number of intromissions, and intromission ratio are mean ± SE.

*$p < 0.05$ and **$p < 0.01$ vs. vehicle.

percentage of animals displaying mounts and intromissions. In fact, the only animal that copulated after the high dose of DEOXY displayed only one intromission (Table 5). No effect was observed on mount, intromission, and ejaculation latencies (data not shown).

Sociosexual interactions. The MANOVA of frequencies of sociosexual interactions for ISO showed significant effects of stimulus animal and dose and an interaction between stimulus animal and dose. The simple main effects were therefore analyzed (data not shown). The interaction between stimulus animal and dose was not significant when the duration of sociosexual behaviors was analyzed. Therefore, no further analysis of durations was done.

Although 200 mg/kg of ISO did not inhibit sexual behavior, it affected sociosexual interactions. The frequency of sniffing and grooming was reduced when the animals were tested with a receptive female. Similarly, the frequency of grooming the partner was reduced after ISO treatment when the animals were tested with both a receptive female and a castrated male (data not shown).

The analysis of simple main effects of stimulus animal in the DEOXY group showed that the frequencies of self-grooming and pursuit were significantly lower when the animals were observed with a castrated male than with a receptive female after saline treatment (MANOVA statistics are summarized in Table 6). Analysis of simple main effects of dose showed that the frequency of sociosexual interactions after a DEOXY dose of 200 mg/kg was significantly affected. Self-grooming and pursuit were reduced when the animals were observed with a receptive female, whereas sniffing was reduced when the animals were tested with both a receptive female and a castrated male (data not shown). A dose of DEOXY (400 mg/kg) that produced an almost complete inhibition of copulation also reduced sociosexual interactions. As can be seen in Figs. 1 and 2, the frequencies of sniffing, rearing, and self-grooming were reduced when the animals were tested with both a receptive female and a castrated male. The frequency of pursuit when tested with a receptive female and that of grooming the partner when tested with a castrated male were also reduced after DEOXY 400 mg/kg in comparison with vehicle injection. These results indicate that social and sexual interactions as well as exploratory behaviors were affected by DEOXY treatment.

To summarize, the analysis of sociosexual interactions showed that vehicle-treated animals pursue the receptive female more than they pursue the castrated male. As a consequence of copulation, they also engage in significantly more grooming than when tested with a castrated male. The doses of the drugs administered that produced a strong inhibition of copulation also drastically affected exploratory behaviors, social interactions with a castrated male, and sociosexual interactions with a receptive female.

TABLE 6

MANOVA STATISTICS ON SOCIOSEXUAL INTERACTIONS AFTER TREATMENT WITH DEOXY

	Pillai's V	df	F	p
Main effects				
Frequency				
Stimulus animal	0.774	7, 12	5.886	0.004
Dose	1.157	14, 62	6.089	<0.001
Stimulus animal × dose	0.737	14, 62	2.588	0.005
Duration				
Stimulus animal	0.704	6, 13	5.174	0.006
Dose	0.988	12, 64	5.210	<0.001
Stimulus animal × dose	0.520	12, 64	1.877	0.054
Simple main effects				
Frequency				
Stimulus animal				
Vehicle	0.656	7, 12	3.264	0.035
DEOXY 200 mg/kg	0.757	7, 12	5.335	0.006
Dose				
Castrated male	0.981	14, 62	4.267	<0.001
Receptive female	1.023	14, 62	4.642	<0.001

For DEOXY 400 mg/kg, it was impossible to perform multivariate tests because of zero error variance in some variables; therefore, the Mann–Whitney U-test was performed for each variable. No significance was obtained (all ps > 0.5). The simple main effects of the duration of sociosexual behaviors were not analyzed because there was no significant interaction between stimulus animal and dose.

Lickometer

All drugs tested significantly reduced drinking behavior (see Table 7). The same doses that inhibited sexual behavior (THIP 16 mg/kg, VAL 400 mg/kg, GAG 100 mg/kg, and DEOXY 400 mg/kg; $n = 10$ for each group) produced a drastic reduction of drinking behavior.

DISCUSSION

We have previously suggested that some GABAergic drugs affect sexual behavior only indirectly, via an impair-

TABLE 7

DRINKING BEHAVIOR IN WATER-DEPRIVED ANIMALS TREATED WITH DIFFERENT GABAERGIC COMPOUNDS

	Number of licks
Vehicle	1,361 ± 40
THIP 16	111 ± 111*
GAG 100	678 ± 206*
VAL 400	383 ± 91*
DEOXY 400	203 ± 127*

Drug doses are expressed in mg/kg; $n = 10$ for each drug.

*$p < 0.05$ vs. vehicle.

ment of motor execution (7,30). The results from the present experiment support and extend this hypothesis. The effective doses reduce ambulatory activity and frequently also motor coordination, as evaluated by open field activity and a treadmill test in this laboratory (7). However, it is not certain that a motor impairment can explain all behavioral effects of the drugs. The reduction of sexual behavior was always associated with reduced social interactions and exploratory behaviors. This could suggest that the drugs have a general inhibitory action on behavior, perhaps independent of their motor effects, reducing sensitivity to environmental stimuli. The results of the licking experiment suggest that this reduced sensitivity is not specific to sexually relevant stimuli. Even such a powerful incentive as water lost its capacity to activate the appropriate behavior in water-deprived rats after treatment with the drugs in doses that inhibited sexual behavior.

Effects similar to those reported here have previously been found with dopamine antagonists (3,8). These drugs reduce sexual behavior and ambulatory activity and induce motor incoordination, in addition to inhibiting exploratory behavior. To explain these effects, it was suggested that dopaminergic activation is permissive to the initiation of sexual behavior. This proposal is supported by recent data showing that rats that copulate shortly after castration, in contrast to noncopulating rats, release dopamine in the medial preoptic area in response to a receptive female (14). There is extensive evidence showing that enhanced GABAergic activity inhibits dopaminergic systems (20,25,35). It is possible that the similar effects of GABAergic agents and dopamine antagonists are the result of a common action. Interestingly, large doses of dopamine antagonists also inhibit drinking in thirsty rats (2).

Unfortunately, this explanation does not apply to the effects of GABA synthesis inhibitors. We have previously reported that picrotoxin, in subconvulsive doses, also inhibits male sexual behavior, whereas no effect was obtained with bicuculline (4,6). There is also evidence showing that some GABA agonists and antagonists have similar effects on ambulatory activity (6), motor coordination, and analgesia (19,37). Some studies have also found that baclofen has proconvulsive effects (12,28). Currently, no convincing explanation is available, but it can be speculated that any modification of GABAergic activity outside the physiological range disrupts neuronal functioning.

The only GABAergic drug tested so far that inhibits sexual interactions without modifying social or exploratory behaviors is the $GABA_B$ agonist baclofen (29). Males treated with baclofen spend significantly less time pursuing the female and hence do not engage in copulatory behavior. Pursuit of the female is a crucial component of sociosexual interactions, allowing the male to pass from the precopulatory to the copulatory phase (21). Male rats with lesions of the medial preoptic area, a brain structure involved in the control of male sexual behavior [e.g., (31)], showed reduced pursuit of the female (22,33) and did not engage in sexual behavior. These effects are strikingly similar to those found after baclofen treatment (29). It might be possible to propose that this drug modifies activity in the preoptic area. Indeed, in the female rat, it has been reported that systemic baclofen much reduces serotonin (5-HT) turnover in this area; at the same time, noradrenaline function was much increased (26). The biochemical changes observed in that study were closely correlated with the appearance of lordosis behavior. This does not mean, of course, that the effects of the systemically administered drugs are limited to the preoptic area. Because baclofen is the only GABAergic compound that has a specific effect upon sexual behavior, it could be argued that the $GABA_B$ receptor is directly involved in the mechanisms that control the initiation of that behavior. However, a $GABA_B$ antagonist has been reported to be without effect on male sexual behavior (32). This observation makes the physiological significance of the $GABA_B$ receptor in the control of sexual behavior uncertain.

As described in the introduction, the behavioral effects observed after baclofen administration are most probably mediated by the $GABA_B$ receptor, because they are stereospecific (29) and blocked by the $GABA_B$ antagonist CGP35348 (32). In contrast, neither the inhibitory effects observed on sexual behavior after administration of $GABA_A$ agonists (5,6) nor those of GABA transaminase inhibitors on locomotor activity (5) were blocked by bicuculline, suggesting that the $GABA_A$ receptors are of slight importance in the control of ambulatory activity and sexual behavior. Pharmacological and behavioral studies suggest the existence of GABA receptor sites different from the $GABA_A$ and $GABA_B$ receptors [for a review, see (30)]. For example, the GABA uptake inhibitor SKF-89976A alone produced a higher antinociceptive response than did specific stimulation of the $GABA_A$ or $GABA_B$ receptors by THIP or baclofen or by combined treatment with the two agonists (38). Similarly, SKF-89976A and γ-vinyl GABA had an anticonvulsant effect, whereas THIP and baclofen lacked any protective effect against pentylenetetrazol-induced seizures (38). It is possible, then, that some of the behavioral effects of GABAergic compounds observed in the present study might be mediated by GABA receptors different from the $GABA_A$ or $GABA_B$ sites. Further studies combining drugs that increase GABA levels (e.g., GTIs) and compounds that specifically block the different GABA receptors, as well as studies in which GABA levels are reduced in combination with specific GABA receptor agonists, are required to fully understand the contribution of each receptor subtype when GABA levels are altered. Clearly, much additional work is needed before a complete understanding of the effects of systemically administered GABAergic agents can be gained.

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