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Sexual Attraction and Copulation in Male Rats: Effects of the Dopamine Agonist SND 919

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FERRARI, F. AND D. GIULIANI. *Sexual attraction and copulation in male rats: Effects of the dopamine agonist SND 919.* PHARMACOL BIOCHEM BEHAV **50**(1) 29-34, 1995.—Behavioral differences towards receptive females, involving latency to the first contact, amicable behavior, genital exploration, and copulatory pattern, were seen in sexually active (A), sluggish (S), and inactive (I) male rats classified on the basis of 11 consecutive mating tests. The D_2 dopamine agonist SND 919 (1 mg/kg) was intraperitoneally administered to the three groups 25 min before a 12th test; the drug stimulated the copulatory behavior of A and S but not of I rats in which it diminished genital exploration and amicable behavior. In a 13th test, conducted 1 week later, 31% of I initiated mating, 16% of them reaching ejaculation. The stimulant effect of SND 919 on copulation in A rats was confirmed in further experiments where it was injected at 0.1 mg/kg, a dose selective for the D_2 autoreceptors.

SND 919 D_2 dopamine receptors D_2 dopamine autoreceptors Sexual behavior Male rats

THE COPULATORY behavior of sexually normal adult male rats is characterized by a series of alternate mounts and intromissions, well defined in number for each animal, which culminate in ejaculation. It is known, however, that inexperienced laboratory rats take time to establish their copulatory pattern (6). Usually, six to seven successive mating tests, once or twice a week, are necessary to determine each rat's individual profile, as represented by a number of parameters that then remain virtually unchanged for a long time. As a result of this stability, the effects of drugs that potentially inhibit or stimulate male sexual behavior can be verified (5,11,20). We have observed that even in the initial training tests there are numerous differences between the animals as regards not only copulatory activity but also certain manifestations of interest towards the female that are not explicitly sexual. Because, for the purpose of the copulatory tests, it is possible to select rats that are sexually active (A), sluggish (S), and inactive (I), the present study was undertaken to compare their general pattern of behavior when tested with receptive females. Moreover, in view of the well-documented key role of dopamine (DA) in the modulation of behavior, and in particular of sexual behavior (1,3,9,10,15,21), we wanted to investigate the effects produced on A, S, and I rats by an acute injection of the selective D_2 DA stimulant, SND 919 (13,18). This new drug, which seems to differ from other nonspecific DA agonists in that it is deprived of D_1 DA and α_2 adrenergic activity, potently elicits penile erections in male rats in the absence of females over a wide dose range (13). Moreover, from 0.05 to 1 mg/kg intraperitoneally (IP), it induces a bell-shaped dose-response curve for the stretching yawning syndrome (13,18), which is indicative of D_2 autoreceptor stimulation (8,10,18,22).

METHOD

Adult male Wistar rats (Morini, San Polo D'Enza, Italy), initial weight 300-350 g, were employed. They were housed in groups of five with food and water ad lib, in a quiet, air-conditioned room (22 ± 2°C, 60% humidity) on a normal 12 L : 12 D cycle (lights on from 0600 to 1800 h). Ovariectomized females of the same strain and weight, used as mating stimulus, were brought into estrus with estradiol benzoate (30 μg, SC) followed 48 h later by progesterone (0.5 mg, SC) and used 5-7 h after progesterone injection. Only animals with high receptivity were used.

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Two series of experiments were performed. Both were carried out between 0900 and 1300 h in a dimly lit room to avoid the anxiogenic influence of bright illumination inhibiting animal interactions (14). The male rats were transferred singly to an observation cage (50 × 40 × 40 cm), into which, after a 10-min adaptation period, a receptive female was introduced. Pairs of experienced observers, sitting about 1.5 m from a cage, independently recorded a male rat's behavior towards a female. Male copulatory behavior was evaluated according to Dewsbury (5) and the following were scored: mount and intromission latencies (ML and IL) (the time from the introduction of the female until the first mount and intromission, respectively); mount and intromission frequency (MF and IF) (the number of mounts and intromissions preceding ejaculation); ejaculation latency (EL) (the interval between the first intromission to ejaculation); postejaculatory interval (PEI) (the time between the first ejaculation and the next intromission). Tests were discontinued when IL or PEI were > 15 min or EL was > 30 min. In this case, ML, IL, and PEI values were arbitrarily put at 900 s and EL at 1800 s. In addition, in the first experiment (Experiment 1), the following parameters were evaluated: a) latency to the first contact (CL) as the time from the introduction of the female on the opposite side of the cage until the first voluntary contact by the male; b) total time spent in genital exploration by the male (GET), which was recorded from the introduction of the female until the first ejaculation or the end of the test, in the event of its being discontinued; c) amicable behavior time (ABT) as the total time spent by the male in partner grooming, nuzzling and crawling (19).

Experiment 1

Initially, a large number of animals (n = 38) was submitted to twice-weekly tests at 3-day intervals. After the 5th test, the animals were tested only once a week. On the basis of the data collected for each animal's copulatory behavior in the first 11 consecutive tests, the animals were assigned to A, S, or I groups. A (n = 10) comprised rats that always reached ejaculation from the 4th to the 11th test; S (n = 6) comprised those that did not initiate their copulatory activity before the 6th test but ejaculated and resumed copulatory activity after PEI at least in the 10th and 11th tests; I (n = 13) were those that never mounted or intromitted. The animals with inconsistent copulatory behavior were discarded. All the rats were intraperitoneally (IP) injected with SND 919 (1 mg/kg) before a 12th test, and a final 13th test was conducted 7 days after treatment. Data obtained on male copulatory behavior, CL, GET, and ABT from the three groups were collected for all tests but, for brevity, the results are presented only for the 2nd, 5th, 7th, 11th, 12th, and 13th tests, which were the most representative of the patterns observed.

Experiment 2

Only A rats (n = 12) were selected, namely, those that completed at least the last four preliminary mating tests out of the seven conducted at 3-day intervals. After having verified the consistency of their copulatory behavior in the 6th and 7th tests, the animals were divided into two groups (not statistically different for any of the parameters considered) that were IP injected with SND 919 at 0.1 mg/kg or saline 25 min before the 8th copulatory test.

Drugs and Treatments

SND 919 (Boehringer Ingelheim, Ingelheim am Rhein, Germany) was freshly dissolved in saline at a concentration that allowed the administration of 1 ml/kg by IP route. The doses used (0.1 and 1 mg/kg) and pretreatment time (25 min before the tests) were chosen on the basis of our previous experiments (13). Estradiol benzoate and progesterone (Sigma Chemical, Co., St. Louis, MO) were dissolved in corn oil and injected in a volume of 0.2 ml/rat.

Statistical Evaluation

Data, which are presented as means ± SEM, were analyzed using Student's t-test, Student's t-test for paired data, ANOVA for repeated measurements followed by Student's t-test for paired data, ANOVA followed by Student-Newman-Keuls test and chi squares, with the level of significance set at $p < 0.05$, where appropriate.

RESULTS

Experiment 1

As previously mentioned, after the 11th test the male rats were assigned, on the basis of their copulatory behavior hitherto, to I, S, or A groups. Figure 1 shows that the I rats always differed in their CL with respect to the other two groups and, despite the wide variation in the parameter in question, always took longer to contact their partners. In S rats, the repetition of the test reduced CL, $F(5, 25) = 2.8$, $p = 0.03$, which was significantly lower in the 11th with the respect to the 2nd test; CL also diminished in the A rats from the 5th test onwards, $F(5, 45) = 13.5$, $p < 0.001$. SND 919 (1 mg/kg, IP) 25 min before the 12th test, did not produce any significant effect on CL.

The results obtained on GET for the same animals are

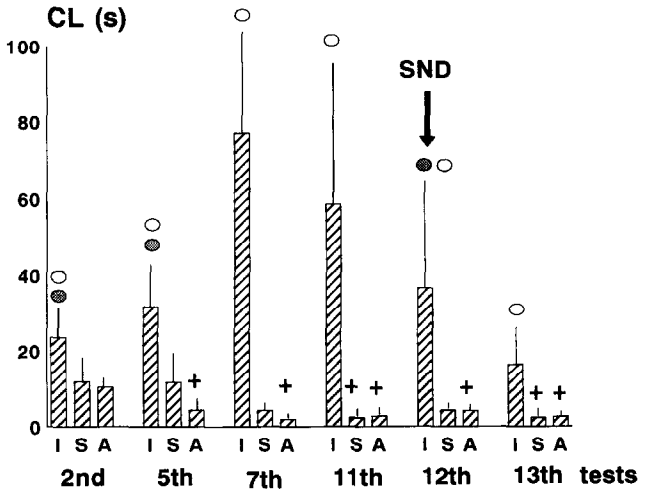

FIG. 1. Effect of training and SND 919 on the latency to the 1st contact with the female by male rats. Sexually inactive (I), sluggish (S), and active (A) rats were selected on the basis of 11 consecutive copulatory tests. Histograms for the latency to the 1st contact (CL) are the means ± SEM of the values per treatment group. Number of rats per treatment group: I = 13, S = 6, and A = 10. SND 919 (SND) was IP injected at 1 mg/kg, 25 min before the 12th test. ● Significantly different from the S group; ○ significantly different from the A group (ANOVA followed by the Student-Newman-Keuls test); + Significantly different from the same animals in the 2nd test (ANOVA for repeated measurements followed by Student's t-test for paired data).

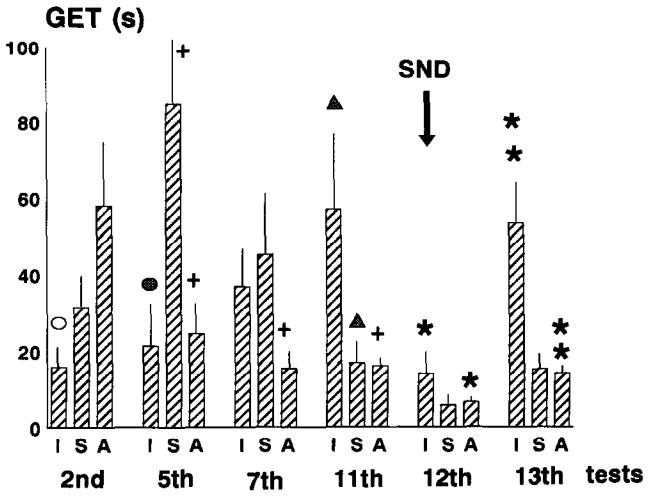

FIG. 2. Effect of training and SND 919 on genital exploration of male rats. The sexually inactive (I), sluggish (S), and active (A) rats were the same animals as reported in Fig. 1. Histograms for genital exploration time (GET) are the means ± SEM of the total time spent in this activity, during the test, by the animals of the three groups. ● Significantly different from the S group; ○ significantly different from the A group (ANOVA followed by the Student-Newman-Keuls test); + significantly different from the same animals in the 2nd test; ▲ significantly different from the same animals in the 5th test; * significantly different from the same animals in the 11th test, ** significantly different from the same animals in the 12th test (ANOVA for repeated measurements followed by Student's *t*-test for paired data).

reported in Fig. 2. In the I group, GET was consistently low until the 5th test, differing significantly from that of the A group in the 2nd and from that of the S group in the 5th test. It gradually increased during the following tests, $F(3, 36) = 2.9, p = 0.04$, so that in the 11th it was higher than in the 5th. In the S group, GET increased from the 2nd to the 5th test, where it was higher (although not significantly) than that of the A group, then decreased in the 11th test, $F(3, 15) = 3.8$, $p = 0.03$. Finally, in the A group, GET reached its highest value in the 2nd test then decreased from the 5th test onwards, $F(3, 27) = 3.9, p = 0.01$.

SND 919 injection produced marked differences of behavior in the I, $F(2, 24) = 5.1, p = 0.01$, and A groups, $F(2, 18) = 5.2, p = 0.01$, whose GET reduced significantly in the 12th with respect to the 11th test and returned to basal level in the 13th. The same phenomenon was observed in the S group, although the level of significance was not reached, probably owing to the limited number of animals.

Figure 3 reports the results on ABT. In the I group, this parameter was in general higher than that of the other two groups, clearly differing from them in the 7th and 11th tests. In the S group, ABT was almost constant in the 2nd and 5th test but decreased sharply in the 7th and 11th tests, $F(3, 15) = 9.3$, $p < 0.001$; a similar pattern was seen in the A group, where it gradually decreased after the 2nd test, $F(3, 27) = 3.9, p = 0.01$, and stabilized from the 7th test onwards. In no group was any significant modification obtained in the 12th test after SND 919 treatment, but in the 13th test ABT increased in the I animals with respect to their 12th test, $F(2, 24) = 4.1, p = 0.02$.

The copulatory behavior of all the animals is reported in Table 1. Until the 11th test, while I rats constantly failed to mount or intromit, the A and S groups mainly differed in their ability to perform mating, as is evident from the respective percentages of rats mounting, intromitting, and ejaculating in the 2nd, 5th, and 7th tests. However, when completely stabilized, their copulatory behavior was very similar.

SND 919 injection did not modify the pattern of the I rats ($n = 13$) in the 12th test but, unexpectedly, in the 13th test four animals initiated mating and mounted, two of them intromitting and reaching ejaculation. The D_2 agonist produced immediate profound behavioral modifications in the S group, IF, and EL, decreasing significantly in the 12th test and returning to basal levels in the 13th test, $F(2, 10) = 15.7$, $p < 0.001$, for IF and, $F(2, 10) = 6.2$ $p = 0.01$ for EL; the same effect was obtained in the A group, $F(2, 18) = 23.6$, $p < 0.001$, for IF; $F(2, 18) = 10.8$, $p < 0.001$, for EL.

Experiment 2

Table 2 shows the influence of SND 919 at 0.1 mg/kg on the copulatory performance of A rats. In the 8th test, despite its known sedative effect (13,18), the drug significantly diminished MF, IF, and EL with respect to the 7th test.

DISCUSSION

First of all, our findings confirm what had been previously observed, that there are considerable differences between rats of the same strain housed in identical conditions and in exclusively male groups as regards their behavior towards receptive females. These differences, which were apparent from the very first tests and were borne out subsequently between the animals categorized as A, S, and I, do not refer solely to copulation but include other behaviors not explicitly involved in this activity. During the present experiments, we were surprised at

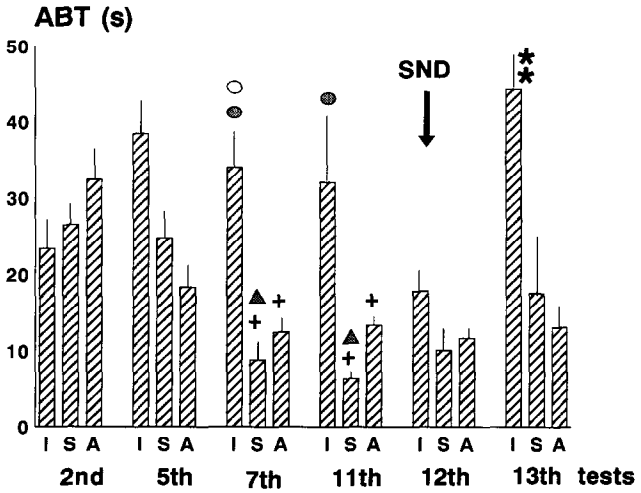

FIG. 3. Effect of training and SND 919 on amicable behavior of male rats. The sexually inactive (I), sluggish (S), and active (A) rats were the same animals as reported in Figs. 1 and 2. Histograms for amicable behavior time (ABT) are the means ± SEM of the total time spent in this activity, during the test, by the animals of the three groups. ● Significantly different from the S group; ○ significantly different from the A group (ANOVA followed by the Student-Newman-Keuls test); + significantly different from the same animals in the 2nd test; ▲ significantly different from the same animals in the 5th test; ** significantly different from the same animals in the 12th test (ANOVA for repeated measurements followed by Student's *t*-test for paired data).

TABLE 1

EFFECT OF SND 919 ON COPULATORY BEHAVIOR OF MALE RATS

Animals	No. Test	ML (s)	IL (s)	MF (No.)	IF (No.)	EL (s)	PEI (s)
I	2th–11th	900 ± 0	900 ± 0	0	0	> 900	> 900
I	12th	900 ± 0	900 ± 0	0	0	> 900	> 900
I	13th	60 ± 24 (4/13)	49–122 (2/13)	5.5 ± 3.2	16–9	1051-660 (2/13)	360–395 (2/13)
S	2th–5th	900 ± 0	900 ± 0	0	0	> 900	> 900
S	7th	46 ± 12.9 (5/6)	180 ± 61 (5/6)	20.3 ± 6.6	12.4 ± 0.7	596.7 ± 152.2 (5/6)	366 ± 17 (4/6)
S	11th	35 ± 11.2 (6/6)	122 ± 31 (6/6)	7.6 ± 2	10.3 ± 0.8	547 ± 160 (6/6)	385 ± 14.2 (6/6)
S	12th	275 ± 166 (6/6)	313 ± 183 (6/6)	2.3 ± 0.8	4.6 ± 0.5*	148 ± 45.4* (6/6)	367 ± 19.3 (6/6)
S	13th	95.6 ± 51.6 (6/6)	281 ± 150 (6/6)	22.3 ± 11	8.3 ± 0.4†	395.3 ± 83.4† (6/6)	360 ± 33 (6/6)
A	2th	214 ± 117 (8/10)‡	253 ± 111 (8/10)‡	23 ± 5.0	12 ± 2.5	903 ± 147 (4/10)	451 ± 47 (4/10)
A	5th	140 ± 51 (10/10)‡	189 ± 55 (10/10)‡	22 ± 2.5	15.8 ± 1.3	1001 ± 93‡ (10/10)‡	428 ± 48 (10/10)
A	7th	29.5 ± 12 (10/10)	73.6 ± 32 (10/10)	10.1 ± 1.7	9.3 ± 0.7	439 ± 28 (10/10)	324 ± 18.2 (10/10)
A	11th	36.2 ± 19.9 (10/10)	63.7 ± 27 (10/10)	8.5 ± 1.2	11.8 ± 1.4	594 ± 76 (10/10)	359 ± 26 (10/10)
A	12th	168 ± 106 (10/10)	213 ± 114 (10/10)	3.2 ± 0.9	5.6 ± 0.6*	193 ± 36* (10/10)	342 ± 23 (10/10)
A	13th	59 ± 33 (10/10)	172 ± 97 (10/10)	14.9 ± 7.1	10.2 ± 0.9†	353 ± 52† (10/10)	343 ± 22 (10/10)

SND 919 (1 mg/kg, IP) was administered 25 min before the 12th test. I = sexually inactive (n = 13), S = sexually sluggish (n = 6) and A = sexually active (n = 10) are the same animals as reported in the figures. Values are the means ± SEM of the following parameters: ML, IL, EL = latency to the 1st mount, intromission, and ejaculation, respectively; MF, IF = mount and intromission frequency, respectively; PEI = postejaculatory interval, for the rats presenting the behavior in question (in parentheses).

*Significantly different from the same animals in the 11th test; †Significantly different from the same animals in the 12th test (ANOVA for repeated measurements followed by Student's t-test for paired data).

‡Significantly different from S in the same test (chi-squares).

the extremely low overall incidence of copulatory behavior observed, and we are as yet unable to offer an explanation for this. One possibility could be the fact that our rats, which, as is known, are nocturnal animals, were housed on a normal light cycle and tested with lights on. It must, however, be remembered that different percentages for A, S, and I rats have been found by us in the past, performing copulatory tests in the same experimental conditions (11).

Already in their first approach to the females, the I rats behaved differently from the other two groups and they evinced almost total sexual disinterest towards the partners, at least until the 11th test. As already mentioned, the A and S

TABLE 2

EFFECT OF SND 919 ON THE COPULATORY BEHAVIOR OF SEXUALLY ACTIVE MALE RATS

Test	Treatment (mg/kg, IP)	ML (s)	IL (s)	MF (No.)	IF (No.)	EL (s)	PEI (s)
7th	–	64.2 ± 29.5	79.1 ± 33	10.3 ± 1.0	10.7 ± 0.8	456 ± 40	368 ± 33
8th	saline	59 ± 26	74.3 ± 26	10.2 ± 0.8	11.8 ± 1.0	448 ± 28	370 ± 23
7th	–	42.0 ± 16.5	72 ± 27	9.3 ± 0.8	10.1 ± 0.8	415 ± 29	375 ± 23
8th	SND 919, 0.1	104 ± 61	136 ± 67	6.3 ± 0.5*†	5.7 ± 0.6*†	222 ± 25*†	331 ± 21

SND 919 (0.1 mg/kg IP) was administered 25 min before the 8th test. For the parameters, see Table 1. Each value is the mean ± SEM of the data for six animals. The two groups of the 7th test did not differ significantly from one another ($p > 0.05$, Student's t-test)

*Significantly different from the same animals in the 7th test (Student's t-test for paired data).

†Significantly different from saline-treated rats in the 8th test (Student's t-test).

rats, on the other hand, differed mainly in the time they took to stabilize their mating pattern, the S rats requiring a greater number of training sessions. The male copulatory pattern explains the variability of GET values during the tests; in fact, as emerges from a comparative analysis of mating behavior and GET, high values of the latter parameter precede the initiation of copulatory activity during the training sessions in A and S rats, fall off rapidly when mating begins, and reduce further if MF and IF are intensified by prolonged training or by treatment with stimulants. Paradoxically, therefore, low GET can reflect two opposite situations, namely, indifference or high sexual drive culminating in copulation. This is clearly visible in the 5th and 12th tests, where I and A rats presented similar GET. There again, given a longer training period, the possibility that even some of the I rats might have initiated mating cannot be excluded, for a high GET value was observed only at the 11th test.

A second significant finding is that treatment with SND 919 modified rat behavior, depending on the category of the animal. In particular, in the 12th test, while drug injection did not immediately affect the inability to copulate of I rats, it potently reduced some mating parameters of A and S rats, an effect that is interpretable as sexual stimulation (20). This effect was confirmed in the Experiment 2, where a dose more selective for D_2 autoreceptors was administered to the animals. It should be pointed out that both doses of SND 919 used are likely to act, to varying degrees, on D_2 DA autoreceptors, because they were both seen to elicit a significant increase of stretching-yawning (13,18).

Previous studies have shown that other DA agonists similarly potentiate male rat copulatory behavior (1,11,21). Some authors have proposed that this facilitation is a nonspecific effect attributable to psychomotor stimulation (1). The present results with SND 919, which is a D_2 receptor agonist, and, in particular, those obtained in Experiment 2 with a low dose considered to be highly selective for the D_2 autoreceptors (13,18), suggest that the D_2 receptor is presumibly responsible for stimulating the coital urge. It is well established that D_2 autoreceptor activation also leads to hypomotility (7): clear sedation was observed particularly in SND 919 (0.1 mg/kg)-treated rats both in past experiments (13,18) and in the present study during the pretreatment time (data not reported). Accordingly, psychomotor stimulation can be ruled out as a determining factor of sexual stimulation. The involvement of D_2 autoreceptors may also justify the tendency of ML and IL to increase in some rats of both S and A groups and also their unmodified PEI. Because the data on SND 919 confirm those obtained on copulatory behavior with B-HT 920 (11), a putative D_2 autoreceptor stimulant, which never elicits stereotyped behavior or hypermotility in normosensitive animals (2,17), it is likely that the two D_2 agonists share the same neurocheminal activity in affecting ejaculatory mechanisms. It seems, on the contrary, that arousal mechanisms are unaffected by these DA agonists, for no reduction was seen in ML, IL, and PEI. It has been demonstrated that ejaculation and sexual arousal mechanisms are independent aspects of sexual behavior and are differently modulated by pharmacological agents (4). Our findings, like most of those in this field, were obtained after systemic administration of the drugs; we cannot, therefore, discount an eventual peripheral component of the effect, which seems to us to be important irrespective of the specific mechanism involved.

The results on I rats in the 12th and 13th tests are more difficult to interpret. On the whole, SND 919 does not appear to have had any immediate influence on male sexual behavior, the significant decrease in GET and the concomitant drop in ABT in the 12th test, both of which return to basal values in the 13th test, unaccompanied by any sign of sexual activity, probably reflect the reported sedative effect of the drug. Indeed, our SND 919-treated I rats were seen to yawn repeatedly during the test and yawning associated with sedation, has been ascribed to D_2 autoreceptor stimulation (8,10,12,18,22). In agreement with a possible (although curious) relationship between sexual excitement and yawning is the report that this latter syndrome is typically observable in rhesus monkeys during coitus (16). Considering the unexpected initiation of copulatory activity in some I rats during the 13th test, it is tempting to hypothesize, albeit prematurely, that once the sedative effect of SND 919 has worn off, the drug triggers or enhances a mechanism of sexual stimulation in these animals, too. Experiments are now being conducted to verify this possibility.

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