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Fluprazine Hydrochloride Decreases Copulation in Male Rats

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FLANNELLY, K J , H L LIM, M DIAMOND, D C BLANCHARD AND R. J BLANCHARD *Fluprazine Hydrochloride decreases copulation in male rats* PHARMACOL BIOCHEM BEHAV 22(1) 1–4, 1985 —The copulatory performance of 18 sexually experienced male rats was tested 30 min after IP injection of the phenylpiperazine, Fluprazine Hydrochloride (4 and 8 mg/kg), or saline solution Three 20-min tests with an estrous female were conducted at weekly intervals Each drug dose produced a significant depressive effect on copulatory behavior (latency to and frequency of intromissions and ejaculations) without affecting social investigation The increased latencies to mounting and intromission observed at both doses suggest that the primary action of the Fluprazine Hydrochloride is to interfere with the transition from social to copulatory patterns. Although the drug's mode of action is currently unknown, the present findings suggest that it operates on some common mediator of sexual and aggressive behavior

Anti-androgen Copulation Fluprazine Hydrochloride Rats Phenylpiperazine Sexual behavior Social investigation

SEXUAL and aggressive behavior in animals are closely linked [17]. For many animals, aggression clearly serves specific reproductive functions related to mate selection and the exclusion of sexual competitors [4] In many rodents, for example, territorial aggression among males increases toward the start of the reproductive period [14] and copulation itself further enhances aggression [13]. Although there is only little evidence of overlap in the neural systems controlling aggression and sex [2], the common hormonal mediation of the two is well recognized for males.

While various hormones are known or suspected to modulate sexual and aggressive behavior, gonadal androgens play a predominant role Castration produces a dramatic decrease in both over time, but the rate of decline appears to be dependent upon degree of prior sexual [7] or aggressive experience, as well as situation variables [6,16]. Sexually experienced male rats may continue to copulate, albeit at a declining rate, for weeks or months following castration [7]. With previous aggressive experience [16,22], a substantial postcastration maintenance of aggression is seen in male rats and mice.

In contrast to the rather slow decline in territorial aggression or offensive attack seen following castration [3], Olivier [18] has reported findings on a new class of drug, the phenylpiperazines, which dramatically reduce the aggression of territorial residents within several minutes. The drug DU27725, a member of this class, was reported to specifically suppress offensive behavior; it had little or no effect on general activity or social interaction and did not seem to substantially alter defensive behavior. A related compound, (DU27716) Fluprazine Hydrochloride, has been shown to produce the same dramatic reduction in offensive behavior [19, 20, 26]. The structural formula for DU27716 is shown in Fig 1 It is presumed that the drug acts on specific neural systems involved in offense, but not much is known about these systems [1] and even less is known about the drug's mode of action.

Because of the close association of offense and sexual behavior, we decided to test the effects of Fluprazine Hydrochloride on copulatory performance. If the drug acted specifically on neural systems involved in offense, no effect on copulation would be expected. To the degree that Fluprazine Hydrochloride affects copulation, however, this would suggest that it operated on some factor common to both sexual and aggressive behaviors

METHOD

Subjects were 18 male rats, 120–150 days old (442–583 g) of the Long-Evans strain All were socially reared until adulthood, then given repeated exposure to estrous females to assure copulatory experience. Each subject was housed separately for several weeks prior to testing. Twelve ovariectomized, adult females were used as stimulus lures. Estrous was induced by injections of 20 μg of estradiol benzoate in oil 48 and 24 hr prior to testing and 0.5 mg of progesterone 4 hr before testing.

After 6–8 repeated 1-hr exposures to estrous females, males were given three 20-min tests once a week to assure stability of baseline copulatory performance. All 18 subjects were then tested under each of three treatment conditions: 4 mg/kg and 8 mg/kg of Fluprazine Hydrochloride (dissolved in

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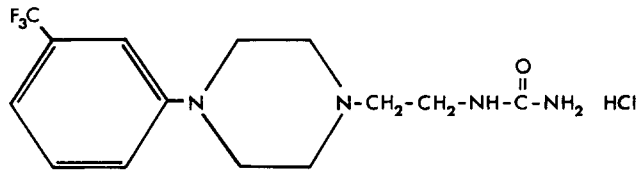

FIG 1 Structural formula for Fluprazine Hydrochloride [2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl] urea hydrochloride

distilled water with the pH adjusted to 7), and a (0 9%) saline control. The selection of these doses was based on Olivier's findings that they significantly reduced offense without impairing social or nonsocial behaviors The volume of the saline injections was equivalent to that used for drug administration. Injections were given IP 30 min before testing Tests were conducted at weekly intervals with order of testing under each drug dose determined by a 3×3 Latin square design using six replications of the same square.

Males were individually tested in a 56×51×41 cm stainless steel cage, with each subject placed into the cage, 5 min before the start of the session. The test was begun by putting an estrous female into the cage

During testing the frequency of the following behaviors were recorded. anogenital sniffing, sniffing (any part of the female other than the perineum), vaginal licking, paws on (abortive mounts in which the male placed his forepaws on the female's back but does not make pelvic thrusts), mounts (with pelvic thrusts), intromissions, and ejaculations [9, 10, 21] Latency from the start of testing to the following behaviors were also taken· initiation of social contact (sniffing, licking or copulatory behavior), paws on (or other copulatory act), mount (with or without intromission) and intromission [10,12] All behaviors were recorded using a push-button keyboard which activated the pens of a 20-channel Esterline-Angus event recorder. In addition to the foregoing measures, standard copulatory measures of Ejaculation latency (the time from first intromission to ejaculation) and the Post Ejaculatory Interval (PEI—time from ejaculation to next intromission) for each copulatory series were also taken from the tape record [21]

The proportion of subjects which engaged in various behaviors under each drug condition was analyzed separately for each measure using a Cochran's Q test Latency and frequency data were analyzed by ANOVA for a 3×3 Latin square design with six replications on the same square. This permitted a seperation of drug effects, the effects of repeated testing regardless of drug dose received on each test, and the overall effect of order in which subjects received drug and saline treatment (3 orders were used) Replications was a statistical but not a methodological factor, replications are inherent in the use of the latin-square design since the number of subjects must be some multiple of the number of treatments [27]. Neuman-Keuls tests were used to make post-hoc paired comparisons between treatment conditions

RESULTS

Table 1 shows the copulatory performance of subjects during the three weekly baseline sessions prior to treatment tests As presented in the table, the mean frequency of mounts, intromissions and ejaculations for each 20-min session was quite stable across all three weeks, as was the

TABLE 1

MEAN (S E) COPULATORY PERFORMANCE OF ALL SUBJECTS DURING THE LAST THREE WEEKS OF TRAINING PRIOR TO DRUG TREATMENT

Measure	Weeks 1	2	3
Latency to			
Paws on	13 9	14 2	9 1
	(2 0)	(1 8)	(1 9)
Mount	90 6	35 4	14 8
	(67 6)	(8 9)	(2 8)
Intromission	116 7	60 7	56 7
	(67 2)	(11 8)	(12 7)
Frequency of			
Mounts	11 2	9 1	9 9
	(2 0)	(2 2)	(2 8)
Intromissions	10 7	11 1	10 3
	(1 0)	(0 6)	(0 7)
Ejaculations	2 2	2 2	2 0
	(0 2)	(0 2)	(0 2)
Ejaculation latency*	300 8	358 6	359 9
	(40 5)	(61 2)	(87 3)
Post Ejaculatory Interval*	347 6	331 6	354 9
	(19 5)	(24 1)	(22 4)

Latency measures and PEI are given in seconds
*Score for first copulatory series only

PEI for the first copulatory series. Mean ejaculatory latency for the first copulatory series was nearly identical for weeks 2 and 3 Latency measures generally tended to decrease across sessions

The effects of drug treatment on copulation can be seen in Tables 2 and 3 As observed in Table 2, drug treatment elevated the latencies of all measures of copulatory performance, but did not affect latency to initiate social contact Latencies to paws on, mount, and intromission increased significantly across treatments ($p<0$ 01), with greater suppression occurring at the higher dose. Comparison of these data with those in Table 1 reveals that subject performance in the saline condition was comparable to that during their last week of training.

Both drug doses significantly increased the frequency of body sniffing with respect to controls ($p<0$ 01) Mean frequencies of body sniffing were 7 8, 24 9 and 32 7 respectively, for saline, 4 mg/kg and 8 mg/kg of Fluprazine Hydrochloride No significant drug effect was found for differences in frequency of anogenital sniffing, vaginal licking or paws-on across conditions A significant effect ($p<0.01$) of repeated testing, regardless of order of drug administration or dose, was found for the frequency of vaginal licking, with an increase in licking during the final test (mean=8 0) compared to the first two tests (means=2 5 and 2 7, respectively) The same effect was found for the mean frequency of anogenital sniffing which increased significantly across tests (means =1 9, 3 0, 4 1). The absence of order effects for any measure indicates no carry-over effects of drug treatment across tests

Frequency of mounts was affected only by the higher dose of Fluprazine Hydrochloride and overall differences in mounting were not significant across treatment conditions

TABLE 2

MEAN (S E.) LATENCY TO VARIOUS MEASURES OF SOCIAL AND COPULATORY ACTIVITY OF DRUG TREATED AND UNTREATED MALES

Measure	Saline	4.0 mg/kg	8.0 mg/kg
Latency to			
Social Contact	7.6	7 2	13 1
	(0 2)	(2 0)	(2.3)
Paws on*	13 7	69 8	417 5
	(3 4)	(27 4)	(109 1)
Mount*	15 4	250.8	774 7
	(3 5)	(104 8)	(121 4)
Intromission*	52 8	400 8	988 0
	(26 8)	(120 1)	(99 1)

All measures are given in seconds
*Significant difference across conditions (ANOVA), $p<0$ 01

TABLE 3

COPULATORY PERFORMANCE OF SUBJECTS ADMINISTERED 4 0 AND 8 0 mg/kg FLUPRAZINE HYDROCHLORIDE OR SALINE

Measure	Saline	4.0 mg/kg	8 0 mg/kg
Frequency of			
Mounts	8 0	9.2	2 4
	(1 1)	(2 8)	(0.9)
Intromissions*	10 4	6 0	0.7
	(1 0)	(1.2)	(0.4)
Ejaculations*	2.0	1 1	0.1
	(0 2)	(0 2)	(0.1)
% of Subjects achieving.			
Intromission†	100 0	77 8	22.2
1 Ejaculation†	94.4	61 1	5 6
2 Ejaculations†	77 8	44.4	0 0

Standard errors are given in parentheses Mean frequencies are based on performance of all subjects under each treatment condition.
*Significant difference across conditions (ANOVA), $p<0$ 01
†Significant difference across conditions (Cochran Q-test), $p<0$ 001

(Table 3). Nevertheless, Fluprazaine Hydrochloride clearly reduced the frequency of intromissions and ejaculations ($p<0$ 01) and this suppressive effect was significantly greater at the higher dosage Again no carry-over effects of treatment were in evidence.

The inhibitory effects of both drug doses can also be seen in terms of the percentage of subjects performing each behavior category. Cochran Q tests revealed a significant overall treatment effect in terms of the number of males which intromitted or ejaculated. The single male which did ejaculate at the 8 mg/kg dose had a ejaculation latency comparable to the mean ejaculation latency of the 11 males who did so at the lower dose (mean=406.3 sec). This was substantially higher than control values, however (mean=228.1 sec). Interestingly, the PEI for the subjects which resumed copulation after their first ejaculation did not differ greatly between the saline (mean PEI=326.4, S. E =24.6) and the 4 mg/kg dose (mean PEI=386.9, S.E. =9.8) conditions. These values are comparable to the PEI values of subjects during the last 3 weeks of training

DISCUSSION

These data show that Fluprazine Hydrochloride is markedly effective in suppressing copulatory behavior. The magnitude of suppression observed is comparable to its inhibitory effect on offensive atttack [19,20] Although these data show that the drug's effect is not specific to offense, its action here is specific in the sense that it inhibited copulation without reducing other social behaviors Instead, certain measures of social investigation actually increased under drug treatment—sniffing, in particular. Sexually experienced males typically sniff estrous females relatively little [8, 10, 25], with sniffing normally being but a brief prelude to copulation if the female is in estrous [24]. The elevation of social investigation seen following drug treatment, then, probably represents the continued social arousal of noncopulating subjects throughout the test. Diamond *et al.*[11] have reported similarly that differential effects of drug treatment may be manifest upon various socio-sexual behavioral parameters.

It appears that Fluprazine primarily acts to inhibit the initiation of copulation. It may prevent the triggering of the intromission and ejaculation mechanism [21], or simply interfere with the transition from social to copulatory behavior. This view is consistent with Olivier *et al.*'s interpretation of its effect on aggressive behavior, as well. Related work in our laboratory has found the compound to substantially prolong attack latencies in male mice [22].

Rather than its being specifically anti-aggressive, Fluprazine Hydrochloride may interfere with some common physiological substrate of copulation and offensive attack. If so, it does so more effectively than other anti-androgens which have been tested [9, 11, 15, 23]. While its action is relatively quick (<30 min), its length of action has not been investigated. The present subjects showed full recovery of copulatory performance within a week's time. Other data from our laboratory indicate that the drug's action does not extend beyond 1 or 2 days, and it is, perhaps, considerably shorter

Whatever its mechanism of action the behavioral effects of Fluprazine are profound. Copulatory behavior was all but abolished at the 8 mg/kg dose despite the considerable sexual experience of the subjects tested The fast action of the compound stands in marked contrast to the effects of castration [7] or the action of anti-androgens such as cyproterone and cyproterone acetate [11,15] or progesterone [9]. The present

findings may have implications for the use of Fluprazine Hydrochloride for humans, under circumstances where cyproterone is now the drug of choice [5]. A thorough examination of the reversibility of its action following chronic exposure would be important in this regard

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