

Yohimbine Attenuates Aging-Induced Sexual Deficiencies in Male Rats

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SMITH, E. R. AND J. M. DAVIDSON. *Yohimbine attenuates aging-induced sexual deficiencies in male rats.* *PHYSIOL BEHAV* 47(4) 631-634, 1990.—Since yohimbine strongly stimulates sexual motivation/arousal in rats, it was of interest to determine whether the arousal deficit in aging male rats could be reversed by yohimbine. Three groups from each of two ages (approximately 3 and 15 months) received vehicle, 1 or 4 mg/kg yohimbine. In the mounting test (for arousal/motivation), 1 mg/kg yohimbine significantly increased mount frequency in middle-aged rats, though not to the level of the young animals. In the mating test, the percentage of aging rats intromitting and displaying ejaculatory behavior was less than in the young group, but after 1 mg/kg yohimbine, these behaviors were significantly increased. Vehicle-treated middle-aged rats which had mated showed significantly elevated intercopulatory intervals and ejaculatory behavior latencies. These measures were reduced by 1 mg/kg yohimbine. While behavioral facilitation following yohimbine in aging rats did not reach that of young yohimbine-treated rats, they reached levels at or close to those of nontreated young animals. We conclude that yohimbine treatment can improve age-related deficits seen to occur in aging male rat sexual behavior.

Yohimbine Alpha₂-adrenoreceptor antagonists Sexual behavior Ejaculation Sexual arousal Genital anesthesia
Mounting behavior Aging

AGE-RELATED sexual dysfunction is common to both human and animal species (7, 8, 10, 17). In the male rat, deficits of sexual arousal/motivation occur as early as one year of age. This form of sexual deficit is apparently not reversible by administration of exogenous testosterone (12). While peripheral changes do occur in sexual aging, the major change mentioned is of CNS origin. Previous work shows that aged, sexually inactive male rats have depleted hypothalamic beta-endorphin activity and reduced septal and midbrain LHRH content in comparison to age-matched sexually active males (10).

Sexual arousal/motivation can be enhanced in normal male rats by yohimbine treatment and considerably improved in a variety of hyposexual conditions (4-6, 16). We have associated this particular prosexual effect with the release of norepinephrine by inhibition of alpha₂-adrenoreceptors (14,16). Furthermore the stimulatory effects of alpha₂-adrenergic blockade on sexual arousal do not require the presence of testosterone (6).

Since yohimbine alone can restore an impressive degree of arousal in castrated rats, it seemed that the drug was accessing an androgen-independent portion of the sexual behavior system which might be amenable to the improvement of age-related sexual dysfunction. In this context, Greenberg (13) has shown that alpha₂-adrenergic receptor numbers are decreased in brains from aged rats and that synthesis of this receptor may also be impaired. Accordingly, the present study examined the effect of yohimbine on mating behavior in middle-aged males and compared the results to those observed in young males.

METHOD

Animals

Forty-two male Long-Evans retired breeders (Simonsen Labs,

Gilroy, CA) were obtained at nine months of age and caged undisturbed until 14 months of age ("middle age"). Sixty sexually naive male rats of the same strain, 2 months old ("young"), were acquired when the retired breeders reached middle age. All received preexperimental mating behavior tests and those included in the studies showed complete mating behavior on at least four occasions. Only three in each batch did not meet this criterion. Rats were housed 2-3 per cage, provided food and water ad lib and maintained in a reversed 14:10 hour light:dark cycle ("lights off" 1100 hr, "lights on" 2100 hr).

Drugs

Yohimbine was obtained commercially (Sigma, St. Louis, MO). Immediately prior to testing, the drug was dissolved in distilled water in a volume of 1.0 ml/kg b.wt. Doses used in all tests were 0, 1 and 4 mg/kg b.wt.; all were injected intraperitoneally (IP).

Stimulus females used in mating behavior and mounting tests were rendered receptive and proceptive via SC injections of 150 µg estradiol benzoate 48 hr before and 750 µg progesterone 4-6 hr before testing (both in 0.15 ml sesame oil).

Mounting Test

To assess sexual arousal/motivation, 39 middle-aged and 36 young males were used. Males of both ages formed three equal groups randomly balanced for mount latency, intromission latency, and intercopulatory interval. Immediately following injection of vehicle, 1 or 4 mg/kg yohimbine, the penis was locally anesthetized with the topical anesthetic Pontocaine (tetracaine

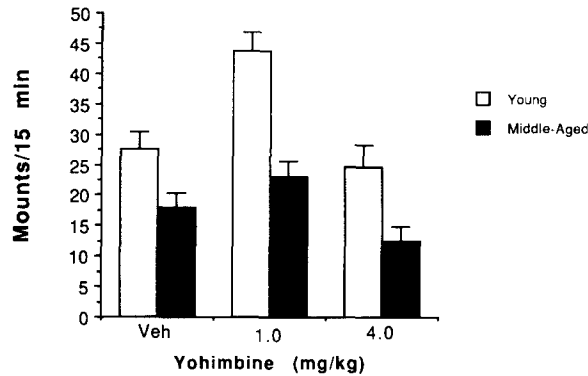


FIG. 1. Comparison of the effects of various doses of yohimbine on mounting behavior in young and middle-aged male rats following genital anesthetization; data are presented as mean \pm SE in this and Fig. 3; No. of rats = 12 for all young groups and 13 for all middle-aged groups.

hydrochloride) as described previously (11). Briefly, Pontocaine was liberally swabbed on the glans and shaft of the penis for 1.5 min twice, separated by at least 5 min. The procedure renders the male incapable of achieving intromissive and ejaculatory behavior. The male was placed in the semicircular observation cage and a receptive female was introduced 25 min postinjection. All mounts with pelvic thrusts were recorded throughout the 15-min test period. Testing was performed in the "lights off" period.

Mating Behavior Test

Thirty-six middle-aged and 37 young males used in the preceding experiment were allowed to remain undisturbed for one month, then given one recorded mating test and each age group was divided into three groups, balanced for behavioral parameters and prior drug treatment. Rats received either vehicle, 1 or 4 mg/kg yohimbine IP and mating tests initiated 20 min later. Males were placed in the observation cage 3–5 min before a receptive/proceptive female was introduced and observations began. The male was allowed to mate until the beginning of the second copulatory series, or for 15 min if no intromission occurred or for 30 min after the first intromission, if no ejaculatory behavior was displayed.

The following parameters were recorded: mount latency (ML), the time from onset of the test to the first mount with or without penile insertion; intromission latency (IL), the time from the introduction of the female to the first intromission; ejaculation latency (EL), time from the first intromission to ejaculatory behavior; postejaculatory interval (PEI), time from ejaculatory behavior to the first intromission of the second copulatory series; mount frequency (MF), the number of mounts prior to ejaculatory behavior; intromission frequency (IF), the number of intromissions before ejaculatory behavior; copulatory efficiency (CE), the number of intromissions divided by the total number of mounts with and without penile insertion; and intercopulatory interval (ICI), the average time between intromissions ($ICI = EL/IF + 1$), or if no ejaculatory behavior occurred, $ICI = 30/IF$. If the male failed to intromit by seven minutes, the female was replaced by another. Testing was performed in the "lights off" period.

Data Analysis

Data for percent of animals responding were analyzed using the nonparametric Fisher exact probability test (15). Other analyses

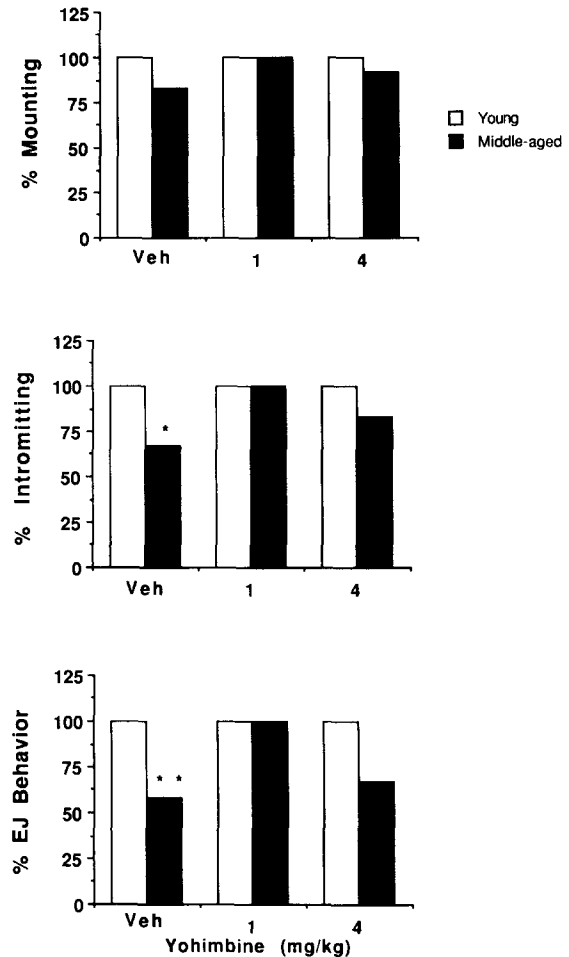


FIG. 2. The percentage of animals mounting, intromitting and displaying ejaculatory (EJ) behavior following yohimbine treatment in young and middle-aged male rats; * $p=0.05$, ** $p=0.025$; No. of rats = 12 (young-vehicle, young-4.0 mg and all middle-aged groups) and 13 (young-1.0 mg group).

were performed using two-way analysis of variance (3×2) followed by post hoc Newman-Keuls tests. Only data from animals positive for the behavior being analyzed were used.

RESULTS

Mating Test

The effects of yohimbine treatment on mounting behavior in young and middle-aged genitally anesthetized males are presented in Fig. 1. Two-way analysis of variance showed a highly significant effect of drug treatment, $F(2,69) = 14.38$, $p < 0.000$, and age, $F(1,69) = 37.22$, $p < 0.000$, while there was no significant treatment by age interaction, $F(2,69) = 2.10$, $p = 0.13$. Newman-Keuls tests indicated a significant difference between drug treatments; specifically the 1 mg dose increased mounting behavior when compared to the vehicle and to 4 mg/kg ($p < 0.05$) dose. In addition, all the young males mounted significantly more than the middle-aged males at all doses ($p < 0.05$). Simple main effects were as follows: drug treatment-young, $F(2,69) = 12.56$, $p < 0.000$; drug treatment-middle aged, $F(2,69) = 3.55$, $p = 0.034$; vehicle-

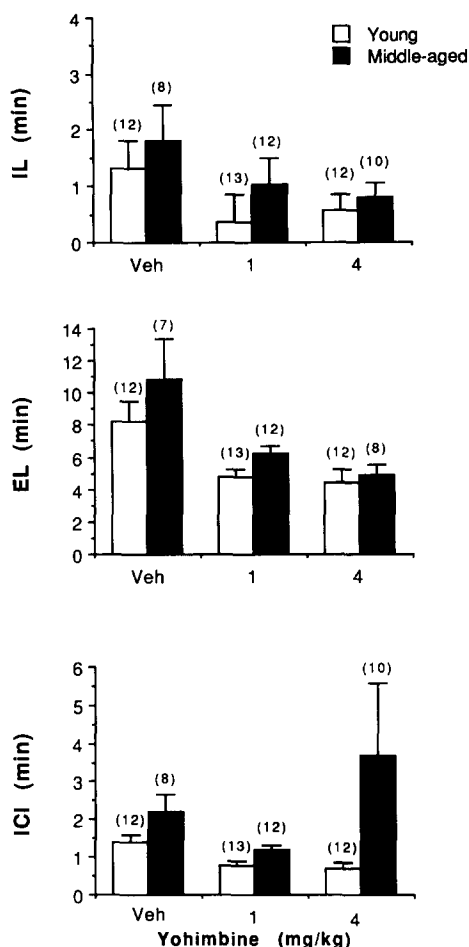


FIG. 3. Comparison of the effects of yohimbine on various parameters of mating behavior in young and middle-aged male rats; intromission latency (IL), ejaculatory behavior latency (EL) and intercopolatory interval (ICI); numbers in parentheses = No. of rats positive for the behavior.

age, $F(1,69) = 5.72, p = 0.02$; 1.0 mg/kg-age, $F(1,69) = 26.59, p < 0.000$, and 4.0 mg/kg-age, $F(1,69) = 9.12, p = 0.004$.

Thus, in the mounting test, 1 mg/kg yohimbine treatment in middle-age males stimulated mounting behavior but not to levels seen in young males. In fact, the older males exhibited a trend towards inhibition of behavior at the high dose.

Mating Test

The percentages of animals displaying mounting, intromission and ejaculatory behavior following vehicle, 1 or 4 mg/kg yohimbine are shown in Fig. 2. Middle-aged males evidenced significantly less intromissive and ejaculatory behavior than young males following vehicle injections (Fisher's test; intromissive behavior, $p = 0.05$; ejaculatory behavior, $p = 0.025$) while 1 mg/kg yohimbine in middle-aged males significantly stimulated ejaculatory behavior when compared to vehicle ($p = 0.025$) and the 4 mg dose ($p = 0.05$).

For animals positive for the specific mating behavior parameter being analyzed, results of the effects of drug treatment for both age groups are presented in Fig. 3 and Table 1. Two-way analysis of variance showed significant effects for the following parameters:

IL [drug treatment: $F(2,61) = 3.24, p = 0.046$]; EL [drug treatment: $F(2,58) = 11.78, p = 0.0001$; age: $F(1,58) = 3.84, p = 0.05$]; ICI [age: $F(1,61) = 5.68, p = 0.02$] and CE [age: $F(1,64) = 7.47, p = 0.008$]. The remaining behavioral measures showed no significant differences nor was there any significant treatment by age interaction for any measure. Post hoc Newman-Keuls indicated that the 1 and 4 mg doses of yohimbine significantly reduced EL ($p < 0.05$) when compared to vehicle.

Middle-aged males had a significantly ($p < 0.05$) elevated EL and ICI, and a significantly reduced ($p < 0.05$) CE as compared to young males. Significant simple main effects were as follows: EL [drug treatment-young: $F(2,58) = 3.54, p = 0.035$; drug treatment-middle aged: $F(2,58) = 8.43, p = 0.001$; age-vehicle: $F(1,58) = 4.57, p = 0.037$]; ICI [age-4.0 mg/kg: $F(1,61) = 8.66, p = 0.005$] and CE [age-vehicle: $F(1,64) = 4.59, p = 0.04$].

DISCUSSION

The main conclusion of this study is that a mild acute IP dose of yohimbine in middle-aged male rats significantly and substantially increases the numbers of males displaying ejaculatory behavior, and improves other measures of sexual behavior. The degree of change is quite substantial, reaching the level of the untreated young rats. Prosexual effects were found both in the mounting and the mating test. Trends and various significant results indicate that in middle-aged males, 4 mg/kg yohimbine may have an inhibitory effect on sex behavior. At this dose, the percent of animals displaying ejaculatory behavior was significantly less than at the 1 mg dose and not different from vehicle values while the rate of mating (ICI) was significantly slower. In a previous dose-response experiment with yohimbine (16), using nonaged males, the 4 mg dose was stimulatory, as in the present study of young males, whereas inhibition was observed only at the 8 mg dose. Thus, it seems that sexual behavior in the aging male is more sensitive to the inhibitory effects of yohimbine than in the young male.

Since we did not test doses below 1 mg/kg, more study is required to determine if the yohimbine dose-response curve truly shifts to the left. However, in a recent study using apomorphine in young and middle-aged males, we have shown an increased sensitivity to the inhibitory effects of high doses of this dopamine agonist on sexual behavior in aging males without a concomitant increased sensitivity to the stimulatory effects of lower doses of the drug (3). A similar situation may exist for yohimbine.

That yohimbine restores sex behavior to a considerable extent, both in aging and castration (6), suggests a possible common factor. The active principle in yohimbine's restoration of sexual behavior, presumably norepinephrine (16), seems mutual to restoration of these two specific sexual dysfunctions. If so, in the chain of sexual events, we could speculate that the yohimbine mechanism for enhancing arousal occurs downstream to the point where testosterone is involved (6), and below or at the level of aging-induced deficiencies.

That the only known major sexual effects of yohimbine on rats are arousal/motivation suggests that yohimbine acts, in this context, on cerebral structures. Accordingly, we hypothesize that the cerebrally oriented sexual deficiencies of aging male rats are, to a considerable degree, reversed by activation of α_2 -adrenergic blockade and subsequent increased noradrenergic transmission. Another alternative would be that the α_2 -adrenoreceptors affected reside and act sexually on other types of neurons.

In general, it needs to be emphasized that yohimbine stimulation of male sexual behavior is unlikely to be unique. For instance, RDS-127, a dopamine and serotonin_{1A} agonist, also stimulates sex behavior in castrates (2). Various agents differentially influ-

TABLE 1
EFFECTS OF AGE AND YOHIMBINE TREATMENT ON THE PARAMETERS OF MATING
BEHAVIOR (MEAN \pm SE)

Treatment and Age	ML (min)	CE*	PEI (min)	IF (No.)	MF (No.)
Vehicle					
Young	0.57 \pm 0.39 (12)†	0.68 \pm 0.06 (12)	6.31 \pm 0.29 (12)	4.9 \pm 0.3 (12)	3.7 \pm 1.0 (12)
Middle aged	0.83 \pm 0.32 (10)	0.47 \pm 0.10 (10)	6.98 \pm 0.59 (7)	4.7 \pm 0.5 (7)	4.6 \pm 1.8 (7)
1.0 mg/kg					
Young	0.16 \pm 0.06 (13)	0.77 \pm 0.04 (13)	5.82 \pm 0.40 (13)	5.5 \pm 0.5 (13)	1.9 \pm 0.4 (13)
Middle aged	0.26 \pm 0.06 (12)	0.64 \pm 0.05 (12)	6.58 \pm 0.50 (12)	4.9 \pm 0.8 (12)	3.2 \pm 0.5 (12)
4.0 mg/kg					
Young	0.18 \pm 0.08 (12)	0.71 \pm 0.05 (12)	5.69 \pm 0.89 (12)	5.8 \pm 1.2 (12)	2.8 \pm 0.6 (12)
Middle aged	0.70 \pm 0.28 (11)	0.61 \pm 0.09 (11)	5.52 \pm 0.78 (8)	5.0 \pm 0.8 (8)	2.5 \pm 0.6 (8)

*ANOVA (3 \times 2); F(1,64) = 7.47, p = 0.008, significant for age only.

†Number of rats.

encing different neurotransmitters have similar effects on sex behavior, such as PCPA, naloxone, gaba antagonists and oxytocin (1,9). Such redundancies may well be common in the normal regulation of sex behavior.

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