Sexual Incentive Motivation and Copulatory Behavior in Male Rats Treated With the Adrenergic α2-Adrenoceptor Agonists Tasipimidine and Fadolmidine: Implications for Treatment of Premature Ejaculation

Jyrki Lehtimäki, MSci

Elisa Ventura-Aquino, MD, PhD

Xi Chu, PhD

Raúl G. Paredes, PhD

Anders Ågmo, PhD

Abstract

Background: Premature ejaculation is the most common sexual dysfunction in young men, and it often leads to reduced relationship satisfaction and quality of life.

Aim: To determine the role of central and peripheral α_2-adrenoceptors in the control of ejaculation and sexual incentive motivation in rats.

Methods: Sexual incentive motivation was studied in a large arena in which a male subject could choose between approaching and remaining close to a sexually receptive female or another male. Sexual behavior was studied in standard observation cages in which a male was allowed to freely interact with a receptive female for 30 min. Two highly selective agonists at the α_2-adrenoceptors, tasipimidine and fadolmidine, were administered before the tests. Low peripheral doses of fadolmidine has been reported to have effects mainly outside of the central nervous system, whereas at large doses also the central effects are evident.

Outcomes: The time spent close to the receptive female in relation to the time spent with the male and measures of ambulatory activity were obtained from the test for sexual incentive motivation, while the habitual parameters of sexual behavior were recorded with the copulation test.

Results: Tasipimidine prolonged ejaculation latency and the interintromission interval at the dose of 200 μg/kg when data from fast-ejaculating rats were used. No other sexual parameter was modified. A dose of 100 μg/kg was ineffective. There was no consistent effect on sexual incentive motivation, although modest sedation was observed. Fadolmidine, a drug that does not easily penetrate the blood–brain barrier, had no effect on sexual incentive motivation at any of the doses used (3, 30, and 100 μg/kg). The largest dose had clear sedative effects. The lower doses had no systematic effect on sexual behavior, not even when only fast or very fast ejaculating males were analyzed.

Clinical Translation: The findings are relevant to the search for treatments for premature ejaculation that are specific enough to selectively delay ejaculation.

Strengths & Limitations: The procedures used here are standard in the field and yield the most reliable data. Whether the effects observed in male rats are directly transferrable to men can only be determined through clinical studies.

Conclusion: The observation that drugs acting at central but not peripheral α_2-adrenoceptors prolong ejaculation latency without affecting any other parameter of sexual behavior or sexual incentive motivation suggests that this kind of drug may be suitable for treating premature ejaculation.

Keywords: premature ejaculation, α_2-adrenoceptor, tasipimidine, fadolmidine

Introduction

Premature ejaculation is the most common sexual dysfunction among young men.[1,2] According to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), the dysfunction is defined as recurrent ejaculation within 1 min of vaginal penetration and before the person wishes it to occur.[3] In the International Classification of Diseases 11th Revision (ICD-11) the dysfunction is called early ejaculation when ejaculation occurs within less than 3 min of vaginal penetration.[4] In addition, the problem should have lasted for several months and be associated with clinically significant distress according to both the DSM-5 and ICD-11.

Because of the reduced satisfaction associated with sexual activities and the often deteriorated quality of relationships caused by premature ejaculation, many of those experiencing the dysfunction seek efficient treatment.[5,6] Some psychotherapeutic approaches exist, and there is some data suggesting that they may have positive effects, particularly for acquired premature ejaculation and when combined with drug treatment.[7] However, pharmacological treatment appears to be more attractive, particularly for patients with lifelong premature ejaculation. Several types of drugs are currently used for delaying ejaculation in patients diagnosed with premature ejaculation.

Specific serotonin reuptake inhibitors (SSRIs) have been employed off-label for several years, and good results have been obtained with paroxetine and citalopram, among others.[8,9] The disadvantages with these classical SSRIs are that they need to be taken chronically for good effect and their inhibitory actions on other sexual responses, such as desire and erection.[10] A more recent SSRI, dapoxetine, is effective when used on demand, and it was the first drug registered as a treatment for premature ejaculation in the European Union.[11] Among other putatively efficient drugs are the opioid agonist tramadol [12], phosphodiesterase-5 inhibitors [13], and others with less established efficacy.[14,15]

Ejaculation is not entirely a central nervous process but rather a series of events in the reproductive organs. The first part of ejaculation, seminal emission from the epididymis and *vas*

deferens to the urethra and contraction of the prostate and seminal vesicles, depends on activity in the sympathetic nervous system. The process is initiated by stimulation of α_1-adrenoceptors in the distal epididymis.[16] Likewise, contractions in the *vas deferens*, seminal vesicles, and prostate are mediated by this receptor.[17,18] Sato et al. (2017) have reported that silodosin, a highly selective α_{1A}-adrenoceptor antagonist, improved premature ejaculation profiles and increased intravaginal ejaculation latency in acquired premature ejaculation patients.[19] Considering that silodosin mainly acts on peripheral adrenoceptors, this observation suggests that these receptors may be a target for the treatment of premature ejaculation. It is also known that presynaptic α_2-adrenoceceptors located on the noradrenergic nerve endings in the structures involved in seminal emission and ejaculation mediate prejunctional inhibition of transmitter release.[20-22.]

In line with the role attributed to the sympathetic nervous system in ejaculation, evidence has accumulated suggesting that premature ejaculation may be associated with high activity in the sympathetic nervous system. An early study showed that men with premature ejaculation showed a faster and larger increase in heart rate than controls during exposure to a pornographic video combined with vibrotactile stimulation of the penis.[23] It was suggested that premature sympathetic activation might be the cause of early ejaculation. Greater sympathetic reactivity in men with premature ejaculation was also reported in a study of reactive hyperemia using peripheral arterial tonometry.[24] Moreover, heart rate recovery after intense exercise has been found to be slower in men diagnosed with premature ejaculation than in controls, again suggesting an overactive sympathetic system.[25] Finally, a study of the sympathetic skin response on the penis showed that it was enhanced in men suffering from premature ejaculation.[26] All these observations coincide with data from male rats. Males with short ejaculation latency have higher concentrations of noradrenaline than rats with long ejaculation latencies.[27] Since circulating noradrenaline is derived from the peripheral nervous system (and the adrenals), it can be assumed that high activity in the sympathetic nervous system is related to fast ejaculation in rats as well.[28]

In addition to being widely expressed in the reproductive tract, adrenoceptors are expressed in many brain areas relevant for sexual behavior and for the central control of erection and ejaculation .[29,30] Indeed, it has been reported that drugs acting on adrenoceptors may alter sexual responses in male rats and dogs.[31-34] A highly specific α_2-adrenoceptor agonist, dexmedetomidine, has been found to enhance ejaculation latency in male rats without affecting any other aspect of sexual behavior when administered in a low dose. Likewise, the drug had no sedative effect at the doses used, and it failed to modify sexual incentive motivation.[35] The specific effect on ejaculation latency makes dexmedetomidine an potential alternative for treating premature ejaculation. However, dexmedetomidine has limited bioavailability after oral administration in man.[36] This prompted us to study the effects of a novel orally active α_2-adrenoceptor agonist tasipimidine, on male rats' sexual behavior and ejaculation parameters. Tasipimidine, developed for situational anxiety and fear in dogs, is a specific and subtype selective α_{2A}-adrenoceptor full agonist having binding affinity and functional EC50 values at nanomolar range in various in vitro biochemical and cell assays and ex vivo models. In vivo studies with peripheral dosing have shown that tasipimidine causes sedation in rats and mice measured by acoustic startle reflex and spontaneous motility assays, respectively (unpublished data).[37] Pharmacodynamic and pharmacokinetic data show that oral bioavailability of tasipimidine is limited particularly in rats, thus we decided to use subcutaneous doses to confirm that pharmacological active exposure is achieved.

α_2-adrenoceptor agonists, such as dexmedetomidine and fadolmidine, reduce the contractile response of *vas deferens* preparations *in ex vivo* setups and thus could possibly delay or reduce the peripheral responses involved in seminal emission.[38] Thus, it is possible that the actions of dexmedetomidine and other α_2-adrenoceptors agonists are mediated, at least partially, by peripheral α_2-adrenoceptors. The peripheral mode of action, especially for α_2-adrenoceptor agonists, might be attractive to avoid centrally mediated effects like sedation. To evaluate the role of peripheral adrenoceptors we studied the effects of fadolmidine, a full α_2-adrenoceptor agonist,

112 invented and developed by Orio Corporation for the spinal analgesia with limited penetrance over
113 the blood–brain barrier. Fadolmidine has been shown to selectively activate peripheral α_2-
114 adrenoceptor when low systemic doses are used.[38,39]
115
116 **Materials and methods**
117 *Animals*
118 For the tasipimidine study, 12 male Wistar Han IGS rats (350 g on arrival,) were purchased
119 from Charles River (Sulzfeld, Germany). The international genetic standardization program (IGS)
120 assures that rats have similar genetic variability regardless of the breeding colony from which they
121 are obtained. Several additional males and females of the same strain and from the same provider
122 were used as copulation partners or as incentives in the sexual incentive motivation test. The
123 animals were housed in same-sex pairs in Makrolon® IV cages in a room with controlled
124 temperature (21 ± 1 °C) and humidity (50 ± 10 %). Lights were on from 23:00 to 11:00.
125 Commercial rat pellets and tap water were available ad libitum. All experimental procedures were
126 approved by the Norwegian Food Safety Authority (ID 3327) and were in agreement with the
127 European Union council directive 2010/63/EU.
128 For the fadolmidine study, thirty experimentally and drug-naive male Wistar rats (from the
129 animal facilities at the Institute of Neurobiology, National Autonomous University of Mexico,
130 Campus Juriquilla) were used. Some other males of the same strain and from the same provider
131 were used as neutral incentives in the experiments. The weight of the male rats was 380–490 g
132 during the treatment period. Twelve females (250–300 g) were used either as incentives in the
133 motivation tests or as copulation partners in the copulatory behavior tests. The rats were housed in
134 groups of 2–4 in acrylic cages in a temperature controlled animal room at 21ºC ± 1 ºC and a relative
135 humidity of 60 % ± 10% with a reversed 12 h light/dark cycle (lights on 20:00–08:00), with free
136 access to water and food (standard certified rat pellets). All experimentation was done in

accordance with the "*Reglamento de la Ley General de Salud en Materia de Investigación para la Salud, NOM-062-ZOO-1999*" of the Mexican Health Ministry, which follows National Institutes of Health (NIH) guidelines. The study protocol was approved by the Institute of Neurobiology animal care committee.

Drugs

Tasipimidine (2-(5-methoxy-3,4-dihydro-1H-isochromen-1-yl)-4,5-dihydro-1H-imidazole) and fadolmidine (3-(1H-imidazol-4-ylmethyl)-indan-5-ol) were synthesized by Orion Corporation, Orion Pharma, Espoo, Finland). Tasipimidine was dissolved in 0.05 M citrate buffer (pH 4.4) shortly before use and administered subcutaneously in a volume of 1.0 ml/kg. The low pH of this preparation did not cause any observable discomfort to the animals. Two subcutaneous doses of tasipimidine were used (100 and 200 µg/kg). The lower dose was below sedative level, whereas the larger dose was chosen to also have a mild sedative effect (unpublished observation). Fadolmidine was freshly prepared in physiological saline before the experiments. Three subcutaneous doses of fadolmidine (3, 30, and 100 µg/kg) or vehicle were used. The lower doses (3 and 30 µg/kg) were earlier shown to have most of their effects in the periphery, whereas the largest dose (100µg/kg) was chosen to also have central effects.[37,38] Each rat was treated with each drug dose or vehicle weekly, in randomized order. The drugs were administered 15 min before the start of behavioral observations. This preinjection time is based on pharmacodynamic data. [37,38.]

Procedure

The female rats were ovariectomized under anesthesia with isoflurane (tasipimidine study) or a cocktail of ketamine (95 mg/kg) and xylazine (12 mg/kg, both IP; fadolmidine study) two weeks before use. To assure maximum receptivity and proceptivity, they were further treated hormonally.[35, 40] Two alternative procedures were used. For the tasipimidine study, a 5-mm-long

silicone capsule (medical grade Silastic tubing, 0.0625 in. inner diameter, 0.125 in. outer diameter, Degania Silicone, Degania Bet, Israel) filled with 10 % 17β-estradiol in cholesterol, both from Sigma, St. Louis, MO, USA, was implanted subcutaneously in conjunction with the ovariectomy. The capsules were sealed with medical-grade adhesive silicone (Nusil Silicone Technology, Carpinteria, CA USA). In addition, the females were given progesterone (Sigma-Aldrich, St Louis, MO, USA), dissolved in peanut oil (Apoteksproduskjon, Oslo, Norway), in a dose of 1 mg/rat about 3.5 h prior to testing by subcutaneous injection in a volume of 0.2 ml/rat. For the fadolmidine study, sexual receptivity was induced by the sequential administration of estradiol benzoate (25 μg/rat) and progesterone (1 mg/rat). Both hormones (Sigma-Aldrich, St Louis, MO, USA) were dissolved in corn oil and administered subcutaneously in a volume of 0.2 ml/rat 48 and 4 h before the tests, respectively. Both of these treatments assure maximum receptivity and a high level of proceptivity. Gross observation of female behavior did not reveal any difference in response to the experimental males, regardless of drug and dose.

The test for sexual incentive motivation has been described in detail elsewhere.[41,42] Briefly, it consists of a large, oval arena (100 x 50 cm) surrounded by 45-cm-high walls. In these walls are two diagonally opposed openings (25 x 25 cm), and behind each of these openings is a small cage housing the incentive animal (intact male and sexually receptive female, respectively). A wire mesh separated these cages from the arena. In front of each of the lateral cages, a virtual zone (the incentive zone) measuring 21 x 30 cm was defined. A videotracking system (Ethovision, Noldus, Wageningen, the Netherlands) recorded the experimental subject's position with a frequency of 5 Hz. The subject was considered to be within a zone whenever the videotracking system determined that the subject's point of gravity was inside. The room was illuminated by dim white light (about 5 lx in the arena). In addition to recording the time spent in the incentive zones and the number of visits to them, the system calculated the distance moved, the time spent moving, and the speed of movement. We also calculated a preference score (time spent in the incentive zone adjacent to the

female incentive / (time spent in the incentive zone adjacent to the female incentive + the time spent in the incentive zone adjacent to the male incentive)).

Sexual behavior was observed in rectangular arenas (40 x 60 cm, 40-cm-high walls) with a Plexiglas front, located in a room different from the incentive motivation setup. Behavioral items were analyzed with Observer XT 12.5 software (Noldus). The light intensity was about 60 lx. The following behavioral features were recorded: mount latency, time from introduction of the female to the first mount with pelvic thrusting; intromission latency, time from introduction of the female to the first vaginal penetration (intromission); ejaculation latency, time from the first intromission until ejaculation; postejaculatory interval, time from ejaculation until the next intromission; number of mounts; and number of intromissions. In addition, we calculated the interintromission interval (the ejaculation latency divided by the number of intromissions) and the intromission ratio (the number of intromissions / (the number of intromissions + the number of mounts).

The experimental males were subjected to three pre-experimental tests for sexual behavior. These tests were ended at the first post-ejaculatory interval. In the event that the male failed to ejaculate, the test lasted for 30 min after the first intromission. If the male did not perform intromissions, the test was ended 15 min after the introduction of the female. Only males that ejaculated in at least two of these tests were used for drug treatment.

Following these pretests, the males were familiarized to the incentive motivation arena during three 10-min sessions, separated by 48 h. Before each test, the arena was carefully cleaned with a 0.1% solution of glacial acetic acid in water. One week following the last familiarization session, drug treatment was begun. Fifteen min after drug injection, the subject was put in the center of the incentive motivation arena. The experimenter immediately left the room and did not return until the end of the 10-min observation period. The subject was then gently removed, and the next subject was introduced into the arena. Immediately after this test, the subject was transferred to the room where sexual behavior was observed. There it was placed in an observation arena, and a

sexually receptive female was introduced 5 min later. Sexual behavior was observed for a period of 30 min. The sequence of events for each subject at each test is illustrated in Fig. 1.

Statistics

Data from the sexual incentive motivation test were analyzed either with a one-factor analysis of variance (ANOVA) for repeated measures (preference score, distance moved, time moving, speed of movement) or two-factor ANOVA for repeated measures on both factors. In the latter case, one factor was incentive (male, female) and the other was treatment. The variables analyzed in this way were time spent in the incentive zones, the number of visits to the incentive zones, and the mean duration of each visit. Sex behavior data were analyzed either with one-factor or repeated measures ANOVA, or, when the data deviated from normality according to the Shapiro–Wilk test, with Friedman's ANOVA. Post hoc comparisons were made with Tukey's honestly significant difference (HSD) test or the Wilcoxon test with the Bonferroni correction for multiple comparisons, as appropriate. A two-tailed α value < 0.05 was considered significant.

Whenever parametric ANOVA was used, data were checked for sphericity with the Mauchly test. In case of non-sphericity ($p < 0.05$), the degrees of freedom for the *F*-test were adjusted according to the Huynh-Feldt procedure, Effect sizes are expressed as partial eta squared (η_p^2) when parametric ANOVA was used, and as Kendall's W when the non-parametric Friedman ANOVA was used. The Statistical Package for the Social Sciences (IBM SPSS), v. 26, was used for all analyses.

One or two males failed to resume copulation after the 1st ejaculation, and several males did not continue until a 3rd. Therefore, data are presented only for the 1st and 2nd ejaculatory series. No substitution was made for missing data, meaning that all analyses and all data reported are based on observed behavior.

The methods and results sections of this paper comply with the ARRIVE guidelines.

Results

Tasipimidine

Sexual incentive motivation

There was an effect of treatment on the preference score ($F_{2,22}$) = 4.30, p = 0.026, η_p^2 = 0.281). It was reduced after the 100 µg/kg dose, but not after the largest dose according to the Tukey's HSD test (Fig. 2 A). Analysis of the time spent in the incentive zones revealed that the time spent in the female incentive zone was far greater than the time spent in the male incentive zone ($F_{1,11}$) = 104.38, $p < 0.001$, $\eta_p^2 = 0.905$). There was also a treatment effect ($F_{2,22}$ = 5.42, p = 0.012, $\eta_p^2 = 0.528$), and the interaction treatment x incentive was also significant ($F_{2,22}$ = 5.42, p = 0.012, $\eta_p^2 = 0.478$. The Tukey's test showed that the time spent with the female incentive was reduced after the 200 µg/kg dose when compared to vehicle. No other effect was found (Fig. 2 B). The number of visits to the incentive zones differed between incentives ($F_{1,11}$ = 36.95, $p < 0.001$, η_p^2 = 0.771) such that the number of visits to the female incentive zone was larger than the number of visits to the male zone. There was no effect of treatment ($F_{2,22}$ = 0.88, NS) and no interaction treatment x incentive ($F_{2,22}$ = 1.31, NS; Supplementary Fig. 1 A). This was also the case for the mean duration of visits to the incentives. There was no effect of treatment ($F_{2,22}$ = 0.24, NS), but the visits to the female incentive zones were longer than the visits to the male incentive zone ($F_{1,11}$ = 29.78, $p < 0.001$ $\eta_p^2 = 0.730$). There was also an interaction between treatment and incentive ($F_{2,22}$ = 5.72, p = 0.010, $\eta_p^2 = 0.342$). When comparing the mean duration within each of the incentives, the mean duration of visits to the male incentive zone did not vary between doses of tasipimidine. On the contrary, the mean duration of visits to the female incentive zone was reduced after 100 µg/kg compared to vehicle. Data are shown in Supplementary Fig. 1 B.

General activity

There was a treatment effect on all indices of ambulatory activity (distance moved, $F_{2,22}$ = 3.75, p = 0.04, η_p^2 = 0.254; velocity of movement while moving, $F_{2,22}$ = 3.76, p = 0.04, η_p^2 = 0.255; time moving, $F_{2,22}$ = 5.84, p = 0.009, η_p^2 = 0.347). Only the 200-µg/kg dose reduced activity according to the Tukey's test. Data are shown in Fig. 3.

Sexual behavior

As can be seen in Table 1, there were few effects of treatment. In the first ejaculatory series, the interintromission interval was affected by the drug ($\chi^2_{(2)}$ = 9.45, p = 0.009, W = 0.430). It was extended after 100 µg/kg tasipimidine, but not after the larger dose. This may seem strange, as the mean interval after the 200-µg/kg dose (53 s) was longer than that after 100 µg/kg (45 s). However, two of the rats had shorter intervals after the former dose compared to only one after the latter. Therefore, the larger difference was non-significant according to the Bonferroni-corrected Wilcoxon test. The intromission ratio was also modified by the drug ($F_{2,22}$ = 3.76, p = 0.039, η_p^2 = 0.255). The 200-µg/kg dose reduced the intromission ratio, whereas the lower dose had no effect. The drug was entirely without effect in the second ejaculatory series. When behavior during the entire 30-min test was evaluated, it was found that the number of intromissions and ejaculations was reduced ($F_{2,22}$ = 4.37, p = 0.025, η_p^2 = 0.284 and $F_{2,22}$ = 4.41, p = 0.025, η_p^2 =0.286 respectively). The Tukey's HSD test established that only the 200-µg/kg dose was effective.

It has repeatedly been suggested that male rats with short ejaculation latencies could be considered a model of premature ejaculation.[43,44] Regardless of the validity of that argument, we decided to analyze the effects of tasipimidine in animals with an ejaculation latency after vehicle below the 75th percentile. In these animals, there was indeed a treatment effect on the ejaculation latency ($\chi^2_{(2)}$ = 7.75, p = 0.021, W = 0.484). It was longer after the largest dose, according to the Wilcoxon test with Bonferroni correction. The interintromission interval was also affected by the drug ($\chi^2_{(2)}$ = 9.25, p = 0.010, W = 0.578). Both doses increased this interval, again confirmed by

Wilcoxon test with Bonferroni correction. There was no drug effect in the second ejaculatory series, but the drug affected the number of intromissions ($F_{2,16} = 4.87$, $p = 0.022$, $\eta_p^2 = 0.378$) and ejaculations ($F_{2,16} = 7.94$, $p = 0.004$, $\eta_p^2 = 0.498$) performed during the entire test. The Tukey's HSD test showed that the 200 µg/kg dose reduced this number. Data are shown in Table 2.

Fadolmidine

Sexual incentive motivation

Fadolmidine did not modify the preference score ($F_{3,57} = 1.56$, NS). Data are shown in Fig. 4 A. Likewise, the time spent in the incentive zones (sexually receptive female and intact male) was not affected by the drug ($F_{3,57} = 0.10$, NS). There was a large difference between incentives ($F_{1,19} = 52.54$, $p < 0.001$, $\eta_p^2 = 0.734$) in that the males spent far more time in the vicinity of the receptive female than in the vicinity of another male. The interaction treatment x incentive was not significant ($F_{3,57} = 1.56$, NS; Fig. 4 B).

An ANOVA of the number of visits to the incentive zones revealed an effect of treatment ($F_{2.266,57} = 17.94$, $p < 0.001$, $\eta_p^2 = 0.486$) and of incentive ($F_{1,19} = 11.72$; $p = 0.003$, $\eta_p^2 = 0.382$). Since these data did not satisfy the sphericity assumption, the Huynh-Feldt correction was applied. *Post hoc* analyses found that the dose of 100 µg/kg produced a reduction in the number of visits to both incentives when compared to vehicle. The interaction incentive x treatment was non-significant ($F_{2.628,57} = 1.73$, NS). Data are illustrated in Supplementary Fig 2 A.

Also here, the Mauchly test showed lack of sphericity, and the degrees of freedom were adjusted accordingly. The mean duration of visits to the incentive zone was longer in the female than in the male zone ($F_{1,19} = 22.79$, $p < 0.001$, $\eta_p^2 = 0.545$). There was also an effect of treatment ($F_{1.401,57} = 15.28$, $p < 0.001$, $\eta_p^2 = 0.446$). The largest dose increased the duration of visits

to both incentives according to the Tukey's test. There was no interaction incentive x treatment ($F_{1.498,57} = 0.89$, NS; Supplementary Fig. 2 B).

General activity

Data from all three indices of ambulatory activity failed the sphericity test. According to the repeated measures ANOVA with Huynh-Feldt correction, the treatment had a robust effect on ambulatory activity during the test. There were significant effects on the distance moved ($F_{2.136,57} = 21.59$, $p < 0.001$, $\eta_p^2 = 0.532$), the mean velocity of movement while moving ($F_{2.15,57} = 21.98$, $p < 0.001$, $\eta_p^2 = 0.536$), and the time spent moving ($F_{1.972,57} = 25.81$, $p < 0.001$, $\eta_p^2 = 0.576$). The Tukey's HSD test showed that the largest dose, 100 µg/kg, reduced all three indices of ambulatory activity. Data are shown in Fig. 5.

Sexual behavior

Fadolmidine had almost no effect on sexual behavior at any of the doses used. In fact, only the first postejaculatory interval was modified by the drug ($F_{3,57} = 7.68$, $p < 0.001$, $\eta_p^2 = 0.299$). The Tukey's HSD test showed that the interval was longer after the administration of 100 µg/kg of fadolmidine than after vehicle. Notably, there was no effect on ejaculation latency, either with the first ($\chi^2_{(3)} = 0.60$; NS) or second ($F_{3,54} = 1.85$; NS) ejaculation. Data is shown in Table 3.

We then analyzed the behavior in the 15 males with an ejaculation latency below the 75th percentile. In these males, the first postejaculatory interval was prolonged ($F_{3,42} = 7.26$; $p < 0.001$, $\eta_p^2 = 0.342$). Moreover, the number of ejaculations performed during the 30 min test was reduced ($F_{3,42} = 3.14$; $p = 0.035$, $\eta_p^2 = 0.183$). The Tukey's HSD test showed that these effects were limited to the 100 µg/kg dose. The number of mounts in the first ejaculatory series ($\chi^2_{(3)} = 8.41$; $p = 0.038$, W = 0.187) and in the entire test ($F_{3,42} = 2.97$, $p = 0.043$, $\eta_p^2 = 0.175$) were also modified by

fadolmidine. Here, the effect was found after the 30 µg/kg dose according to the Tukey's HSD test. No other statistically significant effect was obtained. Data is found in Table 4.

In an effort to further explore the potential effects of fadolmidine, we selected the five males with an ejaculation latency after vehicle below the 25th percentile (189.5 s), that is, very fast ejaculating males. The only significant effect obtained in the first ejaculatory series was on ejaculation latency ($F_{3,12} = 3.63$; $p = 0.045$, $\eta_p^2 = 0.476$). However, the Tukey's test failed to confirm significant differences between vehicle and any of the fadolmidine doses. In the second ejaculatory series, there were effects on the interintromission interval ($F_{3,9} = 4.05$; $p = 0.045$, $\eta_p^2 = 0.574$) as well as on the intromission ratio ($F_{3,12} = 3.53$; $p = 0.048$, $\eta_p^2 = 0.469$). The Tukey's test showed that the interintromission interval was prolonged after the 100 µg/kg dose and that the intromission ratio was reduced after that dose. The apparent increase in ejaculation latency in the second series failed to reach significance ($F_{3,12} = 2.75$; NS). See Table 5 for the data from this subset of males. It appears that fadolmidine was unable to affect sexual behavior in doses without sedative effects, even in very fast ejaculating males.

Discussion

Two novel α_2-adrenoceptor agonists, fadolmidine and tasipimidine, were studied in rat models of sexual incentive motivation and sexual behavior with the purpose to evaluate the potential treatment benefits of these sympatholytic compounds on premature ejaculation.

Tasipimidine had no consistent effect on sexual incentive motivation. There was a small, albeit significant, effect on the preference score after 100 µg/kg and a small reduction of the time spent in the female incentive zone after 200 µg/kg. We have earlier argued that any functionally significant effect on sexual incentive motivation must simultaneously affect both the preference score and the time spent with the female.[45] An increased preference score may be the result either of reduced time spent with the non-sexual incentive or increased time spent with the sexual incentive.

Only in the latter case can the increased score be interpreted as an indication of increased sexual motivation. Therefore, the time spent with the incentives also needs to be evaluated. However, an increase in time spent with the sexual incentive is not sufficient by itself. It could be due to an increase in sociability, i.e. simultaneous increase in time spent with both incentives. To rule out these alternative explanations, both the preference score and the time spent with the sexual incentive must be enhanced in order to propose that sexual motivation was increased. We suggest that tasipimidine, in the doses employed here, does not alter motivation in any functionally relevant way. On the contrary, the drug had a consistent but small effect on measures of ambulatory activity after the largest dose. The sedative actions of α_2-adrenoceptor agonists are well known, and the present observation was expected.

Tasipimidine had modest effects on sexual behavior. In fact, when all subjects were included in the analysis, the drug had minor, erratic effects in the first ejaculatory series and none in the second series. The 200 μg/kg dose reduced the number of mounts and intromissions displayed during the 30-min test, which suggests an inhibitory action at this dose. One possible explanation is that the motor slowing caused by the drug indirectly affected sexual behavior. Even though this possibility cannot be ruled out, earlier data show that motor slowing must be substantial before it affects sexual behavior.[46,47]

When the analysis of sexual behavior was limited to subjects with short ejaculation latencies, a somewhat different picture emerged. The interintromission interval in the first ejaculatory series was prolonged after both doses, and ejaculation latency was enhanced. Since the number of intromissions remained unaffected, the long interintromission interval was a direct consequence of the long ejaculation latency. The fact that this effect was not obtained when we also included males with long ejaculation latencies suggests that tasipimidine affects the ease of achieving ejaculation only in animals ejaculating rapidly. Considering the supposition already mentioned, that such animals represent a good model of premature ejaculation,[42,43] these results

indicate that tasipimidine is a potential candidate for an appropriate treatment of premature ejaculation. The slight sedative effect observed after treatment with the dose of 200 μg/kg is probably not a major concern, especially considering sympathetic overactivity as a potential cause of premature ejaculation. Obviously, further studies, including a replication of the results reported here, are needed before any firm conclusion can be reached.

Fadolmidine, an α_2-adrenoceptor agonists with a peripheral site of action had no effect on sexual incentive motivation, but the drug clearly reduced ambulatory activity after the largest dose. Even though fadolmidine does not easily cross the blood–brain barrier, some penetration does occur after large peripheral doses, as reported earlier.[37,38] Sedation, here manifested as reduced ambulatory activity, is considered a typical, centrally mediated response to α_2-adrenoceptor agonists. The only effect observed on sexual behavior when the entire sample was analyzed was a modest increase in the first postejaculatory interval after the largest dose. It is likely that this effect is a consequence of reduced general activity. When we limited the analysis to males with short or very short ejaculation latencies after vehicle treatment, we again found that all drug effects on sexual behavior were most likely consequences of sedation. An increased interintromission interval is certainly an indication of the slowing of the pace of sexual interaction. An enhanced postejaculatory interval is also suggestive of a sedative effect, manifested in reduced recovery of responsivity to the female. The lower number of ejaculations in the test, caused by the largest dose of fadolmidine, could be a direct consequence of the prolonged period of inactivity following the first ejaculation.

The reduced intromission ratio in the second ejaculatory series found after 100 μg/kg fadolmidine in the very fast ejaculating animals is more difficult to attribute to a sedative effect of the drug. This ratio represents the ease of achieving vaginal penetration, which is dependent on contraction of the ischiocavernosus muscle,[48] in addition to increased intracavernous pressure.[49] The latter of these processes may be affected by α_2-adrenoceptors, but there are no data from rats

showing that they are. However, studies of human cavernous tissue show that α receptors are expressed on smooth muscle cells, and that they may control intracavernous muscle tone.[50] Indeed, it has been reported that α_2-adrenoceptor antagonists enhance nitric oxide release, thereby facilitating erection.[51] Considering that the effect on intromission ratio was only observed in the subsample of very fast ejaculating rats—and then only in the second ejaculatory series—we suggest that it cannot be of any major functional significance.

Doses of fadolmidine that can be considered to have no or little effect on the central nervous system were clearly unable to alter any aspect of male rat sexual behavior. This suggests that the effects obtained with tasipimidine in the present study and those reported earlier for dexmedetomidine most likely depend on actions within the central nervous system.[35] It may also be noted that a peripheral α_2-adrenoceptor antagonist, vatinoxan (also known as L 659066 or MK-467), did not have any effect on sexual behavior or sexual incentive motivation in male rats.[31] The combination of these observations indicates that the peripheral α_2-adrenoceptors are of slight or no importance in the regulation of sexual behavior in these rat models.

The neural control of ejaculation involves both central and peripheral systems. In fact, ejaculation is associated with activity in several brain structures, such as the preoptic area, lateral hypothalamus, paraventricular nucleus, and locus ceruleus.[52] Some of the descending neurons from these structures are noradrenergic.[53] Moreover, neurons in a small area of the lumbar spinal cord are necessary, as selective lesions of these neurons abolishes ejaculation in rats.[54,55] Adrenergic drugs are known to alter several aspects of sexual behavior after administration directly into the brain, showing that central nervous adrenergic mechanisms can modify that behavior.[56] However, none of the previous studies has reported a specific effect on ejaculation latency. In the case of drugs acting at the α_2-adrenoceptor, it has been reported that the agonist clonidine causes a generalized inhibition of sexual behavior when administered to the preoptic area.[57] Curiously, in the same study it was found that the antagonist yohimbine had no effect in that same area. Further,

Yonezawa et al. (1986)[33] showed that intracerebroventricularly administered clonidine in dogs produced a dose-related inhibition of ejaculation but not significant inhibition of erection by manual stimulation of the penis. This ejaculatory disturbance was antagonized by yohimbine.

It is known that α_1-adrenoceptors do not modify sexual behavior at doses strongly reducing contraction of the *vas deferens* and the seminal vesicle.[58] Indeed, the highly specific α_1-adrenoceptor antagonist tamsulosin completely blocked the expulsion of seminal plugs at a dose leaving sexual behavior unaffected. The latter effect clearly shows that peripheral responses to sympathetic activation were strongly reduced, and this had no consequence for ejaculation latency. It seems safe to conclude that peripheral adrenoceptors are not a major player in the control of male rat sexual behavior. Whether these observations in rats can be generalized to humans remains an open question. Whereas reduced seminal output after treatment with α_1-adrenoceptor antagonists is evident in both rats and men, effects on ejaculation latency may be limited to humans. There are actually some reports suggesting that α_1-adrenoceptor antagonists can indeed prolong ejaculation latency in men diagnosed with premature ejaculation. Silodosin, a highly specific α_{1A}-adrenoceptor antagonist, has been found to enhance intravaginal ejaculation latency in men with acquired premature ejaculation.[19,59] The drug also reduced semen volume and resulted in anejaculation in some men. This effect has also been found after treatment with another α_1-adrenoceptor antagonist, tamsulosin.[60] In fact, five days of treatment with a rather large dose (0.8 mg daily) of tamsulosin caused anejaculation in 35% of the male participants. It is not known whether these effects are mediated by peripheral or central adrenoceptors. Although it is believed that the beneficial effects of α_1-adrenoceptor antagonists for patients suffering from prostatic hyperplasia are mediated by adrenoceptors in the urethrogenital tract, a central site of action cannot be excluded.[61,62] This also applies to the possible effects on premature ejaculation.

α_2-adrenoceptors are widely distributed in many organs. They are also widely distributed in the brain.[63]. Thus, any drug acting at these receptors may have a multitude of actions. In the present

458 communication, we have focused on actions on sexual motivation and behavior as well as on
459 measures of locomotor activity. Reductions in the latter were interpreted as signs of sedation. All
460 other possible actions were ignored. Nevertheless, the data reported here can be considered to
461 provide reliable information on the effects of the drugs on sexual functions, particularly since we
462 have been able to distinguish effects on these functions from sedative actions. The real
463 generalizability of these findings from rats to humans can only be established in clinical studies.
464 Any good treatment of premature ejaculation should enhance ejaculation latency, preferably without
465 altering any other parameter of sexual behavior. It is also desirable that such a drug does not affect
466 sexual motivation. Aphrodisiac properties could compromise the clinical acceptability of the drug
467 and lead to various kinds of abuse. In addition, since anxiety responses are frequently observed in
468 premature ejaculation patients,[64] a treatment that not only enhances the intravaginal ejaculation
469 latency but also reduces anxiety would be most suitable. Dexmedetomidine has been shown to be
470 anxiolytic in laboratory animal models[65] as well as in humans[66] and is used to treat noise-associated
471 acute anxiety and fear in dogs.[67] Based on the results of tasipimidine in this particular study and an
472 earlier study with dexmedetomidine[35] in male rats, it is suggested that these α_2-adrenoceptor
473 agonists might have therapeutic value for the symptomatic treatment of premature ejaculation. Lack
474 of efficacy of fadolmidine on ejaculation latency suggests that the peripheral mode of action for
475 theα_2-adrenoceptor agonist is not adequate to increase the ejaculation latency but the central mode
476 of action is needed. Whether similar specificity of action, or any action at all, may be obtained in
477 men remains to be studied.

Table 1. Parameters of copulatory behavior after subcutaneous treatment with vehicle or two doses of the α_2-adrenoceptor agonist tasipimidine. Data are mean ± SEM. *, different from vehicle, $p < 0.05$. N = 12.[a], ejaculation latency in s / number of intromissions; [b], the number of intromissions / number of mounts + number of intromissions.

Parameter	Vehicle	100 µg/kg	200 µg/kg
Latency to 1st mount (s)	11.08 ± 2.31	10.00 ± 1.66	68.50 ± 43.61
Latency to 1st intromission (s)	17.75 ± 4.68	15.67 ± 2.76	77.75 ± 47.30
Number of mounts 1st series	10.00 ± 3.31	13.67 ± 4.72	11.58 ± 2.59
Number of intromissions 1st series	12.75 ± 2.35	11.33 ±1.10	11.17 ± 1.74
Ejaculation latency 1st series (s)	323.58 ± 73.89	492.25 ± 143.82	537.64 ± 127.08
Postejaculatory interval 1st series (s)	254.67 ± 25.77	276.82 ± 15.31	275.78 ± 13.38
Interintromission[a] interval 1st series (s)	29.58 ± 8.65	44.90 ± 15.22*	53.46 ± 14.69
Intromission ratio[b] 1st series	0.64 ± 0.06	0.58 ± 0.06	0.51 ± 0.04*
Number of mounts 2nd series	7.50 ± 1.62	4.42 ± 1.32	5.33 ± 1.63
Number of intromissions 2nd series	5.58 ± 0.85	3.75 ± 0.65	4.00 ± 0.81
Ejaculation latency 2nd series (s)	145.83 ± 24.23	140.00 ± 23.73	160.13 ± 26.49
Postejaculatory interval 2nd series (s)	322.75 ± 15.83	351.70 ± 14.33	365.75 ± 14.46
Interintromission interval 2nd series (s)	28.41 ± 3.58	34.70 ± 6.68	31.51 ± 6.13
Intromission ratio 2nd series	0.46 ± 0.05	0.51 ± 0.09	0.51 ± 0.08
Number of mounts in the test	29.33 ± 3.65	32.42 ± 7.10	22.00 ± 4.50

Number of intromissions in test	28.58 ± 3.26	21.33 ± 2.08	20.50 ± 3.41*
Number of ejaculations in test	3.58 ± 0.31	3.08 ± 0.36	2.50 ± 0.40*
Intromission ratio in the test	0.50 ± 0.05	0.47 ± 0.07	0.50 ± 0.04

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Table 2. Parameters of copulatory behavior after subcutaneous treatment with vehicle or two doses of the α_2-adrenoceptor agonist tasipimidine in the 9 males having a vehicle ejaculation latency below percentile 75 and displaying at least one ejaculation after all treatments. Data are mean ± SEM. *, different from vehicle, $p < 0.05$, **, $p < 0.01$. [a], ejaculation latency in s / number of intromissions; [b], the number of intromissions / number of mounts + number of intromissions.

Parameter	Vehicle	100 µg/kg	200 µg/kg
Number of intromissions 1st series	10.00 ± 1.26	11.1 ± 0.90	9.6 ± 1.92
Ejaculation latency 1st series (s)	201.11 ± 44.89	285.78 ± 50.95	535.13 ± 165.81*
Interintromission interval 1st series (s)	20.91 ± 3.72	24.70± 3.15*	59.11 ± 19.18*
Intromission ratio 1st series	0.70 ± 0.06	0.63 ± 0.07	0.52 ± 0.06
Number of intromissions 2nd series	5.56 ± 0.83	4.56 ± 0.56	3.33 ± 0.94
Ejaculation latency 2nd series (s)	136.89 ± 30.24	120.22 ± 14.66	127.00 ± 20.67
Interintromission interval 2nd series (s)	24.71 ± 3.00	29.72 ± 4.98	29.66 ± 8.20
Intromission ratio 2nd series	0.49 ± 0.07	0.58 ± 0.10	0.59 ± 0.11
Number of mounts in the test	26.89 ± 4.13	32.00 ± 8.81	18.11 ± 5.28
Number of intromissions in test	27.33 ± 2.67	23.33 ± 2.11	18.22 ± 3.88*
Number of ejaculations in test	4.00 ± 0.29	3.67 ± 0.24	2.56 ± 0.50**
Intromission ratio in the test	0.51 ± 0.06	0.51 ± 0.10	0.53 ± 0.06

Table 3. Copulatory behavior expressed as mean ± SEM after subcutaneous treatment with vehicle, 3, 30 and 100 µg/kg of the α_2-adrenoceptor agonist fadolmidine. ***, $p < 0.001$, different from vehicle, Tukey's HSD test (N = 20). [a], ejaculation latency in s / number of intromissions; [b], the number of intromissions / number of mounts + number of intromissions.

Parameter	Vehicle	3 µg/kg	30 µg/kg	100 µg/kg
Latency to 1st mount (s)	5.05 ± 0.88	8.80 ± 3.57	7.60 ± 4.00	11.30 ± 3.76
Latency to 1st intromission (s)	6.60 ± 0.91	27.35 ± 12.02	14.45 ± 5.08	15.25 ± 5.50
Number of mounts 1st series	16.88 ± 1.73	22.95 ± 4.09	22.85 ± 2.92	17.70 ± 1.68
Number of intromissions 1st series	11.50 ± 0.84	12.15 ± 0.98	13.10 ± 0.90	10.65 ± 0.82
Ejaculation latency 1st series (s)	280.90 ± 30.23	296.53± 33.83	344.30 ± 59.34	323.30 ± 35.29
Postejaculatory interval 1st series (s)	366.85 ± 9.32	386.53 ± 16.12	385.85 ± 15.11	454.80 ±13.86***
Interintromission interval 1st series (s)	24.35 ± 1.62	24.88 ± 2.69	27.15 ± 4.46	29.83 ± 2.80
Intromission ratio 1st series	0.72 ± 0.03	0.69 ± 0.05	0.65 ± 0.05	0.64 ± 0.04
Number of mounts 2nd series	9.20 ± 1.27	10.53 ± 1.47	9.68 ± 1.60	11.50 ± 1.23
Number of intromissions 2nd series	6.05 ± 0.58	5.79 ± 0.60	6.10 ± 0.63	5.95 ± 0.49
Ejaculation latency 2nd series (s)	158.25 ± 24.32	189.79± 29.52	138.37 ± 23.32	196.05 ± 23.77
Postejaculatory interval 2nd series (s)	39.79 ± 12.32	492.47± 41.72	427.05 ± 14.22	472.67 ± 11.42
Interintromission interval 2nd series (s)	26.84 ± 3.24	33.50 ± 6.70	21.64 ± 2.19	35.35 ± 4.41
Intromission ratio 2nd series	0.70 ± 0.04	0.66 ± 0.06	0.71 ± 0.05	0.56 ± 0.04
Number of mounts in the test	36.85 ± 2.25	43.80 ± 4.17	44.80 ± 3.62	37.30 ± 2.72
Number of intromissions in test	25.20 ± 1.29	24.55 ± 1.89	26.45 ± 1.96	20.75 ± 1.20
Number of ejaculations in test	3.10 ± 0.12	2.70 ± 0.21	3.00 ± 0.18	2.70 ± 0.13
Intromission ratio in the test	0.70 ± 0.02	0.63 ± 0.05	0.62 ± 0.04	0.58 ± 0.03

494 Table 4. Parameters of copulatory behavior after subcutaneous treatment with vehicle or 3, 30 and 100
495 µg/kg of the α_2-adrenoceptor agonist fadolmidine in the 15 males with a vehicle ejaculation latency below
496 percentile 75 (400 s). Data are mean ± SEM. Different from vehicle, *, $p < 0.05$; ***, $p < 0.001$; Tukey's HSD
497 test. [a], ejaculation latency in s / number of intromissions; [b], the number of intromissions / number of
498 mounts + number of intromissions.

Parameter	Vehicle	3 µg/kg	30 µg/kg	100 µg/kg
Latency to 1st mount (s)	4.67 ± 1.07	8.93 ± 4.49	3.87 ± 0.83	12.53 ± 4.97
Latency to 1st intromission (s)	5.93 ± 1.08	19.20 ± 10.48	12.27 ± 4.81	17.40 ± 7.29
Number of mounts 1st series	13.20 ± 0.95	21.00 ± 3.65	22.53 ± 2.98*	16.93 ± 1.65
Number of intromissions 1st series	9.87 ± 0.60	12.67 ± 1.14	13.13 ± 1.03	10.27 ± 0.83
Ejaculation latency 1st series (s)	217.60 ± 17.90	314.87 ± 40.31	302.87 ± 44.52	321.47 ± 39.14
Postejaculatory interval 1st series (s)	368.1 ± 10.98	390 ± 20.35	383.4 ± 18.22	465.9 ± 16.42***
Interintromission interval 1st series (s)	22.84 ± 1.94	26.37 ± 3.29	24.93 ± 4.45	31.19 ± 3.39
Intromission ratio 1st series	0.76 ± 0.3	0.71 ± 0.05	0.64 ± 0.05	0.64 ± 0.04
Number of mounts 2nd series	8.00 ± 0.66	11.47 ± 1.75	10.20 ± 1.98	11.47 ± 1.41
Number of intromissions 2nd series	5.73 ± 0.59	5.93 ± 0.74	6.13 ± 0.75	5.80 ± 0.60
Ejaculation latency 2nd series (s)	121.73 ± 13.83	188.10 ± 34.42	135.13 ± 27.43	199.87 ± 30.54
Postejaculatory interval 2nd series (s)	445.73 ± 14.30	491.36 ± 55.01	429.93 ± 15.45	486.30 ± 12.09
Interintromission interval 2nd series (s)	23.14 ± 3.11	29.20 ± 4.87	21.18 ± 2.61	36.13 ± 4.96
Intromission ratio 2nd series	0.73 ± 0.05	0.62 ± 0.07	0.69 ± 0.06	0.54 ± 0.04

Number of mounts in the test	32.60 ± 1.50	43.40 ± 4.32	45.73 ± 4.56*****	36.80 ± 3.47
Number of intromissions in test	24.07 ± 1.57	26.07 ± 1.97	27.27 ± 2.38	20.20 ± 1.42
Number of ejaculations in test	3.27 ± 0.12	2.80 ± 0.17	3.06 ± 0.15	2.67 ± 0.16*****
Intromission ratio in the test	0.74 ± 0.03	0.65 ± 0.05	0.63 ± 0.04	0.57 ± 0.03

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500 Table 5. Parameters of copulatory behavior expressed as mean ± SEM in the 5 males with the first
501 ejaculation latency after vehicle below percentile 25 (189.5 s) after subcutaneous treatment with vehicle, 3,
502 30 and 100 µg/kg of the α_2-adrenoceptor agonist fadolmidine. *, different from vehicle, $p < 0.05$, **, $p <$
503 0.01; Tukey's HSD test. [a], ejaculation latency in s / number of intromissions; [b], the number of intromissions /
504 number of mounts + number of intromissions.

Parameter	Vehicle	3 µg/kg	30 µg/kg	100 µg/kg
Latency to 1st mount (s)	5.00 ± 2.02	6.40 ± 2.01	5.20 ± 1.77	19.20 ± 14.77
Latency to 1st intromission (s)	6.00 ± 1.84	7.80 ± 3.37	23.20 ± 13.07	27.60 ± 22.17
Number of mounts 1st series	10.80 ± 0.58	23.20 ± 6.53	26.00 ± 8.42	16.20 ± 1.93
Number of intromissions 1st series	8.80 ± 0.86	13.60 ± 2.29	12.20 ± 1.43	10.40 ± 1.50
Ejaculation latency 1st series (s)	159.40 ± 14.38	354.00 ± 61.34	258.60 ± 56.86	363.40 ± 74.69
Postejaculatory interval 1st series (s)	359.60 ± 19.28	407.4 ± 35.50	404.20 ± 34.69	485.00 ± 34.08
Interintromission interval 1st series (s)	19.16 ± 3.12	26.74 ± 4.05	21.72 ± 0.4.68	33.03 ± 4.71
Intromission ratio 1st series	0.81 ± 0.05	0.69 ± 0.10	0.59 ± 0.11	0.64 ± 0.06
Number of mounts 2nd series	6.40 ± 0.40	12.80 ± 3.43	9.60 ± 1.80	11.20 ± 1.59
Number of intromissions 2nd series	5.40 ± 0.40	6.06 ± 2.16	5.80 ± 0.73	4.60 ± 0.40
Ejaculation latency 2nd series (s)	92.60 ± 4.46	209.80 ± 66.14	113.00 ± 21.29	200.40 ± 34.64
Postejaculatory interval 2nd series (s)	468.80 ± 29.83	373.33 ± 64.22	445.60 ± 27.16	492.33 ± 21.70
Interintromission interval 2nd series (s)	17.46 ± 1.25	33.89 ± 8.03	20.25 ± 3.55	43.46 ± 5.77**
Intromission ratio 2nd series	0.84 ± 0.01	0.55 ± 0.12	0.66 ± 0.07	0.45 ± 0.07****

Number of mounts in the test	29.80 ± 2.54	47.40 ± 6.53	50.60 ± 8.56	34.20 ± 2.92
Number of intromissions in test	23.60 ± 2.66	26.60 ± 3.68	28.40 ± 4.02	18.80 ± 0.58
Number of ejaculations in test	3.40 ± 0.24	2.80 ± 0.37	3.20 ± 0.20	2.80 ± 0.37
Intromission ratio in the test	0.78 ± 0.03	0.57 ±0.05	0.62 ± 0.11	0.57 ± 0.06

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671

Figure 1

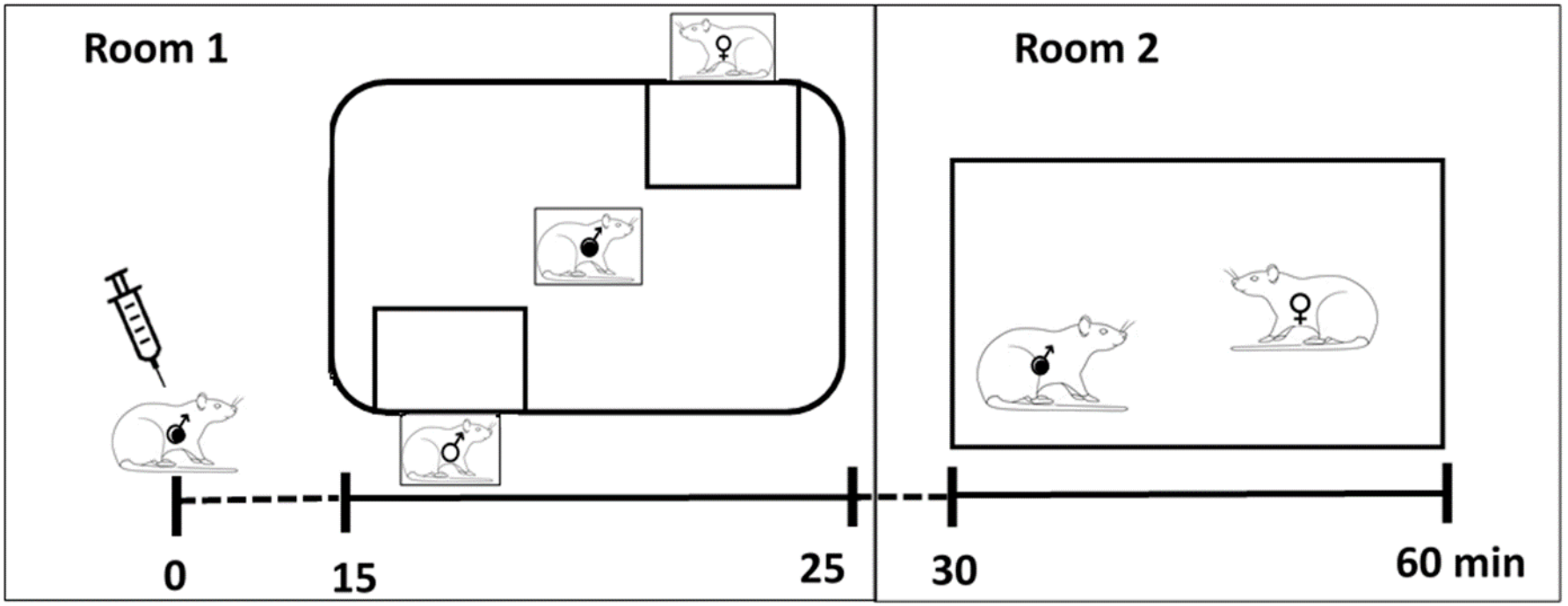
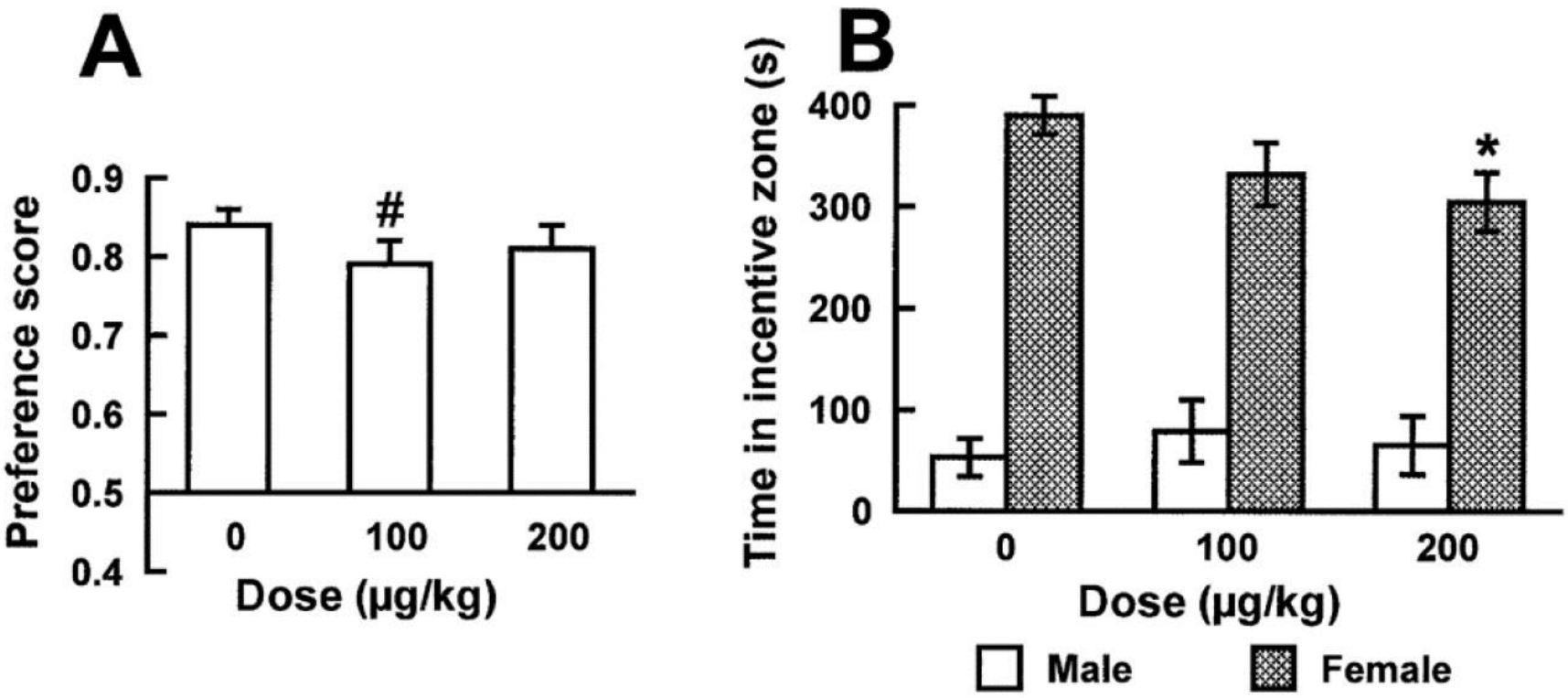


Figure 2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0.7
0.6
0.5
0.4
#
0
100
200
Dose (µg/kg)
B
Time in incentive zone (s)
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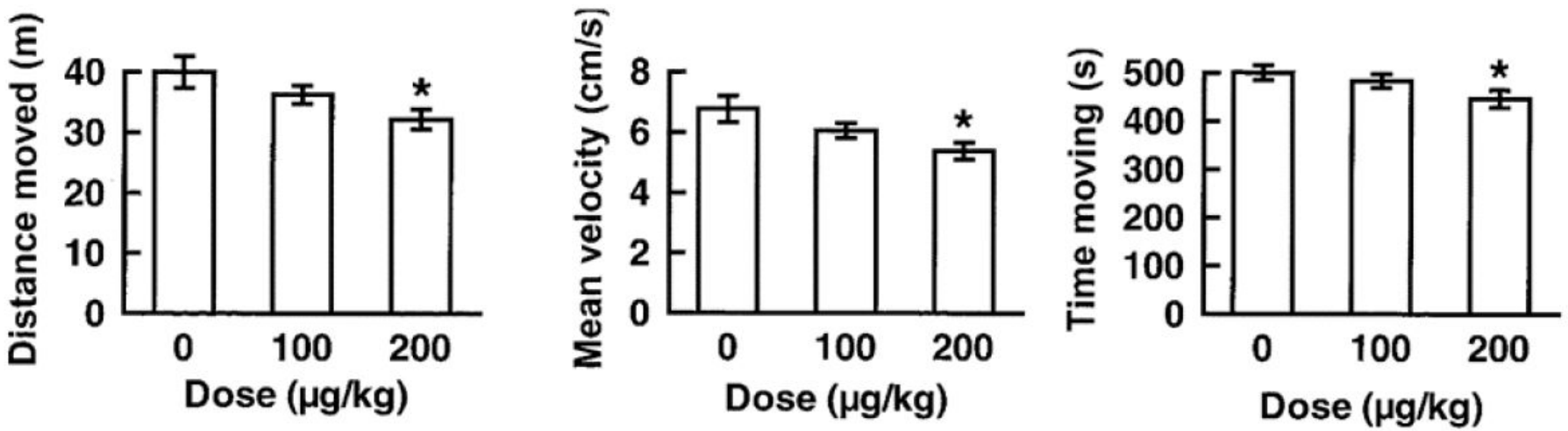
300
200
100
0
*
0
100
200
Dose (µg/kg)
Male
Female

Figure 3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*
0
100
200
Dose (µg/kg)
Mean velocity (cm/s)
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6
4
2
0
*
0
100
200
Dose (µg/kg)
Time moving (s)
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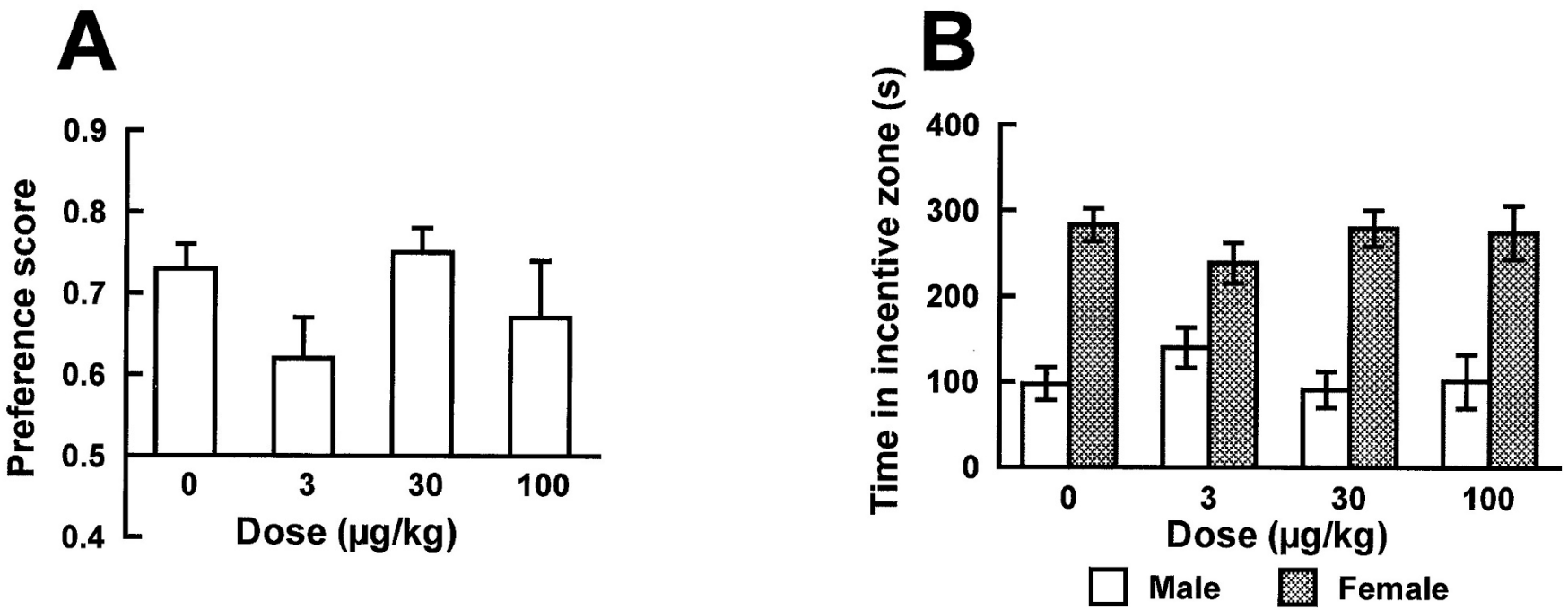
400
300
200
100
0
*
0
100
200
Dose (µg/kg)

Figure 4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Dose (µg/kg)
B
Time in incentive zone (s)
400
300
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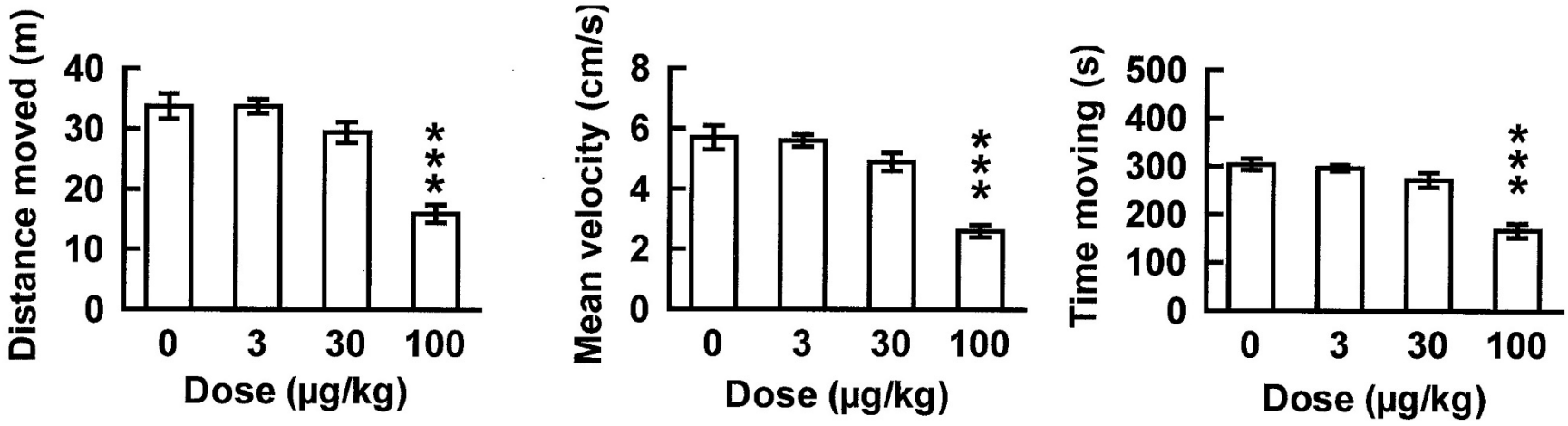
0
3
30
100
Dose (µg/kg)
Male
Female

Figure 5


Distance moved (m)
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Dose (µg/kg)
Mean velocity (cm/s)
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2
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0
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Dose (µg/kg)
Time moving (s)
500
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Dose (µg/kg)