eNeurobiología

Vol. 14 | Núm. 35 | ISSN 2007-3054 | DOI: 10.25009/eb.v14i35.2621

Recibido: 07/08/2023 | Aceptado: 28/08/2023 | Publicado: 29/09/2023

Research article

New study to verify predictive validity criteria in an animal model for evaluating premature ejaculation: the impact of sertraline and fluoxetine

Nuevo estudio para confirmar el criterio de validez predictiva en un modelo animal para evaluar la eyaculación precoz: efectos de la sertralina y la fluoxetina

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Vol. 14 | Núm. 35 | ISSN 2007-3054 | DOI: 10.25009/eb.v14i35.2621
Recibido: 07/08/2023 | Aceptado: 28/08/2023 | Publicado: 29/09/2023

Abstract
Premature ejaculation (PE) is a common sexual dysfunction worldwide, and animal models are used to characterize it. We developed a paradigm called the multiple partner choice arena (MPCA), consisting of 4 acrylic cylinders placed crosswise. Each cylinder contains a sexually experienced male rat, and a receptive female rat resides in the central space, able to choose any cylinder for copulation. We have suggested the MPCA as an animal model for assessing PE be-cause male rats in this paradigm display behavior similar to PE in men, achieving ejaculation in less time. This study aims to demonstrate whether the MPCA satisfies the predictive validity criterion, in which the effects of drugs in the animal model should closely resemble those observed in human medical disorders, such as PE. We investigated the effect of 2 SSRIs (SSRI), sertraline and fluoxetine, on male rats' rapid ejaculation in the MPCA. Two groups of male rats (n=8) were tested: saline, sertraline, and fluoxetine. Weekly doses of 1, 3, and 10 mg/kg/day for sertraline and fluoxetine were administered, while the saline group received only the vehicle. Each test involved four males from the same group, treated with identical drug doses, until achieving the first ejaculation in the MPCA. Fluoxetine and sertraline, at the 10 mg/kg dose, significantly prolonged the ejaculation latency. For this reason, the MPCA satisfies the predictive validity criterion as an animal model of PE.

Keywords: Sertraline, fluoxetine, selective serotonin reuptake inhibitors, premature ejaculation, animal model.

Resumen
La eyaculación precoz (EP) es una disfunción sexual de los hombres muy común en todo el mundo. Nosotros desarrollamos un paradigma llamado arena de selección múltiple de pareja (ASMP), compuesta por 4 cilindros acrílicos colocados en forma de cruz. Cada cilindro contiene una rata macho y una rata hembra, colocada en el espacio central, la cual puede elegir un macho con quien copular. Hemos propuesto la ASMP como un modelo animal para evaluar la EP, ya que en ella las ratas macho eyaculan rápidamente, como ocurre en los hombres con EP. Nuestro objetivo fue determinar si la ASMP cumple con el criterio de validez predictiva, que propone que los fármacos deben tener efectos similares en el modelo animal, a los trastornos que ocurren en el humano, como la EP. Analizamos el efecto de dos inhibidores selectivos de la recaptura de serotonina: sertralina y fluoxetina sobre la eyaculación rápida que presentan las ratas macho en la ASMP. Dos grupos de ratas (n= 8): fueron tratadas semanalmente con dosis semanales y progresivas de 1, 3 y 10 mg/kg/día de sertralina o fluoxetina; el grupo control solo recibió el vehículo (grupo Salina). Cada prueba involucró a 4 machos del mismo grupo, tratados con dosis idénticas del medicamento, hasta que lograron la primera eyaculación en la ASMP. Sólo la dosis de 10 mg/kg de fluoxetina y sertralina incrementó significativamente la latencia de eyaculación, por lo que la ASMP satisfizo el criterio de validez predictiva como un modelo animal de la EP.

Palabras clave: Sertralina, fluoxetina, inhibidor selectivo de la recaptura de serotonina, eyaculación prematura, modelo animal.

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I. Introduction

Premature ejaculation implies the semen emission that may occur immediately after vaginal penetration during coitus, or even before that, in the most severe cases. This condition may occur at any time during the man's sexually active life, and it is the most common sexual dysfunction worldwide. It has a prevalence of 20% in young men and increases to 75% in men over 60.[1,2] The next is some of the physiological evidence of ejaculation that has allowed us to analyze the origin of premature ejaculation.

Ejaculation, as the culmination of sexual behavior, is regulated by a medullary center known as the spinal ejaculation generator. This center coordinates sympathetic, parasympathetic, and motor functions to induce the two phases of ejaculation: emission and expulsion.[3,4] The emission of spermatozoa from the testes and the expulsion of seminal fluid from the seminal vesicles and the prostate is induced by the sympathetic efferent neurons of the thoracolumbar region and parasympathetic efferent neurons of the parasympathetic sacral nucleus.1,3,4 This generating center has descending excitatory and inhibitory regulation from supraspinal sites. They include the median preoptic area, the hypothalamic paraventricular nucleus, and the paragigantocellular Nucleus. The median preoptic area and hypothalamic paraventricular nucleus exert an excitatory influence on ejaculation, while the paragigantocellular nucleus exerts an inhibitory influence on the spinal generator center of ejaculation.[5-8]

In the median preoptic area, which is the regulatory site of male sexual behavior, dopamine facilitates the ejaculatory reflex from D2 receptors. At the same time, in the paragigantocellular nucleus, serotonergic neurons inhibit ejaculation.[5,8-11] When these neurons release serotonin into their synaptic space, this neurotransmitter interacts with the 5-HT2C receptor of the postsynaptic neuron, which causes the inhibition of ejaculation. In contrast, the absence of serotonin facilitates the ejaculatory process.[8,9] Considering these pieces of evidence, Waldinger has proposed that men's premature ejaculation may be caused by the hyposensitivity of the 5-HT2C receptor and /or the hypersensitivity of the 5-HT1A autoreceptor in the paragigantocellular nucleus.[1,8,9]

Different definitions of premature ejaculation disorder have been proposed by specialists varying the duration of intra-vaginal ejaculation latencies. However, there is no consensus on an ideal definition and diagnostic criteria for premature ejaculation.[1,2,7,9,12] All premature ejaculation definitions were based on ejaculation time, the inability to control ejaculation, and the negative impact on the individual and their partner.[1,2,7-9,12] A clinical differentiation has been made between primary or lifelong versus secondary or acquired premature ejaculation. With lifelong premature ejaculation, the patient has experienced premature ejaculation since the beginning of sexual life, and it occurs in the absence of organic illnesses. On the other hand, men with acquired premature ejaculation have experienced normal ejaculations in the past, and this dysfunction usually occurs after an identifiable medical, psychological, or interpersonal cause.[1,2,7,8,10-13] Thus, subjective control of men on their ejaculation during intercourse, the level of satisfaction reached, and anxiety and emotional disturbances after sexual intercourse are claimed as relevant components of this disorder. Long-life or acquired premature ejaculation elicits different emotional, cognitive, and behavioral consequences in men suggesting that neurobiological substrates between the two classifications may differ.[1,2,7,8,10-13]

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The treatment for premature ejaculation currently uses SSRIs, which increase the synaptic levels of this neurotransmitter.[14] It is assumed that the ejaculatory process inhibition is due to their effect on the 5-HT2C receptor.[5-8] Additionally, dapoxetine is the primary SSRIs employed for premature ejaculation treatment but has disadvantages such as cost and potential side effects for some men.[6, 8,14,15] Therefore, it would be beneficial to find alternative medications for men who are sensitive to dapoxetine or lack sufficient economic resources to purchase it.

The rat is one of the most widely used animals for studying some sexual dysfunctions in men since its sexual behavior is easily identifiable by stereotyped patterns and parameters.[16-18] Usually, the sexual behavior of rats is evaluated in a standard arena, which consists of a rectangular closed box or a cylinder made of transparent acrylic, where a female and a male mate, are observed to quantify the patterns and parameters of their sexual behavior.[16,19]

Recently, we developed a device of four acrylic cylinders assembled in a closed circle, called the multiple partner choice arena (MPCA) to evaluate the sexual behavior of male rats. In this device, a receptive female, placed in the central compartment at the beginning of the test, can move freely through small holes in the base of each cylinder, allowing her to choose any male to copulate with him.[20]

In the MPCA, male rats behave as rapid ejaculators since their ejaculation latency is significantly reduced, compared to the same males tested in a standard arena.[21] Similarly, the number of intromission preceding the ejaculation is significantly reduced in the MPCA, compared to that obtained by the same male rats tested in a standard arena.[21] Due to this behavior of male rats, it has been proposed that MPCA could be more useful to study premature ejaculation dysfunction, than the standard arena.[22,23] It's interesting to note that the two parameters of the male sexual behavior, which are involved in the ejaculatory threshold of the rat, namely ejaculation latency and number of intromissions preceding ejaculation,[24] are altered by the testing conditions of the MPCA.[22,23]

According to the criteria established for an animal model for studying neurobehavioral disorders, it must meet at least one of the following criteria: face validity, construct validity, and predictive validity.[25] The more criteria the model fulfills, the more useful and powerful it becomes. In previous studies, we demonstrated that the MPCA satisfies these three criteria, including the predictive validity,[22,23] which assesses the ability of the animal model to respond to treatments used to simulate the modeled phenomenon.[25] In this regard, we were able to demonstrate that the MPCA satisfies this criterion because the SSRI dapoxetine, which is the most used drug in the treatment of premature ejaculation in men, was able to significantly prolong the rapid ejaculation exhibited by male rats in this arena.[14,23]

To further validate the predictive validity criterion and strengthen the power of our model, we decided to analyze whether other SSRIs, such as sertraline and fluoxetine, are capable of delaying rapid ejaculation in male rats when evaluated in the MPCA, as they do in men with premature ejaculation.[6,8,10] These SSRIs have been shown to increase the intravaginal ejaculatory latency time in men with premature ejaculation.[6,14,26] Therefore, the objective of our study was to confirm that the MPCA satisfies the criterion of predictive validity by assessing whether sertraline and fluoxetine can significantly delay rapid ejaculation in male rats when evaluated in the MPCA.

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2. Materials and methods

2.1 Animals

Fifty male rats (weight: 350 g) and 15 females (weight: 250 g) of the Wistar strain, were used from the UAM-Xochimilco's Vivarium. The animals were housed in polysulfone cages (43 x 53 x 20 cm), in groups of 5 rats per cage. They were kept in a room with positive pressure, and regular conditions of humidity (45 to 55%) and temperature (21°C), under inverted light/dark photoperiod of 12/12 h (lights on at 4 pm). Intake water was purified by ozone and UV light, and Purina Mills® 5001 food was supplied ad libitum.

The 15 females used as stimulus rats were bilaterally ovariectomized under isoflurane anesthesia (2.5-5%, inhalation) via a single back incision. They were anesthetized with 4-5% isoflurane® (Pisa), inhaled with a VetEquip IMPAC 6 device, and an oxygen/CO2 flow of 0.5-1.0 L/min. During surgery, eye lubricant was applied to prevent retinal blindness. Tramadol (Psicofarma) 5 mg/Kg subcutaneously was injected as an analgesic. Rats were allowed to recover for at least 15 days before any behavioral testing. After this period, the stimulus females were brought into estrous on the day of the behavioral test, administering subcutaneously, 10 µg of estradiol benzoate (Sigma-Aldrich), and 0.5 mg of progesterone (Sigma-Aldrich), 48 h and 4 h, respectively, before the test.

All the procedures were carried out in the unit for the production and experimentation of laboratory animals-vivarium, of the UAM-Xochimilco; the procedures were approved by the Internal Committee for the Use of Laboratory Animals of the UAM-X (CICUAL), by the organization and procedures manual, and with the official mexican standard for the use and care of animals (NOM-062-ZOO-1999).

2.2. Selection of the sexually experienced male rats

Four 15 min tests of male sexual behavior were performed, one each week, in a standard arena (a closed acrylic cylinder, of 50 cm diameter x 40 cm height). It has been shown that with these tests, males acquire enough sexual experience to maintain a stable ejaculatory frequency in subsequent sexual behavior tests, which reduces the variations that usually occur in this parameter.[27] Twenty-four sexually expert male rats were selected, presenting at least two ejaculations at the fourth test, which lasted 15 min. The 26 male rats discarded were used for educational purposes.

2.3. Habituation of the males and females to the MPCA

Once the 24 sexually expert males were selected, they were habituated to the MPCA. Four males were placed inside the cylinders of the MPCA (one per cylinder), and a sexually receptive female, hormonally treated as mentioned before, was placed in the central chamber of the arena, during a 15-minute habituation test. At the end of the test, these four males were replaced with a new group of four males and a new receptive female. This procedure was carried out until the 24 sexually expert males were submitted to the habituation test. During four consecutive weeks, these males were habituated in this form.

2.4. Drugs

Sertraline (AMSA) and fluoxetine (Medimart), at doses of 1, 3, and 10 mg/kg, dissolved in 2 ml of saline solution, were orally administered in a single dose using an 8 cm stainless steel esophageal cannula, 60 minutes before each test. Male rats in the sertraline and fluoxetine groups (n = 8) received one of the following doses weekly: 1, 3, or 10 mg/kg for three consecutive weeks, with the dose progressively increasing. To

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compare the effect of sertraline and fluoxetine on the sexual behavior of male rats obtained in the MPCA arena with those obtained in a standard arena, as Mos *et al.*[28] reported, we employed the same doses and treatment regime they used. Rats in the control group received only the vehicle (2 ml of saline solution; saline group, n = 8), and they were evaluated on the same days as the experimental groups. The four tests of the saline group were done to check if the rats had already acquired sufficient sexual experience.

2.5. Testing male sexual behavior in the multiple partner choice arena

The MPCA consisted of 4 transparent acrylic cylinders (50 x 40 cm), placed crosswise and joined by their walls; each cylinder had a 3 x 5 cm opening at its base, through which only the female rat could pass due to its smaller size. The entries of the cylinders were oriented towards the central compartment, where the experimental female was placed, so that she could choose, a male to copulate with (Figure 1).

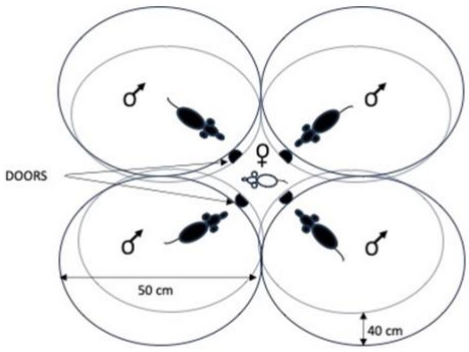

Figure 1. Top view of the multiple partner choice arena (MPCA) constructed with four acrylic cylinders arranged in a closed circle.

In every test, four male rats, from the same group of drugs and doses, were placed individually in the cylinders. When a sexually receptive female was introduced into the central compartment of the MPCA, the behavioral test started until each male reached the first ejaculation, and the following sexual parameters were registered: intromission latency, ejaculation latency (EL), number of mounts (NM), number of intromissions (NI), inter-intromission interval (III=EL/NI), inter-copulatory interval (ICI=LE/NM+NI), detailed in Olayo-Lortia et al.[22]

The main parameter used to assess predictive validity criteria was the ejaculation latency of the male rat, as this parameter is equivalent to the intravaginal ejaculation latency time recorded in men with premature ejaculation.[6,29] The second

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parameter was the number of intromissions preceding ejaculation since, in male rats, the ejaculatory threshold depends on both the ejaculation latency and the number of intromissions leading up to ejaculation.[24] It is believed that a male rat with high sexual motivation will require fewer intromissions and less time to ejaculate.[27]

During the behavioral test, each male rat had a maximum of 300 seconds, the average time a sexually expert male needs to achieve the first ejaculation.[29] The total time the female spent with each male during the test was calculated by adding the partial time the femalespent in each visit with a particular male. Every time a male ejaculated the first time, the mating test ended for this male, and the entry to his cylinder was blocked as soon as the female left it. In this situation, she could only interact with the males that had yet to ejaculate. This procedure was repeated until all the males ejaculated.

All behavioral tests for habituation, male selection, and mating tests, were conducted during the dark phase of the photoperiod cycle (from 4 am to 4 pm) under dim red light.

3. Statistical analysis

The mean ± standard error of the mean (S.E.M.) of the different parameters of the sexual behavior for each of the treatment groups, were statistically compared using a Kruskal-Wallis ANOVA test followed by the post-hoc Dunn's multiple comparisons tests. All statistical analyses were performed at a significance level of $p < 0.01$ and $p < 0.05$, with the Number Cruncher Statistical Systems (NCSS, 2020) software. The non-parametric Kruskal-Wallis ANOVA test was applied because the data for most of the three groups for each sexual parameter did not exhibit a normal distribution when tested for normality using the NCSS program.

4. Results

Table 1 and Figure 2 show the mean ± S.E.M. of the male rat sexual behavior parameters, obtained by saline, sertraline, and fluoxetine groups, and by the different doses (1, 3, and 10 mg/kg). Since, in most of the parameters, the averages of the fluoxetine group at the 10 mg/kg dose were significantly different from those of the saline group they were plotted in Figure 2 for better visualization. In each group, one male was excluded due to notably different sexual behavior, characterized by either longer ejaculation latencies or excessive mounts. As a result, each group consisted of seven male rats, and the three excluded males were euthanized.

The sertraline group increased intromission latency with all three doses compared to the saline group, but only the 10 mg/kg dose showed a significant increase in this sexual parameter (saline: 6.9 ± 0.86 s and sertraline: 17.6 ± 4.52 s; $p < 0.05$, Dunn's post-hoc analysis). The fluoxetine group, when compared to the saline group, also increased intromission latency with all three doses, although these increases were not statistically significant (Table 1 and Figure 2).

In the case of the inter-intromission interval, a decrease was observed at a dose of 1 mg/kg in both the sertraline and fluoxetine groups compared to the saline group, but it was not statistically significant. However, in the 3 and 10 mg/kg doses, an increase was observed in both the sertraline and fluoxetine groups compared to the saline group. Nevertheless, these differences were only statistically significant with the 10 mg/kg dose of fluoxetine (saline: 7.4 ± 0.84 s and fluoxetine: 17.1 ± 2.63 s, $p < 0.05$; Dunn's post-hoc analysis, Figure 2).

As occurred with the inter-intromission interval, when comparing the inter-copulatory interval time of the saline group, both sertraline and fluoxetine groups initially showed a decrease in its duration at

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a dose of 1 mg/kg, but this change was not statistically significant. However, there was an increase with higher doses (3 and 10 mg/kg), and these differences were statistically significant only with the 10 mg/kg dose of fluoxetine (saline: 5.43 ± 2.15 s; fluoxetine: 11.4 ± 1.78 s, $p < 0.05$; Dunn's post-hoc analysis, Figure 2).

Compared to the saline group at the 1 mg/kg dose of sertraline, ejaculation latency decreased and increased with fluoxetine, but these changes were not statistically significant. With the 3 mg/kg dose, both SSRI drugs increased ejaculation latency, compared to the means of the saline group, but once again, these changes were not statistically significant. When comparing the means obtained with the 10 mg/kg dose, significant differences were observed between the saline and sertraline groups (46 ± 6.33 s and 124.7 ± 27.37 s, respectively; $p < 0.05$; Dunn's post-hoc analysis) and between the saline and fluoxetine groups (46 ± 6.33 s and 199.6 ± 38.4 s, respectively, $p < 0.05$; Dunn's post-hoc analysis, Figure 2).

Sexual Parameters	Dose		
	Groups	1 mg / kg	3 mg / kg
Intromission Latency (seconds)	Saline	15.3 ± 3.36	14.0 ± 4.42
	Sertraline	15.7 ± 6.06	30.3 ± 9.03
	Fluoxetine	29.4 ± 9.65	17.3 ± 5.99
	Kruskall-Wallis Probability	H (2) = 1.763 p = 0.207	H (2) = 2.837 p = 0.121
Inter-Intromission Latency (seconds)	Saline	24.1 ± 6.01	13.3 ± 4.77
	Sertraline	11.6 ± 2.11	19.5 ± 4.00
	Fluoxetine	16.0 ± 4.53	14.2 ± 2.38
	Kruskall-Wallis Probability	H (2) = 2.245 p = 0.155	H (2) = 2.545 p = 0.140
Inter-Copulatory Latency (seconds)	Saline	12.1 ± 3.5	5.7 ± 1.18
	Sertraline	6.1 ± 1.1	8.6 ± 2.34
	Fluoxetine	8.4 ± 2.12	9.5 ± 1.38
	Kruskall-Wallis Probability	H (2) = 2.427 p = 0.149	H (2) = 3.614 p = 0.082
Ejaculation Latency (seconds)	Saline	135 ± 34.8	57.7 ± 13.26
	Sertraline	86 ± 29.4	183.9 ± 54.88
	Fluoxetine	148 ± 57.2	154.7 ± 54.64

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	Kruskall-Wallis Probability	H (2) = 1.075 p = 0.292	H (2) = 4.316 p = 0.058
Number of Intromissions	Saline	6.1 ± 1.22	5.6 ± 1.34
	Sertraline	8.0 ± 2.35	9.7 ± 2.57
	Fluoxetine	6.9 ± 1.59	9.1 ± 1.92
	Kruskall-Wallis Probability	H (2) = 0.126 p = 0.469	H (2) = 2.489 p = 0.144

Table 1. Mean ± S.E.M. of the following parameters of male sexual behavior: intromission latency, inter-intromission interval, inter-copulatory interval, ejaculation latency, and number of intromissions, obtained from 3 groups of male rats (n = 7), after receiving a weekly administration of 1 or 3 mg/kg of sertraline or fluoxetine in each treatment, or just the vehicle (saline). The parameters of the sexual behavior for each of the treatment groups, were statistically compared using a Kruskal-Wallis ANOVA test followed by the post-hoc Dunn's multiple comparisons test, but no significant differences were found.

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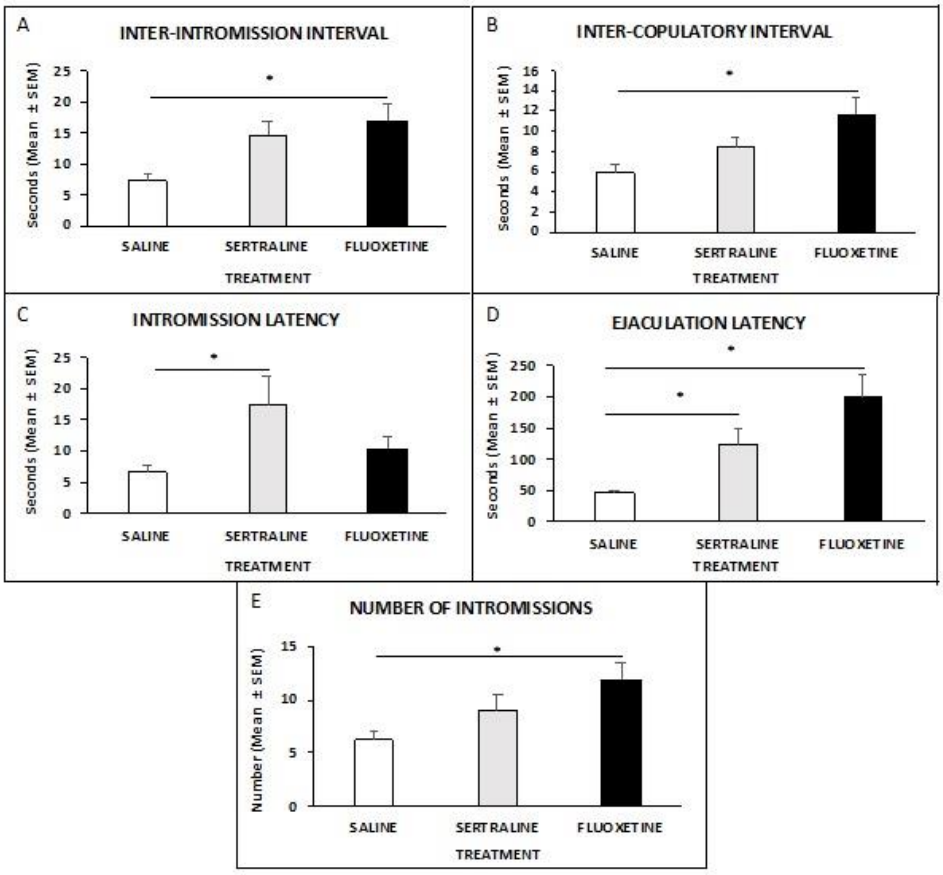

Figure 2. Mean ± S.E.M. of male sexual behavior parameters obtained from 3 groups of male rats (n = 7), after receiving the administration of 10 mg/kg of sertraline or 10 mg/kg of fluoxetine, or just the vehicle (saline solution). (A) inter-intromission interval: H (2) = 8.53, $p < 0.014$; (B) inter-copulatory interval: H (2) = 7.88, $p < 0.019$; (C) intromission latency: H (2) = 7.13, $p < 0.028$; (D) ejaculation latency: H (2) = 14.43, $p < 0.001$ and (E) number of intromissions: H (2) = 6.68, $p < 0.035$. * $p < 0.05$ and ** $p < 0.01$, after applying a Kruskal-Wallis ANOVA test followed by the post hoc Dunn's multiple comparisons test.

5. Discussion

The standard arena has commonly been used as an animal model to evaluate drugs that could be useful for treating premature ejaculation.[29] However, in this arena, male rats do not spontaneously behave as rapid ejaculators. Instead, in this model, a drug is considered to have potential for use in the treatment of premature ejaculation when it increases the ejaculatory threshold in the male rat, either by prolonging its ejaculation latency or increasing the number of intromissions preceding ejaculation, or both.[29] Hypothetically, the MPCA should meet the predictive validity criterion as an animal model for assessing premature ejaculation if

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drugs commonly used in the treatment of premature ejaculation in men are capable of significantly delaying rapid ejaculation in male rats.

Based on our results, both SSRIs, fluoxetine, and sertraline, at a dosage of 10 mg/kg, caused a statistically significant increase in ejaculation latency ($p < 0.05$) in the male rats tested in the MPCA. However, at this dosage, fluoxetine displayed higher efficacy than sertraline because it also produced a signi-ficant increase in other sexual parameters, including the number of intromissions preceding ejaculation, the inter-intromission interval, and the inter-copulatory interval, all of which were not observed with sertraline. On the other hand, the only sexual parameter in which sertraline showed a significant increase ($p < 0.05$) with the highest dose was intromission latency (Figure 2).

In men with premature ejaculation, it has been demonstrated that both SSRIs, fluoxetine, and sertraline, significantly increase intravaginal ejaculation latency time,[11,12] by reducing serotonin reuptake via the blockade of 5-HT transporters and for this reason, they are used to treat PE.[6] However, sertraline prolongs intravaginal ejaculation latency time even more than fluoxetine.[26] This contrasts with our findings in male rats under MPCA conditions, where fluoxetine proved more effective than sertraline in prolonging rapid ejaculation of male rats at the higher dose. Moreover, our results differ from those obtained in the study by Mos et al.[28] conducted in a standard arena. They only observed a significant increase in ejaculation latency with a 3 mg/kg dose of sertraline, while a significant reduction in this parameter was registered with the same dose of fluoxetine. Furthermore, at a dose of 10 mg/kg, they did not find significant differences in this sexual parameter. These discrepancies suggest that different outcomes may arise when testing the ejaculatory threshold in male rats using the same SSRIs in the MPCA versus the standard arena. Consequently, our findings suggest that MPCA conditions may be more sensitive in detecting changes in male sexual behavior in rats that were not observed in the standard arena.

It is possible that the rapid ejaculation observed in male rats in the MPCA may be attributed to the fact that in this arena, the female has the option to move away from the male and exit his compartment at any time, a behavior that doesn't occur in the standard arena. Therefore, it appears plausible that under MPCA conditions, males may be motivated to ejaculate quickly to prevent the females from leaving their space. In this regard, the rapid ejaculation displayed by male rats in MPCA conditions resembles acquired premature ejaculation in men, which is often influenced by the presence of anxiety or stressful factors during sexual intercourse.[1-3,14]

Another important parameter of male sexual behavior to analyze is the number of intromissions preceding ejaculation, as it plays a role in the rat's ejaculatory threshold.[24] Our results demonstrated that fluoxetine caused a significant increase ($p < 0.05$) in the number of intromissions at a dose of 10 mg/kg, compared to saline, which was not observed in the sertraline group at the same dose. Additionally, our findings in the MPCA setting differed from those reported by Mos *et al.*,[28] where none of the two doses used (3, and 10 mg/kg) showed significant diffe-rences between the control group (0 mg/kg) and fluoxetine group. However, the sertraline group at a dose of 3 mg/kg did show a significant increase in this parameter in the Mos *et al.* study.[28] We propose that the higher number of intromissions observed in male rats at the higher dose of fluoxetine under the MPCA testing conditions is a consequence of the increase in ejaculation latency. This

Vol. 14 | Núm. 35 | ISSN 2007-3054 | DOI: 10.25009/eb.v14i35.2621
Recibido: 07/08/2023 | Aceptado: 28/08/2023 | Publicado: 29/09/2023

extended time to ejaculate provides them with more opportunities to perform additional intromissions.

On the other hand, the fact that fluoxetine, unlike sertraline, significantly increased the number of intromissions, inter-intromission, and inter-copulatory intervals at the higher dose could be attributed to fluoxetine's ability to increase extracellular levels of other neurotransmitters in various brain regions, in addition to serotonin levels. For example, acute fluoxetine administration has been shown to raise not only extracellular serotonin levels but also norepinephrine levels in the median preoptic area of the rat hypothalamus,30 which is the brain region involved in regulating male rat sexual behavior.[5,24] Furthermore, Clark et al.31 reported that the administration of methoxamine, an α1-adrenergic receptor agonist, to male rats resulted in reduced penile erection, leading to an increase in the number of intromissions, inter-copulatory interval, inter-intromission interval, and ejaculation latency, similar to what we demonstrated in this study with fluoxetine at a dose of 10 mg/kg (Figure 2). Moreover, at the peripheral level, Seo *et al.*,[32] have also found that fluoxetine can delay ejaculation in male rats by preventing the contraction of the vas deferens induced by adrenaline. So far, there is no evidence that sertraline has the same effects as fluoxetine on ejaculation. All these findings suggest that fluoxetine, in addition to increasing extracellular serotonin levels, exerts various effects at both central and peripheral levels that may negatively influence the ejaculatory capacity of male rats.

6. Conclusions

The MPCA was able to satisfy the predictive validity criterion since both SSRIs, fluoxetine and sertraline, at the highest dose, were able to significantly increase the rapid ejaculation exhibited by male rats in this arena, just as they do in men with premature ejaculation. However, in comparison to the effect of sertraline, fluoxetine was able to prolong the ejaculatory threshold further because it was also capable of significantly increasing the number of intromissions preceding ejaculation, the inter-intromission interval, and the inter-copulatory interval in male rats under the conditions of the MPCA. These results suggest that the conditions of this arena likely make other peripheral effects of fluoxetine more apparent than those of sertraline.

Together with dapoxetine, there are now three SSRIs that have been able to prolong the rapid ejaculation exhibited by male rats when their sexual behavior is evaluated in the MPCA. This supports the idea that this arena may be a useful animal model for testing drugs that could potentially be used to treat premature ejaculation in men.

7. Declaration of conflicts of interest

The authors declare that there is no conflict of interest of any kind.

8. Financing

This article was funded by the Division of Biological and Health Sciences of the Iztapalapa and Xochimilco Units, as well as by the unit of production and experimentation of laboratory animals - Xochimilco Unit, Mexico City.

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Recibido: 07/08/2023 | Aceptado: 28/08/2023 | Publicado: 29/09/2023

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