Escitalopram in combination with drug X inhibits sexual behavior after an aggressive encounter

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Abstract

The most common male sexual disorder is premature ejaculation (PE). PE lacks an efficient on-demand treatment. At the moment, topical anesthetic agents and SSRIs are the most used to treat PE, but they lack efficiency and bear several side-effects. It has been recently shown that the addition of the 5-HT1a-receptor antagonist drug X to a SSRI acutely inhibits sexual behavior in Wistar male rats. In order to confirm the potential of drug X in combination with a different SSRI to be a treatment for PE, we tested the combination of escitalopram with drug X in a dose-dependent manner. Groningen wild-type rats were sexually trained for 6 weeks and underwent the resident-intruder test. Hereafter, the drug combinations were tested in a dose-dependent manner for aggressive and sexual behavior. The results showed that escitalopram together with either 20 or 10 mg/kg drug X inhibits sexual behavior after an aggressive encounter in comparison to the basal sexual behavior of the rats. Furthermore, the resident-intruder test seems to have an impact on sexual behavior, since the rats of all drug combinations showed less sexual behavior in comparison to the basal sexual behavior. This could perhaps be caused by the perceived stress of the aggressive encounter or sexual satiety. Although our results were impacted by the aggressive encounter, we can conclude that escitalopram together with drug X seems to inhibit sexual behavior. Therefore, this implies that this combination perhaps could work as a on-demand treatment for PE. In order to confirm this, the combination should be tested on sexual behavior without the aggressive encounter.

Introduction

Premature ejaculation (PE) is considered to be the most common male sexual disorder and affects up to 30% of the male population (Crowdis et al., 2023). PE is characterized by persistent early ejaculations, i.e. ejaculations that occur within 1 minute during vaginal penetration and before the individual wishes to ejaculate. Furthermore, PE can cause significant depression and distress for patients (Crowdis et al., 2023; Waldinger, 2016). In order to treat PE, multiple treatments have been developed, however, the current existing treatments have several disadvantages (Crowdis et al., 2023). An example of treatments is psychological treatments, which seem promising, but are not on-demand. This type of treatment requires time in order for the patient to undergo therapy and develop necessary skills and changes in way of thinking (Althof, 2016). One way of elevating the success rate for treating PE is to add a pharmacological treatment (Crowdis et al., 2023). However, current pharmacological treatments have several disadvantages and side-effects (Olivier et al., 2023). An example of on-demand

pharmacological treatments is topical anesthetic agents, which seem effective in reducing premature ejaculations. Nevertheless, they bear undesirable side effects, including erection loss and numbness in both the penile shaft and the vagina (Shah et al., 2023). Next, there are also multiple drugs used as treatment of which the biggest group belongs to selective serotonin reuptake inhibitors (SSRIs) (Crowdis et al., 2023; Olivier et al., 2023). An increase in serotonin neurotransmission is linked to the inhibition of sexual behavior and can delay the occurrence of an ejaculation (Olivier et al., 2006). Therefore, SSRIs are used to treat PE, because they can increase serotonin transmission via the blockade of the serotonergic transporter. The increase of serotonin only occurs after chronic administration of SSRIs (Bijlsma et al., 2014; Esquivel-Franco et al., 2018; Olivier et al., 2006). Thus in order to treat PE effectively, SSRIs need to be administered daily (Crowding et al., 2023). However, several side effects of chronic SSRI treatment, like gastrointestinal disturbances, decreased libido, anorgasmia, weight gain and disturbed sleep, result in discontinuous usage of the SSRIs, which decreases their efficacy as a treatment for PE (Ferguson, 2001). Therefore, the on-demand SSRI dapoxetine was developed as a treatment for PE, but the discontinuation rate was high, due to costs, side effects and not being able to cure PE (Porst & Burri, 2019). The flaws of the current treatments make clear that there is a need for an efficient and safe on-demand treatment.

In order to show that PE can be considered to be abnormal, a survey was conducted by Waldinger et al. (2009) about the biological variation in ejaculation latency in human males. The survey showed that PE indeed was outside the normal range and furthermore, the survey showed that there is a biological variation in ejaculation latency among human males. The same biological variation and distribution curve in humans also exist in a stable manner in sex-trained male rats (Pattij et al., 2005). Therefore, possible treatments for PE can be tested in sex-trained male rats (Bijlsma et al., 2014; Pattij et al., 2005). Sex training, consisting of multiple 30-minute sex sessions, is needed to establish stable endophenotypes. The endophenotypes can be subdivided into ‘sluggish’ (0-1 ejaculations), ‘normal’ (1-3 ejaculations) and ‘rapid’ (4-5 ejaculations) ejaculators (Olivier et al., 2006). The rapid ejaculators can be used as an animal model for humans suffering from PE (Bijlsma et al., 2014; Pattij et al., 2005).

This animal model for PE has been used to examine the relation between serotonin and sexual behavior, and to examine treatments for PE (Bijlsma et al., 2014; Esquivel-Franco et al., 2018; Olivier et al., 2023). The sexual side effects of SSRIs can be both found in men and rats, and include increased ejaculation latency, less ejaculations and erectile dysfunction (Bijlsma et al., 2014; Esquivel-Franco et al., 2018; Higgins et al., 2010). Animal models have helped to determine possible mechanisms via which serotonin affects sexual behavior. Several serotonin receptors are thought to be involved in the sexual effects of SSRIs, like 5-HT1a, 5-HT1b and 5-HT2c (Bijlsma et al., 2014). Whereas 5-HT1b and 5-HT2c receptors inhibit sexual behavior as shown by their respective agonists (Olivier & Olivier, 2022; Popova & Amstislavskaya, 2002), the 5-HT1a receptor has pro-sexual behavioral effects as shown by its agonist 8-OH-DPAT (Olivier & Olivier, 2022). Administration of 8-OH-DPAT increases the amount of ejaculations and decreases ejaculation latency, amount of mounts and amount of intromissions in rats (Olivier & Olivier, 2022). In contrast, the antagonist of 5-HT1a receptor WAY100,635 on itself has no effect on sexual behavior in rats (Esquivel-Franco et al., 2018). However, research on a faster

onset of SSRIs in depressive patients revealed that although the 5-HT1a antagonist DU125530 does not accelerate the onset of antidepressant effects of SSRIs (Scorza et al., 2012), it does hold potential to be an on-demand treatment for PE due to its effect on ejaculation latency (Olivier et al., 2023). Namely, a treatment with a 5-HT1a antagonist together with an SSRI delayed ejaculation in Wistar rats (Ahlenius & Larsson, 1999; Olivier et al., 2023). The opposing effects on ejaculation latency of the agonists and of antagonists of 5-HT1a receptor in combination with a SSRI show that the 5-HT1a receptor could be a target for treating PE. This is further illustrated by the research of Olivier et al. (2023). They used rapid ejaculators in order to see the effect of the 5-HT1a antagonist DU125530 together with the SSRI paroxetine, which combination they called Enduro. The results showed that the highest used concentrations of Enduro resulted in higher mount, intromission and ejaculation latencies, a higher amount of mounts, a lower amount of ejaculations and a lower intromission ratio, but not an altered amount of intromissions when administered orally (Olivier et al., 2023). In order to confirm whether a 5-HT1a antagonist together with an SSRI holds potential to be an on-demand treatment for PE, we wanted to test a different combination than tested by Olivier et al. (2023). Research has shown that different SSRIs can have different efficiencies on delaying ejaculations (Olivier et al., 2006). Therefore, we wanted to test the combination of escitalopram together with the 5-HT1a antagonist drug X on sexual behavior. It was namely shown that when citalopram, of which the active compound escitalopram is, was co-administered with 5-HT1a antagonist WAY-100635, this strongly delayed ejaculations (Olivier et al., 2006). We were given the opportunity to test the combination of escitalopram and drug X, because it was also used to test its effect on aggressive behavior. Therefore, right after testing the aggressive behavior, we looked at the sexual behavior of the male rats that were given the treatment. We tested right after aggressive behavior, because this could confirm that the combination can work as a on-demand treatment for PE. We hypothesized that similar results would be found as in the paper of Olivier et al. (2023) in a dose-dependent manner. Therefore, the expectation is that the highest dosage of drug X will result in the highest inhibition of sexual behavior.

2. Materials and Methods

2.1 Animals

All male and female rats were wild type Groningen rats. All rats had ad libitum access to food and water, and received wooden gnawing blocks and plastic tubes for cage enrichment. Outside of experimental procedures, the rats were housed in groups of 3 per cage and at a reversed 12h/12h light dark rhythm (lights were off at 9:00 a.m. and on at 9:00 p.m.). All experiments were conducted according to the law on Animal Experiments of the Netherlands and were approved by the Institutional Animal Care and Use committee of the University of Groningen.

The groups used for the experiments are visualized in figure 1. 24 male rats were used as subjects for all experiments. For the resident-intruder test, all 24 male rats were used as intruders. Sexual behavior was tested by exposing 24 male rats to an ovariectomized female rat. All rats were born in the animal facility from the University of Groningen, where the

experiments were also conducted. After basal sexual testing and aggression testing, different combinations of drugs were tested. Therefore, 1 hour before the start of the resident-intruder test, the male rats were injected intraperitoneal with a combination of drugs. The combinations of drugs consisted of 0 or 5 mg/kg escitalopram with 0, 5 or 10 mg/kg drug X. After ending of the resident-intruder tests, all intruders were removed and were taken care of. Hereafter, the female rats were placed back in the cage and sexual behavior was recorded.

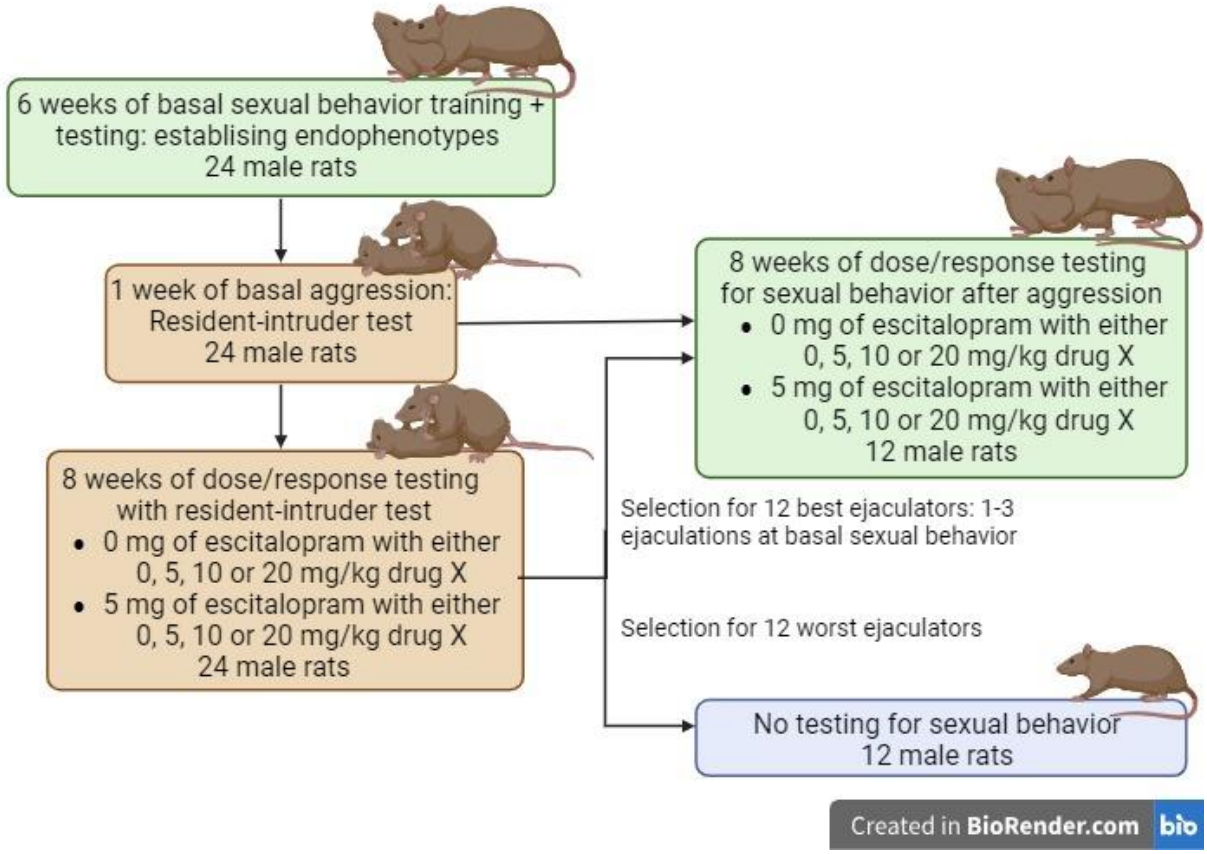

Figure 1: An overview of all groups used in the experiments. The drug testing of the aggressive and sexual behavior is done in the same 8 weeks.

2.2 Drugs

For the drug testing, the SSRI escitalopram and 5HT1a antagonist drug X were used. A combination of escitalopram and the 5HT1a receptor antagonist drug X were administered intraperitoneal 1 hour before the intruder-resident test. An overview of the concentrations of the drugs is stated in table 1. Drug X was suspended in saline in order to be able to administer drug X intraperitoneal. Drug X was synthesized by Syncom, Groningen, Netherlands. Saline was used as the vehicle treatment of 0 mg/kg drug X. Escitalopram was dissolved in saline. Saline was also used as vehicle treatment of 0 mg escitalopram.
Each male rat received every combination of drugs in a random manner over the 8 weeks of the experiment.

Table 1: the concentrations of escitalopram and drug X used in the dose-response tests.

Concentration escitalopram (mg)	Concentration drug X (mg/kg)
0	0
5	0
0	5
5	5
0	10
5	10
0	20
5	20

2.3 Tubal ligation and estradiol administration in female rats

Female rats received tubal ligation as previously described in the paper of Olivier et al. (2023). For the sexual behavioral sessions, the female rats received 0.1 mL estradiol (50 µg in 0.1 mL walnut oil) 36 hours prior to the sessions. Estradiol was administered a maximum of 1 time per every 2 weeks. The estrus females were used a maximum of 2 times per experimental day once every 2 weeks.

2.4 Behavioral observations

Both the sexual behavior and aggressive behavior tests were assessed in a lighted room between 9:00 a.m. and 4:00 p.m.. The training and testing took place in iron rectangular boxes (57 cm × 82 cm × 39 cm) with a plexiglass wall in the front to have non-paced mating. Non-paced mating takes place in a closed box, therefore the female cannot escape sexual interactions. This allows the male to control the rate of sexual interactions with the female (Ventura-Aquino & Paredes, 2023). The boxes contained wooden chips and bedding material during the basal sexual behavior testing. During the basal aggression behavior testing and drug testing, the boxes also contained wooden gnawing blocks and allowed access to food and water. For the basal sexual behavior training and testing, the session started with 10 minutes of habituation of the male rat. After this, the estrus female rat was placed in the cage for 30 minutes. During these 30 minutes, the sexual behavior of the male was recorded and scored. The male rats underwent training in order to establish their own endophenotype (Olivier et al., 2023). Endophenotypes are the stable sexual phenotypes of rats, which establish after at least four successive weeks of the 30-minute sex session. The endophenotypes were subdivided into ‘sluggish’ (0-1 ejaculations), ‘normal’ (1-3 ejaculations) and ‘rapid’ (4-5 ejaculations) ejaculators (Olivier et al., 2006). The male rats were trained in 6 weeks to establish their endophenotype.

Each week 1 training session per animal was conducted. The amount of ejaculations during the sessions from the fourth till the sixth week were used to determine the best and worst ejaculators. The 12 best ejaculators are later used for the drug testing on sexual behavior. After the basal sexual behavior testing, the basal aggression behavior was tested with the resident-intruder test (Koolhaas et al., 2013). The resident male was put in a cage together with a female 1 week prior to the test. The female was removed from the cage 1 hour prior to the test and then the intruder male was added. The aggressive behavior was trained 3 times by allowing the resident male to perform a clinch attack on the intruder male. Hereafter, the intruder male was removed from the cage. This training happened in 3 successive days. On the fourth day, the aggressive behavior of the resident male was tested by either scoring for 10 minutes or 10 minutes after the first clinch attack. This fourth session is used for the basal aggressive behavior. The basal aggressive behavior testing was conducted in 1 week. After the basal test sessions, both the behavior during the resident-intruder test as well as the behavior during a sexual behavior session were scored for each rat for all drug combinations. An overview of all experiments and their respective method are visualized in figure 2.

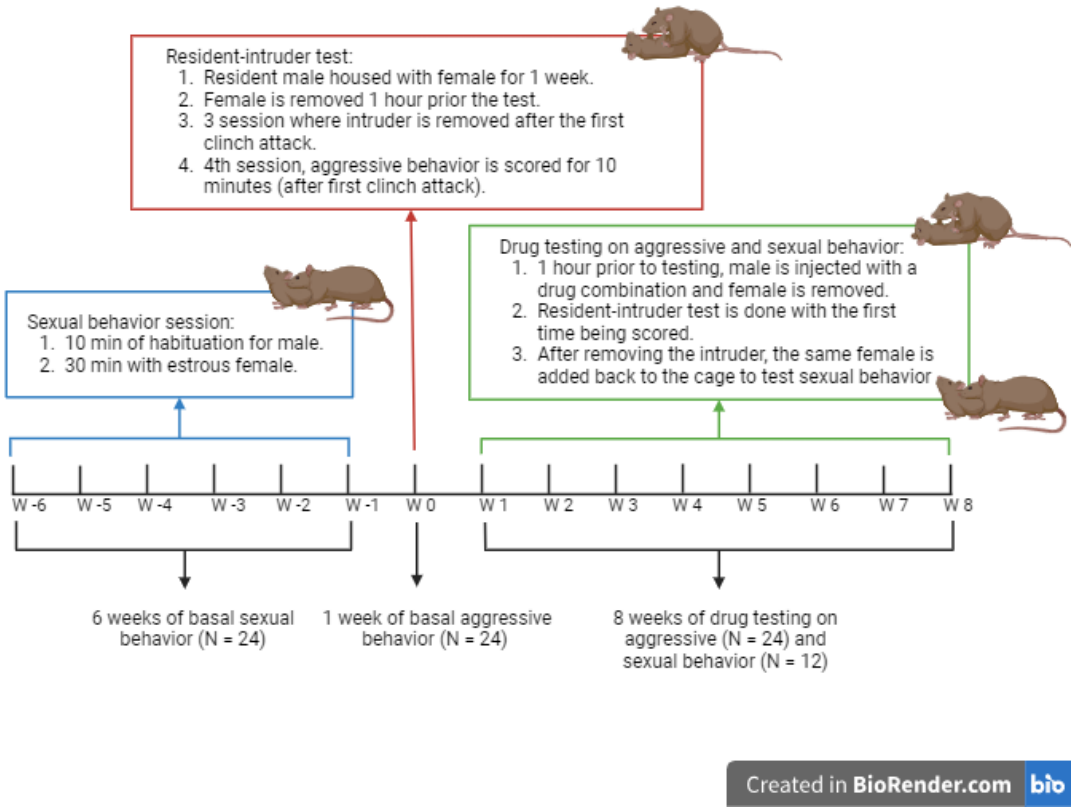

Figure 2: The timeline and method of all experiments done.

A more detailed overview of one week during the drug testing is visualized in figure 3. The male rats were habituated for 1 week with a female. After living together for a week, the female was removed from the box, while the male received the drug combination and underwent the resident-intruder test. Hereafter, only the 12 males that were selected as the best ejaculators, underwent the sexual behavior test. After the resident-intruder test ended and the intruder was taken care of, the known female, which the males were habituated with for 1 week, was placed

back into the respective cages of the 12 selected males. The males were scored for 30 minutes for sexual behavior. Only in week 2, the males received a different female due to miscommunication. In week 7, 1 male received a new female, since its female was not in estrous anymore. Furthermore, during the experiments, 2 intruder male rats were wounded and euthanized. Moreover, 1 resident male rat was euthanized, because this male wounded an intruder deadly. Due to ethical reasons, this male was excluded from further experiments. Therefore, the sample size for the drug testing on sexual behavior were 11 subjects.

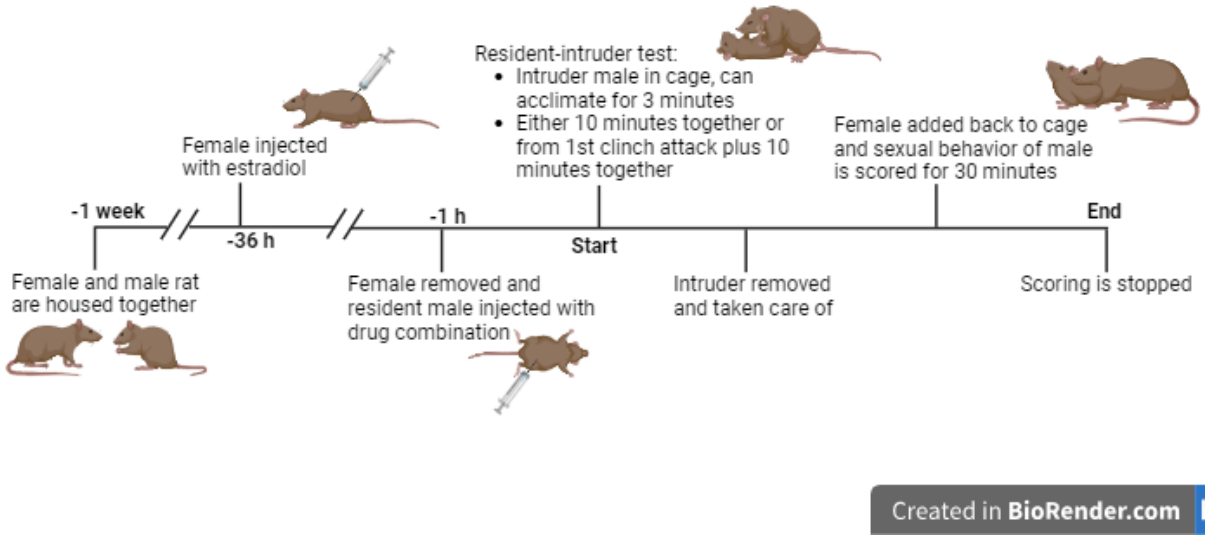

Figure 3: The timeline for the drug testing sessions.

For our research, only the sexual behavior during the drug testing is scored and analyzed. For the sexual behavior, the timing and amounts of mounts, intromissions and ejaculations were scored with the softwares The Observer XT version 14 and Boris version 7. From the scoring, the amounts of mounts, intromissions and ejaculations, and the latencies of the first sexual behavior and of the first ejaculations were calculated. Furthermore, the intromission rate (Intromission rate = (#intromissions / (#intromissions + #mounts)) * 100%) was calculated. The post-ejaculatory interval was also calculated. However, there were only 9 rats that displayed sexual behavior after their ejaculation from the drug testing. Therefore, there were not enough data points and we decided to not include the post-ejaculatory interval in our results.

2.5 Statistical analysis

After collecting the data, the intromission ratio was set to 0 for all rats that did not display any intromissions. When the rat did not display any mounts or ejaculations, their respective latencies were set to 3600 minutes. In order to see what the effect is of drug X in combination with escitalopram, a summation of all scores was made for the 30 minutes and a mean ± SEM were calculated using R. A Shapiro-Wilk test was performed to test for normality. Moreover, a Levene's Test for Homogeneity of Variance was performed to test for equal variances within the groups. Mounts, intromissions, ejaculations, intromission ratio, ejaculation latency had a non-significant p-value, which meant an equal variance between groups. Therefore, a One-way

repeated measures Anova was performed for these parameters. The drug effects of each treatment and basal sex were compared using the paired t-tests with bonferroni correction. For the latency to first sexual performance the Levene's test for Homogeneity of variances revealed a significant p-value, which meant that the Anova assumptions were violated. Therefore, a Friedman's Anova test was performed. The drug effects of each treatment and basal sexual behavior were compared using a wilcoxon bonferroni test. A p-value of 0.05 was considered significant. Biorender was used for a graphical overview of the experiment.

3. Results

3.1 Mounts

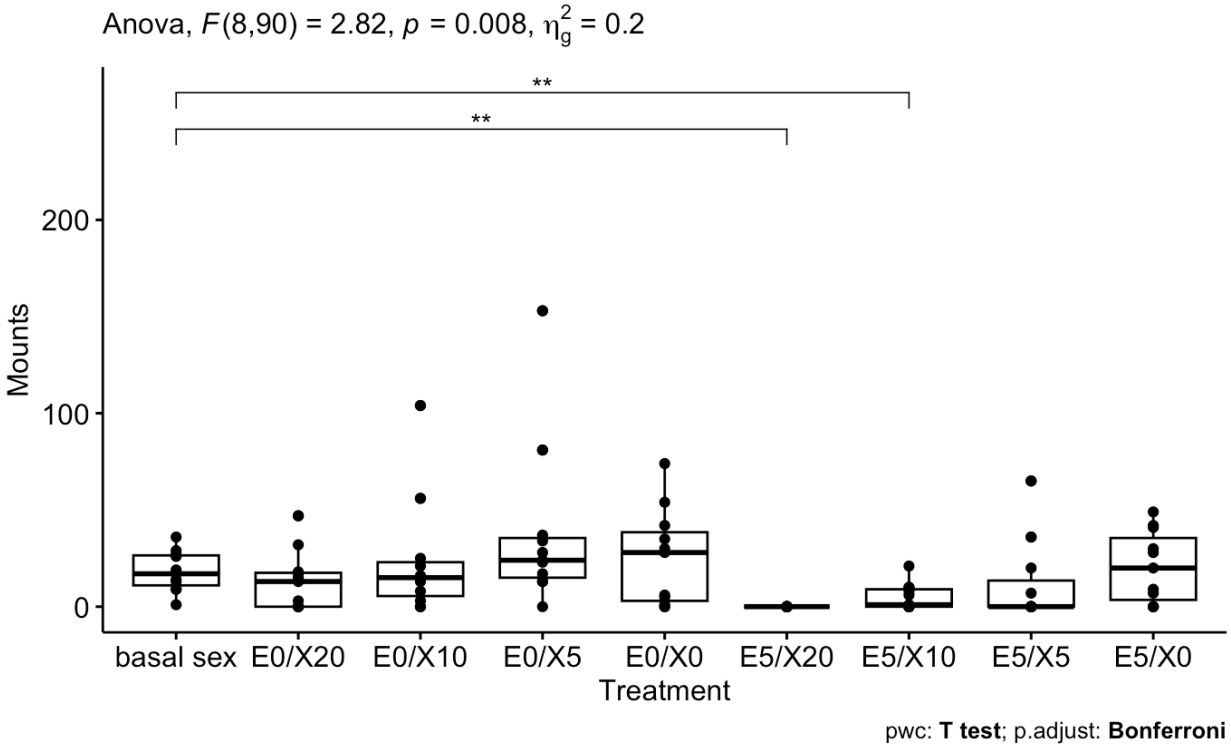

*Figure 4: The amount of mounts during the 30-minute sex session of male Groningen wild type rats (n = 11), which received a combination of escitalopram (E) and drug X (X). Data is shown as a boxplot with median, maximum, minimum and outliers. After the drug, the respective concentration is stated in mg/kg, for example E0 means 0 mg/kg escitalopram, etc. The rats received the treatments 1 hour intraperitoneal before the start of the resident-intruder test, after this test, the sexual behavior was scored. **Significant difference < 0.01.*

There was an overall significant effect on the amount of mounts [Figure 4; F(8,90) = 2.82; p =0.008] due to the drug treatment. Compared with the amount of mounts found in week 6 of the basal sexual behavior scoring, there was a significant reduction in the amount of mounts after administration of 5 mg/kg escitalopram combined with 20 mg/kg drug X (p = 0.006). The mean

amount of mounts from the basal sex was 18.2 ± 10.4, whereas the mean amount of mounts after a treatment of 5 mg/kg escitalopram combined with 20 mg/kg drug X was 0.0 ± 0.0. In addition, there was also a significant reduction found after 5 mg/kg escitalopram combined with 10 mg/kg drug X, where the mean amount of mounts was 5.1 ± 6.7, in comparison to week 6 of the basal sexual behavior (p = 0.010). The other groups were not significantly different from the basal sexual behavior and from each other. However, there is a tendency for a trend visible in figure 4 when only drug X is given. When 20 mg/kg drug X is given, the mean amount of mounts is 13.2 ± 15.3. This mean increases with lowering the concentration of drug X to 25 ± 25 mounts when the saline treatment for drug X is given.

3.2 Intromissions

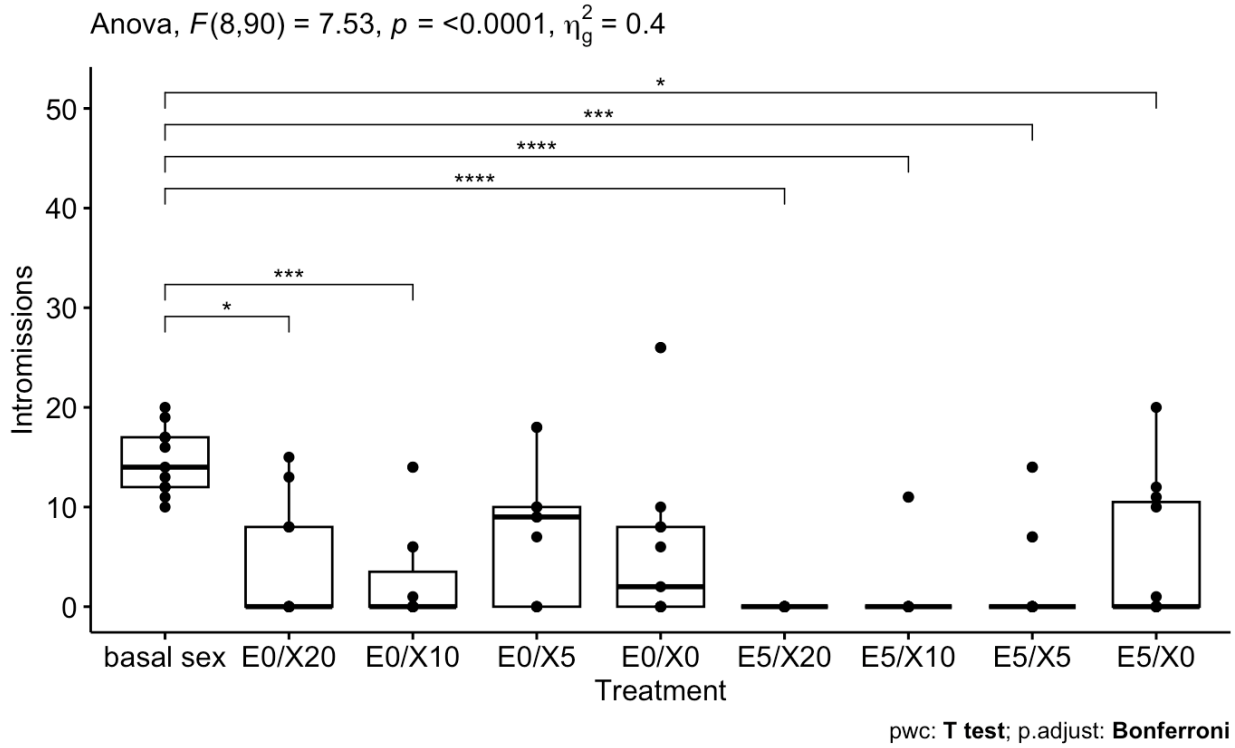

*Figure 5: The amount of intromissions during the 30-minute sex session of male Groningen wild type rats (n = 11), which received a combination of escitalopram (E) and drug X (X). Data is shown as a boxplot with median, maximum, minimum and outliers. After the drug, the respective concentration is stated in mg/kg, for example E0 means 0 mg/kg escitalopram, etc. The rats received the treatments 1 hour intraperitoneal before the start of the resident-intruder test, after this test, the sexual behavior was scored. *Significant difference < 0.05, **Significant difference < 0.01, ***Significant difference < 0.001, ****Significant difference < 0.0001.*

There was an overall significant effect on amount of intromissions [Figure 5; F(8,90) = 7.53; p = <0.0001] after the drug treatments. The rats from the basal sexual behavior had a mean of 14.6 ± 3.4 intromissions. When comparing multiple groups with the amount of intromissions found in week 6 of basal sexual behavior, these groups all had a significant reduction. This is also visible

in figure 5. Firstly, this applies to the treatments 0 mg/kg escitalopram combined with 20 mg/kg drug X (p = 0.014) with a mean of 4 ± 5.9 intromissions and 0 mg/kg escitalopram combined with 10 mg/kg drug X (p = 0.0005) with a mean of 2.5 ± 4.5 intromissions. Lastly, also a reduction in the amount of intromissions is found when basal sexual behavior is compared with 5 mg/kg escitalopram combined with either 20 mg/kg drug X (p < 0.0001), 10 mg/kg drug X (p < 0.0001), 5 mg/kg drug X (p = 0.0001) or with 0 mg/kg drug X (p = 0.034). The means of 5 mg/kg escitalopram with drug X were 0.0 ± 0.0 intromissions for 20 mg/kg drug X, 1.0 ± 3.3 intromissions for 10 mg/kg drug X, 1.9 ± 4.5 intromissions for 5 mg/kg drug X and 4.9 ± 7.1 intromissions for 0 mg/kg drug X. In contrast to the differences with basal sexual behavior, neither of the groups from the drug testing significantly differed from each other.

3.3 Latency to first mount or intromission

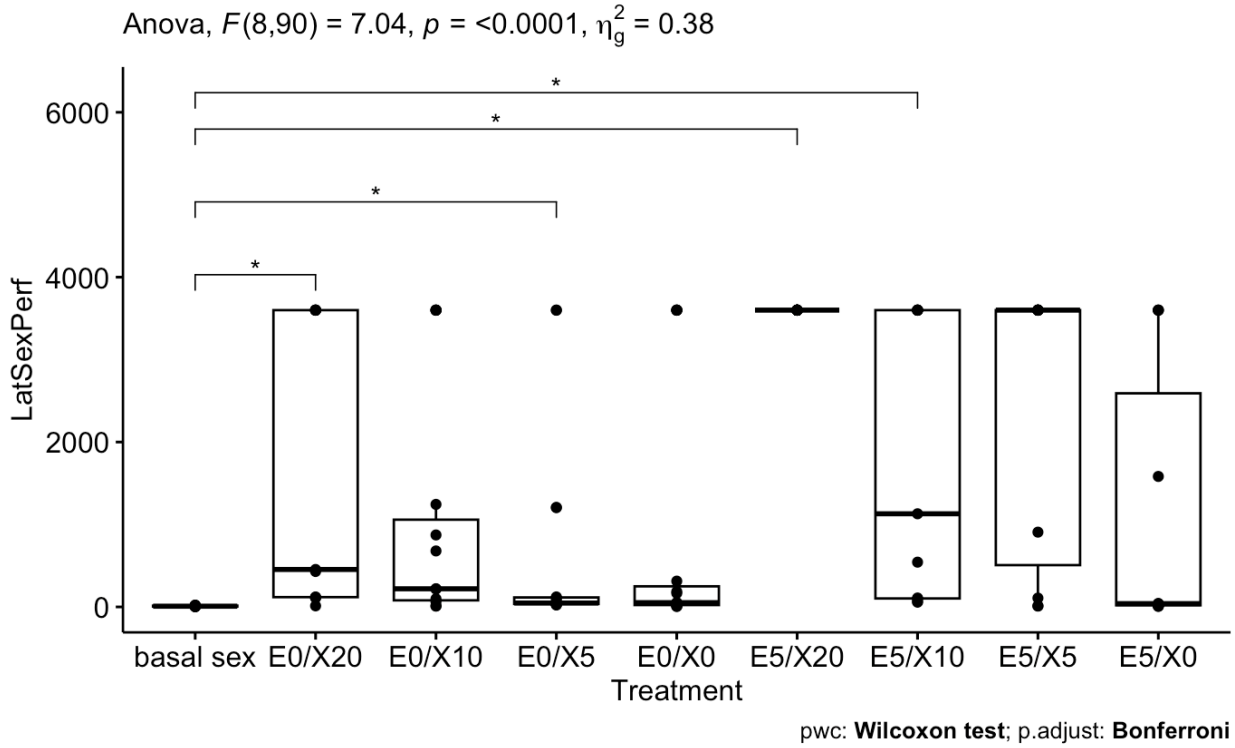

*Figure 6: The latency to perform the first sexual performance (LatSexPerf), either a mount or an intromission, in seconds during the 30-minute sex session of male Groningen wild type rats (n = 11), which received a combination of escitalopram (E) and drug X (X). Data is shown as a boxplot with median, maximum, minimum and outliers. After the drug, the respective concentration is stated in mg/kg, for example E0 means 0 mg/kg escitalopram, etc. The rats received the treatments 1 hour intraperitoneal before the start of the resident-intruder test, after this test, the sexual behavior was scored. *Significant difference < 0.05.*

There was an overall significant effect on the latency to first mount or intromission [Figure 6; χ^2(8) = 43.91; p= <0.0001] after the drug treatments. Almost all rats during all treatments had a mount as their first sexual performance, except for 2 rats during the basal sexual behavior,

who did an intromission as first sexual performance. When comparing the latency to the first mount or intromission of the basal sexual behavior with 0 mg/kg escitalopram combined with 20 mg/kg drug X, a significant increase in latency is found (p = 0.035) from a mean of 10.7 ± 6.4 to a mean of 1749.4 ± 1776.7 seconds. Also when comparing the latency of basal sexual behavior with 0 mg/kg escitalopram combined with 5 mg/kg drug X, a significant increase to a mean of 729.7 ± 1422.3 seconds is found (p = 0.035). Furthermore, a significant increase in latency is found when 5 mg/kg escitalopram is combined with either 20 or 10 mg/kg drug X in comparison to the basal sexual behavior (p=0.035). Noticeably is that the 5 mg/kg escitalopram combination with 20 mg/kg drug X results in a latency of 3600 ± 0 seconds, meaning that no sexual behavior took place during the scored 30 minutes. 5 mg/kg escitalopram with 10 mg/kg drug X resulted in a latency of 1818 ± 1733.0 seconds. All other groups were not significantly different from each other.

3.4 Intromission ratio

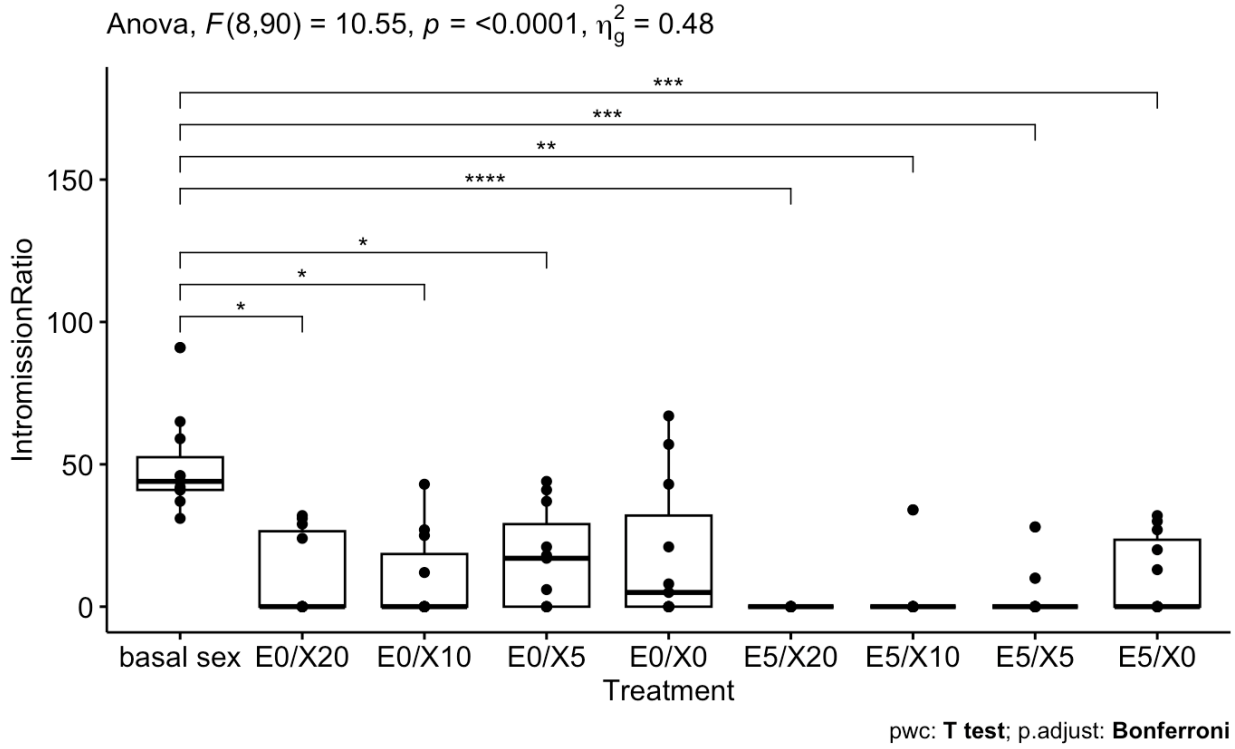

*Figure 7: The intromission ratio in percentage during the 30-minute sex session of male Groningen wild type rats (n = 11), which received a combination of escitalopram (E) and drug X (X). Data is shown as a boxplot with median, maximum, minimum and outliers. After the drug, the respective concentration is stated in mg/kg, for example E0 means 0 mg/kg escitalopram, etc. The rats received the treatments 1 hour intraperitoneal before the start of the resident-intruder test, after this test, the sexual behavior was scored. *Significant difference < 0.05, **Significant difference < 0.01, ***Significant difference < 0.001, ****Significant difference < 0.0001.*

There was an overall significant effect on intromission ratio [Figure 7; F(8,90) = 10.55; p = <0.0001] found after the drug treatments. A significantly higher intromission ratio of 49.4 ± 16.8 % was found in the basal sexual behavior group in comparison to the intromission ratios of 0 mg/kg escitalopram combined with either 20 mg/kg drug X (p = 0.016), 10 mg/kg drug X (p = 0.013) or 5 mg/kg drug X (p = 0.029). The intromission ratios of the drug treatments were respectively 10.5 ± 14.8 %, 9.7 ± 15.2 % and 16.7 ± 17.3 %. In addition, also a significantly higher intromission ratio was found in the basal sexual behavior group when comparing 5 mg/kg escitalopram combined with 20 mg/kg (p < 0.0001), 10 mg/kg (p = 0.002), 5 mg/kg (p = 0.0006) and 0 mg/kg (p = 0.001) drug X. The intromission ratios for 5 mg/kg escitalopram with drug X are 0.0 ± 0.0 for 20 mg/kg drug X, 3.1 ± 10.3 for 10 mg/kg drug X, 3.5 ± 8.7 for 5 mg/kg drug X and 11.1 ± 13.7 for 0 mg/kg drug X. Comparisons between other groups did not yield a significant difference.

3.5 Ejaculations

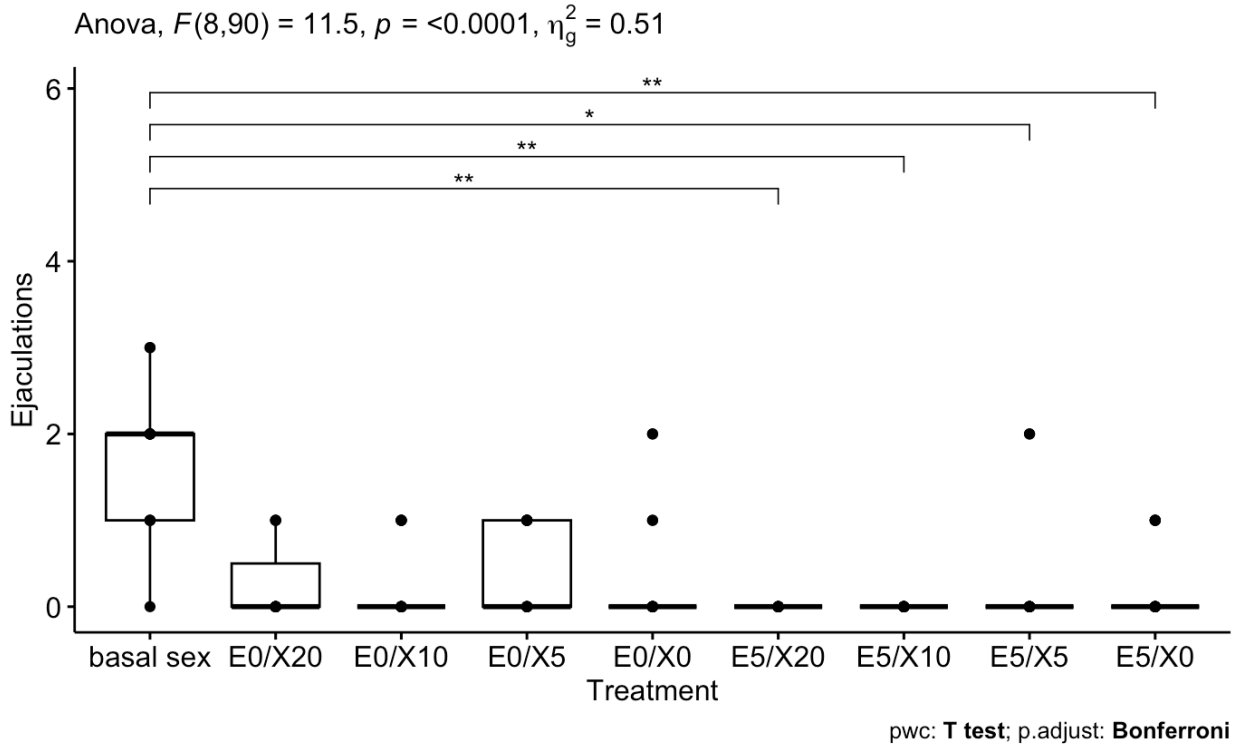

*Figure 8: The amount of ejaculations during the 30-minute sex session of male Groningen wild type rats (n = 11), which received a combination of escitalopram (E) and drug X (X). Data is shown as a boxplot with median, maximum, minimum and outliers. After the drug, the respective concentration is stated in mg/kg, for example E0 means 0 mg/kg escitalopram, etc. The rats received the treatments 1 hour intraperitoneal before the start of the resident-intruder test, after this test, the sexual behavior was scored. *Significant difference < 0.05, **Significant difference < 0.01, ***Significant difference < 0.001, ****Significant difference < 0.0001.*

There was a significant effect found on amount of ejaculations [Figure 8; F(8,90) = 11.5; p = <0.0001] after the drug treatments. There is a significant decrease found in the amount of ejaculations when comparing the basal sexual behavior group with 5 mg/kg escitalopram combined with either 20 mg/kg drug X or 10 mg/kg drug X (p = 0.003). Both treatments resulted in 0 ± 0 ejaculations, meaning that no ejaculations took place during the scored 30 minutes, whereas the basal sexual behavior group had 1.7 ± 0.9 ejaculations. Furthermore, the treatments with 5 mg/kg escitalopram combined with either 5 mg/kg drug X (p = 0.02) or 0 mg/kg drug X (p = 0.01) also resulted in a significant lower amount of ejaculations of respectively 0.182 ± 0.6 and 0.182 ± 0.4 ejaculations. Other groups did not have a significant difference in the amount of ejaculations. However, from the 88 measurements for the drug treatments only 15 rats in total ejaculated. This is a lower ratio than the 10 out of 11 rats ejaculating during week 6 of the basal sexual behavior.

3.6 Ejaculation latency

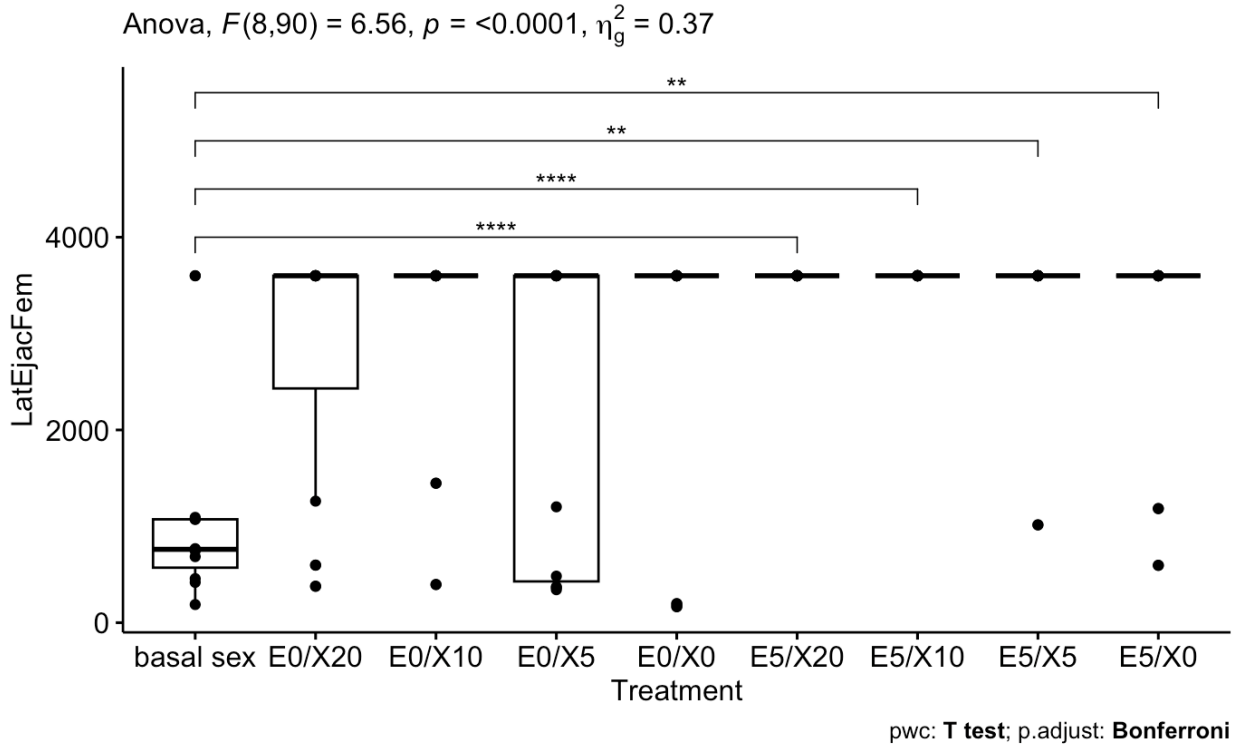

*Figure 9: The ejaculation latency from the moment the female was put in the cage (LatEjacFem) in seconds during the 30-minute sex session of male Groningen wild type rats (n = 11), which received a combination of escitalopram (E) and drug X (X). Data is shown as a boxplot with median, maximum, minimum and outliers. After the drug, the respective concentration is stated in mg/kg, for example E0 means 0 mg/kg escitalopram, etc. The rats received the treatments 1 hour intraperitoneal before the start of the resident-intruder test, after this test, the sexual behavior was scored. *Significant difference < 0.05, **Significant difference < 0.01, ***Significant difference < 0.001, ****Significant difference < 0.0001.*

There was an overall significant effect on ejaculation latency [Figure 9; $F(8,90) = 6.56$; $p = <0.0001$] due to the drug treatments. The mean ejaculation latency of the basal sexual behavior is 987.3 ± 913.5 seconds. The ejaculation latency was significantly increased when 5 mg/kg escitalopram combined with 20 mg/kg drug X or with 10 mg/kg drug X was compared with basal sexual behavior ($p = 0.00009$). Both treatments resulted in a mean ejaculation latency of 3600 ± 0.0 seconds. Furthermore, the ejaculation latency significantly increased to 3365.0 ± 779.4 seconds for 5 mg/kg escitalopram combined with 5 mg/kg drug X ($p = 0.002$) and to 3107.2 ± 1104.3 seconds for 5 mg/kg escitalopram combined with 0 mg/kg drug X ($p = 0.009$) in comparison to basal sexual behavior. There were no other significant differences found between any of the groups of the drug treatments.

Discussion

The aim of this research was to confirm whether drug X also has potential with other SSRIs to be a treatment for PE. Therefore, we tested escitalopram together with different concentrations of drug X. The drug treatments did not show significant differences when compared among each other, but when comparing the treatments with the sexual behavior scored at week 6 of the basal sexual training, there were significant differences. Despite not being significant amongst each other, the current results show that drug X alone has the tendency to lower sexual behavior in a dose-dependent manner. This effect is mostly visible in the amount of mounts, amount of intromissions and latency to perform the first sexual behavior. Furthermore, when drug X is combined with escitalopram, this seems to inhibit sexual behavior more than when only drug X was administered. Especially the treatment of 5 mg/kg escitalopram combined with 20 mg/kg drug X seems to fully inhibit sexual behavior, since there was no sexual behavior observed during the 30-minute sex session. Furthermore, the treatment of 5 mg/kg escitalopram combined with 10 mg/kg drug X also lowers sexual behavior, with only a few rats displaying sexual behavior. When comparing these two treatments with basal sexual behavior, both treatments significantly lower sexual behavior.

A proposed mechanism via which drug X together with escitalopram inhibit sexual behavior, is by the increase of serotonin in the synaptic cleft and the influence on multiple serotonin receptors. There are 14 distinct 5-HT receptors, of which multiple are thought to be involved with sexual behavior (Olivier & Olivier, 2022). First, 5-HT1a receptor has pro-sexual effects and is found both presynaptic as postsynaptic. The presynaptic 5-HT1a receptor inhibits the firing of the neuron when activated (Olivier & Olivier, 2022). Both the pre- and post-synaptic 5-HT1a receptors are thought to be involved with pro-sexual behavior (Esquivel-Franco et al., 2020). Drug X is a 5-HT1a antagonist, which blocks both the pre- and postsynaptic 5-HT1a receptors (Scorza et al., 2012). Other 5-HT receptors that are thought to be involved are 5-HT1b and 5-HT2c, which are both thought to elicit an inhibitory effect on sexual behavior (Bijlsma et al., 2014; Olivier & Olivier, 2022; Popova & Amstislavskaya, 2002). When drug X blocks the 5-HT1a receptors, the neuron will continue to fire and serotonin will be increased in the synaptic cleft. However, without the SSRI, the serotonin transporters will take up most of the serotonin and therefore, only drug X will not elicit a high response on sexual behavior. Yet our results show a

tendence for a dose-dependent response to drug X. Therefore, we argue that a high dose of drug X will increase serotonin in such an amount that the 5-HT1b and 5-HT2c receptors will be activated and inhibit sexual behavior. Our results also show that when drug X is co-administered with a SSRI, the inhibition of sexual behavior is higher in comparison to administration of only drug X. We postulate that this happens due to the increase of serotonin in the synaptic cleft due to the blockage of serotonin transporters by the SSRI (Olivier et al., 2023). This results in more stimulation of the sex-inhibiting 5-HT1b and 5-HT2c receptors. Furthermore, by blocking the 5-HT1a receptors, serotonin will not activate these receptors and therefore, their pro-sexual effect will be blocked.

Drug X in combination with escitalopram seems to have an inhibiting effect on sexual behavior. However, the aggressive encounter seems to impact the sexual behavior, since the amount of sexual behavior at each treatment combination is lower in comparison to basal sexual behavior. Furthermore, we hypothesized that we would replicate the results of Olivier et al. (2023), but our results are not completely in line with each other. There are multiple differences between the studies that perhaps can account for the difference in results and might explain the overall decrease in sexual behavior found in our results.

The first difference between the studies is that during the experiments of Olivier et al. (2023), the rats received their treatments orally, whereas during the current experiments, the rats received their treatment intraperitoneally. The latter was necessary, because Groningen wild type rats are too aggressive to receive the treatments orally. Since the rats received the treatments peritoneally, we lowered our concentrations, because the intraperitoneal way of administration might result in a higher absorption of the drugs (Al Shoyaib et al., 2019). When our experiments finished, two at random selected rats were sacrificed, because multiple rats had small, palpable irregularities in their abdomen. The abdomen of the rats contained encapsulated drug particles. Therefore, it is difficult to say how much of the drugs were absorbed into the body of the rat. However, since the sexual behavior is inhibited in a dose-dependent manner, this might indicate that at least some of the drugs was absorbed by the rats.

The second difference is that during the current experiments Groningen wild type rats were used. All of our rats did not have more than 3 ejaculations during the 30-minute training sessions for sexual behavior, whereas the Wister rats of Olivier et al. (2023) were rapidly ejaculating rats. Therefore, it might be that our rats exhibited a lower sexual baseline than the Wistar rats. In order to determine whether Groningen wild type rats exhibit the same distribution curve in ejaculation frequencies as Wister rats (Bijlsma et al., 2014) and thus can be used as a realistic model for premature ejaculation, a larger sample size is needed.

Thirdly, a large difference between the studies is the aggressive encounter. This encounter seems to have an impact on our results, since the sexual behavior after all treatments is decreased in comparison to the basal sexual behavior. The impact of the aggressive encounter is especially visible in the intromission ratio and the amount of ejaculations, which were lower independently of the received treatment. However, not much research has been conducted on the effects of an aggressive encounter immediately before the assessment of sexual behavior. Therefore, it is difficult to determine how the aggressive encounter might impact sexual behavior. Nevertheless, it has been shown that sexual and aggressive behavior share overlap in brain regions and are both influenced by serotonin and the serotonin receptors 5-HT1a, 5-HT1b

and 5-HT2c (Mbiydzenyuy et al., 2024; Oliver & Olivier, 2022). Future research could focus on the underlying mechanism via which aggressive behavior could impact sexual behavior. The aggressive encounter in our experiment is in the form of the resident-intruder test. The resident-intruder test is a form of social stress for the rats (Koolhaas et al., 2013). Stress can impact sexual behavior by increasing latencies to first mount, intromission and ejaculation (Hawley et al., 2011; Retana-Márquez et al., 2003). Furthermore, the frequency of ejaculations could be reduced (Retana-Márquez et al., 2003). Therefore, the stress inflicted by the resident-intruder test might account for some of the impairments seen for sexual behavior. We were not expecting the resident-intruder test to be of impact on sexual behavior, since it is ensured that the resident rats are the winners in the test. Therefore, the aggressive encounter might be rewarding for the resident (Koolhaas et al., 2013). Also, the winners in a resident-intruder test do not experience a decrease in testosterone levels, which the losers do experience (Koolhaas et al., 1980). Therefore, we expected that the rats do not exhibit reduced testosterone levels due to chronic stress inflicted by the repetitive resident-intruder test (Retana-Márquez et al., 2003). Testosterone deficiencies can result in impaired sexual behavior (Rodríguez-Nieto et al., 2021). Ensuring the winning of the resident would counter this, therefore, it is likely that lowered testosterone due to the resident-intruder test is not the cause of the lowered sexual behavior. However, measuring the testosterone levels of the rats at the resident-intruder test and at the sexual behavior session could rule this out. It would be important to take the normal spontaneous release of testosterone into account during these measurements (Shulman & Spritzer, 2014).
Lastly, the set-up of the resident-intruder test might also partially account for the lower amount of sexual behavior shown during the observed 30 minutes. The female rats, which are used during the testing of sexual behavior, are housed beforehand with the males. The female rats receive estradiol 36 hours before the testing. However, the females are becoming receptive 16 hours after the estradiol injection and after 24 hours all females should be receptive (Green et al., 1970). This would mean that the rat couples would have had at least 8 hours to copulate with each other. Rats copulate usually up to 8 ejaculations before they are sexually satiated (Bialy et al., 2019) and all male rats will reach sexual satiety within 4 hours of *ad libitum* copulation (Phillips-Farfán & Fernández-Guasti, 2009). After sexual satiety is reached, most males are likely not to copulate for the next 24 hours. Another representation of sexual satiety is a longer post-ejaculatory interval (Phillips-Farfán & Fernández-Guasti, 2009). We did assess the post-ejaculatory interval, however, as mentioned in the methods, we did not have enough data points to show valid post-ejaculatory intervals per treatment. In order to counteract the possible confounding effect of sexual satiety, we could allow male rats to only mate an estrous female after the aggressive test and thus introduce a new, receptive female after the resident-intruder test. Even if the males would be sexually satiated, it is expected that they will mate with a new receptive female. This effects is also known as the Coolidge effect (Tlachi-López et al., 2012).

The currents results show that drug X in combination with escitalopram inhibits sexual behavior. This implies that this combination might be an on-demand treatment for PE. However, our results are impacted by the above-mentioned confounding factors. Therefore, we cannot conclude the precise effect and how much sexual behavior is inhibited by drug X in combination with escitalopram. Furthermore, since the social stress due to the resident-intruder test impacts

sexual behavior and PE is associated with social phobia (Corretti et al., 2006; Rajkumar & Kumaran, 2015; Tignol et al., 2006), future research could focus on whether the drug treatment is effective in stressed or anxious rapid ejaculating rats. This would further prove the potential of a 5-HT1a antagonist with a SSRI to be a treatment for PE.

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