Sexual Behaviour in Male Serotonin Transporter Knockout Rats.

Jolijn Hogenbirk

S2890739

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MSc Biomedical Sciences – Neuroscience

Supervisor: Jocelien Olivier

University of Groningen

Abstract

Premature ejaculation is the most common sexual dysfunction in males. This study aimed to investigate the effectiveness of on-demand treatment, combination therapy of a 5-HT1A antagonist *(DU125530)* together with SSRI *(paroxetine)* administration on sexual behaviour. Additionally, the sexual behaviour in serotonin transport (SERT) knockout rats was investigated over time. The neurotransmitter serotonin plays an important role in social behaviour, including sexual behaviour. In general, it is stated that a decrease in serotonin facilitates sexual behaviour, while an increase in serotonin inhibits sexual behaviour. A rat model in which the serotonin levels are disrupted is the serotonin transporter knockout ($SERT^{-/-}$) rat. Due to the high levels of extracellular serotonin, it is expected that sexual behaviour in rats with a lack of the serotonin transporter will be decreased. To investigate this, $SERT^{-/-}$ rats were assessed on their frequency and latencies of mounts, intromissions, and ejaculations for 30 minutes over 10 weeks and compared to wildtype rats. Results show that $SERT^{-/-}$ rats stabilize their sexual behaviour on a lower ejaculations frequency than the $SERT^{+/+}$ rats do. However, both the $SERT^{+/+}$ rats and the $SERT^{-/-}$ rats are able to stabilize their sexual behaviour after 1 week, Additionally, the latency until the first ejaculation is also longer for $SERT^{-/-}$ rats compared with the $SERT^{+/+}$. These findings indicate that the sexual behaviour in the $SERT^{-/-}$ rats is lower than that of the $SERT^{+/+}$ rats, with a consistent lower frequency of ejaculations for the $SERT^{-/-}$ rats compared with $SERT^{+/+}$ rats. These results confirm previous findings which stated that serotonin plays a crucial role in sexual behaviour, indicating that a disrupted serotonin transporter, results in a reduced sexual behaviour, likely due to high levels of extracellular serotonin. In regard to the pharmaceutical experiment there are no results yet available because the experiments are still ongoing.

Keywords: serotonin, serotonergic dysfunction, serotonin transporter knockout rat, selective serotonin reuptake inhibitor (SSRI), sexual behaviour male, sexual dysfunction, premature ejaculation, rodent model.

Introduction

The most common sexual dysfunction in males is premature ejaculation (PE), even though it is very likely to be under-diagnosed (Butcher et al., 2020; Carson & Gunn, 2006; Du et al., 2019; Hutchinson et al., 2012; Jern et al., 2015; Park et al., 2017). Estimates are that more than 30% of the males worldwide have been affected currently or in the past (Butcher et al., 2020; Carson & Gunn, 2006; Coskuner & Ozkan, 2021; Du et al., 2019; Park et al., 2017). Clinical research has led to an evidence-based definition for the different types of PE by the Society for Sexual Medicine (ISSM) (McMahon et al., 2008; Serefoglu et al., 2014; M.D. Waldinger, 2014). According to the generally accepted classification of PE there are two distinguished types (Coskuner & Ozkan, 2021; M.D. Waldinger, 2018). The main distinguishing feature between the two classifications of PE is the time of onset of symptoms (Serefoglu et al., 2014). In an article by Serefoglu et al from 2014 the PE types were described as follows: Primary PE or otherwise referred to as lifelong PE (L-PE) is present since the first sexual encounter. This early ejaculation happens nearly every sexual intercourse between 30-60 seconds after vaginal penetration in the majority of the cases (80%), or between 1 or 2 minutes (20%). The other classified form is secondary or acquired PE (A-PE). In which the premature ejaculation developed later in life after having normal ejaculation latencies previously. In this case the ejaculation generally occurs within 3 minutes (Ciocanel et al., 2019; Serefoglu et al., 2014). It is important to note that people suffering from PE cannot postpone ejaculation in nearly all attempts with vaginal penetration (Butcher et al., 2020; Serefoglu et al., 2014).

Additionally, people can experience personal distress due to the early ejaculation. This can have negative consequences such as distress, frustration and/or avoidance of sexual relations (Butcher et al., 2020; Serefoglu et al., 2014). Furthermore, it can also negatively influence self-esteem (Ciocanel et al., 2019; Tan et al., 2012) or can even cause mental health problems (Ciocanel et al., 2019; Feldman et al., 1994; D. Rowland et al., 2004; D. L. Rowland et al., 2007; Symonds et al., 2003).

In general, it has been found that sexual dysfunction has an impact on the lives of individuals and general wellbeing (Christensen et al., 2011; Ciocanel et al., 2019). It has been associated with low self-esteem (Ciocanel et al., 2019; Tan et al., 2012), mental health problems (Ciocanel et al., 2019; Feldman et al., 1994; D. Rowland et al., 2004; D. L. Rowland et al., 2007; Symonds et al., 2003), interpersonal and intimacy problems (Ciocanel et al., 2019; D. Rowland et al., 2004; D. L. Rowland et al., 2007; Symonds et al., 2003; Tan et al., 2012), a decreased sexual functioning and satisfaction (Ciocanel et al., 2019; Serefoglu et al., 2014) and a decrease in quality of life (Althof et al., 2010; Ciocanel et al., 2019; McMahon et al., 2008; D. L. Rowland et al., 2007) . Therefore, PE is an important clinical condition to treat given its considerable impact on quality of life (Du et al., 2019; Hutchinson et al., 2012).

So far, the management of PE has been challenging. Sexual dysfunctions are a complex medical issue with biological, psychological and social influences (Berman, 2005; Ciocanel et al., 2019; Lewis et al., 2004; Thomas & Thurston, 2016). Currently, there is very few medications specifically indicated for the treatment of PE. There are multiple psychosexual and pharmacological treatments for PE developed, including psychosexual counselling and daily on-demand pharmacotherapy. These are either done alone or in combination as an integrated treatment program (Althof et al., 2014; McMahon, 2015). However, psychotherapy alone has not been found to be very effective (Butcher et al., 2020). The development for an optimal treatment is complex. There is evidence that pharmacologic interventions or combined therapies are more effective than non-pharmacologic interventions for treating sexual dysfunctions (Ciocanel et al., 2019). Other treatment approached for PE include topical anaesthetics (Wyllie & Powell, 2012) and acupuncture (Sunay et al., 2011), with varying degrees of success (Jern et al., 2015). The pharmacotherapy attributed for PE mainly targets multiple neurotransmitters and receptors involved in the control of ejaculation. These targets include serotonin, dopamine, oxytocin, norepinephrine, gamma amino-butyric acid (GABA) and nitric oxide (NO) (McMahon, 2015). Serotonin (5-HT) is found to play a key role in the male sexual behaviour. A decrease in serotonin results in a facilitation of the sexual behaviour, while an increase inhibits the

sexual behaviour (Esquivel-Franco et al., 2020). In a study performed by Waldinger et al. (1998) it was suggested that serotonin receptor subtypes might be involved in premature ejaculation (Marcel D. Waldinger et al., 1998). It had been suggested that PE could be caused by hyposensitivity of 5-HT1C receptors (Marcel D. Waldinger et al., 1998). Additionally, it was found that activation of 5-HT1A receptors by the selective 5-HT1A receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylaminotetralin) shortens the ejaculation latency time and reduces the number of intermissions preceding the ejaculation (Ahlenius et al., 1981; Marcel D. Waldinger et al., 1998). These findings suggest that PE might also be caused by the hypersensitive 5-HT1A receptor (Marcel D. Waldinger et al., 1998).

At the moment there are different types of pharmacological treatments that improved the time to ejaculation by 1-5 minutes. These treatments include Selective Serotonin Reuptake Inhibitors (referred to as SSRIs, which include paroxetine, citalopram, sertraline, fluoxetine and dapoxetine), TCAs (oral and nasal clomipramine), topical anaesthetics (lidocaine gel and topical eutectic mixture for PE), PDE5-Is and opioid analgesics (Tramadol) (Ciocanel et al., 2019). However, these drugs were associated with adverse effects, which are stated below. It is important to present different treatment methods to individual patients so they may consider the risks and benefits of treatment differently (Ciocanel et al., 2019). With a topical agent side effects can include loss of sensitivity, loss of erection and irritation (Ciocanel et al., 2019; Martyn-St James et al., 2016). Additionally, PDE5-Is were associated with increased risk of flushing, headache and palpitation (Ciocanel et al., 2019; Martyn-St James et al., 2017). It was found that TCAs can cause local irritation in the nose in cases of nasal administration (Ciocanel et al., 2019; Cooper et al., 2015). Other medication, such as long-acting SSRIs have shown to be linked to headache, decreased libido, nausea, dry mouth, diarrhoea, dizziness, insomnia and drowsiness (Ciocanel et al., 2019; Cooper et al., 2015; De Hong et al., 2014). And lastly Tramadol could be linked to somnolence, pruritus, erectile dysfunction, nausea, headache, dry mouth, dizziness, vomiting and constipation (Ciocanel et al., 2019; Martyn-St James et al., 2015).

Research into the inhibiting effect of SSRI on sexual behaviour found that SSRIs are successfully capable to delay ejaculation time. An example is paroxetine treatment, which causes ejaculation retardation (Marcel D. Waldinger et al., 1998). Continuous paroxetine treatment repeatedly showed to be the most effective SSRI in terms of ejaculation-delaying potency (Jern et al., 2015; Mcmahon & Porst, 2011; M. D. Waldinger et al., 2004). However, it is important to note that people have different tolerability for different SSRIs, making it more or less likely to discontinue therapy (Jern et al., 2015). In the study of Jern et al. in 2015, it was found that dapoxetine (SSRI particularly developed for PE) users had a discontinuation rate of 70.6%, which was significantly higher than the discontinuation of paroxetine (28.8%). However, the discontinuation rate of paroxetine was greater than that of sertraline and citalopram. The higher discontinuation rate is likely due to the greater likeliness of debilitating side effects in PE treatment (Jern et al., 2015; Mullins et al., 2005). Even though dapoxetine has a high discontinuation rate, there is an advantage of 'on-demand' therapy due to the short-acting nature of this SSRI (Hutchinson et al., 2012). This means it is possible to take it a few hours before the expected sexual encounter, which reduces the possibility of adverse effects. However, even though dapoxetine has been developed as an 'on-demand' SSRI treatment for PE (Hutchinson et al., 2012; Jern et al., 2015; Yue et al., 2015), the efficacy of dapoxetine is still lower than paroxetine. Therefore it is needed to investigate whether a better treatment strategy can be devleope to treat PE on-demand.

A possible candidate for 'on-demand' treatment could be paroxetine. After administration of paroxetine there is an initial peak of serotonin release. This is due to the blockage of the serotonin reuptake. Which is rapidly followed by a decreased serotonergic neurotransmission (Waldinger et al., 2004). This decrease serotonergic neurotransmission is caused by the activation of presynaptic 5-HT1A auto receptors, which causes inhibition of the serotonergic neuron. Resulting in less serotonin release in the synaptic cleft. Over time, chronic paroxetine administration delays the ejaculation not only due to the increased amount of serotonergic neurotransmission, but also due to desensitization of presynaptic 5-HT1A autoreceptors and post-synaptic -HT2C receptors (B. Olivier et al., 1998; Marcel

D. Waldinger et al., 1998, 2004). This desensitization of the 5-HT1A autoreceptor causes a lack of inhibition on the serotonergic neuron. Therefore, serotonin will be released in the synaptic cleft, resulting in high concentrations of serotonin in the synaptic cleft. For a visual representation of this mechanism, see Appendix I. Due to the minimal changes in the serotonin neurotransmission and the absence of 5-HT1A receptor desensitization after acute (on-demand) treatment of paroxetine, it unlikely that on-demand treatment of paroxetine induces a strong delaying effect on the ejaculation (Marcel D. Waldinger et al., 2004). Previous studies found that it is possible to administer an SSRI, citalopram, in combination with an 5-HT1A antagonist, WAY100635. This study found that by administering it simultaneously there was an acute rising effect on the serotonin levels. This acute effect is due to the blockage of serotonin reptake in combination with the blockage of inhibition of the serotonergic neuron, which in turn causes the serotonergic neuron to release more serotonin in the synaptic cleft. Due to these mechanisms there is an acute high level of serotonin in the synpatic cleft (de Jong et al., 2005). In another study they looked at the sexual behavoiur when administering the SSRI paroxetine in combination with WAY100365. They found a significant delay in mounting behavior and ejaculation time (Looney et al., 2005). Recently, in the lab of Jocelien Olivier another 5-HT1A antagonist (DU125530) was combined with the SSRI paroxetine to investigated as a possible on-demand treatment for premature ejaculation. The combination of the two compounds was able to significanly increase the ejaculation latency *(Jocelien Olivier et al., unpublished)*.

The mechanism behind the continuous SSRIs treatment is due to an inhibition of the serotonin reuptake, which result in a higher amount of serotonin in the synaptic cleft. This increase in serotonin causes disrupted sexual behaviour, resulting in for instance an increased ejaculation time. A similar effect is also seen in Serotonin transporter (SERT) knockout rat model. This is a rat model in which the serotonin transporters are missing since conception. These rats showed reduced sexual behaviour and altered 5-HT1A receptor functioning. Desensitization of these receptors may hinder the sexual performance (Chan et al., 2011). The availability of the $SERT^{-/-}$ rodent model makes it possible to study the SERT gene function and the consequences of life-long absence of SERT in for instance sexual

behaviour (Chan et al., 2011; Esquivel-Franco et al., 2020). It is found that there is a significant reduction in the sexual performance of these rats. Especially the number of ejaculations, intromissions and intromission ratio was significantly lower in $SERT^{-/-}$ rats compared to $SERT^{+/+}$ or $SERT^{+/-}$ (Chan et al., 2011; Esquivel-Franco et al., 2020). Similar results were also found in the study by Geng et al. in 2019. This decreased sexual performance in the $SERT^{-/-}$ rats resembles the chronic administration of SSRIs, which induces sexual dysfunction. Therefore, the $SERT^{-/-}$ rat model might be used to understand the role of serotonergic dysfunction and sexual dysfunction, but also for studying sexual dysfunction in men using SSRIs (Chan et al., 2011; Esquivel-Franco et al., 2020).

The main goal of this study is to study the effectiveness of combination therapy of 5-HT1A antagonist *(DU125530*) together with SSRI *(paroxetine)* administration. Additionally, the 5-HT1A antagonist will also be administered by itself. Previously, a similar experiment has been performed with the 5-HT1A antagonist, WAY100635, in SERT rats. The administration of the 5-HT1A antagonist by itself inhibited the sexual behaviour, with a stronger effect in the $SERT^{-/-}$ rat (Chan et al., 2011).

Due to the similarities between chronic SSRI treatment and the $SERT^{-/-}$ rats it is hypothesized that treatment of a $SERT^{+/+}$ with SSRI *(paroxetine)* in combination with DU125530 would result in the same effect as $SERT^{-/-}$ with only DU125530 treatment. Therefore, the research question is: 'Does administration of solely the DU125530, a 5-HT1A antagonist, induces an inhibiting effect of the sexual behaviour in $SERT^{-/-}$ rats?'. Additionally, the sexual behaviour differences between the $SERT^{+/+}$ and $SERT^{-/-}$ rats in their ejaculation, intromission and mount latency and frequency will be investigated. Lastly, the sexual behaviour over time will be investigated to study how it stabilizes in $SERT^{+/+}$ and $SERT^{-/-}$ rats during training of sexual behaviour. It is expected that the $SERT^{-/-}$ will have lower sexual behaviour, indicated by lower ejaculation frequency and a longer latency.

Method

Experimental animals:

SERT knockout rats (Sic6a4^{1Hubr}) are used for this experiment. These Wistar rats were bred in the animal facility of the University of Groningen by crossing SERT$^{+/-}$ males and SERT$^{+/-}$ females, which in turn resulted in male and female SERT$^{+/+}$, SERT$^{+/-}$, SERT$^{-/-}$ rats. The animals that are used in this experiment are 12-week-old male Wistar Unilever rats (n=32, 16 SERT$^{+/+}$ and 16 SERT$^{-/-}$) and female Wistar rats (n=32, either SERT$^{+/+}$ or SERT$^{+/-}$). The rats were housed with four individuals per cage and had *ad libitum* food and water access. For cage enrichment wood gnawing blocks were available for the rats. In order to test the rats during their active period of the day, rats were housed under reversed dark-light conditions 12h light: 12h dark: lights off from 8:00 AM to 8:00 PM. Cages were cleaned at least once every seven days. All experiments were performed in accordance with the governmental guidelines for the care and use of laboratory animals (Central Committee Animal Testing).

Female rats:

Prior to the start of the training phase the females underwent a double tubal ligation surgery to prevent pregnancy. For this surgery females were anaesthetized (Isoflurane) and administered pain relief subcutaneously (Fynadine, 0.1 mg/100g) directly before surgery and 24 and 48 hours after surgery. The females were at least twelve weeks old before undergoing surgery. After the surgery, the females had at least two weeks of recovery prior to the start of the experiment. During the experiment the females were made intentionally receptive for sexual behaviour by inducing the oestrous. The oestrous of the females is induced by injecting oestradiol (50µg in 0.1 ml oil, subcutaan (S.C.) in the neck, 36-38 hours before the test), which makes them receptive for copulation. During the training phase a females participated once per two weeks, and no more than two times per experimental day.

Behavioural tests:

The sexual behaviour training sessions and the pharmaceutical experiments are carried out in a red-light room between 10:00 AM– 04:00 PM. During the experiment the male rats were placed 20-30 minutes prior the start of the experiment into the experimental arena in order to habituate to the

environment. However, during the training phase the males are only habituated 10-15 minutes. After the habituation the females were placed in the cage. The receptive female is placed into the cage with the male for 30 minutes. During this period the sexual behaviour of the male rat is observed and recorded with a camera. The following parameters were deduced: number of mounts, intromissions and ejaculations and the latency time of the first mount, intromission and ejaculation were scored using The ObserverXT software, version 14. The rats were trained on their sexual behaviour once a week for 10 weeks in a row. In a previous study it was found that it takes around 4 weeks for the sexual behaviour of the rats to stabilize (Chan et al., 2011). Therefore, the number of mounts and intromissions is no longer scored after weeks 5, only the ejaculations. After the training phase 20 male rats with stable sexual behaviour with a reliable ejaculation frequency were selected for the pharmaceutical experiment.

Pharmaceutical experiment:

Once every four days the rats weighted and received a SSRI, called Paroxetine (10 mg/kg) or a vehicle combined with 0, 7.5, 15 or 30 mg/kg DU125530 in a randomized design, *see Table 1*. The effects of DU125530 without Paroxetine will also be investigated. Paroxetine is diluted in saline (0.9% NaCL) and S.C. administered. DU125530 was suspended in gelatine mannitol (0.5%gelatine / 5%mannitol) and intra-peritonealy (I.P.) administered. Both drugs were administered one hour before the start of the experiment (first DU125530, then paroxetine). At least four days of drug washout was used between the pharmacological testing.

Paroxetine	DU125530
0	0
0	7.5
0	15
0	30
10	0
10	7.5
10	15
10	30

Table 1: Combinations of doses concentrations for pharmaceutical trials

Data analyses:

The data is expressed as mean ± SEM. The data was normally distributed (Kolmogorov-Smirnov normality test) an analysed with parametric tests. The data of the training phase by performing a two-way ANOVA. The two-way ANOVA was performed to see whether $SERT^{+/+}$ and $SERT^{-/-}$ show a difference in sexual performance over time. This will be assessed by focussing on the

frequency and latency of mounts, intromissions and ejaculations. The factors that were considered in the data analysis were the genotypes and the elapsed weeks of training. Furthermore, the post hoc Bonferroni's multiple comparison test was performed to investigate effects between the different genotypes. Additionally, a post hoc Tukey's multiple comparison test was performed to analyse the differences between the weeks within one genotype. The data of the pharmaceutical experiment was also analysed with a two-way ANOVA. The different doses are compared within one genotype. The effect of these doses is compared with a Bonferroni's multiple comparison test. Additionally, the difference in response to the drug between the genotypes is also investigated. This is performed by doing a two-way ANOVA, with a post hoc Bonferroni's multiple comparison test(

It is important to note that the latency of the ejaculation (EL) was calculated by subtracting the latency of the first sexual activity (mount or intromission) from the latency of the first ejaculation. Resulting in the amount of time it takes between the first sexual activity to the first ejaculation. In case a rat did not ejaculate within 30 minutes, the time is noted as 1800 sec till the first ejaculation. On the other hand, for the latency of the first mount (ML) and the latency to the first intromission (IL), the latency to the first sexual activity was not subtracted from the latency to the first mount/intromission.

For frequency, the total number of ejaculations is scored over the 30 minutes. For the frequency of mounts and intromissions, only the frequency till the first ejaculation was used. In order to calculate the efficiency of the amount of mounts and intromissions the ratio of the sexual behaviours is calculated. In order to acquire the ratio, the following calculation is performed; #intromissions / (#mounts + # intromissions) * 100 = Intromission ratio.

Lastly, the post ejaculatory interval (PEI) was calculated, using the time from the first ejaculation and the time of the first mount/intromission (whichever occurs first) after that previous ejaculation. A mixed-effects analysis was performed, seeing there are missing data points due to lack of ejaculations.

The statistical analysis was performed in GraphPad Prism 8.0.1 for Windows, GraphPad Software Inc., La Jolla, USA, www.graphpad.com. A p-value of 0.05 or smaller was considered significant.

Results

The result section only entails the data of the training phase. The data of the pharmaceutical experiments was not yet available because the experiments were still ongoing.

Sexual stability in ejaculation frequency:

The sexual performance of the animals stabilizes over time. In *Figure 1 and Figure 2A* the frequency of ejaculations over the weeks are shown. Additionally, in *Appendix VI* the number of rats ejaculating specific amounts is represented per week. The frequency ejaculations in $SERT^{+/+}$ animals seems stable from week 2 onwards. The two-way ANOVA indicated that ejaculation frequencies of $SERT^{+/+}$ rats were significantly different than those of the $SERT^{-/-}$ rats ($F_{(1, 30)} = 60.16$; $p < 0.0001$). This effect can be seen from week 1 onwards to week 7 *(Appendix II)*. In week 8 and 9 there seems to be a small reduction in ejaculation rates of the $SERT^{+/+}$, therefore the frequency of ejaculations did not significantly differ between the genotypes. However, in week 10 the significant difference in ejaculation frequency is again acquired *(Appendix II)*.

It can be seen that the animals ejaculate more often after training, therefore the frequency of ejaculations increases with the subsequent experiments. In the first experiments only 7 out of the 32 animals did ejaculate during the 30 minutes training, of which none were $SERT^{-/-}$ rats. This indicates that only 22% of all the animals were able to ejaculate in week 1. However, this is significantly increased in week 2, in which 29 out of 32 animals were able to ejaculate within the 30 minutes, entailing almost 91% of the animals. There were no animals that were not able to ejaculate within the 10 weeks of training. In week 10, $SERT^{+/+}$ animals ejaculated on average 3.75 times, while the $SERT^{-/-}$ rats ejaculated on average 2.5 times. This indicates that $SERT^{-/-}$ rats stabilized their ejaculations at approximately 67% of the level of the $SERT^{+/+}$ rats.

Sexual behaviour male Serotonin Transporter Knockout rats over time.

In addition to the difference between genotypes, a significant difference in ejaculation frequency between the weeks was found ($F_{(5.290, 158.7)} = 14.62$; $p < 0.0001$). A post hoc revealed that the $SERT^{+/+}$ rats had significant difference for week 1 compared to the other weeks. Indicating that at least one week was necessary to stabilize the ejaculation frequency of the $SERT^{+/+}$ animals *(Appendix IVA)*. For $SERT^{-/-}$ rats, no rats ejaculated in the first week *(figure 2A)*, but increased their ejaculation frequency in week 2. Resulting in a significant difference between week 1 and the other weeks *(Appendix IV-A)*. Additionally, there was also a significant difference between week 3 and week 10 in the $SERT^{-/-}$ rats.

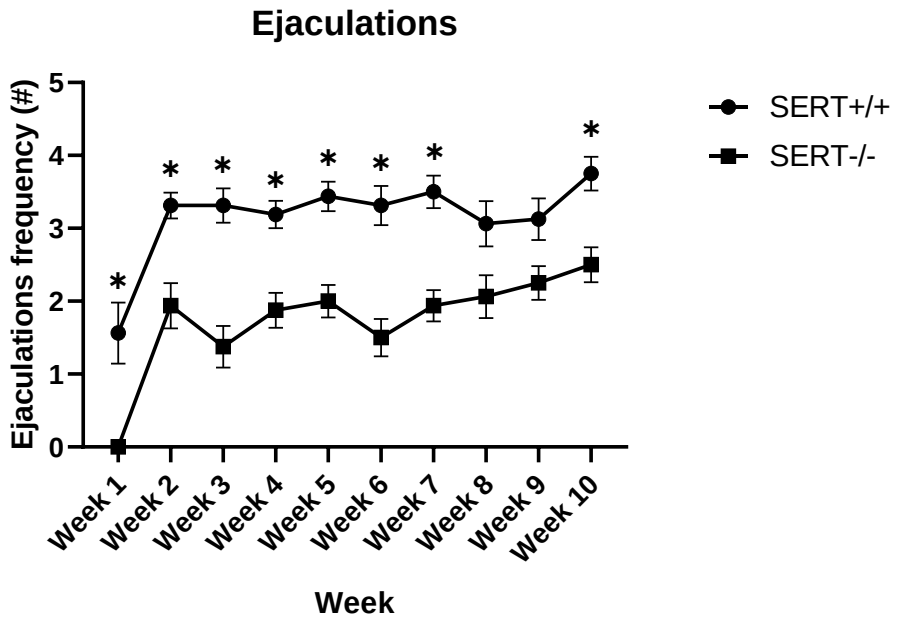


*Figure 1. Distribution and development of the number of ejaculations of the male rats tested over 10 weeks of training. Data are given as mean frequency of ejaculation ±SEM. n = 32 ($SERT^{+/+}$ n =16, $SERT^{-/-}$ n=16). * Indicates significant difference between the genotypes, p < 0.05*

Sexual stability in mount and intromission frequency:

In *Figure 2B and Figure 2C* the number of mounts and intromissions are presented. When performing a two-way ANOVA, a significant difference was found between the genotypes (Mount: $F_{(1,30)} = 25.48$; $p < 0.0001$; Intromissions: $F_{(1, 30)} = 15.05$; $p = 0.0005$). A subsequent post hoc revealed that both the mount and intromission frequency before the first ejaculation did not significantly differ in week 1. However, there were significant differences between the genotypes in week 2-5 *(Appendix IV-B and Appendix IV-C)*. $SERT^{-/-}$ rats had a significant higher frequency of mounts and intromissions

before the first ejaculation than the $SERT^{+/+}$ rats. The average number of mounts and intromissions is shown in *Appendix II*. In week 5, the $SERT^{+/+}$ rats have only 12% of the number of mounts, and 68% of the number of intromissions compared with $SERT^{-/-}$ rats. No significant differences between the different weeks were found within one genotype for the number of mounts or intromissions. In *Figure 2H* the efficiency of mounts and intromissions is presented. A two-way ANOVA was performed. The intromission ratio indicated a significant difference in genotype ($F_{(1, 30)}$ = 10,37; p = 0.0031). Next to the significant difference between the genotypes, there is also a significant difference found between the weeks ($F_{(4, 119)}$ = 2,853; p = 0.0267). However, a subsequent post hoc did not reveal further significance.

Sexual stability in ejaculation latency:

The data of the first ejaculation latency is presented in *Figure 2D.* When performing a two-way ANOVA on latency until the first ejaculation a significant effect of genotype was found ($F_{(1, 30)}$ = 61.57; p < 0.0001). The post hoc found that $SERT^{+/+}$ rats and $SERT^{-/-}$ rats significantly differed in every week *(Appendix III)*. The latency between the first sexual activity and the first ejaculation is significantly more for the $SERT^{-/-}$ rats compared to the $SERT^{+/+}$ rats. Additionally, the two-way ANOVA indicated a significant difference between the weeks *(*$F_{(2.962, 88.86)}$ = 37.12; *p < 0.0001)*. A subsequent post hoc revealed that week 1 significantly differed from the other weeks for both the $SERT^{+/+}$ and the $SERT^{-/-}$ rats *(Appendix V-A)*, the latency till ejaculating was much longer in the first week compared to the other weeks.

Sexual stability in mount and intromission latency:

In *Figure 2E and Figure 2F* the data of the first mount and first intromission latency is presented. A two-way ANOVA was performed in order to investigate whether a genotype difference is present for the first mount and intromission. No significant differences were found for the mount latency *(Appendix III)*. Furthermore, no significant differences were found between genotypes for the intromission latency *(Appendix III)*. There were no significant differences found between the weeks 9for mounts ($F_{(4, 72)}$ = 2.121; p = 0.0869*) (Appendix V-B)*. However, the two-way ANOVA did find a

significant difference between the weeks for the intromissions ($F_{(1.002,\ 18.04)} = 5.467$; $p = 0.0310$. However, a post hoc revealed no significant differences between the weeks for either genotype *(Appendix V-C).*

Post ejaculatory interval

The post ejaculatory interval (PEI) is presented in *Figure 2G.* In week 1 no time was registered for the $SERT^{-/-}$ rats. This was due to the fact that there were no ejaculations perceived in these rats, making the PEI unavailable. The statistical analyses was performed with the absence of ejaculation of $SERT^{-/-}$ rats in week 1. Therefore, the mixed-effect analysis was performed over week 2-5. Within those weeks no significant effect of genotype could be found, nor was there a significant difference found between the weeks.

Sexual behaviour male Serotonin Transporter Knockout rats over time.

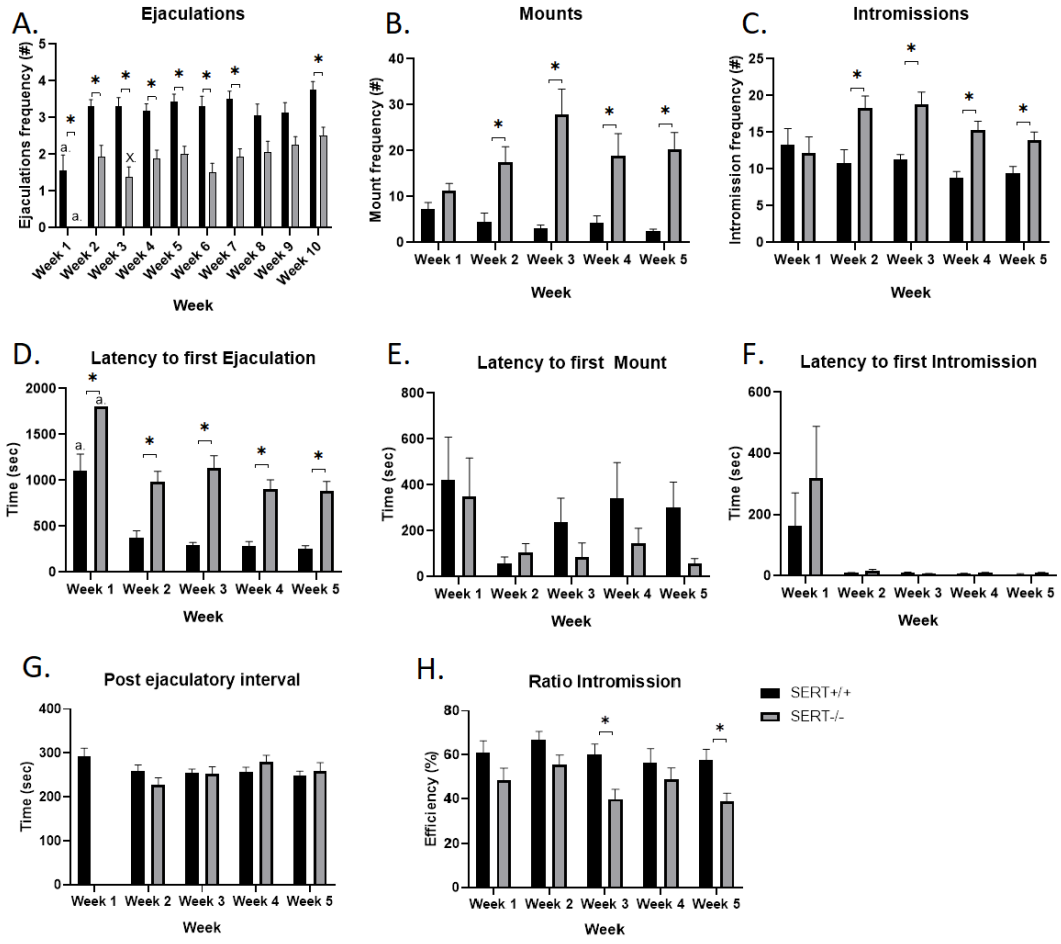


Figure 2. Sexual behaviour of male rats during the training phase. n=32 (SERT$^{+/+}$ n =16, SERT$^{-/-}$ n=16). Data are given as mean ±SEM. Further details of the statistical analyses of the frequency are provided in Table 2 and Appendix II. Details for the statistical analysis of the latency are provided in Table 3 and Appendix III.
***A.** The total number of ejaculations within 30 minutes over a period of 10 weeks.*
***B.** The total amount of mounts till the first ejaculation over a period of 5 weeks.*
***C.** The total amount of intromissions till the first ejaculation over a period of 5 weeks.*
***D.** The Latency to the first ejaculation (EL) has been calculated by subtracting the latency of the first sexual activity from the latency of the ejaculation. Indicating the time it took from the first sexual activity till the first ejaculation.*
***E.** The latency of the first mount (ML).*
***F.** latency to the first intromission (IL).*
***G.** Post Ejaculatory interval (PEI). The time between the first ejaculation and the subsequent first sexual activity over a period of 5 weeks.*
***H.** Ratio Intromission. Indicating the efficiency of intromissions till the first ejaculation*
** indicates significant difference between the genotypes. Symbol a. indicates the value being significantly different compared to all the other weeks within this genotype. Symbol X. indicated the value being significantly different compared to week 10.*

Discussion

This studied aimed to investigate the differences in sexual behaviour between $SERT^{+/+}$ rats and $SERT^{-/-}$ rats. Previous studies have found the lack of serotonin transporters increases the extracellular levels of serotonin in the $SERT^{-/-}$ rats, resulting in ninefold higher levels of extracellular 5-HT in male the $SERT^{-/-}$ rats than in the $SERT^{+/+}$ rats (Homberg et al., 2007; J. D. A. Olivier et al., 2008). The lack of the SERT caused a significant reduction in the number of ejaculations compared to control rats. Additionally, the latency until the first ejaculation was significantly increased in $SERT^{-/-}$ rats compared to $SERT^{+/+}$ rats in the first 5 weeks of training.

Additionally, the frequency of ejaculations in week 1 was significantly different to all the other weeks for both genotypes. This could be an indication that this week is needed to get experience on sexual behaviour. In the second week the training for sexual behaviour has been established for both genotypes, resulting in no significant differences between the weeks after the first week. This finding indicates that the sexual behaviour is stabilized for both genotypes from week 2 onwards. Even though sexual behaviour stabilizes for both genotypes, the ejaculation frequency of $SERT^{-/-}$ rats maintains significantly lower than that of the $SERT^{+/+}$ rats. A remarkable finding in this experiment is the lack of animals that did not ejaculate within the experiments. In an unpublished study by Esquivel-Franco it was reported that 5% of the $SERT^{+/+}$ rats and 20% of the $SERT^{-/-}$ rats did not ejaculate in a 6-week training period (Esquivel-Franco et al., unpublished). This finding was not supported in the current study. All animals in this experiment ejaculated at least once within 5 weeks of training, *see Appendix VI*.

The frequency of the sexual behaviour data for mounts and intromissions till the first ejaculation indicated that $SERT^{-/-}$ rats had significantly higher frequencies than the $SERT^{+/+}$ rats do, except for week 1. After week 1, there is an increase in mount and intromission frequency visible, mainly for the $SERT^{-/-}$ rats. Indicating that the $SERT^{-/-}$ rats need higher amount of stimulation to reach ejaculation. Overall, difference among the two genotypes show that the $SERT^{-/-}$ rats have a significantly

lower basal sexual behaviour than the $SERT^{+/+}$ rats. This difference remains stable even when training the rats on sexual behaviour. These findings support previously findings that serotonin can facilitate sexual behaviour, seeing that a lack of serotonin transporters results in a reduction of sexual behaviour. The latencies for the first mount and intromission had no significant differences.

In an unpublished study by Esquivel-Franco it was stated that if the lower sexual behaviour of the $SERT^{-/-}$ rats was caused by slow learning, repeated exposure to sexual experiences would let the difference disappear. However, the differences between ejaculation frequency maintained even after training, indicating that the differences in ejaculation frequency are not due to a slower learning process (Esquivel-Franco et al., unpublished). Regarding the post ejaculatory interval (PEI), there are no significant differences found between the genotypes nor between the weeks, indicating that the $SERT^{-/-}$ rat do not need significantly more time to resume sexual behaviour after the first ejaculation. Nevertheless, it is important to point out that the data analysis of the mounts and intromissions only include the first 5 weeks of training. It could be valuable in future research to score mounts and intromissions for week 6 till 10 as well to see whether other differences or similarities between the genotypes and weeks arise within this timeframe.

The $SERT^{+/+}$ rats have a significantly higher ejaculation frequency compared to $SERT^{-/-}$ rats in almost all the weeks, except for week 8 and 9. In week 8 and 9 some of the females were less receptive, resulting in a lower frequency of ejaculations than in the previous weeks. This reduced receptivity might be caused by an unsuccessful S.C. injection with oestradiol. Another explanation could be a variability in the injection time of oestradiol in relation to the time of testing. However, this is not the case seeing there was no relationship between time of day and unreceptive females. Nevertheless, the presence of these unreceptive females could have influenced the results in these weeks.

Previously studies have shown that the $SERT^{-/-}$ animals have less sensitive 5-HT1A receptors (Homberg et al., 2007; J. D. A. Olivier et al., 2008). This effect of a desensitized 5-HT1A receptor can be mimicked in $SERT^{+/+}$ rat by chronic administration of SSRIs, resulting in an increase in extracellular

serotonin levels (de Jong et al., 2005; Berend Olivier et al., 2010; Marcel D. Waldinger et al., 1998). It is found that 5-HT1A receptors play an important role in sexual behaviour (Snoeren et al., 2014). A reduction in 5HT1A receptor sensitivity and increase in extracellular serotonin levels in the $SERT^{-/-}$ rat may play a factor in the decreased sexual function in the $SERT^{-/-}$ rat (Esquivel-Franco et al., 2020). This effect is also supported by other studies, in which they showed that desensitization of the 5-HT1A receptor in $SERT^{-/-}$ rat is related to hindered sexual performance (Chan et al., 2011; Berend Olivier et al., 2010). Other possible serotonergic receptors that play a role in the decreased sexual behaviour in the $SERT^{-/-}$ rats remain to be studied.

This study indicates that the $SERT^{-/-}$ male rats can be used as a good resemblance for an animal model on chronic treatment of SSRIs. Such a model may be useful to study sexual dysfunction, such as premature ejaculation, delayed ejaculation or diminished pro-sexual behaviour). Additionally, this animal model can also be useful in the development of new antidepressant drugs, by getting a better understanding of the innerworkings of the serotonergic system.

Unfortunately, due to a delay in breeding there was too little time to perform and analyse the pharmaceutical experiments. The pharmacological studies are therefore not included in this paper.

Conclusion

$SERT^{+/+}$ and $SERT^{-/-}$ rats differ significantly in their ejaculation latency and copulation frequency, probably due to an increase in extracellular serotonin levels in combination with a desensitisation of the 5-HT1A receptor. It is important to note that the $SERT^{-/-}$ rats can stabilize their sexual behaviour, after one week of training, just as the $SERT^{+/+}$ rats do. Nevertheless, the stabilized ejaculation frequency is significantly lower than that of the $SERT^{+/+}$ rats. Additionally, the mount and intromission frequencies are significantly higher in the $SERT^{-/-}$ rats compared to control. Indicating that the $SERT^{-/-}$ rats need higher amounts of stimulation in order to ejaculate. The $SERT^{-/-}$ rats model can be used to further understand disturbances in the serotonergic system and consequential influence on sexual behaviour.

Reference list

Ahlenius, S., Larsson, K., Svensson, L., Hjorth, S., Carlsson, A., Lindberg, P., Wikström, H., Sanchez, D., Arvidsson, L. E., Hacksell, U., & Nilsson, J. L. G. (1981). Effects of a new type of 5-HT receptor agonist on male rat sexual behavior. *Pharmacology, Biochemistry and Behavior*, *15*(5), 785–792. https://doi.org/10.1016/0091-3057(81)90023-X

Althof, S. E., Abdo, C. H. N., Dean, J., Hackett, G., McCabe, M., McMahon, C. G., Rosen, R. C., Sadovsky, R., Waldinger, M., Becher, E., Broderick, G. A., Buvat, J., Goldstein, I., El-Meliegy, A. I., Giuliano, F., Hellstrom, W. J. G., Incrocci, L., Jannini, E. A., Park, K., … Tan, H. M. (2010). International Society for Sexual Medicine's Guidelines for the Diagnosis and Treatment of Premature Ejaculation. *Journal of Sexual Medicine*, *7*(9), 2947–2969. https://doi.org/10.1111/j.1743-6109.2010.01975.x

Althof, S. E., Mcmahon, C. G., Waldinger, M. D., Serefoglu, E. C., Shindel, A. W., Adaikan, P. G., Becher, E., Dean, J., Giuliano, F., Hellstrom, W. J. G., Giraldi, A., Glina, S., Incrocci, L., Jannini, E., Mccabe, M., Parish, S., Rowland, D., Segraves, R. T., Sharlip, I., & Torres, L. O. (2014). An Update of the International Society of Sexual Medicine's Guidelines for the Diagnosis and Treatment of Premature Ejaculation (PE). *Sexual Medicine*, *2*(2), 60–90. https://doi.org/10.1002/sm2.28

Berman, J. R. (2005). Physiology of female sexual function and dysfunction. *International Journal of Impotence Research*, *17*(SUPPL. S1), 44–51. https://doi.org/10.1038/sj.ijir.3901428

Butcher, M. J., Zubert, T., Christiansen, K., Carranza, A., Pawlicki, P., & Seibel, S. (2020). Topical Agents for Premature Ejaculation: A Review. *Sexual Medicine Reviews*, *8*(1), 92–99. https://doi.org/10.1016/j.sxmr.2019.03.003

Byers, E. S., & Grenier, G. (2003). Premature or Rapid Ejaculation: Heterosexual Couples' Perceptions of Men's Ejaculatory Behavior. *Archives of Sexual Behavior*, *32*(3), 261–270. https://doi.org/10.1023/A:1023417718557

Carson, C., & Gunn, K. (2006). Premature ejaculation: Definition and prevalence. *International Journal of Impotence Research*, *18*(SUPPL. 1). https://doi.org/10.1038/sj.ijir.3901507

Chan, J. S. W., Snoeren, E. M. S., Cuppen, E., Waldinger, M. D., Olivier, B., & Oosting, R. S. (2011). The Serotonin Transporter Plays an Important Role in Male Sexual Behavior: A Study in Serotonin Transporter Knockout Rats. *Journal of Sexual Medicine*, *8*(1), 97–108. https://doi.org/10.1111/j.1743-6109.2010.01961.x

Christensen, B. S., Grønbæk, M., Osler, M., Pedersen, B. V., Graugaard, C., & Frisch, M. (2011). Associations between physical and mental health problems and sexual dysfunctions in sexually active danes. *Journal of Sexual Medicine*, *8*(7), 1890–1902. https://doi.org/10.1111/j.1743-6109.2010.02145.x

Ciocanel, O., Power, K., & Eriksen, A. (2019). Interventions to Treat Erectile Dysfunction and Premature Ejaculation: An Overview of Systematic Reviews. *Sexual Medicine*, *7*(3), 251–269. https://doi.org/10.1016/j.esxm.2019.06.001

Cooper, K., James, M. M. S., Kaltenthaler, E., Dickinson, K., & Cantrell, A. (2015). Interventions to treat premature ejaculation: A systematic review short report. *Health Technology Assessment*, *19*(21), 1–180. https://doi.org/10.3310/hta19210

Coskuner, E. R., & Ozkan, B. (2021). Premature ejaculation and endocrine disorders: A literature review. *World Journal of Men's Health*, *39*(1), 38–51. https://doi.org/10.5534/WJMH.200184

De Hong, C., Ren, L. L., Yu, H., & Qiang, W. (2014). The role of dapoxetine hydrochloride on-demand for the treatment of men with premature ejaculation. *Scientific Reports*, *4*, 1–7. https://doi.org/10.1038/srep07269

de Jong, T. R., Pattij, T., Veening, J. G., Waldinger, M. D., Cools, A. R., & Olivier, B. (2005). Effects of chronic selective serotonin reuptake inhibitors on 8-OH-DPAT-induced facilitation of ejaculation in rats: comparison of fluvoxamine and paroxetine. *Psychopharmacology*, *179*(2), 509–515.

https://doi.org/10.1007/s00213-005-2186-6

de Jong, T. R., Pattij, T., Veening, J. G., Dederen, P. J. W. C., Waldinger, M. D., Cools, A. R., & Olivier, B. (2005). Citalopram combined with WAY 100635 inhibits ejaculation and ejaculation-related Fos immunoreactivity. European Journal of Pharmacology, 509(1), 49–59. https://doi.org/10.1016/j.ejphar.2004.12.024

Du, Y., Jiang, Y., Zhang, J., Tian, G., Zhang, N., Wu, D., & Bai, X. (2019). Efficacy and safety of on-demand dapoxetine in treatment of patients with premature ejaculation: A meta-analysis. *Medical Science Monitor*, *25*, 4225–4232. https://doi.org/10.12659/MSM.913606

El-Hamd, M. A., Saleh, R., & Majzoub, A. (2019). Premature ejaculation: an update on definition and pathophysiology. *Asian Journal of Andrology*, *21*, 425–442. https://doi.org/10.4103/aja.aja

Esquivel-Franco, D., Wiegersma, M., Janssen, J., Colaprete, C., Wesselink, L., Olivier, B., & Olivier, J. (2020). PS-3-9 Sexual Behavior in Male Serotonin Transporter Knockout Rats. *The Journal of Sexual Medicine*, *17*(6), S131. https://doi.org/10.1016/j.jsxm.2020.04.041

Feldman, H. A., Irwin, G., Hatzichristou, D. G., Krane, R. J., & McKinlay, J. B. (1994). Impotence and its medical and psychosocial correlates: results of the massachusetts male aging study. In *The Journal of Urology* (Vol. 151, pp. 54–61).

Geng, H., Peng, D., Huang, Y., Tang, D., Gao, J., Zhang, Y., & Zhang, X. (2019). Changes in sexual performance and biochemical characterisation of functional neural regions: A study in serotonin transporter knockout male rats. *Andrologia*, *51*(7), 1–9. https://doi.org/10.1111/and.13291

Homberg, J. R., Olivier, J. D. A., Smits, B. M. G., Mul, J. D., Mudde, J., Verheul, M., Nieuwenhuizen, O. F. M., Cools, A. R., Ronken, E., Cremers, T., Schoffelmeer, A. N. M., Ellenbroek, B. A., & Cuppen, E. (2007). Characterization of the serotonin transporter knockout rat: A selective change in the functioning of the serotonergic system. *Neuroscience*, *146*(4), 1662–1676. https://doi.org/10.1016/j.neuroscience.2007.03.030

Hutchinson, K., Cruickshank, K., & Wylie, K. (2012). A benefit-risk assessment of dapoxetine in the treatment of premature ejaculation. *Drug Safety*, *35*(5), 359–372. https://doi.org/10.2165/11598150-000000000-00000

Jern, P., Johansson, A., Piha, J., Westberg, L., & Santtila, P. (2015). Antidepressant treatment of premature ejaculation: Discontinuation rates and prevalence of side effects for dapoxetine and paroxetine in a naturalistic setting. *International Journal of Impotence Research*, *27*(2), 75–80. https://doi.org/10.1038/ijir.2014.37

Lewis, R. W., Fugl-Meyer, K. S., Bosch, R., Fugl-Meyer, A. R., Laumann, E. O., Lizza, E., & Martin-Morales, A. (2004). Epidemiology/risk factors of sexual dysfunction. *Journal of Sexual Medicine*, *1*(1), 35–39. https://doi.org/10.1111/j.1743-6109.2004.10106.x

Martyn-St James, M., Cooper, K., Kaltenthaler, E., Dickinson, K., Cantrell, A., Wylie, K., Frodsham, L., & Hood, C. (2015). Tramadol for premature ejaculation: A systematic review and meta-analysis Sexual function and fertility. *BMC Urology*, *15*(1), 1–11. https://doi.org/10.1186/1471-2490-15-6

Martyn-St James, M., Cooper, K., Ren, K., Kaltenhaler, E., Dickinson, K., Cantrell, A., Wylie, K., Frodsham, L., & Hood, C. (2016). Topical anaesthetics for premature ejaculation: a systematic review and meta-analysis. *Sex Health*, *13*, 114–123.

Martyn-St James, M., Cooper, K., Ren, S., Kaltenthaler, E., Dickinson, K., Cantrell, A., Wylie, K., Frodsham, L., & Hood, C. (2017). Phosphodiesterase Type 5 Inhibitors for Premature Ejaculation: A Systematic Review and Meta-analysis. *European Urology Focus*, *3*(1), 119–129. https://doi.org/10.1016/j.euf.2016.02.001

McMahon, C. G. (2015). Current and Emerging Treatments for Premature Ejaculation. *Sexual Medicine Reviews*, *3*(3), 183–202. https://doi.org/10.1002/smrj.49

McMahon, C. G., Althof, S. E., Waldinger, M. D., Porst, H., Dean, J., Sharlip, I. D., Adaikan, P. G., Becher,

E., Broderick, G. A., Buvat, J., Dabees, K., Giraldi, A., Giuliano, F., Hellstrom, W. J. G., Incrocci, L., Laan, E., Meuleman, E., Perelman, M. A., Rosen, R. C., … Segraves, R. (2008). An evidence-based definition of lifelong premature Ejaculation: Report of the international society for Sexual medicine (ISSM) ad hoc committee for the definition of premature ejaculation. *Journal of Sexual Medicine*, *5*(7), 1590–1606. https://doi.org/10.1111/j.1743-6109.2008.00901.x

Mcmahon, C. G., & Porst, H. (2011). Oral agents for the treatment of premature ejaculation: Review of efficacy and safety in the context of the recent international society for sexual medicine criteria for lifelong premature ejaculation. *Journal of Sexual Medicine*, *8*(10), 2707–2725. https://doi.org/10.1111/j.1743-6109.2011.02386.x

Mullins, C. D., Shaya, F. T., Meng, F., Wang, J., & Harrison, D. (2005). Persistence, switching, and discontinuation rates among patients receiving sertraline, paroxetine, and citalopram. *Pharmacotherapy*, *25*(5 I), 660–667. https://doi.org/10.1592/phco.25.5.660.63590

Looney, C., Thor, K. B., Ricca, D., & Marson, L. (2005). Differential effects of simultaneous or sequential administration of paroxetine and WAY-100,635 on ejaculatory behavior. *Pharmacology Biochemistry and Behavior*, *82*(3), 427–433. https://doi.org/10.1016/j.pbb.2005.09.014

Olivier, B., van Oorschot, R., & Waldinger, M. D. (1998). Serotonin, serotonergic receptors, selective serotonin reuptake inhibitors and sexual behaviour. *International Clinical Psychopharmacology*, *13*(6), S9–S14.

Olivier, Berend, Chan, J. S. W., Snoeren, E. M. S., Olivier, J. D. A., Veening, J. G., Vinkers, C. H., Waldinger, M. D., & Oosting, R. S. (2010). Differences in Sexual Behaviour in Male and Female Rodents: Role of Serotonin. *Current Topics in Behavioural Neurosciences*, *8*, 15–36. https://doi.org/10.1007/7854

Olivier, J. D. A., Van Der Hart, M. G. C., Van Swelm, R. P. L., Dederen, P. J., Homberg, J. R., Cremers, T.,

Deen, P. M. T., Cuppen, E., Cools, A. R., & Ellenbroek, B. A. (2008). A study in male and female 5-HT transporter knockout rats: An animal model for anxiety and depression disorders. *Neuroscience*, *152*(3), 573–584. https://doi.org/10.1016/j.neuroscience.2007.12.032

Park, H. J., Park, N. C., Kim, T. N., Baek, S. R., Lee, K. M., & Choe, S. (2017). Discontinuation of Dapoxetine Treatment in Patients With Premature Ejaculation: A 2-Year Prospective Observational Study. *Sexual Medicine*, *5*(2), e99–e105. https://doi.org/10.1016/j.esxm.2017.02.003

Rowland, D. L., Patrick, D. L., Rothman, M., & Gagnon, D. D. (2007). The Psychological Burden of Premature Ejaculation. *Journal of Urology*, *177*(3), 1065–1070. https://doi.org/10.1016/j.juro.2006.10.025

Rowland, D., Perelman, M., Althof, S., Barada, J., McCullough, A., Bull, S., Jamieson, C., & Ho, K. F. (2004). Self-reported premature ejaculation and aspects of sexual functioning and satisfaction. *Journal of Sexual Medicine*, *1*(2), 225–232. https://doi.org/10.1111/j.1743-6109.2004.04033.x

Serefoglu, E. C., Mcmahon, C. G., Waldinger, M. D., Althof, S. E., Shindel, A., Adaikan, G., Becher, E. F., Dean, J., Giuliano, F., Hellstrom, W. J. G., Giraldi, A., Glina, S., Incrocci, L., Jannini, E., Mccabe, M., Parish, S., Rowland, D., Segraves, R. T., Sharlip, I., & Torres, L. O. (2014). An evidence-based unified definition of lifelong and acquired premature ejaculation: Report of the second international society for sexual medicine Ad Hoc committee for the definition of premature ejaculation. *Journal of Sexual Medicine*, *11*(6), 1423–1441. https://doi.org/10.1111/jsm.12524

Smits, B. M. G., Mudde, J. B., Van De Belt, J., Verheul, M., Olivier, J., Homberg, J., Guryev, V., Cools, A. R., Ellenbroek, B. A., Plasterk, R. H. A., & Cuppen, E. (2006). Generation of gene knockouts and mutant models in the laboratory rat by ENU-driven target-selected mutagenesis. *Pharmacogenetics and Genomics*, *16*(3), 159–169. https://doi.org/10.1097/01.fpc.0000184960.82903.8f

Snoeren, E. M. S., Veening, J. G., Olivier, B., & Oosting, R. S. (2014). Serotonin 1A receptors and sexual behavior in male rats: A review. *Pharmacology Biochemistry and Behavior*, *121*, 102–114. https://doi.org/10.1016/j.pbb.2013.11.007

Sunay, D., Sunay, M., Aydoğmuş, Y., Bağbanci, S., Arslan, H., Karabulut, A., & Emir, L. (2011). Acupuncture versus paroxetine for the treatment of premature ejaculation: A randomized, placebo-controlled clinical trial. *European Urology*, *59*(5), 765–771. https://doi.org/10.1016/j.eururo.2011.01.019

Symonds, T., Roblin, D., Hart, K., & Althof, S. (2003). How does premature ejaculation impact a man's life? *Journal of Sex and Marital Therapy*, *29*(5), 361–370. https://doi.org/10.1080/00926230390224738

Tan, H. M., Tong, S. F., & Ho, C. C. K. (2012). Men's health: Sexual dysfunction, physical, and psychological health-is there a link? *Journal of Sexual Medicine*, *9*(3), 663–671. https://doi.org/10.1111/j.1743-6109.2011.02582.x

Thomas, H. N., & Thurston, R. C. (2016). A biopsychosocial approach to women's sexual function and dysfunction at midlife: A narrative review. *Maturitas*, *87*, 49–60. https://doi.org/10.1016/j.maturitas.2016.02.009

Waldinger, M. D., Zwinderman, A. H., Schweitzer, D. H., & Olivier, B. (2004). Relevance of methodological design for the interpretation of efficacy of drug treatment of premature ejaculation: A systematic review and meta-analysis. *International Journal of Impotence Research*, *16*(4), 369–381. https://doi.org/10.1038/sj.ijir.3901172

Waldinger, M.D. (2014). Pharmacotherapy for premature ejaculation. *Current Opinion in Psychiatry*, *27*(6), 400–405. https://doi.org/10.1097/YCO.0000000000000096

Waldinger, M.D. (2018). Drug treatment options for premature ejaculation. *Expert Opinion on Pharmacotherapy*, *19*(10), 1077–1085. https://doi.org/10.1080/14656566.2018.1494725

Waldinger, Marcel D. (2002). The neurobiological approach to premature ejaculation. *Journal of Urology*, *168*(6), 2359–2367. https://doi.org/10.1016/S0022-5347(05)64146-8

Waldinger, Marcel D., Berendsen, H. . H. G., Blok, B. F. M., Olivier, B., & Holstege, G. (1998). Premature ejaculation and serotonergic antidepressants-induced delayed ejaculation: The involvement of the serotonergic system. *Behavioural Brain Research*, *92*(2), 111–118. https://doi.org/10.1016/S0166-4328(97)00183-6

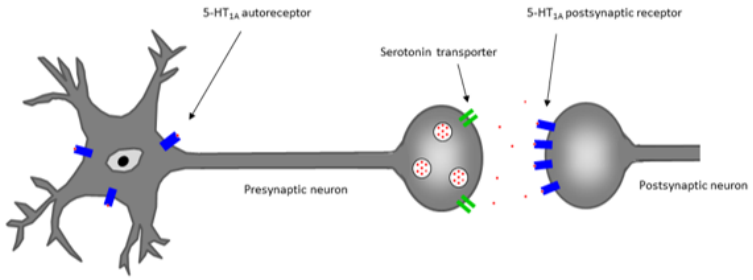
Waldinger, Marcel D., Zwinderman, A. H., & Olivier, B. (2004). On-demand treatment of premature ejaculation with clomipramine and paroxetine: A randomized, double-blind fixed-dose study with stopwatch assessment. *European Urology*, *46*(4), 510–516. https://doi.org/10.1016/j.eururo.2004.05.005

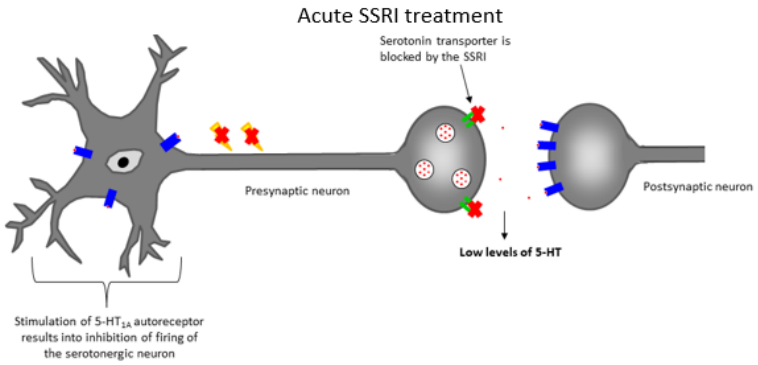
Wyllie, M. G., & Powell, J. A. (2012). The role of local anaesthetics in premature ejaculation. *BJU International*, *110*(11 C). https://doi.org/10.1111/j.1464-410X.2012.11323.x

Yue, F. G., Dong, L., Hu, T. T., & Qu, X. Y. (2015). Efficacy of Dapoxetine for the Treatment of Premature Ejaculation: A Meta-analysis of Randomized Clinical Trials on Intravaginal Ejaculatory Latency Time, Patient-reported Outcomes, and Adverse Events. *Urology*, *85*(4), 856–861. https://doi.org/10.1016/j.urology.2015.01.009

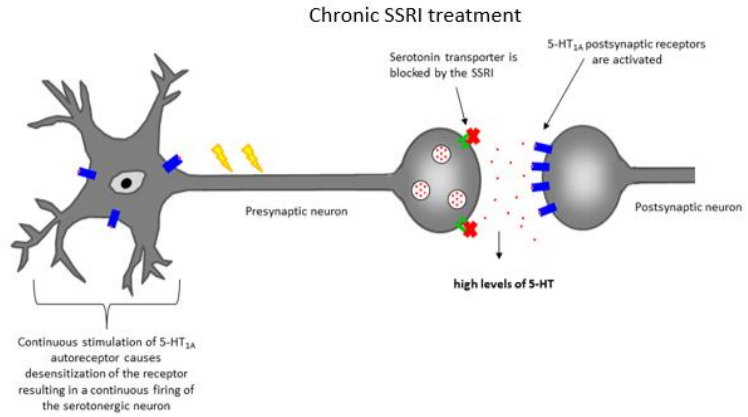
Appendix

Appendix I: Serotonergic neurotransmission

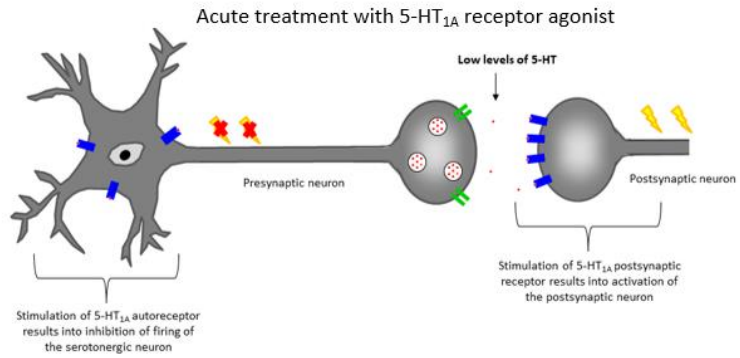




No effect on sexual performance

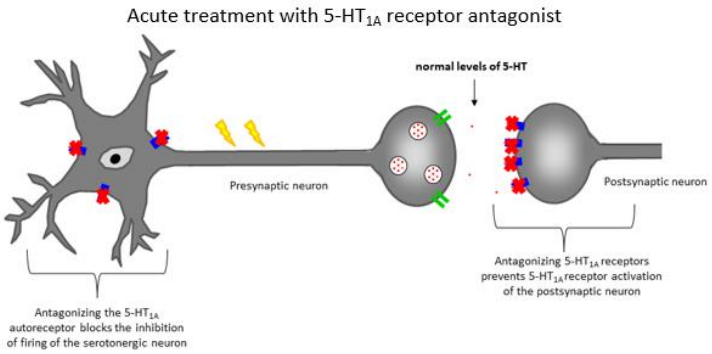


Less ejaculations, increased mounts and intromissions and longer ejaculation latencies

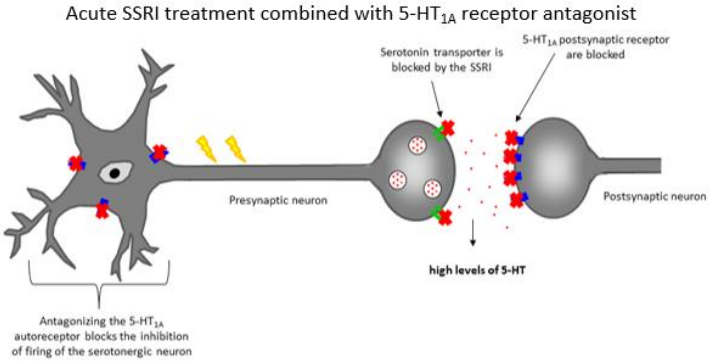


More ejaculations, decreased mounts and intromissions and shorter ejaculation latencies

Sexual behaviour male Serotonin Transporter Knockout rats over time.



No effect on sexual performance, only a high dose will lead to less ejaculations



Less ejaculations, increased mounts and intromissions and longer ejaculation latencies

Appendix II: Frequency – differences between genotypes: post hoc Bonferroni's multiple comparison test

Frequency – Genotype differences *post hoc Bonferroni's multiple comparison test*

Ejaculation

Week	Mean WT	Mean KO	p value	Significant
Week 1	1.563	0.000	0.0198	*
Week 2	3.313	1.938	0.0075	**
Week 3	3.313	1.375	0.0001	***
Week 4	3.188	1.875	0.0017	**
Week 5	3.438	2.000	0.0005	***
Week 6	3.313	1.500	0.0004	***
Week 7	3.500	1.938	0.0002	***
Week 8	3.063	2.063	0.2621	ns
Week 9	3.125	2.250	0.2478	ns
Week 10	3.750	2.500	0.0081	**

Mounts

Week	Mean WT	Mean KO	p value	Significant
Week 1	7.250	11.31	0.2833	ns
Week 2	4.563	17.50	0.0125	*
Week 3	3.000	27.94	0.0019	**
Week 4	4.313	18.94	0.0456	*
Week 5	2.438	20.38	0.0007	***

Intromissions

Week	Mean WT	Mean KO	p value	Significant
Week 1	13.31	12.19	>0.9999	ns
Week 2	10.81	18.25	0.0280	*
Week 3	11.25	18.75	0.0035	**
Week 4	8.813	15.31	0.0008	***
Week 5	9.438	13.94	0.0210	*

Appendix III: Latency – differences between genotypes: post hoc Bonferroni's multiple comparison test

Latency – Genotype differences *post hoc Bonferroni's multiple comparison test*

Ejaculation

Week	Mean WT	Mean KO	p value	Significant
Week 1	1105	1800	0.0082	**
Week 2	373.1	985.9	0.0006	***
Week 3	295.7	1131	<0.0001	****
Week 4	281.6	905.2	<0.0001	****
Week 5	252.2	884.7	<0.0001	****

Mounts

Week	Mean WT	Mean KO	p value	Significant
Week 1	421.1	169.4	0.3575	ns
Week 2	57.06	108.0	>0.9999	ns
Week 3	236.7	86.19	>0.9999	ns
Week 4	343.8	146.8	0.7840	ns
Week 5	301.7	56.61	0.3955	ns

]

Intromissions

Week	Mean WT	Mean KO	p value	Significant
Week 1	163.9	140.5	>0.9999	ns
Week 2	9.024	17.10	0.4243	ns
Week 3	10.04	6.880	>0.9999	ns
Week 4	6.396	9.252	>0.9999	ns
Week 5	5.456	9.352	>0.9999	ns

Appendix IV: Frequency – differences between weeks: post hoc Tukey's multiple comparison test

Appendix IV-A: Frequency differences between weeks - Ejaculations

Frequency – Week differences *post hoc Tukey's multiple comparison test*

Ejaculation

WT	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	1.563	3.313	<0.0001	****
Week 1 vs. Week 3	1.563	3.313	<0.0001	****
Week 1 vs. Week 4	1.563	3.188	<0.0001	****
Week 1 vs. Week 5	1.563	3.438	<0.0001	****
Week 1 vs. Week 6	1.563	3.313	<0.0001	****
Week 1 vs. Week 7	1.563	3.500	<0.0001	****
Week 1 vs. Week 8	1.563	3.063	0.0003	***
Week 1 vs. Week 9	1.563	3.125	0.0001	***
Week 1 vs. Week 10	1.563	3.750	<0.0001	****
Week 2 vs. Week 3	3.313	3.313	>0.9999	ns
Week 2 vs. Week 4	3.313	3.188	>0.9999	ns
Week 2 vs. Week 5	3.313	3.438	>0.9999	ns
Week 2 vs. Week 6	3.313	3.313	>0.9999	ns
Week 2 vs. Week 7	3.313	3.500	>0.9999	ns
Week 2 vs. Week 8	3.313	3.063	0.9990	ns
Week 2 vs. Week 9	3.313	3.125	>0.9999	ns
Week 2 vs. Week 10	3.313	3.750	0.9432	ns
Week 3 vs. Week 4	3.313	3.188	>0.9999	ns
Week 3 vs. Week 5	3.313	3.438	>0.9999	ns
Week 3 vs. Week 6	3.313	3.313	>0.9999	ns
Week 3 vs. Week 7	3.313	3.500	>0.9999	ns
Week 3 vs. Week 8	3.313	3.063	0.9990	ns
Week 3 vs. Week 9	3.313	3.125	>0.9999	ns
Week 3 vs. Week 10	3.313	3.750	0.9432	ns
Week 4 vs. Week 5	3.188	3.438	0.9990	ns
Week 4 vs. Week 6	3.188	3.313	>0.9999	ns
Week 4 vs. Week 7	3.188	3.500	0.9942	ns
Week 4 vs. Week 8	3.188	3.063	>0.9999	ns
Week 4 vs. Week 9	3.188	3.125	>0.9999	ns
Week 4 vs. Week 10	3.188	3.750	0.7812	ns
Week 5 vs. Week 6	3.438	3.313	>0.9999	ns
Week 5 vs. Week 7	3.438	3.500	>0.9999	ns
Week 5 vs. Week 8	3.438	3.063	0.9790	ns
Week 5 vs. Week 9	3.438	3.125	0.9942	ns
Week 5 vs. Week 10	3.438	3.750	0.9942	ns
Week 6 vs. Week 7	3.313	3.500	>0.9999	ns
Week 6 vs. Week 8	3.313	3.063	0.9990	ns
Week 6 vs. Week 9	3.313	3.125	>0.9999	ns
Week 6 vs. Week 10	3.313	3.750	0.9432	ns
Week 7 vs. Week 8	3.500	3.063	0.9432	ns
Week 7 vs. Week 9	3.500	3.125	0.9790	ns
Week 7 vs. Week 10	3.500	3.750	0.9990	ns
Week 8 vs. Week 9	3.063	3.125	>0.9999	ns
Week 8 vs. Week 10	3.063	3.750	0.5244	ns
Week 9 vs. Week 10	3.125	3.750	0.6588	ns

Sexual behaviour male Serotonin Transporter Knockout rats over time.

Frequency – Week differences *post hoc Tukey's multiple comparison test*

Ejaculation

KO	Mean 1	Mean 2	p value	Significant
Week 1 vs. Week 2	0.000	1.938	<0.0001	****
Week 1 vs. Week 3	0.000	1.375	0.0014	**
Week 1 vs. Week 4	0.000	1.875	<0.0001	****
Week 1 vs. Week 5	0.000	2.000	<0.0001	****
Week 1 vs. Week 6	0.000	1.500	0.0003	***
Week 1 vs. Week 7	0.000	1.938	<0.0001	****
Week 1 vs. Week 8	0.000	2.063	<0.0001	****
Week 1 vs. Week 9	0.000	2.250	<0.0001	****
Week 1 vs. Week 10	0.000	2.500	<0.0001	****
Week 2 vs. Week 3	1.938	1.375	0.7812	ns
Week 2 vs. Week 4	1.938	1.875	>0.9999	ns
Week 2 vs. Week 5	1.938	2.000	>0.9999	ns
Week 2 vs. Week 6	1.938	1.500	0.9432	ns
Week 2 vs. Week 7	1.938	1.938	>0.9999	ns
Week 2 vs. Week 8	1.938	2.063	>0.9999	ns
Week 2 vs. Week 9	1.938	2.250	0.9942	ns
Week 2 vs. Week 10	1.938	2.500	0.7812	ns
Week 3 vs. Week 4	1.375	1.875	0.8781	ns
Week 3 vs. Week 5	1.375	2.000	0.6588	ns
Week 3 vs. Week 6	1.375	1.500	>0.9999	ns
Week 3 vs. Week 7	1.375	1.938	0.7812	ns
Week 3 vs. Week 8	1.375	2.063	0.5244	ns
Week 3 vs. Week 9	1.375	2.250	0.1867	ns
Week 3 vs. Week 10	1.375	2.500	0.0227	*
Week 4 vs. Week 5	1.875	2.000	>0.9999	ns
Week 4 vs. Week 6	1.875	1.500	0.9790	ns
Week 4 vs. Week 7	1.875	1.938	>0.9999	ns
Week 4 vs. Week 8	1.875	2.063	>0.9999	ns
Week 4 vs. Week 9	1.875	2.250	0.9790	ns
Week 4 vs. Week 10	1.875	2.500	0.6588	ns
Week 5 vs. Week 6	2.000	1.500	0.8781	ns
Week 5 vs. Week 7	2.000	1.938	>0.9999	ns
Week 5 vs. Week 8	2.000	2.063	>0.9999	ns
Week 5 vs. Week 9	2.000	2.250	0.9990	ns
Week 5 vs. Week 10	2.000	2.500	0.8781	ns
Week 6 vs. Week 7	1.500	1.938	0.9432	ns
Week 6 vs. Week 8	1.500	2.063	0.7812	ns
Week 6 vs. Week 9	1.500	2.250	0.3936	ns
Week 6 vs. Week 10	1.500	2.500	0.0716	ns
Week 7 vs. Week 8	1.938	2.063	>0.9999	ns
Week 7 vs. Week 9	1.938	2.250	0.9942	ns
Week 7 vs. Week 10	1.938	2.500	0.7812	ns
Week 8 vs. Week 9	2.063	2.250	>0.9999	ns
Week 8 vs. Week 10	2.063	2.500	0.9432	ns
Week 9 vs. Week 10	2.250	2.500	0.9990	ns

Appendix IV-B: Frequency differences between weeks - Mounts

Frequency – Week differences *post hoc Tukey's multiple comparison test*

Mounts

WT	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	7.250	4.563	0.4248	ns
Week 1 vs. Week 3	7.250	3.000	0.0705	ns
Week 1 vs. Week 4	7.250	4.313	0.5541	ns
Week 1 vs. Week 5	7.250	2.438	0.0311	*
Week 2 vs. Week 3	4.563	3.000	0.9081	ns
Week 2 vs. Week 4	4.563	4.313	>0.9999	ns
Week 2 vs. Week 5	4.563	2.438	0.7890	ns
Week 3 vs. Week 4	3.000	4.313	0.9401	ns
Week 3 vs. Week 5	3.000	2.438	0.9482	ns
Week 4 vs. Week 5	4.313	2.438	0.7398	ns
KO	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	11.31	17.50	0.4740	ns
Week 1 vs. Week 3	11.31	27.94	0.0841	ns
Week 1 vs. Week 4	11.31	18.94	0.5246	ns
Week 1 vs. Week 5	11.31	20.38	0.2215	ns
Week 2 vs. Week 3	17.50	27.94	0.0584	ns
Week 2 vs. Week 4	17.50	18.94	0.9904	ns
Week 2 vs. Week 5	17.50	20.38	0.9216	ns
Week 3 vs. Week 4	27.94	18.94	0.5009	ns
Week 3 vs. Week 5	27.94	20.38	0.4301	ns
Week 4 vs. Week 5	18.94	20.38	0.9979	ns

Appendix IV-C: Frequency differences between weeks - Intromissions

Frequency – Week differences *post hoc Tukey's multiple comparison test*

Intromissions

WT	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	13.31	10.81	0.8643	ns
Week 1 vs. Week 3	13.31	11.25	0.8878	ns
Week 1 vs. Week 4	13.31	8.813	0.3162	ns
Week 1 vs. Week 5	13.31	9.438	0.2630	ns
Week 2 vs. Week 3	10.81	11.25	0.9992	ns
Week 2 vs. Week 4	10.81	8.813	0.7616	ns
Week 2 vs. Week 5	10.81	9.438	0.9597	ns
Week 3 vs. Week 4	11.25	8.813	0.2445	ns
Week 3 vs. Week 5	11.25	9.438	0.5701	ns
Week 4 vs. Week 5	8.813	9.438	0.9920	ns
KO	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	12.19	18.25	0.2092	ns
Week 1 vs. Week 3	12.19	18.75	0.1400	ns
Week 1 vs. Week 4	12.19	15.31	0.5943	ns
Week 1 vs. Week 5	12.19	13.94	0.9256	ns
Week 2 vs. Week 3	18.25	18.75	0.9996	ns
Week 2 vs. Week 4	18.25	15.31	0.3217	ns
Week 2 vs. Week 5	18.25	13.94	0.2960	ns
Week 3 vs. Week 4	18.75	15.31	0.2521	ns
Week 3 vs. Week 5	18.75	13.94	0.0369	*
Week 4 vs. Week 5	15.31	13.94	0.7698	ns

Appendix V: Latency – differences between weeks: post hoc Tukey's multiple comparison test

Appendix V-A: Latency differences between weeks - Ejaculations

Latency – Week differences *post hoc Tukey's multiple comparison test*

Ejaculation

WT	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	1105	373.1	0.0040	**
Week 1 vs. Week 3	1105	295.7	0.0025	**
Week 1 vs. Week 4	1105	281.6	0.0014	**
Week 1 vs. Week 5	1105	252.2	0.0024	**
Week 2 vs. Week 3	373.1	295.7	0.8369	ns
Week 2 vs. Week 4	373.1	281.6	0.7652	ns
Week 2 vs. Week 5	373.1	252.2	0.6037	ns
Week 3 vs. Week 4	295.7	281.6	0.9990	ns
Week 3 vs. Week 5	295.7	252.2	0.9059	ns
Week 4 vs. Week 5	281.6	252.2	0.9818	ns
KO	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	1800	985.9	<0.0001	****
Week 1 vs. Week 3	1800	1131	0.0017	**
Week 1 vs. Week 4	1800	905.2	<0.0001	****
Week 1 vs. Week 5	1800	884.7	<0.0001	****
Week 2 vs. Week 3	985.9	1131	0.5927	ns
Week 2 vs. Week 4	985.9	905.2	0.9262	ns
Week 2 vs. Week 5	985.9	884.7	0.9330	ns
Week 3 vs. Week 4	1131	905.2	0.5165	ns
Week 3 vs. Week 5	1131	884.7	0.3797	ns
Week 4 vs. Week 5	905.2	884.7	0.9999	ns

Appendix V-B: Latency differences between weeks - Mounts

Latency – Week differences *post hoc Tukey's multiple comparison test*

Mount

WT	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	421.1	57.06	0.1456	ns
Week 1 vs. Week 3	421.1	236.7	0.7610	ns
Week 1 vs. Week 4	421.1	343.8	0.9875	ns
Week 1 vs. Week 5	421.1	301.7	0.9395	ns
Week 2 vs. Week 3	57.06	236.7	0.7777	ns
Week 2 vs. Week 4	57.06	343.8	0.3590	ns
Week 2 vs. Week 5	57.06	301.7	0.5212	ns
Week 3 vs. Week 4	236.7	343.8	0.9586	ns
Week 3 vs. Week 5	236.7	301.7	0.9935	ns
Week 4 vs. Week 5	343.8	301.7	0.9988	ns
KO	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	349.4	108.0	0.5345	ns
Week 1 vs. Week 3	349.4	86.19	0.4472	ns
Week 1 vs. Week 4	349.4	146.8	0.6920	ns
Week 1 vs. Week 5	349.4	56.61	0.3380	ns
Week 2 vs. Week 3	108.0	86.19	>0.9999	ns
Week 2 vs. Week 4	108.0	146.8	0.9991	ns
Week 2 vs. Week 5	108.0	56.61	0.9974	ns
Week 3 vs. Week 4	86.19	146.8	0.9951	ns
Week 3 vs. Week 5	86.19	56.61	0.9997	ns
Week 4 vs. Week 5	146.8	56.61	0.9778	ns

Appendix V-C: Latency differences between weeks - Intromissions

Latency – Week differences *post hoc Tukey's multiple comparison test*

Intromissions

WT	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	163.9	9.024	0.6165	ns
Week 1 vs. Week 3	163.9	10.04	0.6253	ns
Week 1 vs. Week 4	163.9	6.396	0.6042	ns
Week 1 vs. Week 5	163.9	5.456	0.6020	ns
Week 2 vs. Week 3	9.024	10.04	0.9982	ns
Week 2 vs. Week 4	9.024	6.396	0.2662	ns
Week 2 vs. Week 5	9.024	5.456	0.5942	ns
Week 3 vs. Week 4	10.04	6.396	0.8001	ns
Week 3 vs. Week 5	10.04	5.456	0.5980	ns
Week 4 vs. Week 5	6.396	5.456	0.9966	ns
KO	**Mean 1**	**Mean 2**	**p value**	**Significant**
Week 1 vs. Week 2	320.5	17.10	0.4342	ns
Week 1 vs. Week 3	320.5	6.880	0.3924	ns
Week 1 vs. Week 4	320.5	9.252	0.4038	ns
Week 1 vs. Week 5	320.5	9.352	0.3962	ns
Week 2 vs. Week 3	17.10	6.880	0.1910	ns
Week 2 vs. Week 4	17.10	9.252	0.5729	ns
Week 2 vs. Week 5	17.10	9.352	0.5453	ns
Week 3 vs. Week 4	6.880	9.252	0.8644	ns
Week 3 vs. Week 5	6.880	9.352	0.7846	ns
Week 4 vs. Week 5	9.252	9.352	>0.9999	ns

Appendix VI: Frequency – Number of rats ejaculating specific amount per week

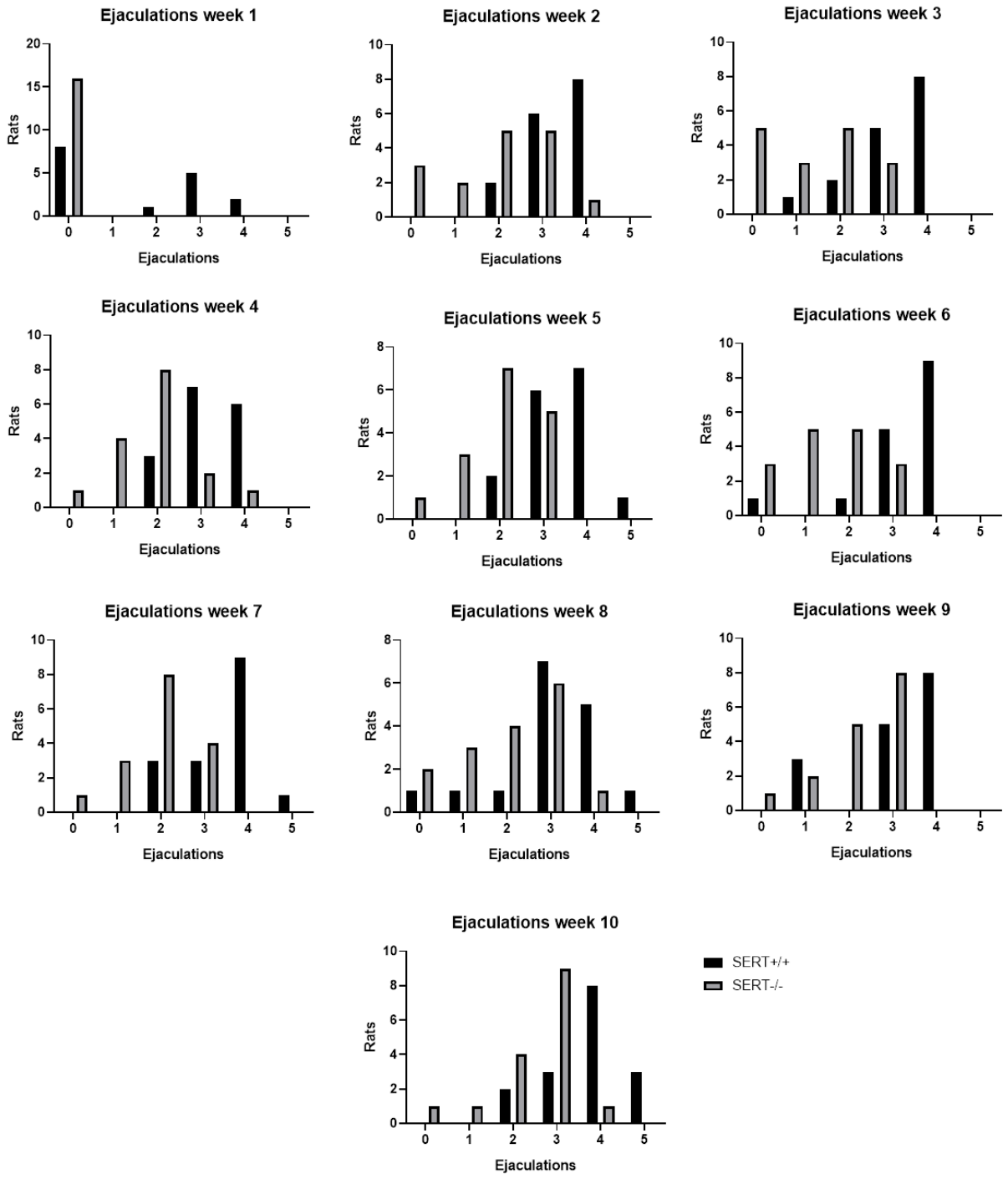


Figure 3: Number of rats per week that ejaculated a specific amount.