ACUTE ADMINISTRATION OF HIGH DOSES OF TRAMADOL STRONGLY INHIBITS SEXUAL BEHAVIOUR IN MALE RATS

Name: Lauren Wesselink

Student number: S2340925

Supervised by: Dr. Ing. J.D.A. Olivier

Report Master Research Project

Biomedical Sciences – University of Groningen

ACUTE ADMINISTRATION OF HIGH DOSES OF TRAMADOL STRONGLY INHIBITS SEXUAL BEHAVIOUR IN MALE RATS

ABSTRACT

INTRODUCTION: Premature ejaculation (PE) is a common male sexual dysfunction. Selective serotonin reuptake inhibitors (SSRI) can be used as a treatment to delay the ejaculation latency. However, chronic use of SSRIs can lead to undesired side effects, which makes acute treatment preferable. Tramadol is mainly used as an analgesic that possess effects as a μ-opioid receptor agonists, SSRI and norepinephrine reuptake inhibitor. Recently, tramadol was shown to be effective in treating PE when administered chronically. The aim of this research is to determine if the acute administration of tramadol is a suitable treatment for PE.

METHODS: Three groups of male wistar rats were studied regarding the serotonin transporter; wildtypes (5-HTT$^{+/+}$), heterozygous (5-HTT$^{+/-}$) and knockouts (5-HTT$^{-/-}$). Sexually experienced male rats received five doses of tramadol (saline, 5, 10, 20, 40 and 50 mg/kg). A receptive female was introduced for 30 minutes to test male sexual behaviour after treatment.

RESULTS: In all groups, high doses of tramadol (40 and 50 mg/kg) significantly inhibited sexual behaviour, including decreases in ejaculation, mount and intromission frequency, an increased ejaculation, mount and intromission latency and a decreased intromission ratio. The post-ejaculatory interval did not differ between genotypes.

DISCUSSION: Acutely administered, high doses of tramadol strongly inhibited sexual behaviour in male rats. Future research will focus on the role of both the 5-HT$_{1A}$- and μ-opioid receptors in the tramadol-mediated inhibition of sexual behaviour.

KEYWORDS: Premature ejaculation, male sexual behaviour, tramadol, μ-opioid receptor, 5-HT$_{1A}$- receptor

INTRODUCTION

According to the DSM-V, 'sexual dysfunctions are a heterogenous group of disorders that are typically characterized by a clinically significant disturbance in a person's ability to respond sexually or to experience sexual pleasure. Male sexual dysfunctions include delayed ejaculation, erectile disorder, hypoactive sexual desire disorder, premature (early) ejaculation, substance/medication-induced sexual dysfunction, other specified sexual dysfunction, and unspecified sexual dysfunction' (American Psychiatric Association, 2013).

Of all sexual dysfunctions, premature ejaculation (PE) is the most prevalent and affects around 20% to 30% of the male population (Rosen et al., 2004). PE is defined by the International Society for Sexual Medicine (ISSM) as "a male sexual dysfunction characterized by ejaculation which always or nearly always occurs prior to or within the first minute of vaginal penetration and the inability to delay ejaculation on all or nearly all vaginal penetrations, and negative personal consequences, such as distress, bother, frustration and/or the avoidance of sexual intimacy" (McMahon et al., 2008). It has been proposed that PE has psychological and neurobiological components (Morales et al., 2007). Recent studies have shown that selective serotonin reuptake inhibitors (SSRIs) are an "effective" alternative to treat PE (Waldinger et al., 1998; Waldinger et al., 2001; Waldinger & Olivier, 2004).

SSRIs are mainly used as antidepressants due to inhibition of the serotonin uptake pump (Waldinger et al., 1998), leading to an increased level of serotonin in the synaptic cleft. However, the chronic use of SSRIs has been associated with the appearance of some side effects like a decrease in the

ability to reach ejaculation or orgasm (Balon, 2006); thus, resulting in a significant impact on the individuals' life quality which can lead to noncompliance with the treatment (Higgins, Nash, & Lynch, 2010).

High extracellular levels of serotonin (5-HT) inhibit ejaculation and orgasm (Schweitzer et al., 2009). As serotonin is involved in both norepinergic and dopaminergic signaling, it is possible that SSRIs have an effect on sexual behavior through these systems (Bijlsma et al., 2014). It is proposed that chronic SSRI-treatment increases the overall serotonin-mediated tonic inhibition of sexual behavior circuits by increasing serotonergic activity in projection areas (Bijlsma et al., 2014). It has been demonstrated that the serotonergic activation of sexual activity in male rats is mainly based on the effects of 5-HT_{1A} receptor agents (Snoeren et al., 2014), and the potential sexual side effects during chronic SSRI-treatment can be defined by the degree of the 5-HT_{1A} receptor activation (De Jong et al., 2005).

Tramadol is used worldwide as a centrally acting analgesic and it produces anti-nociception by binding to μ-opioid receptors (Hennies, Friderichs, & Schneider, 1988). Research has shown that chronically administered tramadol was effective as a treatment for PE in humans (Eassa & El-Shazly, 2013; Yang et al., 2013). Tramadol is a racemic mixture, which is involved in μ-opioid pathways, enhanced serotonin release by selective sertonergic reuptake inhibition (SSRI) and inhibited norepinephrine reuptake (Matthiesen et al., 1998; Olivier et al., 2016). It was stated that tramadol induces antidepressant-like effects (Rojas-Corrales et al., 2002; Rojas-Corrales et al., 1998).

Previously, Olivier et al. (2017) postulated that tramadol inhibits sexual behavior in male wildtype rats mainly through its SSRI properties. Its μ-opioid receptor agonistic activity was proposed to have a small effect on inhibited sexual behavior. In this research, the role of acute administration of tramadol on inhibited sexual behavior will be further clarified. Sexual behavior of male 5-HT-transporter knockout rats (5-$HTT^{-/-}$) was observed and results were compared to observations of 5-HTT heterozygous rats (5-$HTT^{+/-}$) and wildtypes (5-$HTT^{+/+}$). As SSRIs block serotonin transporters, these 5-HTT models will contribute to unravelling mechanisms preventing sexual inhibition, so only additional effects causing sexual dysfunction were examined.

During acute tramadol treatment, the following results are hypothesized. In 5-$HTT^{+/+}$ ejaculation frequency will decrease and ejaculation latency will increase compared to control (saline-treated). These effects are expected at a high dose of tramadol (50 mg/kg), which are in line with previous research (Olivier et al., 2017). In 5-$HTT^{+/-}$ the ejaculation frequency will slightly decrease and ejaculation latency will slightly increase compared to control, as 5-$HTT^{+/-}$ rats are less sensitive to SSRIs due to the mutation in the serotonin transporter. In 5-$HTT^{-/-}$ it is hypothesized that ejaculation frequency and -latency will not change compared to control. The SSRI-component will not be functioning due to the complete knockout of the serotonin transporter gene. Therefore, the aim of this research is to investigate acute administration of tramadol as a potent treatment for premature ejaculation.

METHODS

Male subjects

Male Wistar rats (6 months old, 350-500 g), were studied, which were wildtypes (referred to as 5-HTT$^{+/+}$), had reduced serotonin transporter functioning (heterozygous, referred to as 5-HTT $^{+/-}$), or lacked the serotonin transporter gene (knockout, referred to as 5-HTT$^{-/-}$).

Female subjects

Female rats were used for sexual behaviour experiments. Double tubal ligations were performed on the female subjects under anaesthesia and pain relief was given until 48 hours after surgery. Prior to the experiment (36 to 48 hours), females were injected subcutaneously with estradiol (50 µg oestradiol dissolved in 0,1 ml walnut-oil with lecithin). A female was considered receptive when lordosis was shown during sexual attempts by a male subject. Females were used for sexual behaviour experiments once every week for a maximum of three times a day with resting periods.

Both male- and female subjects were housed in groups (4-5 per cage) and light-dark conditions were reversed (10 a.m.-10 p.m.). Cages were supplemented with cage enrichments and food and water were provided *ad libitum*. Experiments were performed under red light from 11 a.m. to 5 p.m. All experiments were performed in accordance with the law on animal research and were approved by the ethical committee of the University of Groningen.

Sexual behaviour training

All male rats (5-HTT$^{+/+}$, 5-HTT$^{+/-}$ and 5-HTT$^{-/-}$) were trained to assess and stabilize basal sexual activity. Only male subjects ejaculating on a stable level were included in follow-up experiments (n=12 for all genotypes). A stable ejaculation level was considered when the number of ejaculations during the training session was one or more for at least the last two weeks during training sessions. For wildtypes and heterozygous subjects, only the ones that showed a stable, 'normal' sexual performance (2-3 ejaculations per 30 minutes (Chan et al., 2008)) were selected for follow-up experiments. Sexual behaviour in male rats is characterized by appetitive aspects (latency until first mount) and consummatory aspects (intromission latencies, ejaculation latencies, mount frequencies and intromission frequencies) (Ågmo, 1999; Chan et al., 2008). Subjects were trained once a week for 30 minutes for 6 times in total. First, male rats were given 10 minutes to habituate to the test chamber (60x40x30 cm, supplemented with bedding). Hereafter, sexual behaviour was assessed by introducing a receptive female for 30 minutes (1800 seconds). Scoring sexual behaviour (mount, intromission and ejaculation) was carried out with Noldus Observer XT10.5 (Noldus Information Technology, Wageningen, the Netherlands).

Treatment (dose response curve)

After six weeks of training, five doses of tramadol (5, 10, 20, 40 and 50 mg/kg) and a placebo (saline, 0,9% NaCl) were tested in a randomised design (within subjects, n=12 per group). One week was used as a washout period. After intraperitoneal (IP) injection, subjects were placed in the test chamber for 30 minutes for the subjects to habituate. The sexual behaviour test was performed in the same way as the training. Tramadol was provided as powder in capsules of 50 mg (Tramadolhydrochloride, Centrafarm B.V., Etten-Leur, provided by a local pharmacy). All solutions were prepared on the morning before the first day of experiments. Two consecutive days were used to perform sexual behaviour tests. Subjects performed sexual behaviour tests during alternating time slots.

Data analysis

The number of mounts and intromissions per ejaculation series were scored. Based on these data, the following parameters were determined. Ejaculation latency (EL)(s), which is the time that is needed to ejaculate from the first intromission onwards (first session), or from the moment the male rat attempts to perform sexual behaviour in the sessions hereafter (mount or intromission). Mount latency (ML) (s) or intromission latency (IL) (s) is the time until the first mount or intromission, after introducing a female or after ejaculation. Post ejaculatory interval (PEI) (s) is the time between ejaculation and the first mount or intromission in the session hereafter. Intromission ratio (IR) was used to display effectiveness of sexual behaviour during ejaculation series and was calculated as (number of mounts + number of intromissions) / ejaculation latency. In the case of low or no sexual behaviour, a latency of 1800 s was used where data were missing (EL, ML and IL). If there were no mounts during the ejaculatory serie, but the animal performed an intromission, the intromission latency was also used as the mount latency. When an intromission was performed before a mount, the intromission latency was used as the mount latency. If values were not available due to the fact that animals had not performed behaviour, the data were not considered for the corresponding parameter analysis. Because of this, only the first ejaculatory serie was used to accomplish the data analysis.

Statistical analysis

All data were analysed with Graphpad Prism 5. A Friedman's test was performed. An ANOVA for repeated measurements followed by a post-hoc test (Dunnett) for selected columns were used to analyse the results per dose compared to control. For the post-ejaculatory interval a Kruskal-Wallis test and a two-way ANOVA with a Bonferroni post-hoc test was performed to determine significance, using mean and standard error. Results yielding a p-value <0.05 were considered significant. Data are expressed as mean ± SEM.

RESULTS

Dose response curve

To determine the effects of tramadol (0, 5, 10, 20, 40 and 50 mg/kg) on sexual behaviour, a dose response curve was made and a Spearman's correlation test was performed. Ejaculation frequency is negatively correlated with tramadol treatment in all groups (figure 1; appendix I).

Ejaculation, mount and intromission frequency

Overall, ejaculation frequency was significantly decreased in all genotypes after administrating doses of 40 and 50 mg/kg compared to control. Also, ejaculation frequency was significantly reduced in the $5HTT^{+/-}$ group at dose 20 mg/kg (figure 2a; appendix II). Mount frequency was significantly reduced in all groups at dose 50 mg/kg compared to control. At dose 40 mg/kg, mount frequency was significantly diminished in $5\text{-}HTT^{+/+}$ and $5\text{-}HTT^{-/-}$ (figure 2b; appendix III).

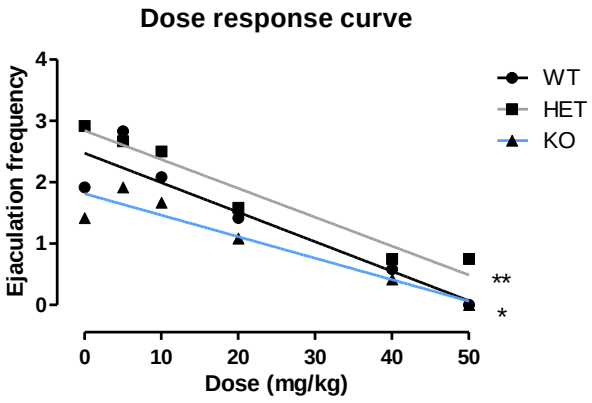


Figure 1. Dose response curve of the effects of tramadol treatment on ejaculation frequency. In all genotypes, the dose was negatively correlated to ejaculation frequency. * = p>0,05 and ** = p>0,01.

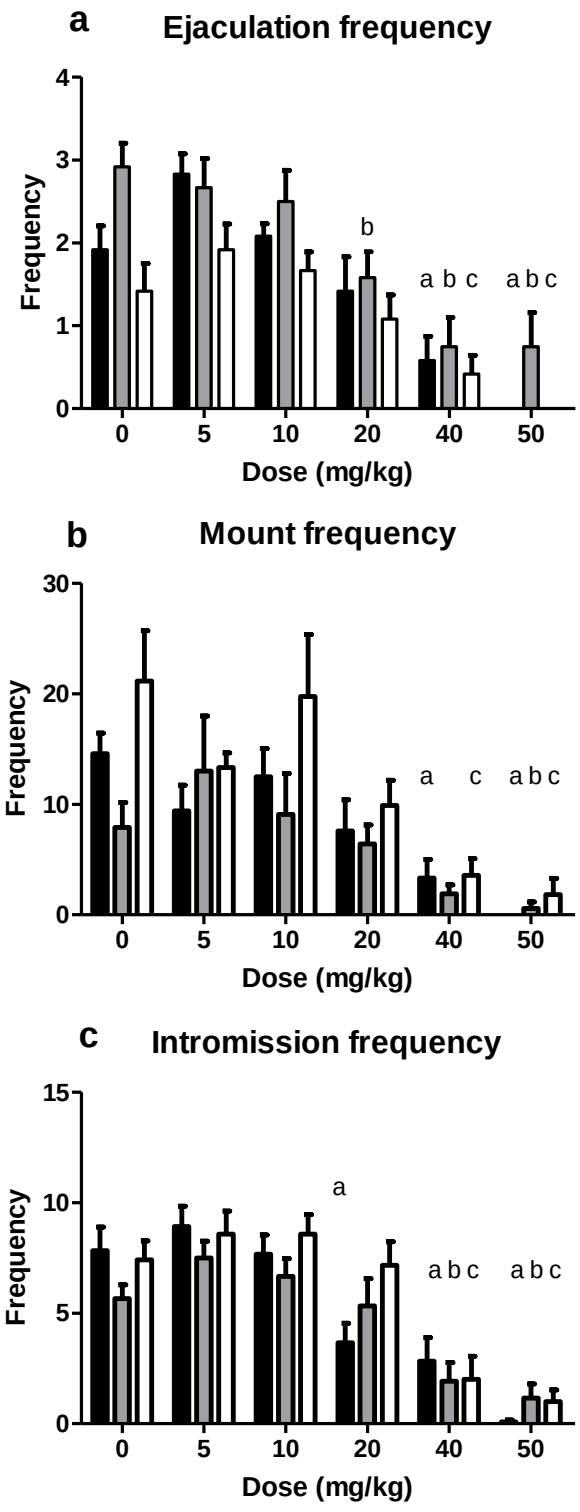


Figure 2. Tramadol-mediated inhibitory effects on ejaculation- (fig 2a), mount- (fig 2b) and intromission frequency (fig 2c) sexual behaviour were shown in all genotypes using high doses. ■ = 5-HTT$^{+/+}$, ■ = 5-HTT$^{+/-}$ and □ = 5-HTT$^{-/-}$. a=significant compared to 5-HTT$^{+/+}$ control, b=significant compared to 5-HTT$^{+/-}$ control, c=significant compared to 5-HTT$^{-/-}$ control. Data are represented as mean ± SEM.

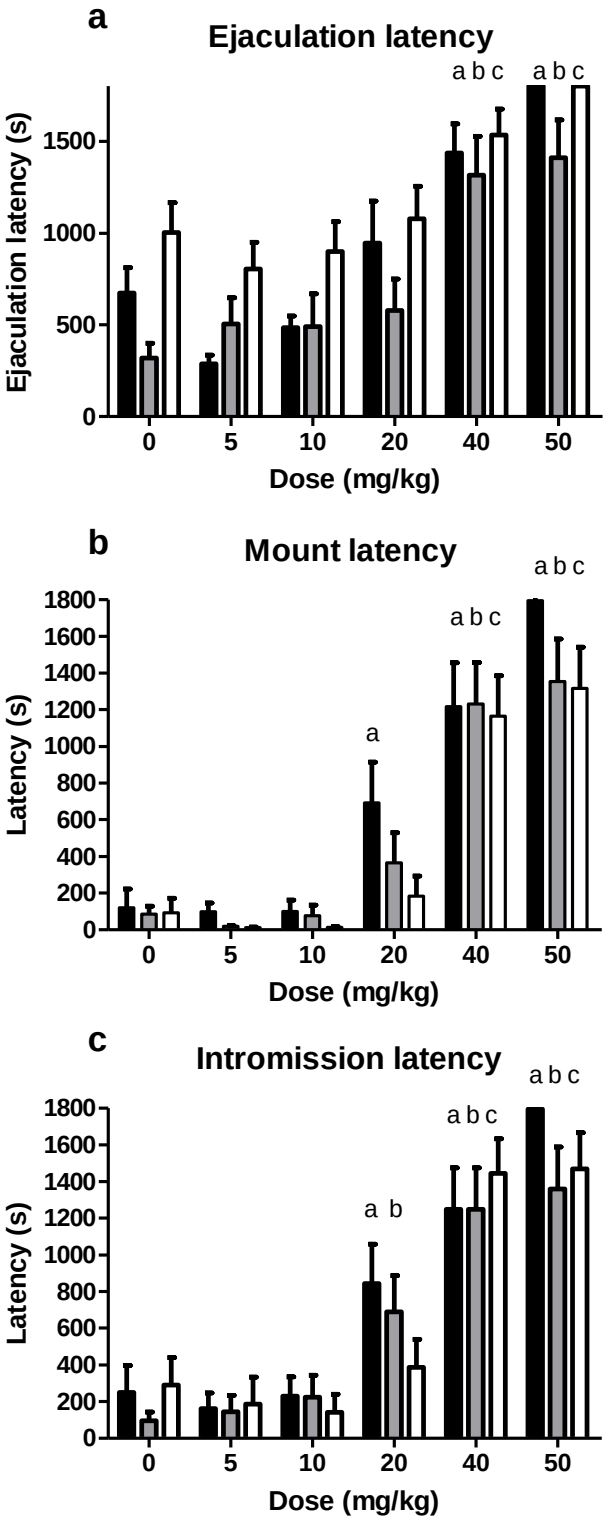


Figure 3. Ejaculation- (fig 3a), mount- (fig 3b) and intromission latency (fig 3c) were all significantly increased in all genotypes after tramadol administration. ■ = 5-HTT$^{+/+}$, ■ = 5-HTT$^{+/-}$ and □ = 5-HTT$^{-/-}$. a=significant compared to 5-HTT$^{+/+}$ control, b=significant compared to 5-HTT$^{+/-}$ control, c=significant compared to 5-HTT$^{-/-}$ control. Data are represented as mean ± SEM.

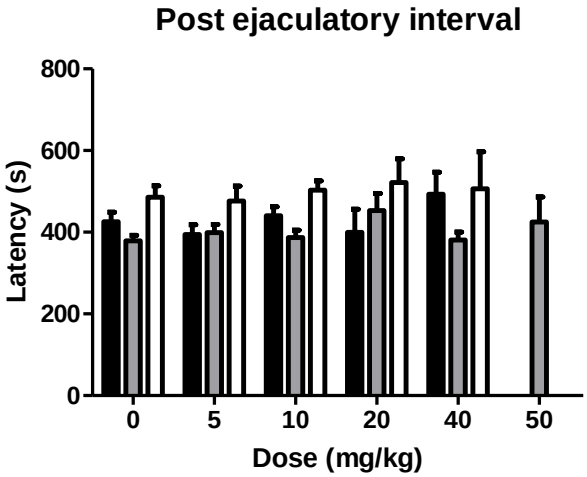


Figure 4. Tramadol treatment did not alter the post-ejaculatory interval compared to control. ■ = 5-HTT$^{+/+}$, ▒ = 5-HTT$^{+/-}$ and □ = 5-HTT$^{-/-}$. Data are represented as mean ± SEM.

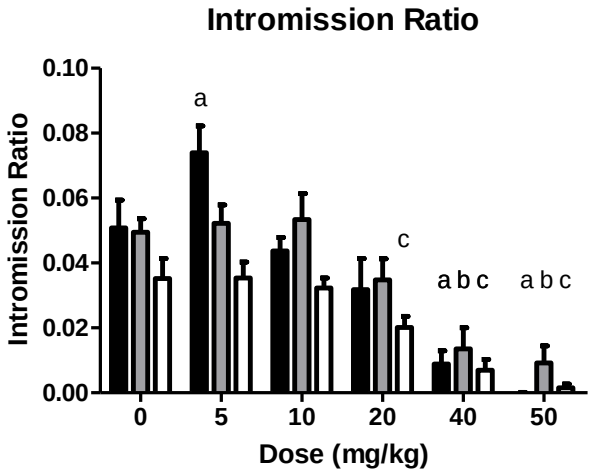


Figure 5. Intromission ratio was significantly decreased in all genotypes after tramadol administration. ■ = 5-HTT+/+ , ▒ = 5-HTT+/- and □ = 5-HTT-/-. a=significant compared to 5-HTT+/+ control, b=significant compared to 5-HTT+/- control, c=significant compared to 5-HTT-/- control. Data are represented as mean ± SEM.

Moreover, intromission frequency was significantly declined in all groups at dose 40 and dose 50 mg/kg compared to control. Dose 20 mg/kg only affected intromission frequency in 5-HTT$^{+/+}$ compared to control (figure 2c; appendix IV).

Ejaculation-, mount- and intromission latency

Ejaculation latency was significantly increased in all genotypes after administrating doses of 40 and 50 mg/kg compared to control (figure 3; appendix V). Mount latency was significantly enhanced in all groups at dose 40 and 50 mg/kg compared to control. Also, administration of dose 20 mg/kg significantly enlarged mount latency in 5-HTT$^{+/+}$ compared to control (figure 3; appendix VI). Furthermore, intromission latency was significantly increased in all groups at dose 40 and dose 50 mg/kg compared to control. Dose 20 mg/kg affected intromission latency in 5-HTT$^{+/+}$ and 5-HTT$^{+/-}$ compared to control (figure 3; appendix VII).

Post-ejaculatory interval

In all groups, there was no dose-dependent effect on PEI compared to control (appendix X). Data is missing at dose 50 mg/kg in both 5-HTT$^{+/+}$ and 5-HTT$^{-/-}$ groups, simply because of the fact that there was no ejaculation, or even no sexual behaviour, in these groups (figure 4; appendix VIII).

Intromission ratio

A significant decline was seen in all groups at dose 40 and dose 50 mg/kg compared to control (figure 5; appendix IX).

Others

Body weights remained stable during treatment (appendix XI). Two types of side effects were reported (appendix XII).

DISCUSSION

Acutely administered, tramadol significantly affected sexual behaviour in male rats at doses 40 mg/kg and 50 mg/kg in all groups (5-HTT$^{+/+}$, 5-HTT$^{+/-}$ and 5-HTT$^{-/-}$). Dose 20 mg/kg was also effective in inhibiting sexual behaviour in some parameters (EF, IF, ML,

IL, IR), but not in all genotypes. After administration of high doses (40 and 50 mg/kg), ejaculation-, mount- and intromission frequency were decreased, indicating that tramadol inhibits all aspects of sexual behaviour.

Moreover, ejaculation latency is significantly increased in all groups, underlining the effect of tramadol as treatment for PE by delaying ejaculation. Mount- and intromission latency were increased, demonstrating that the animals were not motivated or able to perform sexual behaviour. Intromission ratio was decreased, suggesting that the subjects need less mounts and/or intromissions per ejaculation latency to reach ejaculation. Post ejaculatory interval did not differ between groups.
The results are in contrast with our hypothesis, as it was expected that sexual behaviour in wildtypes would be affected by tramadol treatment, heterozygous subjects would slightly be affected and knockouts would not be affected.

Previously, Olivier et al. (2017) found some inhibitory trends on sexual behaviour after tramadol treatment at dose 40 mg/kg. Sexual behaviour was demonstrated to be inhibited at dose 50 mg/kg, which is in line with current research.

The groups consisted of the same size (n=12), but the subjects were not tested with a within-subject design and only had 4 weeks of training.
Previously, it was presumed that high doses of tramadol would not or slightly affect the knockouts, as the mechanism of action was thought to mainly act via the SSRI component and to a smaller extent via the μ-opioid agonistic mechanism (Olivier et al., 2017). Current research shows that sexual behaviour is inhibited in all genotypes (5-HTT+/+, 5-HTT+/- and 5-HTT-/-; dose 40 and 50 mg/kg), so it is likely to assume that these inhibitory effects are mediated via the μ-opioid receptor.

In former research, naloxone, a μ-opioid receptor antagonist, showed no inhibitory effects at low doses (5 and 10 mg/kg), but strongly inhibited sexual behaviour at dose 20 mg/kg, suggesting a possible role of the μ-opioid receptor in inhibiting sexual behaviour. Combined with tramadol (20 mg/kg), sexual behaviour was also inhibited (Olivier et al., 2017), suggesting that the inhibition was caused by antagonizing the μ-opioid receptor or by the SSRI-component of tramadol. Moreover, sexual behaviour was not inhibited after WAY100,635 administration (1,0 mg/kg; 5-HT_{1A} receptor antagonist), but combined treatment with tramadol (25 mg/kg) showed inhibitory effects on EF, ML, IL, EL and PEI. (Olivier et al., 2017). The latter underlines the role of μ-opioid receptor in inhibiting sexual behaviour. Serotonin is often linked to sexual behaviour, but recently it was reviewed that 5-HT_{1A} receptors might not have an essential role in regulating male sexual behaviour under basal conditions. It was suggested that 5-HT_{1A} receptors decrease sexual behaviour when the 5-HT_{1A} receptors are desensitized due to chronically elevated serotonin levels (Snoeren et al., 2014), which might explain why there was no inhibited sexual behaviour after WAY100,635 treatment. Furthermore, it has been shown that the μ-opioid receptor is associated with sexual behaviour and that endogenous opioid release is related to ejaculation (Agmo & Paredes, 1988; Agmo & Berenfeld, 1990).

Overall, it is possible that both the 5-HT_{1A} and μ-opioid pathway (or an interaction of both receptors) are involved in inhibiting sexual behaviour. To further investigate these effects, following research will demonstrate the effects of acute tramadol administration combined with antagonists. To study the effects of the tramadol's SSRI-

component on sexual behaviour, a 5-HT-$_{1A}$ receptor antagonist (WAY100,635) will be administered (0,1; 0,3 and 1 mg/kg) and compared to control. The influence of the μ-opioid receptor on sexual behaviour will be further examined with administration of the μ-opioid receptor antagonist naloxone (5, 10 and 20 mg/kg) compared to control. After defining the optimal dose, both antagonists will be combined with tramadol to further study the underlying effects of both receptors on sexual behaviour and to determine the contribution of both receptors in inhibiting sexual behaviour after acute tramadol administration. In addition, besides the μ-opioid receptor agonist and SSRI-component, tramadol also has norepinephrine reuptake inhibiting effects. As tramadol acts in a synergistic way (Lehmann, 1997), it is important to also take the mechanism of action of norepinephrine into account. Future research on the role of tramadol-mediated norepinephrine reuptake inhibition is recommended.

Furthermore, tramadol is generally well tolerated and the most common side effects include nausea and emesis (Lehmann, 1997). However, due to the high dose of tramadol (40 and 50 mg/kg), it is possible that adverse effects occur, which might account for the inhibited sexual behaviour due to the fact that the subjects were physically unable to perform sexual behaviour. After administrating high doses (40 and 50 mg/kg), it was observed that subjects mainly remained on the same spot in the test chamber and showed no exploratory- or sexual behaviour during habituation or after introducing a female. These possible adverse effects need to be taken into account in future research.

Additionally, as the subjects with a 5-HTT$^{-/-}$ genotype did not overall ejaculate that much, only animals with a stable ejaculation level were taken for current experiments (≥1 ejaculations/30 minutes in training-week 5 and 6). It is questionable whether these animals are suitable as a model for PE. In the 5-HTT$^{+/+}$ and 5-HTT$^{+/-}$ groups, only the 'average ejaculating animals' were used (2-3 ejaculations/30 minutes in training week 5 and 6).

CONCLUSION

The results indicate that tramadol inhibits sexual behaviour after acute administration of high doses (40 and 50 mg/kg). The ejaculation latency was significantly increased, underlining the use of tramadol as a suitable treatment for premature ejaculation. Future research will conclude the role of both 5-HT$_{1A}$- and μ-opioid receptors on inhibiting sexual behaviour after tramadol treatment.

ACKNOWLEDGEMENTS

Special thanks to Marileen Wiegersma for her help during the sexual behaviour training.

REFERENCES

Ågmo, A. (1999). Sexual motivation—an inquiry into events determining the occurrence of sexual behavior. *Behavioural Brain Research*, *105*(1), 129–150. https://doi.org/10.1016/S0166-4328(99)00088-1

Agmo, A., & Paredes, L. (1988). Opioids and Sexual Behavior in the Male Rat. *Pharmacology Biochemistry & Behavior*, *30*, 1021–1034. Retrieved from http://ac.els-cdn.com/0091305788901359/1-s2.0-0091305788901359-main.pdf?_tid=049c2ec0-4c86-11e7-b2f8-00000aab0f01&acdnat=1496952594_d9e371b1d1cc0ea91722d1e278ddaa6f

Agmo, a, & Berenfeld, R. (1990). Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. *Behavioral Neuroscience*, *104*(1), 177–82. https://doi.org/10.1037/0735-

7044.104.1.177
American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders (5th edition)*. Washington.
Balon, R. (2006). SSRI-associated sexual dysfunction. *American Journal of Psychiatry*, *163*(9), 1504–1509. https://doi.org/10.1176/appi.ajp.163.9.1504
Bijlsma, E. Y., Chan, J. S. W., Olivier, B., Veening, J. G., Millan, M. J., Waldinger, M. D., & Oosting, R. S. (2014). Sexual side effects of serotonergic antidepressants: Mediated by inhibition of serotonin on central dopamine release? *Pharmacology Biochemistry and Behavior*, *121*, 88–101. https://doi.org/10.1016/j.pbb.2013.10.004
Chan, J. S. W., Olivier, B., de Jong, T. R., Snoeren, E. M. S., Kooijman, E., van Hasselt, F. N., … Oosting, R. S. (2008). Translational research into sexual disorders: Pharmacology and genomics. *European Journal of Pharmacology*, *585*(2), 426–435. https://doi.org/10.1016/j.ejphar.2008.02.098
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
Eassa, B. I., & El-Shazly, M. A. (2013). Safety and efficacy of tramadol hydrochloride on treatment of premature ejaculation. *Asian Journal of Andrology*, *1596*(10), 138–142. https://doi.org/10.1038/aja.2012.96
Hennies, H. H., Friderichs, E., & Schneider, J. (1988). Receptor binding, analgesic and antitussive potency of tramadol and other selected opioids. *Arzneimittel-Forschung*, *38*(7), 877–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2849950
Higgins, A., Nash, M., & Lynch, A. M. (2010). Antidepressant-associated sexual dysfunction: Impact, effects, and treatment. *Drug, Healthcare and Patient Safety*, *2*(1), 141–150. https://doi.org/10.2147/DHPS.S7634
Lehmann, K. (1997). Tramadol in acute pain. *Drugs*, *53 Suppl 2*, 25–33. Retrieved from http://europepmc.org/abstract/med/9190322
Matthiesen, T., Wö Hrmann, T., Coogan, T. P., & Uragg, H. (1998). The experimental toxicology of tramadol: an overview. *Toxicology Letters*, *95*, 63–71.
McMahon, C. G., Althof, S. E., Waldinger, M. D., Porst, H., Dean, J., Sharlip, I. D., … 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. *The Journal of Sexual Medicine*, *5*(7), 1590–1606. https://doi.org/10.1111/j.1743-6109.2008.00901.x
Morales, A., Barada, J., & Wyllie, M. G. (2007). A review of the current status of topical treatments for premature ejaculation. *BJU International*, *100*(3), 493–501. https://doi.org/10.1111/j.1464-410X.2007.07051.x
Olivier, J. D. A., Esquivel Franco, D. C., Oosting, R., Waldinger, M., Sarnyai, Z., & Olivier, B. (2016). Tramadol: Effects on sexual behavior in male rats are mainly caused by its 5-HT reuptake blocking effects. *Neuropharmacology*, *116*, 50–58. https://doi.org/10.1016/j.neuropharm.2016.11.020
Olivier, J. D. A., Esquivel Franco, D. C., Oosting, R., Waldinger, M., Sarnyai, Z., & Olivier, B. (2017). Tramadol: Effects on sexual behavior in male rats are mainly caused by its 5-HT reuptake blocking effects. *Neuropharmacology*, *116*, 50–58. https://doi.org/10.1016/j.neuropharm.2016.11.020
Rojas-Corrales, M. O., Berrocoso, E., Gibert-Rahola, J., & Micó, J. A. (2002). Antidepressant-like effects of tramadol and other central analgesics with activity on monoamines reuptake, in helpless rats. *Life Sciences*, *72*, 143–152. Retrieved from www.elsevier.com/locate/lifescie
Rojas-Corrales, M. O., Giber-T-Rahola, J., & Mica, J. A. (1998). TRAMADOL INDUCES ANTIDEPRESSANT-TYPE EFFECTS IN MICE. *Life Sciences*, *63*(12), 175–180.
Rosen R, Porst H, Montorsi F. The Premature Ejaculation Prevalence and Attitudes (PEPA) Survey: a Multi-National Survey [Abstract]. Proceedings of the 11th World Congress of the International Society of Sexual and Impotence Research 2004, 17–21 October 2004
Schweitzer, I., Maguire, K., & Ng, C. (2009). Sexual side-effects of contemporary antidepressants: review. *Australian and New Zealand Journal of Psychiatry*, *43*, 795–808.
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
Waldinger, M. D., Hengeveld, M. W., Zwinderman, A. H., & Olivier, B. (1998). Effect of SSRI

Antidepressants on Ejaculation. *Journal of Clinical Psychopharmacology*, *18*(4), 274–281. https://doi.org/10.1097/00004714-199808000-00004

Waldinger, M. D., Hengeveld, M. W., Zwinderman, A. H., & Olivier, B. (1998). Effect of SSRI antidepressants on ejaculation: a double-blind, randomized, placebo-controlled study with fluoxetine, fluvoxamine, paroxetine, and sertraline. *Journal of Clinical Psychopharmacology*, *18*(4), 274–81. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9690692

Waldinger, M. D., & Olivier, B. (2004). Utility of selective serotonin reuptake inhibitors in premature ejaculation. *Current Opinion in Investigational Drugs (London, England : 2000)*, *5*(7), 743–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15298071

Waldinger, M. D., Zwinderman, A. H., & Olivier, B. (2001). SSRIs and Ejaculation: A Double-Blind, Randomized, Fixed-dose Study With Paroxetine and Citalopram. *Journal of Clinical Psychopharmacology*, *21*(6), 1–5. Retrieved from papers://40ab18b2-d275-4dd1-ab17-808c82b9f3da/Paper/p156

Yang, L., Qian, S., Liu, H., Liu, L., Pu, C., Han, P., & Wei, Q. (2013). Role of Tramadol in Premature Ejaculation: A Systematic Review and Meta-Analysis. *Urol Int*, *91*, 197–205. https://doi.org/10.1159/000348826

APPENDIX

Appendix I. R-values of the Spearman's test for correlations for the dose response curve.

Dose-response curve	**5-HTT$^{+/+}$**	**5-HTT$^{+/-}$**	**5-HTT$^{-/-}$**
Spearman's test	r= -0,8286	r= -0,9856	r= -0,8286
Level of significance	p<0,05	p<0,01	p<0,05

Appendix II. Statistical analysis of the ejaculation frequency, all genotypes.

Ejaculation frequency	**Wildtype (5-HTT$^{+/+}$)**		**Heterozygous (5-HTT$^{+/-}$)**		**Knockout (5-HTT$^{-/-}$)**	
Dose (mg/kg)	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0
0	1,917 ± 0,2876		2,917 ± 0,2876		1,417 ± 0,3362	
5	2,833 ± 0,241	ns	2,667 ± 0,3553	ns	1,917 ± 0,3128	ns
10	2,083 ± 0,1486	ns	2,5 ± 0,3794	ns	1,667 ± 0,2247	ns
20	1,417 ± 0,4167	ns	1,583 ± 0,3128	p<0,01	1,083 ± 0,2876	ns
40	0,5833 ± 0,2876	p<0,01	0,75 ± 0,3509	p<0,001	0,4167 ± 0,2289	p<0,05
50	0 ± 0	p<0,001	0,75 ± 0,4106	p<0,001	0 ± 0	p<0,001

Appendix III. Statistical analysis of the mount frequency, all genotypes.

Mount frequency	**Wildtype (5-HTT$^{+/+}$)**		**Heterozygous (5-HTT$^{+/-}$)**		**Knockout (5-HTT$^{-/-}$)**	
Dose (mg/kg)	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0
0	14,58 ± 1,885		7,917 ± 2,258		21,17 ± 4,536	
5	9,417 ± 2,314	ns	13,000 ± 4,998	ns	13,33 ± 1,328	ns
10	12,5 ± 2,575	ns	9,083 ± 3,716	ns	19,75 ± 5,625	ns
20	7,583 ± 2,835	ns	6,417 ± 1,708	ns	9,917 ± 2,261	ns
40	3,333 ± 1,676	p<0,001	1,917 ± 0,7926	ns	3,583 ± 1,495	p<0,01
50	0 ± 0	p<0,001	0,5833 ± 0,5833	ns	1,833 ± 1,481	p<0,001

Appendix IV. Statistical analysis of the intromission frequency, all genotypes.

Intromission frequency	Wildtype (5-HTT$^{+/+}$)		Heterozygous (5-HTT$^{+/-}$)		Knockout (5-HTT$^{-/-}$)	
Dose (mg/kg)	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0
0	7,833 ± 1,0665		5,667 ± 0,6317		7,417 ± 0,8480	
5	8,917 ± 0,933	ns	7,500 ± 0,7538	ns	8,583 ± 1,041	ns
10	7,667 ± 0,8733	ns	6,667 ± 0,8196	ns	8,583 ± 0,8915	ns
20	3,667 ± 0,8819	p<0,01	5,333 ± 1,223	ns	7,167 ± 1,079	ns
40	2,833 ± 1,065	p<0,01	1,917 ± 0,8480	p<0,01	2,000 ± 1,052	p<0,001
50	0,0833 ± 0,0833	p<0,001	1,167 ± 0,6376	p<0,001	1,000 ± 0,5365	p<0,001

Appendix V. Statistical analysis of the ejaculation latency, all genotypes.

Ejaculation latency	Wildtype (5-HTT$^{+/+}$)		Heterozygous (5-HTT$^{+/-}$)		Knockout (5-HTT$^{-/-}$)	
Dose (mg/kg)	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0
0	674,0 ± 138,3		319,0 ± 80,95		1002 ± 163,8	
5	286,5 ± 49,14	ns	504,6 ± 141,9	ns	804,0 ± 146,0	ns
10	485,6 ± 63,14	ns	490,7 ± 178,8	ns	898,7 ± 163,5	ns
20	945,8 ± 229,7	ns	578,0 ± 170,7	ns	1077 ± 177,3	ns
40	1436 ± 159,7	p<0,001	1316 ± 211,4	p<0,001	1535 ± 140,9	p<0,05
50	1800 ± 0	p<0,001	1411 ± 205,7	p<0,001	1800 ± 0	p<0,001

Appendix VI. Statistical analysis of the mount latency, all genotypes.

Mount latency	Wildtype (5-HTT$^{+/+}$)		Heterozygous (5-HTT$^{+/-}$)		Knockout (5-HTT$^{-/-}$)	
Dose (mg/kg)	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0
0	119,8 ± 102,8		84,49 ± 43,88		91,76 ± 80,11	
5	98,49 ± 48,46	ns	18,34 ± 3,471	ns	11,41 ± 2,283	ns
10	99,11 ± 61,66	ns	76,94 ± 57,22	ns	12,11 ± 4,454	ns
20	692,8 ± 222,1	p<0,05	364,7 ± 163,9	ns	184,1 ± 109,2	ns
40	1217 ± 238,9	p<0,001	1230 ± 227,8	p<0,001	1165 ± 220,0	p<0,001
50	1795 ± 5,346	p<0,001	1353 ± 233,4	p<0,001	1316 ± 225,5	p<0,001

Appendix VII. Statistical analysis of the intromission latency, all genotypes.

Intromission latency	Wildtype (5-HTT$^{+/+}$)		Heterozygous (5-HTT$^{+/-}$)		Knockout (5-HTT$^{-/-}$)	
Dose (mg/kg)	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0
0	249,9 ± 147,0		95,93 ± 46,81		290,8 ± 150,0	
5	161,9 ± 84,23	ns	144,7 ± 87,86	ns	186,7 ± 147,2	ns
10	229,3 ± 106,2	ns	223,8 ± 118,6	ns	141,3 ± 98,39	ns
20	843,6 ± 214,5	p<0,05	688,8 ± 197,9	p<0,05	386,4 ± 152,9	ns
40	1249 ± 225,5	p<0,001	1249 ± 226,2	p<0,001	1445 ± 189,7	p<0,001
50	1795 ± 5,346	p<0,001	1360 ± 229,8	p<0,001	1469 ± 198,0	p<0,001

Appendix VIII. Statistical analysis of the post-ejaculatory interval, all genotypes.

Post-ejaculatory interval	5-HTT$^{+/+}$	5-HTT$^{+/-}$	5-HTT$^{-/-}$
Dose (mg/kg)	Mean ± SEM	Mean ± SEM	Mean ± SEM
0	425,9 ± 22,99	378,9 ± 13,65	485,6 ± 28.25
5	394,6 ± 23,65	398,8 ± 20,20	476,3 ± 36,58
10	440,4 ± 21,63	386,8 ± 18,30	503,2 ± 22,26
20	399,7 ± 56,88	452,7 ± 42,6	521,5 ± 58,44
40	493,3 ± 53,64	381,2 ± 18,89	506,1 ± 90,66
50	0 ± 0	424,7 ± 61,66	0 ± 0

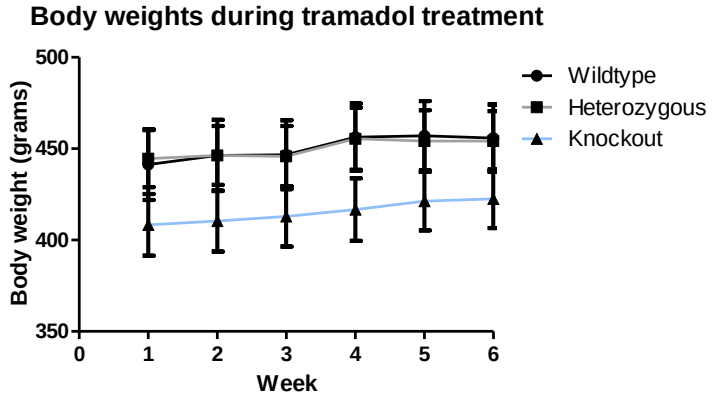
Appendix IX. Statistical analysis of the intromission ratio, all genotypes.

Intromission ratio	Wildtype (5-HTT$^{+/+}$)		Heterozygous (5-HTT$^{+/-}$)		Knockout (5-HTT$^{-/-}$)	
Dose (mg/kg)	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0	Mean ± SEM	ANOVA with Dunnett's Multiple Comparison vs 0
0	0,0508 ± 0,00857		0,0495 ± 0,00411		0,0352 ± 0,00616	
5	0,0740 ± 0,00823	p<0,05	0,0522 ± 0,00570	ns	0,0354 ± 0,00489	ns
10	0,0437 ± 0,00415	ns	0,0534 ± 0,00798	ns	0,0323 ± 0,00312	ns
20	0,0318 ± 0,00959	ns	0,0348 ± 0,00647	ns	0,0201 ± 0,00343	p<0,05
40	0,0089 ± 0,00411	p<0,001	0,0135 ± 0,00650	p<0,001	0,0069 ± 0,00333	p<0,001
50	0 ± 0	p<0,001	0,0092 ± 0,00524	p<0,001	0,0016 ± 0,00110	p<0,001

Appendix X. Statistical analysis of all parameters for all genotypes.

Parameter	5-HTT$^{+/+}$	5-HTT$^{+/-}$	5-HTT$^{-/-}$
Ejaculation frequency	F=37,19	F=28,86	F=28,19
Mount frequency	F=37,4	F=31,54	F=31,7
Intromission frequency	F=31,37	F=33,6	F=31,03
Ejaculation latency	F=35,26	F=19,75	F=26,51
Mount latency	F=37,00	F=19,07	F=38,28
Intromission latency	F=34,99	F=22,88	F=32,93
Intromission ratio	F=42,95	F=39,32	F=41,24
Post-ejaculatory interval (Kruskal-Wallis test)	3,621	2,811	0,9561

Appendix XI. Body weights during acute tramadol treatment



Appendix XII. Reported side effects after acute tramadol treatment

Side effect	Dose (mg/kg)	Genotype	Occurrence
(Epileptic) seizure	50	Wildtype	1
Dragging through test chamber	50	Wildtype	1