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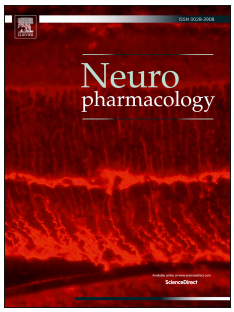

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Tramadol: effects on sexual behavior in male rats are mainly caused by its 5-HT reuptake blocking effects

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Abstract

Tramadol is a well-known and effective analgesic. Recently it was shown that tramadol is also effective in human premature ejaculation. The inhibitory effect of tramadol on the ejaculation latency is probably due to its mechanism of action as a μ-opioid receptor agonist and noradrenaline/serotonin (5-HT) reuptake inhibitor. In order to test this speculation, we tested several doses of tramadol in a rat model of male sexual behavior and investigated two types of drugs interfering with the μ-opioid and the 5-HT system. First the μ-opioid receptor agonist properties of tramadol were tested with naloxone, a μ-opioid receptor antagonist. Second, the effects of WAY100,635, a 5-HT_{1A} receptor antagonist, were tested on the behavioral effects of tramadol. Finally the effects of paroxetine, a selective serotonin reuptake inhibitor, combined with naloxone or WAY100,635 treatment, were compared to the effects of tramadol combined with these drugs.

Results showed that naloxone, at a sexually inactive dose, could only partially antagonize the inhibitory effect of tramadol. Moreover, low and behaviorally inactive doses of WAY100,635, strongly decreased sexual behavior when combined with a behaviorally inactive dose of tramadol. Finally we showed that the effects of paroxetine on sexual behavior resembled the effects of tramadol, indicating that tramadol's inhibitory effects on sexual behavior are primarily and mainly caused by its SSRI properties and that its μ-opioid receptor agonistic activity only contributes marginally. These findings support the hypothesis that tramadol exerts inhibition of premature ejaculations in men by its 5-HT reuptake inhibiting properties.

Key words: tramadol, male sexual behavior, SSRI, naloxone, 5-HT_{1A} receptor

1. Introduction

Tramadol is a centrally acting and clinically approved and used drug for pain treatment (Shipton 2000). Tramadol is a weak μ-opioid receptor agonist, probably acting via its active metabolite O-desmethyltramadol (M_1), which has a 10-fold lower affinity for the μ-opioid receptor than morphine (Frink et al. 1996; Minami et al. 2015). Tramadol is a racemic mixture of two active enantiomers (Frink et al. 1996). The (+)-enantiomer and its metabolite ((+)-M_1) are selective agonists of the μ-opioid receptor and have also serotonergic reuptake inhibitory effects (SSRI); the (-)-enantiomer and the (-)-M_1 metabolite are norepinephrine reuptake inhibitors (Matthiesen et al. 1998). This activity profile suggests antidepressant potency and in animal paradigms, tramadol indeed shows antidepressant activity (Rojas-Corrales et al. 1998; 2002). Recently, tramadol has been shown, as an off-label application, to be effective in premature ejaculation in humans (Eassa and El-Shazly 2013; Yang et al. 2013), comparable to the SSRIs (Waldinger et al. 1998; 2001b; 2001a; Waldinger and Olivier 2004).

The present study was undertaken to investigate the potential 'inhibitory' effect of tramadol on sexual behavior in male rats in analogy to such effects in SSRIs (Bijlsma et al. 2014; Chan et al. 2008). SSRIs have strong inhibitory effects on sexual behavior both in humans (Waldinger et al. 1998; 2001b; 2001a) and rodents (Olivier 2011). These effects are particularly emerging after (sub-) chronic dosing and most SSRIs do not exert strong inhibitory effects on sexual behavior after acute administration in man (Waldinger and Olivier 2004) or rats (Mos et al. 1999; Olivier 2011; Waldinger and Olivier 2004), although acutely sometimes inhibitory effects are reported (Bijlsma et al. 2014; Olivier 2011). Noradrenergic reuptake inhibiting effects are generally not considered to strongly contribute to the inhibitory action on sexual behavior as shown with venlafaxine and other SNRIs (Bijlsma et al. 2014; Segraves and Balon 2014). However, the μ-opioid receptor agonistic activity or the SSRI and SNRI activity in the molecule's action could lead to

an acute sexual inhibitory effect of tramadol as μ-opiate receptor agonists like morphine exert acute inhibitory effects on male sexual behavior in rats (McIntosh et al. 1980; Ågmo and Paredes 1988). In the present studies, we first explored several doses of tramadol (10, 12.5, 20, 25, 40 and 50 mg/kg IP, experiments 1 and 6) on sexual behavior of male rats selected and trained for average sexual activity (2-3 ejaculations per 30-min test at the end of the training). Because only the highest dose of tramadol (50 mg/kg) reduced sexual behavior we tried to antagonize these inhibitory effects with naloxone, an opiate receptor antagonist (experiments 2, 3 and 4). In another set of studies, we selected a non-sexual behavior inhibiting dose of tramadol (25 mg/kg) and combined it with a selected, sexual behavior-inactive dose of the 5-HT_{1A} receptor antagonist WAY100,635 (experiment 5). The idea behind this experiment was based on our previous finding that combining sexually inactive doses of a 5-HT_{1A} receptor antagonist with a sexually inactive dose of an SSRI after acute administration strongly inhibits sexual behavior (de Jong et al. 2005; Olivier 2015). As comparison, we also performed an acute combination study of the SSRI paroxetine and WAY100,635 (experiment 7).

2. *Materials and Methods*

2.1 Animals

One hundred and twenty male Wistar rats (Harlan, Zeist, The Netherlands) ranging from 450-500 g were trained for sexual behavior with a sexually primed intact female rat (50 μg estradiol benzoate in sesame oil saturated with lecithin given 36 hours before testing) once a week for 4 weeks in 30-min tests. Training and sex test were performed under red light and reversed light-dark conditions (12hLight-12hDark: lights off from 6:00 AM to 6:00 PM) in sex test chambers (60cmx 40cmx30cm; rectangular plastic boxes with clear front window and regular bedding material). Bedding of the sex test chambers was not changed during training and testing, to stimulate the sexual behavior due to the pheromones. All tests were performed between 9 AM and 4 PM. After 4 training tests (30 min/training), the male rats were considered sexually trained and classified into average ejaculating (2-3 ejaculations(E)/test), fast ejaculating (>3 E/test), and slow ejaculating (0-1 E/test) groups based on the ejaculations numbers per test (Chan et al. 2008; Olivier et al. 2006; Pattij et al. 2005). A total of 48 rats with an average number of ejaculations have been selected and used during all subsequent experiments. In all individual experiments at least N=8 rats per dose of a drug were used and rats were maximally used once a week to guarantee sufficient washout of drugs. Rats had ad libitum access to food and water. All experiments were conducted in accordance with the governmental guidelines for care and use of laboratory animals and was approved by the Ethical Committee for Animal research of the Faculties of Veterinary Medicine, Pharmaceutical Sciences, Chemistry and Biology at Utrecht University. All efforts were made to minimize the amount of animals and their suffering.

2.2 Drug treatment and behavioral experiments

Care was taken that animals did not receive the same drugs or vehicle during all these experiments, which were run over a couple of months. For the pharmacological tests, male rats were given a 30-min habituation time in the test chambers. All drugs were injected IP 30 minutes before introduction of the female rat. Double injections were given immediately after each other. All tests were performed between 9:00 AM-16:00 PM. Behavioral observations over 30-minutes after introduction of the female were analyzed using Noldus Observer® (Noldus Information Technology, Wageningen, the Netherlands). **The number of ejaculations/test (E) were scored and f**rom these data the following parameters of the first ejaculation series were deduced (Chan et al. 2011): latency (s) to first mount (ML), latency (s) to first intromission (IL), number of mounts (M), number of intromissions (I), and latency (s) to the first ejaculation (EL). After ejaculation, the post ejaculatory latency (PEL) was calculated, using the time from the first ejaculation and the time of the first mount/intromission (whichever occurred first) of the second ejaculation series. **Intromission ratio (IR)** was calculated as: IR = *(#I/(#*I + *#*M*)) * 100%.*. In the present study the main results are deduced from the effects of the treatment (vehicle or dose of a drug) on the first ejaculation series, which includes the first post-ejaculatory latency. In order to study drugs, it is important to have comparable pharmacodynamics and kinetics, and thus a fixed test duration of 30 minutes (1800 sec) is chosen. Because some treatments cause low sexual activities (e.g. zero ejaculations) some animals actually cannot be used for statistics. Artificial maximum values of 1800 sec (i.e. the test duration) for some latencies (ejaculation latency, mount and intromission latency, post-ejaculatory latency) are used, although this is certainly a matter of dispute. The mount and intromission data from these non-ejaculating animals are also problematic because it is actually unknown whether a rat may eventually ejaculate. These data may be considered artificially and are questionable for statistical analyses. In some experiments where the

drug inhibited ejaculatory behavior, few or no animal achieve a second ejaculation making statistical analyses of the second ejaculatory series impossible, If in our experiments a drug blocked ejaculation in the majority of animals data values of 1800 sec were imputed for EL, ML, IL and PEL and also include the frequency values (MF, IF) for all animals for statistical purposes. In such cases, the strong inhibitory drug effects warrant the use of these values. We chose to skip statistical analyses if less than 50% of animals in a certain drug-treated group were left for second ES parameters. All tables only show the results for the first Ejaculation Series. Specifics for a certain experiment are described in the legends of the respective tables.

2.3 Statistical analyses

The data was separated into ejaculations series. One-way ANOVA and Bonferroni post-hoc statistical analysis was used to analyze the data. All data were analyzed using SPSS 16.0 software (LEAD technologies, Chicago, USA). Level of significance was set at $p < 0.05$. Data are expressed as mean ± SEM.

2.4 Drugs

Tramadol hydrochloride (obtained from DMI, UK), naloxone hydrochloride and WAY100635 maleate were bought from Sigma-Aldrich. Paroxetine hydrochloride was prepared from tablets obtained from a local pharmacy, grinded and suspended in saline. We have extensive evidence that the paroxetine used in this way has an excellent bioavailability that is comparable to that of the hydrochloride salt of paroxetine alone (Bijlsma et al. 2014; Chan et al. 2008). All drugs were dissolved in 0.9% NaCl (saline), and each solution was freshly prepared for each testing day. All drugs were administered IP.

2.5 Pharmacological studies:

The following studies have been performed:

Study 1: 48 selected average ejaculating male rats were randomly divided into 4 groups of N=12 each. Groups received vehicle (saline), 10, 20 or 40 mg/kg tramadol (tramadol hydrochloride). Because we physically could not test 48 animals in one test-day, we performed testing over two consecutive days and animals and treatment were randomized over these two days. In the next experiments (studies 2-6) a lower number of animals was used. They were randomly chosen from the 48 rats available with the restriction that animals never got the same treatment more than once and all 48 animals underwent approx. the same number of experimental tests.

Study 2: 24 animals were treated with vehicle + saline (N=8), vehicle + tramadol (50 mg/kg: N=8) and a group with naloxone (10 mg/kg + tramadol 50 mg/kg; N=8). Testing was performed on one testing day

Study 3: 32 rats were either treated with vehicle + vehicle (N=8), vehicle + 5 mg/kg Naloxone (N=8), vehicle + naloxone (10 mg/kg; N=8) and vehicle + paroxetine (10 mg/kg; N=8). Testing was performed on one testing day

Study 4: 24 rats were either treated with vehicle (vehicle + vehicle; N=8), vehicle + naloxone (20 mg/kg; N=8), or tramadol (50 mg/kg) + naloxone (20 mg/kg; N=8). Testing was performed on one testing day

Study 5: 24 rats were either treated with vehicle + vehicle (N=8), WAY100,635 (1 mg/kg) + vehicle (N=8) or WAY 100635 (1 mg/kg) + tramadol (25 mg/kg; N=8). Testing was performed on one testing day

Study 6: 24 rats were either treated with vehicle (N=8), tramadol (12.5 mg/kg; N=8) or tramadol (25 mg/kg; N=8). Testing was performed on one testing day

Study 7: all 48 rats were either treated with vehicle + vehicle (N=8), vehicle + WAY100635 (0.1 mg/kg; N=8), vehicle + WAY100635 (0.3 mg/kg; N=8), vehicle + paroxetine (10 mg/kg; N=8), WAY 100635 (0.1 mg/kg) + paroxetine (10 mg/kg; N=8) or WAY100635 (0.3 mg/kg) + paroxetine (10 mg/kg; N=8). This experiment was performed over two consecutive days (like in study 1).

3. Results

3.1 Dose-response study of tramadol

In the first dose-response study (0, 10, 20, and 40 mg/kg) tramadol had no significant effects on any aspects of male sexual behavior (Fig. 1/Suppl. table 1). Although there were tendencies that the 40 mg/kg dose had some lowering effects on various aspects of the sexual behavior, none of the parameters differed significantly from vehicle. Therefore, we decided to use a higher dose of tramadol in the next experiment. This 50-mg/kg tramadol dose vs. vehicle experiment (Fig 2/ Suppl. table 2), tramadol dramatically reduced sexual behavior. Most animals refrained from any sexual activity, most clearly seen by the absence of any ejaculation, long latencies before the first mount and intromission in a limited number of animals.

3.2 Antagonism of tramadol's sexual effects by naloxone.

Naloxone (5 and 10 mg/kg: Suppl. table 3) had no significant effects on sexual behavior. The 10-mg/kg dose of naloxone had a very limited partial antagonizing effect on the inhibitory effects of 50-mg/kg tramadol (Fig 2, suppl. table 2). The latency to the first mount (M_1) and first intromission (I_1) were significantly shorter after adding naloxone to tramadol than after tramadol alone. Also the total number of mounts (M) and intromissions (I) were significantly enhanced.

An attempt to further antagonize tramadol's inhibitory effect by increasing the naloxone dose to 20 mg/kg failed, because that dose of naloxone itself strongly inhibited sexual behavior (Fig.3, suppl. table 4) and was also not able to antagonize tramadol's 50-mg/kg effects.

3.2 Combination of tramadol and paroxetine with the 5-HT_{1A} receptor antagonist WAY100,635.

Paroxetine (10 mg/kg IP) alone has no consistent inhibitory effect on sexual behavior when given acutely (suppl. table 3 (last column) and suppl. table 7). But, when this dose was combined with, by themselves inactive doses of the 5-HT_{1A} receptor antagonist WAY100,635. (1.0 mg/kg (Fig. 4, suppl. table 5); 0.1 and 0.3 mg/kg (Fig. 5; suppl. table 7)) sexual behavior was strongly inhibited (Fig. 4 and Fig. 5 and Suppl. tables 5 and 7).

When 1.0-mg/kg WAY100,635, that alone has no effect on sexual behavior (Fig.4 and Suppl. table 5), was combined with a dose of tramadol (25 mg/kg) that on itself had no significant effect on sexual behavior (suppl. table 6), sexual behavior was severely reduced (Fig. 4; Suppl. table 5).

4. Discussion

Acutely administered, tramadol has, up to 40-mg/kg (IP) no significant effects on sexual behavior of 'normally' ejaculating male rats, although some inhibitory trends were seen at the 40-mg/kg dose. At 50-mg/kg IP, however, tramadol strongly inhibited sexual behavior, reducing it almost to zero. Because tramadol, via its enantiomers and active metabolites, exerts opiate receptor agonistic and 5-HT reuptake inhibiting effects, it was tried to unravel whether tramadol's effects on sexual behavior were related to either of these mechanisms. Naloxone, a μ-opiate receptor antagonist had at low doses (5 and 10 mg/kg) no intrinsic effects on sexual behavior. At 20-mg/kg, however, naloxone strongly inhibited sexual behavior itself making this dose unfit to try to antagonize tramadol's inhibitory effects. Naloxone (10 mg/kg) had some minor, but significant, antagonizing effects on the inhibitory effects of 50-mg/kg tramadol, suggesting that the μ-opioid receptor may play a minor role in this effect. Morphine, a μ-opiate receptor agonist inhibits male sexual behavior in rats (McIntosh et al. 1980; Ågmo and Paredes 1988), an effect that could be completely antagonized by naloxone. Although the intrinsic effects of naloxone on male sexual behavior are somewhat controversial (Gessa et al. 1979; McIntosh et al. 1980; Myers and Baum 1979), in our hands doses of 5 and 10 mg/kg are behaviorally silent, whereas the 20-mg/kg dose appeared inhibitory. The dose-response curve of tramadol shows a steep decrease in sexual behavior between the 40 and 50-mg/kg doses. Although part of this inhibition is due to blockade of the μ-opioid receptor, antagonism of this effect by naloxone cannot completely overcome the tramadol-induced inhibition of sexual behavior. The remaining inhibitory effects might be due to stronger SSRI effects at the 50-mg/kg dose or to other effects exerted by tramadol at higher doses, e.g. norepinephrine-reuptake inhibition or other mechanisms (see Minami et al. 2015).

It is postulated that the potential inhibitory action of SSRIs on sexual behavior may be mediated via 5-HT_{1A} receptors (de Jong et al. 2005; Olivier 2011). Blocking this receptor in the presence of

an SSRI strongly (and dose-dependently) inhibits sexual behavior, even at doses of the SSRI (10 mg/kg IP paroxetine) that acutely do not exert intrinsic inhibitory activity. WAY100,635, a potent and selective 5-HT_{1A} receptor antagonist has no intrinsic activity in sexual behavior, but strongly decreases sexual activities when it (at doses of 0.1, 0.3 and 1 mg/kg) is combined with 10 mg/kg paroxetine. When tramadol, at a selected dose (25 mg/kg IP) that on itself does not affect sexual behavior, is combined with a selected (1 mg/kg IP) dose of WAY100,635, a strong reduction in sexual behavior is found, supporting the role of the 5-HT reuptake inhibiting mechanism of tramadol in its inhibitory effect in sexual behavior. Drug-discrimination studies in rats (Filip et al. 2004) where a 20-mg/kg dose (IP) of tramadol was trained as discriminative stimulus (DS) versus saline, supported a role for the opiate mechanism in tramadol, because morphine (2 mg/kg) fully substituted for the tramadol cue, whereas the DS could be completely antagonized by naloxone at rather low dosages (ED_{50} around 0.2 mg/kg). Remarkably, neither noradrenaline (NRI), serotonin (SSRI) nor mixed NE/5-HT reuptake blockers (SNRI) were able to substitute for the tramadol DS, whereas NRIs, but not SSRIs were able to shift the dose-response curve to the left. It is well known that SSRIs are notoriously difficult to train as a DS in rats (Olivier 2015) whereas NRIs create trainable cues (Caldarone et al. 2010; Dekeyne et al. 2001). Tramadol, via its (-)-enantiomer and (-)-metabolite, has norepinephrine reuptake inhibiting effects that may contribute to its sexual inhibitory effects at higher doses. In general NRIs (e.g. reboxetine, milnacepram) are not known as antidepressants with strong sexual side effects (Graf et al. 2014; Segraves and Balon 2014); enhancement of NE-neurotransmission could even functionally antagonize the inhibitory actions of SSRIs on sexual behavior (Bijlsma et al. 2014). Based on our data, we postulate that the SSRI component in tramadol is primarily responsible for the inhibitory action on sexual behavior, whereas the μ-opioid agonistic effects might (slightly) contribute to this effect.

From these findings, it is predicted that tramadol, at non-sexual behavior inhibiting doses after acute administration, will exert inhibitory effects after (sub) chronic dosing, in analogy to those properties of SSRIs (Chan et al. 2008). Whether the μ-opioid receptor agonistic properties of tramadol contribute significantly to these effects is as yet unclear and would need more studies. Testing tramadol on sexual behavior in SERT-knockout rats will determine whether the opioid agonistic activity in tramadol exerts sexual inhibitory effects in the absence of the SERT-inhibiting effects of tramadol. These experiments will be performed in the near future.

The SSRI component of tramadol alone seems sufficient (compared to paroxetine) to explain tramadol's inhibitory effects on sexual behavior in humans with premature ejaculation (Eassa and El-Shazly 2013; Yang et al. 2013). Although our data suggests that the μ-opioid component in tramadol might contribute to this effect, the question remains what this means in clinical practice. The human data suggest no clear 'on demand' treatment effects of tramadol, although studies performed up to date have not realistically looked into that aspect.

5. Conclusions

The results indicate that the sexual inhibitory effects of tramadol after acute administration and relatively high doses are mainly mediated via the SSRI component of tramadol, although a small effect of the μ-opioid agonistic mechanism might contribute to this inhibitory effect.

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Figure legends

Figure 1: Sexual behavior of male rats (N=12/ group) treated with vehicle, 10, 20 or 40 mg/kg tramadol. Data are given as mean + SEM. The number of ejaculations per 30 min (A), number of Mounts (B), Intromissions (C), Post-ejaculatory Interval (D) and Copulatory Efficiency (E) of the first Ejaculation Series are given. Detailed statistical analyses (ANOVA) are shown in Suppl. Table 1.

Figure 2: Sexual behavior of male rats (N=8/group) treated with saline + saline, tramadol 50 mg/kg + saline, or tramadol 50 mg/kg + naloxone 10 mg/kg. Data are given as mean + SEM. The number of ejaculations per 30 min (A), number of Mounts (B), Intromissions (C), Post-ejaculatory Interval (D), Copulatory Efficiency (E), Latency to first Mount (F), Latency to first Intromission (G) and Latency to first Ejaculation (H) of the first Ejaculation Series are given. Detailed statistical analyses (ANOVA) are shown in Suppl. Table 2. *: significant difference ($P<0.05$) compared to Vehicle + saline group. §: significant difference between tramadol + saline group and tramadol + naloxone group ($P<0.05$).

Figure 3: Sexual behavior of male rats (N=8/group) treated with saline + saline, naloxone 20 mg/kg + saline, or tramadol 50 mg/kg + naloxone 20 mg/kg. Data are given as mean + SEM. The number of ejaculations per 30 min (A), number of Mounts (B), Intromissions (C), Post-ejaculatory Interval (D), Copulatory Efficiency (E), Latency to first Mount (F), Latency to first Intromission (G) and Latency to first Ejaculation (H) of the first Ejaculation Series are given. Detailed statistical analyses (ANOVA) are shown in Suppl. Table 4. *: significant difference ($P<0.05$) compared to saline + saline group. §: significant difference between naloxone + saline group and tramadol + naloxone group ($P<0.05$).

Figure 4: Sexual behavior of male rats (N=8/group) treated with saline + saline, WAY100,635 (1 mg/kg) mg/kg + saline, or tramadol 25 mg/kg + WAY100,635 1 mg/kg. Data are given as mean + SEM. The number of ejaculations per 30 min (A), number of Mounts (B), Intromissions (C), Post-ejaculatory Interval (D), Copulatory Efficiency (E), Latency to first Mount (F), Latency to first Intromission (G) and Latency to first Ejaculation (H) of the first Ejaculation Series are given. Detailed statistical analyses (ANOVA) are shown in Suppl. Table 5. *: significant difference ($P<0.05$) compared to saline + saline group. §: significant difference between WAY100,635 + saline group and tramadol + WAY100,635 group ($P<0.05$).

Figure 5: Sexual behavior of male rats (N=8/group) treated with saline + saline, WAY100,635 (0.1 mg/kg) + saline, WAY100,635 (0.3 mg/kg) + saline, saline +paroxetine (10 mg/kg), WAY100635 (0.1 mg/kg) + paroxetine (10 mg/kg) and WAY100,635 (0.3 mg/kg) + paroxetine (10 mg/kg). Data are given as mean + SEM. The number of ejaculations per 30 min (A), number of Mounts (B), Intromissions (C), Post-ejaculatory Interval (D), Copulatory Efficiency (E), Latency to first Mount (F), Latency to first Intromission (G) and Latency to first Ejaculation (H) of the first Ejaculation Series are given. Detailed statistical analyses (ANOVA) are shown in Suppl. Table 7. a: significant difference ($P<0.05$) compared to saline + saline group. b: significant difference compared to WAY100,365 (0.1 mg/kg) + saline group ($P<0.05$). c: significant difference compared to WAY100,635 (0.3 mg/kg) + saline group. d: significant difference compared to saline +paroxetine (10 mg/kg) group.

Supplementary data

Suppl. table 1: Effects of Tramadol on Sexual Behavior of male Wistar rats. N=12/group

Dose of tramadol, mg/kg	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	ANOVA significance
Parameters measured	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	
# E	2.4±0.2	2.08±0.3	1.75±0.3	1.42±0.5	F(3,44)=2.222; P =0.178
Latency 1st M (s)	26.1±13.3	7.46±2.1	55.4±145.2	43.2±10.6	F(3,44)=0.848; P=0.475
Latency 1st I (s)	46.5±25.6	62.0±19.4	76.9±116.5	231.3±124.4	F(3,44)= 1.633; P=0.189
# M 1st series	20.3±3.8	16.8±3.6	23.6±6.1	15.1±3.4	F(3,44)=0.739; P=0.534
# I 1st series	7.4±1.0	7.1±0.9	7.5±0.9	5.3±0.8	F(3,44)=1.269; P=0. 297
Latency 1st E (s)	541.9±126.9	642.7±166.5	873.1±170.7	1020.4±210.3	F(3,44)=1.610; P=0.201
PEL_1	428.5±125.7	557.4±167.9	569.4±166.3	1064.8±221.7	F(3,44)=2.606; P=0.064
IR_1	32.7±4.7	33.9±4.2	36.4±6.6	31.0±3.8	F(3,44)=0.204; P=0.893

M=Mount; I= Intromission; E=Ejaculation; PEL=post-ejaculatory interval; # =number; **IR= Intromission Ratio** = [# intromissions / (# intromissions + # mounts)]*100

Suppl. table 2: Effects of Tramadol and Naloxone on Sexual Behavior of male Wistar rats. N=8 /group

Dose of tramadol, mg/kg IP	0 mg/kg + 0 mg/kg A	50 mg/kg + Saline B	50 mg/kg + 10mg/kg Naloxone C	ANOVA significance
Parameters measured	Mean ± SEM	Mean ± SEM	Mean ± SEM	
# E	2.4±0.4	0.0±0.0*	0.8±0.4*	F(2,21)=12.781; P<0.001
Latency 1st M (s)	5.2±1.1	1593.7±203.1*	574.4±272.9 (bc)	F(2,21)=16.789; P<0.001
Latency 1st I (s)	32.7±16.4	1760.2±39.8*	948.4±273.3* (bc)	F(2,21)=29.284; P<0.001
# M 1st series	21.4±7.8	0.5±0.4*	4.5±1.9	F(2,21)=5.767; P=0.010
# I 1st series	7.0±1.7	0.3±0.3*	1.9±1.0*	F(2,21)=9.637; P=0.001
Latency 1st E (s)	598.9±217.8	1800±0.0*	1364.4±256.8*	F(2,21)=9.782; P=0.001
PEL_1	502.9±186.1	1800±0.0*	1447.5±231.1*	F(2,21)=15.327; P<0.001
IR_1	33.5±7.0	20.0±20.0 (n=2)	30.8±8.8 (n=6)	F(2,13)=0.320; P=0.732

M=Mount; I= Intromission; E=Ejaculation; PEL=post-ejaculatory interval; # =number; **IR= Intromission Ratio** = [# intromissions / (# intromissions + # mounts)]*100
* = Significantly (P<0.05) different from 0 mg/kg.
(bc) = significantly (P<0.05) different between 50 mg/kg + saline (B) and 50 mg/kg + 10mg/kg Naloxone (C).

Suppl. table 3: Effects of Naloxone and Paroxetine on Sexual Behavior of male Wistar rats. N=8 animals/group

Dose of drug, mg/kg IP	0 mg/kg (saline + saline)	5 mg/kg Naloxone + Saline	10 mg/kg Naloxone + Saline	10 mg/kg Paroxetine + saline	ANOVA significance
Parameters	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	
# E	1.0±0.5	1.3±0.6	2.3±0.7	1.0±0.5	F(3,28)=1.150; P=0. 346
Latency 1st M (s)	21.5±7.5	12.5±2.8	20.5±8.8	46.9±20.7	F(3,28)=1.557; P=0.222
Latency 1st I (s)	21.5±5.4	27.9±11.9	44.9±26.4	56.2±17.0	F(3,28)=0.835; P=0.486
# M 1st series	8.9±2.2	14.9±5.0	14.1±4.1	12.4±3.6	F(3,28)=0.473; P=0.703
# I 1st series	9.6±2.7	7.9±0.6	8.5±1.8	8.3±4.4	F(3,28)=0.085; P=0. 968
Latency 1st E (s)	319.9±90.2	531.9±144.1	704.4±261.7	725.1±249.0	F(3,28)=0.888; P=0.460
PEL_1	283.2±23.1	120.9±216.1	251.5±53.5	60.4±290.4	F(3,24)=1.820; P=0.170
IR_1	51.2±4.6	41.4±5.3	43.2±5.7	33.8±8.9	F(3,27)=0.788; P=0.511

M=Mount; I= Intromission; E=Ejaculation; PEL=post-ejaculatory interval; # =number; **IR= Intromission Ratio** = [# intromissions / (# intromissions + # mounts)]*100

Suppl. table 4: Effects of Naloxone and Tramadol on Sexual Behavior of male Wistar rats. N=8 animals per group

Dose of drug, mg/kg IP	0 mg/kg (saline + saline) A	20 mg/kg Naloxone + saline B	20 mg/kg Naloxone + 50mg/kg Tramadol C	ANOVA significance
Parameters measured	Mean± SEM	Mean± SEM	Mean± SEM	
# E	2.6±0.3	0.6±0.3*	0.4±0.2*	F(2,21)=26.545; P <0.001
Latency 1st M (s)	8.4±5.0	114.3±60.7	743.5±310.1*	F(2,21)=4.744; P=0.020
Latency 1st I (s)	11.5±2.2	249.6±146.3	1213.8±295.8* ; (bc)	F(2,21)=11.167; P<0.001
# M 1st series	11.9±4.4	29.3±9.6	4.5±1.7	F(2,21)=4.208; P=0.029
# I 1st series	5.6±1.0	5.8±1.2	2.9±1.6 (bc)	F(2,21)=1.618; P=0.222
Latency 1st E (s)	288.0±62.4	1262.5±228.2*	1522.6±171.8*	F(2,21)=14.869;P<0.001
PEL_1	318.5±18.1	1099.8±264.9*	1456.1±225.2*	F(2,21)8.379; P=0.002
IR_1	41.1±7.3	19.2±4.1	26.5±11.3	F(2,18)=2.798; P=0.092

M=Mount; I= Intromission; E=Ejaculation; PEL=post-ejaculatory interval; # =number; **IR= Intromission Ratio** = [# intromissions / (# intromissions + # mounts)]*100
* = Significantly (P<0.05) different from 0 mg/kg.
(bc) = significantly (P<0.05) different between 20 mg/kg Naloxone + saline (B) and 20 mg/kg Naloxone + 50mg/kg Tramadol (C).

Suppl. table 5: Effects of WAY100635 (5-HT$_{1A}$ receptor antagonist) and Tramadol on Sexual Behavior of male Wistar rats. N=8/group

Dose of drug, mg/kg IP	0 mg/kg (saline + saline) A	1 mg/kg WAY100635 + Saline B	1 mg/kg WAY100635+ 25mg/kg Tramadol C	ANOVA significance
Parameters measured	Mean±SEM	Mean±SEM	Mean±SEM	
# E	3.1±0.4	2.4±0.3	0.3 ±0.2* (bc)	F(2,21)=22.582; P<0.001
Latency 1st M (s)	14.9±7.0	18.0±11.5	895.9 ±303.5* (bc)	F(2,17)=9.967; P=0.001
Latency 1st I (s)	50.9±26.0	68.7±42.1	926.7 ±328.7* (bc)	F(2,17)=8.020; P=0.004
# M 1st series	11.6±2.4	12.6±4.6	10.0±5.8	F(2,17)=0.085; P=0.919
# I 1st series	6.1±0.8	5.6±0.7	3.2±1.5	F(2,17)-2.323; P=0.128
Latency 1st E (s)	407.9±110.2	356.7±104.8	1482.0±201.3* (bc)	F(2,17)-19.774; P<0.001
PEL$_1$	305.5±20.5	291.3±17.0	1558.2 ±241.8* (bc)	F(2,17)=31.761; P<0.001
IR$_1$	37.0±5.2	40.8±7.8	31.4±10.5 (n=4)	F(2,15)=0.338; P=0.719

M=Mount; I= Intromission; E=Ejaculation; PEL=post-ejaculatory interval; # =number; **IR= Intromission Ratio** = [# intromissions / (# intromissions + # mounts)]*100
* = Significantly (P<0.05) different from 0 mg/kg.

(bc) = significantly (P<0.05) different between 1 mg/kg WAY100635 + saline (B) and 1 mg/kg WAY100635 + 25mg/kg Tramadol (C).

Suppl. table 6: Effects of Tramadol on Sexual Behavior of male Wistar rats. N=8/group

Dose of drug, mg/kg IP	0 mg/kg (saline)	12.5mg/kg Tramadol	25.0 mg/kg Tramadol	ANOVA significance
Parameters measured	Mean±SEM	Mean±SEM	Mean±SEM	
# E	2.8±0.5	2.3±0.5	1.5±0.5	F(2,21)=1.797; P=0.190
Latency 1st M (s)	4.7±1.5	12.2±3.5	344.0±197.7	F(2,21)=2.880P=0.078
Latency 1st I (s)	29.8±12.2	50.8±16.8	348.6±211.2	F(2,21)=2.110; P=0.145
# M 1st series	23.0±6.8	20.9±7.8	17.4±5.8	F(2,21)0.171; P=0.844
# I 1st series	7.5±1.0	7.0±1.5	5.6±1.6	F(2,21)=0.492; P=0.618
Latency 1st E (s)	566.7±200.2	673.1±215.4	1062.9±252.2	F(2,21)=1.364; P=0.277
PEL_1	473.0±189.9	508.4±185.5	1081.0±272.1	F(2,21)=2.418; P=0.113
IR_1	29.8±5.1	33.7±5.3	25.8±4.9	F(2,21)=0.569; P=0.575

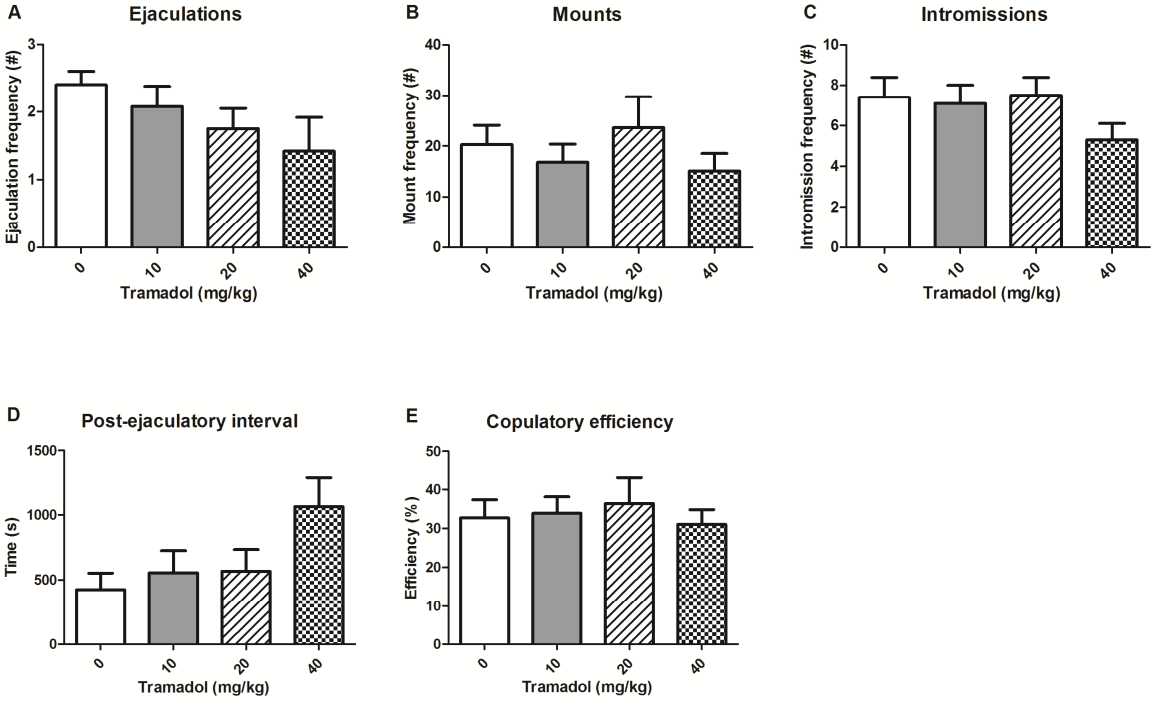
M=Mount; I= Intromission; E=Ejaculation; PEL=post-ejaculatory interval; # =number; **IR= Intromission Ratio** = [# intromissions / (# intromissions + # mounts)]*100

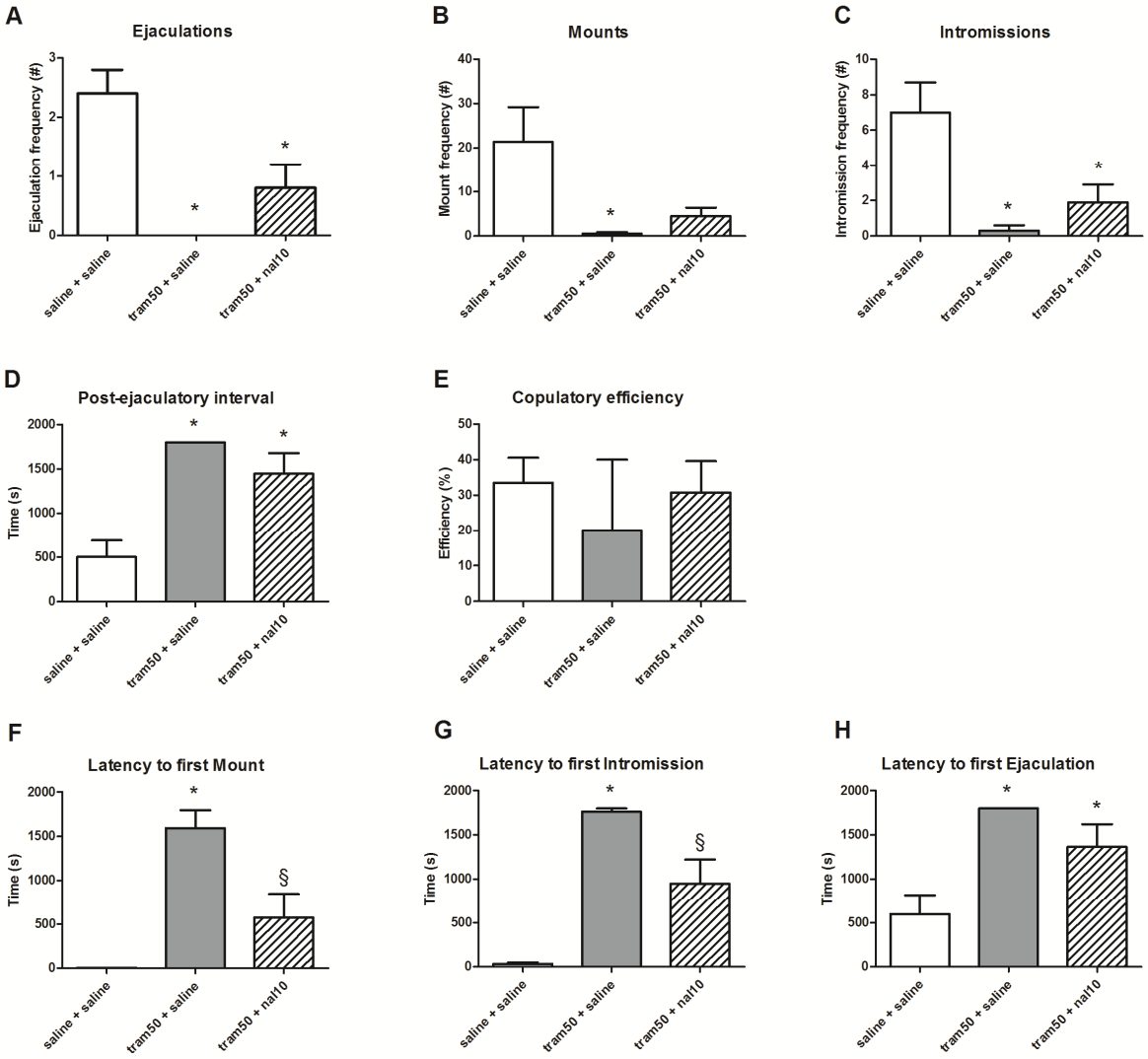
Suppl. table 7: Effects of WAY100635 ($5HT_{1A}$ receptor antagonist) and Paroxetine on Sexual Behavior of male Wistar rats. N=8/group

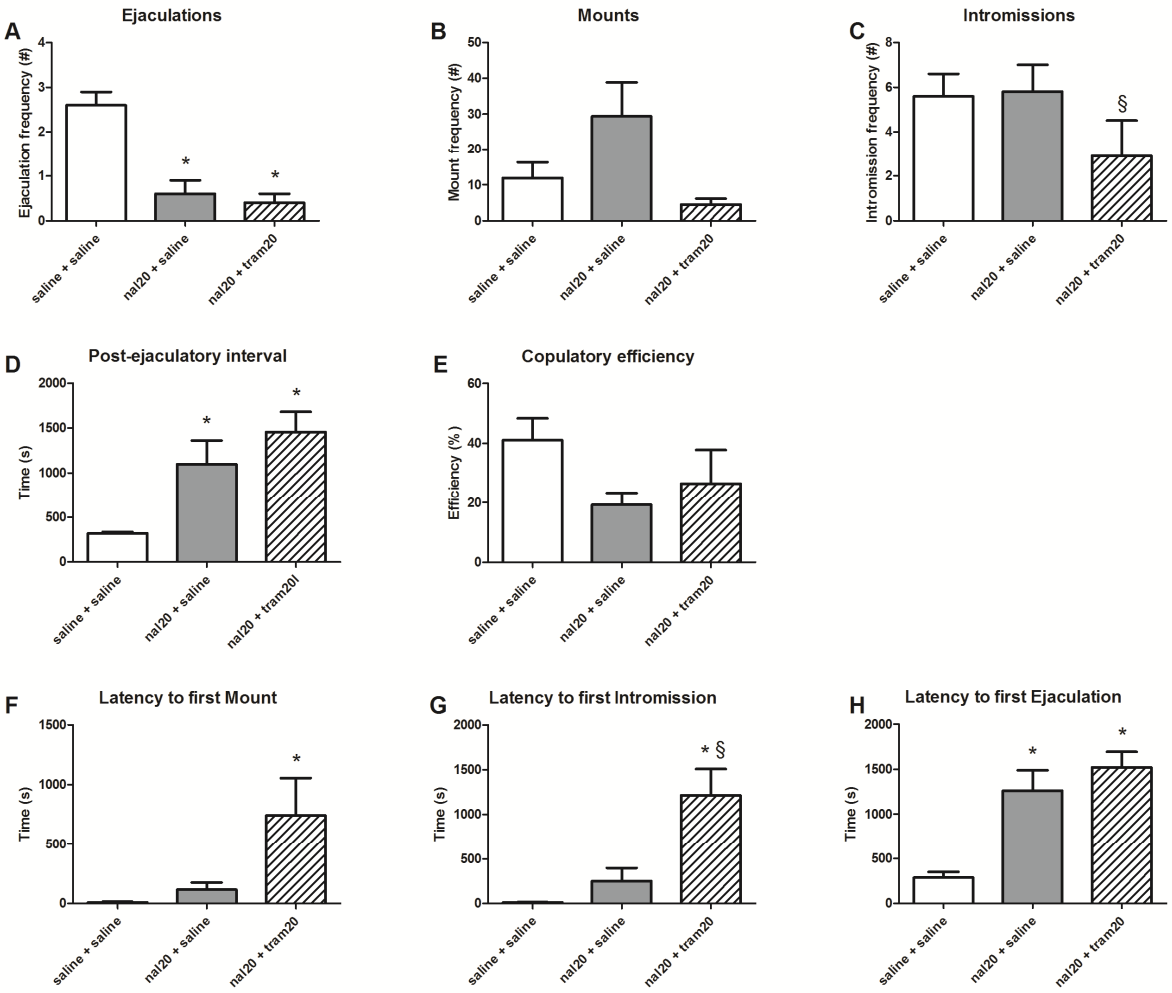
Dose of drug, mg/kg IP	0 mg/kg (saline +saline) A	0.1 mg/kg WAY100635 + saline B	0.3 mg/kg WAY100635 + saline C	Saline + 10mg/kg paroxetine D	0.1 mg/kg WAY100635 + 10mg/kg paroxetine E	0.3 mg/kg WAY100635 + 10mg/kg paroxetine F	ANOVA significance
Parameters measured	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM	
# E	2.4±0.2	1.5±0.5	2.5±0.3	1.5±0.4	0.0±0.0*; b;d	0.0±0.0*;c;d	F(5,42)=13.619; P<0.001
Latency 1st M (s)	19.3±11.1	88.9±74.3	8.1±2.2	54.4±33.8	923.9±286.1*; b;d	1527.2±;222.7* c;d	F(5,42)=17.810; P<0.001
Latency 1st I (s)	25.5±14.4	350.0±213.8	21.0±10.1	451.2±221.3	1415.2±190.3*;b;d	1800.0±0.0*;c;d	F(5,42)=25.780; P<0.001
# M 1st series	15.6±3.9	19.1±7.3	12.4±2.0	13.8±3.6	6.6±2.8	1.1±1.0	F(5,42)=2.696; P=0.034
# I 1st series	10.5±4.7	4.3±0.8	10.0±2.2	4.8±2.1	0.8±0.3	0.0±0.0* ;	F(5,42) =5.700; P=0.007
Latency 1st E (s)	352.9±57.6	1008.3±241.6	501.9±127.4	986.3±44.7	1800.0±0.0*;b;d	1800.0±0.0*;c;d	F(5,42)=16.639; P<0.001
PEL_1	344.3±16.2	693.3±242.9	347.7±20.7	895.9±265.3	1800 .0±0.0*;b;d	1800.0±0.0*;c;d	F(5,42)=20.648; P<0.001
IR_1	40.0±6.8	35.5±11.1	44.6±4.9	31.0±6.3	n=4; 15.7±6.1	n =0	F(5,42)=1.462; P=0.238

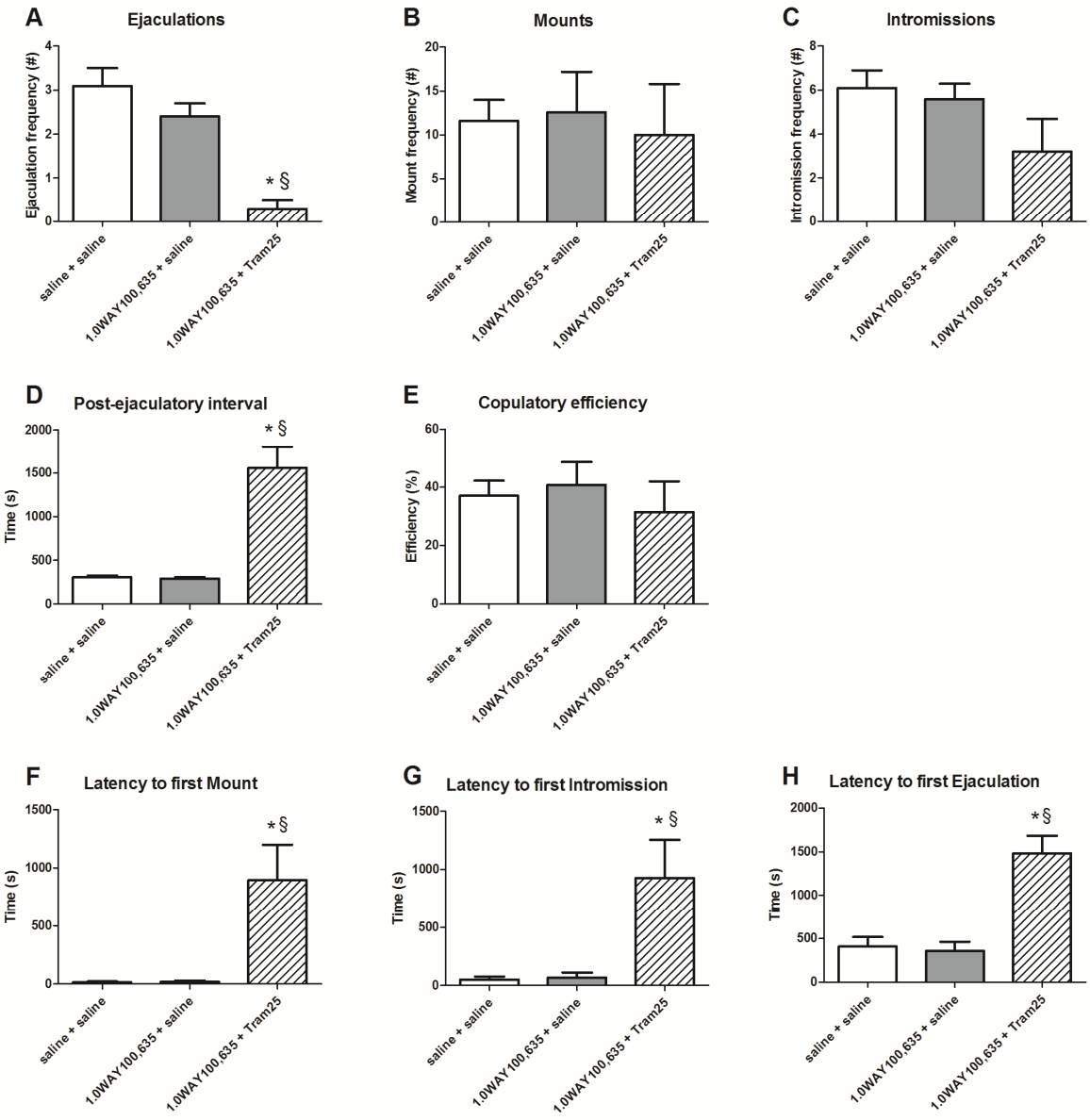
M=Mount; I= Intromission; E=Ejaculation; PEL=post-ejaculatory interval; # =number;
IR= Intromission Ratio = [# intromissions / (# intromissions + # mounts)]*100
* = Significantly (P<0.05) different from 0 mg/kg.

b = significantly (P<0.05) different from 0.1 mg/kg WAY100635 + saline (B); c = significantly (P<0.05) different from 0.3 mg/kg WAY100635 + saline (C); d = significantly (P<0.05) different from Saline + 10mg/kg paroxetine (D)





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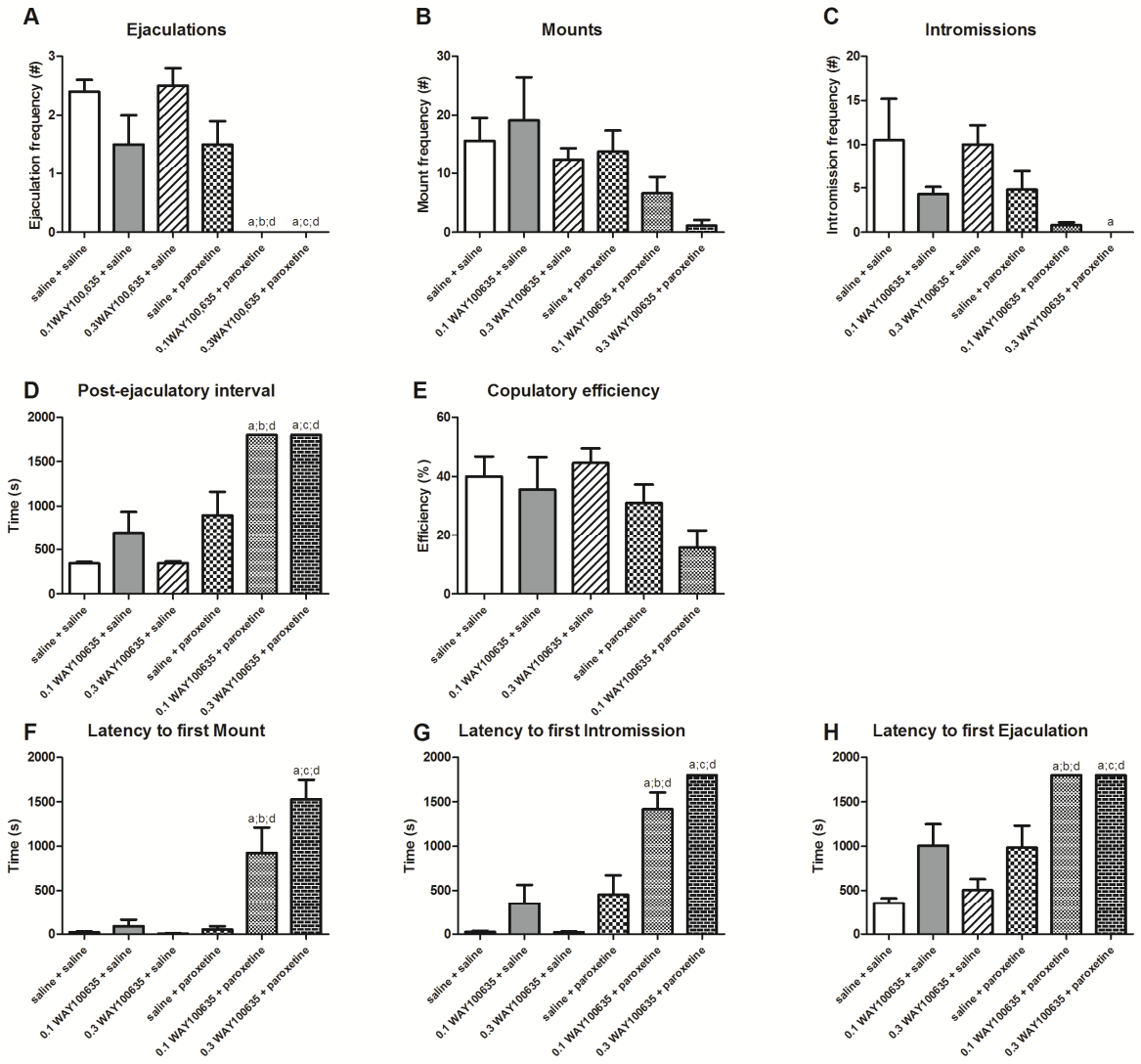

Highlights

- Paroxetine resembled the inhibitory effects of tramadol on sexual behavior
- 5-HT_{1A} receptor antagonist combined with tramadol strongly decreased sexual behavior
- μ-opioid receptor antagonist only partially antagonized the effects of tramadol
- Tramadol's sexual inhibitory effects are primarily caused by its SSRI properties