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A study of time- and sex-dependent effects of vortioxetine on rat sexual behavior: Possible roles of direct receptor modulation

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ABSTRACT

Treatment-related sexual dysfunction is a common side effect of antidepressants and contributes to patient non-compliance or treatment cessation. However, the multimodal antidepressant, vortioxetine, demonstrates low sexual side effects in depressed patients. To investigate the mechanisms involved, sexual behavior was assessed in male and female rats after acute, and repeated (7 and 14 days) treatment with vortioxetine, flesinoxan (a 5-HT_{1A} receptor agonist), CP-94253 (a 5-HT_{1B} receptor agonist), or ondansetron (a 5-HT_3 receptor antagonist). These selective ligands were chosen to simulate vortioxetine's direct modulation of these receptors. Paroxetine was also included in the male study. Acute and repeated treatment with vortioxetine at doses corresponding to clinical levels (based on serotonin transporter occupancy) had minimal effects on sexual behavior in male and female rats. High dose vortioxetine plus flesinoxan (to mimic predicted clinical levels of 5-HT_{1A} receptor occupancy by vortioxetine) facilitated male rat sexual behavior (acutely) while inhibiting female rat proceptive behavior (both acutely and after 14 days treatment). The selective serotonin reuptake inhibitor, paroxetine, inhibited male sexual behavior after repeated administration (7 and 14 days). Flesinoxan alone facilitated male sexual behavior acutely while inhibiting female rat proceptive behavior after repeated administration (7 and 14 days). CP-94253 inhibited sexual behavior in both male and female rats after repeated administration. Ondansetron had no effect on sexual behavior. These findings underline the complex serotonergic regulation of sexual behavior and indicate that the low sexual side effects of vortioxetine found in clinical studies are likely associated with its direct modulation of serotonin receptors.

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1. Introduction

Major depressive disorder (MDD) is one of the most common mental disorders and carries a heavy burden of disability in adults (Global burden of disease study 2013 collaborators, 2015). The selective serotonin (5-HT) reuptake inhibitor (SSRI) and serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressants first launched in the 1980s and 1990s are still the current first-line treatments for MDD. However, there is a pressing need for improved treatment options. Only about 50% of MDD patients achieve clinical remission following initial antidepressant treatment, regardless of the drug chosen (Rush et al., 2006). After four consecutive antidepressant treatment attempts, the overall cumulative remission rate is only about 67% (Rush et al., 2006). In addition, the therapeutic response is often delayed, usually requiring several weeks of treatment. Furthermore, drug-related adverse effects are common (Anderson et al., 2012). There is a high prevalence of persistent sexual dysfunction related to antidepressants in both female and male patients, especially those drugs inhibiting the serotonin transporter (SERT) (Serretti and Chiesa, 2009). This adverse effect is a common reason for treatment cessation or noncompliance in patients (Ashton et al., 2005). Therefore, more efficacious MDD treatments with fewer sexual side effects would be extremely valuable.

Several clinical studies examined the treatment-emergent

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sexual dysfunction associated with the multimodal antidepressant, vortioxetine. There was a relatively low incidence of self-reported sexual dysfunction in MDD patients receiving vortioxetine treatment (Baldwin et al., 2016; Sanchez et al., 2015). A study by Jacobsen and colleagues focused on adult MDD patients who experienced treatment-emergent sexual dysfunction despite reduction in depressive mood during SSRI treatment. This study demonstrated that switching to vortioxetine resulted in a greater improvement in sexual function than switching to another SSRI, citalopram (Jacobsen et al., 2015). Furthermore, a recent pooled analysis concluded that there was no increased risk for patients to develop treatment-emergent sexual dysfunction when treated with vortioxetine at any dose, compared to patients that received placebo. In contrast, the risk was increased in patients treated with 60 mg/day duloxetine (Jacobsen et al., 2016). It is plausible that the distinct mechanisms of action of these different classes of antidepressants may underlie their differential effects on sexual function.

The aim of the present study was to use a preclinical model to investigate which of vortioxetine's mechanisms may be related to the low incidence of sexual dysfunction observed in clinical studies. Animal models are critical to obtain objective and quantitative measures of sexual function and to establish the putative roles of different 5-HT receptor subtypes. Vortioxetine is an antidepressant with a multimodal mechanism of action (i.e., an antidepressant exerting its pharmacological actions through two or more different target classes (Zohar et al., 2014)). In addition to being a SERT inhibitor, vortioxetine is a 5-HT_{1A} receptor agonist, a 5-HT_{1B} receptor partial agonist, and a 5-HT_{1D}, 5-HT_3 and 5-HT_7 receptor antagonist (Sanchez et al., 2015). Clinical and preclinical research indicates that several of these 5-HT receptor subtypes are involved in regulating sexual behavior, and the effects of these receptor systems are sex-dependent (Olivier et al., 2011). Although the acute effects of 5-HT_{1A} and 5-HT_{1B} receptor modulation have been reported, there is a paucity of data regarding their chronic effects. As antidepressants typically require several weeks to exert effects in patients, it is important to assess sexual behavior after chronic modulation of the aforementioned receptors. To this end, we examined sexual behaviors in male and female rats after acute, subchronic (7 days) and chronic (14 days) vortioxetine treatment. In addition, the putative roles of 5-HT_{1A}, 5-HT_{1B} and 5-HT_3 receptors on sexual behavior were assessed by examining the effects of selective ligands (flesinoxan, CP-94253, and ondansetron, respectively) following the same treatment schedule.

In the present study, doses of vortioxetine were chosen based on clinical practice. The low vortioxetine dose (corresponding to 5 mg per day clinical dose) aimed to achieve 50% SERT occupancy. The high vortioxetine dose (corresponding to 20 mg per day clinical dose) aimed to achieve 90% SERT occupancy (Sanchez et al., 2015). Notably, it has been reported that the affinity of vortioxetine for the rat 5-HT_{1A} receptor is markedly lower than its affinity for the human 5-HT_{1A} receptor (Sanchez et al., 2015). Since 5-HT_{1A} receptors are involved in the modulation of some aspects of sexual function (Giuliano and Clement, 2005; Landen et al., 1999; Snoeren et al., 2014a, b), a high dose vortioxetine plus flesinoxan (a 5-HT_{1A} receptor agonist) group was included in this study to better mimic the effects of highest dose of vortioxetine in humans. Doses of flesinoxan, CP-94253, and ondansetron were chosen based on results from pilot studies, to match the levels of receptor occupancy at these targets by vortioxetine at clinically relevant doses (du Jardin et al., 2014; Leiser et al., 2014).

Reference compounds were included in this study. Paroxetine, a standard SSRI, was included as a positive control for sexual dysfunction in male rats. However, although paroxetine has been shown to inhibit male sexual behavior after chronic administration, it does not affect sexual behavior in female Wistar rats. In contrast, acute 8-OH-DPAT inhibits female sexual behavior (Snoeren et al., 2011). Therefore, acute administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT was included in the female study, to ensure deficits in sexual dysfunction could be detected. Sexual behaviors were assessed in male rats focusing on mounts, intromissions and ejaculations (Bijlsma et al., 2014; Chan et al., 2009). In female rats, sexual behaviors were assessed in a paced mating paradigm (Snoeren et al., 2011), focusing on proceptive behaviors rather than receptive behaviors. The proceptive behaviors (characterized by 'ear wiggling', darting and hopping) are more related to sexual motivation, while the often measured lordosis is more of a direct assessment for copulatory function (Martinez and Paredes, 2001).

2. Material and methods

2.1. Animals

Male and female Wistar Crl/Wu rats (Charles River, Germany) were group-housed (4/cage) with a reversed light cycle (lights off from 6:00 a.m. to 6:00 p.m.), upon arrival at approximately 10 weeks of age. Animals had *ad libitum* access to rodent chow and tap water. The study was reviewed and approved by Utrecht University's animal welfare committee.

2.2. Study design

Rats were randomly allocated to the following treatment groups (n = 12–16 per group, Table 1): vehicle (Purina 5001 chow and saline s.c.), low dose vortioxetine (1 mg/kg oral gavage once followed by p.o. administration via food pellet [176 mg vortioxetine per kg Purina 5001 chow, equivalent to approximately 1 mg/kg/day]), high dose vortioxetine (10 mg/kg oral gavage followed by p.o. administration via food pellet [600 mg vortioxetine per kg Purina 5001 chow, equivalent to approximately 10 mg/kg/day]), high dose vortioxetine (same treatment regimen as above) + flesinoxan (2.5 mg/kg b.i.d, s.c.), flesinoxan (2.5 mg/kg b.i.d., s.c.), CP-94253 (5 mg/kg daily, s.c.), and ondansetron (1 mg/kg b.i.d., s.c.). The initial doses of vortioxetine were given by p.o. gavage 60min before the test, as rats would not eat enough drug containing food pellets immediately on first exposure (due to neophobia). Paroxetine (10 mg/kg once a day p.o. gavage in males) and 8-OH-DPAT (0.1 mg/kg s.c. in females on the days of behavioral test, 30min before test) were included as they are known to inhibit sexual behaviors in male and female rats, respectively. All drugs were synthesized by H. Lundbeck A/S (Valby, Denmark), except CP-94253 (Tocris, Minneapolis, MN), ondansetron and paroxetine (Sigma-Aldrich, St. Louis, MO). *Ex vivo* autoradiography was used to measure SERT occupancy to confirm target engagement of vortioxetine and paroxetine. Sexual behaviors were assessed after the first drug treatment (acute), 7 days (subchronic) and 14 days (chronic) of treatment for all groups, except the female 8-OH-DPAT group. This group only received 8-OH-DPAT on the days of behavioral test, 30 min prior to the test sessions.

2.3. Rodent sexual behavior test

The sexual behavior in male and female rats was assessed using the testing paradigm established previously (Chan et al., 2009; Snoeren et al., 2011).

2.3.1. Training and selection of males

Male rats were first trained weekly for 5 consecutive weeks, by exposing them to an estrous female for 30 min in an observation cage (30 × 40 × 60 cm) with a Plexiglas front. Females were brought into estrus with estradiol (Sigma-Aldrich) (50 μg s.c. in the

Table 1
Summary of the experimental groups in the study. The number of animals assigned to each group and treatment they received are represented in this table. B.i.d.: twice a day; q.d.: once a day; s.c.: subcutaneous; p.o.: oral. VEH: vehicle; VOR: vortioxetine; FLESI: flesinoxan; PAR: paroxetine (male only); DPAT: 8-OH-DPAT (female only, 30min prior to each test); CP: CP-94253; OND: ondansetron.

Sex	Groups	N	Treatment
Male	VEH	16	Purina 5001 chow + saline (1 mL/kg, b.i.d., s.c.)
	VOR (low)	16	Low dose vortioxetine (1 mg/kg oral gavage × 1, followed by vortioxetine containing food at equivalent dose)
	VOR (high)	16	High dose vortioxetine (10 mg/kg oral gavage × 1, followed by vortioxetine containing food at equivalent dose)
	VOR (high) + FLESI	16	High dose vortioxetine (same as above) + Flesinoxan (5-HT_{1A} agonist, 2.5 mg/kg, b.i.d., s.c.)
	PAR	16	Paroxetine (10 mg/kg, q.d., p.o. gavage)
	FLESI	16	Flesinoxan (5-HT_{1A} agonist, 2.5 mg/kg, b.i.d., s.c.)
	CP	16	CP-94251 (5-HT_{1B} agonist, 5 mg/kg, q.d., s.c.)
	OND	16	Ondansetron (5-HT_3 antagonist, 1 mg/kg, b.i.d., s.c.)
Female	VEH	12	Purina 5001 chow + saline (1 mL/kg, b.i.d., s.c.)
	VOR (low)	16	Low dose vortioxetine (1 mg/kg oral gavage × 1, followed by vortioxetine containing food at equivalent dose)
	VOR (high)	16	High dose vortioxetine (10 mg/kg oral gavage × 1, followed by vortioxetine containing food at equivalent dose)
	VOR (high) + FLESI	13	High dose vortioxetine (same as above) + Flesinoxan (5-HT_{1A} agonist, 2.5 mg/kg, b.i.d., s.c.)
	DPAT	15	8-OH-DPAT (5-HT_{1A} agonist, 0.1 mg/kg, s.c., 30min prior to sexual behavioral test)
	FLESI	13	Flesinoxan (5-HT_{1A} agonist, 2.5 mg/kg, b.i.d., s.c.)
	CP	15	CP-94251 (5-HT_{1B} agonist, 5 mg/kg, q.d., s.c.)
	OND	13	Ondansetron (5-HT_3 antagonist, 1 mg/kg, b.i.d., s.c.)

nape of the neck 36 h prior to the session). The number of ejaculations over the last two training sessions were used to designate male rats as low, medium (normal), or high performers. Only medium and high performers (2 or more ejaculations in a 30 min session) were randomly allocated to aforementioned treatment groups.

2.3.2. Male sexual behavior test

Tests were conducted between 9:00 a.m. and 4:00 p.m. in the dark phase under dim red light. A male rat was first habituated to the observation cage for 30 min, and then an estrous female was introduced for the 30-min test session. These test sessions were videotaped and the following male behaviors were scored using the Noldus Observer 5.0 program (Wageningen, The Netherlands): number of mounts before first ejaculation (no vaginal penetration), number of intromissions (prior to first ejaculation, vaginal penetration), number of ejaculations (for the entire 30 min), and latency to first ejaculation. The male intromission rate (%) was calculated as number of intromissions × 100/(number of intromissions + number of mounts). A reduction in male sexual behavior generally results in increased number of mounts before first ejaculation, fewer ejaculations, increased latency to first ejaculation, and/or lower intromission rate.

2.3.3. Female paced mating test

A two-compartment test cage was used (male compartment 43 × 26 × 38 cm, female compartment 15 × 26 × 38 cm), divided by a transparent plastic wall containing three holes (4 cm diameter) through which only the smaller females can pass. An estrous female rat was first allowed to freely explore both compartments of the test cage for 5 min. Then the holes were blocked and the female rat was placed in the female compartment. A drug naïve, sexually experienced male rat was placed in the male compartment. The rats can smell, hear, and see each other for another 25 min (habituation). Afterward, the barrier to those connecting holes was removed and the test session was videotaped for the next 30 min. The Noldus Observer 5.0 program was used to score the following female behaviors: number of darts, time in male compartment, percent exits after mount (the total number of exits from male compartment to female compartment within 120s of a mount × 100/total number of mounts), percent exits after intromission (the number of exits within 120s of an intromission × 100/the total number of intromissions). In addition, the latencies for the female to re-enter the male compartment after an exit − (contact-return latency - CRL) after mounts and after intromissions were also measured. A reduction in female proceptive behavior may thus be characterized by fewer darts, less time in the male compartment, higher percent exits after mounts or intromissions, and/or increased contact-return latency after mounts or intromissions. As a measure of locomotor activity, the number of entries into the male compartment was analyzed.

2.4. Autoradiographic assessment of SERT occupancy

SERT occupancy by vortioxetine or paroxetine was measured in a separate cohort of rats that underwent the same treatment regimen, using *ex vivo* autoradiography method as detailed elsewhere (du Jardin et al., 2014; Leiser et al., 2014; Pehrson et al., 2013).

2.5. Statistical analysis

Statistical analysis was conducted using GraphPad Prism 7 software (La Jolla, CA). Sexual behaviors were analyzed with repeated measures two-way ANOVA: drug as the between subject factor and treatment length as the within subject factor. Overall significant differences were followed up with *post-hoc* protected Dunnett's test, comparing different treatments to vehicle for each test session. Significance was defined *a priori* as $p < 0.05$ for all statistical tests.

3. Results

3.1. Sexual behavior in male and female rats were affected by different treatments

The statistical analysis results are summarized in Table 2. There were significant differences in measures of male sexual behavior, including number of mounts before first ejaculation (significant drug main effect and drug × treatment length interaction effect), number of ejaculations (significant drug main effect), latency to first ejaculation (significant drug main effect and drug × treatment length interaction effect), and intromission rate (significant drug main effect and drug × treatment length interaction effect). Significant differences were also observed in some measures of female sexual behavior, including number of darts (significant drug main effect and drug × treatment length interaction effect), percent exits after mount (significant drug and treatment length main effects, as

Table 2

Summary of results from statistical analysis. Data were analyzed using repeated measures two-way ANOVA. When there was an overall significant difference ($p < 0.05$), *post-hoc* Dunnett's test was conducted to compare each treatment group to the vehicle group. VEH: vehicle; VOR: vortioxetine; FLESI: flesinoxan; PAR: paroxetine (male only); DPAT: 8-OH-DPAT (female only, acute^: administrated 30min prior to each test session); CP: CP-94253; OND: ondansetron. Bold font: significant differences. N.S.: not significant. ‡: Animals in some groups were not scored in these measures and lead to smaller dataset for repeated measures analysis.

	Overall Analysis			*Post-Hoc* Analysis							
Parameter	Drug (Between subject)	Treatment length (Within subject)	Interaction	VOR (low)	VOR (high)	VOR (high) + FLESI	PAR (male only)	DPAT (female only, acute ^)	FLESI	CP	OND
Male											
Number of mounts before 1st ejaculation	**$F_{(7, 120)} = 2.5$, $p < 0.05$**	$F_{(2, 240)} = 1.4$, $p = 0.24$	**$F_{(14, 240)} = 2.1$, $p < 0.05$**	n.s.	n.s.	n.s.	**$p < 0.05$ chronic**		n.s.	n.s.	n.s.
Total number of ejaculations	**$F_{(7, 120)} = 10.9$, $p < 0.01$**	$F_{(2, 240)} = 1.4$, $p = 0.25$	$F_{(14, 240)} = 1.7$, $p = 0.06$	n.s.	n.s.	n.s.	**$p < 0.05$ subchronic & chronic**		n.s.	**$p < 0.01$ subchronic; $p < 0.05$ chronic**	n.s.
Latency to 1st ejaculation	**$F_{(7, 120)} = 11.4$, $p < 0.01$**	$F_{(2, 240)} = 1.3$, $p = 0.28$	**$F_{(14, 240)} = 1.8$, $p < 0.05$**	n.s.	n.s.	**$p < 0.05$ acute**	**$p < 0.01$ subchronic; $p < 0.05$ chronic**		**$p < 0.05$ acute**	**$p < 0.01$ chronic**	n.s.
Intromission rate	**$F_{(7, 120)} = 4.0$, $p < 0.01$**	$F_{(2, 240)} = 0.2$, $p = 0.85$	**$F_{(14, 240)} = 2.7$, $p < 0.01$**	n.s.	n.s.	n.s.	**$p < 0.05$ chronic**		**$p < 0.05$ subchronic**	n.s.	n.s.
Female											
Number of darts	**$F_{(7, 105)} = 7.3$, $p < 0.01$**	$F_{(2, 210)} = 0.2$, $p = 0.85$	**$F_{(14, 210)} = 3.1$, $p < 0.01$**	n.s.	n.s.	**$p < 0.01$ acute; $p < 0.05$ chronic**		**$p < 0.05$ day 14**	**$p < 0.01$ subchronic; $p < 0.05$ chronic**	**$p < 0.05$ chronic**	n.s.
Time in male compartment (sec)	$F_{(7, 104)} = 0.6$, $p = 0.8$	$F_{(2, 208)} = 0$, $p = 0.96$	$F_{(14, 208)} = 1.2$, $p = 0.31$								
Percent exits after mount	**$F_{(7, 78)} = 3.6$, $p < 0.05$**	**$F_{(2, 156)} = 6.6$, $p < 0.01$**	**$F_{(14, 156)} = 2.2$, $p < 0.05$**	n.s.	n.s.	n.s.		n.s.	**$p < 0.01$ subchronic**	n.s.	n.s.
Percent exit after intromission‡	**$F_{(5, 54)} = 2.6$, $p < 0.05$**	**$F_{(2, 108)} = 4.1$, $p < 0.05$**	$F_{(10, 108)} = 0.5$, $p = 0.91$	n.s.	n.s.			n.s.		**$p < 0.05$ acute**	n.s.
Contact-return latency after mount‡	$F_{(5, 54)} = 1.4$, $p = 0.25$	$F_{(2, 108)} = 1.5$, $p = 0.23$	$F_{(10, 108)} = 1.1$, $p = 0.37$								
Contact-return Latency after intromission‡	$F_{(5, 45)} = 0.8$, $p = 0.55$	$F_{(2, 90)} = 0.2$, $p = 0.84$	$F_{(10, 90)} = 0.9$, $p = 0.51$								
Number of entries to male compartment	**$F_{(7, 105)} = 2.9$, $p < 0.01$**	**$F_{(2, 210)} = 3.6$, $p < 0.05$**	$F_{(14, 210)} = 1$, $p = 0.44$	n.s.	n.s.	n.s.		n.s.	n.s.	**$p < 0.05$ acute**	n.s.

well as drug × treatment length interaction effect), and percent exits after intromission (significant drug and treatment length main effects). No overall significant difference was detected in other measures of female sexual behavior, such as time in male compartment, contact-return latency after mount, or contact-return latency after intromission. In the following sections, results of vortioxetine treatment and different receptor modulators are illustrated in separate figures for clarity.

3.2. The effects of vortioxetine

The effects of vortioxetine (low dose, high dose, high dose + flesinoxan) and reference compounds (paroxetine in male and 8-OH-DPAT in female) are illustrated in Fig. 1 and Table 3.

Vortioxetine (either low or high dose, VOR) did not significantly alter male sexual behavior compared to vehicle after acute, 7 days (subchronic) or 14 days (chronic) of treatment, in terms of number of mounts before first ejaculation (Fig. 1A), number of ejaculations (Fig. 1B), latency to first ejaculation (Fig. 1C), or intromission rate (Fig. 1D). A treatment length-dependent effect of high dose vortioxetine + flesinoxan (FLESI) was observed on the latency to first ejaculation, which was reduced after acute treatment, but not after 7 days or 14 days administration. Consistent with previous reports, paroxetine (PAR) negatively affected male sexual behavior in a treatment length-dependent manner. Specifically, paroxetine increased the number of mounts before first ejaculation after chronic treatment, reduced the total number of ejaculations after subchronic and chronic treatment, increased the latency to first ejaculation after subchronic and chronic treatment, and reduced the intromission rate after chronic treatment.

Similarly, low or high dose vortioxetine did not change proceptive behavior in female rats, compared to vehicle (number of darts, Fig. 1E; percent exits after mount, Fig. 1F; time in male compartment, percent exits after intromission, contact-return latency (CRL) after mount, or CRL after intromission, Table 3). High dose vortioxetine + flesinoxan reduced the number of darts in a treatment length-dependent manner (Fig. 1E), after acute and chronic treatment. Consistent with previous reports, 8-OH-DPAT negatively impacted female proceptive behavior (reduced number of darts after each acute treatment, reached significant level in the

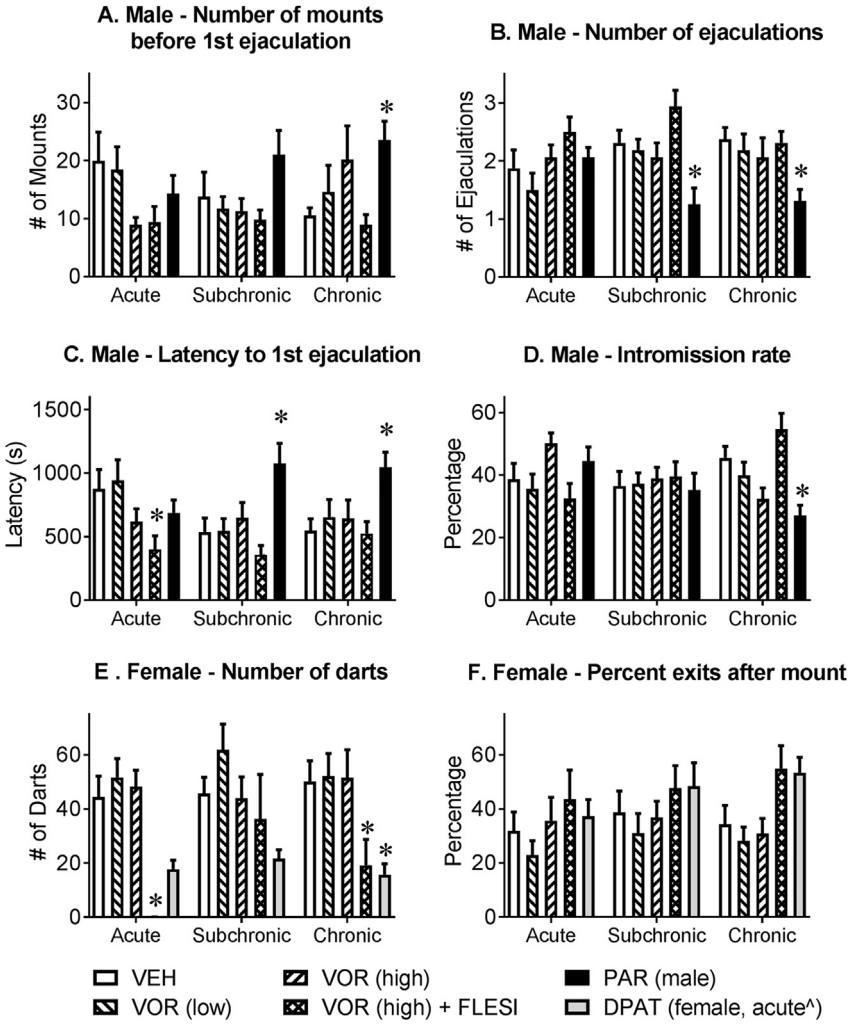

Fig. 1. The effects of vortioxetine on sexual behavior in male and female rats. Rats received 14 days of vehicle (VEH), low dose vortioxetine (VOR), high dose vortioxetine, high dose vortioxetine plus flesinoxan (FLESI) or paroxetine (PAR, male only). Sexual behavior was measured after first (acute) and repeated (7 days-subchronic, 14 days - chronic) treatment. A group of female rats received 8-OH-DPAT (DPAT) 30 min prior to each test session (acute) to demonstrate the assay can detect an inhibitory effect on female proceptive behavior. The number of mounts before first ejaculation (Panel A), number of ejaculations (Panel B), latency to first ejaculation (Panel C) and intromission rate (Panel D) were assessed for male sexual behavior. The number of darts (Panel E) and percent exits after mount (Panel F) were assessed for female proceptive behavior. Data are shown as mean ± standard error, n = 12–16 per group. '*' indicate significant difference between a treatment group and the vehicle group ($p < 0.05$) in *post-hoc* analysis, following significant overall effect. [In legend, acute ^: 8-OH-DPAT was given 30min prior to a testing session.].

Day 14 test, Fig. 1E). These treatments did not cause any significant change in general locomotor activity, assessed as the number of entries to the male compartment (Table 3).

3.3. The effects of the 5-HT_{1A} receptor agonist flesinoxan

The effects of flesinoxan (FLESI) on rat sexual behavior are illustrated in Fig. 2 and Table 3. Flesinoxan's effects in males depended on the treatment length. Flesinoxan reduced the latency to first ejaculation (Fig. 2C) after acute administration, and increased the intromission rate after subchronic treatment (Fig. 2D). However, chronic flesinoxan did not significantly alter these measures compared to vehicle controls.

In female rats, the effects of flesinoxan were also treatment length-dependent. Flesinoxan reduced the number of darts after subchronic and chronic treatment relative to vehicle controls (Fig. 2E), but not after acute administration. After subchronic treatment, flesinoxan also increased percent exits after mount (Fig. 2F). These effects were not accompanied by any change in general locomotor activity, assessed as the number of entries to the male compartment (Table 3). These data indicate an inhibitory effect of flesinoxan on female proceptive behavior.

3.4. The effects of the 5-HT_{1B} receptor agonist CP-94253

The effects of CP-94253 (CP) on rat sexual behavior are illustrated in Fig. 3 and Table 3. In male rats, CP-94253 reduced the number of ejaculations after subchronic and chronic treatment (Fig. 3B) and increased the latency to first ejaculation after chronic treatment (Fig. 3C), suggesting it has treatment length-dependent inhibitory effects on male sexual behavior.

In female rats, CP-94253 also exhibited treatment length-dependent inhibitory effects. It reduced the number of darts after chronic treatment (Fig. 3E) and increased the percent exits after intromission following acute treatment (Table 3). These inhibitory effects on sexual behavior were not associated with any reduction in locomotor activity, as the number of entries to the male compartment was increased after acute treatment. There was no significant difference in this measure after chronic treatment (Table 3).

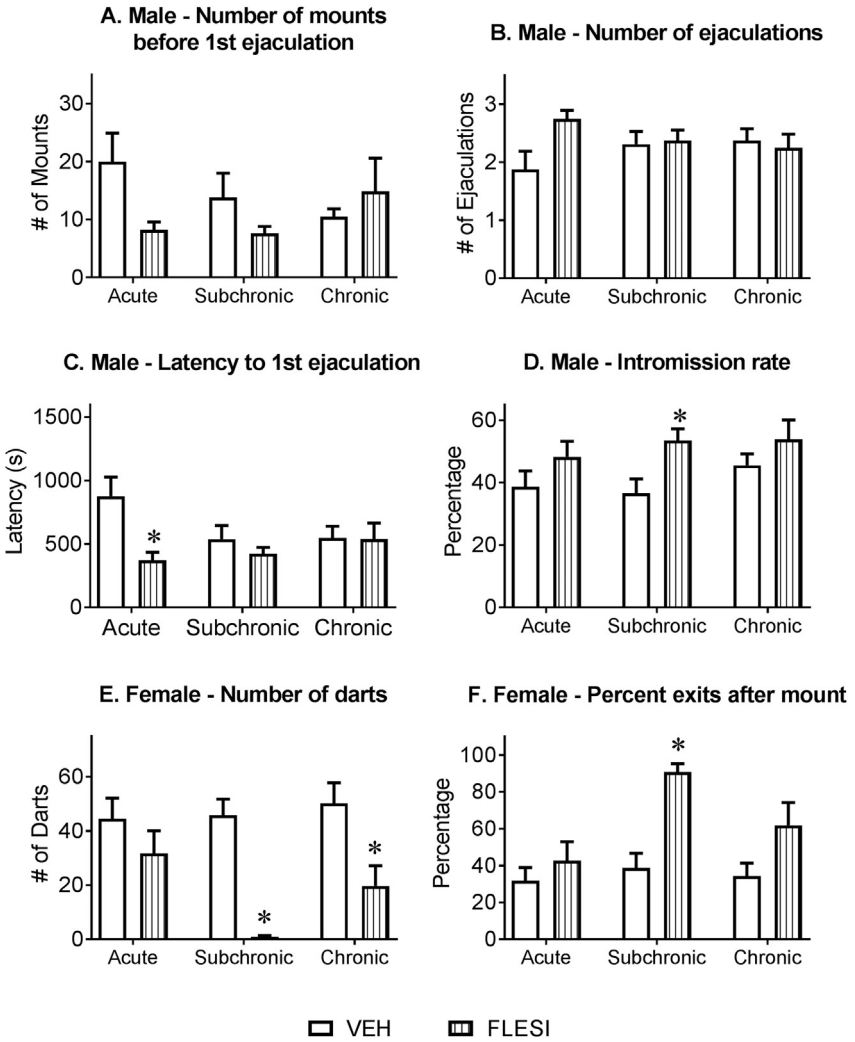

Fig. 2. The effects of 5-HT_{1A} receptor agonism on sexual behavior in male and female rats. Rats received 14 days of vehicle (VEH) or flesinoxan (FLESI) and their sexual behavior was measured after first (acute) and repeated (7 days-subchronic, 14 days - chronic) treatment. The number of mounts before first ejaculation (Panel A), number of ejaculations (Panel B), latency to first ejaculation (Panel C) and intromission rate (Panel D) were assessed for male sexual behavior. The number of darts (Panel E) and percent exits after mount (Panel F) were assessed for female proceptive behavior. Data are shown as mean ± standard error, n = 12–16 per group. '*' indicate significant difference between flesinoxan and vehicle ($p < 0.05$) in *post-hoc* analysis, following significant overall effect.

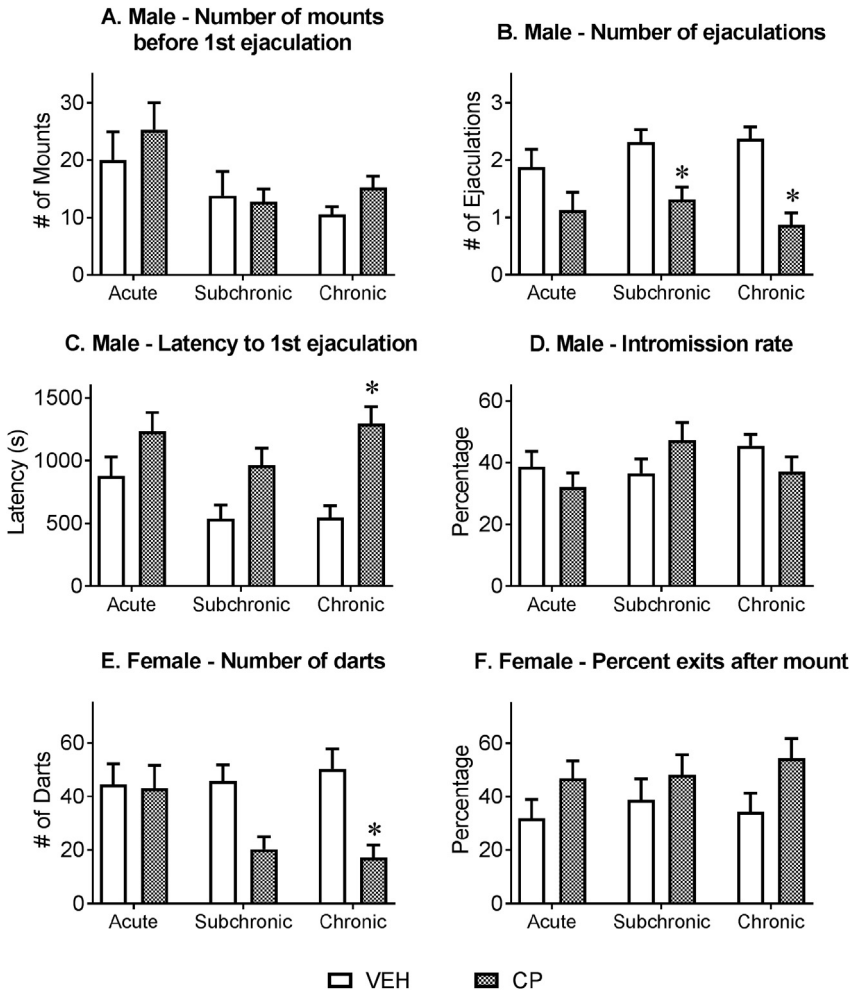

Fig. 3. The effects of 5-HT$_{1B}$ receptor agonism on sexual behavior in male and female rats. Rats received 14 days of vehicle (VEH) or CP-94253 (CP) and their sexual behavior was measured after first (acute) and repeated (7 days-subchronic, 14 days - chronic) treatment. The number of mounts before first ejaculation (Panel A), number of ejaculations (Panel B), latency to first ejaculation (Panel C) and intromission rate (Panel D) were assessed for male sexual behavior. The number of darts (Panel E) and percent exits after mount (Panel F) were assessed for female proceptive behavior. Data are shown as mean ± standard error, n = 12–16 per group. '*' indicate significant difference between CP-94253 and vehicle ($p < 0.05$) in *post-hoc* analysis, following significant overall effect.

3.5. *The effects of the 5-HT$_3$ receptor antagonist ondansetron*

Ondansetron (OND) did not significantly alter any sexual behavior in either male or female rats (Fig. 4 and Table 3).

3.6. *SERT occupancies of vortioxetine and paroxetine*

In the present study, vortioxetine treatments achieved levels of SERT occupancy that are similar to those observed in humans (Stenkrona et al., 2013). The high dose of vortioxetine fully occupied SERT (defined as 80% or greater occupancy) in male (Fig. 5A) and female (Fig. 5B) rats, while the low dose of vortioxetine achieved 40–50% SERT occupancy. Paroxetine at the dose used also achieved full SERT occupancy in male rats.

4. Discussion

Sexual dysfunction is a common reason for MDD patients to stop taking antidepressants, such as SSRIs (Serretti and Chiesa, 2009). However, clinical studies indicate a multimodal antidepressant, vortioxetine, causes a low degree of sexual dysfunction (Baldwin et al., 2016; Jacobsen et al., 2016). The current study investigated the mechanisms that might contribute to this observation in a preclinical model. The effects of vortioxetine on sexual behavior were assessed in both male and female rats after acute, subchronic and chronic treatment. In addition, the effects of 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_3$ receptors were measured after acute, subchronic and chronic treatment with selective ligands in both sexes. Each of the receptors chosen for study is directly modulated by vortioxetine. As antidepressants typically require several weeks to exert their effects, it is important to differentiate the effects of acute and chronic modulation of 5-HT receptors. To the best of our knowledge, this is the first report addressing the effect of subchronic and chronic administration of a selective 5-HT$_{1B}$ receptor agonist or a 5-HT$_3$ receptor antagonist on male and female rat sexual behavior.

4.1. *Effects of antidepressants on sexual behavior*

At a dose fully occupying SERT (similar to the level of SERT occupancy observed in the clinic) (Meyer et al., 2001), paroxetine inhibits male sexual behavior after chronic administration but not after acute treatment. This is consistent with previous findings and confirms the validity of using paroxetine in male rats as a model for SSRI-induced sexual dysfunction. We did not test paroxetine in

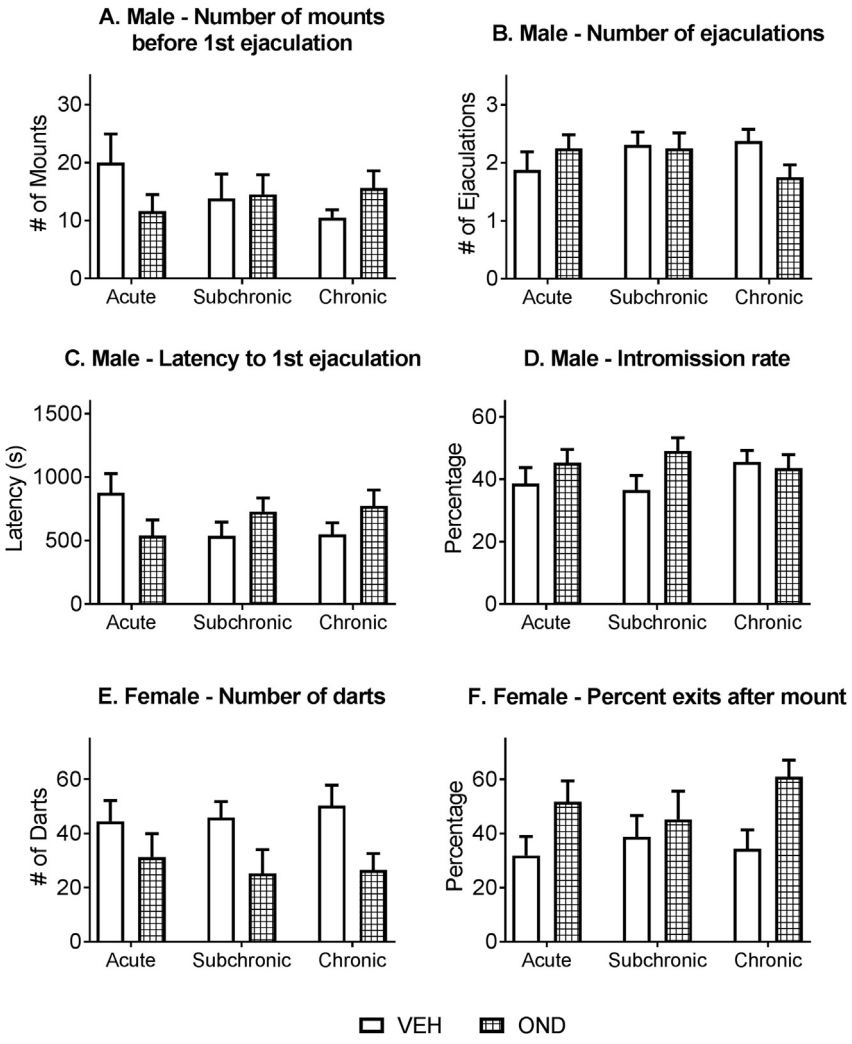

Fig. 4. The effects of 5-HT$_3$ receptor antagonism on sexual behavior in male and female rats. Rats received 14 days of vehicle (VEH) or ondansetron (OND) and their sexual behavior was measured after first (acute) and repeated (7 days-subchronic, 14 days - chronic) treatment. The number of mounts before first ejaculation (Panel A), number of ejaculations (Panel B), latency to first ejaculation (Panel C) and intromission rate (Panel D) were assessed for male sexual behavior. The number of darts (Panel E) and percent exits after mount (Panel F) were assessed for female proceptive behavior. Data are shown as mean ± standard error, n = 12–16 per group.

Table 3
Summary of other measures of female sexual behavior. Acute CP-94253 increased the percentage of exits from male compartment after intromission (bold font, *). No other significant difference was detected in female sexual behavior. Data were shown as mean ± standard error, n = 12–16 per group. VEH: vehicle; VOR: vortioxetine; FLESI: flesinoxan; DPAT: 8-OH-DPAT (acute^: administrated 30min prior to each test session); CP: CP-94253; OND: ondansetron. No Score: Too few data points were present, due to either a lack of mount or lack of intromission.

Treatment length	VEH	VOR (low)	VOR (high)	VOR (high) + FLESI	DPAT (acute^)	FLESI	CP	OND
Time in male compartment (sec)								
Acute	1198 ± 65	1096 ± 71	1042 ± 69	1132 ± 107	1146 ± 106	928 ± 64	918 ± 68	998 ± 80
Subchronic	1029 ± 69	1033 ± 88	1117 ± 69	949 ± 131	1012 ± 116	1119 ± 124	979 ± 104	1134 ± 87
Chronic	993 ± 119	990 ± 84	1123 ± 101	1182 ± 118	1148 ± 69	949 ± 105	984 ± 111	1035 ± 107
Percent exits after intromission								
Acute	44 ± 6	56 ± 9	54 ± 7	63 ± 13	61 ± 8	66 ± 5	**71 ± 4***	68 ± 8
Subchronic	59 ± 6	68 ± 7	66 ± 6	75 ± 8	57 ± 9	87 ± 8	76 ± 5	76 ± 6
Chronic	64 ± 8	62 ± 8	70 ± 8	67 ± 12	65 ± 6	78 ± 6	74 ± 6	85 ± 6
Contact-return latency (CRL) after mount (sec)								
Acute	123 ± 54	92 ± 15	125 ± 39	No Score	173 ± 48	195 ± 45	154 ± 29	222 ± 52
Subchronic	72 ± 15	89 ± 24	124 ± 27	184 ± 35	181 ± 60	No Score	258 ± 64	89 ± 15
Chronic	82 ± 23	117 ± 30	216 ± 73	264 ± 75	156 ± 59	140 ± 48	280 ± 65	220 ± 49
Contact-return latency (CRL) after intromission (sec)								
Acute	142 ± 45	108 ± 24	99 ± 32	No Score	232 ± 75	210 ± 46	154 ± 29	252 ± 75
Subchronic	107 ± 36	102 ± 28	133 ± 36	239 ± 128	179 ± 67	No Score	263 ± 76	113 ± 20
Chronic	115 ± 25	116 ± 27	218 ± 84	311 ± 142	126 ± 57	133 ± 34	246 ± 74	190 ± 33

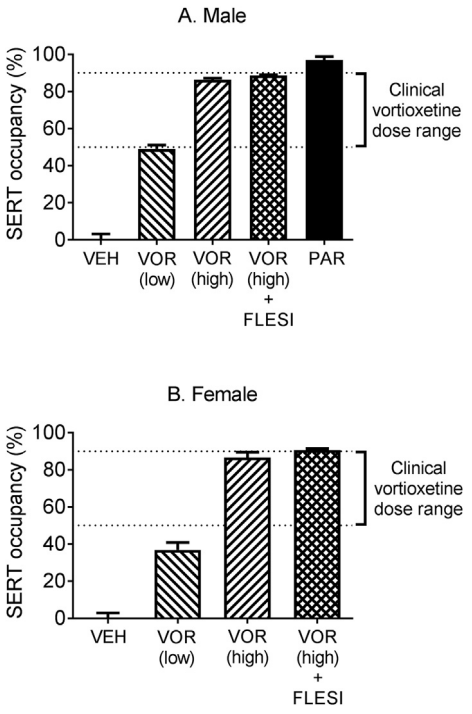

Fig. 5. SERT (serotonin transporter) occupancy levels of antidepressant in male and female rats. SERT occupancy levels were measured using *ex vivo* autoradiography in a separate cohort of rats (male- Panel A, female- Panel B) received 14 days of following treatments: vehicle (VEH), low dose vortioxetine (VOR), high dose vortioxetine, or high dose vortioxetine plus flesinoxan (FLESI) treatment. An additional group of male rats received 14 days of paroxetine (PAR). Between the dotted lines is the range of SERT occupancy levels achieved by vortioxetine clinically. Data are shown as mean ± standard error, n = 4–12 per group.

female rats, as it did not inhibit female sexual behavior in our earlier study (Snoeren et al., 2011). Previous studies have demonstrated that acute treatment with the SSRI fluoxetine inhibits female sexual activity in a strain-dependent manner (Miryala et al., 2013; Sarkar et al., 2008), at doses higher than those required to fully occupy the serotonin transporters. We have also found that high doses (20 mg/kg and greater) of paroxetine abolish sexual behavior in females (unpublished data).

In contrast, vortioxetine alone did not inhibit sexual behavior in male or female rats, including the high dose that resulted in a full SERT occupancy, equivalent to that of paroxetine. We hypothesize that the difference between paroxetine and vortioxetine results from the latter's direct modulation of serotonin receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_3 and 5-HT_7) at therapeutic doses, as previous studies have demonstrated the roles of specific 5-HT subtypes in regulating sexual behavior and sexual motivation (Maswood et al., 1998; Mendelson and Gorzalka, 1990; Snoeren et al., 2014a; Tanco et al., 1994).

Because the affinity of vortioxetine for the human 5-HT_{1A} receptor is markedly higher than its affinity for rat 5-HT_{1A} receptor, we also administered flesinoxan (5-HT_{1A} receptor agonist) together with the high dose of vortioxetine, to mimic the predicted occupancy of 5-HT_{1A} receptors in humans by the highest clinical dose. This combination facilitated male sexual behavior in a treatment length-dependent manner (i.e. reduced the latency to first ejaculation after acute treatment) and reduced female proceptive behavior (the number of darts, after acute and chronic treatment). It has yet to be determined if vortioxetine specifically alters female sexual motivation in humans.

4.2. Effects of 5-HT_{1A} receptor agonism on sexual behavior

Acute flesinoxan administration facilitates ejaculation in males, consistent with previous studies (Ahlenius et al., 1989; Mendelson and Gorzalka, 1986), but this effect was not detected after chronic administration. In females, 5-HT_{1A} receptor activation leads to inhibition of proceptive behavior (e.g. the number of darts and percent of exits after mount) after repeated treatment. These observations suggest that in addition to inhibiting lordosis in female rats (Mendelson and Gorzalka, 1986), acute treatment with a 5-HT_{1A} receptor agonist also inhibits proceptive behavior. These treatment length-dependent effects of 5-HT_{1A} stimulation in both male and female rats may suggest that the receptor desensitization may be involved (Hensler, 2003). An earlier study (Landen et al., 1999) reported that combined treatment of buspirone (a 5-HT_{1A} receptor partial agonist and antagonist for several dopamine receptor subtypes) and an SSRI improved sexual function of depressed patients, and this effect was more pronounced in women than in men. It is likely that modulation of both dopamine and 5-HT receptors underlie buspirone's effects. A recent study demonstrated that vilazodone (a SERT inhibitor and a 5-HT_{1A} receptor partial agonist) improved sexual function in both women and men, compared to SSRI citalopram (Clayton et al., 2015). Therefore, 5-HT_{1A} is likely to play a role in regulating sex functions in humans, even though the exact nature of this role (facilitation or inhibition) has yet to be determined.

4.3. Effects of 5-HT_{1B} receptor agonism on sexual behavior

Data from the present study indicate that 5-HT_{1B} receptor activation mediates an overall inhibitory effect on sexual behavior in both male and female rodents. Previous studies reported inhibition of male rat sexual behavior after single administration of non-selective 5-HT_{1B} receptor agonists, such as RU 24969 (an agonist for both 5-HT_{1A} and 5-HT_{1B} receptors), TFMPP (an agonist for 5-HT_{2C} and 5-HT_{1B} receptors, in addition to reversing the direction of serotonin transportation) and mCPP (an agonist with higher affinities for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} than for 5-HT_{1B} receptors, a reuptake inhibitor and releasing agent for serotonin) (Fernandez-Guasti et al., 1989). Similar effects were also observed after acute anpirtoline (a 5-HT_{1B} receptor agonist and 5-HT_3 receptor antagonist) treatment (Ahlenius and Larsson, 1998; Hillegaart and Ahlenius, 1998). The levels of 5-HT_{1B} receptor occupancy of these compounds are unknown. CP-94253 is a more selective and longer-acting 5-HT_{1B} receptor agonist, and the dose tested in the current study corresponds to approximately 80% 5-HT_{1B} receptor occupancy (unpublished data). Overall, our data are consistent with the notion that 5-HT_{1B} receptors exert an inhibitory control over male rat sexual behavior. Given the differences in dosing regimen, target profiles of ligands, and parameters measured, it is not possible to directly compare the current study with previous studies in female rats.

4.4. Effects of 5-HT_3 receptor antagonism on sexual behavior

The selective 5-HT_3 receptor antagonist, ondansetron, had no significant effect on sexual behavior in either sex in the current study, at a dose corresponding to approximately 60% brain 5-HT_3 receptor occupancy after acute administration (du Jardin et al., 2014). This is consistent with earlier studies demonstrating that acute 5-HT_3 receptor modulation has very limited effect on male and female rat sexual behavior (Tanco et al., 1993, 1994). However, direct infusion of the 5-HT_3 receptor antagonist tropisetron into the ventromedial nucleus (VMN) of the hypothalamus was shown to reduce the lordosis to mount ratio in female rats (Maswood et al.,

1998). Several factors raise the possibility that this observed effect may be mediated by other targets: the dose of tropisetron used in this study was very high compared to its affinity for the 5-HT_3 receptor (Dumuis et al., 1992), tropisetron also modulates other receptors (Papke et al., 2004), and the expression level of the 5-HT_3 receptor in VMN is low (Kow et al., 1992). Even though it has been suggested that 5-HT_3 receptor antagonism could be useful for treatment of SSRI-induced sexual dysfunction in patients (Berk et al., 2000), there is currently no substantive clinical evidence to support this hypothesis.

4.5. Potential impact of locomotor activity on sexual behavior

Gross changes in general locomotor activity after drug treatment could confound some measures of sexual behavior in rats. To this end, we analyzed the number of entries to the male compartment by females. Acute CP-94253 increased this measure, which suggests an increase in locomotor activity. No significant change was detected at other time points or with other treatments. These data suggest that changes in female proceptive behavior are not associated with a reduction in locomotor activity. A previous study demonstrated that 8-OH-DPAT inhibits female sexual behavior without reducing locomotor activity (Kishitake and Yamanouchi, 2003). Published literature suggests that acute flesinoxan administration at similar doses as used in the current study has equivocal effects on locomotion, with some studies suggesting that flesinoxan increases (Suwabe et al., 2000) or decreases (Ahlenius et al., 1991) locomotor activity in rats. In addition, acute CP-94253 at the dose used in the present study did not affect locomotor activity (Przegalinski et al., 2007). Taken as a whole, it seems that the absent or modest effects of these agents on general locomotor activity do not correlate with their effects on sexual behavior.

4.6. Mechanisms potentially contributing to vortioxetine's lack of effect on sexual behavior

The overall absence of vortioxetine's effect on sexual behavior may be a result of the direct modulation of different 5-HT receptors balancing out the effect of serotonin transporter inhibition. Although other serotonin receptors, such as those of 5-HT_2 family, are involved in regulating sexual behavior (Angoa-Perez and Kuhn, 2015; Uphouse, 2014), neither vortioxetine nor paroxetine directly modulate these receptors. Therefore, vortioxetine and paroxetine may activate these receptors via a global increase of serotonin (through SERT inhibition) to the same degree. Thus an indirect effect mediated by elevated serotonin tone is unlikely to explain the difference between these two antidepressants. We propose that the direct modulations of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_3, and 5-HT_7 receptors are the underlying mechanisms that differentiate vortioxetine from paroxetine.

5-HT_{1A} receptor agonists are consistently found to counteract SSRI-induced suppression of ejaculatory behavior in male rats (Oosting et al., 2016; Snoeren et al., 2014b). While 5-HT_{1A} receptor stimulation may contribute to the overall effect of vortioxetine, this mechanism alone is unlikely to account for the difference between vortioxetine and paroxetine in male rats. First, the level of rat 5-HT_{1A} receptor occupancy by vortioxetine alone, even at the high dose, is modest (du Jardin et al., 2014). Second, the effect of this stimulation in male rats disappears after chronic administration.

Notably, the high dose vortioxetine (corresponding to approximately 70% 5-HT_{1B} receptor occupancy and full SERT occupancy) did not affect sexual behavior of male and female rats. In contrast, the selective 5-HT_{1B} receptor agonist CP-94253 inhibited sexual behavior in both male and female rats, the time course of which is consistent with that of paroxetine in males. It is plausible that the inhibitory effect of paroxetine may be mediated by activating 5-HT_{1B} receptors, result from an increased serotonin level following SERT inhibition. In contrast, vortioxetine is reported to be a partial 5-HT_{1B} receptor agonist in *in vitro* functional assays (Mork et al., 2012), and is able to reverse the effect of CP-94253 in electrophysiology studies (El Mansari et al., 2015). Thus, as a partial agonist, vortioxetine may functionally act as an antagonist at this receptor under increased 5-HT tone following the inhibition of SERT, and limit this negative effect on sexual behavior. This hypothesis is further supported by studies reporting that acute treatment with a 5-HT_{1B} receptor antagonist fully blocked the inhibitory effect of 5-HTP administration on ejaculation in male rats (Ahlenius and Larsson, 1998). To test this hypothesis directly, a study may be carried out to assess the overall effect of a 5-HT_{1B} antagonist in combination with paroxetine, or to measure the effect of a 5-HT_{1B} receptor agonist in combination with vortioxetine treatment.

Vortioxetine, even at the low dose tested in the current study, fully occupies 5-HT_3 receptors (Sanchez et al., 2015). Nevertheless, rat sexual behavior was not affected by vortioxetine at either the low or the high dose, in either male or female subjects. In addition, the selective 5-HT_3 antagonist ondensetron did not affect sex behavior in either male or female rats, even after chronic treatment. These data are consistent with earlier reports that 5-HT_3 receptors are not involved in regulating sexual behavior (Tanco et al., 1993, 1994). Thus, it is not likely that 5-HT_3 receptor antagonism is the essential mechanism for vortioxetine's lack of effects on sexual behavior.

Direct modulation of other 5-HT receptors may also contribute to the overall lack of effects induced by vortioxetine, i.e., antagonizing 5-HT_{1D} and 5-HT_7 receptors. While the involvement of 5-HT_{1D} receptors in regulating sexual activity is unclear, a 5-HT_7 receptor antagonist was shown to facilitate sexual behavior in female rats (Siddiqui et al., 2007). It is also possible that the low sexual effects of vortioxetine are mediated by the combination of direct modulation of different 5-HT receptors. It will be interesting to test vortioxetine in SERT knockout rats, which provide a platform to study the putative modulatory effects of the non-SERT mechanisms.

In conclusion, the present study demonstrates that acute and repeated treatment with vortioxetine, at doses corresponding to the clinical levels of target engagement, does not affect sexual behavior in either male or female rats. High dose vortioxetine plus a 5-HT_{1A} receptor agonist, and modulation of 5-HT_{1A} or 5-HT_{1B} receptors cause sex- and treatment length-dependent effects. These findings underline the complex serotonergic regulation of sexual behavior, and suggest direct receptor modulation contributes to the low sexual side effect of vortioxetine.

Disclosure

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