Sex, aggression and pair-bond : a study on the serotonergic regulation of female sexual function in the marmoset monkey

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CHAPTER 2

Flibanserin and 8-OH-DPAT implicate serotonin in association between female marmoset monkey sexual behavior and changes in pair-bond quality

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ABSTRACT

Introduction. Psychopathological origins of personally distressing, hypoactive sexual desire disorder (HSDD) in women are unknown, but are generally attributed to an inhibitory neural regulator, serotonin (5-HT). Flibanserin, a 5-HT_{1A} agonist and 5-HT_{2A} antagonist, shows promise as a treatment for HSDD.

Aim. To test the hypothesis that female marmoset sexual behavior is enhanced by flibanserin and diminished by 8-OH-DPAT, in order to evaluate the efficacy of serotonergic modulation of female sexual behavior in a pairmate social setting comparable to humans.

Methods. Sexual and social behavior were examined in eight female marmoset monkeys receiving daily flibanserin (15 mg/kg), 8-OH-DPAT (0.1 mg/kg), or corresponding vehicle for 15–16 weeks in a counterbalanced, withinsubject design, while housed in long-term, stable male–female pairs.

Main Outcome Measures. Marmoset pairmate interactions, including sexual and social behavior, were scored during weeks 5–6 of daily flibanserin, 8-OH-DPAT or vehicle treatment. 24-hour pharmacokinetic profiles of the drugs and their metabolites, as well as drug-induced acute symptoms of the 5-HT behavioral syndrome were also assessed.

Results. Two-way analysis of variance reveals that flibanserin-treated females attract more male sexual interest ($P = 0.020$) and trigger increased grooming ($P = 0.001$) between partners. In contrast, 8-OH-DPAT-treated females show increased rejection of male sexual advances ($P = 0.024$), a tendency for decreased male sexual interest ($P = 0.080$), and increased aggression with their male pairmates ($P = 0.049$).

Conclusions. While 8-OH-DPAT-treated female marmosets display decreased sexual receptivity and increased aggressive interactions with their male pairmates, flibanserin-treated female marmosets demonstrate increased affiliative behavior with their male pairmates. Such pro-affiliation attributes may underlie flibanserin's effectiveness in treating HSDD in women.

INTRODUCTION

In an estimated 10% of women [1], marked distressand interpersonal difficulty arise from unwanted, persistent or recurrent low sexual desire (hypoactive sexual desire disorder, HSDD; American Psychiatric Association's Diagnostic and Statistical Manual, DSM-IV-TR). Psychopathogenesis of HSDD is not known, but neurotransmitter dysfunction has been proposed involving the excitatory regulators dopamine (DA) and norepinephrine (NE), as well as inhibitory serotonin (5-HT) [2,3]. 5-HT is a key neurotransmitter involved in female sexual inhibition [2]. Pharmacological manipulation of 5-HT commonly results in diminished female sexual satisfaction and activity, particularly in women prescribed selective serotonin reuptake inhibitors (SSRIs) for depression [4]. Animal studies that apply 5-HT receptor subtype specific ligands permit mechanistic examination of 5-HTmediated effects on sexual behavior. There are seven known 5-HT receptor families, each with its own specific brain distribution, as well as effects on behavior and physiology [5]. For example, in rodents, the sexually receptive female lordosis posture is inhibited by 5-HT_{1A} receptor activation [6,7] and 5-HT_3 receptor antagonism [8], but is facilitated by 5-$HT_{2A/C}$ receptor activation [9].

Recently, flibanserin (2H-benzimidazol-2-one, 1,3-dihydro-1-[2-[4-[3-(trifluoromethyl) phenyl]- 1-piperazinyl]ethyl], an agonist of 5-HT_{1A} and antagonist of 5-HT_{2A} receptors [11,12], has been shown to stimulate sexual solicitation and receptivity in female rats [13] and to improve sexual desire in women with major depression [14]. Women with HSDD report increased satisfying sexual events, increased desire and decreased distress following flibanserin treatment [15]. *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin hydrobromide (8-OH-DPAT), however, a selective 5-HT_{1A} receptor agonist [16], diminishes female sexual receptivity in rats [17]. Thus, despite the shared 5-HT_{1A} agonist activity between flibanserin and 8-OH-DPAT, the contrasting effects of the two drugs on rodent female sexual behavior provides us with an opportunity to examine whether such contrasting behavioral outcomes translate to a nonhuman primate model, the common marmoset, in a well-established male– female pairmate social environment.

Female marmoset monkeys present an opportunity to contrast the effects of flibanserin and 8-OH-DPAT in an animal model that readily translates to humans because marmosets form and display modest amounts of sexual behavior [19]. Unlike the multiple-mating social structures of rats and many nonhuman primates, such as macaques and baboons, marmoset sexual behavior most commonly occurs within stable male–female pairs [18,20]. During acceptance or rejection of a pairmate's sexual advances, female marmosets can readily promote, prevent, or terminate sexual interactions [21],

and our recent development of a standardized behavioral testing paradigm permits repeatable, quantitative exploration of neurally active compounds that enhance or diminish female marmoset sexual behavior [19].

AIMS

The aim of the present study was to test the hypothesis that female marmoset sexual behavior is enhanced by flibanserin and diminished by 8-OH-DPAT, in order to evaluate the efficacy of serotonergic modulation of female sexual behavior in a pairmate social setting comparable to humans.

METHODS

Study animals

This study was conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act and its subsequent amendments. All animal procedures were reviewed and approved by the Graduate School Animal Care and Use Committee of the University of Wisconsin-Madison. The Wisconsin National Primate Research Center (WNPRC) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care as part of the University of Wisconsin-Madison Graduate School. Sixteen adult (age 2–5 years) nulliparous captive-born common marmoset *(Callithrix jacchus)* females were pair housed with similarly aged male partners at the WNPRC for 8–20 months before onset of this study. Females were housed with the same male partner for the entire study, as previously described [22], and were ovariectomized and primed with either mid-follicular phase estradiol levels or no hormone [19] before study onset. This model allows us to provide a repeatable estrogen replete (estradiol capsules implanted) or estrogen deficient (empty capsules implanted) hormonal environment in which sex hormone levels are stable and reflect, respectively, the equivalent of an estrogen-dominant, pre-ovulatory stage in the ovarian cycle or a post-menopausal stage.

Experimental design

A counterbalanced, crossover study that applied within-subject comparisons was designed to examine the effects of chronic (15–16 weeks) daily (12:00pm–2:00pm) administration of flibanserin (N = 8; 15 mg/kg, orally (PO) in 1 mL/kg vehicle; Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany), 8-OH-DPAT (N = 8; 0.1 mg/kg in 0.4 mL/kg vehicle, injected subcutaneously (SC); Sigma-Aldrich St. Louis, MO, USA), or

respective vehicles (for flibanserin, 98.5% of 0.5% hydroxycellulose solution and 1.5% of 1% polysorbate 80 solution, 1.0 mL/kg PO; for 8-OH-DPAT, 0.4 mL saline, SC). The study focused on two major behavioral outcomes: (i) sexual and social interactions between pairmates; and (ii) acute manifestation of the 5-HT behavioral syndrome providing quantitative indication of continued drug efficacy.

As flibanserin was not previously administered to female marmosets, we performed an initial dose response study comparing male–female interactions observed after 5–6 weeks of oral dosing of 10 mg/kg (N = 4) or 30 mg/kg (N = 4) flibanserin to behavior observed during baseline male–female interactions made prior to these flibanserin treatments. We observed changes in selected behaviors (Table S1) in our male–female testing paradigm, described in later discussion, 16–24 hours after daily flibanserin administration, compared to baseline. Blood samples assessing pharmacokinetics of both doses (Figure S1) were obtained after 3–4 weeks. As similar results were obtained from both doses for behavioral and pharmacokinetic assessments, we decided to employ an intermediate dose of 15 mg/kg for the main study. The scaled equivalent in humans of 15 mg/kg flibanserin administered to marmosets is ~2.4 mg/kg [23] and approximately similar to ~1.7 mg/kg flibanserin administered to women in clinical trials (100 mg/ day, assuming 60 kg body weight [24].

8-OH-DPAT has previously been administered to marmosets in i.p. injections of 0.3 mg/kg, resulting in pronounced expression of the serotonin behavioral syndrome immediately following each treatment. 8-OH-DPAT at a dose of 0.3 mg/kg also induces scratching and diarrhea [25]. To minimize the latter responses and to remain within a dose range previously used to consistently diminish female sexual behavior in rats (0.025–1.0 mg/kg, SC or IP [6,17,26], we selected 0.1 mg/kg SC for this study.

Bilateral ovariectomy

Females were injected intramuscularly (IM) with ketamine (15 mg/kg), 0.02–0.04 mg/kg atropine and 0.01 mg/kg buprenorphine, and were maintained on isofluorane (2%; 0.6 liter/min oxygen). Each ovary was isolated through a ventral midline incision and exteriorized for visualization of the fallopian tube and ovarian pedicle. Subsequent histological examination confirmed complete ovarian removal.

Estradiol replacement

One week before the start of daily treatment, females were implanted SC with silastic capsules that were either estradiol-filled (N = 4 per active compound/vehicle) or empty (N = 4 per active compound/vehicle). Plasma

estradiol levels were determined every 2 weeks whenever capsules were implanted. Treatment with active compound/vehicle started at (i) either 7 weeks after ovariectomy or 7 weeks after removal of capsules; and (ii) 1 week after implantation or re-implantation of capsules that occurred at 6 weeks after ovariectomy or 6 weeks after removal of previous capsules, resulting in a constant intertreatment interval of 7 weeks. Estradiol status was maintained throughout treatment for each female.

Estradiol levels in blood samples that were collected by femoral puncture [22] were determined using celite column chromatography and a validated estradiol radioimmunoassay (RIA) for marmoset plasma [27]. Assay sensitivity was 4.6 pg/tube (30.4 pg/mL), and intra- and inter-assay assay coefficients of variation (CVs), respectively, were 5.0% and 14.0%.

Ovariectomized females implanted with estradiol-filled capsules (N = 8) had higher ($P < 0.003$) circulating estradiol levels (396.0 30.6 pg/mL) compared to females implanted with empty capsules (67.5 5.2 pg/mL), and circulating estradiol values in the former females were comparable to those previously reported for female marmosets in the mid-follicular phase of the ovarian cycle [19]. Estradiol replacement is thus below the mid-cycle, peri-ovulatory levels used in some previous studies [28] and thus supports modest [19] rather than maximal [29] expression of female marmoset sexual behavior.

Pharmacokinetic assessment of flibanserin and 8-OH-DPAT administration

Treatment-induced systemic exposure to circulating levels of flibanserin and two common flibanserin metabolites, 1-(3-trifluoromethylphenyl) piperazine (TFMPP) and 6-hydroxy-flibanserin (BIMA 23 BS), and to 8-OH-DPAT, was assessed during study weeks 40 to 42 by validated high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Serial blood samples were collected at 0.25, 0.5, 1, 3, 6, and 24 hours after flibanserin or 8-OH-DPAT administration (N = 4 for each compound).

Behavioral observation of sexual and social behavior

In order to stimulate social and sexual interactions upon reunion [19], females andmales were separated for 90 minutes prior to each of four, 30-minute behavioral tests at 7:00am–1:00pm (16–24 hours after daily administration of active compound/ vehicle), 5–6 weeks after treatment onset. This time window for observations was chosen to assess the chronic changes induced by flibanserin and 8-OHDPAT when circulating levels of drugs were minimal, thus avoiding potential acute effects driven by elevated circulating concentrations of the drug preparations and active metabolites.

Potential behavioral changes are thus consequences of longterm adaptation to treatment that may involve changes in gene and protein expression [30].

At the start of the behavioral test, the male was introduced to the female by remote door operation, and behavior was manually and digitally recorded by two observers from behind a one-way window (Table 1). Behavioral tests were reanalyzed in a random fashion from the digitally stored recordings by two observers blinded with respect to treatment. The reanalyzed data were compared with those obtained on the day of the test to confirm final values and to generate behavioral data not originally scored during live observations (for aggression and self-grooming). Inter-observer reliability scores for behavioral data collection averaged 90.6%, and within-observer reliability scores averaged 96.1%.

Monitoring of the 5-HT behavioral syndrome

Locomotor behaviors indicative of the 5-HT behavioral syndrome, i.e., “random rapid limb movements” and “wet-dog shakes” [16,25], were monitored once per week (0.5 hour) at 0–0.5 hour and at 16–24 hours after administration of active compound/vehicle treatment, during weeks 1 to 4 of treatment (Table 1). Females were placed in a test cage for the duration of the test (8-OH-DPAT/vehicle), or returned to their home cage (flibanserin/vehicle). In addition, sprawling behavior (females lying down in prone position, monitored during flibanserin or respective vehicle treatment only), a possible non-locomotor component of the 5-HT behavioral syndrome [32], and self-scratching behavior, a locomotor behavior not specifically linked to 5-HT neurotransmission, were scored.

Data analysis

Observed levels of female proceptive sexual behavior (Table 1) were too infrequent to permit statistical analysis. Female sexual receptivity was quantified by frequency of female acceptance of male ejaculatory mounts. Female refusal of male sexual advances was quantified by frequency of rejection of male mounts and mount attempts. Sexual arousal of the male was quantified by frequency of penile erection. Analyses of all behavior were performed on transformed frequency data square root (1 + x)] to achieve homogeneity of variance and to increase linearity of data. This transformation generates positive numbers, permitting appropriate analysis of behavioral frequency data as square root transformation in which the variance is independent of the mean [33]. Mean values of the frequencies were analyzed by two-way anova incorporating repeated measures design, with Treatment (active compound, vehicle) and Observations (observation 1–4) as within-subject factors. Data

are presented as backtransformed mean values (95% confidence intervals). A P value less than 0.05 was considered significant.

Initial analyses of behavioral data were performed using the same mixed design anova with Estradiol supplementation and Order of treatment as between-subject factors, and Treatment as within-subject factor. As Estradiol supplementation and Order of treatment consistently failed to affect ($P > 0.05$) any behavioral variable, both factors were omitted in the final analyses reported here.

Table 1 Ethogram and mean ± SEM values for behavioral scores during pairmate tests

Behavior	Flibanserin vehicle	Flibanserin	8-OH-DPAT vehicle	8-OH-DPAT
Proceptive female sexual behavior*				
Proceptive tongue flicking	0	0.03 ± 0.06	0	0
Proceptive staring	0	0	0	0
Proceptive freeze posture/Sprawling position	0.16 ± 0.28	0.12 ± 0.14	0.05 ± 0.06	0.06 ± 0.09
Receptive female sexual behaviors*				
Acceptance of mounts	0.61 ± 0.58	0.75 ± 0.75	0.23 ± 0.27	0.64 ± 0.57
Rejection of mount attempts and mounts**	0.19 ± 0.28	0.25 ± 0.29	**0.62 ± 0.75**	**1.49 ± 1.50**
Receptive freeze posture	0.29 ± 0.28	0.45 ± 0.56	0.23 ± 0.27	0.58 ± 0.55
Receptive tongue flicking	0	0	0	0
Receptive head turning/biting	0.40 ± 0.35	0.35 ± 0.30	0.20 ± 0.23	0.29 ± 0.24
Male sexual behaviors*				
Penile erection	0.78 ± 0.65	1.12 ± 0.98	0.89 ± 0.73	1.05 ± 0.46
Mounting	0.61 ± 0.58	0.71 ± 0.69	0.23 ± 0.27	0.64 ± 0.57
Mounting attempts**	0.19 ± 0.28	0.26 ± 0.27	**0.63 ± 0.83**	**1.63 ± 1.59**
Intromitting	0.43 ± 0.37	0.48 ± 0.43	0.17 ± 0.20	0.35 ± 0.27
Ejaculating	0.28 ± 0.31	0.26 ± 0.27	0.17 ± 0.20	0.21 ± 0.15
Social odors*				
Genital investigation by female	0.03 ± 0.06	0.06 ± 0.08	0.28 ± 0.66	0.08 ± 0.17
Genital investigation by male¶	**1.76 ± 1.54**	**2.62 ± 1.89**	1.12 ± 0.74	0.49 ± 0.39
Scent marking by female	2.54 ± 2.53	3.15 ± 2.67	3.50 ± 3.95	4.36 ± 5.17
Scent marking by male	3.52 ± 4.71	3.39 ± 3.07	5.18 ± 5.23	3.69 ± 2.62
Social interactions*				
Grooming by female	1.29 ± 1.57	2.19 ± 2.15	2.77 ± 3.57	1.79 ± 2.60
Grooming by male¶	**2.21 ± 2.02**	**3.97 ± 3.27**	1.09 ± 0.57	0.76 ± 0.47
Total allogrooming¶	**3.94 ± 2.22**	**6.91 ± 3.20**	4.07 ± 3.74	2.71 ± 2.69
Aggression by female	0.94 ± 1.17	0.78 ± 1.07	0.92 ± 1.03	1.66 ± 1.76
Aggression by male	0.06 ± 0.08	0.17 ± 0.23	0.18 ± 0.18	0.53 ± 0.62
Total aggression**	0.99 ± 1.21	0.91 ± 1.27	**1.09 ± 1.14**	**2.23 ± 1.98**
Contact within arm-length	5.54 ± 1.23	5.42 ± 1.04	7.71 ± 2.04	6.27 ± 1.75
Direct body contact/huddling	8.69 ± 1.72	10.37 ± 2.60	9.42 ± 2.84	9.77 ± 2.48
Total contact	14.30 ± 2.07	15.87 ± 3.07	17.62 ± 1.55	16.19 ± 3.30
Initiating contact by female	11.14 ± 5.67	10.99 ± 3.41	20.13 ± 8.08	16.99 ± 7.84
Breaking contact by female	15.60 ± 5.99	15.03 ± 7.71	21.09 ± 6.46	14.27 ± 7.20
Following by female	1.60 ± 1.66	1.67 ± 1.39	3.00 ± 2.52	2.60 ± 2.33
Avoiding contact by female	1.69 ± 1.15	1.53 ± 1.22	1.60 ± 1.00	1.15 ± 0.61
Self-directed behavior by female*				
Self-grooming¶	**2.75 ± 1.86**	**3.96 ± 1.95**	1.00 ± 0.55	1.89 ± 1.25
Genital inspection/Genital self-grooming¶	**1.87 ± 1.58**	**4.18 ± 2.95**	0.45 ± 0.43	0.33 ± 0.23
Scratching	12.74 ± 8.73	11.50 ± 4.94	8.44 ± 5.09	10.95 ± 3.56
Locomotion and movement by female*				
Mobile in locomotion	2.50 ± 0.74	1.93 ± 0.53	3.07 ± 1.15	2.39 ± 1.03
Mobile while stationary	4.45 ± 1.55	5.33 ± 1.64	4.06 ± 1.95	4.14 ± 1.41
Mobile total	6.97 ± 2.14	7.29 ± 1.92	7.25 ± 2.47	6.67 ± 1.63
Immobile, incl. sprawling position	22.72 ± 2.09	22.47 ± 1.77	22.33 ± 2.59	23.14 ± 1.59
Acute behavioral changes†				
Scratching*	7.32 ± 2.40	5.23 ± 2.32	1.50 ± 0.90	5.56 ± 4.49
Random rapid limb movements‡**	0	0	0	**6.89 ± 4.32**
"Wet-dog" shakes‡**	0.24 ± 0.16	0.11 ± 0.18	0	**17.44 ± 11.90**
Latency to sprawling position§¶	**19.47 ± 4.19**	**14.58 ± 4.71**	Not scored	Not scored

A zero value indicates complete absence of behavior. Bold numbers indicate significant changes compared to vehicle treatment. 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin

*Adapted from Stevenson and Poole [21]. Behavior was recorded at 16–24 hours after daily drug/vehicle administration during weeks 5–6 of daily treatment

†Behavior was recorded at 0–0.5 hours after drug/vehicle administration during weeks 1–4 of daily treatment

‡Behavior indicative of the 5-HT behavioral syndrome. Elliott et al. [25]

§Behavior resembling the rodent flat-body posture. Tricklebank et al. [31]

¶Significantly altered by flibanserin administration ($P < 0.05$)

**Significantly altered by 8-OH-DPAT administration ($P < 0.05$)

MAIN OUTCOME MEASURES

Marmoset pairmate interactions, including sexual and social behavior, were scored atweeks 5–6 of daily flibanserin, 8-OH-DPAT or vehicle treatment. In addition, 24-hour pharmacokinetic profiles of flibanserin, TFMPP, BIMA 23 BS, and 8-OH-DPAT, as well as drug-induced acute symptoms of the5-HT behavioral syndrome, were assessed.

RESULTS

Systemic exposure to flibanserin, flibanserin metabolites, and 8-OH-DPAT during chronic drug treatment

Maximal plasma concentrations of flibanserin, TFMPP and BIMA 23 BS (C(max)) of 628 ± 225 ng/mL (mean ± SEM), 46.0 ± 17.0 ng/mL and 149 ± 38.7 ng/mL, respectively, were reached at 0.5–1 hour after oral administration, decreasing rapidly to low levels by 24 hours, providing a circulating half-life for flibanserin of 2.8 hours (Figure 1). For 8-OH-DPAT, C(max) of 69.7 ± 19.8 nmol/L was reached by 0.25–0.5 hour after SC injection, and values dropped rapidly to 3.4 ± 2.6 nmol/L after 3 hours, providing a circulating half-life of 8-OH-DPAT of 0.6 hour (Figure 1). No cumulative effects of chronic 8-OH-DPAT and

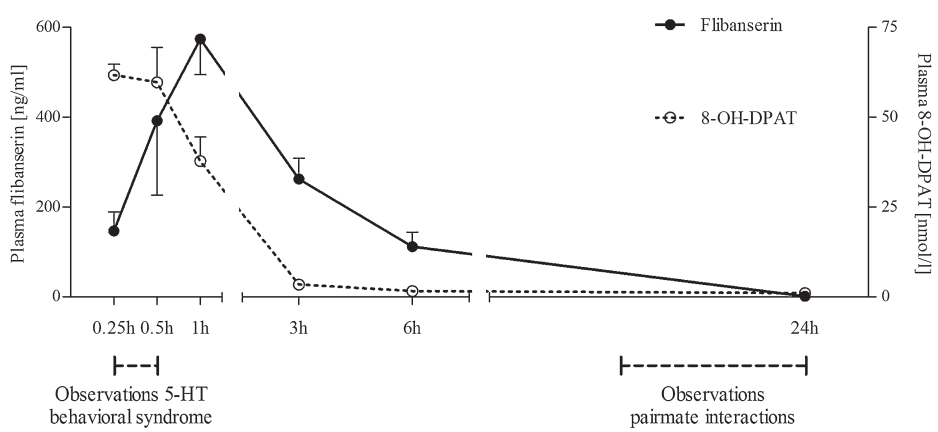

Figure 1. 24-hour pharmacokinetic profiles of flibanserin and 8-OH-DPAT in female marmoset monkeys. Pairmate observations were performed at 16-24 hours after administration, when exposure to circulating drug concentrations was low or absent, while observations of the 5-HT behavioral syndrome was performed at 0-0.5 hour after administration, when circulating drug concentrations were high. 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin; 5-HT, serotonin.

flibanserin administration were apparent. The results confirm a substantial systemic exposure to flibanserin, BIMA 23 BS, TFMPP and 8-OH-DPAT during observations for 5-HT behavioral syndrome, but not during pairmate observations. Thus, treatment-induced changes during pairmate observations are most likely attributable to the long-term consequences of flibanserin and 8-OH-DPAT exposure, and not to acute effects elicited by elevated circulating levels of active drug.

Chronic effects of 8-OH-DPAT and flibanserin on sexual, social and self-directed behavior

Five to six weeks of daily oral administration of flibanserin to female marmosets noticeably increased female genital area self-grooming ($F(1,7) = 31.28$, $P = 0.001$; Figure 2A) and male pairmate sniffing/licking of their female's genital area ($F(1,7) = 8.91$, $P = 0.020$; Figure 2B). No other sexually related behavior was altered by flibanserin (Table 1). In contrast, 5–6 weeks of daily SC administration of 0.1 mg/kg 8-OH-DPAT strikingly increased female rejection of male pairmate mount attempts and mounts compared to corresponding vehicle ($F(1,7) = 8.24$, $P = 0.024$; Figure 3). 8-OH-DPAT-induced female rejection of male sexual advances also increased male attempts to mount their female pairmate ($F(1,7) = 6.93$, $P = 0.034$). The male pairmates

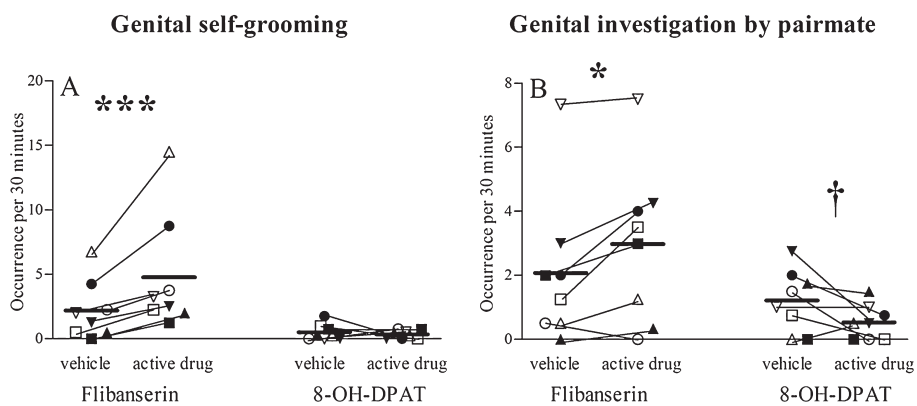

Figure 2. Investigation of female genital area. Frequencies (backtransformed mean) of **(A)** female genital area selfgrooming and **(B)** male inspection of female genital area per 30 minutes during 5–6 weeks following the onset of flibanserin, flibanserin vehicle, 8-OH-DPAT, or 8-OH-DPAT vehicle administration. *** $P = 0.001$ vs. flibanserin vehicle ($F(1,7) = 31.3$), * $P = 0.020$ vs. flibanserin vehicle ($F(1,7) = 8.9$), † $P = 0.080$ vs. 8-OH-DPAT vehicle ($F(1,7) = 4.2$). Each symbol indicates the same individual female marmoset receiving estradiol (solid symbols) or no estradiol (open symbols). 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin.

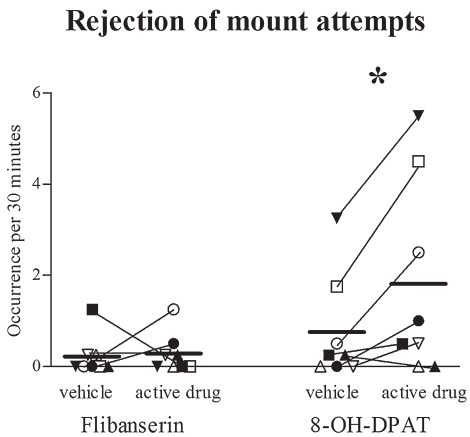

Figure 3. Female sexual rejection. Frequency (backtransformed mean) of female rejection of male mounts and mount attempts per 30 minutes during 5–6 weeks of flibanserin, flibanserin vehicle, 8-OH-DPAT, or 8-OH DPAT vehicle administration. * P = 0.024 vs. 8-OH-DPAT vehicle (F(1,7) = 8.2). Each symbol indicates the same individual female marmoset receiving estradiol (solid symbols) or no estradiol (open symbols). 8-OH-DPAT, *R*-(+)-8-hydroxy-2- (di-n-propylamino)-tetralin.

of 8-OH-DPAT treated females also tended to sniff/lick their female pairmate's genital area less (F(1,7) = 4.20, P = 0.080; Figure 2B), and decreased male genital sniffing was correlated with female rejection of male mount attempts and mounts (r = -0.747, P = 0.033). No other sexually related behavior was altered (Table 1).

Such opposing effects of flibanserin and 8-OHDPAT were also observed in social interactions between male and female pairmates. Flibanserin increased

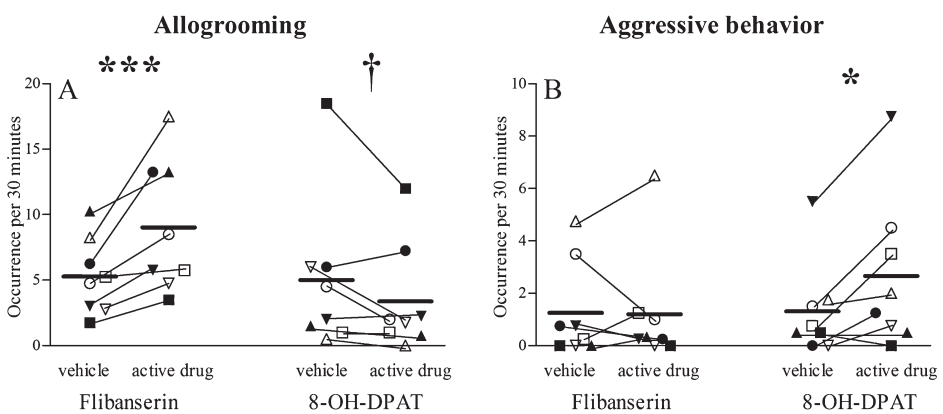

Figure 4. Pairmate allogrooming and aggression. Frequencies (backtransformed mean) of **(A)** allogrooming and **(B)** aggressive interactions between pairmates per 30 minutes during 5–6 weeks of flibanserin, flibanserin vehicle, 8-OH-DPAT, or 8-OH-DPAT vehicle administration. *** P = 0.001 vs. flibanserin vehicle (F(1,7) = 34.2), † P = 0.079 vs. 8-OH-DPAT vehicle (F(1,7) = 4.2), * P = 0.049 vs. 8-OH-DPAT vehicle (F(1,7) = 5.6). Each symbol indicates the same individual female marmoset receiving estradiol (solid symbols) or no estradiol (open symbols). 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin.

allogrooming between pairmates (F(1,7) = 34.25, P = 0.001; Figure 4A), mostly male grooming of their female pairmates (F(1,7) = 6.25, P = 0.041). 8-OH-DPAT, in contrast, tended to diminish allogrooming between males and females (F(1,7) = 4.23, P = 0.079; Figure 4A), including a trend toward diminished male grooming of female pairmates (F(1,7) = 4.92, P = 0.062). Frequency of aggressive interactions between the pairmates was increased by 8-OH-DPAT (F(1,7) = 5.65, P = 0.049; Figure 4B), but not by flibanserin (F(1,7) = 0.09, P = 0.778). This 8-OH-DPAT induced increase in aggression positively correlated with increased female rejection of male mount attempts and mounts (r = 0.941, P < 0.001), and correlated negatively with male genital sniffing (r = -0.834, P = 0.010). No other changes in social behavior were induced by flibanserin or 8-OH-DPAT (Table 1).

Female self-grooming behavior was increased by flibanserin (F(1,7) = 7.13, P = 0.032), but not by 8-OH-DPAT (F(1,7) = 3.02, P = 0.126). There were no other behavioral effects of either drug on self-directed behaviors (Table 1). Estradiol supplementation, in both 8-OH-DPAT and flibanserin groups, was without effect on pairmate behavior.

Effects of flibanserin and 8-OH-DPAT on acute induction of the 5-HT behavioral syndrome

During pairmate observations to assess sexual and social behavior (7:00am–1:00pm, 16–24 hours following daily drug administration), neither 8-OH-DPAT nor flibanserin resulted in females displaying symptoms of the potentially disruptive 5-HT behavioral syndrome.

Directly (0–0.5 hour) following administration, however, flibanserin shortened the latency to the first occurrence of sprawling or prone behavior (Figure 5, F(1,7) = 11.90, P = 0.011), without affecting the frequency of this behavior (F(1,7) = 0.44, P = 0.528). Furthermore, in contrast to 8-OH-DPAT, flibanserin first increased (weeks 1–3) female scratching behavior 0–0.5 hour

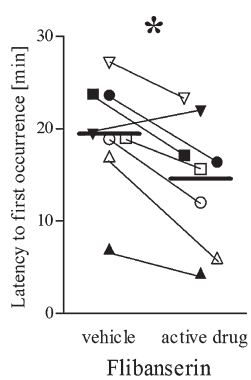

Figure 5. Sprawling behavior. Latency (minutes; backtransformed mean) to the first occurrence of sprawling behavior by female marmosets during pairmate observation after 5–6 weeks of flibanserin or flibanserin vehicle. * P = 0.011 vs. flibanserin vehicle (F(1,7) = 11.90). Each symbol indicates the same individual female marmoset receiving estradiol (solid symbols) or no estradiol (open symbols).

after administration (F(3,21) = 5.64, P = 0.005), and then diminished scratching behavior over the remaining weeks of flibanserin treatment compared to corresponding vehicle treatment (Treatment × Week interaction [F(3,21) = 7.21, P = 0.002]).

In contrast to flibanserin, 8-OH-DPAT consistently induced locomotor components of an acute, transient 5-HT behavioral syndrome in female marmosets 0–0.5 hour after administration, which persisted over the entire time course (15–16 weeks) of chronic treatment. In 8-OH-DPAT-treated female marmosets, the acute 5-HT behavioral syndrome involved displays of "random rapid limb movements" (Figure 6A, F(1,7) = 16.67, P = 0.005) and "wet-dog shakes" (Figure 6B, F(1,7) = 19.81, P = 0.003). In addition, frequency of scratching behavior tended to be elevated 0–0.5 hour after 8-OH-DPAT administration (Figure 6C, F(1,7) = 5.46, P = 0.052), but there was aTreatment × Week interaction (F(1,7) = 15.39, P < 0.001). Post hoc analysis indicated that female marmosets demonstrated an acute onset of scratching behavior during the first week of 8-OH-DPAT treatment (week 1), but not during subsequent weeks. As found with 5-HT behavioral syndrome behaviors, there was no effect of 8-OH-DPAT treatment on scratching behavior at 16–24 hours following drug administration (F(1,7) = 1.75, P = 0.227) when pairmate behavioral observations were conducted.

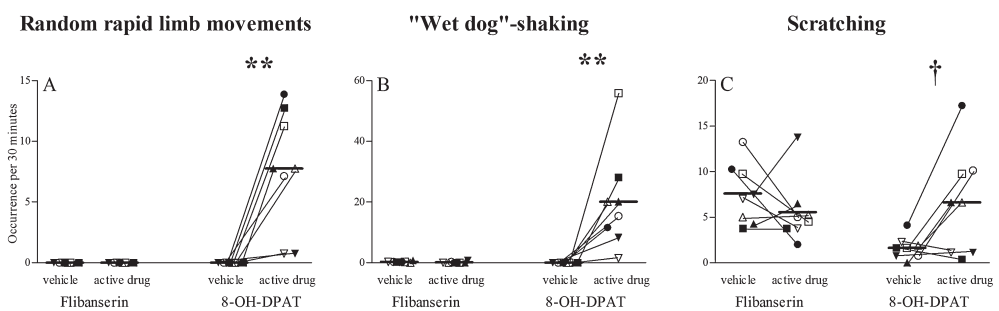

Figure 6. Acute induction of the 5-HT behavioral syndrome. Frequency (backtransformed mean) of female **(A)** "rapid, random limb movements" behavior **(B)** "wet dog shake" behavior, and **(C)** scratching behavior per 30 minutes during 5–6 weeks of flibanserin, flibanserin vehicle, 8-OH-DPAT, or 8-OH-DPAT vehicle and following 0–0.5 hours of treatment administration. ** P < 0.01 vs. 8-OH-DPAT vehicle ("rapid, random limb movements": F(1,7) = 16.7; "wet dog shakes": F(1,7) = 19.8), † P = 0.052 vs. 8-OH-DPAT vehicle (F(1,7) = 5.46). Each symbol indicates the same individual female marmoset receiving estradiol (solid symbols) or no estradiol (open symbols). 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-npropylamino)- tetralin.

DISCUSSION

Despite the prevalence of HSDD among women [1,3], its psychopathogenesis is unknown. Pharmacological manipulation of 5-HT, however, commonly induces an HSDD-like condition, especially in women chronically treated with SSRIs for depression [4], likely involving 5-HT_{1A} receptors [34]. Flibanserin, a 5-HT_{1A} postsynaptic receptor agonist and 5-HT_{2A} antagonist, presents a pharmacological opportunity to ameliorate psychosexual distress in women. Flibanserin improves sexual desire in women with major depression [14], and in women with HSDD it has been demonstrated to increase satisfying sexual events, sexual desire, and decreases distress [15]. Female marmosets, already established as a mechanistic model for neural regulation of female sexual behavior [19,28], form stable, long-term, male–female relationships [18] that allow for the examination of the pharmacological efficacy of flibanserin with regard to female sexual behavior.

Chronic administration of flibanserin to female marmoset pairmates stimulates male inspection of female genital area and increases female genital self-grooming. Female sexual behavior, however, is otherwise unaltered, possibly because we employ well-established male–female pairs without a history of infrequent-to-absent sexual behavior. Enhancement of proceptive and receptive aspects of female marmoset sexual behavior can be quantified using our behavioral testing paradigm, as previously shown following administration of gonadotropin releasing-hormone II and respective analogues [19]. In contrast, 8-OH-DPAT (a 5-HT_{1A} pre- and postsynaptic receptor agonist) administered to female marmoset pairmates tends to diminish male inspection of female genitalia and increases female rejection of male-initiated sexual advances, thus diminishing female sexual receptivity toward their long-standing male pairmate. The comparability of behavioral responses in both estrogen and “no hormone replaced” ovariectomized female marmosets used in this study suggests applicability of our results to both pre- and post-menopausal conditions in women, as found in a previous marmoset study [19].

The differential effects of flibanserin and 8-OH-DPAT on female marmoset sexual behavior resemble findings in female rats. Female rats given flibanserin increase expression of proceptive and receptive sexual behavior and receive increased genital sniffing by the male pairmate [13], whereas female rats given 8-OH-DPAT exhibit diminished female sexual receptivity [17]. These contrasting effects of flibanserin and 8-OH-DPAT on marmoset and rodent behavior are surprising since both compounds are described as 5-HT_{1A} agonists. However, while both flibanserin and 8-OH-DPAT activate postsynaptic 5-HT_{1A} receptors, flibanserin is functional only as a postsynaptic

receptor agonist [15], whereas 8-OH-DPAT activates both pre- and postsynaptic 5-HT_{1A} receptors. This difference in biological action results in fundamental differences in pharmacology and the abilities of flibanserin and 8-OH-DPAT to induce functional changes in a brain region-specific manner. For example, flibanserin inhibits forskolin-stimulated cAMP formation in the cortex, while 8-OHDPAT does not affect cortical cAMP accumulation [10]. Flibanserin decreases neuronal firing rate in the rat cortex regardless of whether the presynaptic receptor-containing dorsal raphé nucleus is intact, while the effects of 8-OH-DPAT are dependent upon intact raphé serotonergic neurons [11]. Taken together, flibanserin and 8-OH-DPAT display a different regional selectivity, and they differentially affect neuronal function in 5-HT projection sites.

These functional differences are particularly evident in pyramidal neurons in the prefrontal cortex, a key site for flibanserin's mode of action in affecting female sexual behavior, as these neurons are an important part of regulatory networks that coordinate the release of 5-HT, DA and NE in a brain region-specific manner [15]. Additional 5-HT_{2A} antagonist effects of flibanserin, which 8-OH-DPAT lacks, have been shown to enhance 5-HT_{1A} agonist effects on pyramidal neurons of the prefrontal cortex, thus creating a biochemical environment in flibanserin-exposed prefrontal cortex that is clearly different from 8-OH-DPAT exposure. 8-OH-DPAT is thus not likely to directly affect cortical neurocircuitries [10], but will suppress female sexual behavior by acting on 5-HT_{1A} receptors in postsynaptic hypothalamic areas, such as the ventromedial hypothalamus [35] and medial preoptic area [36], or in presynaptic raphé nuclei [37]. Thus,flibanserin's effects on female sexual behavior are likely mediated by altering cortical neurocircuitries, while 8-OH-DPAT likely inhibits female sexual behavior by activation of hypothalamic or midbrain 5-HT_{1A} receptor populations. The inhibitory effects of 8-OH-DPAT occur immediately and do not require chronic administration in rats [6]. Chronic administration seems not to lead to either tolerance or sensitization of sexual behavior, at least in male rats [38]. Flibanserin, in contrast, might regulate female sexual behavior by affecting pyramidal neurons in the prefrontal cortex and establishing a new monoamine neurotransmitter balance in a brain region-specific manner [15]. Chronic elevations in prefrontal cortex levels of DA and NE in rats are reported after 21 days of repeated flibanserin administration, consistent with the duration needed for flibanserin to facilitate female sexual behavior in rats [13].

Flibanserin's enhancement of interest in treated female marmosets' genitals is reminiscent of its effects when administered twice daily to female rats (45 mg/kg, PO) for 2–3 weeks. Flibanserintreated female rats attract increased male

inspection of their genital area [13]. In both rats and marmosets, female genital odor is important for activation of male sexual arousal [rat: [39]; marmoset: [40]], particularly, female genital odor from the peri-ovulatory period when female sexual proceptivity and receptivity are maximal [rat: [41]; marmoset: [42]]. 5-HT regulates likely mediators of genital olfactory attractiveness, such as oxytocinergic (OT) and vasopressinergic (AVP) neurons [43] that innervate female external genitalia, possibly in conjunction with the central DA neurotransmitter system [44]. Neuro-regulated changes in vaginal odor may also be a consequence of 5-HT-mediated alterations in genital vasodilatation [44] or salt-water regulation [45]. Increased female interest in genital self-grooming and selfgrooming, in general, could involve 5-HT mediated changes in OT, as central administration of OT (and AVP) to female rats increases selfgrooming, particularly of the genital area [46].

Perhaps the most intriguing finding of this marmoset study, the opposing effects of flibanserin and 8-OH-DPAT on the quality of male–female pair interactions, raises the possibility that female sexuality is strongly influenced by the perceived quality of the relationship with their partner (e.g., [3,47]). Social attachment and maintenance of proximity, allogrooming and pair-bonding, function to facilitate reproduction [48] and sexual behavior may contribute toward maintenance of a close male–female relationship [49]. In marmosets, affiliative behavior between females and males co-occurs with sexual behavior, while aggressive behavior between pairmates impedes sexual interaction [50,51]. Thus, the aggression-inducing effect of 8-OH-DPAT and the affiliation-enhancing effect of flibanserin may create relationship environments that either diminish or facilitate sexual interactions between marmoset pairmates, respectively, likely mediated by neurotransmitter systems implicated in the regulation of social behavior, such as 5-HT [52], DA [53], AVP and OT [54,55]. Flibanserin selectively activates postsynaptic 5-HT_{1A} receptors while antagonizing 5-HT_{2A} receptors [11]. Gerretsen et al. [56] recently reported a negative association between the desire for social relationships in humans and 5-HT_{2A} binding in the prefrontal cortex. Furthermore, flibanserin's distinct receptor binding profile induces long-lasting increases in basal DA in the prefrontal cortex, while not changing basal levels in other tested brain areas, including the hypothalamus and nucleus accumbens [57,58]. If replicated in flibanserin-treated female marmosets, such increases in prefrontal cortex DA would be expected to enhance prefrontal cortex-guided attention to male behavioral cues and to more readily elicit female responses based on previous experience [58]. Improved efficiency of DAmediated neural processing is proposed as one aspect of therapeutic efficacy for a variety of drugs that improve disorders of PFC dysfunction, possibly including HSDD [59,60].

Beyond neurobiological factors that influence sexual function, several studies, including the National and Social Life Survey [61], point out that sexual behavior in women is closely linked to psychosocial factors and quality of their relationship with a partner [47]. The complex interplay of these factors with hormonal and neural environments is a challenge for any animal model of female sexual function. The translation of rodent findings to humans, for example, is difficult in light of their strict circadian hormonal control of female sexual function [62]. Studies in non-primate mammals are thus not likely able to capture the multifactorial environment in which sexual behavior is expressed in nonhuman primates and humans [63]. In contrast, our observations in the marmoset monkey emulate the human situation more closely and highlight the importance of pairbond quality in female sexual behavior. Our findings are likely based within an important set of marmoset-typical characteristics, including welldeveloped social behavior, long-term female–male pairings and a degree of emancipation of female sexual behavior from strict hormonal control [28] that permit a significant influence of the social environment on the shaping of sexual behavior.

The strong and consistent, but transient, expression of a 5-HT behavioral syndrome induced in female marmosets by 8-OH-DPAT administration is likely mediated by activation of postsynaptic 5-HT_{1A} receptors [64,65]. Flibanserin, in contrast, does not elicit shaking or rapid limb movements despite its postsynaptic 5-HT_{1A} agonist characteristic. This may be due to flibanserin's 5-HT_{2A} antagonist property, as 5-HT_{1A} and 5-HT_{2A} receptors are functionally linked and show reciprocal modulation [66]. Indeed, pretreatment with 5-HT_{2A} antagonists reduces 8-OH-DPAT-induced 5-HT behavioral syndrome in rats [31]. It is possible that flibanserin increases the expression of brain-derived neurotrophic factor (BDNF) in the cerebral cortex and hippocampus, which, in rats, diminishes the 5-HT behavioral syndrome induced by 8-OH-DPAT [67]. A shortened latency to sprawl induced by flibanserin may be equivalent to the flat-body posture component of the 5-HT behavioral syndrome [32]. Sprawling, however, is also commonly observed when marmosets solicit allogrooming from a partner [21]. Thus, a shortened latency to sprawling behavior may indicate another pro-social effect of flibanserin rather than a flibanserin-induced component of the 5-HT behavioral syndrome. Neither sprawling in flibanserin treated females, nor the 5-HT behavioral syndrome displayed by 8-OH-DPAT treated females, are likely related to 5-HT toxicity (or 5-HT syndrome) described in humans, a potentially life-threatening condition induced by excess 5-HT [68]. 5-HT toxicity can result from combinations of antidepressant treatments, such as monoamine oxidase inhibitors (MAOIs) and SSRIs, causing clonus (involuntary muscle contraction and relaxation)

and hyperthermia [68] apparently due to 5-HT_2 receptor-mediated effects [69]. Activation of 5-HT_{1A} receptors, in contrast, commonly leads to hypothermia in rodents and humans [70,71]. Flibanserin, a 5-HT_{1A} agonist and 5-HT_{2A} receptor antagonist, and 8-OH-DPAT, a 5-HT_{1A} agonist, are thus both unlikely to cause hyperthermic responses. Furthermore, both flibanserin and 8-OH-DPAT administered to rodents selectively decrease 5-HT synthesis in several brain regions [72], further indicating the unlikelihood of either contributing to 5-HT toxicity.

Similar to a previous observation indicating that low-level estradiol supplementation is not required for the efficacy of gonadotropin-releasing hormone II to stimulate sexual behavior in ovariectomized female marmosets [19], our present study, applying the same design in regard to estradiol replacement, demonstrates that behavioral changes due to chronic flibanserin and 8-OH-DPAT treatments are independent of estradiol status. This finding might be somewhat surprising in light of welldocumented interactions of estrogens with the central serotonin system [73] and contrast with a previous study conducted by Kendrick and Dixson [29]. Kendrick and Dixson [29], however, applied high levels of estradiol supplementation equivalent to pre-ovulatory peak levels (~940 pg/mL). The much lower circulating estradiol levels in our study (average with estradiol supplementation: 396 pg/mL), chosen to facilitate a low to modest baseline of sexual behavior, may be responsible for the lack of obvious estradiol-induced behavioral changes in the present study.

CONCLUSIONS

Our findings are the first to demonstrate differential and potentially bi-directional regulation of female sexual behavior (diminished versus unchanged) and social behavior (diminished versus enhanced) in a nonhuman primate through prolonged serotonergic modulation, supporting the central 5-HT system as a promising target for pharmacotherapy of sexual dysfunction in women. Our model applies species-appropriate settings in which female partners can control sexual and social interactions. We show that female marmoset sexual behavior is suppressed by selective chronic stimulation of 5-HT_{1A} receptors by 8-OH-DPAT. In contrast, through its distinctively different 5-HT receptor binding profile, and potential additional abilities to enhance DA levels (and possibly other neurotransmitters) in a brain region specific manner [57,58], flibanserin increases pro-social interactions in male and female marmoset pairmates without obvious enhancement of female sexual behavior. While these findings suggest that flibanserin's effects may not translate from female marmosets to women in every respect, they do show that flibanserin

enhances species-specific, intimate aspects of partner interactions. These are manifest by increased intimate affiliative engagement between marmoset pairmates and improved sexual relationships between women and their partners [14]. Thus, the putative beneficial effect of flibanserin on sexual well-being in women, otherwise distressed about low sexual desire, may arise from its ability to positively influence the quality of relationship with long-term partners. Our observations of female marmosets may therefore suggest the need to integrate mechanistic, neurochemical explanations of female sexual behavior with holistic views of health psychology, including emotional and relational aspects of female sexuality, in order to successfully contribute novel therapeutic approaches to female sexual well-being.

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SUPPORTING INFORMATION

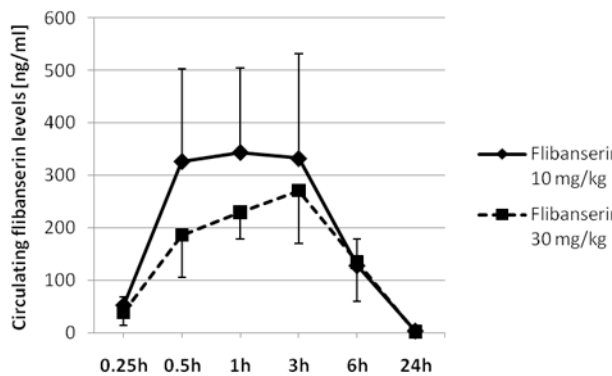

Figure S1. 24-hour pharmacokinetic profiles of 10 mg/kg and 30 mg/kg flibanserin in female marmoset monkeys. Flibanserin was administered PO at doses of 10 mg/kg or 30 mg/kg. Blood samples were taken at 0.25 h, 0.5 h, 1 h, 3 h, 6 h and 24 h after flibanserin administration and analyzed for circulating flibanserin concentrations. Flibanserin doses did not significantly differ ($P > 0.05$; $N = 4$).

Table S1. In a dose-response study, the behavioral effects of 10 mg/kg or 30 mg/kg flibanserin, administered to female marmosets, were compared to respective baseline behavior of each individual. Difference between flibanserin doses were assessed by three-way anova including the dose of flibanserin as between-subject factor.

	Baseline (all)	Flibanserin (all)	Baseline 10	Flibanserin 10	Baseline 30	Flibanserin 30	P-value[1)]
Affiliative Behavior[2)]							
Female Grooms Male	1.72 ± 0.58	2.91 ± 0.76	0.56 ± 0.24	1.81 ± 0.74	2.88 ± 1.07	4.00 ± 1.30	0.037
Female Initiates Contact with Male	10.41 ± 1.05	8.94 ± 1.23	8.81 ± 1.83	7.13 ± 1.59	12.00 ± 0.96	10.75 ± 1.81	n.s.
Female Terminates Contact with Male	18.59 ± 1.80	14.94 ± 1.98	15.00 ± 1.28	8.88 ±1.02	22.19 ± 3.18	21.00 ± 3.21	0.030
Time in Contact	30.41 ± 1.31	24.78 ± 1.74	30.00 ± 1.70	24.19 ± 2.66	30.81 ± 2.05	25.38 ± 2.34	0.020
Sexual Behavior[2)]							
Mount (All)	0.69 ± 0.19	0.66 ± 0.15	0.38 ± 0.22	0.38 ± 0.15	1.00 ± 0.29	0.94 ± 0.25	n.s.
Mount (Ejaculatory)	0.19 ± 0.07	0.19 ± 0.07	0.13 ± 0.09	0.19 ± 0.07	0.25 ± 0.11	0.19 ± 0.07	n.s.
Female Rejection of Male Mounts	0.66 ± 0.24	0.41 ± 0.13	0.25 ± 0.14	0.19 ± 0.14	1.06 ± 0.45	0.63 ± 0.22	n.s.
Self Grooming[2)]							
Non-Genital	4.09 ± 0.80	1.97 ± 0.49	6.31 ± 1.36	2.56 ± 0.85	1.88 ± 0.35	1.38 ± 0.46	0.006
Genital	1.69 ± 0.25	5.31 ± 0.98	1.38 ± 0.29	3.19 ± 0.69	2.00 ± 0.41	7.44 ± 1.71	0.049
Acute Behavior Change[3)]							
Prone/Sprawling Position	0.44 ± 0.16	7.45 ± 1.18	0.19 ± 0.14	7.86 ± 1.89	0.69 ± 0.28	7.03 ± 1.44	0.042
Irregular Locomotion	absent	absent	absent	absent	absent	absent	

[1)] Values reflect Mean ± SEM; Flibanserin 10 = 10 mg/kg; Flibanserin 30 = 30 mg/kg; P-value is shown for main effect of Flibanserin Treatment. There are no significant interaction effects of Flibanserin Treatment x Dose of Flibanserin, indicating no differences between flibanserin doses on marmoset behavior. Effects are reported as significant at $p < 0.05$.

[2)] Behavior was recorded at baseline, and 16-24 hours after daily flibanserin administration during weeks 5-6 of 6 weeks of daily flibanserin treatment.

[3)] Behavior was recorded at baseline, and 0-30 minutes after daily flibanserin administration during weeks 1-6 of 6 weeks of daily flibanserin treatment.