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Flibanserin stimulated partner grooming reflects brain metabolism changes in female marmosets

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Abstract

Introduction—Female Sexual Interest and Arousal Disorder (FSIAD) is personally distressing for women. To better understand the mechanism of the candidate therapeutic, flibanserin, we determined its effects on an index of brain glucose metabolism.

Aim—We hypothesized that chronic treatment with flibanserin would alter metabolism in brain regions associated with serotonergic function and female sexual behavior.

Methods—In a crossover design, eight adult female common marmosets (*Calithrix jacchus*) received daily flibanserin or vehicle. After 7–12 weeks of treatment, the glucose metabolism radiotracer FDG was administered to each female immediately prior to 30 min of interaction with her male pairmate, after which females were anesthetized and imaged by PET. Whole-brain normalized images were analyzed with anatomically defined regions of interest. Whole brain voxelwise mapping was used to explore treatment effects. Correlations were examined between alterations in metabolism and pairmate social grooming.

Main Outcome Measures—Changes in metabolism associated with flibanserin were determined for dorsal raphe (DR), medial prefrontal cortex (mPFC), medial preoptic area of hypothalamus (mPOA), ventromedial nucleus of hypothalamus (VMH), and field CA1 of hippocampus.

Results—In response to chronic flibanserin, metabolism in mPOA declined, and this reduction correlated with increases in pairmate grooming. A cluster of voxels in frontal cortico-limbic regions exhibited reduced metabolism in response to flibanserin and overlapped with a voxel

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CONFLICT OF INTEREST

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cluster in which reductions in metabolism correlated with increases in pairmate grooming. Finally, reductions in mPOA metabolism correlated with increases in metabolism in a cluster of voxels in somatosensory cortex.

Conclusions—Taken together, these results suggest that flibanserin-induced reductions in female mPOA neural activity increase intimate affiliative behavior with male pairmates.

Keywords

Positron emission tomography; Marmoset; Brain; Glucose metabolism; flibanserin; Female sexual behavior

1 Introduction

Hypoactive sexual desire disorder (HSDD), refined by DSM-5 to female sexual interest/arousal disorder (FSIAD) [1], can become personally distressing to women [3,4]. While relationship dissatisfaction [6], together with hormonal, neurobiological and psychosocial factors, all contribute to FSIAD [3], [7], its psychopathogenesis is unknown. Although the neurotransmitter serotonin (5-HT) is a potent inhibitory regulator of female sexual behavior [8], [9], activation of specific 5-HT receptor subtypes can nevertheless facilitate female sexual [9] and social behavior [10], [11]. Pharmacological modulation of 5-HT neurotransmission is a common cause of diminished sexual activity and altered social interactions in women. Multiple clinical reports show that up to 75% of female patients prescribed selective serotonin reuptake inhibitors (SSRIs) experience diminished sexual satisfaction, including delayed or inhibited orgasm, or decreased sexual desire [3,14,16]. While these studies provide evidence for 5-HT involvement in sexual interest/arousal disorder in women, little is known of the underlying neural mechanism. The present study employs a potential 5-HT therapeutic for FSIAD, flibanserin, together with positron emission tomography (PET) neural imaging, to determine neural sites of drug action in female marmoset monkeys associated with flibanserin-induced increases in intimate affiliation with a male partner [11].

Studies in female rodents have shown that 5-HT receptor specific agonists, which act like 5-HT, or antagonists, which block the action of 5-HT, can either facilitate or inhibit sexual activity, depending on the 5-HT receptor subtype activated. Lordosis is inhibited by 5-HT_{1A} receptor activation and 5-HT₃ receptor antagonism [9], but is facilitated by 5-HT_{2A/C} receptor activation [9]. While the inhibitory effect of 5-HT_{1A} agonists on female receptivity is clear, less is known as to where in the brain the receptors involved in this response are located. Lesion studies and local administration of the 5-HT_{1A} receptor agonist R-(+)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) into different brain areas highlight the importance of pre-synaptic 5-HT_{1A} receptors, i.e. those located on the upstream neuron, as well as post-synaptic 5-HT_{1A} receptors, i.e. those located on the downstream neuron. Both pre- and post-synaptic 5-HT_{1A} receptors function in the serotonergic regulation of female sexual behavior, involving a circuitry that comprises the presynaptic raphe nuclei and the postsynaptic prefrontal cortex, ventromedial hypothalamus (VMH), medial preoptic area (mPOA) and peri-aqueductal gray (PAG) [9].

Recently, flibanserin (2H-benzimidazol-2-one, 1,3-dihydro-1-[2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl]), an agonist of post-synaptic 5-HT_{1A} receptor function and an antagonist of 5-HT_{2A} receptors [27], demonstrated the ability to stimulate female sexual behavior in rats [28] and to improve sexual desire in women with HSDD [29]–[33], major depressive disorder [34], or who had become post-menopausal [35]. The sexual impact of flibanserin was serendipitously discovered while studying patients with major depressive disorder. Flibanserin is ineffective as an antidepressant, but reliably increases scores on the Arizona Sexual Experiences Scale for women [34]. In phase-III clinical trials designed to investigate the efficacy of flibanserin as first-line pharmacotherapeutic treatment of HSDD (the “BOUQUET” studies), premenopausal women with HSDD reported increased satisfying sexual events and increased desire following chronic flibanserin treatment [29]–[32]. Despite these positive clinical and animal findings, controversy still surrounds flibanserin as a therapeutic for sexual dysfunction in women [36].

Female marmoset monkeys present an opportune, nonhuman primate model that is utilized to explore sexually relevant pharmacological manipulations that readily translate to humans [11], [37], [38]. Marmosets exhibit primate-typical neural expression and distribution of 5-HT_{1A/2A} receptors [39] and the serotonin transporter [40], show no genetic polymorphism for serotonin transporter expression [41], and provide novel insight into 5-HT-regulated behavior [38]. Thus, they present an increasingly well-characterized translational model for evaluating 5-HT neural regulation of human behavior. Marmosets form long-term social and sexual relationships between male-female pairs [42] in contrast to multiple-mating social structures of rats [44] and many nonhuman primates [45]. By acceptance or rejection of a pairmate’s sexual advances, female marmosets can readily promote, prevent, or terminate sexual interactions, [47]. We have established a marmoset model for female social and sexual function that employs a standardized behavioral testing paradigm, permitting repeatable, quantitative exploration of neurally active compounds [11], [37]. Female marmosets used in this study had no previous history of sexual or reproductive issues, were in well-established male-female pairs, and were in known and controlled estrogenic, dietary, developmental and physical environments. They therefore provided unparalleled, controlled models in which to quantify neural correlates of flibanserin action, and a study design opportunity that cannot be replicated in humans. With the advent of high resolution PET, it has become feasible to study neural responses in small primates such as marmosets [48], [49]. We have shown that repeated, daily administration of the 5-HT_{1A/7} agonist 8-OH-DPAT diminishes female sexual receptivity, and increases antagonistic behavior, while aligning changes in endocrine function and cortical glucose metabolism parameters with sexual and social rejection [11], [48], [50]. Administering flibanserin, in contrast, subtly enhances sex-related behavior in female marmosets, stimulates intimate affiliative behavior with their male pairmates, and increases male pairmate sexual investigation [11]. These differing behavioral consequences of flibanserin and 8-OH-DPAT, i.e. increased affiliation versus aggression respectively [11], may be due in part to flibanserin’s lack of presynaptic agonist action in regions such as the dorsal raphe [57], [58].

In the present study, we extend our previous characterization of alteration of brain cortical glucose metabolism induced by chronic 8-OH-DPAT [48] in order to gain insight into chronic flibanserin-induced neural changes accompanying increased female affiliation and

male sexual investigation. Flibanserin's enhancement of socio-sexual interactions in female marmosets may contribute mechanistic understanding to flibanserin-induced increases in satisfying sexual events and desire in women with sexual interest/arousal disorder. We hypothesized that chronic treatment with flibanserin would alter metabolism in regions associated with serotonergic function and female sexual behavior, namely dorsal raphe (DR), medial prefrontal cortex (mPFC), medial preoptic area of hypothalamus (mPOA), ventromedial nucleus of hypothalamus (VMH), and field CA1 of hippocampus [48].

2 Methods

2.1 Subjects

Eight adult female common marmosets (*Callithrix jacchus*, 2–5 years of age) were pair housed with male pairmates at the Wisconsin National Primate Research Center (WNPRC) for 8–20 months before and throughout the duration of the study, as previously described [11]. Ovaries were removed and subcutaneous silastic capsules implanted to provide either mid-follicular phase estradiol levels (~400 pg/ml; n=4) or no estradiol (~70 pg/ml; n=4) a week before study onset. Estradiol status remained the same throughout the study period for each individual animal. Surgical procedures, estradiol priming and study design with regard to estradiol priming were performed as described in [11]. 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. WNPRC, as part of the University of Wisconsin-Madison Graduate School, is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2 Experimental protocol

A counterbalanced, cross-over study, that applied within-subject comparisons, was designed to examine the effects of chronic (7–12 weeks) daily (12:00 h–14:00 h) administration of flibanserin (15 mg/kg, orally (PO) in 1 ml/kg vehicle; Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany) or vehicle-only (98.5% of 0.5% hydroxycellulose solution and 1.5% of 1% poly-sorbate 80 solution, 1.0 ml/kg PO).

2.3 Behavioral observations of sexual behavior

In order to stimulate a modicum of social and sexual interaction upon reunion [37], females and males were separated for 90 min prior to each of four 30-min behavioral tests at 07:00h–13:00h (16–24 h after daily administration of flibanserin/vehicle), 5–6 weeks after treatment onset, as previously described [11]. Lights were on between 06:30h–18:30h.

2.4 Brain imaging and analysis

Brain imaging and analysis generally followed methods described elsewhere [48], and a detailed description is provided in the online Supplementary Data. Briefly, at 7–12 weeks following start of treatment with either flibanserin or vehicle, subjects were administered the radiotracer FDG, reunited with their male pairmate for 30 minutes, anesthetized, and scanned with PET. MR images were also acquired, and each subject's PET images were

aligned to the group MR template and whole brain normalized. Atlas based ROIs for CA1, DR, mPFC, mPOA, and VMH were delineated on the MR template and applied to the PET images, and paired t-test comparisons of ROI values between conditions with $p < 0.05$ uncorrected were considered significant. Alterations in FDG uptake were correlated by regression through the origin against changes in total grooming between pairmates, the behavior most significantly altered by flibanserin [11]. Voxelwise paired t-tests and linear regressions were performed as well, and multiple-comparison corrected clusters of $p < 0.05$ were considered significant. Post-hoc, alterations in FDG uptake were correlated voxelwise against those seen in the mPOA, an anatomically defined ROI within the hypothalamus, using a seed-based approach [49].

3 Results

3.1 Experimental parameters

As seen in Table 1, experimental parameters were consistent across treatment conditions ($p > 0.38$).

3.2 ROI analysis

Typical MRI and PET images are shown in Figure 1. As shown in Table 2, flibanserin induced ($p < 0.042$) a 5% reduction in whole brain normalized radioactivity in the medial preoptic area (mPOA) compared to vehicle. Furthermore, as Table 3 and Figure 2 illustrate, the difference in FDG uptake in mPOA correlated negatively ($p = 0.019$) with the difference in total grooming behavior between flibanserin and vehicle conditions.

3.3 Voxelwise analysis

As seen in Figure 3 and Table 4, voxelwise analysis identified a significant cluster of voxels with reduced whole brain normalized radioactivity in the flibanserin condition versus vehicle. In addition, voxelwise regression through the origin revealed a significant cluster of voxels in which differences in normalized radioactivity correlated negatively with differences in the frequency of total grooming. In these correlated voxels, female marmosets with the greatest reductions in FDG uptake in the flibanserin condition exhibited greater increases in intimate pairmate grooming. These two statistically defined clusters share common voxels, with 53% of the voxels in the correlation cluster included in the contrast cluster.

Given flibanserin's significant reduction of FDG uptake in the mPOA of female marmosets, together with the correlation of that reduction with flibanserin-increased pairmate grooming, we examined correlations of neural activity between the mPOA and all brain regions using a seed-based approach. A voxelwise regression through the origin revealed a significant somatosensory cortical cluster in which flibanserin-induced differences in FDG uptake were negatively correlated with those seen in mPOA (Figure 4). As expected, a significant cluster was also found centered around the mPOA, where flibanserin-induced differences in FDG uptake were positively associated with those seen within the mPOA ROI itself.

4 Discussion

The current study employed MRI-guided PET imaging to examine the effect of flibanserin on an index of cerebral glucose metabolism in a nonhuman primate model for female sexual and social behavior, the pair-bonded female marmoset [48]. Whole-brain normalized FDG radioactivity measures in anatomically defined ROIs identified a significant decrease in glucose metabolism in response to flibanserin in the mPOA. In addition, such diminished glucose metabolism in the mPOA negatively correlated with flibanserin-mediated increases in grooming between pairmates, demonstrating a predictable increment in pairmate grooming as glucose metabolism in the females' mPOA declined. The mPOA appears to be a key neural area in regulating grooming interactions in pair-bonded marmosets, and possibly primates, generally [52]]. In marmosets, bilateral lesions of the mPOA in either females [47] or males [54], increase grooming among female and male pairmates. Flibanserin-diminished neural activity in the mPOA, found in the present study, may thus contribute to intimate social interactions between females and males already familiar with one another. The accompanying finding of correlated flibanserin-induced alterations in mPOA and somatosensory glucose metabolism in the same female marmosets, particularly somatosensory areas involving genitals, hindlimbs, tail and trunk, suggests drug-induced synchronicity between hypothalamic and somatosensory function. Such concordant neural activity may result from increased engagement in, or increased receipt of, pairmate grooming by female marmosets instead of, or in addition to, a neural basis for increased affiliation, i.e. these neural sites may respond to grooming and/or be involved in initiation of grooming.

Flibanserin is a 5-HT_{1A} agonist and 5-HT_{2A} antagonist. Its 5-HT_{1A} agonist action in the mammalian brain, however, is limited to postsynaptic receptor locations, such as in the prefrontal cortex, and is not found at presynaptic receptors, such as those expressed in the dorsal raphe [58]. In female marmosets, it increases pairmate grooming (an intimate social interaction), while increasing male pairmate inspection of female genitalia, an indication of male sexual interest [11]. These behavioral changes in pair-bonded female marmosets are clinically relevant because of flibanserin's positive impact on women with HSDD and their interactions with long-term male partners. In a series of recent clinical trials, flibanserin improved sexual desire and increased frequency of sexually satisfying events in women with HSDD [29]–[33], suffering from major depression [34], as well in those who were postmenopausal [35]. This 5-HT agonist-antagonist thus appears to have a potential broad application in women for the relief of personally distressing reduction in sexual interest or arousal [1]. There is, however, little mechanistic understanding regarding flibanserin-mediated changes in the women's behavior.

In a recent brain ROI gene expression study of female marmosets engaged in a similar experimental design to the present study, the mPOA demonstrated a pronounced increase in oxytocin gene expression when females were treated with a 5-HT_{1A} agonist, 8-OH-DPAT [59]. Flibanserin-reduced glucose metabolism in the mPOA found in the present study may also be related to its 5-HT_{1A} agonist properties increasing oxytocin. Oxytocin stimulates social grooming and partner preference in female rodents [60]]and female interaction with male partners in marmosets [63]], and is positively associated with social bonding in married

couples [65]. In marmosets, oxytocin is expressed in brain areas overlapping with the mPOA [66]. As activation of 5-HT_{1A} receptors stimulates hypothalamic oxytocin expression in female marmosets [59], the post-synaptic 5-HT_{1A} agonist property of flibanserin may mimic this action, likely contributing to flibanserin-enhanced social grooming in female marmosets [11]. Increased frequency of pairmate grooming may, in turn, induce beta-endorphin release [69]]from neurons that densely target the mPOA [71], potentially diminishing mPOA neural activity, as found in this study. The absence of flibanserin presynaptic 5-HT_{1A} agonist action, such as in the dorsal raphe [57], [58], may contribute to its distinctively different behavioral consequences in comparison to 8-OH-DPAT, i.e., increased affiliation versus aggression, respectively [11].

In addition, voxelwise analysis of flibanserin versus vehicle identifies a significant neural cluster associating diminished cortical glucose metabolism with increased marmoset pairmate grooming. The neural cluster, formed by an ~53% overlap between two separately identified and significant neural clusters within the female marmoset brain, is defined by (1) flibanserin-induced reduction in glucose metabolism, and (2) a negative correlation between flibanserin-induced increase in pairmate social grooming and flibanserin-diminished FDG uptake. The overlap cluster encompasses regions of prefrontal, cingulate and insular cortex; corpus callosum and forceps minor; caudate-putamen and nucleus accumbens (including the oxytocin receptor dense ventral striatum) [73]; and a right-sided caudal extension encompassing the bed nucleus of the stria terminalis and the extended amygdala [74]. Flibanserin-induced changes in pairmate grooming that correlate negatively with increased FDG uptake in this cortico-limbic cluster are perhaps not surprising since primate frontal cortex and amygdala receive substantial innervation from 5-HT neurons originating in the dorsal raphe [76].

Flibanserin-diminished glucose metabolism in the voxelwise-identified cortico-limbic cluster may arise from flibanserin's multiple abilities to (1) diminish pyramidal neuron firing rate in the prefrontal cortex [77], (2) inhibit forskolin-stimulated cAMP formation in the cortex, and (3) with its 5-HT_{2A} antagonistic properties, to diminish 5-HT_{2A}-mediated activity in the prefrontal cortex, a functional change known to increase desire for social relationships in humans [78]. These findings are consistent with the predominant locations of 5-HT_{1A} and 5-HT_{2A} receptors on the axon hillock, and soma and dendrites, respectively, of glutamatergic pyramidal neurons in the cortex and amygdala [80] and with preferential engagement of flibanserin's 1A agonist-2A antagonist actions in the prefrontal cortex [58], likely resulting in diminished glutamate release in cortical and limbic targets [58].

Flibanserin-induced prominence of the cortico-limbic neural cluster in female marmosets could represent a previously identified top-down neural circuit [82] that simultaneously controls 5-HT orchestration of emotional processing [76], dopamine-enabling of reward processing [84], and norepinephrine regulation of arousal and autonomic function [86]. In support of this hypothesis, high concentrations of 5-HT and serotonin transporter are found in the more medial and caudal aspects of the orbitofrontal cortex and these coincide with increased density of innervation from the amygdala within the more caudal division of the orbitofrontal cortex [88]. The frontal cortex directly innervates the dorsal raphe, ventral tegmental area, nucleus accumbens and locus coeruleus. Such 5-HT integrated cortico-

limbic control is consistent with 5-HT-based therapeutic moderation of orbitofrontal cortex function that ameliorates neuropsychiatric disorders, including mood disorders, obsessive-compulsive disorder and impulsive-aggressive disorders [89,90]. Flibanserin certainly regulates the release of 5-HT, dopamine and norepinephrine in the prefrontal cortex [58], [93], maintaining catecholamine elevation long-term [93] and causing widespread diminution of activity in prefrontal pyramidal glutamatergic neurons [58]. In such a scenario, flibanserin modulation of the cortico-limbic environment may enable dopamine-mediated pro-social behaviors [95], such as social grooming..

This study was subject to certain limitations. The small size of the anatomically defined mPOA ROI, 4.6 μ L, is comparable to the spatial resolution of the PET scanner, 5 μ L. We estimate that due to partial volume effects, 29% of the radioactivity calculated in the ROI originated in the ROI itself, and the reported results must therefore be considered best estimates for the delineated ROI [48]. No multiple comparison correction was applied in determining the significance of the results for the 5 ROIs we identified a priori for analysis. Therefore, the mPOA ROI results indicating decreased metabolism in the flibanserin condition, and correlated increases in pairmate grooming, should be viewed as exploratory and require confirmation. Note, however, that the voxelwise analysis results were corrected for multiple comparisons. Additionally, while adequately powered for behavioral outcomes, the number of study subjects was small.

Flibanserin-induced association of intimate social interaction with a male pairmate and diminished glucose metabolism in the identified frontal cortico-limbic cluster of female marmosets is reminiscent of the neural differences in fMRI-derived brain activity between women with and without HSDD [97]. When viewing erotic scenes, women with HSDD demonstrate greater neural activity in the anterior ventromedial prefrontal cortex, lateral orbito-frontal cortex and medial frontal gyrus than women without HSDD. The erotic material was designed to be viewed by women, but elicited muted arousal ratings from HSDD compared to non-HSDD women [97]. Given the locations of increased brain activity in HSDD women, Arnow and colleagues [97] suggest that HSDD may involve undue emphasis on monitoring and evaluating personal performance, with heightened self-focus in sexual situations, a neural processing difference that has long been postulated to underlie sexual arousal disorders in women [99]. It is interesting to speculate that flibanserin-diminished neural activity in similar brain areas in the present study, i.e., the voxelwise-identified cortico-limbic cluster, may translate into more positive female emotional engagement with diminished self-focus, an outcome that may enhance willing engagement in intimate behavior with a partner.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Images aligned to template

Axial, coronal, and sagittal slices for a single female subject (cj1192) showing (A) MRI, (B) whole brain normalized FDG PET radioactivity in the vehicle condition, and (C) in the flibanserin condition. Contour is on template whole brain mask trimmed to avoid contributions from radioactivity uptake outside of the brain.

Figure 2. Increased grooming is associated with reduced glucose metabolism in the medial preoptic area (mPOA) in response to flibanserin

Between-condition difference in normalized FDG uptake in the a priori anatomically defined region of interest plotted against alteration in total grooming events per 30 minute session for the eight subjects. = flibanserin - vehicle. Dashed line shows regression through origin. $F(1,7) = 9.315$, $p = 0.019$.

Figure 3. Neural and behavioral responses to treatment

(A) Orthogonal slices showing significant clusters of voxels overlaid on MRI template. **Red:** significant reduction in FDG radioactivity during flibanserin condition compared to vehicle condition (paired t test, 2-tailed $p_{\text{uncorrected}} < 0.05$, extent 88 μL , $p_{\text{corrected}} = 0.001$). **Green:** significant negative correlation between alterations of FDG radioactivity and total grooming between conditions (regression through origin, 2-tailed $p_{\text{uncorrected}} < 0.05$, extent 57 μL , $p_{\text{corrected}} = 0.011$). **Yellow:** The two clusters overlap (30 μL , overlap / correlation = 53%). Cursor is at voxel having peak t value of flibanserin vs vehicle comparison. The only statistically significant clusters of voxels in the entire brain are shown. (B) The same clusters shown overlaid on 0.62 mm thick coronal slices of the MRI template from +15.5 mm (upper left) to +1.2 mm (lower right) with respect to bregma. Image left = subject right. See also Supplementary Figure 1.

Figure 4. Correlated responses between brain regions

In two clusters of voxels, alterations in FDG uptake correlated across subjects with alterations observed in the medial preoptic area (mPOA) in response to flibanserin. A negative correlation (orange) was seen in bilateral somatosensory cortex (extent = 62 μ L, $p_{\text{corrected}} = 0.007$). A positive correlation (red) was seen in the neighborhood of mPOA itself, as might be expected (extent = 119 μ L, $p_{\text{corrected}} < 0.001$). Regression through origin; 2-tailed $p_{\text{uncorrected}} < 0.05$; clusters are shown on every fourth 0.62 mm thick coronal slice of the MRI template; image left = subject right. See also supplementary Figure 2.

Table 1**Experimental parameters**Mean $\pm$ SD (n=8).

	vehicle	flibanserin	p
age (years)	4.12 $\pm$ 0.58	4.12 $\pm$ 0.77	1.00
weight (g)	426 $\pm$ 56	429 $\pm$ 49	0.81
injected FDG (MBq)	30.4 $\pm$ 6.8	28.3 $\pm$ 3.7	0.38
behavior start - FDG inject (min)	6.0 $\pm$ 1.0	5.9 $\pm$ 0.9	0.83
scan start time	11:18 $\pm$ 1:13	11:16 $\pm$ 1:13	0.96
scan start - last treatment (hr)	21.3 $\pm$ 1.1	20.9 $\pm$ 1.4	0.44
scan start - FDG inject (min)	80.3 $\pm$ 7.4	81.0 $\pm$ 5.4	0.86
scan start - ketamine inject (min)	26.1 $\pm$ 7.0	25.0 $\pm$ 3.1	0.67
WB/(ID/BW) (g/mL)	0.86 $\pm$ 0.20	0.85 $\pm$ 0.11	0.87

p: paired t test 2-tailed. WB = mean radioactivity concentration in whole brain (MBq/mL), ID = injected dose (MBq), and BW = body weight (g).

Table 2
Brain metabolic response to treatment

Flibanserin reduced FDG uptake in only a single ROI: the medial preoptic area (mPOA).

ROI	vehicle	flibanserin	flibanserin - vehicle	p
CA1	0.993 +/- 0.046	0.987 +/- 0.075	-0.005 +/- 0.062	0.813
DR	1.185 +/- 0.117	1.195 +/- 0.090	0.010 +/- 0.064	0.684
mPFC	0.969 +/- 0.057	0.903 +/- 0.079	-0.066 +/- 0.110	0.135
mPOA	1.389 +/- 0.156	1.318 +/- 0.164	-0.071 +/- 0.081	0.042
VMH	1.339 +/- 0.158	1.317 +/- 0.180	-0.022 +/- 0.070	0.405

Whole brain normalized radioactivity measures in anatomically defined regions of interest for vehicle condition, flibanserin condition, and the difference flibanserin - vehicle. p: paired t test, 2-tailed; n = 8, **bold: p < 0.05**.

Table 3
Association between brain and behavioral response to treatment

Change in Total Grooming is negatively correlated with change in FDG uptake in the hypothalamic ROI, medial preoptic area (mPOA).

ROI	F(1,7)	p
CA1	0.146	0.714
DR	0.766	0.410
mPFC	1.237	0.303
mPOA	9.315	0.019
VMH	0.434	0.531

Linear regression through the origin of difference (flibanserin - vehicle) in whole brain normalized FDG vs difference in Total Grooming, p: ANOVA, **bold: p < 0.05.**

Table 4

Significant clusters identified by voxelwise statistical mapping

Clusters in t maps above threshold at $p_{uncorrected} = 0.05$ with extent shown in μL .

Test	μL	$p_{corr.}$	max. t	LR	AP	DV
Contrast: flibanserin - vehicle	88	0.001	12.3	+2.1	+9.3	+11.0
Correlation: FDG vs Total Grooming	57	0.011	-17.3	+4.6	+6.2	+7.2
Correlation: FDG vs FDG mPOA ($r > 0$)	119	<0.001	24.8	+0.0	+5.6	+7.5
($r < 0$)	62	0.007	-5.5	-2.6	+1.9	+16.9

$p_{corr.}$ = p value of cluster corrected for familywise error (SPM8). Position of maximum absolute t value (max. |t|) within cluster indicated in atlas coordinates (mm) [101]: LR = right with respect to midline, AP = anterior w.r.t. bregma, DV = dorsal w.r.t. interaural line, and mPOA = medial preoptic area.