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CHAPTER 6

General Discussion
TABLE OF CONTENTS
1. Overview of the effects of flibanserin and 8-OH-DPAT in the female marmoset monkey
   1.1 Sexual and social behavior
   1.2 HPA axis function
   1.3 Cerebral glucose metabolism
   1.4 Gene transcription in the marmoset brain
2. Potential mechanisms of action of flibanserin and 8-OH-DPAT in the regulation of female sexual and social behavior
   2.1 Monoamine regulatory module
   2.2 HPA axis module
   2.3 Pair-bond, experience and memory module
   2.4 Regulatory module of female sexual and social behavior
3. Flibanserin and hypoactive sexual desire disorder (HSDD) in women: where next?
4. The bouquet finale
5. Disclaimer
6. References
The main goal of this thesis is to investigate serotonergic (5-HT) regulation of female sexual behavior in marmoset monkeys using the 5-HT$_{1A}$ agonist/5-HT$_{2A}$ antagonist flibanserin and the 5-HT$_{1A}$ agonist 8-OH-DPAT. The specific objectives are to assess the effects of chronic flibanserin and 8-OH-DPAT treatments on (1) sexual and social behavior, (2) hypothalamic-pituitary-adrenal (HPA) axis function, (3) brain activity, and (4) gene transcription in the brain.\footnote{Flibanserin data are not available for brain activity (analyses ongoing) and gene transcription (not assessed as per agreement with Boehringer Ingelheim, proprietor and developer of flibanserin).}

1. OVERVIEW OF THE EFFECTS OF FLIBANSERIN AND 8-OH-DPAT IN THE FEMALE MARMOSET MONKEY

Table 1 presents an overview of the effects of repeated daily administration of flibanserin (15 mg/kg, PO) and 8-OH-DPAT (0.1 mg/kg, SC) on ovariectomized female marmosets. More detailed information regarding the timing of experiments with respect to treatment duration and regarding estradiol supplementation is shown in Chapter 1, Figure 7, study design.

1.1 Sexual and social behavior

The main objective in Chapter 2 was to quantify the effects of chronic flibanserin and 8-OH-DPAT on sexual and social interactions between treated females and their untreated male pairmates. Repeated observations of the pairmates’ interactions at reunion following a 90 minute separation revealed remarkable and contrasting differences between flibanserin and 8-OH-DPAT on marmoset pair behavior, despite the drugs’ shared 5-HT$_{1A}$ agonist properties.

Chronic flibanserin treatment increased the female’s sexual attractiveness to her male pairmate (more frequent male inspection of the female’s genital area) and grooming between pairmates. While increased grooming indicates a flibanserin-induced strengthening of the pair bond between marmoset partners, female sexual behavior \textit{per se} was not altered by flibanserin. Chronic flibanserin also enhanced female genital and non-genital self-grooming. Notably, flibanserin administration did not cause an acute occurrence of the serotonin (5-HT) behavioral syndrome, commonly induced by other 5-HT$_{1A}$ agonists, as well as by 5-HT$_{2A/2C}$ agonists \cite{1-3}.

In contrast to flibanserin, 8-OH-DPAT (0.1 mg/kg, SC, 4-16 weeks) transiently induced an acute 5-HT behavioral syndrome, which persisted during the course of chronic administration. Chronic 8-OH-DPAT decreased...
Table 1. Summary of chronic flibanserin and 8-OH-DPAT actions in the female marmoset monkey (cumulated data from Chapters 2-5). ↑, increased function; ↓, decreased function; ---, not changed; n/a, data not available; *, parameter correlated with female sexual receptivity; 8-OH-DPAT, R-(+)-8-hydroxy-2-(di-n-propylamino)tetralin; 5-HT<sub>1A</sub>, serotonin-1A receptor; 5-HT<sub>2A</sub>, serotonin-2A receptor; 5-HT<sub>7</sub>, serotonin-7 receptor; 5-HTT, serotonin transporter; OXT, oxytocin; mPFC, medial prefrontal cortex; mPOA, medial preoptic area; VMH, ventromedial hypothalamic nucleus; CA1, CA1 field of the hippocampus; DRN, dorsal raphé nucleus; mOCC, medial occipital cortex.

<table>
<thead>
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<th>Parameter</th>
<th>Flibanserin</th>
<th>8-OH-DPAT</th>
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female sexual receptivity and increased aggressive interactions between male-female pairmates, while tending to decrease female sexual attractiveness and grooming between pairmates.

The data described in Chapter 2 resemble studies performed in female rodents that show that flibanserin increases the female’s sexual attractiveness and display of proceptive and receptive sexual behavior [4], while 8-OH-DPAT inhibits lordosis [5], an indicator of female sexual receptivity in rodents. Chapter 2 thus presents the first study to confirm the sexual attractiveness-enhancing effects of flibanserin and sexually inhibitory effects of 8-OH-DPAT in a non-human primate. In humans, flibanserin improved satisfying sexual events, desire and the female sexual function index, and reduced sexual distress [6, 7]. While flibanserin did not alter the frequency of female sexual behavior in the marmoset, enhanced female sexual attractiveness and strengthened pair-bond between marmoset pairmates suggest that the observed beneficial effects of flibanserin on female sexual function in humans might be rooted on improved partner interactions and intimacy in the relationship between long-term sexual partners.

1.2 HPA axis function

In Chapter 3, the impact of chronic flibanserin and 8-OH-DPAT on HPA axis function was investigated to delineate the possibility of a HPA axis-mediated mechanism in the serotonergic regulation of female sexual behavior.

Chronic flibanserin neither affected circulating morning basal cortisol levels, nor ACTH and cortisol responses to an acute 5-HT$_{1A}$ agonist challenge. Flibanserin treated females, however, displayed an increased ACTH response at 30 minutes during a restraint test, while cortisol levels were not altered during the 30 minutes of restraint, nor at 3 hours after return to the home cage.

Similar to flibanserin, neither circulating morning basal cortisol levels nor ACTH and cortisol responses to an acute 5-HT$_{1A}$ agonist challenge were altered by chronic 8-OH-DPAT. The restraint-induced increase in ACTH was enhanced by chronic 8-OH-DPAT at both 15 and 30 minutes, while cortisol was elevated at 3 hours after return to the home cage, in comparison to vehicle control treatment. In 8-OH-DPAT, but not in flibanserin treated females, exaggerated ACTH responses at 15 minutes of restraint were associated with reduced female sexual receptivity, reduced sexual attractiveness and increased aggression between pairmates.

Importantly, enhanced ACTH responses to restraint were correlated with increased aggression and reduced sexual receptivity in 8-OH-DPAT treated female marmosets, supporting the hypothesis that increased stress reactivity might contribute to inhibition of sexual behavior. Such correlations were
absent in flibanserin treated females, suggesting no inhibitory role of HPA axis reactivity on female sexual function.

1.3 Cerebral glucose metabolism

In Chapter 4, a PET/MRI functional brain imaging experiment was described that was designed to measure cerebral glucose metabolism, an indicator of neural activity, in chronic 8-OH-DPAT or vehicle treated female marmosets during sexual and social interactions after a 90 minute separation from their male pairmates. Radiolabeled \(^{[18}F\)fluorodeoxyglucose (FDG) was infused to the femoral vein immediately prior to a 30 minute pair test. The females were subsequently imaged by PET under isofluorane anesthesia. Structural MRI scans were recorded and overlaid on the PET images to improve the visualization of anatomical structures. PET scans using 5-HT\(_{1A}\)- and 5-HTT-specific radiotracers were also performed. Data processing and analysis of the latter scans are still ongoing, and the results are not within the scope of this thesis.

8-OH-DPAT decreased glucose metabolism, an indicator of neural activity, in the females’ medial occipital cortex (mOCC) while interacting with their male partners. Decreased neural activity in the mOCC was associated with reduced female sexual receptivity, suggesting 8-OH-DPAT induced altered female perception of interactions with their male pairmate. However, glucose metabolism in pre-defined regions of interest (ROI), the medial prefrontal cortex (mPFC), medial preoptic area (mPOA), ventromedial hypothalamic nucleus (VMH), CA1 field of the hippocampus (CA1) and dorsal raphé nucleus (DRN), hypothesized to play a central role in sexual function and serotonin neurotransmission, were not altered.

1.4 Gene transcription in the marmoset brain

In Chapter 5, the transcriptomic effects of chronic 8-OH-DPAT administration to female marmoset monkeys were reported, complementing an open, hypothesis-generating microarray approach using the marmoset-specific EUMAMA microarray, with a candidate gene approach using real-time quantitative PCR (RT-qPCR) to measure serotonin receptor and transporter transcripts. In keeping experimental parameters consistent between chapters, the same brain regions of interest (mPFC, mPOA, CA1, DRN) as in Chapter 4 were selected, with exception of the VMH, which was not analyzed in Chapter 5.

Chronic 8-OH-DPAT, in a brain region-specific fashion, altered the expression of gene clusters linked to neural development (mPFC, mPOA, DRN), neurotransmission (mPOA), energy production (mPFC, mPOA),
mitochondrial function (CA1, DRN), protein transport (mPOA, DRN), learning and memory (CA1), ion channel activity (CA1), and intracellular signal transduction (DRN), suggesting global changes in gene regulation of neuronal function induced by 8-OH-DPAT that involve changes in cellular metabolism, communication between neurons and axonal growth.

Furthermore, 8-OH-DPAT increased 5-HT transporter (5-HTT, or SERT) mRNA expression in the DRN and oxytocin (OXT) mRNA expression in the mPOA. There was a statistical trend towards increased 5-HT$_{1A}$ expression in the mPFC and towards reduced 5-HT$_7$ expression in the CA1. Activation of 5-HT$_{1A}$ autoreceptors in the DRN by 8-OH-DPAT likely suppressed serotonergic activity [8], which may have triggered a compensatory upregulation of 5-HTT in the DRN and of 5-HT$_{1A}$ in the mPFC to restore the serotonergic tone. In contrast, decreased 5-HT$_7$ expression in the CA1 may reflect a brain region-specific desensitization of 5-HT$_7$ receptors. As 8-OH-DPAT directly interacts with 5-HT$_{1A}$ and 5-HT$_7$ receptors, their brain region-specific expression changes may present the most upstream molecular mechanisms underlying sexual and affiliative suppression induced by chronic 8-OH-DPAT.

While resolution was a limiting factor in the brain imaging study (Chapter 4), the gene expression experiments described in Chapter 5 benefitted from high anatomical precision provided by laser-microdissection techniques, which were used to dissect the ROIs prior to RNA isolation. Both Chapter 4 and Chapter 5, however, indicate that the selected ROIs (mPFC, mPOA, CA1 and DRN) may not comprehensively cover the brain areas involved in 8-OH-DPAT induced modulation of female sexual and social behavior. For example, Chapter 5 highlights increased oxytocin expression in the mPOA, but does not investigate expression levels in oxytocin-rich areas such as the paraventricular and supraoptic nuclei of the hypothalamus. Future studies should also target the expression of oxytocin receptor in marmoset brain areas with high receptor densities, including the basal forebrain cholinergic nuclei, nucleus basalis of Meynert, the diagonal band of Broca, and the amygdala [9, 10], areas that were not part of the pre-selected ROIs.

2. POTENTIAL MECHANISMS OF ACTION OF FLIBANSERIN AND 8-OH-DPAT IN THE REGULATION OF FEMALE SEXUAL AND SOCIAL BEHAVIOR

In assessing how flibanserin and 8-OH-DPAT may affect female sexual function, the data collected in Chapters 2-5 point towards a complex, multimodal mechanism of serotonergic regulation of female sexual function. Thus, flibanserin’s and 8-OH-DPAT’s potential modes of action in regulating female
sexual behavior will be discussed in the framework of a model that involves four distinct, but interactive modules (Figure 1).

The core component of this model entails a hypothalamic unit, referred to as the *regulatory module of female sexual and social behavior*, which integrates relevant sensory information and produces sexual and social behavioral responses. Sexual and social behaviors are joined into a single unit, consistent with the results obtained in Chapter 2 that highlight the significance of pair-bond quality for female sexual behavior. This module is influenced by the other three modules. A *monoamine regulatory module* can exert either excitatory or inhibitory leverage, as outlined in Chapter 1, Figure 3: *Excitatory-Inhibitory model of female sexual behavior* (adapted from [11]). A *HPA axis module* accounts for the potential influence of stress hormones on female sexual behavior, while a *pair-bond, experience and memory module* applies a more integrative, holistic approach and emphasizes the important findings

Figure 1. Four interacting modules through which flibanserin and 8-OH-DPAT modulate female sexual and social behavior. Circle and arrow size indicate relative impact of the respective module on female sexual and social behavior according to the observations described in Chapters 2-5 and additional data reported in [12, 13]. HPA, hypothalamus-pituitary-adrenal; mPFC, medial prefrontal cortex; DRN, dorsal raphé nucleus; VTA, ventral tegmental area; LC, locus coeruleus; PVN, paraventricular hypothalamic nucleus; mPOA, medial preoptic area; VMH, ventromedial hypothalamic nucleus.
of Chapters 2, 4 and 5 that implicate relationship dynamics and cognitive processes in the regulation of female sexual behavior. The four modules are briefly described in the following sections.

2.1 Monoamine regulatory module

Pharmacological approaches based on manipulation of serotonin receptors, particularly of the 1A and 2A subtypes, have recently emerged in animal models of female sexual dysfunction [4, 14, 15] and as putative treatments for HSDD in women [6, 7]. The monoamine regulatory module integrates evidence derived from rodent studies, as modified from Stahl et al [16], in which flibanserin and 8-OH-DPAT selectively alter serotonergic, dopaminergic and noradrenergic neurotransmission in a brain region-specific manner. Pyramidal neurons in the prefrontal cortex (PFC) process sensory and cognitive inputs to the PFC and send glutamatergic projections to, among others, brainstem monoamine centers for 5-HT (DRN), for DA (ventral tegmental area, VTA) and for NE (locus coeruleus, LC). These connections can either be direct, resulting in monoamine release, or indirect via inhibitory GABAergic (γ amino butyric acid) interneurons, in which case monoamine release decreases.

Flibanserin activates 5-HT$_{1A}$ and inhibits 5-HT$_{2A}$ receptors on PFC pyramidal neurons, leading to increased DA and NE, but decreased 5-HT levels in the mPFC [12], purportedly by selective inhibition of pyramidal neurons that excite brainstem serotonergic neurons (decrease in 5-HT) and GABAergic interneurons in the VTA and LC (increase in DA and NE) [16]. Interestingly, we found that neural activity was indeed decreased in the mPFC of flibanserin treated female marmosets [AK Converse and DH Abbott, personal communication], consistent with the hypothesized inhibition of pyramidal neurons by flibanserin.

In contrast to flibanserin, 8-OH-DPAT preferentially binds to 5-HT$_{1A}$ receptors located on GABAergic interneurons, but not pyramidal neurons of the PFC, causing enhanced pyramidal cell firing in the PFC [17]. Increased activation of dopaminergic and noradrenergic neurons in the VTA and LC through these pyramidal neurons would explain the cortical increase in DA and NE that is observed after systemic administration of 8-OH-DPAT [18]. Compared to flibanserin, however, the increase in DA is more modest [11]. 5-HT levels have been shown to remain unaltered [13, 18], possibly due to direct activation of counteractive presynaptic 5-HT$_{1A}$ autoreceptors in the DRN, or due to upregulated 5-HTT function in the DRN in response to chronic 5-HT$_{1A}$ activation, as suggested by the marmoset data described in Chapter 5.

As proposed by the Excitatory-Inhibitory model of female sexual behavior (Chapter 1, Figure 3, [11]), DA and NE activate the regulatory module of
female sexual and social behavior (section 2.4) to facilitate sexual behavior, while 5-HT has an inhibitory effect. Increased DA and NE and decreased 5-HT after flibanserin is thus consistent with increased sexual attractiveness and affiliative behavior observed in flibanserin treated female marmosets (Chapter 2). 8-OH-DPAT likely increases excitatory DA and NE inputs to the regulatory module of female sexual and social behavior, but more moderately compared to flibanserin, without affecting the inhibitory 5-HT input. The expected net effect of 8-OH-DPAT on sexual and social behavior in the female marmoset would thus be slightly pro-sexual and pro-social, which contrasts the results shown in Chapter 2. This discrepancy will be discussed in section 2.4 below.

Projected module effect on sexual and social behavior:
Flibanserin ↑; 8-OH-DPAT (↑)^[2]

2.2 HPA axis module
Pharmacological manipulations of serotonin receptors commonly activate the HPA axis. The HPA axis is a key neuroendocrine component in the physiological response to stress that generally suppresses female reproductive function [19]. Cortisol interacts with the hippocampus and amygdala which indirectly regulate the HPA axis by modulating the processing of stressful information, involving inhibitory GABAergic neuron projections to the PVN [20]. Adrenergic and noradrenergic projections from the nucleus of the solitary tract (NTS) [21, 22] and from the LC [23] can modulate the HPA axis responsiveness to stress.

In Chapter 3, evidence was presented for flibanserin and 8-OH-DPAT induced HPA axis sensitization in response to a stressor. In 8-OH-DPAT, but not in flibanserin treated females, increased ACTH responses to restraint were positively correlated with increased sexual rejection. Furthermore, only 8-OH-DPAT, but not flibanserin, elevated cortisol levels several hours after termination of the stressor.

Chronic flibanserin might enhance the HPA axis response to a stressor by inhibiting the modulatory, inhibitory GABAergic inputs from the hippocampus or amygdala, or by activating the stimulatory noradrenergic inputs from the NTS and LC, causing exaggerated ACTH release to restraint stress. Chronic 8-OH-DPAT might enhance the ACTH response to restraint stress in a similar way as flibanserin by changing modulatory and/or activating inputs to the PVN. Such stress-induced rises in ACTH are likely independent from acute

^[2] Upwards pointing arrows indicate that the module exerts pro-sexual and pro-social influence, while downwards pointing arrows indicate that the module exerts anti-sexual and anti-social influence.
5-HT<sub>1A</sub> actions of flibanserin and 8-OH-DPAT [24]. In contrast to flibanserin, 8-OH-DPAT also elevates cortisol levels following a stressor. Elevated cortisol might suppress female sexual receptivity and induce aggression, consistent with the observation that exaggerated ACTH responses to a stressor correlate with agonistic and sexual rejection behavior in 8-OH-DPAT treated female marmosets (Chapter 3).

**Projected module effect on sexual and social behavior:**
Flibanserin unaltered; 8-OH-DPAT ↓

### 2.3 Pair-bond, experience and memory module

In humans, psychosocial factors such as partnership quality, previous sexual experiences and personal, social and cultural expectations [25-31] are important determinants of women’s sexuality (see Chapter 1). Interpersonal distress is in fact a central component in the diagnosis of hypoactive sexual desire disorder (HSDD) in women [32]. It is in this context that marmoset data on flibanserin’s actions, outlined in Chapters 2-5, may add the most important contribution to understanding flibanserin’s action on HSDD in women. The pair-bond, experience and memory module links the effects of flibanserin and 8-OH-DPAT on female sexual behavior to experimental evidence that partner interactions (Chapter 2), stress reactivity (Chapter 3), cortical glucose metabolism (Chapter 4) and learning and memory processes (Chapter 5) may all partake in regulating female sexual function.

In Chapter 2, it was demonstrated that both flibanserin and 8-OH-DPAT not only affected the treated female’s behavior towards her partner, but also altered the untreated male’s social behavior towards the female, clearly implicating altered relationship dynamics between the partners. As outlined in Chapter 1, sexual behavior in women entails a complex interplay between psychosocial, hormonal and neural factors and requires hypothalamic, limbic and cortical brain structures for its regulation [11].

Similar factors may play a role in the female marmoset monkey. For 8-OH-DPAT, such psychosocial, hormonal and neural factors were determined in Chapters 2-5. Reduced female sexual receptivity was associated with more frequent agonistic interactions between pairmates (Chapter 2), increased endocrine stress reactivity (Chapter 3) and reduced glucose metabolism in the occipital cortex (Chapter 4). Reduced glucose metabolism in the occipital cortex suggests an 8-OH-DPAT-induced impairment in the perception of visual salience in social interactions with the long-term sexual partner. Agonistic social interactions, as well as increased stress reactivity, may cause sexual signals from the male partner to be negatively interpreted and rejected,
thus establishing a negative association with the sexual partner. Chapter 5 highlights that 8-OH-DPAT affects the expression of hippocampal genes that are linked to learning and memory. Learning negative associations with the sexual partner and remembering previous agonistic interactions could indeed impair the pair-bond between marmoset pairmates and negatively affect female sexual function.

For flibanserin, behavioral data indicate a positive shift in social and sexual behavior that is built on improved pair bonds (Chapter 2). Brain activity and transcriptomic data, however, are not yet available.

**Projected module effect on sexual and social behavior:**

Flibanserin ↑; 8-OH-DPAT ↓

2.4 Regulatory module of female sexual and social behavior

This module relates to a key finding described in Chapter 5, that 8-OH-DPAT affects the transcription of hypothalamic oxytocin, to a previous marmoset study employing the same study design [33], and places it in context of a theoretical framework proposed by Choleris et al [34], the four-gene micronet of social behavior regulation, described below.

The hypothalamus, specifically the mPOA and VMH, plays a central role in the regulation of female sexual behavior by integrating sexually-relevant sensory inputs with excitatory and inhibitory neurotransmitter and sex hormone signals, thus controlling female sexual behavior (reviewed in [11, 35]. The essential function of the mPOA and VMH in the generation of sexual behavior in female and male marmoset monkeys is confirmed by lesion and functional imaging studies [36-39]. In rodents, oxytocin immunoreactivity and mRNA expression in the mPOA has been associated with social behavior [40, 41], and infusion of oxytocin into the mPOA increases female sexual receptivity [42]. It is however possible that the detection of oxytocin mRNA in the mPOA may reflect mRNA molecules stored in dendritic projections of oxytocin neurons located in the PVN [43].

The mPOA receives a large number of projections from the PVN, which also sends efferents to the amygdala [44]. The PVN is a major site of oxytocin production, which can act centrally as a neuropeptide through oxytocin receptors expressed in the mPOA and amygdala to regulate maternal, social and sexual behavior [45-49]. Oxytocin thus serves as a prominent connection between the pair-bond, experience and memory module (Section 2.3), and the regulatory module of female sexual and social behavior presented in this section.

Choleris et al [34] propose a four-gene micronet, involving oxytocin,
DISCUSSION

Oxytocin receptor and the estrogen receptors alpha and beta (ERα, ERβ), in the regulation of social recognition and ultimately in the regulation of affiliation, pair bonds, aggression and sex [50]. In this model, estradiol plays a crucial role in the expression of oxytocin in the PVN and PVN dendrites that extend to other hypothalamic areas, and of oxytocin receptors in the amygdala. Signaling through the oxytocin receptor initiates a cascade of events (described in [35, 51]) that facilitate social recognition and promote or prevent the display of sexual behavior.

Chronic 8-OH-DPAT increases the expression of oxytocin in the mPOA (Chapter 5) and likely in the PVN [52]. 8-OH-DPAT, however, downregulates the expression of estrogen receptors [53], which are essential for oxytocin receptor function in the amygdala [34]. Ovariectomy may further impair oxytocin receptor expression [54]. Thus, and despite elevated hypothalamic oxytocin expression, the cumulative effects of chronic administration and ovariectomy might suppress oxytocin signaling through its receptor and cause sexual rejection and aggression between marmoset pairmates (Chapter 2; discussion in Chapter 5). Furthermore, the impact of increased excitatory input from the monoamine regulatory module on the regulatory module of sexual and social behavior, proposed in Section 2.1, may also be without functional consequence due to the lack of downstream mediators.

Although no molecular data are available for the effect of flibanserin on oxytocin and oxytocin receptor expression, there is ample indirect evidence that flibanserin, similar to 8-OH-DPAT, may increase oxytocin in the hypothalamus. Flibanserin supports pro-social and pro-sexual behavior in female marmosets (Chapter 2), behaviors that are strongly associated to increased central oxytocin function [55-57]. Flibanserin also increases genital and non-genital self-grooming in female marmosets (Chapter 2), a behavior that is induced by central oxytocin administration in female rats [58]. Furthermore, flibanserin increases norepinephrine (NE) levels in the mPFC and mPOA in female rats [59]. Oxytocin induces NE release in the VMH [60], while NE conversely induces oxytocin release in the PVN and the supraoptic hypothalamic nucleus (SON) [41]. Unlike 8-OH-DPAT, flibanserin has not been reported to impair oxytocin receptor function. Thus, according to Choleris' model [34], flibanserin-induced increases in oxytocin in the mPOA and PVN could lead to enhanced activation of the amygdala and promote pro-social [41] and pro-sexual behavior [11, 61].

A flibanserin-induced increase in oxytocin signaling may also stimulate sexual and social behavior through the release of gonadotropin-releasing hormone (GnRH). Oxytocinergic neurons in the mPOA and PVN project to GnRH containing neurons in the mPOA to stimulate GnRH release [62]. In the marmoset monkey, GnRH II has been shown to stimulate female sexual
behavior by binding to the GnRH receptor type II, which is expressed in the mPOA and VMH [33]. Noradrenergic, dopaminergic and serotonergic afferents from the brainstem monoamine centers play an important regulatory role on oxytocin and GnRH release in the hypothalamic nuclei [60, 62] and serve thus as interface between the monoamine regulatory module described in Section 2.1, and the regulatory module of female sexual and social behavior.

**Net effect on sexual and social behavior:**

Flibanserin ↑; 8-OH-DPAT ↓

3. **FLIBANSERIN AND HYPOACTIVE SEXUAL DESIRE DISORDER (HSDD) IN WOMEN: WHERE NEXT?**

Parallel to the marmoset study presented in this thesis, flibanserin underwent large Phase III clinical trials termed the **BOUQUET Study Program**, conducted by Boehringer Ingelheim [63]. The seven trials involved over 5,000 pre-menopausal women with HSDD and were conducted in North America and Europe, with the aim to evaluate the efficacy and safety of flibanserin. Trial participants suffered from primary generalized acquired HSDD for a period of more than 24 weeks. Inclusion criteria were, among others, that the participants lived in a stable, communicative, heterosexual relationship of at least one year, with a sexually functioning partner and that they experienced major personal distress due to sexual dysfunction.

The co-primary endpoints of the **BOUQUET** studies were the change from baseline to study end in the number of satisfying sexual events (SSE) in all trials and a sexual desire score measured using a daily electronic diary (eDiary). Sexual desire was measured using both the daily eDiary and the desire domain of the Female Sexual Function Index (FSFI). Secondary endpoints assessed to provide data on sexual functioning and the alleviation of distress, a hallmark of HSDD, using the Female Sexual Function Index (FSFI) and Female Sexual Distress Scale (FSDS-R).

The North American trials demonstrated significantly increased numbers of sexually satisfying events at the highest flibanserin dose of 100 mg/day (from 2.7/month to 4.5/month) compared to 2.7/month versus 3.7/month, respectively, in the placebo group. Flibanserin also improved the secondary endpoints (FSFI, FSDS-R) and was thus effective in decreasing interpersonal distress. The eDiary desire scores were, however, not significantly increased by flibanserin, when compared to placebo treatment [6, 7]. The latter result, together with some reported adverse events (sleepiness, dizziness and fatigue in > 10% of women treated with flibanserin), led to a negative review
for flibanserin by an advisory panel of the US Food and Drug Administration (FDA) in August 2010. Boehringer Ingelheim, as a consequence, withdrew the drug from development in October 2010. Controversy regarding the usability of eDiary and SSE as suitable primary endpoints remains after the withdrawal of flibanserin, as the development of meaningful and valid end points that capture the complexity of women’s sexual responses is unsolved [64].³

The marmoset flibanserin data presented in this thesis suggest the possibility that flibanserin might indirectly affect female sexual function by improving the relationship with a partner. Increased female sexual attractiveness and strengthened pair-bond between marmoset pairmates reflect improved partner interactions and intimacy in the relationship between long-term sexual partners. Allogrooming in nonhuman primates is essential to lasting social bonding and the creation of a psychological environment of trust between partners [66], likely involving central actions of oxytocin. Flibanserin’s main effect in the marmoset study was to increase allogrooming in established, long-term marmoset pairs, while its main effect in the clinical BOUQUET trials was to increase sexual satisfaction and decrease distress in women in a relationship with a long-term sexual partner. Considering these main outcomes, it seems plausible that flibanserin’s therapeutic effect in the treatment of HSDD may be mediated by improving relationship quality and reducing sexual distress between partners. Flibanserin-induced improvements in sexual, social and emotional bonding between partners may underlie the efficacy of flibanserin in increasing sexual satisfaction and decreasing interpersonal distress in women suffering from HSDD.

For future clinical HSDD trials, the marmoset findings suggest that it would be valuable to consider parameters that characterize the intimacy of a relationship and emphasize on the role of the partner as trial end-points, thus exploring novel perspectives in the pharmacotherapeutic treatment of HSDD. In addition, the role of hypothalamic oxytocin in the regulation of female sexual behavior merits special attention in future studies of flibanserin. It would be of great interest to measure oxytocin and oxytocin receptor expression in the available brain tissues of chronic flibanserin treated female marmoset monkeys. Regions of interest should entail PVN, mPOA and amygdala, as well as other brain regions with high oxytocin and oxytocin receptor expression. A large-scale microarray study using the EUMAMA microarray would provide the transcriptomic correlates of the behavioral findings. Furthermore,

³ On July 13, 2012, it was reported that the startup company Sprout Pharmaceuticals, who acquired flibanserin from Boehringer Ingelheim in 2011, plans to resubmit a flibanserin drug application to the FDA in early 2013 without additional clinical studies. Flawed metrics in looking at the data were cited that led the FDA to deny flibanserin approval [65].
neuroimaging tools for oxytocin receptor binding are currently being developed [67] and may soon provide the opportunity to detect oxytocin receptors in vivo in both marmoset and human fibanserin trials.

4. THE BOUQUET FINALE

A bouquet of concluding statements highlights new insights gained from experiments conducted as part of this thesis and their added value to the fibanserin pivotal phase-III clinical trials (BOUQUET Study Program).

- Pair bond quality determines sexual attractiveness and activity in female marmoset monkeys (Chapter 2).
- Chronic 5-HT$_{1A}$ activation by 8-OH-DPAT causes a phenotype characterized by reduced female sexual receptivity and attractiveness, increased aggression between pairmates, increased stress reactivity and reduced neural activity in the medial occipital cortex (Chapters 2, 3, 4).
- Oxytocin may be the pivot of serotonergic regulation of female sexual behavior, pair-bond and pharmacotherapy of HSDD (Chapters 2, 5).
- Marmoset data suggest that fibanserin-induced improvements in partner interactions may underlie increased sexual satisfaction and reduced interpersonal distress in the BOUQUET studies, thus presenting unexplored perspectives to investigate HSDD and its future pharmacotherapy.

5. DISCLAIMER

Experiments described in the thesis were sponsored by Boehringer Ingelheim, proprietor and developer of fibanserin. Selection of experiments, as well as experimental design, was under full control of the Principal Investigators of the studies. Experiments described in Chapters 2, 3 and 4 were conceived and designed by Prof. David Abbott (PI), Dr. Alexander Converse and Yves Aubert. Experiments described in Chapter 5 were conceived and designed by Dr. Nicole Datson (PI) and Yves Aubert.

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