CHAPTER 1

General Introduction

TABLE OF CONTENTS

1. FEMALE SEXUALITY AND FEMALE SEXUAL DYSFUNCTION

Sexual reproduction is one of the most universal, fundamental and essential processes that all species have in common. Sexuality in humans, however, goes far beyond the basic principles of reproduction and entails complex interactions of biological, psychological and sociocultural variables. Few other biological systems are as multifaceted as sexuality. Beyond neurobiological factors that influence sexual function, several studies, including the National Health and Social Life Survey (NHSLS) that was conducted in the United States [1], point out that sexuality in women is closely linked to psychological factors, quality of their relationship with a partner and lifestyle [2-7], and that all are influenced by cultural, family and personal expectations [2, 3; 8-10]. Ultimately, sexuality serves a basic emotional health need for all individuals. Despite this understanding, sexual function in general, and female sexual function in particular, remains a relatively understudied area of scientific and clinical investigation, although pharmacotherapeutic interest has intensified research in recent years [11].

Masters and Johnson (1966) were among the first to describe and characterize female sexual function by proposing a linear, four stage model of the human sexual response. This traditional view divides a woman's sexual response into four sequential stages, starting with spontaneous sexual desire that leads to sexual arousal, and that is then followed by orgasm and resolution [12]. Later observations made it evident that this view of the female sexual response is just one of several normal variants in sexual responses in women [13]. For example, studies of women in established relationships show that desire by itself is not a frequent reason to engage in sexual activity [14], while other reasons such as the nurture of emotional closeness with a partner, the increase of their own sense of well-being, or the avoidance of negative consequences of sexual inactivity are commonly reported to also play a role [2, 3, 9, 10]. Other reports suggest that that female sexual desire and sexual arousal are overlapping concepts that women find difficult to separate [13]. At the same time, functional imaging studies in women reveal that subjectively perceived sexual arousal is often unrelated to objectively measured sexual arousal (reviewed in [6, 7, 15]). These and other observations led to novel views on women's sexuality and spurred the development of alternative models of the female sexual response cycle, centering around sociocultural and relationship factors [2, 8]. The model proposed by Basson (Figure 1, [8]) may be the most accurate for women with a history of sexual problems [16].

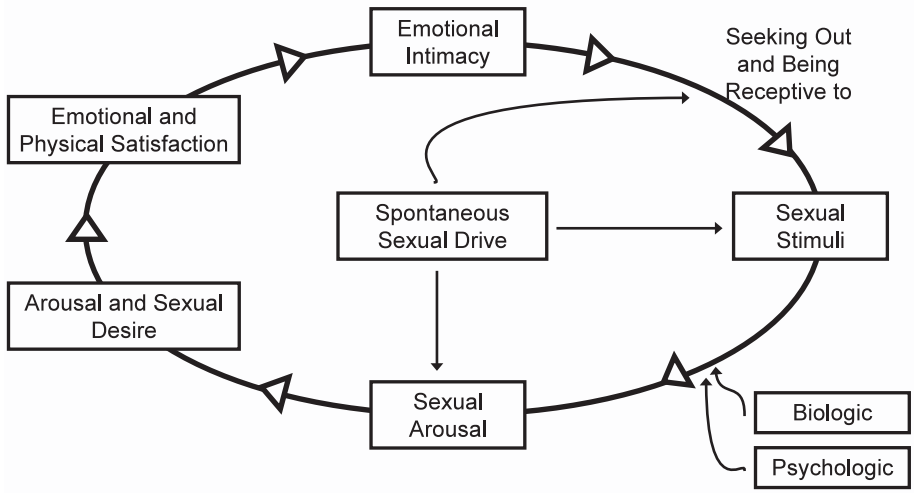


Figure 1. Female sexual response cycle. Adapted from [8].

Following the groundbreaking work by Masters and Johnson, the Diagnostic and Statistical Manual of Mental Disorders (DSM) implemented a classification system for female sexual dysfunctions (FSDs) that is based on the female sexual response stages. Sexual problems are divided into disorders of desire (hypoactive sexual desire disorder (HSDD), sexual aversion), arousal, orgasm and pain (dyspareunia and vaginismus). In the 4th edition (DSM-IV) and text-revised 4th edition (DSM-IV-TR), attention is paid to psychological factors such as interpersonal distress, which must be subjectively perceived by the female patient for the diagnosis of certain sexual dysfunctions, including HSDD. The NHSLS reveals that sexual dysfunction is prevalent across the entire demographic population and found in 43% of the women who participated in the NHSLS study at some point in their lives [1]. Among female sexual desire subgroups, low sexual desire is the most frequently reported complaint in women (32%; Figure 2). Clinically relevant, personally distressing occurrence of HSDD is reported in approximately 1 in 10 women [6, 7, 17]. With the exception of estrogen therapy for impaired genital vasocongestion in response to sexual stimulation and, in some countries, testosterone formulations for reduced desire, there are currently no approved drugs for the treatment of sexual dysfunction in women despite their high prevalence [18].

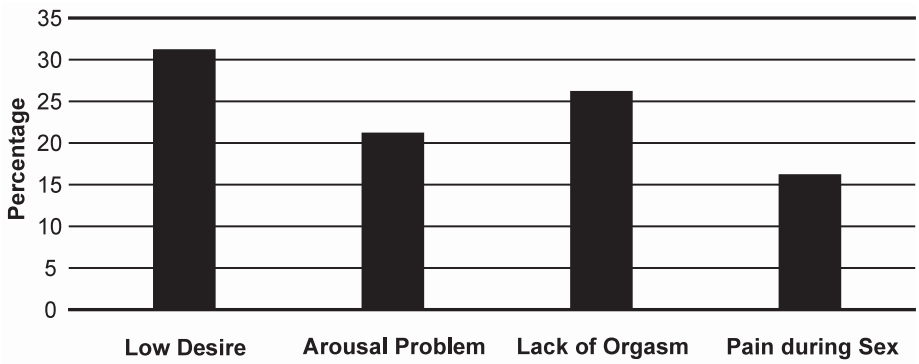


Figure 2. Prevalence of sexual dysfunction in women. Adapted from the National Health and Social Life Survey (NHSLS) [1].

Sexual desire and sexual arousal have traditionally been regarded as two distinct though related phenomena, a concept which seems to fit the sexual experience in men, but less in women [19]. In fact, some women find it difficult to differentiate arousal from desire [13]. Bancroft and Graham [13] describe sexual desire and sexual arousal as overlapping concepts. They suggest that sexual arousal involves (i) information processing of sexual stimuli involving unconscious and conscious cognitive mechanisms, (ii) incentive motivation that includes activation of the dopaminergic incentive motivational system, (iii) induction of generalized central nervous system (CNS) arousal, and (iv) genital response. The state of sexual desire, in contrast, involves only some of these components (usually components (i) and (ii)). The DSM-IV-TR defines HSDD as persistent or recurrent deficiency or absence of sexual fantasies and thoughts, and/or desire for, or receptivity to, sexual activity, which causes personal distress or interpersonal difficulties and is not caused by a medical condition or drug. Distress is an integral part of HSDD diagnosis. A diagnosis of HSDD requires a clinical evaluation, taking into account factors that affect sexual functioning, such as age and the context of the woman's life [20]. Despite of its prevalence, the acronym HSDD is poorly understood by clinicians, and the current DSM-IV-TR definition of HSDD has been criticized. There is an ongoing debate for combining arousal and desire into one disorder, and to include duration, intensity, and frequency to the definition of HSDD in the upcoming DSM-5 edition [20, 21]. The debate, however, goes beyond the scope of this thesis and its outcome neither affects objectives nor results of the experimental chapters presented in this book (Chapters 2-5).

2. THE NEUROBIOLOGY OF FEMALE SEXUAL BEHAVIOR

Following the principle that animal and human behavior is based on an underlying neurobiological substrate, neuroscientists are facing the challenge of dissecting and reducing the multifaceted behavior of female sexuality into separate components that can be studied individually. Health psychologists and ethologists, on the other hand, are equally challenged with the integration of complex etiological factors to explain the behavioral expression of female sexuality. The display of female sexual behavior, as well as the lack thereof, is the net result of motivational states, sensory stimuli, hormonal and neurochemical environments, previous experience, cultural influences and many other factors.

For practical and ethical reasons, many studies attempting to unravel the neurobiology of female sexual behavior have been performed in animals, and mostly in rodents. Pfaff and colleagues succeeded in describing the complete neural circuit of a primary reproductive behavior, lordosis behavior, in the female rat [22]. Lordosis behavior is a sexual response of the female to male tactile stimulation and consists of a ventral arching of the spine, thus allowing for copulation. In short, somatosensory stimulation of the female's flanks, tailbase and perineum by the male's paws and penis activate pressure receptors that signal via ascending spinal neurons to nuclei in the brain stem (medullary reticular formation and lateral vestibular nucleus), midbrain (midbrain reticular formation and periaqueductal gray, PAG) and hypothalamus (medial preoptic area, mPOA, medial anterior and ventromedial hypothalamus, VMH), where estradiol and progesterone are necessary to facilitate the behavior. Integrated signals are projected via the brain stem nuclei to descending pathways of the lumbar spinal cord, leading to the contraction of deep back muscles to elicit lordosis behavior [22]. Further studies in rodents applying lesion, electrical stimulation, tract-tracing and Fos-immunoreactivity (Fos-IR) techniques revealed other brain structures involved in different aspects of female sexual behavior. Following mating, Fos-IR is not only activated in the mPOA, VMH, and PAG, but also in the bed nucleus of the stria terminalis (BNST) and central tegmental field (CTF) [23-27]. Chemosensory investigation of the anogenital area induces Fos-IR in the posteromedial part of the BNST only [28], while vaginocervical stimulation either by a high number of penile intromissions or manual probing induces Fos-IR in the mPOA and BNST, and also in the medial amygdala and parvocellular subparafascicular nucleus [25, 26, 28, 29]. Importantly, combined estrogen and progesterone treatment in female rodents activates Fos-IR in most of the same brain areas that are activated after mating, including the pivotal VMH and mPOA, confirming the high density of estrogen receptors in these areas [30] and suggesting that both hormonal

and environmental signals relevant to sexual function may converge in these brain structures to determine the expression of female sexual behavior.

In female cats, a similar neural circuit to rats has been found to control lordosis behavior. Neurons in the nucleus retroambiguus (NRA), located in the brain stem, send descending projections to lumbosacral motoneurons that control the lower limbs and trunk to produce the copulatory posture. The NRA receives sensory information from the midbrain central gray, which in turn is a projection area for hypothalamic, amygdaloid and cortical neurons. Estradiol regulates the strength of the NRA-lumbosacral connection, explaining why lordosis behavior only occurs when the female cat is in estrous [31].

While experiments as described above are limited in their applicability to animal studies, more recent advances in functional imaging methods provide a window into the conscious human mind and allow for the study of neural correlates of human behavior. Both functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) approaches have attempted to investigate the brain activity during different sexual states, ranging from sexual interest to arousal and orgasm. fMRI studies in women show that several cortical areas (medial prefrontal, anterior cingulate, orbitofrontal, insular, entorhinal and occipitotemporal cortices) as well as the amygdala and ventral striatum are activated in response to the visual presentation of erotic video clips [32, 33]. Interestingly, the peripheral sexual response seems not to be correlated to either the subjective sexual response or brain activation patterns [33]. In a H215O PET study, clitoral stimulation of women by their partners leads to activation of the medial areas of the somatosensory and somatomotor cortices, which represent the pelvic region. These same regions are also activated during orgasm, although in a less pronounced fashion. Orgasm is furthermore characterized by an activated rostromedial cerebellum [31, 34]. Most strikingly, while some brain areas are activated during sexual stimulation and orgasm, much larger areas are deactivated, especially during orgasm. Clitoral stimulation deactivates the amygdala and the posterior temporal lobe mainly in the left hemisphere, and these same structures, together with large areas of the ventral temporal lobe and ventral prefrontal cortex, are also deactivated during orgasm. This study, together with an fMRI study that demonstrates greater activation of the medial frontal and right inferior frontal gyri in women with HSDD when watching erotic video clips [33], suggests that the deactivation of certain neural processes involved in alertness and thought processing is important for the experience of sexual arousal and orgasm, while HSDD patients may allocate more attention to monitoring and evaluating their sexual responses.

Of equal importance as the description of anatomical structures involved in

the regulation of female sexual behavior, including female sexual desire, is the characterization of neurochemical and hormonal mediators that orchestrate the neural activity within and between these brain regions. In a recent review publication [35], Pfaus applies the concept of separate but interactive neural systems for behavioral excitation and inhibition of female sexual behavior (Figure 3) and stresses that behavior can commence either because of direct excitation, or through a process of disinhibition. Neurobiological mechanisms of this dual control model for sexual behavior include, on the excitatory side, the central dopamine (DA) and norepinephrine (NE) neurotransmitter systems, as well as the neuropeptides oxytocin (OT) and melanocortin (MC), and the steroid hormones estradiol (E2) and testosterone (T). DA facilitates sexual excitement via mesolimbic/mesocortical, nigrostriatal and incertohypothalamic pathways. The mesolimbic/mesocortical pathway sends projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc), amygdala and frontal cortex [35]. The medial prefrontal cortex (mPFC) is of particular interest as it is a key region implicated in executive control and behavioral inhibition [36, 37].

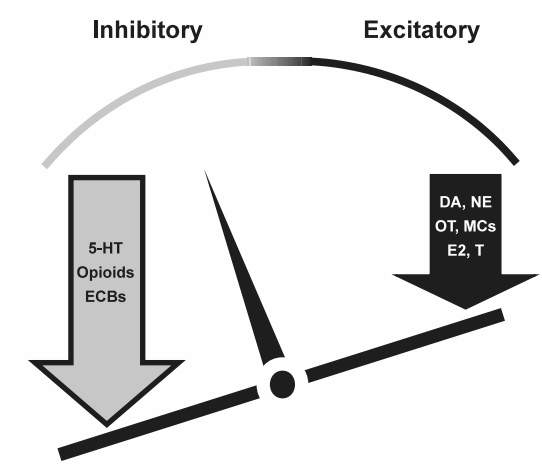


Figure 3. Excitatory-inhibitory model of female sexual behavior. Adapted from [35]. 5-HT, serotonin; ECBs, endogenous cannabinoids; DA, dopamine; NE, norepinephrine; OT, oxytocin; MCs, melanocortins; E2, estradiol; T, testosterone.

The nigrostriatal pathway sends projections from cell bodies in the substantia nigra to the striatum, while in the incertohypothalamic pathway, dopaminergic cell bodies in the zona incerta send projections to the mPOA. In estradiol and progesterone primed ovariectomized female rats, DA levels increase in the NAcc when the females are exposed to a sexually active male, and even more

during copulation. Copulation also elevates DA in the dorsal striatum [38]. DA antagonists generally inhibit proceptive, or appetitive, sexual behavior in female rats [39]. When D1 and D2 agonists are directly administered to the mPOA of estradiol primed ovariectomized rats, proceptive sexual behavior is selectively increased or decreased, depending on the specificity of the administered ligands to D1 or D2 receptors. This study suggests that it is the ratio of DA D1/D2 activity within the mPOA that influences the expression of proceptive sexual behavior [40].

The activation of the melanocortin receptors MC3R and MC4R in the brain increase female proceptive behavior in the rat, without affecting receptive (lordosis) behavior [41]. Peripheral administration of the MC3R and MC4R agonist bremelanotide induces c-fos expression in the mPOA and other hypothalamic and limbic brain regions. Direct infusion of bremelanotide into the mPOA facilitates proceptive, but not receptive, sexual behavior in the female rat, while administration to the VMH is without effect [42]. Subcutaneous administration of bremelanotide to ovariectomized and hormonally primed females also increases DA selectively in the mPOA, but not in the NAcc or VMH, suggesting that bremelanotide's effect on female sexual behavior is mediated by DA in the mPOA, a hypothesis that is supported by the observation that D1 antagonist administration to the mPOA reverses the effect of bremelanotide [Gelez H, Pfaus JG, personal communication].

The central serotonin (5-HT) neurotransmitter system is a key component in sexual inhibition, though there is evidence that activation of certain 5-HT receptor subtypes facilitates female sexual behavior [43-48]. The regulation of female sexual function by the serotonin neurotransmitter system is the main focus of this thesis, and a more detailed introduction to serotonin is presented in section 3.

Alongside the neural control of sexual function, steroid hormones play an important role in the regulation (in rodents) or modulation (in primates) of sexual behavior. Estrogens, progesterone and androgens have been recognized to be excitatory on sexual behavior in both humans and rodents [49], while glucocorticoids secreted in response to a stressful environment generally inhibit sexual behavior [49-52]. Thus, disorders of both ovarian function and the hypothalamic-pituitary-adrenal (HPA) axis, the latter responsible for the secretion of glucocorticoids from the adrenal glands, are associated with decreased sexual desire and arousal in women [53, 54]. In addition to direct endocrine effects on sexual behavior, steroid hormones modulate the central neurotransmitter systems involved in sexual function and may thus indirectly regulate sexual behavior.

3. THE ROLE OF SEROTONIN IN FEMALE SEXUAL FUNCTION

Pharmacological modulation of 5-HT neurotransmission is a common cause of diminished female sexual activity. Multiple clinical reports show that up to 75% of patients prescribed selective serotonin reuptake inhibitors (SSRIs) experience a treatment-induced detriment in sexual satisfaction, such as delayed or inhibited orgasm, or decreased sexual desire [55-60]. These studies provide evidence for the involvement of 5-HT in diminishing sexual behavior in women, however, without elucidating underlying brain circuitries as 5-HT modulates a wide range of neural, neuroendocrine and behavioral functions [61].

To date, there are 14 known 5-HT receptors, each of which exhibits considerable variation in brain distribution and physiological effects upon activation [61]. The 5-HT receptors are grouped in 7 families (5-HT1-7). Except for the 5-HT_3 receptor subtype, which is a ligand-gated ion channel, 5-HT receptors are G-protein coupled receptors. While serotonergic projections in the brain are diffuse, most of them originate from the raphé nuclei, a neuronal cluster located in the midbrain and the brain stem that contains the cell bodies of 5-HT neurons (Figure 4). A negative feedback mechanism regulates the

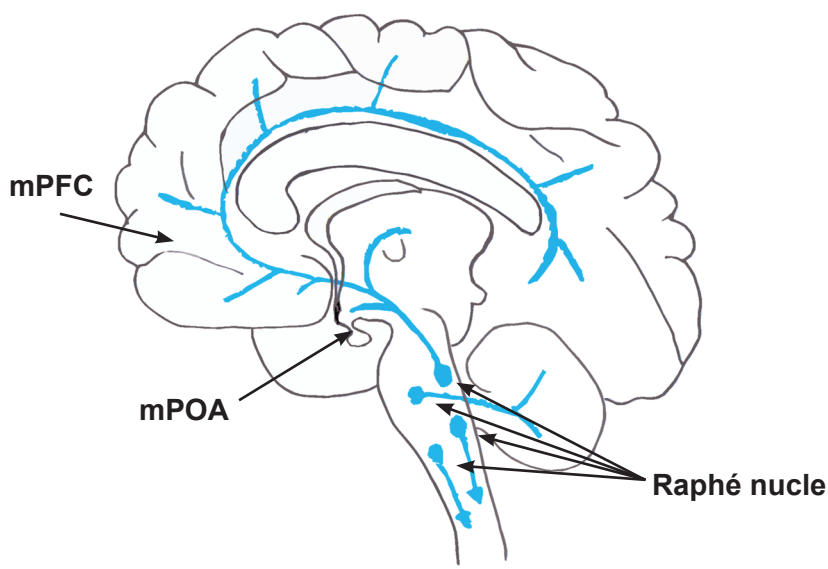


Figure 4. The central serotonin neurotransmitter system. Serotonergic neurons diffusely project from the Raphé nuclei to cortical, limbic and hypothalamic areas. mPFC, medial prefrontal cortex; mPOA, medial preoptic area. Adapted from [35].

activity of serotonergic neurons and acts mostly through presynaptically located autoreceptors of the 5-HT_{1A} and 5-HT_{1B} subtypes. Activation of 5-HT_{1A} receptors located on soma and dendrites opens potassium channels and thus inhibits 5-HT cell firing. Activation of 5-HT_{1B} receptors on the nerve terminals also inhibits 5-HT release. A negative feedback mechanism via postsynaptic 5-HT receptors might also play a role in regulating central 5-HT neurotransmission. This mechanism likely acts via the prefrontal cortex, where an activation of glutamatergic and GABAergic neurons that project back to the raphé nuclei leads to an inhibition of further 5-HT neural firing [62]. Serotonin transporters (5-HTT, or alternatively SERT) also partake in the negative feedback mechanism by actively transporting 5-HT from the synaptic cleft into presynaptic 5-HT neurons. The rapid removal of 5-HT released into the synaptic cleft by 5-HTT allows for the control of magnitude, duration and spatial distribution of signals reaching 5-HT receptors following neuronal stimulation [63].

Serotonin exerts a generally inhibitory tone on female sexual behavior. Studies in female rodents, however, have shown that 5-HT receptor specific agonists or antagonists 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-HT_3 receptor antagonism [43, 44, 47, 64], but is facilitated by 5-$HT_{2A/C}$ receptor activation [46, 65]. While the inhibitory effect of 5-HT_{1A} agonists on female receptivity is clear, less is known as to where in the brain these receptors 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 both pre- and post-synaptic 5-HT_{1A} receptor function in the serotonergic regulation of female sexual behavior, involving a circuitry that comprises the presynaptic raphé nuclei and the postsynaptic VMH, mPOA and PAG [44, 66-71]. The central 5-HT system furthermore interacts with the excitatory DA and NE neurotransmitter systems [72, 73], thus providing the possibility that a manipulation of 5-HT neurotransmission could affect sexual behavior both directly and indirectly by modulation of DA and NE.

The 5-HT system is also intertwined with endocrine systems. Estrogens and glucocorticoids modulate 5-HT receptor density in a receptor subtype and brain region specific manner in both rodents and primates [74-76]. Conversely, the central 5-HT system generally exerts a stimulatory function on HPA axis activity [77-79], and depletion of 5-HT reduces the responsiveness of the HPA axis to stress [80]. Liposits et al. [81] provide an anatomical context for the involvement of 5-HT in activating the HPA axis by showing that serotonergic nerve terminals and corticotropin-releasing hormone (CRH) neurons are

synaptically linked in the paraventricular hypothalamic nucleus (PVN). CRH containing neurons in the PVN that regulate HPA axis activity express 5-HT_{1A} and 5-HT_{2A} receptors [81, 82], and activation of either receptor type stimulates the HPA axis to release ACTH from the pituitary, and cortisol (in primates) or corticosterone (in rodents) from the adrenal cortex [82-85]. Thus, it seems plausible that sexual behavior could be indirectly modulated by 5-HT-regulated HPA axis activity and glucocorticoid secretion.

4. FLIBANSERIN – A NOVEL PHARMACOTHERAPEUTIC APPROACH FOR THE TREATMENT OF HYPOACTIVE SEXUAL DESIRE DISORDER IN WOMEN

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 [86, 87], demonstrates the ability to stimulate female sexual behavior in rats [88] and to improve sexual desire in women with either HSDD or major depressive disorder [89-91]. The positive actions of flibanserin on sexual functioning were first discovered serendipitously while studying flibanserin in patients with major depressive disorder. Flibanserin is ineffective as antidepressant, but increases the scores of women on the Arizona Sexual Experiences Scale [89]. These findings led to further clinical evaluations of flibanserin. In phase-III clinical trials designed to investigate the efficacy of flibanserin as first pharmacotherapeutic treatment of HSDD in women (the *BOUQUET* studies), premenopausal women with HSDD report increased satisfying sexual events, increased desire and decreased distress following chronic flibanserin treatment over several weeks [90, 91]. With exception of the experiments described in this thesis (Chapters 2-5), flibanserin has not been tested in any nonhuman primate.

Flibanserin crosses the blood-brain barrier by diffusion, and brain exposure is therefore expected to be similar to that in blood and peripheral organs. In rodents, flibanserin shows functional preference for cortical 5-HT_{1A} and 5-HT_{2A} receptors over other brain regions [92, 93]. While both flibanserin and 8-OH-DPAT, a prototypical 5-HT_{1A} agonist [94, 95], decrease neuronal firing in the prefrontal cortex (PFC), this decrease is eliminated when the neurons of the dorsal raphé nucleus (DRN) are lesioned in 8-OH-DPAT treated rats, but not in flibanserin treated rats [96]. These findings provide evidence that while 8-OH-DPAT exerts its action on neuronal firing by binding to presynaptic 5-HT_{1A} receptors (autoreceptors), flibanserin seems to activate a postsynaptic mechanism as all 5-HT_{1A} autoreceptors on serotonergic neurons in the DRN have been eliminated by the lesion. In human brain tissue, flibanserin

activates 5-HT_{1A} receptors in the PFC and hippocampus, but does not activate presynaptic 5-HT_{1A} receptors in the DRN [92], thus confirming the postsynaptic binding preference of flibanserin. While in rats flibanserin stimulates female sexual behavior [88], it is of particular interest that 8-OH-DPAT induces the opposite effect and contrasts with flibanserin with regard to female sexual behavior [43, 44, 47].

5. MODELING FEMALE SEXUAL BEHAVIOR IN ANIMALS

Much of our understanding of female sexual function has been gained from the study of animal models that display nonhuman counterparts to desire, arousal, orgasm and satiety [97]. Importantly, these aspects of female sexuality can be assessed in animals both qualitatively and quantitatively. For example, the female rat displays a distinct set of behaviors, referred to as proceptive hops and darts [98], to solicit copulation from the male (sexual proceptivity), while others, such as lordosis, signal her readiness for copulation (sexual receptivity). Sexual proceptivity and receptivity behaviors are flexible in regard to frequency and intensity and can be altered by pharmacological manipulation, a critical prerequisite for a rational approach to pharmacotherapy for female sexual dysfunction.

In animals, sexual desire can be inferred from certain appetitive behaviors that occur during copulation. The occurrence of such behaviors can be experimentally tested and pharmacologically manipulated. For example, female rats that show high motivation to press a lever to gain access to a male, or to run back and forth from behind an opaque barrier, release increased DA in the striatum and nucleus accumbens compared to females with low motivation (reviewed in [97]). The rate at which female rats solicit and pace their copulatory contact with males is another example of an analogous behavior of desire. Pharmacological manipulation of melanocortin and serotonin receptors has been demonstrated to be effective in altering this pacing behavior in female rodents [41, 88]. Furthermore, the willingness to engage in sexual intercourse, expressed as lordosis in female rodents, is mediated by estrogen and progesterone, and modulated by hypothalamic dopamine, norepinephrine, oxytocin and opioid receptor activation [98].

Sexual arousal, as mentioned above in Section 2, additionally involves induction of generalized CNS arousal and genital arousal. While generalized CNS arousal is regulated by the neurotransmitters DA, NE, 5-HT and acetylcholine [99], the genital response includes increased vaginal blood flow, clitoral, labial and vestibular bulb engorgement, and vaginal smooth muscle contraction and relaxation, which are under parasympathetic (cholinergic) and

sympathetic (adrenergic, cholinergic) control and also influenced by vasoactive intestinal polypeptide (VIP), nitric oxide synthase (NOS), neuropeptide Y (NPY), and other mediators (see [97] for a comprehensive review).

Value and importance of animal models to study female sexual function have been recognized, though a vast majority of the studies that aimed to investigate the animal counterparts of human sexual behavior have been performed in rodents. The translation of rodent data to human behavior, however, entails problems. Unlike in humans, certain aspects of female sexual function in rodents are under strict control of hormonal status. Lordosis, for example, is a highly estrogen-dependent behavior. Ovariectomy of the female rat abolishes the behavior, while estradiol and progesterone [100, 101] or estradiol and testosterone replacement [102] restore proceptive and receptive sexual behavior.

In humans, and in primates generally, sex hormones play an important role in the modulation of sexual behavior. They are however not essential [103], and sex in women is abolished neither by menopause nor by a surgical removal of the ovaries [104]. As outlined in Section 1, sexual behavior in women is strongly associated with physical and emotional factors, such as feelings of happiness and satisfaction, quality of their relationship with the partner and lifestyle, and it is influenced by sociocultural, family and personal expectations [2-10]. Sexual behavior in women thus entails the net output of an interplay between hormonal, neural and psychosocial factors and involves, on the neurobiological level, hypothalamic and limbic brain structures that are under close cortical control. In this respect, nonhuman primate models of female sexual function may be a valuable and sensible alternative to rodent models due to their emancipation of sexual behavior from strict hormonal control [103, 105] and their phylogenetic proximity to humans, including advanced corticalization [106, 107]. Considering the advantages of nonhuman primates as animal models for female sexual function and dysfunction, the common marmoset monkey *(Callithrix jacchus)* was chosen as model system for this thesis.

6. THE COMMON MARMOSET MONKEY: A SENSIBLE CHOICE

Figure 5. The common marmoset *(Callithrix jacchus)*. Adult marmosets form stable male-female pair-bonds that are strengthened by affiliative interactions, including allogrooming. Photo: Ivan Mikšík

All experiments presented in this thesis, designed to explore the role of the central 5-HT neurotransmitter system in the regulation of female sexual function, were conducted in the common marmoset monkey (Figure 5). Suitability of the common marmoset to study exogenous compounds regulating female sexual behavior has previously been demonstrated [108]. Unlike the multiple-mating social structures of rats and most nonhuman primates, such as macaques and baboons, marmoset sexual behavior most commonly occurs within stable male-female pairs [109, 110]. The frequency and pattern of sexual behavior can vary greatly between marmoset pairs, as does the quality of their social interactions, as manifest by allogrooming and aggressive behavior [111]. Similar to humans, several aspects in the repertoire of marmoset sexual behavior are controlled by both partners. During most stages of the species-specific sequence of sexual behaviors, including sexual solicitation, acceptance and rejection of the partner's sexual advances, and the execution and permission of an ejaculatory mount, both male and female partner have the ability to promote or to interrupt the sexual interaction. As a further parallel to human sexual physiology, but in contrast to some nonhuman primate [112-115] and many non-primate species [116, 117], the female marmoset's display of sexual

behavior is not limited to the periovulatory period. While marmoset sexual behavior increases across the first half of the ovarian cycle, peaking just prior to ovulation, it also occurs throughout the ovarian cycle, reinforcing the bond between partners [118]. Ovariectomy reduces, but does not abolish sexual behavior or the female's sexual attractiveness to the male [119]. Lesion of the anteromedial hypothalamus, which overlaps with the mPOA, blocks sexual proceptivity in female marmosets, but not sexual receptivity [120]. Finally, the availability of a well-characterized behavioral ethogram of marmoset sexual proceptivity and sexual receptivity [121, 122] permits validated quantification of marmoset sexual behavior.

Female marmosets display sexual proceptivity by rapid protrusion and retraction of the tongue (proceptive tongue flick) and stares directed at the male pairmate while their ear tufts are flattened (proceptive stare). Receptive sexual behaviors include female acceptance of male mounts and mount attempts (accept mount), frequently by pressing herself to the ground in a frozen position (receptive freeze posture, Figure 6), thus permitting the male to intromit his erect penis (erection, intromission), to which the female commonly responds with a head turn and nuzzling or biting of the male (receptive head turn). A successful mount is completed by male ejaculation within the vagina, which is normally followed by the male licking his penis (ejaculation). Grooming behavior between partners (allogrooming) is normally a sign of a stable and close pair-bond between male and female pairmates, while aggressive interactions signal disagreement and conflict between partners. Male marmosets sniff and lick the ano-genital region of the female pairmate (ano-genital sniff/lick), thus obtaining sensory information of the female's ovarian cycle status [123]. Ano-genital sniff/lick also signals sexual interest by the male pairmate (adapted from [121, 122]).

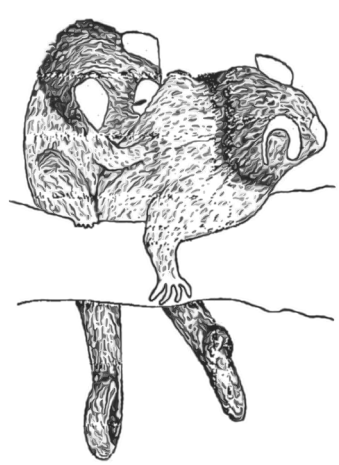


Figure 6. Marmoset copulatory behavior. The female partner (to the right) accpted the male partner's mount attempt. The male is in a mounting position, while the female's posture indicates a receptive freeze posture. Adapted from [121].

7. SCOPE OF THE THESIS

Objectives

The central theme of this thesis is the serotonergic regulation of female sexual behavior in a nonhuman primate that is characterized by a pairmate social setting comparable to humans. The experimental agents employed in the study are (1) flibanserin, a 5-HT1A agonist, 5-HT2A antagonist and putative pharmacotherapeutic treatment for hypoactive sexual desire disorder in women, and (2) 8-OH-DPAT, a prototype 5-HT1A agonist. In the thesis, flibanserin data are presented as they relate to two of the four Objectives key to this thesis (Objectives 1 and 2 in Chapters 2 and 3), while 8-OH-DPAT data are related to all four Objectives (Chapters 2 – 5).

The **First Objective** is to assess the effects of chronic flibanserin and 8-OH-DPAT on sexual and social interactions between female-male marmoset pairs. Based on the results from human clinical trials and rodent experimental data, we hypothesize that female marmoset sexual behavior is facilitated by flibanserin and diminished by 8-OH-DPAT.

The **Second Objective** is to test whether chronic flibanserin and 8-OH-DPAT alter the hypothalamic-pituitary-adrenal axis and thus indirectly modulate female sexual function through a suppressive endocrine mechanism. Central activation of 5-HT1A and 5-HT2A receptors stimulates the HPA axis. We consequently hypothesize that the 5-HT1A agonist 8-OH-DPAT increases HPA axis activity and thus suppresses female sexual behavior, while flibanserin, through concurrent inhibition of 5-HT2A receptors, displays a more moderate effect on HPA axis activity and therefore does not suppress female sexual behavior.

The **Third Objective** is to measure brain activity correlates of 8-OH-DPAT induced alterations of female sexual behavior and social interactions between pairmates. We hypothesize that chronic 8-OH-DPAT alters neural activity in brain regions with high 5-HT1A density, and in regions that have been associated with female sexual behavior: (1) dorsal raphé nucleus (DRN) with its serotonergic cell bodies, (2) medial prefrontal cortex (mPFC) as executive and inhibitory behavioral regulator, (3) medial preoptic area of the hypothalamus (mPOA) as a region essential for the generation of marmoset female sexual behavior, (4) ventromedial hypothalamic nucleus (VMH), a key regulator of female sexual receptivity, and (5) CA1 field of the hippocampus (CA1), involved in sexually relevant memory processes.

The **Fourth Objective** is to investigate the brain region-specific changes in gene expression induced by chronic 8-OH-DPAT to discover neural molecular mechanisms that may underlie suppressed female sexual behavior. We hypothesize that 8-OH-DPAT alters functional gene classes and pathways in

the same brain regions determined in objective 3: (1) DRN, (2) mPFC, (3) mPOA and (4) CA1. The VMH was not studied.

Experimental approach

Sixteen adult common marmoset females will be pair housed with male partners for 8-20 months before study onset. Eight of the 16 females will be assigned to test the effects of flibanserin, while the remaining 8 females will be assigned to test the effects of 8-OH-DPAT. Females will be housed with the same male partner for the entire study and will be ovariectomized and primed with either mid-follicular phase estradiol levels or no estradiol before study onset. Estradiol status will remain the same throughout the study for each individual animal, except for the final 16 weeks prior to brain tissue collection, when all females will be primed with estradiol to increase group size for the gene expression experiment. All in vivo experiments (behavior, Chapter 2; endocrine, Chapter 3; brain imaging, Chapter 4) will be conducted using a counterbalanced, cross-over design that will apply within-subject comparisons to measure the effect of chronic flibanserin and 8-OH-DPAT against respective vehicle treatment. Figure 7 shows a detailed visual representation of the complete study design. The transcriptomic experiment (Chapter 5) will employ a between-subject comparison to measure the effects of 8-OH-DPAT against vehicle treatment.

To investigate the effects of flibanserin and 8-OH-DPAT on pair behavior in **Chapter 2**, behavioral observations will be conducted using a validated experimental setup that encourages a standardized, modest baseline of sexual activity induced by a 90-minute separation of the pairmates prior

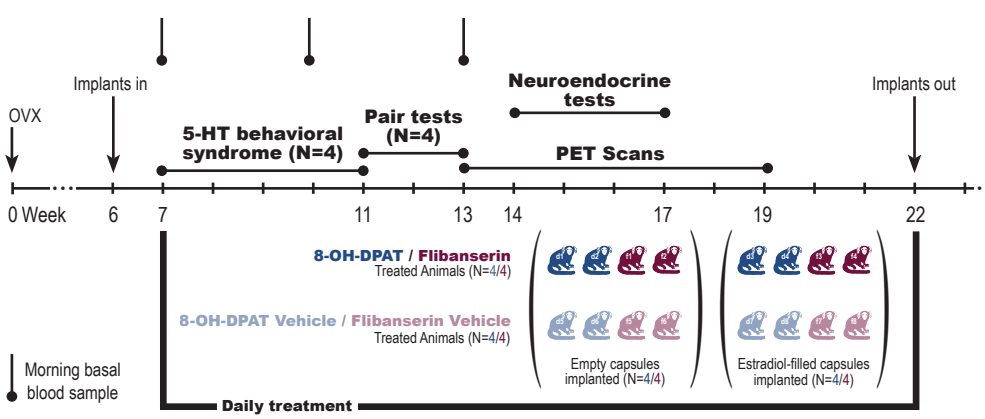
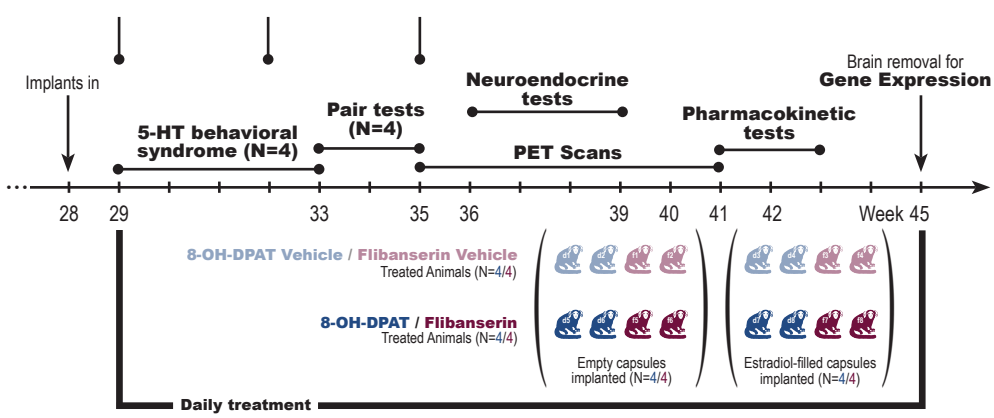


Figure 7. Study design. Drug treatment and estradiol replacement

to a 30-min pair test. In addition to sexual and social behavior observed during the pair tests, additional experiments described in this chapter will also explore behavioral responses to acute flibanserin and 8-OH-DPAT (serotonin behavioral syndrome), and the 24-hour pharmacokinetic profiles of circulating drug levels at 15 min, 30 min, 60 min, 3h, 6h and 24 h of drug administration during the chronic treatment phase.

Chapter 3 will assess female marmoset HPA axis properties following chronic treatment with flibanserin and 8-OH-DPAT. Baseline plasma cortisol levels will be measured before starting chronic treatments, and again after 3 and 6 weeks of daily treatment. Using a 5-HT1A agonist challenge test, it will be determined whether the responsiveness of the HPA axis to 5-HT1A activation will be affected by chronic 5-HT1A activation/5-HT2A inhibition (flibanserin), or by chronic 5-HT1A activation alone (8-OH-DPAT). To further test whether altered HPA axis function may contribute to changes in female interactions with male pairmates, a 30-minute restraint test will be administered to test the marmosets' stress responsiveness after chronic flibanserin and 8-OH-DPAT treatment. Timed blood samples will be drawn for all tests, and plasma ACTH and cortisol levels will be measured using radioimmunoassays.

To associate 8-OH-DPAT induced changes in pair behavior to brain function, a PET imaging experiment will be conducted in **Chapter 4**. Female marmosets will be infused with radiolabeled fluorodeoxyglucose (FDG) immediately prior to a pair test with their male pairmates. The majority of FDG uptake into brain tissue occurs within the first 30 minutes of infusion. The FDG signal will thus reflect glucose metabolism during pair behavior, with the most active brain regions trapping the highest concentration of radiolabeled FDG.



regimens are shown in relation to the timing of experimental events.

After pair test, the females will be anesthetized and imaged by PET. Whole brain normalized PET images will be analyzed with anatomically defined regions of interest derived from overlaid structural MRI images and scanned marmoset brain atlas images, and by whole brain voxelwise mapping. Finally, FDG signal differentials will be correlated with behavioral changes.

In a large-scale gene expression experiment described in **Chapter 5**, transcriptomic changes induced by chronic 8-OH-DPAT will be analyzed in brain tissues of 8-OH-DPAT and vehicle treated female marmosets. Due to great transcriptomic heterogeneity between brain regions, the mPFC, mPOA, CA1 and DRN will be excised using high-precision laser-microdissection. RNA will be isolated, amplified and hybridized to the marmoset-specific EUMAMA microarray. Real-time quantitative PCR (RT-qPCR) will be used to validate the microarray results, and a candidate gene approach will be employed to measure the expression of serotonin receptor and transporter genes by RT-qPCR. Enriched functional gene classes will be determined, possibly representing underlying molecular markers and mechanisms of suppressed female sexual function.

In **Chapter 6**, the combined experimental results from Chapters 2 – 5 will be considered and the abilities of flibanserin and 8-OH-DPAT to regulate female sexual function will be discussed in the context of pharmacological modes of action and future pharmacotherapeutic perspectives for the treatment of hypoactive sexual desire disorder in women.

REFERENCES

1. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA. 1999;281(6):537-44.
2. Tiefer L, Hall M, Tavris C. Beyond dysfunction: a new view of women's sexual problems. J Sex Marital Ther. 2002;28 Suppl 1:225-32.
3. Bancroft J, Loftus J, Long JS. Distress about sex: a national survey of women in heterosexual relationships. Arch Sex Behav. 2003;32(3):193-208.
4. Kolotkin RL, Binks M, Crosby RD, Østbye T, Gress RE, Adams TD. Obesity and sexual quality of life. Obesity (Silver Spring). 2006;14(3):472-9.
5. Hayes RD, Dennerstein L, Bennett CM, Sidat M, Gurrin LC, Fairley CK. Risk factors for female sexual dysfunction in the general population: exploring factors associated with low sexual function and sexual distress. J Sex Med. 2008;5(7):1681-93.
6. Both S, Laan E, Schultz WW. Disorders in sexual desire and sexual arousal in women, a 2010 state of the art. J Psychosom Obstet Gynaecol 2010;31(4):207-18.
7. Laan E, Both S. Sexual desire and arousal disorders in women. Adv Psychosom Med 2011;31:16-34.

8. Basson R. Using a different model for female sexual response to address women's problematic low sexual desire. J Sex Marital Ther. 2001;27(5):395-403.
9. Dennerstein L, Lehert P. Women's sexual functioning, lifestyle, mid-age, and menopause in 12 European countries. Menopause. 2004;11(6 Pt 2):778-85.
10. Marston C, King E. Factors that shape young people's sexual behaviour: a systematic review. Lancet. 2006;368(9547):1581-6.
11. Basson R. Pharmacotherapy for women's sexual dysfunction. Expert Opin Pharmacother. 2009 Jul;10(10):1631-48. Review.
12. Masters, WH, Johnson VE. Human Sexual Response. Toronto; New York: Bantam Books.
13. Bancroft J, Graham CA. The varied nature of women's sexuality: unresolved issues and a theoretical approach. Horm Behav 2011;59(5):717-29.
14. Meana M. Elucidating women's (hetero) sexual desire: definitional challenges and content expansion. J Sex Res 2010;47(2):104-22.
15. Basson R. Recent advances in women's sexual function and dysfunction. Menopause. 2004;11(6 Pt 2):714-25.
16. Sand M, Fisher WA. Women's endorsement of models of female sexual response: the Nurses' Sexuality Study. J Sex Med 2007;4:708-719.
17. Clayton AH. The pathophysiology of hypoactive sexual desire disorder in women. Int J Gynaecol Obstet 2010;110(1):7-11.
18. Clayton AH, Hamilton DV. Female sexual dysfunction. Psychiatr Clin North Am. 2010 Jun;33(2):323-38.
19. Graham CA. The DSM diagnostic criteria for female sexual arousal disorder. Arch Sex Behav 2010;39(2):240-55.
20. Simon JA, 2010, Postgrad Med 122(6):128-36
21. Brotto LA, 2010, Arch Sex Behav 39(2):221-39
22. Pfaff DW. Features of a hormone-driven defined neural circuit for a mammalian behavior. Principles illustrated, neuroendocrine syllogisms, and multiplicative steroid effects. Ann N Y Acad Sci. 1989;563:131-47.
23. Erskine MS. Mating-induced increases in FOS protein in preoptic area and medial amygdala of cycling female rats. Brain Res Bull. 1993;32(5):447-51.
24. Rowe DW, Erskine MS. c-Fos protooncogene activity induced by mating in the preoptic area, hypothalamus and amygdala in the female rat: role of afferent input via the pelvic nerve. Brain Res. 1993;621(1):25-34.
25. Pfaus JG, Kleopoulos SP, Mobbs CV, Gibbs RB, Pfaff DW. Sexual stimulation activates c-fos within estrogen-concentrating regions of the female rat forebrain. Brain Res. 1993;624(1-2):253-67.
26. Tetel MJ, Getzinger MJ, Blaustein JD. Fos expression in the rat brain following vaginal-cervical stimulation by mating and manual probing. J Neuroendocrinol. 1993;5(4):397-404.
27. Polston EK, Erskine MS. Patterns of induction of the immediate-early genes c-fos and egr-1 in the female rat brain following differential amounts of mating stimulation. Neuroendocrinology. 1995;62(4):370-84.

28. Coolen LM, Peters HJ, Veening JG. Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison. Brain Res. 1996;738(1):67-82.
29. Tetel MJ, Getzinger MJ, Blaustein JD. Estradiol and progesterone influence the response of ventromedial hypothalamic neurons to tactile stimuli associated with female reproduction. Brain Res. 1994;646(2):267-72.
30. Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol. 1990;294(1):76-95.
31. Holstege G, Huynh HK. Brain circuits for mating behavior in cats and brain activations and de-activations during sexual stimulation and ejaculation and orgasm in humans. Horm Behav. 2011;59(5):702-7.
32. Karama S, Lecours AR, Leroux JM, Bourgouin P, Beaudoin G, Joubert S, Beauregard M. Areas of brain activation in males and females during viewing of erotic film excerpts. Hum Brain Mapp. 2002;16(1):1-13.
33. Arnow BA, Millheiser L, Garrett A, Lake Polan M, Glover GH, Hill KR, Lightbody A, Watson C, Banner L, Smart T, Buchanan T, Desmond JE. Women with hypoactive sexual desire disorder compared to normal females: a functional magnetic resonance imaging study. Neuroscience. 2009 ;158(2):484-502.
34. Georgiadis JR, Kortekaas R, Kuipers R, Nieuwenburg A, Pruim J, Reinders AA, Holstege G. Regional cerebral blood flow changes associated with clitorally induced orgasm in healthy women. Eur J Neurosci. 2006;24(11):3305-16.
35. Pfaus JG. Pathways of sexual desire. J Sex Med. 2009;6(6):1506-33.
36. Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neurosci Biobehav Rev. 2004;28(7):771-84.
37. Kehagia AA, Murray GK, Robbins TW. Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. Curr Opin Neurobiol. 2010;20(2):199-204.
38. Pfaus JG, Damsma G, Wenkstern D, Fibiger HC. Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. Brain Res. 1995;693(1-2):21-30.
39. Grierson JP, James MD, Pearson JR, Wilson CA. The effect of selective D1 and D2 dopaminergic agents on sexual receptivity in the female rat. Neuropharmacology. 1988;27(2):181-9.
40. Graham MD, Pfaus JG. Differential regulation of female sexual behaviour by dopamine agonists in the medial preoptic area. Pharmacol Biochem Behav. 2010;97(2):284-92.
41. Pfaus JG, Shadiack A, Van Soest T, Tse M, Molinoff P. Selective facilitation of sexual solicitation in the female rat by a melanocortin receptor agonist. Proc Natl Acad Sci U S A. 2004;101(27):10201-4.
42. Pfaus J, Giuliano F, Gelez H. Bremelanotide: an overview of preclinical CNS effects on female sexual function. J Sex Med. 2007;4 Suppl 4:269-79.
43. Ahlenius S, Fernandez-Guasti A, Hjorth S, Larsson K. Suppression of lordosis

behavior by the putative 5-HT receptor agonist 8-OH-DPAT in the rat. Eur J Pharmacol 1986;124:361–3.

44. Uphouse L, Caldarola-Pastuszka M, Montanez S. Intracerebral actions of the 5-HT1A agonists, 8-OH-DPAT and buspirone and of the 5-HT1A partial agonist/antagonist, NAN-190, on female sexual behavior. Neuropharmacology 1992;31:969–81.
45. Maswood N, Caldarola-Pastuszka M, Uphouse L. 5-HT3 receptors in the ventromedial nucleus of the hypothalamus and female sexual behavior. Brain Res 1997;769:13–20.
46. Mendelson SD, Gorzalka BB. A facilitatory role for serotonin in the sexual behavior of the female rat. Pharmacol Biochem Behav 1985;22:1025–33.
47. Haensel SM, Mos J, Olivier B, Slob AK. Sex behavior of male and female Wistar rats affected by the serotonin agonist 8-OH-DPAT. Pharmacol Biochem Behav 1991;40(2):221-8.
48. Olivier B, Chan JS, Snoeren EM, Olivier JD, Veening JG, Vinkers CH, Waldinger MD, Oosting RS. Differences in sexual behaviour in male and female rodents: role of serotonin. Curr Top Behav Neurosci 2011;8:15-36.
49. Becker JB, Breedlove SM, Crews D, McCarthy MM. Behavioral Endocrinology 2nd ed, Cambridge: MIT Press, 2002.
50. De Catanzaro D, Gorzalka BB. Effects of dexamethasone, corticosterone, and ACTH on lordosis in ovariectomized and adrenalectomized-ovariectomized rats. Pharmacol Biochem Behav. 1980;12(2):201-6.
51. Sirinathsinghji DJ, Rees LH, Rivier J, Vale W. Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat. Nature. 1983;305(5931):232-5.
52. Wingfield JC, Sapolsky RM. Reproduction and resistance to stress: when and how. J Neuroendocrinol. 2003;15(8):711-24.
53. Starkman MN, Schteingart DE. Neuropsychiatric manifestations of patients with Cushing's syndrome. Relationship to cortisol and adrenocorticotropic hormone levels. Arch Intern Med. 1981;141(2):215-9.
54. Frohlich P, Meston C. Sexual functioning and self-reported depressive symptoms among college women. J Sex Res. 2002;39(4):321-5.
55. Patterson WM. Fluoxetine-induced sexual dysfunction. J Clin Psychiatry. 1993;54(2):71.
56. Segraves RT. Antidepressant-induced sexual dysfunction. J Clin Psychiatry. 1998;59 Suppl 4:48-54.
57. Rosen RC, Lane RM, Menza M. Effects of SSRIs on sexual function: a critical review. J Clin Psychopharmacol. 1999;19(1):67-85.
58. Kennedy SH, Eisfeld BS, Dickens SE, Bacchiochi JR, Bagby RM. Antidepressant-induced sexual dysfunction during treatment with moclobemide, paroxetine, sertraline, and venlafaxine. J Clin Psychiatry. 2000;61(4):276-81.
59. Clayton AH, Pradko JF, Croft HA, Montano CB, Leadbetter RA, Bolden-Watson C, Bass KI, Donahue RM, Jamerson BD, Metz A. Prevalence of sexual dysfunction among newer antidepressants. J Clin Psychiatry. 2002;63(4):357-66.

60. Clayton A, Keller A, McGarvey EL. Burden of phase-specific sexual dysfunction with SSRIs. J Affect Disord. 2006;91(1):27-32.
61. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999 Aug;38(8):1083-152.
62. Sharp T, Boothman L, Raley J, Quérée P. Important messages in the 'post': recent discoveries in 5-HT neurone feedback control. Trends Pharmacol Sci. 2007;28(12):629-36.
63. Murphy DL, Lerner A, Rudnick G, Lesch KP. Serotonin transporter: gene, genetic disorders, and pharmacogenetics. Mol Interv. 2004;4(2):109-23.
64. Hebert TJ, Menard CS, Dohanich GP. Inhibition of lordosis in female hamsters and rats by 8-OH-DPAT treatment. Physiol Behav. 1995;57(3):523-7.
65. Wilson CA, Hunter AJ. Progesterone stimulates sexual behaviour in female rats by increasing 5-HT activity on 5-HT2 receptors. Brain Res. 1985;333(2):223-9.
66. Powers B, Valenstein ES. Sexual receptivity: facilitation by medial preoptic lesions in female rats. Science. 1972;175(4025):1003-5.
67. Rajendren G, Dudley CA, Moss RL. Role of the ventromedial nucleus of hypothalamus in the male-induced enhancement of lordosis in female rats. Physiol Behav. 1991;50(4):705-10.
68. Uphouse L, Caldarola-Pastuszka M, Moore N. Inhibitory effects of the 5-HT1A agonists, 5-hydroxy- and 5-methoxy-(3-di-n-propylamino)chroman, on female lordosis behavior. Neuropharmacology. 1993;32(7):641-51.
69. Uphouse L, Caldarola-Pastuszka M. Female sexual behavior following intracerebral infusion of the 5-HT1A agonist, 8-OH-DPAT, into the medial preoptic area. Brain Res. 1993;601(1-2):203-8.
70. Uphouse L, Maswood S, Caldarola-Pastuszka M. Agonist activation of 5-HT1A receptors in the median raphe nucleus and female rat lordosis behavior. Brain Res. 1994;668(1-2):271-5.
71. Kakeyama M, Negishi M, Yamanouchi K. Facilitatory effect of ventral cut of dorsal raphe nucleus on lordosis in female rats. Endocr J. 1997;44(4):589-93.
72. Di Giovanni G, Esposito E, Di Matteo V. Role of serotonin in central dopamine dysfunction. CNS Neurosci Ther. 2010;16(3):179-94.
73. Allers KA, Dremencov E, Ceci A, Flik G, Ferger B, Cremers TI, Ittrich C, Sommer B. Acute and repeated flibanserin administration in female rats modulates monoamines differentially across brain areas: a microdialysis study. J Sex Med. 2010;7(5):1757-67
74. De Kloet ER, Sybesma H, Reul HM. Selective control by corticosterone of serotonin1 receptor capacity in raphe-hippocampal system. Neuroendocrinology. 1986;42(6):513-21.
75. Sumner BE, Fink G. The density of 5-hydoxytryptamine2A receptors in forebrain is increased at pro-oestrus in intact female rats. Neurosci Lett. 1997;234(1):7-10.
76. Lu NZ, Bethea CL. Ovarian steroid regulation of 5-HT1A receptor binding and G protein activation in female monkeys. Neuropsychopharmacology. 2002;27(1):12-24.

77. Feldman S., Conforti N., and Melamed E, 1987. Paraventricular nucleus serotonin mediates neurally stimulated adrenocortical secretion. Brain Res Bull. 1987;18:165-8.
78. Dinan TG. Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. Life Sciences. 1996;58:1683-1694.
79. Jorgensen HS. Studies on the neuroendocrine role of serotonin. Dan Med Bull. 2007;54;266-88.
80. Feldman S, Weidenfeld J. The excitatory effects of the amygdala on hypothalamo-pituitary-adrenocortical responses are mediated by hypothalamic norepinephrine, serotonin, and CRF-41. Brain Res Bull. 1998;45(4):389-93.
81. Liposits Z, Phelix C, Paull WK. Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. A light and electron microscopic immunocytochemical study. Histochemistry. 1987;86(6):541-9.
82. Zhang Y, Damjanoska KJ, Carrasco GA, Dudas B, D'Souza DN, Tetzlaff J, Garcia F, Hanley NR, Scripathirathan K, Petersen BR, Gray TS, Battaglia G, Muma NA, Van de Kar LD. Evidence that 5-HT2A receptors in the hypothalamic paraventricular nucleus mediate neuroendocrine responses to (-)DOI. J Neurosci. 2002;22(21):9635-42.
83. Rittenhouse PA, Bakkum EA, Levy AD, Li Q, Carnes M, van de Kar LD. Evidence that ACTH secretion is regulated by serotonin2A/2C (5-HT2A/2C) receptors. J Pharmacol Exp Ther. 1994;271(3):1647-55.
84. Van de Kar LD, Javed A, Zhang Y, Serres F, Raap DK, Gray TS. 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. J Neurosci. 2012;21(10):3572-9.
85. Osei-Owusu P, James A, Crane J, Scrogin KE. 5-Hydroxytryptamine 1A receptors in the paraventricular nucleus of the hypothalamus mediate oxytocin and adrenocorticotropin hormone release and some behavioral components of the serotonin syndrome. J Pharmacol Exp Ther. 2005;313(3):1324-30.
86. Borsini F, Giraldo E, Monferini E, Antonini G, Parenti M, Bietti G, Donetti A. BIMT 17, a 5-HT2A receptor antagonist and 5-HT1A receptor full agonist in rat cerebral cortex. Naunyn Schmiedebergs Arch Pharmacol. 1995;352:276-82.
87. Borsini F, Evans K, Jason K, Rohde F, Alexander B, Pollentier S. Pharmacology of flibanserin. CNS Drug Rev. 2002;8:117-42.
88. Gelez H, Allers K, Sommer B, Giuliano F. Chronic Flibanserin treatment increases solicitations in the female rat. J Sex Med. 2010;7 (Suppl. 3):118.
89. Kennedy S. Flibanserin: Initial evidence of efficacy on sexual dysfunction, in patients with major depressive disorder. J Sex Med. 2010;7:3449-59.
90. Derogatis LR, Komer L, Katz M, Moreau M, Kimura T, Garcia Jr M, Wunderlich G, Pyke R. Treatment of Hypoactive Sexual Desire Disorder in premenopausal 22 women: Efficacy of flibanserin in the VIOLET study. J Sex Med. 2012;9(4):1074-85

91. Thorp J, Simon J, Dattani D, Taylor L, Kimura T, Garcia Jr M, Lesko L, Pyke R. Treatment of Hypoactive Sexual Desire Disorder in premenopausal women: Efficacy of flibanserin in the DAISY study. J Sex Med. 2012;9(3):793-804.
92. Marazziti D, Palego L, Giromella A, Mazzoni MR, Borsini F, Mayer N, Naccarato AG, Lucacchini A, Cassano GB. Region-dependent effects of flibanserin and buspirone on adenylyl cyclase activity in the human brain. Int J Neuropsychopharmacol 2002;5:131–40.
93. Scandroglio A, Monferini E, Borsini F. Ex vivo binding of flibanserin to serotonin 5-HT1A and 5-HT2A receptors. Pharmacol Res 2001;43:179–83.
94. Arvidsson LE, Hacksell U, Nilsson JL, Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikstrom H. 8-Hydroxy-2- (di-n-propylamino)tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. J Med Chem 1981;24: 921–3.
95. Middlemiss DN, Fozard JR. 8-Hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT1 recognition site. Eur J Pharmacol. 1983;90(1):151-3.
96. Borsini F, Ceci A, Bietti G, Donetti A. BIMT 17, a 5-HT1A receptor agonist/5-HT2A receptor antagonist, directly activates postsynaptic 5-HT inhibitory responses in the rat cerebral cortex. Naunyn Schmiedebergs Arch Pharmacol. 1995;352:283–90.
97. Giraldi A, Marson L, Nappi R, Pfaus J, Traish AM, Vardi Y, Goldstein I. Physiology of female sexual function: animal models. J Sex Med. 2004;1(3):237-53.
98. Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. Horm Behav. 1976;7(1):105-38.
99. Pfaff D, Ribeiro A, Matthews J, Kow LM. Concepts and mechanisms of generalized central nervous system arousal. Ann N Y Acad Sci 2008;1129:11-25.
100. Bolling JL, Blandau RJ. The estrogen-progesterone induction of mating responses in the spayed female rat. Endocrinology 1939;25:359–64.
101. Pfaff DW. Estrogens and brain function. New York: Springer; 1980.
102. Jones SL, Pfaus JG. Sexual behavior in ovariectomized Wistar rats following varied doses of testosterone with or without estrogen, and the effects of repeated exposure to testosterone. Meeting of the Canadian Association for Neuroscience, Montréal, QC. 2008.
103. Wallen K. Desire and ability: hormones and the regulation of female sexual behavior. Neurosci Biobehav Rev. 1990;14(2):233-41.
104. Dennerstein L, Koochaki P, Barton I, Graziottin A. Hypoactive sexual desire disorder in menopausal women: a survey of Western European women. J Sex Med. 2006;3(2):212-22.
105. Wallen K. Sex and context: hormones and primate sexual motivation. Horm Behav. 2001;40(2):339-57.
106. Stephan H, Evolution of Primate Brains: A comparative anatomical investigation; in The Functional and evolutionary biology of primates, edited by Tuttle R, 3rd edition, 2009, ISBN: 978-0-202-36139-0
107. Hofman MA, Neuronal correlates of corticalization in mammals: A theory, J Theor Biol, 1985.
108. Barnett DK, Bunnell TM, Millar RP, Ab-

bott DH. Gonadotropin-releasing hormone II stimulates female sexual behavior in marmoset monkeys. Endocrinology. 2006;147(1):615-23.

109. Evans S, Poole TB. Long-term changes and maintenance of the pair-bond in common marmosets, Callithrix jacchus jacchus. Folia Primatol. 1984;42:33–41.
110. Abbott DH, Barnett DK, Colman RJ, Yamamoto ME, Schultz-Darken NJ. Aspects of common marmoset basic biology and life history important for biomedical research. Comp Med. 2003;53(4):339-50.
111. Dixson AF, Lunn SF. Post-partum changes in hormones and sexual behaviour in captive groups of marmosets (Callithrix jacchus). Physiol Behav. 1987;41(6):577-83.
112. Chalmers NR, Rowell TE. Behaviour and female reproductive cycles in a captive group of Mangabeys. Folia Primatol (Basel). 1971;14(1):1-14.
113. Wilson MI. Characterization of the oestrous cycle and mating season of squirrel monkeys from copulatory behaviour. J Reprod Fertil. 1977;51(1):57-63.
114. Nadler RD. Sexual behavior of captive orangutans. Arch Sex Behav. 1977;6(6):457-75.
115. Van Horn RN and Eaton GG, In: The Study of Prosimian Behavior, ed. by Doyle GA and Martin RD, New York: Academic Press, 1979, pp. 79-122.
116. Feder HH, In: Handbook of Sexology, vol. 2, Genetics, Hormones and Behaviour, ed. by Money J and Mustaph H, New York: Elsevier, 1978, pp. 393-411.
117. Lisk, RD, In: Biological Determinants of Sexual Behaviour, ed. by Hutchison JB, Chichester: John Wiley, 1978, pp. 425-466.
118. Kendrick KM, Dixson AF. The effect of the ovarian cycle on the sexual behaviour of the common marmoset (Callithrix jacchus). Physiol Behav. 1983;30(5):735-42.
119. Kendrick KM, Dixson AF. Ovariectomy does not abolish proceptive behaviour cyclicity in the common marmoset (Callithrix jacchus). J Endocrinol. 1984;101(2):155-62.
120. Kendrick KM, Dixson AF. Anteromedial hypothalamic lesions block proceptivity but not receptivity in the female common marmoset (Callithrix jacchus). Brain Res. 1986;375(2):221-9.
121. Stevenson MF, Poole TB. An ethogram of the common marmoset (Calithrix jacchus jacchus): general behavioural repertoire. Anim Behav. 1976;24(2):428-51.
122. Dixson AF, In: Rylands AB, ed. Marmoset, tamarins: systematic, behavior and ecology. Oxford: Oxford University Press: 164-175, 1993.
123. Smith TE, Abbott DH. Behavioral discrimination between circumgenital odor from peri-ovulatory dominant and anovulatory female common marmosets (Callithrix jacchus). Am J Primatol 1998;46(4):265-84.