In search for animal models of female sexual dysfunction

Eelke Snoeren

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In search for animal models of female sexual dysfunction

Op zoek naar diermodellen voor vrouwelijke seksuele disfunctie

(met een samenvatting in het Nederlands)

Proefschrift

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door

Eelke Mirthe Simone Snoeren
geboren op 24 juli 1983 te Oirschot

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Table of Contents

General introduction

1

Eelke Snoeren

Introduction

This thesis focuses on the development of an animal model for female sexual dysfunction (FSD). FSD is a disorder that affects between 33-48% of the population in the USA and in Europe.[1-3] According to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV),[4] FSD can be divided in four main categories: low sexual desire, low arousal, orgasmic disorders, and sexual pain. Each is defined as "persistent or recurrent" and causes "marked distress or interpersonal difficulty". The majority of sexual dysfunction surveys identify low sexual desire/interest and sexual arousal disorder as the most common problems in women.[5, 6] These sexual disturbances can have multiple causes, like low hormonal levels,[7, 8] different diseases (anxiety, depression), but also medication. Antidepressants (SSRIs), for instance, can cause sexual dysfunctions in men and women.[9-11] Although a large population of women suffers from FSD, the disorder is not well studied, in particular in comparison with male sexual dysfunction.
Research with patients can have ethical and practical limitations, and therefore, animal models are required for neuromolecular, neuroanatomical and psychopharmacological research into female sexual behavior and its pathology.

Method

Throughout this thesis female sexual behavior was measured in a paced mating setup. The test was performed in a cage with two compartments divided by a sheet with three little holes (4 cm diameter). One compartment was empty and one room contained a sexually active male rat. The female could choose between the compartments because of her smaller size than the male. The most important factor in this paced mating sex test is the possibility of the female rat to control her sexual behavior. In female sexual behavior research, it is important to use a paced mating set up, because non-paced mating is not rewarding.[12, 13] As soon as the female is able to pace her interactions, conditioned place preference is shown.[12] What the exact component of paced mating is that is rewarding is unclear.
By creating the right environment for a female rat to have rewarding sexual experiences, the outcome of sex experiments will not be influenced anymore by aversive environmental cues, and thus it enables the measurement of the real female's sexual functioning.

Aim of the thesis

The goal of this thesis was the development of animal models of FSD. Pharmacological studies were necessary to test the developed animal models for their usefulness in future research.
In **chapter 2**, a review about the existing literature regarding the role of 5-HT_{1A} receptors in sexual behavior is presented. The 5-HT_{1A} receptor was chosen as central topic for this review because of its important role in the regulation of sexual behavior. Both, human and animal studies are discussed here to give a broad overview of male and female sexual behavior.

In **chapter 3,** we have investigated whether stable differences (endophenotypes) in sexual behavior exist between individual female rats. Female rats with low sexual behavior may model women suffering from hyposexual desire or arousal disorders. In the same chapter, the effects of some drugs acting on the 5-HT system or the dopaminergic system are presented.
In **chapter 4,** we used hormonally sub-primed rats as a model for FSD. Female rats were ovariectomized and manually primed with estradiol to develop a rat with lower levels of sexual functioning. To validate this model, the effects of testosterone and vardenafil (PDE-5 inhibitor) alone and in combination were tested.

Chronic SSRIs (selective serotonin reuptake inhibitors) are used for the treatment of depression and their use may affect sexual functioning in women. However it is unclear whether the sexual complaint under SSRI users is the result of the drug use or one of the symptoms of depression.
In **chapter 5,** we used the serotonin transporter (SERT) knockout rats to investigate the importance of the SERT in the female sexual behavior. Furthermore, we studied the function of 5-HT_{1A} receptors in these females.
Surprisingly, there was no effect of the absence of SERTs on sexual activity in female rats. Maybe, there are adaptive changes in these animals, such as desensitization of the 5-HT_{1A} receptors, that prevents the development of sexual dysfunctions.
Therefore, we tested, in **chapter 6,** the effects of chronic paroxetine (SSRI) treatment in ovariectomized female rats. We used both sub-primed and fully-primed females and injected the paroxetine for in total 56 days. After this period of chronic injections, we also screened the females for 5-HT_{1A} receptor desensitization.
In the last part, **chapter 7,** all preceding chapters are summarized, integrated and discussed. Also the perspectives for future research are presented.

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Serotonin 1A receptors and sexual behavior: a review

2

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Ronald Oosting

Abstract

Serotonin plays an important role in both male and female sexual behavior. Many studies are performed on the role of 5-HT_{1A} receptors in sexual behavior. Overall, it seems that 5-HT_{1A} receptors are not involved under normal circumstances, but become more important under conditions with elevated serotonin levels. 5-HT_{1A} receptor agonists facilitate sexual behavior in males, and inhibit female sexual activity. This seems quite conflicting, but could be due to the different elements of sexual behavior. There could be different mechanisms and subregions involved in the different elements of sexual behavior, like ejaculation and arousal behavior. These mechanisms might be similiar in males and females.

The aim of this review is to give a broad overview of many research performed on the role of 5-HT_{1A} receptors in sexual behavior. Both, human and animal studies will be discussed of male and female sexual behavior. This review may therefore contribute to future research and potential treatments for humans with sexual dysfunctions.

Introduction

In this review we will focus on research performed on the role of 5-HT_{1A} receptors in sexual behavior. Both, human and animal studies will be discussed to give a broad overview of male and female sexual behavior. This review may therefore contribute to future research and potential treatments for humans with sexual dysfunctions. Rats are mostly used as animal model to study sexual behavior. In rats, sexual behavior is an interaction between male and female rats in which they perform several kinds of behavioral movements to influence the opposite sex and achieve sexual excitement. The interplay starts with appetitive behavior, such as sniffing the anogenital regions to obtain pheromonal cues of sexual receptivity. During estrus, the female rat displays receptive behavior (lordosis) and a variety of complex solicitation behaviors which triggers copulatory behavior from the male. During lordosis, the female displays a hollow back and deflects her tail to one side allowing the male access to her vagina. Solicitation, or proceptive, behavior (hopping, darting and ear wiggling) is defined as species-typical behaviors that signal readiness to mate and to govern the timing of sexual stimulation received by the female. Thus, hopping, darting and ear wiggling have been used as an index of feminine sexual motivation.[2] Male rat sexual behavior consists of repeated intromissions and mounts followed by ejaculations (figure 1). Intromissions are characterized as mounts including pelvic thrusting. Ejaculation consists of two phases, emissions (secretion and movement of seminal fluids to the urethra) and expulsion (forceful ejection of urethral contents), which have been demonstrated to follow a highly synchronized series of events in humans[3] and in rats.[4] In rats, usually 10 to 20 intromissions are needed during a short period (ca. 2-10 minutes) to reach an ejaculation. After an ejaculation a post ejaculatory interval (PEI) of about 5 minutes is started, which can be described as the resting period preceding the

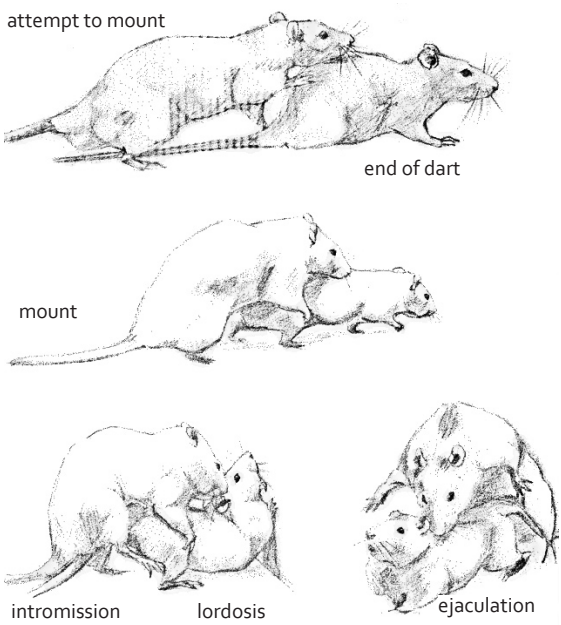

Figure 1: Sexual behavior male and female rats: mount, intromission, ejaculation, dart and lordosis are shown in drawings by P.J.A. Timmermans. Mount = jump on the female without pelvic thrusting; intromission = mount with pelvic thrusting; ejaculation = orgasm with sperm emission and expulsion; dart = a run through the cage with a sudden stop and the hind body down to allow the male to mount; lordosis = an arched back and deflection of her tail to one side allowing the male access to her vagina. Movie of all behaviors can be found on http://www.youtube.com/watch?v=HogcqAIb9wI

next ejaculation cycle (figure 2). During this period, the male rats produce ultrasonic vocalizations to scare away the female rat.
In addition, female's pacing is a function of solicitation behaviors. This is the ability of the female rat to control the timing of the receipt of sexual stimulation, as a pattern of approach and withdrawal from the male. The female rat can decide to go to the male and have sex or escape to a "safe" place where the male cannot reach her. (This will only be seen in experimental environments with escape possibilities) The display of this behavior is directly dependent upon the intensity of the coital stimulation (mounts, intromissions and ejaculations) received immediately prior to the solicitation behaviors. The rate of approaches toward the male is decreasing with the increasing intensity of the stimulus from the male.[5] During mating, the intermittent display of solicitation behaviors directly determines the type and timing of copulatory stimulation that the female receives. McClintock et al.[6] reported that 90% of intromissions were preceded by approach/runaway solicitations by the female, while only 3% of intromissions were generated when the male approached the female. The strong correlation between the amount of darts and male sexual behavior (mounts, intromissions and ejaculations) was also proven by our data.[7]
In animal research, several behavioral measurements are used as parameters for female and male sexual behavior. For the females, receptive behavior is represented by lordosis behavior, which can be quantified by using lordosis quotient or lordosis score. The lordosis quotient is the percentage of time the female exhibited lordosis in response to a sexual contact with the male rat. The lordosis score is the intensity of the lordosis responses (figure 3), scored on a 4-point scale (0-3; Hardy et al.[1]). Solicitation behavior is measured by the amount of hops and darts female rats perform in the presence of a sexual active male rat. Measurements of paced mating capacity are contact-return latencies and percentages of exits after stimuli. For males, the mount-, intromission- and ejaculation frequencies, latencies and the PEI are usually used as parameters to quantify male sexual behavior.[8] Ejaculation latency is usually defined as the time between the first intromission and ejaculation.

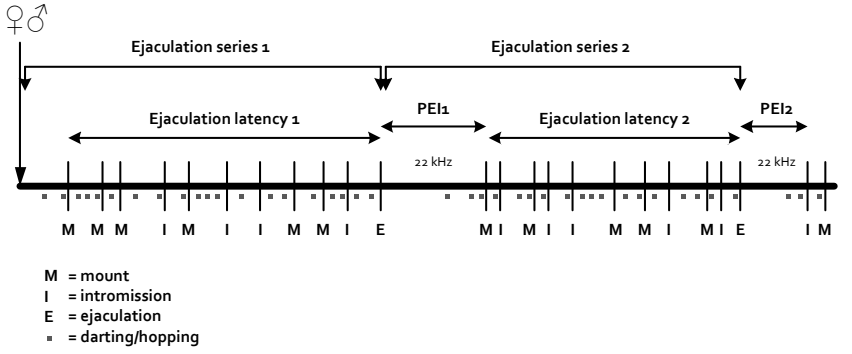

Figure 2: Sexual response cycle of male and female sexual behavior. There is a sequence of many ejaculation series within a 30 minutes period. Ejaculation latency is the time between the first male behavior and ejaculation within 1 series. PEI=post ejaculatory interval, which is the time to first male behavior after an ejaculation. Male rats emit ultrasonic vocalizations of 22 kHz during a post ejaculatory interval.

Brain regions, serotonin and sexual behavior

Sexual behavior is regulated by several brain areas. Studies with lesions, electrical stimulations, tract-tracing, and Fos-immunoreactivity (Fos-IR), performed in different species, give a nice overview of these functional regions. Here we will shortly discuss these brain areas involved in sexual functioning before we will focus on the serotonergic influence and especially the 5-HT_{1A} receptor.

Brain regions and male sexual behavior

Fos-IR quantifies the amount of immediate early gene c-fos and indentifies cells and extended circuits that become activated in response to various stimuli.[9] Fos-IR in the brain increases consistently in response to the different aspects of male sexual behavior, like anogenital investigations (chemosensory investigation of the female) and consummatory behaviors as mounts, intromissions, and ejaculations. Anogenital investigation by itself induces neural activity (by Fos-IR) in the posteromedial part of BNST (BNSTpm) and the posterodorsal part of medial amygdala (MeApd),[10, 11] brain areas that receive chemosensory signals processed through the accessory olfactory bulbs.[12] Consummatory behavior increases neural activity in the parvocellular subparafascicular nucleus (SPFp), a brain region that receives genital sensory inputs[13] and in turn projects to the medial preoptic nucleus (MPN) and posterior nucleus of the amygdala (PA).[10] In addition, differently located neurons in the BNSTpm and the lateral part of the MeApd are also activated following consummatory behavior compared to anogenital investigation, indicating that the MeApd and BNSTpm consist of functionally different subregions receiving either olfactory or genital sensory inputs.[10] The MPN receives both olfactory and genital sensory signals.[10, 14]

To distinguish between the different elements of copulatory behavior, Coolen et al.[15] demonstrated with 5-HT_{1A} receptor agonist ±8-OH-DPAT (a drug with stimulating effects on the number of ejaculations and decreasing the amount of mounts and intromissions necessary to achieve the ejaculation) that the lateral zone of the MeApd, specific rostral and caudoventral portions of the BNSTpm, posterodorsal preoptic nucleus (PD), and the medial portion of SPFp are activated by ejaculations alone. In contrast, brain areas related to mounts and intromissions are the MPN, medial MeApd and caudodorsal part of the BNSTpm.

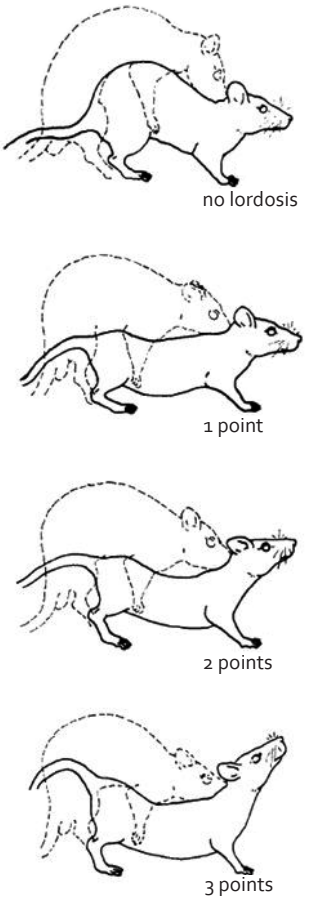

Figure 3: Lordosis score is the intensity of the lordosis responses scored on a 4-point scale (0-3; Hardy et al. 19711)

The Fos-IR data combined with lesion and tract-tracing studies, have led to the theory that the medial preoptic area (MPOA) integrates sensory information and genital stimulation to promote copulation and ejaculation.[14, 16-18] The sensory information enters from the vomeronasal organ, MeApd and BNSTpm, while genital stimulation enters the lumbosacral spinal cord to ascend from there towards the SPFp.[14, 16-18] After an ejaculation, areas like MeApd, BNSTpm, and SPFp are involved in the regulation of the post ejaculatory interval.[15, 19]
The SPFp is also an important factor in the regulation of sexual behavior, because it processes auditory signals through connections with the auditory cortex,[20] medial geniculate body,[21] inferior colliculus,[13] and other brainstem areas related to the auditory system[22] and may be involved in processing sensory cues via connections with the spinal cord,[13] amygdalo-striatal region[23] and hypothalamic regions, including the ventromedial nucleus (VMN),[21] paraventricular nucleus (PVN)[24] and MPOA.[16] The medial part of the SPFp is mostly involved in sexual behavior[25] and is connected to LSt neurons in spinal cord, MPN, MPOA, PD, and lateral part of nPGi. It appears that the medial SPFp conveys copulation-related information to these areas, which in turn provide feedback to the medial SPFp.[26] Furthermore, the SPFp receives inputs from the motor cortex that is involved in the control of locomotor patterns associated with ejaculation.[26]
Overall, these brain areas are a complex interconnected network that regulates sexual behavior. For the ejaculation there is a very important spinal ejaculation generator located lateral to the central canal in lamina X and in the medial portion of lamina VII of L_3 and L_4 of the lumbar spinal cord. These lumbar spinothalamic (LSt) neurons project to the medial SPFp and are specifically activated during ejaculation but not with other components of male sexual behavior.[27] Lesions of these neurons cause dramatic disruptions in ejaculatory behavior.[27]
The ejaculatory reflex is complex and involves multiple afferent and efferent systems. The afferent stimuli may involve sensory, visceral, propioceptive, and somatic inputs. It is possible that LSt cells receive stimuli related to onset of ejaculation and, in turn, trigger the ejaculatory reflex. The efferent site of the reflex involves a complex control of sympathetic and parasympathetic systems.[27]
In summary, ejaculation is a spinal reflex controlled by the spinal ejaculation generator which is modulated by sensory input from the pelvis and descending input from inhibitory and excitatory centers in the brainstem and the hypothalamus. Allard et al.[28] suggest that these supraspinal centers are controlled by cortico-limbic centers which are responsible for switching on the state of sexual excitement. During sexual intercourse, the cortico-limbic centers inhibit and activate the inhibitory and excitatory centers respectively, shifting the supraspinal tone to the spinal ejaculation generator from overall inhibitory to excitatory.

Brain regions and female sexual behavior

The activation of Fos-IR following copulatory behavior has also been studied in female rodents. Several studies have reported the induction of Fos-IR following mating in the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), medial amygdala (MeA), central tegmental field (CTF), ventromedial hypothalamic nucleus (VMN), and periaquaductal gray (PAG).[29-33] Just as in males, chemosensory investigation induced Fos-IR in the female BNSTpm,[34] in contrast to the MeApd

which is not activated in females,[33, 34] whereas it is activated in males. Vaginocervical stimulation by high numbers of intromissions or manual stimulations induced Fos-IR in the MPOA, BNST, SPFp, and MeA.[30, 33-35] If ejaculations were achieved, activation of Fos-IR in the MPN, BNSTpm, MeApd and SPFp were found.[30-32, 34] Furthermore, it was reported that vaginocervical stimulation, by intromissions and ejaculations of the male partner or by manual probing, was followed by a much stronger induction of Fos-IR than after lordosis behavior induced by flank stimulation.[30, 32, 33]

This suggests that males and females show comparable induction of Fos-IR after mating, but in females, additional brain areas are activated, including the ventrolateral part of VMN (VMNvl), the caudoventral part of VMN (VMNcv) and ventral premammillary nucleus (PMV).[34] Interestingly, most of the areas activated after mating (MPN, BNSTpm, PD, VMNcv and PMV) also showed Fos-IR after treatment with estrogen and progesterone,[34] possibly reflecting that the areas contain large amounts of estrogen receptors.[36]

The major site for regulation of lordosis behavior is the VMN. Lesions of this area dramatically reduce lordosis,[37] while electrical stimulation facilitated the expression of lordosis.[38] Coolen et al.[34] showed that lordosis behavior in response to mounts activates Fos-IR in the VMNvl. Intromissions, however, cause stronger induction of Fos-IR, although these were not accompanied by a higher expression of lordosis behavior. [30, 32] Therefore, it is suggested that the induction of Fos-IR in VMNvl is not solely a reflection of vaginocervical sensory stimulation and not a translation of motor activity related to lordosis behavior. The stronger staining intensity may reflect a stronger activation of the Fos-IR neurons, which possibly reflects some aspects of the internal motivational state of the female concerning the display of lordosis behavior, because repetitive mating enhanced lordosis behavior, suggesting an increase in motivation of the female to perform lordosis.[39]

In comparison to male rats, the SPFp in females receives direct and indirect inputs from the lumbosacral spinal cord,[13, 40] at levels that receive input from the pelvic nerve.[41, 42] This shows another similarity between male and female rats; the importance of the medial SPFp in collaboration with the spinal cord.

Overall, we can conclude that neural circuits underlying sexual behavior in males and females appear to be similar in terms of integration of sensory information. In males, however, the MPN may be regarded as an important brain region for the integration of sensory and hormonal stimulation leading to the onset of male sexual behavior. While in females, the VMNvl appears to be involved in the integration of stimuli leading to the onset of lordosis behavior.

The serotonergic system

Sexual behavior in both genders is affected by different hormones and neurotransmitters. Both from human and animal studies it is clear that 5-HT plays a very important role in sexual activity.

The serotonergic neurotransmitter system exists of the endogenous ligand, 5-hydroxytryptamine (5-HT, serotonin) and 14 structurally, functionally and pharmacologically distinct 5-HT receptor subtypes. The receptors can be subdivided into seven families, namely $5\text{-HT}_{1\text{-}7}$, with all different and limited distributions in the nervous system.[43] Except for the 5-HT_3 receptor subtype, which is a ligand-gated ion channel, 5-HT receptors are 7-transmembrane receptors and act via G-proteins.

The most important source of 5-HT neuronal pathways in the forebrain are the raphé nuclei (RN), which consist of dense clusters of 5-HT cell bodies. The serotonin neurons are regulated via a negative feedback mechanism.[44, 45] This feedback mechanism acts through different presynaptic 5-HT autoreceptors (5-HT_{1A} receptors, 5-HT_{1B} receptors and serotonin transporters (SERT)). The 5-HT_{1A} receptors, located on the soma and dendrites of 5-HT neurons, upon activation open potassium channels through G-protein coupling, which inhibit 5-HT cell firing. Activation of 5-HT_{1B} receptors, located on the nerve terminals, also inhibit 5-HT release. Furthermore, 5-HT_{1D} and 5-HT_{5A} autoreceptors are involved at the level of the soma, dendrites and nerve terminals (figure 4).[46-48]
Later, with the discovery that 5-HT_{2A} and 5-HT_{2C} receptors, which also inhibit 5-HT firing, the existence of negative feedback via postsynaptic heteroreceptors, not expressed by 5-HT neurons, was discovered.[49-51] Other postsynaptic 5-HT receptors that could be involved in 5-HT feedback mechanisms are 5-HT_{1A}, 5-HT_{1B}, 5-HT_4, and 5-HT_7 receptor subtypes. The mechanisms of the postsynaptic feedback systems are still unclear, but it might act via the prefrontal cortex where it activates glutamatergic and GABAergic neurons that project back to the raphé nucleus to inhibit 5-HT neural firing. (reviewed in Sharp et al.[51]) The last receptor involved in the feedback mechanism is the serotonin transporter (SERT) which is responsible for the active transport of serotonin into neurons. SERTs are situated in perisynaptic membranes of nerve terminals and in dendritic arbors of serotonin-containing cells in the midbrain and brain stem raphé nuclei. The role of SERTs are the mediation of rapid removal and recycling of released serotonin following neuronal stimulation, which means that it has a critical role in the homeostatic regulation of the magnitude, duration, and spatial distribution of signals reaching serotonin receptors.[52]

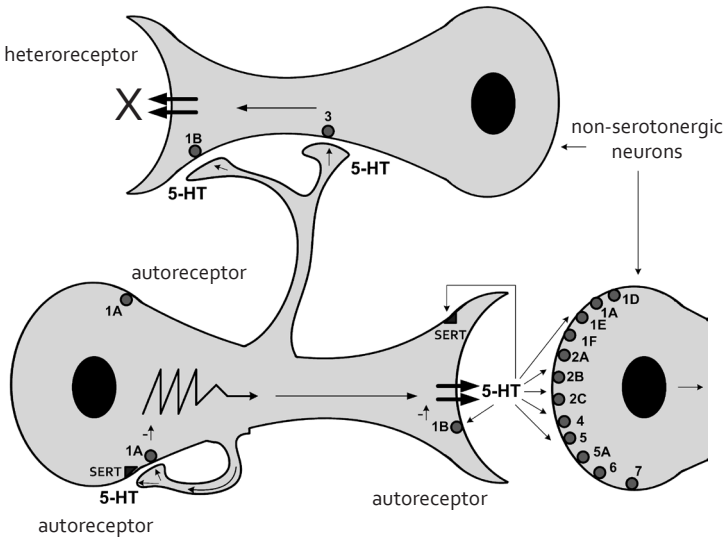

Figure 4: The serotonin (5-HT) system with the location of 5-HT 1A, 1B, 1D, 1E, 1F, 2A, 2B, 2C, 4, 5, 5A, 6, 7, and serotonin transporter (SERT) subtypes on the neurons and their effect on serotonin release.

The expression of Fos-IR can also be studied in response to psychoactive drugs in order to locate brain areas affected by them. Increased 5-HT neurotransmission induces Fos-IR in the cortex, BNSTdl, MPOA, PVNmp, PVNpm, VMN, dorsomedial hypothalamic nucleus, MeA, etc.[53-55]
The sensitivity of Fos-IR to both sexual behavior and serotonergic drugs can be used as tool to study the role of the serotonergic system in sexual behavior and the brain areas that are involved. Then, with psychoactive agents it is possible to investigate the inhibitory or facilitatory function of the systems and the specific receptor subtypes.
Serotonergic fibers have been found in all spinal cord areas containing sensory axons and motor neurons involved in ejaculation. They are present in the dorsal and ventral horns, dorsal commissural grey and thoracolumbar intermediolateral cell column (IML) and sacral parasympathetic nucleus (SPN) of the lumbosacral spinal cord.[56] In addition, serotonergic postsynaptic receptors have been found in the area where LSt cells are located.[57] This suggests a role of serotonin in ejaculation via these possible connections in the spinal cord. But these serotonergic connections are also found in supraspinal areas. In the nucleus paragigantocellularis (nPGI), an area in the ventrolateral medulla of the brainstem, serotonergic neurons are found to innervate the bulbospongiosus muscles involved in the inhibition of ejaculation.[57] The MPOA, which is discussed before, might lower the ejaculatory threshold by removing the tonic serotonergic inhibition exerted by the nPGI.[58-60] Another serotonergic innervation exists in the anterior lateral hypothalamic area (LHA). Lesions of the LHA strongly affect the occurence of ejaculations, but it does not affect mounts and intromissions,[61] showing the excitatory role of this brain region in the regulation of ejaculation. This effect is caused by serotonin, since serotonin is released in the anterior lateral hypothalamus (LHA) at the time of ejaculation[62] and injections of selective serotonin reuptake inhibitors into the LHA increased the latencies to mounts, intromissions and ejaculations.[62]

The main focus of interest of this review is the 5-HT_{1A} receptor. This receptor is one of the most extensively studied 5-HT receptors and seems to have a facilitatory effect on male sexual behavior, while it regulates an inhibition in female sexual behavior. Interestingly, it is suggested that 5-HT_{1A} receptors are not critical for the performance of sexual behavior when serotonin levels are normal, but become extremely important when the serotonin levels are elevated. Together, this makes the 5-HT_{1A} receptors an interesting subject to study sexual behavior.
5-HT_{1A} receptors are widely distributed throughout the brain. For example, the hippocampus contains a high density of 5-HT_1 receptors, most of which belong to the 5-HT_{1A} receptor subtype.[63] Other brain areas that are highly populated by 5-HT_{1A} receptors are the septal regions, MeA, raphé nuclei (particularly the dorsal raphé), neocortex, hypothalamus and substantia gelatinosa of the spinal cord.[63] Activation of 5-HT_{1A} autoreceptors in the dorsal and median raphé nuclei with the agonist (±)8-OH-DPAT decreases 5-HT levels in several brain areas like hippocampus, globus pallidus, frontal cortex, nucleus accumbens (NAc) and septum.[64] The two important subregions of the raphé nuclei, dorsal raphé nucleus (DRN) and median raphé nucleus (MRN), are important in serotonergic projections to forebrain areas, whereas the spinal cord and hindbrain, on the other hand, are mainly innervated by the caudal

group of the raphé nuclei which can be divided in raphé magnus nucleus, raphé obscures nucleus and raphé pallidus nucleus.[65, 66] The efferent connections of the dorsal and median raphé nuclei follow a pathway almost identical until the hypothalamic region, but the anatomical destination of their terminal projections presents clearly some specificity, which probably parallels some specific function in the central nervous system. The MRN fibers mainly distribute to midline/paramidline structures and innervate the cortex entorhinalis, the hippocampus, the neocortex and the mammillary bodies. DRN fibers, on the other hand, primarily project laterally to such sites as the substantia nigra pars compacta, amygdala, striatum, BNST, lateral preoptic area, substantia innominata, magnocellular preoptic nucleus, NAc etc.[67-70] Thereby, the ascending serotonergic system may be subdivided into a mesolimbic pathway originating from the MRN and a mesostriatal pathway projecting from the DRN. The DRN and MRN also project to the same areas, but it is generally the case that they project to different parts of a structure. (summarized in Vertes et al.[70]) (figure 5)

5-HT_{1A} RECEPTORS AND MALE SEXUAL BEHAVIOR

Rats

Overall, it is assumed that serotonergic activation inhibits male sexual activity. In rats, the 5-HT_{1A} receptor agonist (±)8-OH-DPAT and the active enantiomer (+)8-OH-DPAT decreases the number of mounts and intromissions needed to achieve ejaculations.[71-74] In addition, ejaculation latencies and post-ejaculatory intervals are reduced. Other 5-HT_{1A} receptor agonists have comparable effects as 8-OH-DPAT, but might slightly differ in the efficacy. The same effects were found for non-selective 5-HT_{1A} receptor agonists (buspirone,[75] LY-228,729,[76] and ipsapirone[77]) and selective 5-HT_{1A} receptor agonists (flesinoxan,[78-80] LY-293,284,[81] and indorenate[82]). Interestingly, flesinoxan does not affect the number of mounts and intromissions to the same amount as (±)8-OH-DPAT. The latencies to mounts, intromissions and ejaculation were significantly higher with flesinoxan. This effect was mainly seen in the first ejaculation series, and suggests that flesinoxan has prosexual effects on male sexual behavior without inducing premature ejaculations as (±)8-OH-DPAT.[80] Administration of FG5893, a 5-HT_{1A} receptor agonist and 5-HT_2 receptor antagonist, caused a decrease in the number of mounts and intromissions to ejaculation and a drop in ejaculation latency.[83] (The effects of the different compounds are summarized in table 1.)

The specific role of 5-HT_{1A} receptors in this facilitatory effect on male sexual activity is supported by 5-HT_{1A} receptor antagonists that selectively attenuate the effects of agonists. Different compounds have been studied; e.g. pindolol[83] (non-selective 5-HT_{1A} and β-receptor blocker), (S)-UH-301[71] (5-HT_{1A} receptor antagonist), NAD-299 (selective 5-HT_{1A} receptor antagonist)[73] and WAY-100635.[84]

Importantly, these 5-HT_{1A} receptor antagonists, by itself, have no intrinsic effects on male rat mount, intromission or ejaculatory behavior,[73, 84, 85] indicating that under normal basal circumstances 5-HT_{1A} receptors do not play a crucial role in sexual behavior.

However, 5-HT_{1A} receptors turn out to become important when activated by 5-HT_{1A} receptor agonists and under conditions with high extracellular 5-HT levels, such as

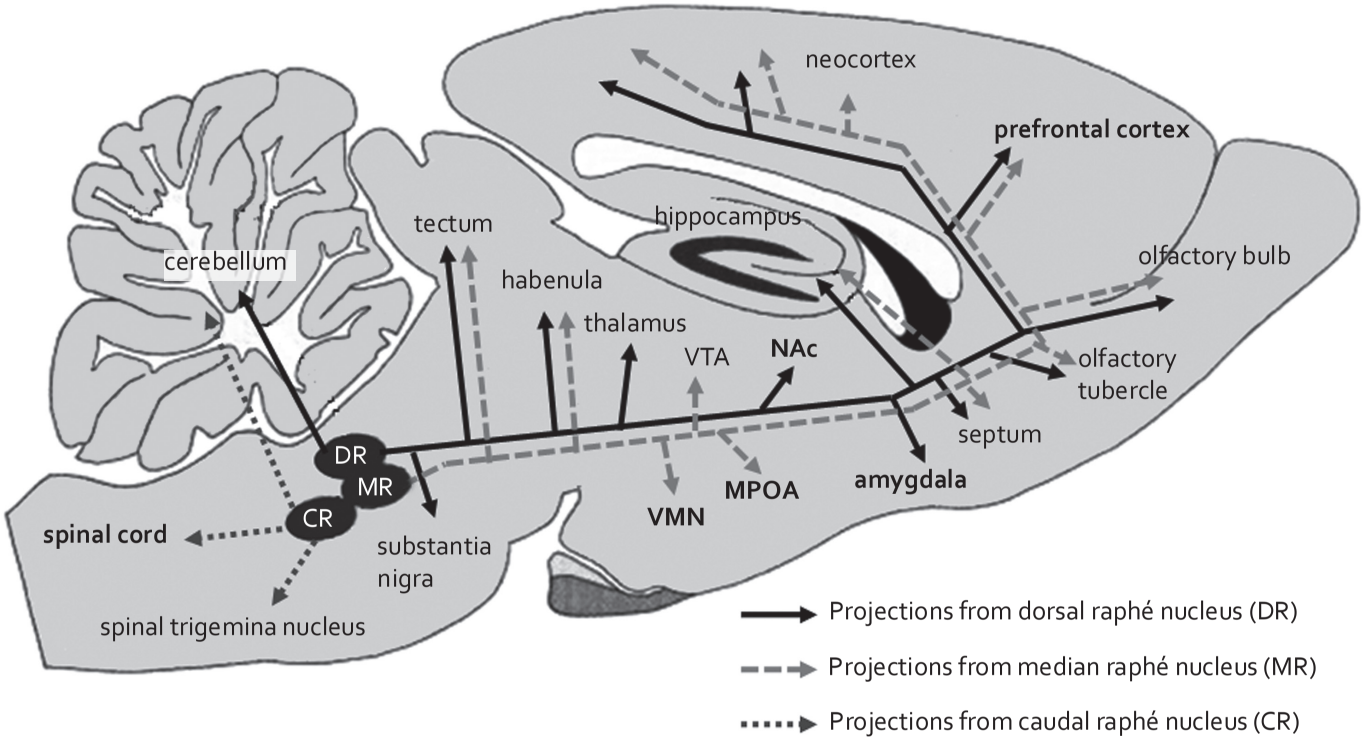

Figure 5: serotonin projections from the raphé nucleus to other brain areas in rats. In black the areas are shown that are involved in female and male sexual behavior (VMN=ventral medial nucleus of hypothalamus, MPOA= medial preoptic area, amygdale, nucleus accumbens and prefrontal cortex) and in grey the other projection areas.

induced by selective serotonin reuptake inhibitors (SSRIs). SSRIs prevent reuptake of 5-HT from the synaptic cleft into the presynaptic serotonergic neuron by blocking 5-HT transporters. This leads to elevated extracellular 5-HT levels which, in turn, stimulate autoreceptors and postsynaptic 5-HT receptors. Chronic SSRI treatment often causes sexual dysfunctions in men, like delayed ejaculation and the inability to ejaculate.[86] This problem is exacerbated by a combination treatment with 5-HT_{1A} receptor antagonist WAY-100635 that strongly enhances the increased ejaculatory threshold caused by both chronic and acute SSRIs.[85, 87] This could be mediated by the blockade of 5-HT_{1A} autoreceptors that normally limit the increase in 5-HT levels, or by blockade of postsynaptic 5-HT_{1A} receptors that lower the ejaculatory threshold. A comparable effect of WAY-100635 was seen in serotonin transporter (SERT) knockout rats; WAY-100635 caused a larger decrease in ejaculation frequencies in homozygous knockout than in wildtype rats.[88] In addition, a combination of increased 5-HT levels and blocked 5-HT_{1A} receptor functioning strongly inhibits ejaculation. Chronic SSRI treatment also reduced the facilitation of ejaculation induced by (±)8-OH-DPAT.[89] It is suggested that 5-HT_{1A} receptor desensitization is the underlying factor.[90, 91] These findings imply that 5-HT_{1A} receptor activation becomes increasingly important to reach the threshold to ejaculate when serotonin levels are elevated.

In conclusion, under normal circumstances serotonin levels are not high enough to induce an effect of 5-HT_{1A} receptors on male sexual behavior. If serotonin levels are elevated, the role of 5-HT_{1A} receptors becomes important. Therefore, one would suspect that acute SSRI treatment elevates serotonin levels and thereby have a pro-sexual effect. However the opposite effect is found, while co-treatment with 5-HT_{1A} receptor antagonists and a SSRI causes a strong inhibition of sexual behavior. This suggests that 5-HT neurons are not silent during sexual behavior, because then serotonin transporter blockers and 5-HT_{1A} receptor antagonists should not have an inhibiting effect. Therefore, it seems that there is some activity in the 5-HT neurons during sexual behavior. Lesioning of serotonin neurons increases sexual behavior.[92] This is probably due to the lack of serotonin inhibition on postsynaptic neurons.

5-HT_{1A} receptors, brain areas and sexual behavior

The question now is where the 5HT_{1A} receptors involved in various aspects of sexual behavior are localized. As mentioned before, many brain areas contain 5-HT_{1A} receptors and could therefore be involved in this mechanism. High levels of serotonin normally inhibit male sexual behavior[93] although activation of 5-HT_{1A} receptors facilitates sex. One possibility is that 5-HT_{1A} receptor agonists may act via their effect on inhibitory autoreceptors, which decrease 5-HT release.[94] In fact, local administration of (±)8-OH-DPAT into the median raphé nucleus (MRN) reduced ejaculation latencies and intromission frequencies,[95] although injection of (±)8-OH-DPAT in the dorsal raphé nucleus (DRN) failed to affect male sexual behavior.[95, 96] As mentioned before, this variation in effect could be due to the differences in projection areas of the DRN and MRN.

On the other hand, the facilitating effects can also be caused by activation of postsynaptic receptors in target areas involved in sexual behavior (figure 5). Intrathecal administration of (±)8-OH-DPAT,[97, 98] lisuride (dopamine and 5-HT_{1A} receptor partial agonist)[99] and buspirone[75] caused a reduction in ejaculation latency, intromission

frequency and intervals between copulatory behavior. Since there are no serotonergic cell bodies and no 5-HT_{1A} autoreceptors located in the spinal cord, spinal postsynaptic 5-HT_{1A} receptors probably mediate these effects. Contrarily, the effect of Intrathecal 5-HT_{1A} receptor agonists on post ejaculatory intervals (PEI) was very low, suggesting that this is not regulated at the level of the spinal cord.[75] This is in line with the suggestions from the introduction, that different subregions in the central nervous system have different roles in regulating sexual behavior. And thus, communication between the subregions is necessary to regulate sexual behavior.
Other target areas are also involved in male sexual behavior. Local administration of 5-HT_{1A} receptor agonists into nucleus accumbens (NAc),[95, 96] MPOA and medial amygdala (MeA)[100] all facilitate male sexual behavior. Local injection of (±)8-OH-DPAT into the nucleus accumbens[95, 96] reduced ejaculation latencies and number of intromissions prior to ejaculations. Microinjections of (±)8-OH-DPAT into the medial amygdala decrease the latency for intromission and ejaculation and shortens the duration of post ejaculatory intervals.[100] Local injections of (±)8-OH-DPAT in MPOA decrease ejaculation latencies[96] and disruption of presynaptic receptors (with the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT)) does not change these (±)8-OH-DPAT effects.[94] Overall, this suggests the role of 5-HT_{1A} receptors in different brain areas in male sexual behavior.

5-HT1A receptors, sexual behavior and monoamines
5-HT_{1A} receptor activation by systemic or local administration of 5-HT_{1A} receptor agonists leads to changes in dopamine and noradrenaline levels in various parts of the central nervous system.[101, 102] The question is to what extent the ejaculation stimulating effects of 5-HT_{1A} receptor activation are mediated by changes in other monoaminergic systems than serotonin.
Lorrain et al.[101] showed that systemic administration of (±)8-OH-DPAT decreases extracellular 5-HT in the MPOA. On the other hand, (±)8-OH-DPAT administration directly in the MPOA causes an increase in extracellular 5-HT in this brain area, indicating that 5-HT levels in the MPOA do not affect male sexual behavior. The stimulatory effects of (±)8-OHDPAT in the MPOA may be explained by its effect on extracellular dopamine in this brain area,[101, 103] which may indirectly act via 5-HT_{2A} receptors on dopaminergic neurons by disinhibiting dopamine release. The effects can be partially blocked by the dopamine D_2 receptor antagonist reclopride.[101, 104]
The hypothesis that serotonin is not very important in the MPOA is supported by findings that microinjections of a 5-HT reuptake blocker, in this brain area, does not affect any parameter of male rat sexual behavior.[62] Also the role of dopamine in the stimulating effect of 5-HT_{1A} receptors on male sexual behavior remains unclear. Haloperidol (dopamine D_2 receptor antagonist), for instance, induced no attenuation of the effects of (±)8-OH-DPAT,[105] but this might indicate that other subtypes of dopamine receptors are involved in this specific area.

Another monoaminergic mechanism that could be involved in 5-HT_{1A} receptors mediated effect on sexual behavior is the noradrenergic system. In 1986, Fernandez-Guasti et al.[102] already showed the possible interaction effects between serotonin and noradrenaline. The facilitating effects of 5-$HT_{1A/2}$ receptor agonist 5-MeODMT or lisuride (dopamine and 5-HT_{1A} receptor partial agonist) on male sexual behavior

(the ejaculatory threshold was reduced) was abolished by DSP4-induced noradrenaline depletion. Interestingly, 5-MeODMT also had an inhibitory effect on male sexual behavior, because it increased intromission and ejaculation latencies. This effect was not different in DSP4-treated males. More research is needed to clarify this possible interaction between noradrenaline and 5-HT_{1A} receptors.

Mice

Only a few studies describe the effect of 5-HT_{1A} receptor agonists on male sexual behavior in mice. In these studies, arousal tests were performed measuring the time male mice spend nearby a wall that separates the male from a receptive female mouse. Popova et al.[106] showed that (±)8-OH-DPAT caused a decrease in sexual motivation and arousal in response to the exposure of a female mouse. This effect was blocked by the 5-HT_{1A} receptor antagonist p-MPPI, which had no effect on its own.[106] Comparable results were found with other 5-HT_{1A} receptor agonists (flesinoxan and ipsapirone).[107] The ejaculation latency is increased in mice after (±)8-OH-DPAT administration.[108] This is interesting, because it is completely opposite to the effects on sexual behavior in male rat studies, suggesting species differences in the role of 5-HT_{1A} receptors in sexual behavior.

Other animals

The role of 5-HT_{1A} receptors has also been investigated in other species, like rabbits, dogs and ferrets. Interestingly, no effect of (±)8-OH-DPAT on sexual motivation in rabbits is seen, as with lisuride.[109] Because rabbits ejaculate upon every intromission, parameters like ejaculation latency and intromission frequency cannot be used. Also in ferrets no effect of low doses (±)8-OH-DPAT is seen on masculine sexual behavior.[110] Effects of 5-HT_{1A} receptors on sexual behavior could, therefore, depend on whether the species require several intromissions to ejaculate or if the male ejaculates after a single intromission.

Monkeys

Pomerantz et al.[111] studied the effects of 5-HT_{1A} receptor agonists in rhesus monkeys and showed that (±)8-OH-DPAT and ipsapirone lowered the ejaculatory threshold by reducing the ejaculation latency and intromission frequency. Interestingly, low doses, but not high doses, of (±)8-OH-DPAT facilitated male sexual behavior. But high doses of (±)8-OH-DPAT could cause a serotonergic syndrome, which might affect the ability to perform sexual behavior.

This again suggests differences between species. Another difference is the wider range in sexual behavior that is stimulated in rats compared to rhesus monkeys. In monkeys, 5-HT_{1A} receptor stimulation was limited to reduction in ejaculation latency and intromission frequency, while in rats 5-HT_{1A} receptor stimulation also reduced the post ejaculatory and intercopulatory intervals.

Human

As mentioned in the introduction, SSRIs are used as treatment for men with premature ejaculations (PE). After (sub) chronic administration, studies have shown that selective serotonin reuptake inhibitors cause an increase in extracellular serotonin levels at synaptic terminals. Activation of 5-HT_{1A} autoreceptors in the raphé

nuclei, in turn, inhibits 5-HT firing and, hence, 5-HT release in terminal synapses. Therefore, weak or delayed clinical SSRI effects might be due to feedback inhibition of 5-HT release. Human studies have shown that chronic SSRI use causes 5-HT_{1A} receptor desensitization.[112, 113] It is even proposed from rat studies that the receptor desensitization may be partly responsible for the delay in therapeutic effects of SSRIs on depression.[114, 115] It would be logical that co-administration of 5-HT_{1A} receptor antagonists would mimic these desensitization effects, and thereby accelerate the process. Studies with pindolol combined with paroxetine showed an increase in the intravaginal ejaculation latency time (IELT) in men with PE.[116] This suggests a role for 5-HT_{1A} receptors in ejaculation performance in men.
On the contrary, it would be logical that co-treatment with 5-HT_{1A} receptor agonists would reduce sexual side effects of SSRIs. This is, in fact, shown by co-administration of buspirone (partial 5-HT_{1A} receptor agonist) that improved SSRI-induced sexual dysfunctions.[117] This might occur by stimulating presynaptic autoreceptors that moderate 5-HT release. An old study of Othmer et al.[118] even showed beneficial effects of buspirone administration alone on sexual functioning in patients suffering generalized anxiety disorder. However, this study was limited to 10 patients.

Discussion

The experiments with 5-HT_{1A} receptor antagonists in rats show that under basal conditions, 5-HT_{1A} receptors have probably no important role in the regulation of male sexual behavior. The 5-HT_{1A} receptors become important under situations with chronically elevated serotonin levels. Under such conditions, desensitization of 5-HT_{1A} receptors and thereby decreases in sexual behavior become apparent. It seems that there is balance between facilitatory and inhibitory systems in the regulation of sexual behavior. It could be that during chronic elevated serotonin levels, the inhibitory systems become more active than the facilitatory systems.

Studies with 5-HT_{1A} receptor agonists administered in specific brain areas demonstrate that the stimulatory effects of these agonists on male sexual behavior are mediated by many different brain areas, including the MPOA, MeA and NAc (figure 3). In these brain areas, different aspects of male sexual behavior can be regulated in specific brain subregions.

An interesting observation is that the effects of 5-HT_{1A} receptor agonists on ejaculatory behavior are not universal, but species dependent. Systemic administration of (±)8-OH-DPAT facilitates ejaculation in rats, but it inhibits in mice, rabbits, dogs, and ferrets. A possible explanation for this difference could be different distribution of postsynaptic 5-HT_{1A} receptors in the brain and spinal cord areas, because 5-HT_{1A} autoreceptors probably have the same location and function in different mammalian species.[119] Overall, we hypothesize that rats are the best animal model for research into the role of the 5HT_{1A} receptor in male sexual behavior, because of the similarities in pharmacology between male rats and humans.

On the whole, 5-HT_{1A} receptors are involved in the regulation of male sexual behavior under certain circumstances. But many questions remain still unanswered. Untill now, studies have focused on a few brain areas, but there are probably more

regions involved in male sexual behavior. In addition, there are, most likely, several interactions between neurotransmitter systems in the brain. Therefore, more research is needed before we have a complete picture of the regulation of male sexual behavior.

5-HT_{1A} RECEPTORS AND FEMALE SEXUAL BEHAVIOR

Rats

Serotonin is thought to exert a dual role in the control of female sexual behavior with 5-HT_{1A} receptors acting to inhibit and 5-HT_{2} receptors to facilitate lordosis upon either activation. 5-HT_{1A} receptors are the most investigated receptors in female sexual behavior research. Almost all of these studies are performed in the rats. Many studies can be found in literature that showed an inhibiting effect of 5-HT_{1A} receptor agonists on receptive[72,120-123] and proceptive behaviors[7] (summarized in table 1). This effect is seen in both ovariectomized females primed with estrogen (EB) and progesterone (P) and intact cycling females. Both, (±)8-OH-DPAT[72, 120, 122, 123] and buspirone[120] decrease lordosis quotients (LQ) in female rats.[120] Uphouse et al.[124] also showed differences between rat strains, whereas the sensitivity to (±)8-OH-DPAT after EB-priming is higher in Sprague-Dawleys than in Fischer female rats.
That the inhibiting effects of 5-HT_{1A} agonists on female sexual activity were really due to activation of the 5-HT_{1A} receptor was shown in experiments in which the effects of (±)8-OH-DPAT were antagonized by 5-HT_{1A} receptor antagonists, such as WAY-100635[126] and (S)-UH-301.[71]

In males, 5-HT_{1A} receptors do not play a crucial role in ejaculation under basal circumstances, because antagonists, by itself, do not have an effect on copulation. In females, on the other hand, results about the effect of 5-HT_{1A} receptor antagonists alone are controversial. Kishitake et al.[127] reported that systemic treatment with WAY-100635 facilitates lordosis in female rats, while other studies showed that WAY-100635 did not affect lordosis and proceptive behavior.[126, 128] These differences in outcome may be due to the fact that Kishitake et al.[127] used females in a low estrus state (sub-primed) and Uphouse et al.[128] fully-primed females. There might be a variation in response during the different stages of the estrus cycle, if 5-HT_{1A} receptors are only important during the beginning of the estrus cycle.

5-HT_{1A} receptors, brain areas and sexual behavior

The observation that stimulation of 5-HT_{1A} receptors disrupts female sexual behavior is without doubt, but the location of these 5-HT_{1A} receptors effects is unknown.
As mentioned before, one such brain area with an intense distribution of 5-HT_{1A} receptors is the dorsal raphé nucleus (DRN). Studies have shown an inhibiting role for this brain area, since lesions in the DRN increase lordosis.[129-131] Nevertheless, the inhibiting effects of 5-HT_{1A} receptor agonist (±)8-OH-DPAT are not produced by acting in the DRN, because (±)8-OH-DPAT was still active in DRN-lesioned rats.[132] Local injections of (±)8-OH-DPAT in the DRN also did not affect lordosis behavior.[133] Infusion of (±)8-OH-DPAT in the medial raphé nucleus (MRN), on the other hand, decreased lordosis responses. This suggests that facilitatory and inhibitory effect of 5-HT on lordosis could be derived from different subsets of midbrain raphé neurons,[134] which

Males						
Drug	**5-HT$_{1A}$ receptor**	**Other receptors**	**#M/I**	**EL**	**Extra**	**References**
(±)8-OH-DPAT	non-selective agonist	5-HT$_7$ receptor agonist	↓	↓		71-74
Buspirone	non-selective agonist		↓	↓		75
LY-228,729	non-selective agonist	5-HT$_{1D}$ receptor agonist	↓	↓		76
Flesinoxan	selective agonist		~	↓		78-80
LY-293,284	selective agonist		↓	↓		81
Ipsapirone	selective partial agonist		↓	↓		77
Indorenate	agonist		↓	↓		82
FG5893	non-selective agonist	5-HT$_2$ receptor antagonist	↓	↓		83
Pindolol	non-selective antagonist	β-receptor blocker	~	~	attenuate effect agonist	83
(S)-UH-301	antagonist		~	~	attenuate effect agonist	71
NAD-299	selective antagonist		~	~	attenuate effect agonist	73
WAY-100635	selective antagonist		~	~	- attenuate effect agonist - strengthen effect SSRI	84 85,87
Females						
Drug	**5-HT$_{1A}$ receptor**	**Other receptors**	**LQ**	**Darts**	**Extra**	**References**
(±)8-OH-DPAT	non-selective agonist	5-HT$_7$ receptor agonist	↓	↓		120,122-124
Buspirone	non-selective agonist		↓	↓		120
WAY-100635	selective antagonist		↑/-	~	attenuate effect agonist	126
(S)-UH-301	antagonist		~	~	attenuate effect agonist	71

Table 1: The effects of 5-HT1A receptor agents on male and female sexual behavior.

could also be explained by the differences in projection areas of these raphé nuclei. Another brain area with high levels of 5HT$_{1A}$ receptors and involvement in the regulation of female sexual activity is the ventral medial hypothalamic nucleus (VMN). Lesions of the VMN reduce lordosis, suggesting a facilitatory role of this brain area in female sexual behavior.[39] Local (±)8-OH-DPAT injections in the VMN also suppresses lordosis behavior.[135-138] Furthermore, this effect can be attenuated by several 5-HT$_{1A}$ receptor antagonists like WAY-100635,[25] WAY-100135 and propanolol.[136] Other 5-HT$_{1A}$ receptor agonists, 5-MEO-DPAC and 5-OH-DPAC, also decreased lordosis per mount (L/M) ratios in female rats when injected in the VMN, even though the effect was less clear than with (±)8-OH-DPAT.[138] Together, this suggests a role for 5-HT$_{1A}$ receptors in the VMN in the regulation of lordosis behavior in female rat.
Other possible brain areas that are involved in female sexual behavior are the MPOA and midbrain central gray (MCG). In contrast to the VMN, the MPOA is assumed to be inhibitory in the control of lordosis behavior, because MPOA lesions cause an increase in lordosis responses.[139] Electrical stimulation of this brain area, on the other hand, reduce lordotic behavior in female rats.[140] Interestingly, bilateral MPOA lesions caused a decrease in proceptive behavior (darts and hops) and paced mating behavior by prolonging contact-return latencies and percentage of exits.[141, 142] This suggests a stimulating role of the MPOA in the regulations of proceptive behavior. Involvement of 5-HT$_{1A}$ receptors in this brain area is suggested by Uphouse et al.[143] who showed a reduction in lordosis reflexes in response to direct (±)8-OH-DPAT infusions. But the dose of (±)8-OH-DPAT needed to inhibit lordosis was higher in the MPOA than in the VMN. Which could also mean that the (±)8-OH-DPAT has to diffuse to the VMN to induce an inhibiting effect on lordosis. Furthermore, the females showed more rejections after microinjection of (±)8-OH-DPAT into the MPOA. Interestingly, the local (±)8-OH-DPAT injections did not change the number of darts and hops in females. Again, this suggests the involvement of different subregions in the brain for different elements of sexual behavior.
The MCG is an area that is required for the VMN's facilitation of the lordosis reflex. Lordosis-relevant sensory information travels within the anterolateral column of the spinal cord to terminate widely throughout several structures, including the MCG.[144] Microinjection of (±)8-OH-DPAT into the MCG caused an inhibition of lordosis responses, but did not affect the number of male rejections.[133] In combination with the fact that the MCG is involved in the motor systems of the lordosis reflex, it is logical to suggest that (±)8-OH-DPAT affected the completion of the lordosis reflex.

5-HT$_{1A}$ receptors, sexual behavior and monoamines

Whether other monoaminergic neurotransmitter systems are involved in the effects of 5-HT$_{1A}$ receptors on female sexual behavior is still unclear. Some studies show beneficial effects of dopamine on receptive behavior,[145, 146] while other show inhibiting effects.[147, 148] Grierson et al.[149] suggested that dopamine D$_2$ receptors are more involved in sexual behaviors than dopamine D$_1$ receptors and additionally that low doses of dopaminergic agents act via presynaptic receptors, and therefore inhibit dopamine release and stimulate female sexual behavior, while high doses inhibit lordosis via postsynaptic receptors. The dopamine D$_2$ receptor antagonist haloperidol does not prevent the lordosis-inhibitory effects of (±)8-OH-DPAT or lisuride.[123]
Although not tested in rats on sexual behavior, the 5-HT$_{1A}$ receptor agonist fliban-

serin show possible interactions with other monoamines. Allers et al.[150] showed that acute systemic administration of flibanserin (non-selective 5-HT_{1A} receptor agonist) increased extracellular dopamine levels in the mPFC and MPOA. Repeated treatment with flibanserin also elevated dopamine levels in mPFC, but attenuated the effect in the MPOA. Repeated flibanserin treatment did not change the amount of extracellular dopamine in the NAc, whereas acute administration even had a tendency to decrease dopamine levels. Another study showed that this effect was due to 5-HT_{1A} receptors, because WAY-100635 was able to antagonize all effects.[151] Overall, this phenomenon suggests a possible interaction of 5-HT_{1A} receptors and dopamine in the brain in the modulation of sexual behavior.
The same results were seen with flibanserin treatment and noradrenaline. Several reports indicate that noradrenaline may be the mediator of the stimulatory effect on sexual receptivity of a number of hormones.[152, 153] Local injections in different brain areas reveal controversial effects,[154-156] but it might be caused by different noradrenergic (NE) receptors. The general hypothesis is that β-adrenoceptors inhibit lordosis, while α_2-adrenoceptors facilitate sexual behavior.[157] More research is needed to clarify the role of the noradrenergic systems, but the study with chronic flibanserin treatment indicates an interaction of 5-HT_{1A} receptors and NE release.
Administration of flibanserin caused an elevation in extracellular NE levels in the mPFC, MPOA and NAc.[150] The effect of repeated flibanserin on NE levels was different in the individual brain regions; increases in mPFC and NAc and no change in MPOA.[150] It is therefore logic to conclude that possible interactions of the 5-HT_{1A} receptor systems with other monoaminergic mechanisms (dopamine and noradrenaline) are region specific. In males, as illustration, it is shown that dopamine certainly is involved locally in the MPOA in sexual behavior,[101, 103] whereas other brain areas remain uncertain.

Overall, serotonin exerts a dual role in the control of female sexual behavior with activation of 5-HT_{1A} receptors inhibiting and activation of 5-HT_2 receptors facilitating lordosis. Mendelson et al.[158] have suggested that it is the relative balance between 5-HT's activation of these systems that determines if sexual activity will or will not occur. Since intact pro-estrus rats show lordosis behavior, it is reasonable to assume that during naturally occurring sexual receptivity, the inhibitory system, mediated via 5-HT_{1A} receptors, is suppressed in favor of the facilitatory system. Furthermore, this hypothesis is confirmed by our studies with SERT (serotonin transporter) knockout rats[125] and chronic paroxetine (serotonin selective reuptake inhibitor) treatment (submitted) which showed that the induced 5-HT_{1A} receptor desensitization does not affect proceptive and receptive behavior in female rats. The balance is not in favor of the 5-HT_{1A} receptor system in these situations and might underlie the lack of sexual dysfunctions during chronic elevated serotonin levels.

The mechanisms behind 5-HT_{1A} receptor inhibiting effects are unclear. As mentioned before, 5-HT_{1A} receptors belong to the superfamily of G-protein-coupled receptors, and activation of the receptor is generally negatively associated with adenylyl cyclase or opening of K+ channels.[43, 159] Agents that decrease adenylyl cyclase are known to inhibit lordosis behavior, and therefore 5-HT_{1A} receptors could act via this system. This was supported by a study that showed that cAMP increasing agents

attenuated the inhibiting effects of (±)8-OH-DPAT.[160] However, 5-HT_{1A} receptors have also been suggested to couple to multiple second messenger systems,[159, 161, 162] so it remains unclear what effect is responsible for the inhibiting effect on lordosis behavior.

Other animals

In male studies, other animals are used as model too. However, in female studies, the role of 5-HT_{1A} receptors is not much explored in different species than the rat. Only ferrets have been used as animal model. In rats, lordosis is characterized as a reflexive curvature of the back to mounting and flank palpation by a male. Ferrets, on the other hand, display an unresisting posture in response to neck gripping, mounting and intromitting from a male. Administration of (±)8-OH-DPAT caused a stimulation of receptive behavior, which is an opposite effect than in female rats.[110] If some of the inhibitory effects of (±)8-OH-DPAT on lordosis in the rat are associated with disruption of a reflex arc, it is not surprising to have a different effect on the ferrets receptive response, which involves no such reflex.
On the other hand, opposing results in male studies suggested differences between species and their regulation of sexual behavior. This might also be true for female sexual behavior. But more research in different species is needed to clarify this hypothesis.

Human

After describing the effect of 5-HT_{1A} receptors on their sexual behavior in animals, again the interesting question arises: what about sexual behavior in women? In men, more knowledge about 5-HT_{1A} receptors is derived from SSRI studies that were used as treatment for premature ejaculations. However, this treatment is not effective in women and therefore there is no information about these systems. Only in rats, as mentioned before, it is shown that chronic SSRI treatment causes 5-HT_{1A} receptor desensitization, which might underlie the lack of sexual dysfunctions. However, in human studies it is suggested that women using SSRIs complain more about sexual disorders than non-users,[163, 164] but whether this is really due to the chronic drug use is questionable, whereas depression can also cause sexual problems.[165, 166] But co-treatment of SSRIs with the non-selective 5-HT_{1A} receptor agonist Buspirone seems to reduce the SSRI-induced sexual side effects,[167] suggesting a role of 5-HT_{1A} receptors in the regulation of sexual behavior in women.

Discussion

In conclusion, upon activation of 5-HT_{1A} receptors have an inhibitory role in female sexual behavior. Many brain areas are possibly involved in this regulation mechanism, but unfortunately, only the MPOA, MCG and VMN are well studied in females. In males, other brain regions like the NAc and MeA are also involved (figure 5). And therefore, there is no reason to assume that this is different in the female sexual system. Hopefully, more research will be performed in the future to unravel all complicated systems interfering in sexual activity in normal females, but also those suffering from sexual dysfunction.
The results of the role of the MPOA in female sexual behavior is controversial; an inhibiting role in lordosis behavior versus a stimulating role in proceptive behavior.

This suggests that different mechanisms are involved in receptive and proceptive behavior. Whether these differences are universal or locally in the MPOA are not clear yet.

Just as with males, we have shown differences in 5-HT_{1A} receptor involvement in sexual behavior between species, although in females only rats and ferrets have been used, which makes it difficult to draw final conclusions. It is even not sure whether mechanisms involved in sexual behavior in female rats is similar by organized as in women. In this case, it is questionable whether rats are a good model for women with female sexual dysfunctions.
Like mentioned before, there is also a difference in sensitivity to 5-HT_{1A} receptor agonists between different rat strain. But the general inhibitory effect is similar. For sure, more research to this phenomenon is needed before conclusions can be drawn.

In addition, we would like to address the hormonal menstrual cycle as another important factor in human female sexual behavior. In this review we did not discuss the effect of hormone levels on the function of 5-HT_{1A} receptors. We only mentioned the possibility that 5-HT_{1A} receptors are only crucial in regulating female sexual behavior throughout basal circumstances during relatively low hormonal levels, while 5-HT_{1A} receptor might be less important during relatively high hormonal levels. When the extracellular serotonin levels are high, 5-HT_{1A} receptors are important during all hormonal levels. This is an interesting observable fact that could affect more processes in female sexual behavior. In future research, the hormonal effect on 5-HT levels and female sexual behavior should be taken into account.

General discussion

Overall, it seems that there are similarities between male and female sexual behavior and the role of serotonin, and especially 5-HT_{1A} receptors. In both, males and females, it seems that 5-HT_{1A} receptors are only crucial in regulating sexual behavior when serotonin levels are high. This conclusion can be drawn out from the observation that 5-HT_{1A} receptor antagonists are not effective when injected alone in wildtype rats. Only when serotonin levels are high, like after chronic SSRI treatment or in SERT knockout rats, 5-HT_{1A} receptor antagonists have an (extra) effect. In females, this effect could be different in situations where gonadal hormone levels are low.

Another similarity between males and females is the involvement of the same brain areas in sexual behavior. In both genders, the role of 5-HT_{1A} autoreceptors in the MRN seems to be more important than in the DRN. In addition, the MPOA plays an important role in both male and female sexual behavior. This is probably also true for brain areas like NAc and medial amygdala (MeA), but more research is needed in these areas. Conditioned place preference tests[168] could be a good method to investigate these areas, whereas the NAc plays an important role in the rewarding system.[169]

However, there are also contrasts between male and female rats; 5-HT_{1A} receptor agonists facilitate ejaculation in males and inhibit lordosis and darts in females. This seems quite conflicting, but is this true? Is the regulation of sexual behavior really opposite in male and female rats? Could it be possible that these systems have more in common than it might seem?

The tradition is to use different measurement scales to evaluate male and female sexual behavior. The main parameters in male rats are latency to and frequency of copulation, and post ejaculatory intervals, which are parameters that involve the orgasm, and not arousal of sexual behavior. In females, on the other hand, the main measurements are lordosis frequencies and darts and hops. These behaviors are typically arousal measurements. It is so far impossible to measure orgasm in female rats (if this phenomenon exists), which would be more comparable to male sexual behavior. Therefore, we suggest that these sexual systems, orgasm and arousal, consist of two complete different mechanisms (figure 6). The observation in the introduction that different subregions are involved in the different aspects of sexual behavior also suggests this hypothesis.

In addition, it is shown that (±)8-OH-DPAT decrease the amount of mounts and intromissions in male rats, next to the increase in ejaculation latencies. These parameters in males represent arousal behavior better than orgasms. As a result, we can conclude that there is no difference between males and females in sexual behavior. The results should only be separated into arousal behavior on one hand and orgasms in the other. In this vision, 5-HT_{1A} receptor agonists decrease sexual arousal in both male and female rats and would thereby suggest similarities between different genders instead of differences (figure 6). Unfortunately, no studies have been focused on sexual arousal in males.

That, at the moment, studies in male rats only focus on ejaculation behavior and female research on arousal is logical if we look at human behavior. Premature ejaculation and erectile dysfunctions are the main problems in men, while arousal and desire disorders are most common in women. Orgasmic disorder is sometimes even not mentioned in the list of female sexual dysfunction, which suggests that women can still enjoy sex without orgasm. Men, in most cases, prefer ejaculations to increase their quality of life. Therefore, research on ejaculatory behavior in men and sexual arousal research in women is most important. However, it can be helpful for the general knowledge of sexual behaviors in both genders if more research will be performed on male sexual arousal and, if possible, on female orgasm.

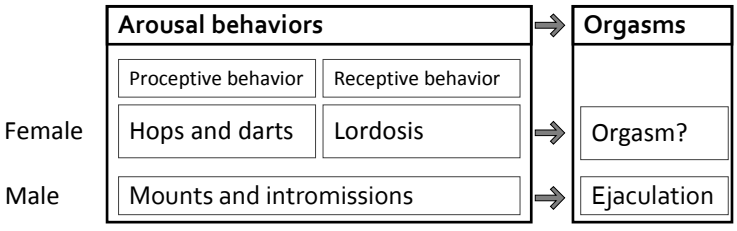

Figure 6: Hypothesis about the similarities between male and female rat sexual behavior.

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A new female rat animal model for hypoactive sexual desire disorder; behavioral and pharmacological evidence

3

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Abstract

Introduction. Female Sexual Dysfunction (FSD) affects 33-48% of women. Female rats with low sexual activity might model FSD.

Aim. In this study, we have investigated whether in a population of normal female rats, subpopulations of rats exist with different levels of sexual behavior.

Methods. Sexually experienced, intact, estradiol-primed female rats were placed in an empty compartment adjacent to a compartment with a male. The females were allowed, during 30 minutes, to switch between the compartments via a hole through which only the females could pass (paced mating). Next, we investigated the acute effects on female sexual behavior of apomorphine, a D_1 and D_2 type dopamine receptor agonist, (±)8-OH-DPAT, a 5-HT_{1A} receptor agonist, and paroxetine, a selective serotonin reuptake inhibitor.

Main outcome measures. Time spent in compartments, proceptive behaviors, contact-return latencies and percentages of exits were quantified.

Results. Based on their behavior in the paced mating sex test, estradiol-primed, intact female rats can be divided in three groups: those that mostly avoid the male, a large middle group, and those that mostly approach the male. The avoiders also showed significantly less proceptive behavior than the male-approachers. The sexual behavior of the females was relatively stable over time, suggesting the existence of different endophenotypes in female rats. Apomorphine and (±)8-OH-DPAT had an inhibiting effect on sexual behavior, but only females dosed with apomorphine showed a different response in avoiders and approachers; more inhibiting effect in avoiders than approachers. Paroxetine had no effect on proceptive behavior.

Discussion. The stable, male-avoiding behavior of some females might correspond to the characteristics of women with FSD. Therefore, these avoiders are a promising new model for Female Sexual Dysfunction, specifically for sexual desire and/or arousal disorders. Furthermore, the apomorphine data suggest that differences in the dopamine system may (partly) underlie the differences in sexual behaviors between avoiders and approachers.

Introduction

The prevalence of Female Sexual Dysfunction (FSD) in the human population ranges from 33-48% in the USA and in Europe.[1-7] According to DSM-IV-TR,[8] FSD can be divided in four main categories: low sexual desire, low arousal, orgasmic disorders, and sexual pain. Each is defined as "persistent or recurrent" and causes "marked distress or interpersonal difficulty". The majority of sexual dysfunction surveys identify low sexual desire/interest and sexual arousal disorder as the most common problems.[3-7, 9] Currently, there are several validated animal models employed to examine sexual behavior in female rats.[10, 11] In non-paced mating tests, male and female rats are placed together to quantify proceptive (hopping, darting, ear wiggling, and approach) and receptive behavior (lordosis quotient and quality). Paced mating tests, using bi-level chambers or multiple compartments, allow the female rats to control ("pace") the sexual encounters.[12-14] Additional parameters like percentages of exits and contact-return latencies can also be quantified in this test. In appetitive tests, such as odor/partner/place preference and operant tests, the motivation of female rats to engage in sexual behavior is tested.[15-20]

In our laboratory, we are currently using a model of male sexual dysfunction.[21, 22] After four to five consecutive weekly 30-min tests where males freely interact with sexually receptive females, male Wistar rats fall into three endophenotypic groups of consistently sluggish, normal, and rapid ejaculating rats.[22] Sluggish rats have 0-1 ejaculations, normal rats have 2-3 ejaculations, and rapid rats have up to 5 ejaculations in each test. Based on the similarities between human and rat male sexual behavior, we proposed that sluggish rats could be preclinical models for anorgasmia, delayed ejaculation, or low desire disorders in men whereas rapid ejaculating rats could be a model for premature ejaculation.[22]

In our 'male experiments', we noticed repeatedly that some estradiol-primed female rats displayed more rejective behavior than others. This rejective behavior consisted of intermittent bouts of fleeing, boxing, kicking, vocal outbursts and/or adopting a supine position, before resuming copulation. Those females were clearly receptive, since they showed a stable and full lordosis response to mounting males. These observations suggest that individual differences in sexual motivation and behavior exist in both male and female rat population. As with the male rats, female rats may also have stable sexual endophenotypic groups displaying hypoactive, normal, or hyperactive sexual behavior, and this could be the basis of a new and promising animal model to examine the biological mechanisms of FSD.

The first goal of this experiment was to investigate the existence of stable individual differences in female rat sexual behavior in a paced mating paradigm. To exclude the role of the male rat, we also tested the preference of females for certain endophenotypic rats.[21, 22] The second goal was to investigate the biological mechanisms of FSD. Therefore, we administered a 5-HT_{1A} receptor agonist ((±)8-OH-DPAT), a D_1 and D_2 type dopamine receptor agonist (apomorphine) and a selective serotonin reuptake inhibitor (SSRI, paroxetine) in both hypoactive and normal sexually behaving females. We selected these inhibiting drugs, because there might be a limit of time approachers can spend with the male rat (ceiling effect). Stimulatory drugs would therefore not show the differences in response

of avoiders and approachers, because the potential effect is than not visible in approachers. Inhibiting drugs, on the other hand, would be visible in both groups.
The choice for apomorphine, (±)8-OH-DPAT and paroxetine were based on studies described in literature. Several studies showed the inhibiting effect of apomorphine on female sexual behavior, by a decrease in proceptive behavior,[23] lordosis[24-26] and incentive motivation[23] after administration of dopamine increasing agents. Also the inhibiting effect of (±)8-OH-DPAT on lordosis behavior[27-29] was described in literature in the past. Paroxetine blocks the serotonin transporter (SERT) and thereby induces an increase in extracellular serotonin levels. No other rat studies with paroxetine are published, but in women it causes sexual dysfunctions, just as other SSRIs.[30, 31] Therefore, it would be interesting to see the effect of acute paroxetine treatment in our female rats.

Materials and Methods

Animals

Adult female (3 months of age at the beginning of the experiment) and stimulus male outbred Wistar rats (Harlan, Zeist, The Netherlands) were used. The animals were housed in the Central Animal Laboratory of the University of Utrecht. All rats were adapted to the laboratory environmental condition and a reversed 12/12-h light/dark cycle (lights off at 7 am). Standard food and water were available ad libitum. Male and female rats were housed in separate rooms in groups of four per Macrolon type-IV cage.

Sexual receptivity was induced in intact female rats by subcutaneous administration of 50 µg estradiol benzoate (EB) dissolved in 0.1 ml sesame oil saturated with phosphatidylcholine, 36 hours prior to testing. This high dose of estradiol also prevents pregnancies in intact female rats. The use of these females primed with only estradiol is based on a experiment done in our laboratory showing that progesterone is not necessary to perform full receptivity (data not shown).
Sexually experienced active male rats with various sexual endophenotypes (sluggish, normal and fast ejaculators) were used as stimulus animals. In experiment 1, the males were selected based on their sexual endophenotype (see Pattij et al.[22]). In experiment 2, 3, and 4 the males with all sexual endophenotypes were used as stimulus animals. They were randomly chosen from a large group of males that represented all endophenotypes. Every test, a different male was presented to the female.
All experiments were carried out in accordance with institutional, national and international guidelines for animal care and the Dutch law concerning animal welfare.

Behavioral Procedure

All experimental procedures were conducted under red light during the dark phase of the reversed light/dark cycle. Before the females were tested in the male preference/paced mating tests, they had at least three non-paced sexual encounters in which the female had access to a different male every test. Non-paced mating was performed as described previously.[22] The training was done to rule out possible effects of sexual inexperience.

Male preference test: Four transparent cylinders (diameter 60 cm, height 40 cm) were assembled together, forming a compartment in the center where the female was placed in the beginning of the test. The bottom of each cylinder contained a small hole (3 cm from the floor, diameter of 4 cm), which allowed only the (smaller) female to move freely. At the beginning of the test (experiment 1), one sluggish, one normal, and one fast male were placed in separate cylinders of the test cage. The fourth cylinder was empty. The female was placed in the central compartment with the holes of the cylinders closed. After 30 minutes of habituation, in which the rats could only smell, see and hear each other, the holes were opened allowing the female to move freely during the 30 minute test. The tests were recorded on video. The number of female proceptive sexual behaviors (darting and hopping) and the time that the female spends in each compartment were measured. To evaluate the preference for a particular male, the male with which the female spent most time was considered to be the preferred male of the test.
Paced mating sex test: The test cage was divided into two compartments: a "male compartment" of 43 x 26 x 38 cm and a "female-only compartment" of 15 x 26 x 38 cm. The compartments were divided by a transparent plastic wall containing three holes (4 cm diameter) through which only the smaller females could pass. First, female rats were allowed to freely explore both compartments of the test cage for five minutes. Then, a blockade was used to block the holes and the female rat was placed in the "female-only", and the male was placed in the "male compartment". The rats could smell, hear, and see each other for another 25 minutes of habituation. Females were tested once a week (or less). For each sex test a female had to perform, a different male (with all endophenotypes) was used.
After this habituation period the blockade was removed and the behavior was videotaped for the next 30 minutes. Event recording software (Observer 5.0, Noldus, the Netherlands) was used to score female proceptive behavior (number of darts and hops), percentages of exits, contact-return latencies (CRL) and male sexual behavior (number of mounts, intromissions and ejaculations), as well as the time spent by the female in either compartment.
The percentage of exits after mount was calculated as described before by Guarraci et al.[32]: the total number of exits after the received mount (when the female escapes to her own compartment after the mount with a time limit of 120 seconds) divided by the total number of mount times 100%. The percentage of exits after intromission is the number of exits after intromissions divided by total number of intromissions times 100%. The same measurement was done for ejaculations. After an escape within 120 seconds, the average time the female needs to enter the male compartment again is called contact-return latency.

Drugs

(±)-8-Hydroxy-2-(dipropylamino)tetralin hydrobromide ((±)8-OH-DPAT), R-(-)-Apomorphine hydrochloride hemihydrate (Sigma-Aldrich, Steinheim, Germany) and Paroxetine hydrochloride hemihydrate (obtained via a local pharmacy, the Netherlands) were dissolved/suspended in saline, 0,1% ascorbinic acid and 0,5% methyl cellulose respectively. (±)8-OH-DPAT (-10 min, s.c.), apomorphine (-15 min, s.c.) and paroxetine (-1 hour, p.o.) were injected in a volume of 2 ml/kg before the paced mating sex tests.

Experiments

Experiment 1: The preference for certain endophenotypic males was investigated in 12 receptive female rats. They were all tested once in the male preference test.
Experiment 2: Basal levels of sexual behavior were measured in 110 receptive female rats in the paced mating sex test. Next, to test whether this behavior was of a temporary or more permanent nature, 20 female rats were selected from the left and right portion of the graph and classified as "approachers" (n=8) and "avoiders" (n=12) (figure 2). These females participated in two additional tests. The time interval between the 1st and the 2nd test was about 3 months, and between the 2nd and 3rd test 1 month.
Experiment 3: To replicate our data of experiment 2, we tested another 90 female rats in the paced mating paradigm. In this experiment, all females were tested 3 times with one-week intervals to check the stability of the sexual behavior.
Experiment 4: For this experiment, 30 "avoiders" (<600 seconds spent with the male) and 30 "approachers" (>600 seconds) were selected from experiment 3 to investigate putative neurochemical mechanisms involved in FSD. In total 6 groups (n=10/group) were formed, 3 "avoider" and 3 "approacher" groups and tested in the paced mating sex test. Doses of the D_1 and D_2 type dopamine receptor agonist apomorphine (0, 0.125 and 0.5 mg/kg), the 5-HT_{1A} receptor agonist (±)8-OH-DPAT (0, 0.3 and 0.4 mg/kg), and SSRI paroxetine (0, 10 and 20 mg/kg) were administered in both the "avoiders" and "approachers". The drugs were chosen to explore a wide range of possible mechanisms underlying FSD.

Data analysis

A combined histogram of the data of experiment 2 and 3 was created with SPSS. There was no homogeneity of variances found in our data. Therefore, all behavioral data were analyzed using the non-parametric Kruskall-Wallis test. Further post hoc analysis was performed with Mann-Whitney test (SPSS). The level of significance was set at $p<0.05$.
Pearson's product moment correlation coefficient was calculated to analyze the correlation between test 2 and 3 of the time spent in the male compartment, and the total number of proceptive behaviors. Furthermore, the correlation was used to analyze the correlation between the amount of darts and the received male behaviors. Correlations were considered relevant when significant ($p<0.05$) and larger than 0.5.

Main outcome measures

Time spent in compartments, proceptive behaviors (darts and hops), contact-return latencies and percentages of exits were quantified.

Results

Experiment 1:

As shown in figure 1a, the time a female spent in each compartment was not significantly different between certain endophenotypic male rats. However, the females significantly spent more time in the empty compartment compared to all other

compartments (Z=-2.425, p=0.015 compared to sluggish; Z=-3.406, p=0.001 to normal and Z=-3.349, p=0.001 to fast). There was no difference in number of proceptive behaviors with a certain endophenotypic male (figure 1b). The number of darts and hops was divided by the time spent with the male to correct for the influence that the time has on the proceptive behavior in this compartment.
Interestingly, the female rats appeared to prefer the compartment of first choice. Therefore the differences between the 1st, 2nd and 3rd choice have been calculated. The first choice did not depend on the endophenotype of the male rat. But there was a significant difference in time spent with their first and the third choice male (Z=-3.464, p<0.001) (figure 1c). The number of darts and hops (corrected by the time spent in the compartment) did not differ between the males (figure 1d).

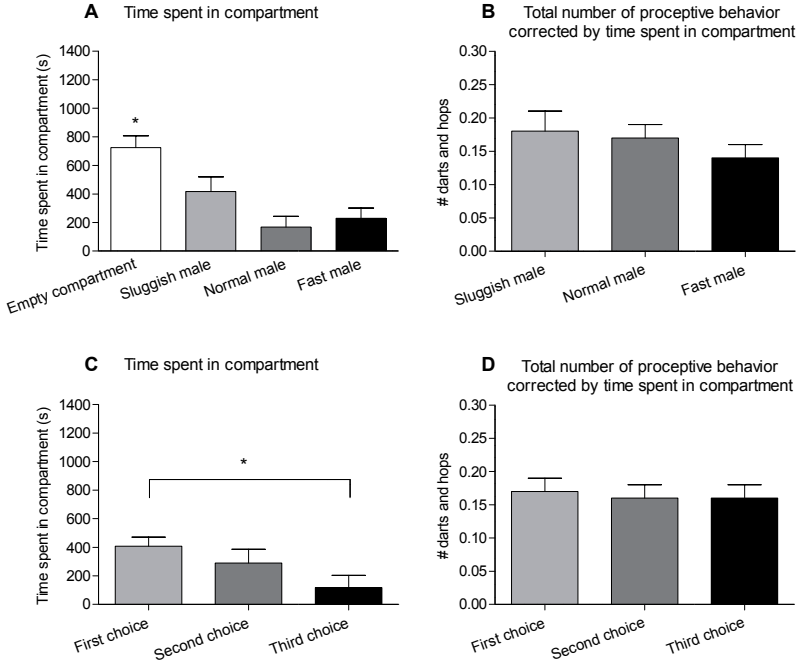

Figure 1: Preference test in estradiol-primed female Wistar rats during a 30-min sexual behavior test. Comparison between sluggish, normal and fast males for time spent in compartment (A) and proceptive behavior (corrected by the time spent in the compartment) (B) and comparison between the first, second and third choice of visit for time spent in compartment (C) and proceptive behavior (D). Data are medians ± standard error of the median; *p<0.05

Experiments 2 and 3:

In experiment 2 and 3, a total of 200 female rats were tested. Figure 2a shows a frequency histogram for the time spent with the male. At least three different subpopulations of female rats can be recognized: rats that mostly avoid the male (avoiders), rats that mostly approach the male (approachers) and a large middle group. The number of proceptive behavior resulted in the same kind of histogram (figure 2b).

In experiment 2, we selected 12 approachers and 8 avoiders (the extremes out of the total population), based on the average time spent in male compartment: 1433.7
± 90.0 and 163. 8 ± 60.9 sec, respectively for two similar follow-up tests. The non-parametric Mann-Whitney test revealed that avoiders spent significantly less time in the male compartment in all tests (test 1: Z=-3.703, p<0.001; test 2: Z=-3.420, p=0.001; and test 3: Z=-2.349, p=0.015) compared to approachers (figure 3a). In addition (figure 3b), avoiders showed significantly less proceptive behavior than the approachers in test 1 and 3 (test 1: Z=-2.667, p=0.008; test 3: Z=-2.857, p=0.004), and marginally significant in test 2 (Z=-1.930, p=0.054). Approachers darted and hopped more in the male compartment in all three tests (test 1: Z=-3.626, p<0.001; test 2: Z=-3.032, p=0.002; test 3: Z=-2.905, p=0.004), whereas avoiders darted more in the female compartment in all three tests (test 1: Z=-3.579, p<0.001; test 2: Z=-3.176, p=0.001; test 3: -2.023, p=0.043) (data not shown).

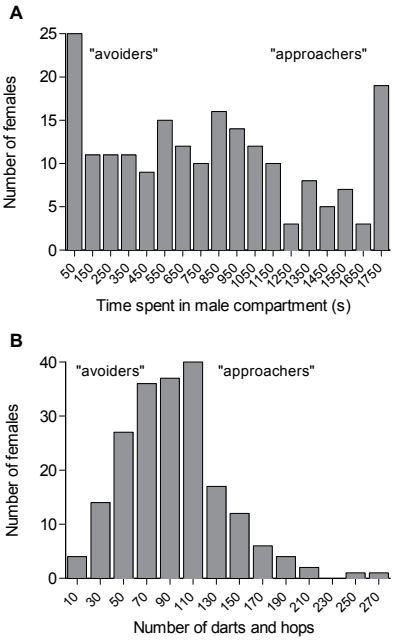

Figure 2: Histogram of (A) time spent (in seconds) in the male compartment, as opposed to the empty compartment and (B) number of darts and hops by 200 estradiol-primed adult female Wistar rats (combination of experiment 2 and 3). The Y-axis represents the number of females in each category; the X-axis the time categories split in intervals of 100 s (0-100 s, 101-200 s, 201- 300 s etc) and 20 proceptive behaviors (0-20, 21-40, 41-60 etc).

Experiment 3:

To strengthen our initial findings, we replicated our experiment with a new population of female rats. In this experiment we used a cut-off of 600 seconds; rats that spent less than 600 seconds with the male were considered avoiders, and rats that spent more than 600 seconds were considered approachers. In both, experiment 2 and 3 the prevalence of avoiders based on these cut-off points was 37% and 48 %, respectively. From the initial 90 animals in experiment 3, we selected 30 avoiders and 30 approachers for further testing. The time spent in the male compartment was again significantly different during all 3 tests between the avoiders and approachers (test 1: Z=-4.257, p<0.001; test 2: Z=-7.823, p<0.001; test 3: Z=-6.528, p<0.001) (figure 3c). The female rats also showed the same amount of proceptive behavior in this replication experiment (figure 3d). Male-avoiders showed significantly less hops and darts than the male-approachers (test 1: Z=-2.546, p=0.011; test 2: Z=-5.056, p<0.001; test 3: Z=-3.912, p<0.001), but darted significantly more in the female compartment (data not shown) (test1: Z=-3.509, p<0.001; test2: Z=-5.225, p<0.001; test 3: Z=-4.783, p<0.001). Figure 4 shows correlations between behaviors of individual animals during test 2 and test 3. In particular the time spent with the male is quite stable between the tests (figure 4a, $r^2 = 0.54$, p<0.001),

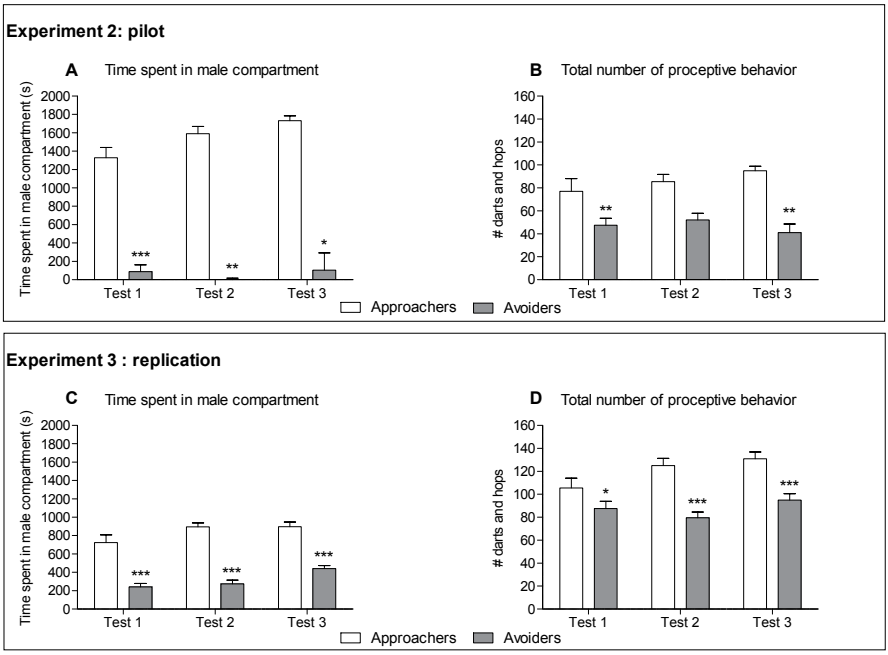

Figure 3: Consecutive 30-min sexual behavior tests in approaching and avoiding adult estradiol-primed female Wistar rats: (A) time spent in the male compartment (s) and (B) total number of proceptive behavior in experiment 2 and (C) time spent in male compartment and (D) proceptive behavior in experiment 3. Data are medians ± standard error of the median; ; *p<0.05, **p<0.01, ***p<0.001

while the number of darts and hops shows more variability (figure 4b, r^2=0.17, n.s.). To strengthen our results, we have tested two additional parameters (percentages of exits and contact-return latencies (CRL)) in this experiment as well. There was no difference in percentage of exits after mount, intromission and ejaculation between avoiders and approachers (table 1), but the avoiders had a significant longer CRL after intromission (test 1: Z=-3.788, p<0.001; test 2: Z=-5.510, p<0.001; test 3: Z=-3.766, p<0.001) and ejaculation (test 1: n.s.; test 2: Z=-3.995, p<0.001; test 3: Z=-3.920, p<0.001) than the approachers (table 1). The CRL after mount was only different in the first test (Z=-2.814, p=0.005).

The last parameter that we have calculated was the amount of male behaviors (mount, intromission and ejaculations) the females received. As shown in table 1, male-avoiders received significantly less mounts (test 1: Z=-3.286, p=0.001; test 2: Z=-4.824, p<0.001; test 3: Z=-2.952, p=0.003), intromissions (test 1: Z=-3.854, p<0.001; test 2: Z=-4.693, p<0.001; test 3: Z=-2.809, p=0.005) and ejaculations (test 1: Z=-2.468, p=0.014; test 2: Z=-2.160, p=0.031; test 3: n.s.) in all tests compared to the male-approachers. The amount of mounts and intromissions are strongly correlated with the amount of darts and hops (test 1: mounts: r^2=0.71, p<0.001; intromissions: r^2=0.36, p=0.004; test 2: mounts: r^2=0.61, p<0.001; intromissions: r^2=0.34, p=0.007; test 3: mounts: r^2=0.64, p<0.001; intromissions: r^2=0.35, p=0.006).

	Test 1		Test 2		Test 3	
	Avoiders	Approachers	Avoiders	Approachers	Avoiders	Approachers
# Mounts	8.0±1,4***	20.0±3.0	7.5±1.5***	29.5±3.1	16.5±2.1**	28.0±2.7
# Intromissions	8.0±0.9***	13.0±0.8	12.0±0.9***	18.0±0.6	15.0±0.8**	20.0±0.8
# Ejaculations	2.0±0.2*	2.0±0.1	2.0±0.1*	3.0±0.1	3.0±0.1	3.0±0.1
% of exits after mounts	87.5±2.4	89.7±3.0	85.7±2.2	87.7±2.3	92.6±1.0	92.0±1.3
% of exits after intromissions	87.5±2.2	90.0±0.8	91.7±0.7	92.9±1.0	93.3±0.4	94.4±0.5
% of exits after ejaculations	50.0±9.1	50.0±2.3	50.0±2.3	66.7±3.1	66.7±1.7	66.7±1.1
CRL after mounts	32.1±5.9**	22.3±1.5	19.9±2.5	21.4±2.4	25.4±1.8	19.9±2.1
CRL after intromissions	44.4±4.5***	24.8±2.5	42.6±4.5***	19.3±1.4	29.5±2.5***	20.3±1.3
CRL after ejaculation	129.3±11.8***	80.8±9.7	187.2±13.0***	90.9±6.0	166.2±8.7***	91.1±6.1

Table 1: Total number of received mounts, intromissions and ejaculation and percentages of exits and contact-return latencies (CRL) after mounts, intromissions and ejaculation in avoiders and approachers during three 30 min tests. Data are medians ± standard error of median; *p<0.05, **p<0.01, ***p<0.001 compared to approachers.

Experiment 4:

Both doses of apomorphine (0.125 mg/kg and 0.5 mg/kg) induced a drop in both time spent with the male (figure 5a) and proceptive behavior (figure 5b) in the avoiders (time spent in male compartment: χ^2=10.653, p=0.005, proceptive behavior: χ^2=14.685, p=0.001), whereas only 0.5 mg/kg apomorphine had a significant inhibiting effect on proceptive behavior in the approachers (χ^2=16.265, p<0.001). Apomorphine did also show a slight decrease in time spent in the male compartment of the approachers, but this effect was not significant.

0.3 mg/kg and 0.4 mg/kg (±)8-OH-DPAT did not affect the time spent in male compartment in the avoiders, but did decrease this parameter in the approachers (χ^2=7.311, p=0.026) (figure 5c). (±)8-OH-DPAT dramatically decreased the total number of darts and hops in both endophenotypes (avoiders: χ^2=19.494, p<0.001; approachers: χ^2=19.658, p<0.001) (figure 5d).

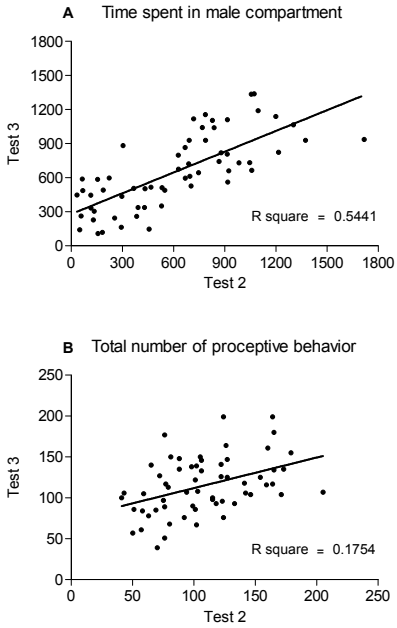

Figure 4: Correlations of (A) time spent in the male compartment (s) and (B) total number of proceptive behavior between test 2 and 3.

Furthermore, 10 mg/kg and 20 mg/kg paroxetine did not have a significant effect on time spent in male compartment (figure 5e) and total number of darts and hops (figure 5f).

Discussion

Our experiments clearly indicate the existence of subpopulations of female rats with differences in sexual behavior. We showed that the parameter "time spent in male compartment" can be used to divide groups of female rats: avoiders and approachers. This parameter was also the most reproducible at the level of the individual rat (see figure 4a), while avoiders and approachers clearly differed at group level in proceptive behavior. At the individual level this behavior was less stable between tests (see figure 4b) and thus this parameter has less discriminative power.

In the present report, we used quite arbitrary cut-offs for defining avoiders and approachers. In experiment 3, we used a cut-off of 600 seconds. These wider selection criteria were chosen primarily for practical reasons. A selection of animals with more extreme behavior would be ideal, but then initially a much larger group of females needs to be screened to get enough animals for follow-up experiments. It is arguable that in our experiment the avoider population is also containing normal

behaving females, but even with this high cut-off, we were able to detect differences in response to a low dose of apomorphine between avoiders and approachers.
Proceptive behavior (hops and darts) is regarded as a measure of the intensity of estrus responsiveness and a signal of readiness to mate.[12] Overall, avoiders darted significantly less than approachers, which indicates that these rats are not that ready for mating: a reminiscent of low arousal FSD rather than low sexual desire in women.[33] Strangely, avoiders darted quite substantially in the female compartment. Therefore, we suggest that although both male-avoiders and approachers are receptive, the higher libido of approachers allows the proximity of the male rat, which in turn stimulates darting. McClintock et al.[34] already showed that 90% of intromissions are preceded by approach solicitations by the female, while only 3% occurred when the male approached the female. A strong correlation between the amount of darts and male sexual behavior (mounts, intromissions and ejaculations) was also proven by our data.
The persistent tendency to avoid the male compartment in some individuals might correspond to the avoidance of sexual interactions by women suffering from low sexual desire FSD.[33, 35] Where many women suffer from combinations, it is hard to distinguish between the different subtypes of FSD.[9, 33] However, the observation that the time spent with the male is a more relevant parameter for the division in subpopulations, we suggest that our model approaches low sexual desire disorder more. Other parameters as contact-return latencies (CRL) and percentage of exits could help to distinguish between the subtypes of FSD. Whether longer CRLs reflect sexual satiety or increased aversion to excessive stimulation is not known. Erskine et al.[12] suggested that the change in percentage of exits reflects the female's short-term response to the intensity of the copulatory stimulus, while CRL is a direct measure of the female's motivation to reinitiate mating. This hypothesis was strengthened by our results and other studies by Guarraci et al.[32, 36] Based on this idea, there is a reason to suggest that our male-avoiders suffer from sexual desire disorder, because they are different in contact-return latencies and not in percentages of exits. However, it is hard to distinguish between all subtypes of FSD in addition to the fact that women can suffer from combinations.
Some approachers, on the other hand, spent practically all of the time in the male compartment. Those females could perhaps represent an endophenotype different from the middle group (or intermediate females), and possibly model intense arousal feelings or even sexual addiction or compulsivity. Women with this latter disorder are driven by the need to make personal contact or the need for self-validation, motivated by the mood improvement and increase in arousal.[37] However, this is still a hypothesis that should be investigated in the future. Our experiment focused on the avoiders as animal model for FSD.
Therefore, we have tested the effect of different drugs in our animal model: a D_1 and D_2 type dopamine receptor agonist (apomorphine), a 5-HT_{1A} receptor agonist ((±)8-OH-DPAT), and a SSRI (paroxetine). Apomorphine showed a different effect on sexual behavior in avoiders compared to approachers. Both doses of apomorphine decreased the time spent with the male and number of darts and hops in the avoiders, whereas only the highest dose affected the proceptive behaviors in the approachers. This suggests that avoiders are more sensitive to dopamine agonists compared to approachers.

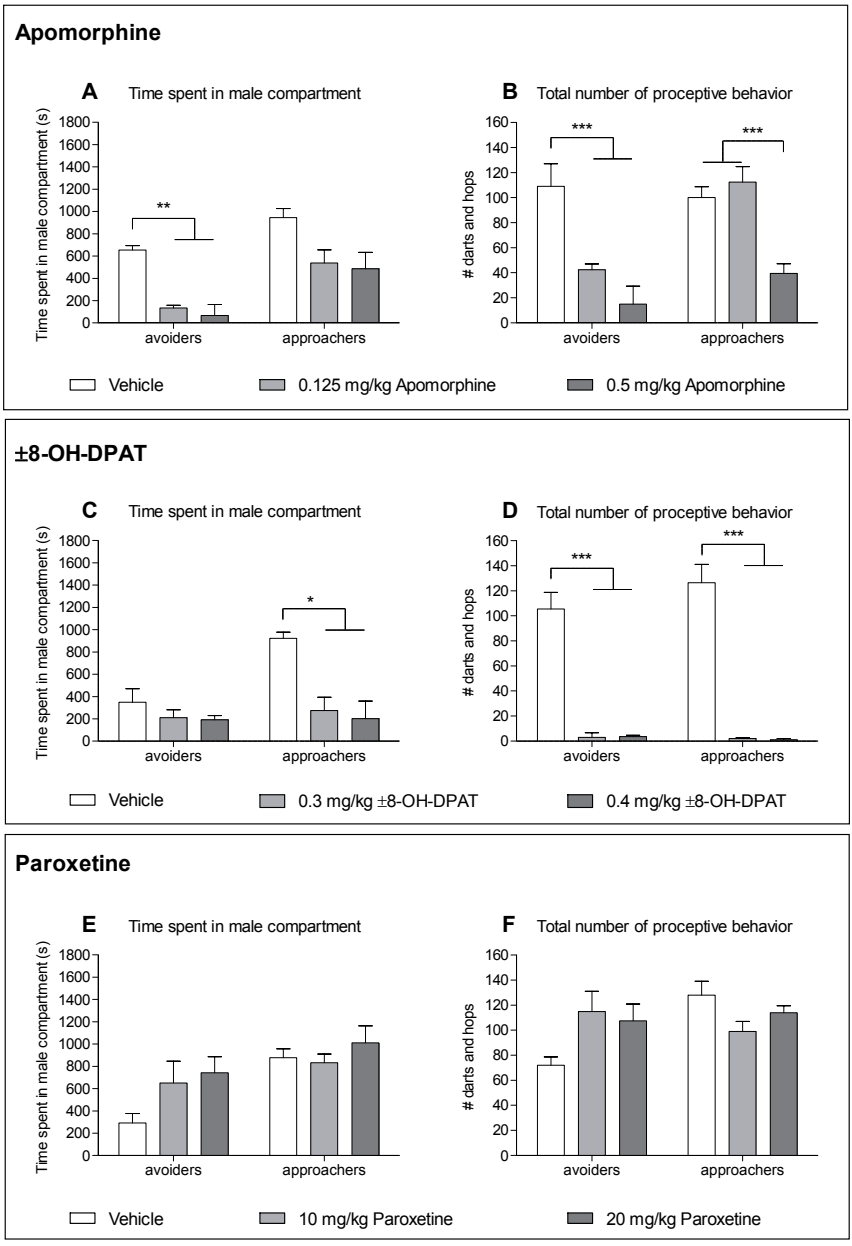

Figure 5: Time spent in the male compartment (s) and total number of proceptive behavior in avoider and approacher intact estradiol-primed female Wistar rats after administration of (A/B) apomorphine (s.c.), (C/D) (±)8-OH-DPAT (sc) and (E/F) paroxetine (p.o.). Data are medians ± standard error of the median; *p<0.05, **p<0.01, ***p<0.001

Apomorphine is a non-selective dopamine receptor agonist with a slightly higher affinity for D_2-like than D_1-like dopamine receptors. The precise involvement of dopamine in female sexual behavior is less clear. The inhibiting effect of apomorphine was in line with other studies that showed a decrease in proceptive behavior,[23] lordosis[24-26] and incentive motivation[23] after administration of dopamine increasing agents. Even though, some other studies have shown the opposite effect[38, 39] or no effect of lordosis behavior.[23] Grierson et al.[40] suggested that dopamine D_2 receptors are more involved in female sexual behaviors than dopamine D_1 receptors and additionally that low doses of dopaminergic agents work via presynaptic receptors, and therefore inhibit dopamine release, and stimulate sexual behavior, while high doses inhibit lordosis via postsynaptic receptors. For that reason, we suggest that the high sensitivity to apomorphine in male-avoiders is mainly caused by dopamine D_2 receptors. Further investigation is needed to discover the biological mechanisms underlying this sensitivity. One possible explanation could be the existence of a higher density in dopamine D_2 receptors in avoiders compared to approachers.

(±)8-OH-DPAT, on the other hand, showed no differences in effect between avoiders and approachers. The 5-HT_{1A} receptor agonist significantly decreased time spent in the male compartment in approachers only, while it inhibited darts and hops in both avoiders and approachers. Other studies have shown the inhibiting effect of (±)8-OH-DPAT on lordosis behavior.[27-29] The lack of effect on time spent in the male compartment in avoiders could be due to an already reached floor effect.

Therefore, we conclude that the 5-HT_{1A} receptor is involved in female sexual behavior, but that the receptor is not affected in females with FSD. There is no 5-HT_{1A} receptor desensitization in avoiders, as we showed following chronic paroxetine treatment (chapter 6) and in serotonin transporter (SERT) knockout rats.[41]

Finally, acutely administered paroxetine did not affect sexual behavior in both avoiders and approachers. Paroxetine is a selective serotonin reuptake inhibitor (SSRI) acting on the SERT. No other rat studies with paroxetine are published, but in women it causes sexual dysfunctions, just as other SSRIs.[30, 31] However, this effect is only seen after chronic and not after acute treatment. In our experiment paroxetine was administered acutely, which could explain the differences in results. A study of Sarkar et al.[42] found an inhibitory effect of acute fluoxetine treatment (10 mg/kg) on lordosis, whereas subchronic (10 days) treatment did not affect it. High doses (20 mg/kg) of fluoxetine reduced proceptive behavior both after acute and subchronic administration. On the other hand, Matuszczyk et al.[43] reported reduced proceptive and receptive behavior in female rats following chronic treatment with 10 mg/kg fluoxetine (SSRI). In their study, the inhibitory effects on sexual activity became most prominent after 21 to 28 days of treatment. Nonetheless, there are no studies performed with acute and chronic paroxetine treatments in female rats yet. Based on our results, we conclude that acute paroxetine administration is not affecting sexual behavior in female rats and that there is no difference in sensitivity of the serotonin transporter between avoiders and approachers.

Experiment 1 was performed to investigate the dependence of the female sexual activity on the performance of the male rat. Previously, we showed that male rats can be divided based on their number of ejaculations in a 30 minute sex test, in sluggish, normal and fast. The results of our experiment showed that the male's sexual performance is irrelevant for the sexual activity of the female. There was no preference for a certain endophenotypic male rat (figure 1). However, female rats preferred the male of first choice over the others, but the endophenotype of this male rat was not important.

Sexual receptivity can be induced with different strategies. One possibility is the use of ovariectomized females, and thereby inducing estrus by injecting a combination of estradiol and progesterone. The dosage of progesterone is important in the full induction of receptivity.[14, 44, 45] Another option is administration of only estradiol in intact females, like in this study. This strategy was based on our experiences with sex studies in male rats, where females were used as stimulus rat. Injections with high doses of estradiol (50 µg) induce fully receptive behavior,[41, 46, 47] since the ovaries still produce progesterone and testosterone. Furthermore, intact females, in contrast to OVX females, have a normal cycle and therefore the normal changes in gene expression caused by estrogen. On the whole, in all our female rats, full receptivity was induced and thereby we can conclude that the male-avoiding behavior was not due to lack of receptivity. We should consider the possibility that the lower levels of sexual activity of the avoiders could be due to a differential sensitivity to estradiol. Of course, this would not be different in ovariectomized hormone-primed females. An advantage of using intact females, instead of ovariectomized (OVX) rats, is that their normal hormone production and their effects on the body are intact.

At last, we would like to point out some limitations of this animal model. First, the endophenotypes are based on the parameter time spent in the male compartment. Despite the fact that our results show clear differences in time with the male that is stable over time, this parameter could be influenced by social avoidance and thereby may interfere with the quantifications of the sexual behaviors. However, our results show that time spent with the male is also accompanied by the number of proceptive behaviors and paced mating measures. Together, this suggests that our parameters measure sexual activity and that time spent with the male can be used as selection criterion. In future, more experiment can be done to investigate this appearance more, like partner preference tests etc. Furthermore, there could be several reasons for the existence of male-avoiding behavior in female rats, for instance differential sensitivity to olfactory cues, major histocompatibility complex (MHCs) or to vaginocervical stimulation (some females may find male sexual stimulation more aversive than other females). In addition, it could be that the avoiders had more or less maternal care after birth, different locomotor activity or social deficits. All these possibilities can be studied in future to reveal the underlying mechanisms causing FSD in the male-avoiders.

A second limitation is that the test takes a lot of time before a selection of endophenotypes for further testing can be made. And finally, a substantial number of female rats is needed to select enough male-avoiders. On the other hand, a strong advantage of this animal model is the possibility to investigate "dysfunctional" avoiders parallel to "healthy" approachers. Overall, both the advantages and limitations of this model for FSD should be taken in account.

Conclusion

Overall, our results suggest that different sexual endophenotypes are present in female Wistar rats and that the avoiders can be used as an animal model for FSD. With no effective treatment for FSD available, this animal model would provide a way to search for such a treatment, to assess possible sexual side effects of pharmacological compounds, and to study the neurobiological background of FSD. Our study showed a possible difference in sensitivity to dopamine agonists between avoiders and approachers.

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Combination of testosterone and vardenafil increases female sexual functioning in sub-primed rats

4

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Abstract

Introduction. Hypoactive sexual desire disorder (HSDD) is a common problem in women and may have a negative impact on their quality of life. A recent clinical study shows an increase in sexual drive of HSDD women after co-treatment of testosterone and vardenafil (PDE-5 inhibitor).

Aim. In this study we investigated the effect of testosterone and vardenafil on sexual activity in female rats.

Methods. Ovariectomized female rats, sub-primed with only estradiol and fully-primed with estradiol and progesterone, were tested in a paced-mating sex test and sexual behaviors were quantified. The effect of testosterone (100 and 300 µg, s.c.) and vardenafil (10 mg/kg p.o.) alone and testosterone (300 µg, s.c.) in combination with vardenafil (3 and 10 mg/kg, p.o.) were tested. We also studied the effects of testosterone (300 µg, s.c.) + intracerebroventricular (i.c.v.) injections of vardenafil (25 and 50 µg) on sexual activity.

Main outcome measures. Proceptive (darts and hops), receptive (lordosis), and paced mating (percentages after exits and contact-return latencies) behaviors were quantified.

Results. No effect of testosterone and vardenafil alone was found, but co-treatment of testosterone and vardenafil (p.o.) caused a significant increase in proceptive and receptive behavior in the sub-primed female rats. Testosterone and vardenafil did not affect fully-primed females. I.c.v. administration of vardenafil combined with systemic testosterone, on the other hand, had no effect on sexual activity in both sub-primed and fully-primed female rats.

Discussion. We conclude that co-treatment of subcutaneous testosterone and oral vardenafil increase sexual activity. The beneficial effects of co-treatment of testosterone and vardenafil may be due to local action in the vaginal area or a combination of peripheral and brain actions. Overall, we support the human finding that combination treatment of testosterone and vardenafil could be used as a new treatment for women with HSDD.

Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV [1]), female sexual dysfunction (FSD) can be divided in four main categories: low sexual desire, low arousal, orgasmic disorders, and sexual pain. Each is defined as "persistent or recurrent" and causes "marked distress or interpersonal difficulty". The prevalence of FSD in the human population ranges from 33-48% in the USA and in Europe.[2-8] The majority of sexual dysfunction surveys identify low sexual desire/interest and sexual arousal disorder as the most common problems.[4-8]

The biological mechanisms underlying the different types of FSD are not well known, and may differ between women. Especially decreased libido and lack of sexual arousal may be due to hormonal changes. The menopause, for instance, induces a decline in estrogen and androgen levels. Furthermore, surgeries like ovariectomy and hysterectomy, cause a drop in hormone levels[9, 10] and may lead to FSD. A few double-blind studies have shown that hormone replacement therapy could have beneficial effects on sexuality in these women.[11, 12] Testosterone, delivered by transdermal patches, can improve sexual function and decrease distress.[13] Sometimes testosterone is administered together with estrogens. Unfortunately, this beneficial effect is only seen in women suffering from FSD after ovariectomy surgery or menopause[14] and not in other forms of FSD.

For men with erectile dysfunctions, phosphodiesterase type 5 (PDE-5) inhibitors, like sildenafil and vardenafil, are effective treatments.[15, 16] Whether these medications are beneficial in women with sexual disorders is unclear. Some studies showed an increase in clitoral sensitivity,[17] arousal, and frequency of sexual fantasies, sexual intercourse, and orgasm.[18] There is only one randomized and placebo-controlled clinical trial performed and this trail showed no improvement of vardenafil on sexual response among women with sexual arousal disorder.[19] Overall, no clear proof of an effect of PDE-5 inhibitors on female sexual functioning is available.

Recently Van der Made et al.[20] showed positive effects of testosterone combined with vardenafil on females with low sexual desire disorders. They showed that the combination of testosterone and vardenafil enhances their sexual motivation during exposure to erotic visual stimuli. This effect was only seen in females suffering from FSD and not in healthy women. Thereby, this research offers an interesting potential treatment for women with FSD.

As animal model of FSD, we developed a so-called 'sub-primed' model in which female rats are ovariectomized and administered with low doses of estradiol. As control, 'fully-primed' rats are injected with both estradiol and progesterone.

Based on the study of Van der Made et al.,[20] we performed an experiment in which we investigated the effect of testosterone and vardenafil alone and combined in sub- and fully-primed rats. We have found that the combined treatment increased sexual excitement in the sub-primed female rats. Next, we investigated whether this stimulatory effect was due to a central or peripheral action of vardenafil.

Materials and methods

Animals

For the first three experiments, Wistar female rats (n=45, 3 months of age at the beginning of the experiment) and stimulus Wistar male rats (n=45, 6 months of age) were used (Harlan, Zeist, The Netherlands). In the last experiment, new females and males (both n=45, 3 months of age) were used. All animals were housed in the Central Animal Laboratory of the Utrecht University. The rats were adapted to the laboratory environmental condition and a reversed 12/12-h light/dark cycle (lights off at 7 am). Standard food and water were available ad libitum. Furthermore, male and female rats were housed in separate rooms in groups of four per Macrolon type-IV cage. The male rats were sexually trained with a different set of females before the experiments.

The female rats were bilaterally ovariectomized under isoflurane anesthesia 14 days before the start of the experiment. Sexual receptivity was induced by subcutaneous administration of estradiol benzoate (2 or 5 µg EB) alone or 5 µg estradiol in combination with progesterone (500 µg P). The hormones were dissolved in 0.1 ml sesame oil saturated with phosphatidylcholine 36 (EB) and 4 (P) hours prior to testing. The combination of estradiol and progesterone (fully-priming) induces full receptive and proceptive behavior in females, whereas a single injection with estradiol (sub-priming) induces low levels of receptivity. The sub-priming females could therefore model female sexual dysfunction.

All experiments were carried out in accordance with institutional, national and international guidelines for animal care and the Dutch law concerning welfare.

Drugs

Testosterone propionate (Spruyt hillen, IJsselstein, the Netherlands) was dissolved in 0.1 ml sesame oil saturated with phosphatidylcholine. Vardenafil (Levitra®, Bayer, Leverkusen, Germany) was suspended in 0.5% methylcellulose. Testosterone (s.c.) was injected in a volume of 0.1 ml 4 hours before the paced mating sex test whereas vardenafil (p.o.) was administered in a volume of 4 ml/kg 1 hour before the sex tests.

For experiment 4 (i.c.v. experiment), vardenafil was dissolved in a drop of dimethyl sulphoxide followed by dilution in Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM $CaCl_2$, and 1 mM $MgCl_2$). The drug was injected at a volume of 2 µl per rat, 10 minutes before the sex tests.

Intracerebroventricular (i.c.v.) cannula implantation

For experiment 4, a 3.7 mm guide cannula (Bilaney Consultants GmbH, Düsseldorf, Germany) was implanted in the right lateral ventricle. The coordinates of the ventricles were set at -0.92 mm anterior-posterior, +1.5 mm medial-lateral, and -3.7 mm dorsal-ventral (from bregma and skull). The cannulas were anchored in place with three screws and dental cement on the skull. The females had one week to recover from surgery before the sex tests started. For microinjections, an internal cannula (extended 5.0 mm below the tip of the guide cannula) was connected by polyethylene tubing to a 10 µl Hamilton syringe. Over a 1 minute period, the solution was manually administered.

Test cage

The test cage was divided into two compartments: a "male compartment" of 45 x 26 x 38 cm and a "female-only" compartment" of 20 x 26 x 38 cm. The compartments were divided by a transparent plastic wall containing three holes (4 cm diameter each) through which only the females could pass. Another wall with air holes was used to block the three holes. This wall was present during the habituation phase of the sex test.

Behavioral procedure

All tests were conducted under red light during the dark phase of the light/dark cycle. The behavioral procedure was performed exactly the same as in our previous studies.[21, 22] The paced mating 30 minute sex tests were videotaped for event recording of female receptive behavior (lordosis), proceptive behavior (number of darts and hops), percentages of exits, contact-return latencies, male sexual behavior (number of mounts, intromissions and ejaculations), as well as time spent in the male compartment. The measurements of the behaviors were also the same as in our previous studies.[21, 22]

Experimental setup

Experiment 1: Dose finding of estradiol and progesterone

First, four groups of rats received four doses of estradiol (0, 2 µg, 5 µg and 10 µg, s.c.) 36 hours before the experiment. Then, they received again 5 µg estradiol (36 hours before the experiment), but also two doses of progesterone (200 µg and 500 µg, s.c.) 4 hours before the sex test. The females were tested in a within-subject Latin square design with one-week intervals.

Experiment 2: Dose finding of testosterone

Three groups of females (n=15) received a different dose of testosterone (0, 100 µg and 300 µg) during all tests. The females were tested in three hormone-primed situations at one-week intervals (within-animal with a Latin square design): in non-estrus, sub-primed (2 µg estradiol) and fully-primed (5 µg estradiol and 500 µg progesterone).

Experiment 3: Effect of vardenafil

The females were divided in three hormone groups (n=15): non-estrus, sub-primed (2 µg estradiol) and fully-primed (5 µg estradiol and 500 µg progesterone). Each group received 4 different treatments: vehicle + vehicle, 300 µg testosterone (TP) + vehicle, vehicle + 10 mg/kg vardenafil and 300 µg testosterone + 10 mg/kg vardenafil. The tests were performed with one-week intervals in a within-animal design (Latin square).

Experiment 4: Dose finding of vardenafil

The females were divided in three hormone groups (n=15): non-estrus, sub-primed (5 µg estradiol) and fully-primed (5 µg estradiol and 500 µg progesterone). In this experiment, the hormone treatments remained unchanged, whereas the three treatments (vehicle + vehicle, 300 µg testosterone + 3 mg/kg vardenafil and 300 µg testosterone + 10 mg/kg vardenafil) were given in a within-animal design (Latin square). The tests were performed with one-week intervals.

Experiment 5: i.c.v. administration of vardenafil

In this experiment, the hormone treatments remained unchanged (sub-primed (5 µg estradiol) and fully-primed (5 µg estradiol and 500 µg progesterone)), whereas the three treatments were given in within-animal design (Latin square). All females received vehicle + vehicle, 300 µg testosterone (s.c.) + 25 µg vardenafil (i.c.v.) and 300 µg testosterone (s.c.) + 50 µg vardenafil (i.c.v.). The tests were performed with one-week intervals. The doses of vardenafil were based on a recent study of Sanna et al.[23]

Vardenafil alone was not tested, because it did not have an effect by itself in experiment 4.

Data analysis

The levels of proceptive behaviors, time spent with the males, percentages of exits, and contact-return latencies were analyzed by ANOVA mixed models. Further post hoc analysis was done with one-way ANOVA using Bonferroni correction.

The lordosis data was analyzed with the non-parametric Kruskall-Wallis test, because there was no homogeneity of variances. Further post hoc analysis was performed with a Mann-Whitney U-test. The level of significance was set at $p<0.05$.

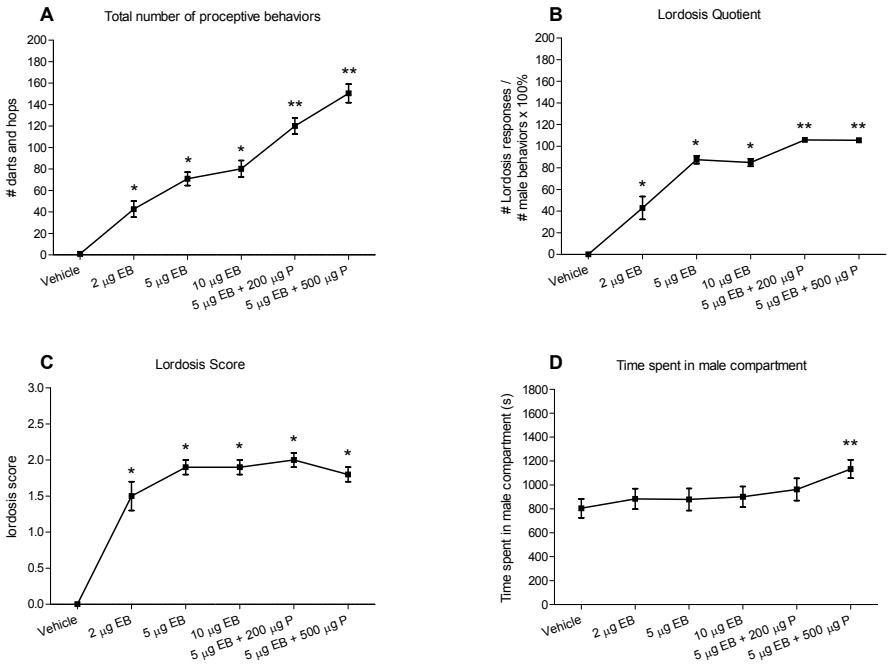

Figure 1: levels of sexual behavior after administration of estradiol (EB) and progesterone (P): (A) total number of proceptive behavior (darting and hopping); (B) lordosis quotient; (C) lordosis score; and (D) time spent in male compartment (s). All in OVX Wistar females rats during a 30 min test. Data are median± SEM for lordosis data and means ± SEM for the rest; *p<0.05, **also significantly different (P<0.05) from EB-primed females.

Main outcome measures

Proceptive, receptive and paced mating behaviors were quantified.

Results

Experiment 1: Dose finding of estradiol and progesterone

Estradiol treatment showed a dose dependent increase in total number of darts and hops in female rats ($F_{(3,6)}$=34.762, p<0.001) (figure 1a). All doses were significantly different from the vehicle treatment and 2 µg was also different from 5 µg and 10 µg estradiol. A similar pattern was seen on lordosis score (χ^2=62.505, p<0.001) (figure 1c). 2, 5 and 10 µg estradiol also increased the lordosis quotient (χ^2=15.888, p=0.001) in the females (figure 1b). No effect was found on time spent in the male compartment (figure 1d) and percentages after mounts and intromissions (table 1a). Only 5 µg estradiol showed a slight decrease in contact-return latency (CRL) after mounts compared to 2 µg estradiol, but this effect was not significant ($F_{(2,5)}$=2.942, p=0.067). The vehicle group was not analyzed, whereas the females did not perform enough sexual behaviors to calculate the percentage of exits and CRL.

Estradiol and progesterone also showed a significantly dose-response increase in proceptive behavior ($F_{(2,5)}$=28.066, p<0.001) (figure 1a) and lordosis quotient (χ^2=8.798, p=0.012) (figure 1b). 500 µg progesterone (in combination with estradiol) showed an increase compared to 200 µg on number of darts and hops. No effect

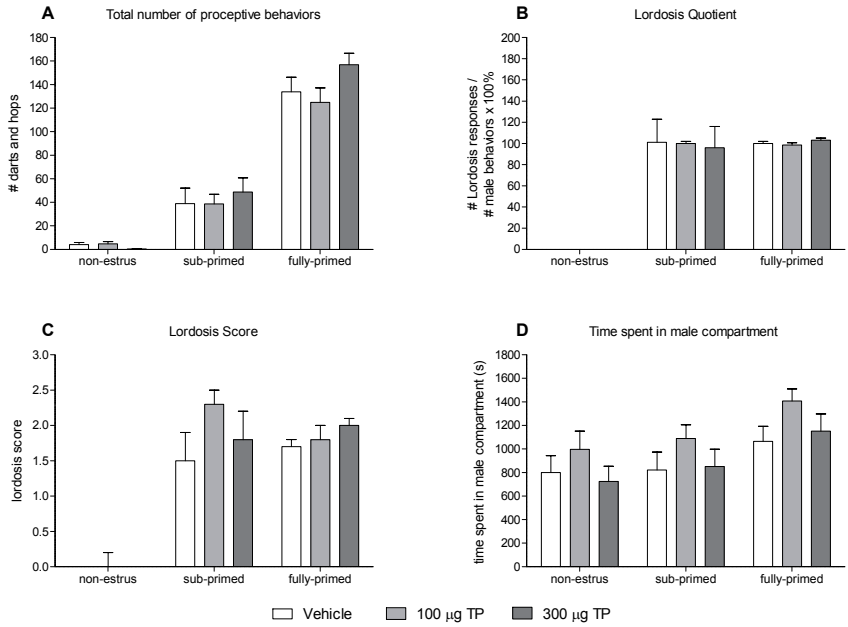

Figure 2: levels of sexual behavior after administration of 100 and 300 µg testosterone propionate (TP, s.c.): (A) total number of proceptive behavior (darting and hopping); (B) lordosis quotient; (C) lordosis score; and (D) time spent in male compartment (s). All in OVX Wistar females rats during a 30 min test. Sub-primed females were hormonally primed with 2 µg estradiol and fully-primed with 5 µg estradiol plus 500 µg progesterone. The non-estrus group was not primed. Data are median± SEM for lordosis data and means ± SEM for the rest; *p<0.05.

was found on lordosis score (figure 1c) and only a small effect of 500 µg progesterone compared to vehicle ($F_{(2,5)}$=4.879, p=0.010) on time spent with the male rat (figure 1d) was found. The percentage of exits after mounts and intromissions were not different with or without progesterone treatment (table 1a), but 200 µg and 500 µg progesterone significantly reduced the CRL after both mounts ($F_{(2,5)}$=6.328, p=0.003) and intromissions ($F_{(2,5)}$=34.106, p<0.001).

Experiment 2: Dose finding of testosterone

Testosterone propionate did not have any effect on proceptive behavior (figure 2a), lordosis quotient (figure 2b) or lordosis score (figure 2c) of non-estrus, sub-primed and fully-primed females. Furthermore, there was no effect on time spent in the male compartment (figure 2d) and percentages of exits after mounts and intromissions (table 1b). (CRLs of the sub-primed group could not be calculated because their low sexual behavior). No interaction effects between testosterone and hormone treatments were found.

In agreement with experiment 1, the non-estrus, sub-primed and fully-primed groups still differed from each other. Sub-primed rats darted significantly more than non-estrus and less than fully-primed females ($F_{(2,11)}$=176,120, p<0.001). There was no effect in lordosis quotient and lordosis score between sub-primed and fully-primed rats, but there was a significant increase compared to non-estrus

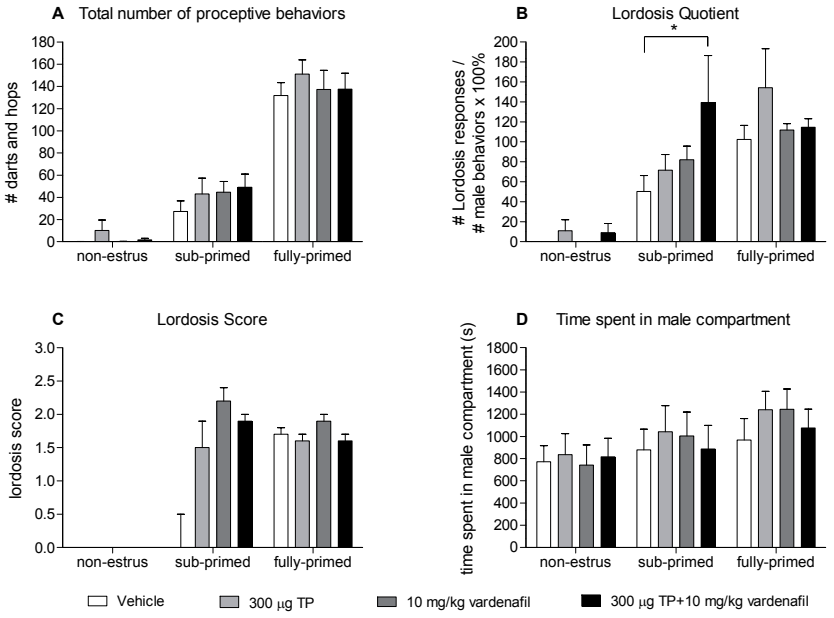

Figure 3: levels of sexual behavior after administration of 300 µg testosterone propionate (TP, s.c.), 10 mg/kg vardenafil or 300 µg TP (s.c.) and 10 mg/kg vardenafil (p.o.): (A) total number of proceptive behavior (darting and hopping); (B) lordosis quotient; (C) lordosis score; and (D) time spent in male compartment (s). All in OVX Wistar females rats during a 30 min test. Sub-primed females were hormonally primed with 2 µg estradiol and fully-primed with 5 µg estradiol plus 500 µg progesterone. The non-estrus group was not primed. Data are median± SEM for lordosis data and means ± SEM for the rest; *p<0.05.

A	Percentages of exits after mounts	Percentages of exits after intromissions	CRL after mounts	CRL after intromissions
Vehicle	-	-	-	-
2 µg EB	14.63±3.48	24.72±4.91	87.45±14.70	114.66±15.95
5 µg EB	13.76±3.43	22.25±4.23	41.43±4.45	119.44±14.14
10 µg EB	13.67±3.20	32.78±4.77	53.99±7.33	77.54±5.52
5 µg EB + 200 µg P	18.28±3.64	37.38±5.74	35.34±5.16*	74.06±20.51*
5 µg EB + 500 µg P	16.30±3.26	34.37±5.36	20.81±1.77*	24.95±1.69*

B	Percentages of exits after mounts		Percentages of exits after intromissions		CRL after mounts		CRL after intromissions	
	Sub-primed	Fully-primed	Sub-primed	Fully-primed	Sub-primed	Fully-primed	Sub-primed	Fully-primed
Experiment 2:								
Vehicle	5.13±3.45	9.85±5.21	37.00±13.25	47.08±9.67	-	87.62±60.65	-	34.12±5.33
100 µg TP	2.00±1.37	3.62±2.69	15.67±7.34	16.00±8.62	-	23.95±11.83	-	41.19±7.22
300 µg TP	23.56±9.43*	17.10±3.60	50.78±13.40	45.57±10.52	-	35.71±7.55	-	35.82±11.98
Experiment 3:								
Vehicle	16.67±11.24	7.60±5.72	27.46±13.16	20.96±9.71	-	29.17±5.37	80.27±13.56	41.12±6.15
300 µg TP	10.43±7.27	7.68±5.32	52.50±11.32	21.17±11.28	61.54±12.16	28.36±6.35	151.98±28.47	47.40±7.02
10 mg/kg vardenafil	3.16±2.12	8.20±6.26	31.25±11.11	23.03±8.44	58.37±16.80	104.01±39.64	172.66±35.23	36.00±6.93
300 µg TP + 10 mg/kg vardenafil	11.25±6.75	7.18±4.00	36.81±12.07	25.09±8.97	83.43±17.55	46.99±9.82	108.07±9.20	52.61±10.91
Experiment 4:								
Vehicle	38.91±9.37	25.63±10.80	38.89±10.80	46.29±12.71	86.97±31.92	50.10±13.50	95.97±25.47	26.53±6.98
300 µg TP + 3 mg/kg vardenafil	33.65±9.64	26.15±10.87	41.95±12.26	26.46±12.24	149.62±35.26	34.37±13.83	140.27±28.45	12.46±8.17
300 µg TP + 10 mg/kg vardenafil	31.12±9.64	39.29±10.45	45.71±13.01	45.66±12.29	73.18±32.08	44.66±13.52	85.64±29.90	38.00±6.72*
Experiment 5:								
Vehicle	69.42±10.85	75.67±8.82	78.84±8.92	90.07±8.11	34.36±12.58	45.68±17.31	33.04±10.23	40.42±14.25
300 µg TP + 25 µg vardenafil	62.86±10.90	72.32±8.81	77.49±8.96	74.89±8.11	60.26±12.92	75.94±17.31	60.90±10.24	58.79±14.25
300 µg TP + 50 µg vardenafil	64.84±11.67	59.34±8.81	63.00±9.32	77.22±8.23	46.23±12.58	31.93±18.81	39.05±12.17	34.25±16.24

Table 1: Percentages of exits and contact-return latencies (CRL) after mounts and intromissions during 30 min tests. The table shows (A) data of experiment 1 and (B) data of experiment 2, 3, 4 and 5 in both sub-primed and fully-primed OVX females. Non-estrus females (without hormonal priming) did not receive mounts and intromissions and were therefore not included in the table. Also, experiment 2 did not have enough data points to calculate CRL. Data are means ± SEM; *p<0.05.

(lordosis score: χ^2=37.977, p<0.001; lordosis quotient: χ^2=41.368, p<0.001). The time spent with the male was significantly different between non-estrus and fully-primed treatment ($F_{(2,11)}$=12.180, p<0.001).

Experiment 3: Effect of vardenafil

None of the treatments (testosterone and vardenafil alone, or in combination) had an effect on proceptive behaviors (figure 3a), lordosis score (figure 3c) or time spent with the male (figure 3d). There was also no effect on percentages of exits or contact-return latencies after mounts or intromissions (table 1b). Only the combination treatment of testosterone and 10 mg/kg vardenafil had a stimulating effect on lordosis quotient compared to vehicle (Z=-2.561, p=0.010) (figure 3b).
Sub-primed rats darted significantly more than non-estrus and less than fully-primed females ($F_{(2,14)}$= 163.33, p<0.001). The same was found for lordosis quotient (χ^2=69.415, p<0.001).

Experiment 4: Dose finding of vardenafil

In this experiment, a higher dose of estradiol (5 µg instead of 2 µg) was used in the sub-primed females. Figure 4a shows the stimulating effect of testosterone combined with 10 mg/kg vardenafil on number of darts and hops in the sub-primed group ($F_{(2,5)}$=4.226, p=0.026). In the non-estrus and fully-primed group, no in-

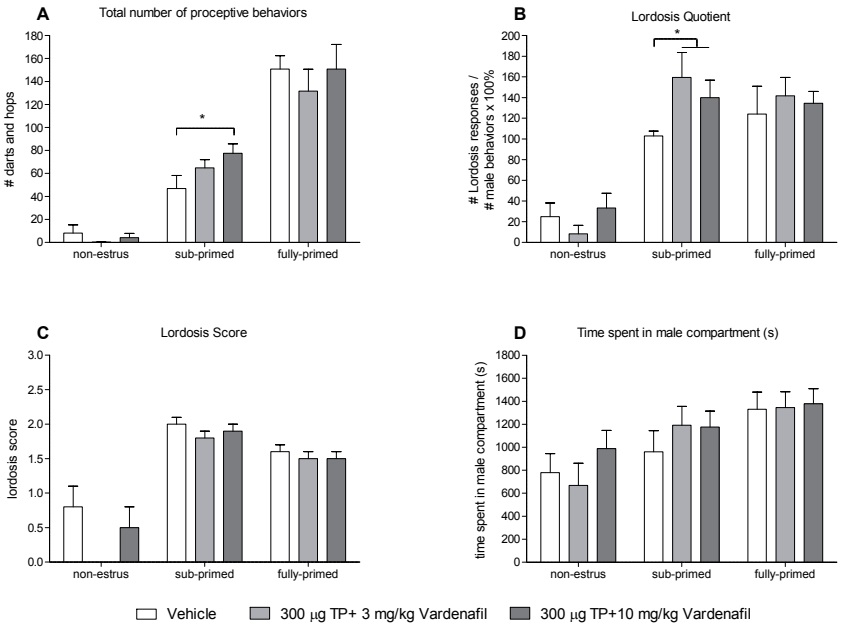

Figure 4: levels of sexual behavior after co-administration of 300 µg testosterone propionate (TP, s.c.) with 3 and 10 mg/kg vardenafil (p.o.): (A) total number of proceptive behavior (darting and hopping); (B) lordosis quotient; (C) lordosis score; and (D) time spent in male compartment (s). All in OVX Wistar females rats during a 30 min test. Sub-primed females were hormonally primed with 5 µg estradiol and fully-primed with 5 µg estradiol plus 500 µg progesterone. The non-estrus group was not primed. Data are median± SEM for lordosis data and means ± SEM for the rest; *p<0.05.

creasing effect was found. Testosterone and vardenafil also increased the lordosis quotient (χ^2=8.503, p=0.014) in the sub-primed group (figure 4b). However, this effect was also induced by a lower dose of vardenafil (3 mg/kg: Z=-2.601, p=0.009; 10 mg/kg: Z=-2.403, p=0.016). No significant effect were found in lordosis score (figure 4c), time spent with the male (figure 4d) and percentages of exits after mounts and intromissions (table 1b). Only in the fully-primed females, the rats had significantly longer CRL after intromissions with 10 mg/kg vardenafil compared to 3 mg/kg vardenafil ($F_{(2,5)}$=5.492, p=0.038). Both treatments did not differ from the vehicle group and thus the relevance of this observation is questionable.

Experiment 5: i.c.v. administration of vardenafil

Testosterone administration combined with intracerebroventricular (i.c.v.) injections with vardenafil did not affect the number of darts in hops in sub- and fully-primed females (figure 5a). Furthermore, there was no effect of the local infusions on lordosis quotient (figure 5b), lordosis score (figure 5c) and time spent in male compartment (figure 5d). Percentages of exits and contact-return latencies after mounts and intromissions were also not affected in this experiment (table 1b).
The absence of effect was not due to cannula misplacements, because the positions of all cannulas were checked and approved.

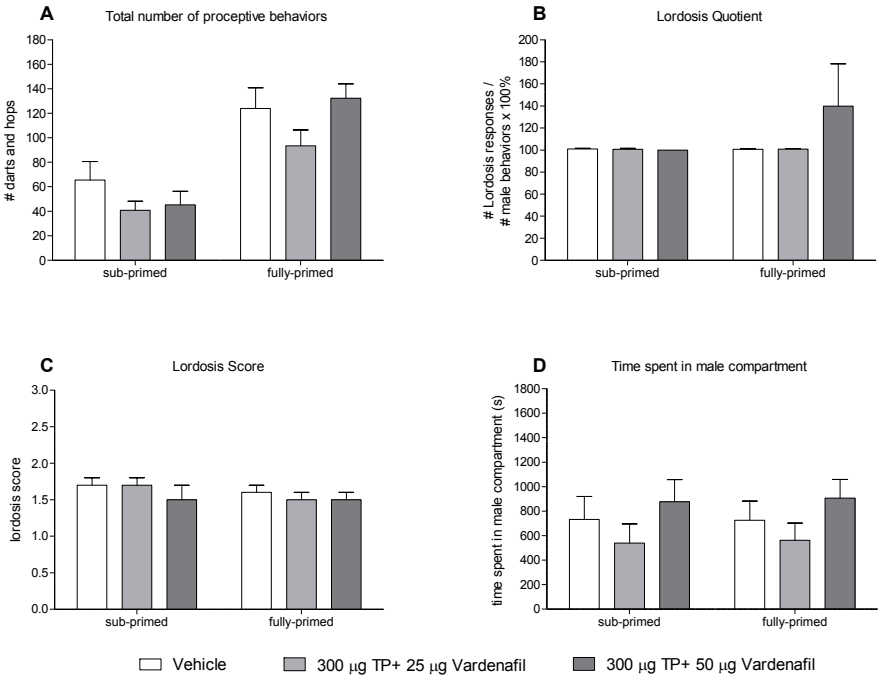

Figure 5: levels of sexual behavior after local intracerebralventricular (i.c.v.) administration of 25 and 50 µg vardenafil in combination with 300 µg testosterone propionate (TP, s.c.): (A) total number of proceptive behavior (darting and hopping); (B) lordosis quotient; (C) lordosis score; and (D) time spent in male compartment (s). All in OVX Wistar females rats during a 30 min test. Sub-primed females were hormonally primed with 5 µg estradiol and fully-primed with 5 µg estradiol plus 500 µg progesterone. Data are median± SEM for lordosis data and means ± SEM for the rest; *p<0.05.

Discussion

The most important finding in this experiment was the stimulating effect of a combination treatment of testosterone and vardenafil on sexual activity in dysfunctional female rats. Both proceptive and receptive behaviors were improved by this combination treatment, while the effects were not seen in fully-primed rats. This finding is in line with the study of Van der Made et al.[20] that showed the same results in women suffering from desire disorders, strongly supporting that a combination of testosterone and vardenafil is a new treatment for FSD.

Interestingly, the stimulating effect on proceptive behavior of the combination treatment of testosterone and vardenafil was only found in females sub-primed with 5 µg estradiol, and not with 2 µg estradiol, while the effect was present on lordosis quotients with both doses of estradiol. This may suggest that a minimum amount of hormonal priming is needed before darts and hops can be affected by stimulating drugs. But because of the effects on lordosis quotient, it is reasonable to suggest that the combination treatment has already some prosexual effects on sub-primed females injected with only 2 µg estradiol.

In experiment 4, we did not test vardenafil or testosterone alone. So formally, we can not conclude that a combination of these drugs is needed to stimulate sexual behavior in sub-primed rats. However, based on the following two arguments, we are convinced that both drugs are needed for stimulation of female sexual behavior. First, in the experiment with 5 µg estradiol (experiment 4) the combination of testosterone and 3 mg/kg vardenafil had no effect on darts and hops. Therefore, it is very likely that testosterone alone under these estradiol conditions would also be without an effect. The second argument is coming from experiment 3 (2 µg estradiol), in which 10 mg/kg vardenafil did not have an effect on lordosis, while the combination treatment with testosterone had.

An important question now is what testosterone is doing that enables vardenafil to stimulate sexual behavior. In humans, it is described that testosterone induces an increase in preconscious sexual attention,[24] and thereby creates the right environment for vardenafil to stimulate sexual motivation during exposure to erotic stimuli. Testosterone was given 4 hours before the sex test, while vardenafil was given 30 minutes before. In the human study by van der Made et al.,[20] it was shown that this time window is essential. It is likely that testosterone binds to androgen receptors, which leads to changes in gene expression,[25] which subsequently enables the behavioral effects of vardenafil.

But what is the working mechanism of vardenafil? In males, vardenafil induces smooth muscle relaxation in the corpora cavernosa by inhibition of PDE-5.[26-28] This effect is arranged via the nitric oxide (NO) system that is produced in the nerve terminals after activation of NO synthase. The released NO acts primarily through triggering an increase in second messenger cyclic guanosine monophosphate (cGMP), which in turn causes smooth muscle relaxation.[26] In addition, as shown by Sanna et al.,[23] PDE-5 inhibitors may also regulate sexual excitement by an effect on the brain. Micro-injections with PDE-5 inhibitors (sildenafil, vardenafil and tadalafil) into the lateral ventricles and the caudal ventral tegmental area (VTA) caused an increase of noncontact erections in males. This effect was accompanied with an increase in extracellular dopamine levels in the nucleus accumbens. Other studies revealed that

PDE-5 inhibitors can also prolong dopamine behavioral effects.[29, 30] Together, these results suggest that PDE-5 inhibitors injected in the brain induce penile erection by increasing the activity of mesolimbic dopaminergic neurons. This seems a plausible theory, as dopamine is known to be involved in the translation of motivational aspects of natural stimuli into goal-directed behaviors.[31] But how does vardenafil work in females? One possibility is that vardenafil increases blood flow to the vaginal area, in analogy with the effects of this drug on the male penis. Corpora cavernosa tissue is also present in the glans clitoris of women.[32] PDE-5 inhibitors may, therefore, also affect female sexual excitement via relaxation of corpora cavernosa.[26]
As we showed in experiment 5, i.c.v. administration of vardenafil (two different doses were tested that have been reported to be effective in males[23]) did not stimulate sexual behavior in the female rats. This suggests that in females the prosexual effects of vardenafil are not regulated in the brain, but is solely a peripheral effect.

But overall, how relevant are the observed effects of testosterone and vardenafil? We found that the combination of 300 µg testosterone and 10 mg/kg vardenafil almost doubled the total amount of darts and hops (figure 3a) compared to vehicle treatment. However, this effect is still lower than the amount of darts and hops in the fully-primed rats. We assume that the effect of treatment can be further optimized by using different doses of testosterone and vardenafil and by changing the time window between testosterone and vardenafil administration.
Our results show that the combination treatment is only effective in sub-primed females. This is in agreement with the study of Van der Made et al.[20] and suggests that testosterone can only establish an increase in sensitivity for sexual stimuli in females with initial low levels of sexual attention. Therefore, the combination of testosterone and vardenafil will probably only be beneficial in women with hyposexual desire disorders and not in healthy volunteers. And thus, this treatment may not only be beneficial in females with low hormonal levels, but also in women suffering from FSD not caused by hormonal deficiency.
Furthermore, it should be taken into account that testosterone can induce side effect as increased facial oiliness, acne, deepening voice, hostility, weight gain, alopecia, elevated liver functions, lower HDL levels, and carcinoma.[33, 34] However, this is only reported after repeated intake of testosterone. In this study, acute administration of testosterone combined with vardenafil showed prosexual effects, indicating that the treatment can be taken on demand. This lowers the amount of testosterone intake and the chance of developing side effects. Furthermore, combination treatments with other drugs might lower the amount of testosterone necessary to induce the prosexual effects, with fewer side effects as a result.
At last, we would like to discuss the animal model of FSD that we have used in these experiments. In experiment 1, we showed that sub-primed female rat performed only a low amount of proceptive and receptive behavior. The fully-primed rats, on the other hand, had a much higher level of sexual behaviors. Therefore, we suggest that sub-primed females model FSD, whereas fully-primed females model normal sexual acting woman.
Interestingly, we did not find relevant differences in time spent in male compartment between non-estrus, sub-primed and fully-primed females. In a previous study, we have used this parameter to select "sexual dysfunctional" females from

a rat population.[22] This seems quite arbitrary, but we hypothesize that time spent in male compartment is only important for sexual behavior in hormonally fully-primed females. The sexual dysfunctional rats (male-avoiders) from the other study were intact females primed with a high dose of estradiol. Now, the male rats will smell the estrus female and will try to copulate. Rejective behavior by male-avoiders will not stop the male from copulation, because he also receives the estrus signals/odors. Therefore, the only way for the female to escape from sexual behavior is to run away to her own compartment. The non-estrus females from current experiments, on the other hand, are not hormonally primed. The males will not try to copulate, because they will not be confused with "receptive" odors. In addition, the non-estrus females do not have a reason to escape from the male anymore, and may stay based on social behavior.

Conclusion

We conclude that co-treatment of subcutaneous testosterone and oral vardenafil increase sexual activity in an animal model for FSD. The beneficial effects of co-treatment of testosterone and vardenafil may be due to an action locally in the vaginal area or in combination with certain activated brain mechanisms. Overall, we conclude that the combination treatment of TP and vardenafil could be used as a new treatment for women with HSDD.

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Serotonin transporter null mutation and sexual behavior in female rats: 5-HT_{1A} receptor desensitization

5

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Abstract

Introduction. Serotonin plays a key role in sexual behavior. In serotonin transporter (SERT) knockout rats (-/-), basal extracellular 5-HT levels are considerably increased, indicating a serotonergic disturbance. Heterozygous SERT(+/-) rats express 50% of SERT in comparison to wildtype rats and may therefore model the s/s phenotype of the human SERT promoter (5-HTTLPR) polymorphism.

Aim. In the present study we used both homozygote and heterozygote SERT knockout and wildtype rats (+/+) to study the putative role of the SERT in female sexual behavior.

Methods. Female rats were brought into estrus by hormonal injections before the paced mating sex tests. The effects of the 5-HT_{1A}/5-HT_{7} receptor agonist (±)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide ((±)8-OH-DPAT) (0.03-1 mg/kg s.c.) and the 5-HT_{1A} receptor antagonist WAY-100635 (0.1-1 mg/kg i.p.) on sexual behaviors of females were tested separately and in a selected combination of both in all three genotypes.

Main outcome measures. Proceptive (darting and hopping) and receptive (lordosis) behaviors were quantified.

Results. Basal proceptive and receptive sexual activities were not different between SERT+/+, +/- and -/- female rats. The dose-effect curve after (±)8-OH-DPAT for these activities was clearly shifted to the right in SERT-/- animals compared to other genotypes. WAY-100635 alone had no effect on sexual behavior in any genotype, but was able to antagonize the (±)8-OH-DPAT-induced decrease in sexual activities indicating the involvement of the 5-HT_{1A} receptor.

Conclusions. The absence (-/-) or reduced (+/-) expression of SERT does not affect basal sexual activity in female rats in a paced mating situation. The data indicate desensitized 5-HT_{1A} receptors in the SERT-/-, but not in the SERT+/- females. Under normal basal conditions, desensitized 5-HT_{1A} receptors apparently do not play a role in female sexual behavior of the SERT-/-. However, upon activation of the 5-HT_{1A} receptor in "normal" females (SERT+/+ and SERT+/-), a hyposexual behavior is induced.

Introduction

The prevalence of female sexual dysfunction (FSD) in the human population ranges from 33-48% in the USA and in Europe.[1-7] According to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV),[8] FSD can be divided in four main categories: low sexual desire, low arousal, orgasmic disorders, and sexual pain. Each is defined as "persistent or recurrent" and causes "marked distress or interpersonal difficulty". The majority of sexual dysfunction surveys identify low sexual desire/interest and sexual arousal disorder as the most common problems.[3-7] Large percentages (36-56%) of females using selective serotonin reuptake inhibitors (SSRIs), mostly for depressive symptoms, complain about sexual dysfunction.[9-11] The problem with the interpretation of these studies is that a large part of non-users also suffer from FSD, and in addition one of the symptoms of depression is lack of sexual desire.

The serotonin system plays an important role in sexual behavior in rats.[12-15] Serotonin (5-HT) produces its effects through 14 different receptors. Agonists of one of these receptors, the 5-HT$_{1A}$ receptor, inhibit lordosis, the receptive behavior of female rats,[16-20] while activation of other 5-HT receptors, like 5-HT$_{2A}$ and/or 5-HT$_{2C}$ receptors, facilitates female sexual behavior.[14, 21] Animal studies investigating sexual side effects of SSRIs on females are rather limited and produce conflicting results. Matuszczyk et al.[22] reported reduced proceptive (darts and hops) and receptive (lordosis) behavior in female rats following chronic treatment with 10 mg/kg fluoxetine (SSRI). In their study, the inhibitory effects on sexual activity became most prominent after 21-28 days of treatment. On the other hand, Sarkar et al.[23] found an inhibitory effect of acute fluoxetine treatment (10 mg/kg) on lordosis, whereas subchronic (10 days) treatment did not affect it. High doses (20 mg/kg) of fluoxetine reduced proceptive behavior both after acute and subchronic administration. Thus, the role of the serotonin transporter (SERT) in female sexual behavior is less clear and needs more investigation.

In this study we examined the role of SERT in female sexual behavior using SERT knockout rats. These rats have no functional SERT mRNA and functional SERT protein[24] due to a premature stop codon in the SERT gene induced by N-ethyl-N-nitrosounea (ENU) mutagenesis.[25] As a result, 5-HT neural regulation is severely affected: 5-HT tissue levels and evoked 5-HT release are significantly reduced, while basal extracellular 5-HT levels are ninefold increased.[24] Furthermore, the heterozygous knockout (SERT+/-) rats express around 50% of the SERT compared to wildtype rats and therefore, these rats might model the s/s genotype of the human SERT polymorphism, because carriers of the s-allele show reduced mRNA and protein expression.[26-29] However, other studies were unable to find a correlation between the SERT linked promoter region and expression levels.[30, 31]

We hypothesized that SERT-/- females, and to a lesser extent also the SERT+/- females, show a decreased sexual activity, either because these animals are more anxious/depressed than wildtype rats[32] and/or because they model chronic SSRI usage. In this study, we compared basal sexual behavior of both the SERT-/- and SERT+/- with that of SERT+/+ rats. In addition, given the considerations that SERT-/- rats might model chronic SSRI administration and that desensitization of 5-HT$_{1A}$ receptors following chronic SSRI treatment may underlie the sexual side effects of this

drug class in males,[33] we tested (±)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide ((±)8-OH-DPAT), a 5-HT_{1A}/5-HT_{7} receptor agonist, and WAY-100635, a selective 5-HT_{1A} receptor antagonist alone and in combination with (±)8-OH-DPAT, on sexual behavior of females of the three genotypes. We tested the sexual behavior of the females in a paced mating situation in which the female controls (paces) the sexual interaction with the male.[34] Thereby, many parameters can be studied; e.g. percentages of exits, contact-return latencies and time spent with the male. In this experiment we mainly focused on the proceptive (darts and hops) and receptive (lordosis) female behaviors.

Materials and Methods

Animals

Serotonin transporter knockout rats (Slc6a4^{1Hubr}) on a Wistar rat genetic background were generated by ENU-induced mutagenesis.[25, 35] All females were derived from crossings between heterozygous rats and were genotyped as described previously.[36] The animals were housed in the Central Animal Laboratory of Utrecht University. In all experiments, SERT+/+, SERT+/- and SERT -/- female rats were compared. Wistar male rats (Harlan, Zeist, The Netherlands) were used as stimulus rats. All rats were housed under reversed 12/12-h light/dark cycle (lights off at 7 am). Male and female rats were housed in separate rooms in groups of four per Macrolon type-IV cage. The male rats were sexually trained before the experiments. In experiment 1 (first part) and 2, sexual receptivity was induced in intact female rats by subcutaneous administration of 50 µg estradiol benzoate (EB) dissolved in 0.1 ml sesame oil saturated with phosphatidylcholine 36 hours prior to testing. This regime induces full receptive and proceptive behavior and prevents pregnancy. In the second part of experiment 1, the female rats were bilaterally ovariectomized under isoflurane anesthesia 14 days before the

	Percentages of exits after mounts			Percentages of exits after intromissions			CRL after mounts			CRL after intromissions		
	SERT+/+	SERT+/-	SERT-/-	SERT+/+	SERT+/-	SERT-/-	SERT+/+	SERT+/-	SERT-/-	SERT+/+	SERT+/-	SERT-/-
Intact females:												
Test 1	75.41±5.21	84.11±2.72	68.64±6.80	83.01±3.49	82.19±3.37	68.86±5.77	32.31±3.93	44.42±4.66	47.99±10.5	41.27±5.16	62.81±6.03	61.21±10.29
Test 2	80.27±5.51	89.47±1.90	69.70±6.15	79.88±4.03	89.47±0.78	69.09±6.71	36.13±6.76	39.05±6.26	45.29±7.81	38.08±4.68	49.62±11.9	59.97±10.7
Test 3	79.92±4.21	80.58±3.66	67.64±6.18	84.96±3.80	89.70±1.37	77.03±5.68	40.63±5.98	35.19±4.37	41.20±6.71	47.87±9.08	40.49±3.68	56.19±7.59
OVX females:												
EB	35.25±7.06	43.41±7.14	36.79±7.51	45.30±7.06	38.04±6.33	44.26±6.63	29.93±5.20	20.44±3.49	14.10±1.24	49.31±15.6	32.03±4.05	35.90±3.56
EB+P	38.90±5.75	30.59±6.97	37.49±6.77	27.78±5.92	33.59±6.52	37.18±5.28	26.99±8.39	9.32±1.66	12.63±2.29	35.72±9.56	25.32±5.10	43.48±11.3

Table 1: Percentages of exits and contact-return latencies (CRL) after mounts and intromissions in SERT+/+, SERT+/- and SERT-/- female rats during 30 min test. The table shows both intact and OVX females. Non-estrus females did not receive mounts and intromissions and was therefore not included in the table. Data are means ± SEM; *p<0.05.

start of the experiments. Sexual receptivity was induced in these females by subcutaneous administration of 5 µg estradiol (EB) alone or 5 µg estradiol plus 500 µg progesterone (P).
All experiments were carried out in accordance with institutional, national and international guidelines for animal care and the Dutch law concerning animal welfare.

Test cage

The test cage was divided into two compartments: a "male compartment" of 45 x 26 x 38 cm and a "female-only" compartment" of 20 x 26 x 38 cm. The compartments were divided by a transparent plastic wall containing three holes (4 cm) through which only the smaller females could pass. A blockade with ten, equally spaced 5 mm holes was used during the habituation phase of the sex test (see below).

Behavioral procedure

All tests were conducted under red light during the dark phase of the light/dark cycle. First, female rats were allowed to freely explore both compartments of the test cage for five minutes. Then the holes were blocked and the female rat was placed in the "female-only" compartment, and the male rat was placed in the "male compartment". The rats could smell, hear, and see each other for 25 minutes. For every sex test a female had to perform, a different male was used.
After this habituation period, the blockade was removed and the behavior was videotaped for the next 30 minutes. Event recording software (Observer 5.0, Noldus, the Netherlands) was used to score female receptive behavior (lordosis) and to score proceptive behavior (number of darts and hops), percentages of exits, contact-return latencies, male sexual behavior (number of mounts, intromissions and ejaculations),[28, 33] as well as time spent in the male compartment.
The lordosis responses was scored on a 4-point scale (0-3;[37]). The lordosis quotient is the percentage of times the female rat exhibited lordosis (scores 1, 2 or 3) in response to a sexual contact with the male rat. The lordosis score is the average of the intensities (0, 1, 2 or 3) of the lordosis responses.
The percentage of exits after mounts was calculated as the total number of exits after mounts (when the female escapes to her own compartment within 120 seconds after the mount) divided by the total number of mounts times 100%. The percentage of exits after intromission is the number of exits after intromissions divided by total number of intromissions times 100%. After an escape within 120 seconds, the average time the female needs to enter the male compartment again is called contact-return latency.

Drugs

(±)8-OH-DPAT and WAY-100635 maleate salt (Sigma-Aldrich, Steinheim, Germany) were dissolved in saline. (±)8-OH-DPAT (s.c.) and WAY-100635 (i.p.) were injected in a volume of 2 ml/kg 10 minutes and 30 minutes before the paced mating sex tests, respectively.

Experiment 1:
Basal levels of sexual behavior were measured in intact and ovariectomized SERT+/+, +/-, and -/- female rats. Intact rats were tested three times with one-week intervals. Lordosis responses were only scored during the third test.
OVX females were tested in a within-animal Latin square design using three hormonal priming situations: in non-estrus (N), with 5 µg estradiol alone (sub-primed) and 5 µg estradiol plus 500 µg progesterone (fully-primed). The tests were performed with one-week intervals. (For group sizes see legends of the figures.)

Experiment 2:
For this experiment, intact female rats were selected from experiment 1 with the least variation (most stable) in proceptive behaviors over the 3 preceding tests. Doses of the 5-HT_{1A} receptor agonist (±)8-OH-DPAT (0, 0.03, 0.1, 0.3 and 1 mg/kg), the 5-HT_{1A} receptor antagonist WAY-100635 (0, 0.1, 0.3 and 1 mg/kg) and the combination of selected doses of (±)8-OH-DPAT (0.3 mg/kg) and WAY-100635 (0.3 mg/kg) were administered at one-week intervals using a within-animal Latin square design. The doses used of (±)8-OH-DPAT and WAY-100635 were based on studies performed with male rats.[33,38,39] Furthermore, the (±)8-OH-DPAT dose used in the combination treatment was chosen based on the results of the prior experiment in which a clear inhibition in proceptive behavior was found in wildtype rats while knockout rats showed desensitization.

Data analysis

The basal levels of proceptive behaviors, time spent with the males, percentages of exits and contact-return latencies of the intact females were analyzed by repeated measures analysis of variance (ANOVA) to analyze genotype differences. Basal levels of all parameters in OVX females of experiment 1 and the parameters in experiment 2 were analyzed by a 3x3 (OVX females) 3x5 ((±)8-OH-DPAT) and 3x4 (WAY-100635 and the combination) ANOVA (mixed models), because a within-subject Latin square design was used. Next, per drug dose, a one-way ANOVA followed by post hoc analysis using Bonferroni correction was performed.
The lordosis data (in all experiments) was analyzed with the non-parametric Kruskall-Wallis test, because there was no homogeneity of variance. Further post hoc analysis was performed with Mann-Whitney U-test. The level of significance was set at $p<0.05$.
Pearson's product moment correlation coefficient was calculated to analyze the correlation between the number of male sexual behaviors (mount, intromissions and ejaculations) received and the number of proceptive behaviors executed by these females. Correlations were considered relevant when significant ($p<0.05$) and larger than 0.5. SPSS version 14.0 was used for these analyses.

Main outcome measures

Proceptive (darting and hopping) and receptive (lordosis) behaviors were quantified.

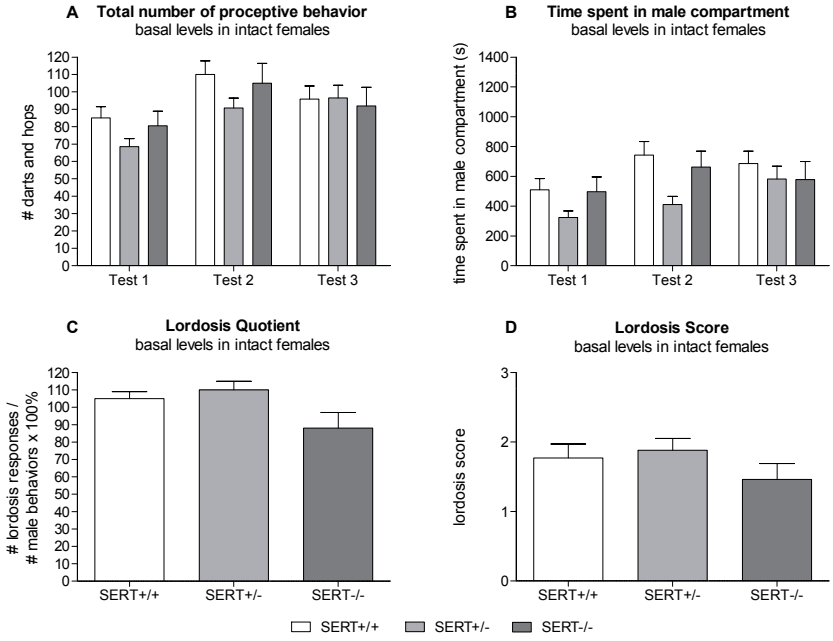

Figure 1: Basal levels of sexual behavior in intact females during 30-minute test: (A) total number of proceptive behavior (darting and hopping) (B) time spent in male compartment (s); (C) lordosis quotient in percentages and (D) lordosis scores. All in serotonin transporter (SERT)+/+ (n=27), SERT+/- (n=30) and SERT-/- (n=24) female Wistar rats. Data are means ± standard error of the mean; *P<0.05.

Results

Experiment 1:

SERT+/+, SERT+/- and SERT-/- intact female rats showed no differences in proceptive behavior (darting and hopping (figure 1A)), as measured in three successive tests with 1-week intervals. Furthermore, the time spent in the male compartment was similar between the different genotypes (figure 1B). Both the amount of proceptive behavior and the time spent in the male compartment were similar over time. Also the percentage of exits from the male compartment and the contact-return latencies after mounts and intermission did not differ between the genotypes (table 1). The lordosis responses were additionally scored during test 3. No differences in lordosis quotient and lordosis score were found between the three genotypes (figure 1C and 1D).

There was no interaction effect on the basal levels of proceptive behavior in SERT+/+, SERT+/- and SERT-/- OVX females under the different hormonal-priming conditions. However, there was a genotype effect in where the fully-primed SERT-/- females darted ±30% less than the wildtypes and heterozygotes ($F_{(2,11)}$=5.31, p=0.007) (figure 2A). The OVX female rats showed no differences in time spent in the male compartment in the hormonally sub- and fully-primed groups (figure 2B). However, the SERT-/- females that were in non-estrus spent significantly more time with the male than SERT+/+ and SERT+/- rats ($F_{(2,59)}$ =8.968, p<0.001). No dif-

ferences were found between SERT+/+, SERT+/-, and SERT-/- rats in percentages of exits and contact-return latencies after mounts and intromissions (table 1). Only for the EB+P group there was a trend towards decreased contact-return latencies after mounts in SERT-/- females, however, this effect was not significant (p=0.061). These parameters could not be calculated for non-estrus conditions, because these females did not receive mounts and/or intromissions.
The receptive behavior (lordosis score and lordosis quotient) was equal between SERT+/+, SERT+/-, and SERT-/- females, in both the EB sub-primed and EB+P fully-primed tests (figure 2C and 2D). Only in the non-estrus group, the SERT-/- rats showed a significantly higher lordosis quotient compared to SERT+/- (Z=-2.550, p=0.030). However, we consider this a chance finding since we have no explanation for this effect under hormonal unprimed conditions.

Experiment 2, pharmacology:

(±)8-OH-DPAT did not affect the time spent in the male compartment by the (intact) females of the three genotypes compared to the vehicle (figure 3A). However, proceptive behavior was affected dose dependently by (±)8-OH-DPAT (mixed models, $F_{(4,20)}$=110.92, p<0.001), and the genotype ($F_{(8,20)}$= 4.59, p<0.001). The SERT-/- rat showed a clear right-shift in the dose-response curve of (±)8-OH-DPAT compared to the other genotypes.

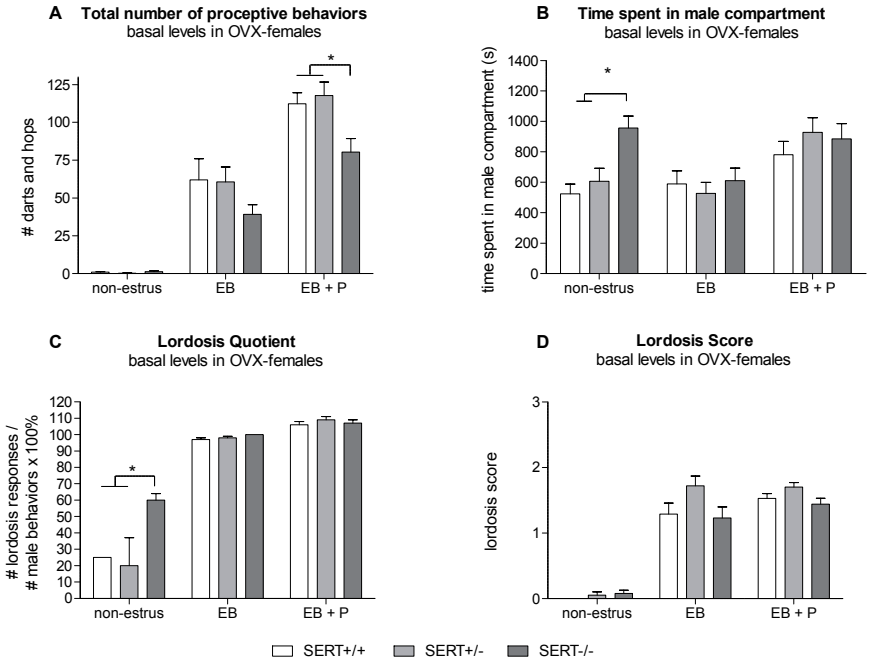

Figure 2: Basal levels of sexual behavior in OVX females during 30-minute test: (A) total number of proceptive behavior (darting and hopping) (B) time spent in male compartment (s); (C) lordosis quotient in percentages and (D) lordosis scores. All in serotonin transporter (SERT)+/+ (n=20), SERT+/- (n=20) and SERT-/- (n=20) female Wistar rats. Data are means ± standard error of the mean; *P<0.05.

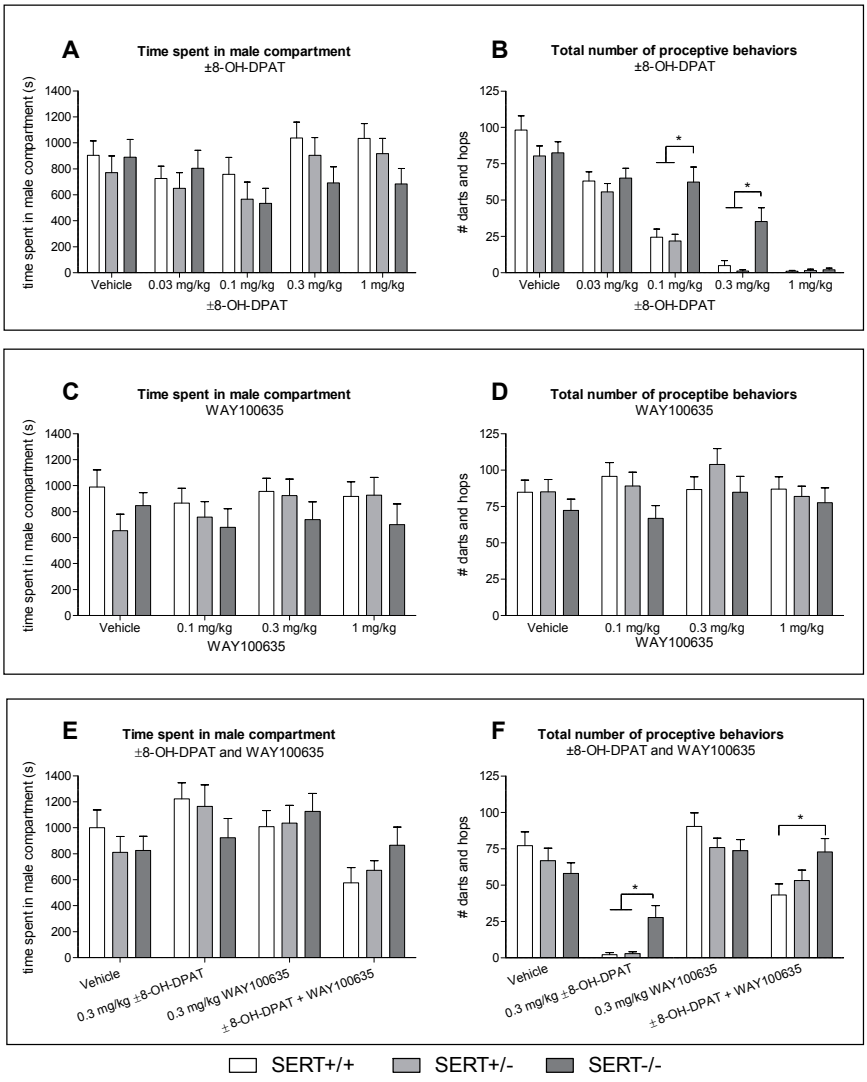

Figure 3: Time spent in the male compartment (s) and total number of proceptive behavior in serotonin transporter (SERT)+/+ (n=17), SERT+/- (n=18) and SERT-/- (n=15) female Wistar rats after administration of (A/B) ±8-OH-DPAT (s.c.), (C/D) WAY-100635 (i.p.) and (E/F) co-administration of 0.3 mg/kg ±8-OH-DPAT and 0.3 mg/kg WAY-100635. . Data are means ± standard error of the mean; *P<0.05.

Further analysis revealed a significant inhibition of darts and hops in SERT+/+ ($F_{(4,85)}$=48.33, p<0.001) and SERT +/- ($F_{(4,90)}$=60.62, p<0.001) female rats in all doses higher than 0.03 mg/kg (±)8-OH-DPAT compared to the vehicle treatment. In SERT-/- rats, only 0.3 mg/kg and 1 mg/kg (±)8-OH-DPAT caused a significant decrease in proceptive behavior ($F_{(4,85)}$=16.13, p<0.001) (figure 3B).

High doses of (±)8-OH-DPAT can induce the serotonin syndrome consisting of, among others, a flattened body posture. (±)8-OH-DPAT induced some flattened

body posture in the highest dose (1 mg/kg), but in the dose of 0.3 mg/kg, this effect was very limited and the rats could still perform normal walking behavior. This was observed for all three genotypes (data not shown).
WAY-100635 administration alone had no effect compared to vehicle on any parameter. There was also no difference between the different genotypes (figures 3C/D).
A combination treatment of 0.3 mg/kg WAY-100635 and 0.3 mg/kg (±)8-OH-DPAT did not have any effect compared to vehicle on time spent with the male rat. However, WAY-100635 significantly attenuated the effect of (±)8-OH-DPAT on proceptive behavior in SERT+/+ ($F_{(3,67)}$=26.17, p<0.001), SERT+/- ($F_{(3,68)}$=24.99, p<0.001) and SERT-/- ($F_{(3,56)}$=6.99, p<0.001) female rats (figure 3F). In contrast to SERT-/- and +/- rats, the decrease in proceptive behavior by (±)8-OH-DPAT was not completely normalized by WAY-100635 in the SERT+/+ females.
We also scored the male sexual behavior towards the females of the three genotypes. Male sexual parameters (mounts, intromissions and ejaculations) did not differ towards the females of the three genotypes under vehicle conditions. (±)8-OH-DPAT administration to the females decreased the number of mounts, intromissions and ejaculations by the males. Highly significant correlations exist (r^2=0.709, p<0.001 for SERT+/+, r^2=0.632, p<0.001 for SERT+/- and r^2=0.646, p<0.001 for SERT-/-) between proceptive behavior and the number of male sexual behaviors.

Discussion

Our results with the SERT knockout rats suggest that the serotonin transporter is not essential for normal female sexual behavior in a paced mating situation. Both proceptive and receptive behavior of the SERT knockout (+/- and -/-) was normal in both intact and OVX females. Only the number of darts/hops by fully-primed OVX SERT-/- rats showed a significant, but small reduction. This finding is opposite to what we hypothesized on the basis of reports from women on SSRIs, and on a rather limited set of animal studies in which inhibition of female sexual behavior by antidepressants were reported.[9-11, 22, 23, 32] Therefore, the question arises to what extent the SERT-/- female rat models chronic SSRI usage in women. It is assumed that in human patients on SSRIs 60-80% of the available SERT binding sites are occupied.[40] So, at least in humans, even a partial blockade of SERT may lead to a decrease in female sexual behavior.
The normal sexual behavior of SERT-/- female rats may point to compensatory mechanisms in SERT-/- rats that prevent sexual dysfunctions. There is evidence that altered 5-HT neurotransmission during development in animals will probably have effects on brain development,[41, 42] which differs from the impact of chronic SSRI treatment in adults.[43] Alternatively, it could be hypothesized that the serotonergic system does not play a role in the basic regulation of sexual functions and behavior and that only under 'challenged' conditions (like giving a stressor or a 5-HT_{1A} receptor agonist) the 5-HT system is involved.
In experiment 2, we studied the effects of the 5-HT_{1A}/5-HT_7 receptor agonist (±)8-OH-DPAT alone and in combination with the selective 5-HT_{1A} receptor antagonist WAY-100635. It is known that both activation of the 5-HT_{1A} and the 5-HT_7

receptor inhibits female sexual activity.[16, 17, 44, 45] In this study, (±)8-OH-DPAT inhibited proceptive behaviors in all three genotypes. However, the SERT-/- rat showed a clear right-shift in the dose-response curve, indicative for desensitization of one of the mentioned 5-HT receptors. In SERT-/- and SERT+/- rats, the inhibitory effects of (±)8-OH-DPAT could be completely blocked by co-administration of WAY-100635, while in wildtypes the inhibitory effects of (±)8-OH-DPAT were only partially blocked. This suggests the importance of the 5-HT$_{1A}$ receptor in the effects of (±)8-OH-DPAT. Desensitization can be caused by a decrease in the amounts of functional receptors. Thereby, a lower dose of WAY-100635 will be sufficient to fully attenuate the effect of (±)8-OH-DPAT in SERT-/- compared to SERT+/+ rats. However, we cannot exclude the possibility that this difference in effect is due to inhibiting effects of 5-HT$_7$ receptors on female sexual behavior.[44] Other studies, in support, have also shown a change in function of 5-HT$_{1A}$ receptors in SERT-/- rats.[39] High doses of (±)8-OH-DPAT are known to cause the serotonin syndrome like flattened body posture. This might indicate that the decrease in proceptive behavior is caused by the inability to move properly. However, flattened body posture was not, or very limited, present in the females injected with 0.3 mg/kg and all females did show normal activity. Therefore, it is likely that the decrease in darts and hops is not due to loss of motor function, but due to a decrease in sexual motivation.

Finally, it was found that administration of WAY-100635 alone did not affect female sexual function in any of the three genotypes. This agrees with other studies where WAY-100635 alone did not affect female sexual behavior.[18, 46] WAY-100635 is a silent 5-HT$_{1A}$ receptor antagonist in that it prevents effects of 5-HT$_{1A}$ receptor agonists, but has no effect in the absence of the agonist.[47, 48] WAY-100635 can therefore be used as a tool to assess the presence or absence of tonic activation of 5-HT$_{1A}$ receptors.[47, 49] The lack of effects of WAY-100635 on female sexual activity clearly indicates that activation of 5-HT$_{1A}$ receptors by endogenous 5-HT does not play a role in this behavior in fully-primed females, although we can not exclude the possibility that 5-HT$_{1A}$ receptors may play a role under hormonal sub-primed conditions, however, this was not investigated.

In male sexual activity, the 5-HT$_{1A}$ receptor plays a more important role. SERT-/- male rats showed a reduction in sexual function compared to wildtypes, as well as a desensitization of the 5-HT$_{1A}$ receptor.[50] Earlier, de Jong et al.[33] also demonstrated in males a role of 5-HT$_{1A}$ receptor desensitization in the sexual side effects of chronic SSRI usage. In addition, in males, (±)8-OH-DPAT stimulates sexual activity.[51] Therefore, our results confirm the general pattern that different mechanisms or differential activation of serotonergic substrates are involved in the brain for male and female rat sexual behaviors.

In the present study we used a paced mating procedure. While proceptive behavior was clearly affected by (±)8-OH-DPAT, the time spent in the male compartment was unaffected. Moreover, the time spent with the male rat was equal over all tests. This strengthens the comparison between the genotypes and the effect of (±)8-OH-DPAT on proceptive behavior and excludes interpretations of the female behavior in terms of time (not) spent with the male. Furthermore, the time spent with the male probably reflect social interaction with the male rather than her willingness to have sex, since non-estrus females spent a similar amount of time with the males as females in estrus. Interestingly, non-estrus SERT-/- females showed even an increased

time with the males. We have no explanation for this observation. The time spent in the male compartment is an important parameter to quantify, because any social avoidance may severely interfere with the quantifications of the sexual behaviors.
The sexual interaction between rats has a fixed order. Seventy percent of the proceptive behavior (darts and hops) of the female was shown to be correlated with the number of mounts and intromissions by the male. This means that female sexual behavior is not completely depending on the performance of the male rat. The remaining variation in male sexual activity may be partly due to the innate sex drive of each animal. Furthermore, additional cues of interactions (sound, smell) between the male and female are probably also involved.
In most studies in literature ovariectomized females are used. Here we compared intact females, who had received a high dose of only estradiol, with fully-primed and sub-primed OVX females. There were no clear quantitative differences between sexual behavior of intact females and fully-primed OVX females, especially in SERT+/+ and SERT+/- rats. Fully-primed OVX SERT -/- females showed a significant, but small reduction in the number of darts and hops. This decrease was not seen in intact SERT -/- animals. Furthermore, only a difference in percentages of exits is seen between intact and OVX females. Table 1 show that intact females escape more after mounts and intromissions than OVX females, suggesting that our priming strategy of intact females with a high dose of estradiol still leads to normal behavioral responses.

Conclusions

In conclusion, the absence (-/-) or reduced (+/-) expression of SERT does not affect basal sexual activity in female rats in a paced mating situation. This result was rather unexpected in view of the sexual side effects observed after SSRI treatment as found both in humans and in rats.[9-11, 22, 23] We found, however, desensitized 5-HT_{1A} receptors in the SERT-/- females. Under normal conditions, such desensitized 5-HT_{1A} receptors apparently do not play a role in female sexual behavior.

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Chronic paroxetine treatment does not affect sexual behavior in hormonally sub-primed female rats despite 5-HT_{1A} receptor desensitization

6

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Abstract

Introduction. Selective serotonin reuptake inhibitors (SSRIs) cause sexual dysfunctions in humans. However, because SSRIs are given for the depression, it is unclear whether the problems are caused by the drug, by the depression itself, or an interaction between both. Serotonin transporter knockout rats, a genetic homologue of long-term SSRI treatment, do not display decreased female sexual behavior.

Aim. The present study investigated the effects of chronic paroxetine treatment on sexual behavior in female rats. Furthermore, we tested whether 5-HT_{1A} receptors were desensitized in these females.

Methods. Ovariectomized female rats, either sub-primed with estradiol or fully-primed with estradiol and progesterone, were tested in a paced mating test. Proceptive (darting and hopping), receptive (lordosis) and paced mating related (percentages of exits and contact-return latencies) behaviors were quantified during the course of 56 days of chronic paroxetine treatment (10 mg/kg and 20 mg/kg per day). The 5-HT_{1A}/5-HT_{7} receptor agonist (±)8-OH-DPAT alone and in combination with the selective 5-HT_{1A} receptor antagonist WAY-100635 was administered to study putative 5-HT_{1A} desensitization in the same females.

Main outcome measures. Proceptive, receptive and paced mating behaviors were quantified.

Results. Acute and chronic paroxetine treatment did not change proceptive and receptive behaviors in both sub- and fully-primed female rats. In all groups, (±)8-OH-DPAT showed a clear dose dependent inhibition of sexual behaviors in vehicle-treated females and a right-shifted dose-response effect in the paroxetine-treated rats. WAY-100635 attenuated the inhibiting effect of the 5-HT_{1A} receptor agonist in all females. These data suggest 5-HT_{1A} receptor desensitization after chronic paroxetine treatment.

Conclusions. Chronic paroxetine treatment does not cause sexual side effects in sub- or fully hormonally primed female rats. Furthermore, chronic treatment causes adaptive changes in the serotonin system such as desensitization of 5-HT_{1A} receptors, which may counteract the inhibiting effects of increased extracellular serotonin levels in the chronic paroxetine treated rats.

Introduction

A large percentages (36-56%) of females using selective serotonin reuptake inhibitors (SSRIs), mostly for depressive symptoms, complain about sexual dysfunctions.[1-5] Whether these problems are solely due to the medication, is uncertain, because low sexual desire could also be a result of the depression alone.[6] Common problems with depression are lack of energy, lowered self-esteem, inability to experience pleasure, irritability and social withdrawal. Consequently, one can expect a high prevalence of sexual dysfunctions in depressed patients compared to controls. A study of Casper et al.[6] in patients with depressive disorders reported lack of sexual interest, characterized by loss of libido or decrease in sexual desire or potency, in 72% of women with untreated major depression. Other studies revealed that the prevalence of sexual dysfunctions is higher in depressed patients who received drug treatment, compared to patients without treatment.[7] However, none of the studies compared sexual behavior before and after treatment in the same patients, which makes it difficult to determine the real cause for their sexual problems. Furthermore, the reported incidence of sexual dysfunctions may be influenced by many factors. First, the method to obtain information about their sex life varies over studies. And second, cultural and social factors might play an important role in the experience of sexual dysfunctions, such as the expectations people have to their sexual performance and their willingness to discuss such issues with a physician. The way of defining sexual dysfunction is always subjective and depends on the individual's idea of what is normal. (see the review by Montgomery et al.[8])

Animal studies make it possible to study the mechanism underlying sexual (side) effects of SSRIs. So far, only a few studies describe the effects of SSRIs on female rats. The outcome of these studies is rather conflicting. Matuszczyk et al.[9] reported a slight reduction in receptive and proceptive behavior after 21 days of chronic fluoxetine (10 mg/kg) treatment in fully-primed ovariectomized rats. In contrast, Sarkar et al.[10] showed that acute administration of fluoxetine (10 and 20 mg/kg) in ovariectomized females decreased receptive behavior (lordosis) whereas the decrease after 10 days of treatment was much smaller. Repeated fluoxetine administration has a modest effect on sexual behavior in intact naturally cycling females.[11]

SSRIs prevent reuptake of 5-HT from the synaptic cleft into the presynaptic serotonergic neuron by blocking 5-HT transporters (SERT). This leads to elevated extracellular 5-HT levels which, in turn, stimulate autoreceptors and postsynaptic 5-HT receptors. In a previous study, we investigated the importance of the serotonin transporter (SERT) on sexual behavior using SERT knockout rats.[12] The main difference between SERT knockout rats and rats that are chronically administered with SSRIs is that the knockouts have an altered serotonergic system from conception. We found no differences in sexual behavior between SERT knockout and wildtype rats, indicating that the SERT are not essential in the regulation of female sexual behavior under normal conditions. However, it is possible that during embryonic/postnatal development, the SERT knockout required adaptations that prevent the development of sexual dysfunction. One of such adaptations may be a desensitized 5-HT_{1A} receptor system, as we were able to show.

To complicate matters, chronic fluoxetine treatment might affect female estrus cyclicity. Some studies found no effect of SSRI treatment on the estrus cycle[9, 12-14] while others showed a disruption of the cycle after chronic fluoxetine treatment.[15,16] Remarkably, all studies with disruption of the estrus cycle are performed in Fischer female rats, while the other studies are performed in, respectively, Wistar, Long-Evans and Sprague-Dawley females. This may suggest strain differences in responses to fluoxetine.
In the present study we investigated the effects (up to nine weeks) of chronic treatment with paroxetine on female sexual behavior. We used ovariectomized rats that were brought upon experiment into estrus using estradiol and progesterone. In this way we were able to investigate the direct effects of this SSRI on sexual behavior without its potential disrupting effect on estrus cyclicity. Furthermore, we used two different groups of hormonally primed females: sub-primed, with only 5 µg estradiol and fully-primed, with both 5 µg estradiol and 500 µg progesterone. The sub-primed females show significant lower levels of proceptive and receptive behavior and could therefore model female sexual dysfunction. By using the sub-primed and fully-primed females, we are able to differentiate the effects of chronic paroxetine treatment between normal functioning and sexual disrupted females.
Paroxetine was chosen because this SSRI has the biggest impact on sexual functioning in men and women compared to other SSRIs[1]. Furthermore, we studied the possible existence of 5-HT_{1A} receptor desensitization in chronically treated female rats.

Materials and Methods

Animals

Wistar female rats (n=60, 3 months of age at the start of the experiment) and sexually experienced Wistar male rats (n=60, 3 months of age at the start of the experiment) were used in this experiment (Harlan, Zeist, The Netherlands). All animals were housed in the Central Animal Laboratory of Utrecht University under a reversed 12/12-h light/dark cycle (lights off at 7 am). The male and female rats were housed in separate rooms in groups of four per Macrolon type-IV cage. Standard food and water were available ad libitum.
The female rats were bilaterally ovariectomized under isoflurane anesthesia 14 days before the start of the experiment. Sexual receptivity was induced by subcutaneous administration of estradiol benzoate (5 µg EB) alone or estradiol in combination with progesterone (500 µg P). The hormones were dissolved in 0.1 ml sesame oil saturated with phosphatidylcholine and injected 36 (EB) and 4 (P) hours prior to testing.
All experiments were carried out in accordance with institutional, national and international guidelines for animal care and the Dutch law concerning welfare.

Drugs

Paroxetine hydrochloride hemihydrate (Safron, Nieuwerkerk a/d IJssel, the Netherlands) was dissolved in water (vehicle) and administered daily in a volume of 4 ml/kg (p.o.). On test days, the paroxetine was injected 1 hour prior to the paced mating sex tests. On non-test days, the paroxetine was injected around 11 am.

	Percentages of exits after mounts			Percentages of exits after intromissions			CRL after mounts			CRL after intromissions		
	vehicle	10 mg/kg paroxetine	20 mg/kg paroxetine	vehicle	10 mg/kg paroxetine	20 mg/kg paroxetine	vehicle	10 mg/kg paroxetine	20 mg/kg paroxetine	vehicle	10 mg/kg paroxetine	20 mg/kg paroxetine
Sub-primed:												
Acute	18.1±12.0	53.0±11.6	54.0±11.7	31.0±13.4	60.9±8.9	63.9±6.1	130.2±23.4	89.4±22.3	101.4±54.4	44.7±0.7	152.8±40.1	481.0±178.0
7 days	56.3±11.8	44.4±13.7	66.3±6.6	60.4±11.1	61.3±11.3	70.1±6.4	35.3±8.7	42.1±6.4	37.4±7.0	44.0±9.8	68.4±9.4	99.0±33.7
14 days	41.7±14.3	80.5±5.8	71.9±5.6	78.0±3.6	72.6±9.5	78.8±5.2	37.1±5.4	39.1±5.9	32.0±7.4	62.0±8.4	38.6±4.0	60.5±13.8
21 days	84.2±4.1	85.8±4.1	90.6±2.5	86.6±1.3	86.7±1.7	78.3±5.2	29.0±4.6	26.4±2.7	24.5±4.4	47.1±4.9	36.3±3.1	32.7±5.3
Fully-primed:												
Acute	66.2±5.5	43.9±10.8	77.8±9.1	79.8±4.5	56.7±11.3	76.4±4.0	90.9±33.9	39.0±7.3	51.1±25.3	97.9±24.4	54.4±10.5	55.9±33.6
7 days	70.6±6.7	58.9±13.1	76.8±8.0	74.6±7.0	69.9±11.0	73.6±9.9	44.5±9.3	24.5±4.5	52.5±22.9	41.9±5.4	53.6±23.5	35.6±8.3
14 days	83.8±6.6	80.0±10.4	86.0±7.0	83.4±9.3	79.7±10.1	83.4±5.8	29.9±6.0	32.8±5.9	44.5±10.9	34.3±4.9	29.2±3.1	43.8±13.9
21 days	87.0±5.4	83.5±5.7	76.4±8.8	84.4±9.4	84.4±4.9	88.3±2.6	25.7±4.7	31.8±12.3	19.2±4.5	24.2±3.6	22.8±2.7	25.2±3.8

Table 1: Percentages of exits and contact-return latencies (CRL) after mounts and intromissions in female rats treated with vehicle, 10 mg/kg paroxetine or 20 mg/kg paroxetine for 21 days. The table shows sub-primed and fully-primed OVX females during 30 minute test. Data are means ± SEM; *p<0.05.

(±)-8-Hydroxy-2-(dipropylamino)tetralin hydrobromide ((±)8-OH-DPAT) and the maleate salt of WAY-100635 (Sigma-Aldrich, Steinheim, Germany) were dissolved in saline. (±)8-OH-DPAT (s.c.) and WAY-100635 (i.p.) were injected in a volume of 2 ml/kg 10 minutes and 30 minutes, resp. before the paced mating sex tests.

Test cage

The test cage was divided into two compartments: a "male compartment" of 45 x 26 x 38 cm and a "female-only" compartment" of 20 x 26 x 38 cm. The compartments were divided by a transparent plastic wall containing three holes (4 cm diameter each) through which only the females could pass. Another wall with ten, equally spaced, 5 mm diameter air holes was used to block the holes. This wall was present during the habituation phase of the sex test.

Behavioral procedure

All tests were conducted under red light during the dark phase of the light/dark cycle. The behavioral procedure was performed exactly the same as in our previous studies.[12, 17] The paced mating 30 minute sex tests were videotaped for event recording of female receptive behavior (lordosis), proceptive behavior (number of darts and hops), percentages of exits, contact-return latencies, male sexual behavior (number of mounts, intromissions and ejaculations), as well as time spent in the male compartment. The measurements of the behaviors were also the same as in our previous studies.[12, 17]

Experimental setup

Part I: Both, the sub-primed and fully-primed OVX females were divided into three treatment groups (n=10 per group). All rats received a daily oral injection with vehicle or paroxetine (10 and 20 mg/kg) for 10 weeks. Basal levels of sexual behavior were measured in a paced mating test (acutely, and on day 7, 14 and 21).

Part II: Next we investigated in these animals, while continuing the paroxetine treatment, the existence of 5-HT_{1A} receptor desensitization. Based on a within-subject Latin square design, all rats were (once a week) administered with vehicle, 0.1 mg/kg (±)8-OH-DPAT, 0.3 mg/kg (±)8-OH-DPAT or a combination of 0.3 mg/kg (±)8-OH-DPAT and 0.3 mg/kg WAY-100635. The doses of (±)8-OH-DPAT and WAY-100635 were based on our previous study performed in wildtype and SERT knockout rats on the same background strain.[12] WAY-100635 alone was not tested this time, because it did show any effects on female sexual behavior in previous studies.[12] The sexual activity was observed in the paced mating test.

Data analysis

The levels of proceptive behaviors, time spent with the males, percentages of exits, and contact-return latencies of part I were analyzed by repeated measures analysis of variance (AVOVA) to examine differences in effect of chronic paroxetine treatment. Further post hoc analysis was done in one-way ANOVA using Bonferroni correction.

All behavioral parameters of part II were analyzed by a 4x3 ANOVA (mixed models). Next, per drug dose, a one-way ANOVA was performed followed by a post hoc analysis using Bonferroni correction.

The lordosis data (in both experiments) were analyzed with the non-parametric Kruskall-Wallis test, because there was no homogeneity of variances. Further post hoc analysis was performed with a Mann-Whitney U-test. The level of significance was set at $p<0.05$.

Main outcome measures

Proceptive, receptive and paced mating behaviors were quantified.

Results

Acute treatment with 10 mg/kg and 20 mg/kg paroxetine had no effect on time spent in male compartment in both sub-primed and fully-primed females (figure 1a/b). There was no interaction effect of chronic paroxetine treatment for 7, 14 and 21 days with the time spent with the male. In both, the sub-primed and fully-primed females, no effect of acute paroxetine treatment was found on the number of proceptive behaviors (figure 1c/d). However, there was an interaction effect of paroxetine treatment with the subchronic treatment (repeated measures: $F_{(2,26)}=5.155$, $p=0.013$). Further analysis revealed that 10 and 20 mg/kg paroxetine significantly ($F_{(2,26)}=5.773$, $p=0.008$) decreased the number of darts and hops in the

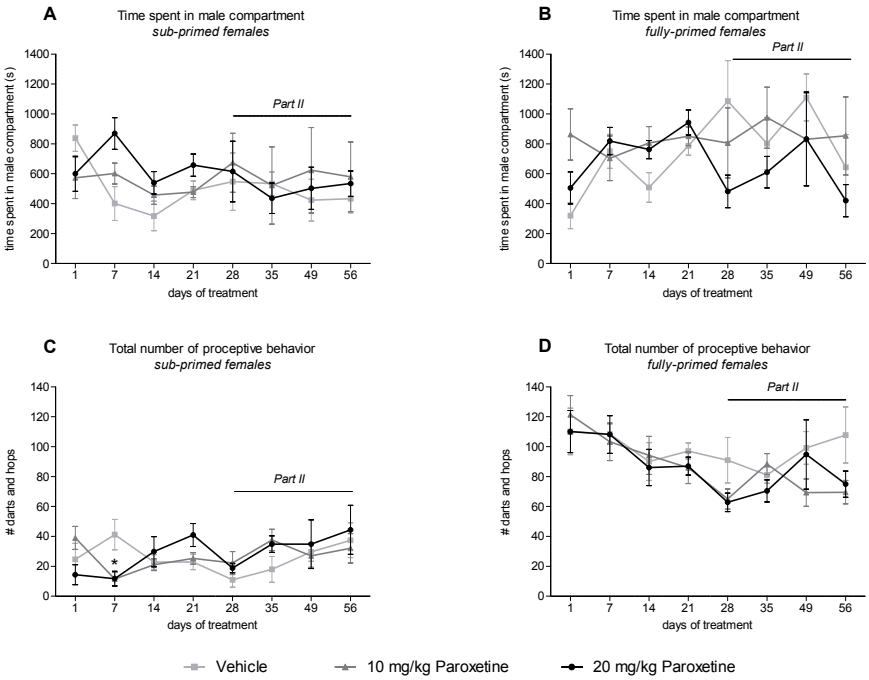

Figure 1: basal levels of sexual behavior in OVX Wistar females during 30 min test: (A) time spent in male compartment (s) in sub-primed females; (B) time spent in male compartment (s) in fully-primed females; (C) total number of proceptive behavior (darting and hopping) in sub-primed females; and (D) total number of proceptive behavior in fully-primed females. All in vehicle, 10 mg/kg and 20 mg/kg paroxetine treated rats. Day 1 till 21 was based on n=10 per group, day 28 till 56 (from experiment part II) on n=4. Data are means ± SEM; *p<0.05.

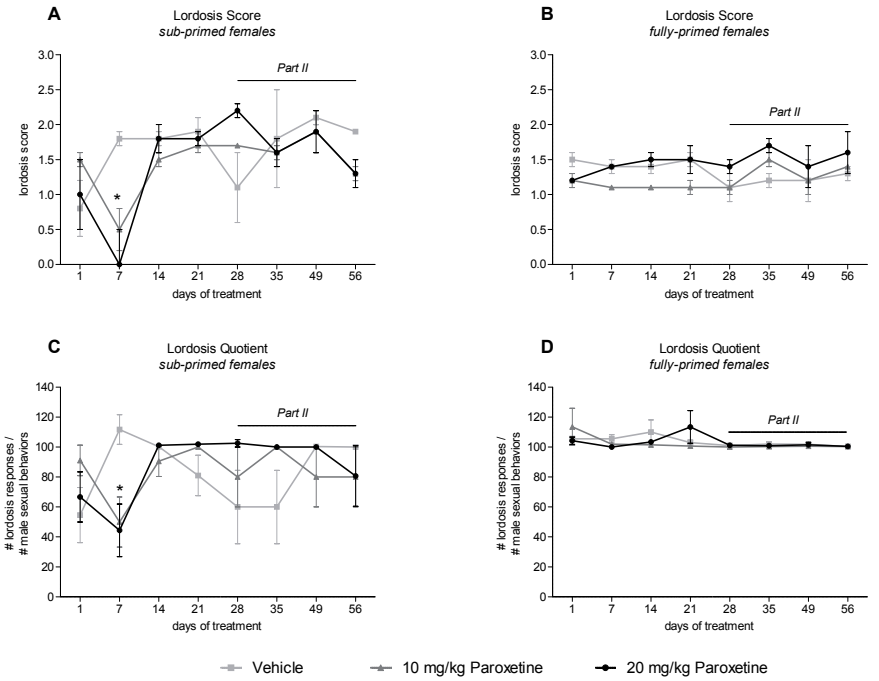

Figure 2: basal levels of sexual behavior in OVX Wistar females during 30 min test: (A) lordosis score in sub-primed females; (B) lordosis score in fully-primed females; (C) lordosis quotient in sub-primed females; and (D) lordosis quotient in fully-primed females. All in vehicle, 10 mg/kg and 20 mg/kg paroxetine treated rats. Day 1 till 21 was based on n=10 per group, day 28 till 56 (from experiment part II) on n=4. Data are means ± SEM; *p<0.05.

sub-primed females after 7 days of treatment compared to vehicle (figure 1c). This effect was not longer present at later time points. In the fully-primed group, there were no effects of paroxetine. Similar results were found on receptive behavior: There was no effect of paroxetine treatment in the fully-primed females (figure 2b/d), but a decrease in lordosis score (figure 2a)(Kruskal-Wallis test, Z=-2.936, p=0.003 in 10 mg/kg paroxetine) and lordosis quotient (figure 2c)(Z=-2.593, p=0.01 in 10 mg/kg paroxetine and Z=-2.702, p=0.007 in 20 mg/kg paroxetine) in sub-primed females only after 7 days of paroxetine treatment. Again, this effect disappeared after longer treatment.

After the three weeks of treatment, the females were still injected daily with paroxetine for the second part of our experiment in which we tested (within-subject design) a 5-HT_{1A} receptor agonist. In the figures 1 and 2 we included the behaviors of the vehicle treated rats of part II, and therefore, the group size of animals for this later time point is only 4 and too small to perform meaningful statistics. However, from these graphs one can conclude that even after 56 days of paroxetine treatment the sexual behaviors of the females are not changed.

Both, acute and chronic paroxetine treatment did not affect paced mating in all female rats. No differences in percentages of exits after mounts and intromissions were shown. Just as in contact-return latencies (CRL) after mounts and intromissions in both sub-primed and fully-primed females (table 1).

5-HT$_{1A}$ receptor desensitization

Next, we investigated the role of 5-HT$_{1A}$ receptors on female sexual behavior in the chronically paroxetine treated rats. Therefore, we administered two doses of (±)8-OH-DPAT alone and in combination with WAY-100635.

There was no effect of (±)8-OH-DPAT on time spent in male compartment in all paroxetine treated sub-primed females (figure 3a). However, in the fully-primed females, (±)8-OH-DPAT reduced the time spent in the male compartment in all females ($F_{(3,14)}$=4.600, p=0.005) (figure 3b). This effect was attenuated by the co-administration of WAY-100635 in the paroxetine treated groups. Further analysis revealed that there was a significant difference between the experimental groups that had received paroxetine and the vehicle control group during the combination test ($F_{(2,25)}$=6.133, p=0.007).

(±)8-OH-DPAT dose dependently decreased the amount of proceptive behavior in both sub-primed ($F_{(3,14)}$=26.686, p<0.001) (figure 3c) and fully-primed ($F_{(3,14)}$=83.054, p<0.001) females (figure 3d). Co-administration of WAY-100635 attenuated the in-

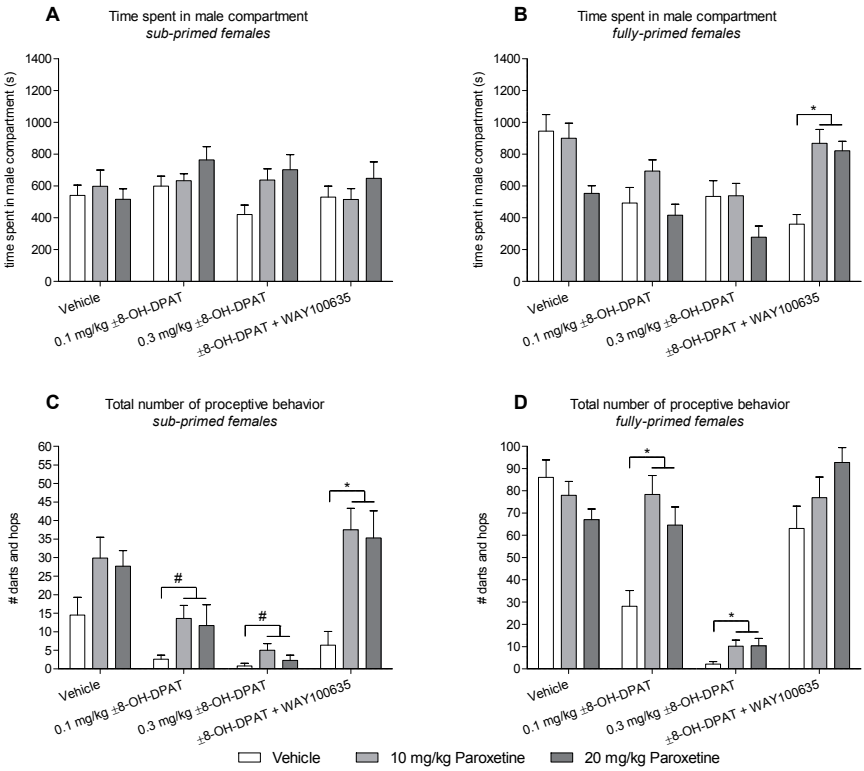

Figure 3: levels of sexual behavior after administration of 0.1 mg/kg and 0.3 mg/kg ±8-OH-DPAT (s.c.), and co-administration of 0.3 mg/kg ±8-OH-DPAT (s.c.) and 0.3 mg/kg WAY-100635 (i.p.): (A) time spent in male compartment (s) in sub-primed females; (B) time spent in male compartment (s) in fully-primed females; (C) total number of proceptive behavior (darting and hopping) in sub-primed females; and (D) total number of proceptive behavior in fully-primed females. All in vehicle, 10 mg/kg and 20 mg/kg paroxetine treated OVX Wistar females rats during a 30 min test. Data are means ± SEM; *p<0.05 and #p<0.10.

hibiting effects of (±)8-OH-DPAT. There was a drug interaction effect with paroxetine treatment in both sub-primed ($F_{(6,14)}$=2.552, p=0.028) and fully-primed ($F_{(6,14)}$=5.296, p<0.001) females, suggesting 5-HT_{1A} receptor desensitization in females chronically treated with 10 and 20 mg/kg paroxetine. Post hoc analysis showed that this difference was approaching significance in the sub-primed females (0.1 mg/kg (±)8-OH-DPAT: $F_{(2,26)}$=2.535, p=0.09 and 0.3 mg/kg (±)8-OH-DPAT: $F_{(2,26)}$=2.551, p=0.09), and was significant in fully-primed females (0.1 mg/kg (±)8-OH-DPAT: $F_{(2,25)}$=11.243, p<0.001 and 0.3 mg/kg (±)8-OH-DPAT: $F_{(2,25)}$=3.675, p=0.040).
Similar results were found in lordosis score; 0.3 mg/kg (±)8-OH-DPAT, but not 0.1 mg/kg, significantly decreased the lordosis score in all sub-primed females (vehicle: Z=-1.985, p=0.047; 10 mg/kg paroxetine: Z=-2.368, p=0.018; 20 mg/kg paroxetine: Z=-2.805, p=0.005) (figure 4a). This effect was attenuated after co-administration with WAY-100635, with a bigger effect in paroxetine treated females than in vehicle treated rats (10 mg/kg paroxetine: Z=-1.809, p=0.071; 20 mg/kg paroxetine: Z=-1.951, p=0.051). (±)8-OH-DPAT did not affect the lordosis quotient in sub-primed females (figure 4c). However, there was again a significant difference with combination treatment between the paroxetine treated females and the vehicle group (10 mg/kg paroxetine: Z=-2.517, p=0.012; 20 mg/kg paroxetine: Z=-2.405, p=0.016). In fully-primed females, 0.3 mg/kg (±)8-OH-DPAT only affected

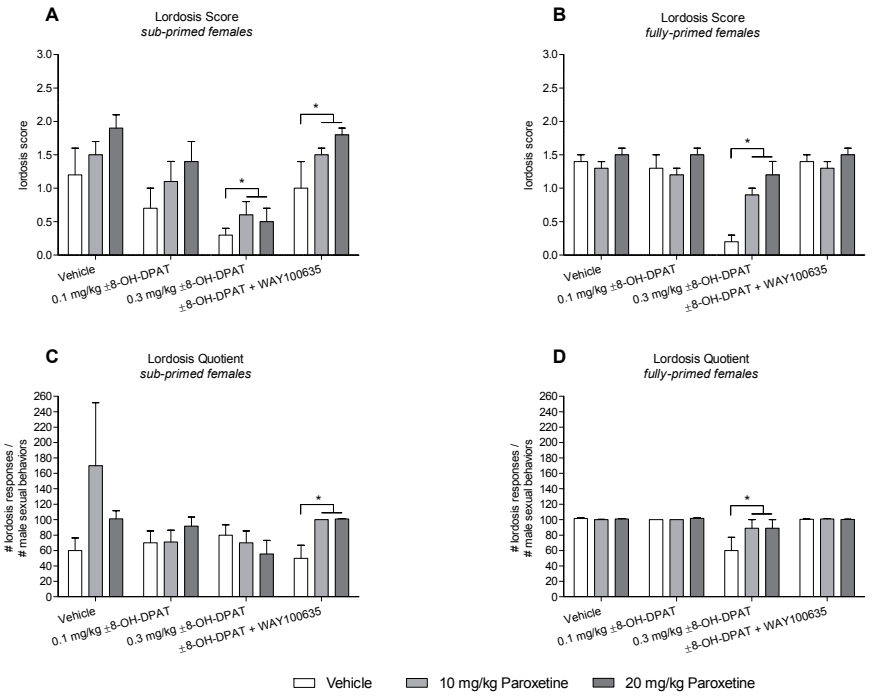

Figure 4: levels of sexual behavior after administration of 0.1 mg/kg and 0.3 mg/kg ±8-OH-DPAT (s.c.), and co-administration of 0.3 mg/kg ±8-OH-DPAT (s.c.) and 0.3 mg/kg WAY-100635 (i.p.): (A) mean lordosis score in sub-primed females; (B) mean lordosis score in fully-primed females; (C) lordosis quotient in sub-primed females; and (D) lordosis quotient in fully-primed females. All in vehicle, 10 mg/kg and 20 mg/kg paroxetine treated OVX Wistar females rats during a 30 min test. Data are median ± SEM; *p<0.05.

the lordosis score (figure 4b) and lordosis quotient (figure 4d) of vehicle treated females (LS: Z=-3.880, p<0.001; LQ: Z=- 2.743, p=0.006), while the paroxetine treated females were unaffected by (±)8-OH-DPAT. The difference between vehicle and paroxetine treatment was significant (10 mg/kg paroxetine: Z=-2.489, p=0.013; 20 mg/kg paroxetine: Z=-2.505, p=0.012). The decreased lordosis score and lordosis quotient was again attenuated by co-treatment with WAY-100635. These experiments combined suggest 5-HT_{1A} receptor desensitization.
The effects of (±)8-OH-DPAT on paced mating could not be calculated, because there were not enough mounts and intromissions received to measure the percentage of exits and contact-return latencies.

Discussion

Our main finding is that, both, acute and chronic paroxetine treatment does not affect sexual behavior in hormonally primed female rats. This result is in line with our previous study in which we showed that serotonin transporter (SERT) knockout rats have normal sexual behavior compared to wildtype females.[12] Together, these studies suggest that, under normal conditions, the SERT is not essential in the regulation of female sexual behavior.
However, our data conflicts with some other studies. As already mentioned in the introduction, Sarkar et al.[10] found inhibiting effects of acute SSRI treatment, which was attenuated after 10 days of treatment. Differences in rat strain (Fischer vs. Wistar) and the type of SSRI (fluoxetine vs. paroxetine) may be responsible for the differences in outcome of the Sarkar study and our study.
It is suggested that under normal circumstances, the relative balance between 5-HT's activation of the inhibitory (via 5-HT_{1A} receptors) and facilitatory (via 5-$HT_{2A/2C}$ receptors) systems determines whether sexual activity will or will not occur.[18] Since intact pro-estrus rats show lordosis behavior, it is reasonable to assume that during naturally occurring sexual receptivity, the inhibitory system is suppressed in favor of the facilitatory system.[18] Our results suggest that the acute elevation of 5-HT levels after paroxetine treatment does not affect this balance in the Wistar rats, whereas it might have changed the balance (in favor of the inhibiting system) in Fischer rats. Variation in 5-HT sensitivity between different strains have been reported both in sexual behaviors,[11, 19] but also in receptor densities in the brain.[20]
Our results also conflict with the study of Matuszczyk et al.[9] who reported a slight reduction in receptive and proceptive behavior after 21 days of chronic fluoxetine (10 mg/kg) treatment in Wistar females. Fluoxetine and paroxetine are both SSRIs, but with different characteristics. SSRIs could differ in functionalities caused by different affinities for the SERT or the ability to influence other receptor systems.[20, 21] Paroxetine, for instance, has also a modest affinity for the noradrenalin transporter. These different characteristics might have caused the dissimilarities in results compared to studies with other SSRIs. Another explanation for the lack of an effect of chronic paroxetine may be that we used the wrong dosage. We used 10 and 20 mg/kg. These dosages were inhibitory in our male rat studies,[22, 23] making this rather unlikely.
Our data showed a slight decrease in proceptive and receptive behavior in the sub-primed females after 7 days of paroxetine treatment, which was attenuated after

longer treatment. It may be that the paroxetine treatment reduced the levels of circulating estrogen injected 36 hours before the sex test, as has been reported by Taylor et al.[24], which might have affected the level of receptivity of the sub-primed subchronic paroxetine treated females to an even lower amount. If this suggestion is correct, then why is this inhibition not seen after 3 weeks of paroxetine treatment? As we showed in this study, chronic paroxetine treatment led to 5-HT_{1A} receptor desensitization. Therefore, we suggest that after one week of paroxetine treatment the 5-HT_{1A} receptor desensitization is less pronounced and thus the inhibitory activity of this receptor on sexual behavior may be more visible at the one week than at the three week time point. Furthermore, there was no effect of 7 days of paroxetine treatment in the fully-primed females. The most likely explanation is that co-priming with progesterone is so strong that inhibitory effects of a possible reduction in circulating estrogen are overruled.

Overall, our experiments clearly show that chronic paroxetine treatment does not cause sexual dysfunctions in OVX female Wistar rats, while others using different rat strains and different SSRIs have reported female sexual side effects of antidepressants. Many female SSRI users complain about sexual dysfunctions.[1-5] However, it is uncertain whether these dysfunctions are due to their antidepressant drug use, because depression by itself can also cause sexual disturbances.[6,7] The major problems with the currently available studies on SSRI induced sexual side effects are the lack of validated rating scales, failure to include a baseline assessment and/or a placebo control group, and lack of randomization and/or blinding. In this respect, research on sexual side effects of SSRIs in healthy women is highly relevant. Unfortunately, only two studies have investigated the effects of SSRIs in healthy female volunteers,[25,26] but one of them has also been criticized for its methodology.[27] Therefore, the effects of SSRIs in women remain blurred; suggesting that more research of this type on healthy women should be performed in order to clarify the real (side) effects of chronic SSRI use on female sexual performance. With respect to this serious limitation of available human data, and acknowledging that 1) female animal research is most likely inadequate to investigate female orgasm, and 2) that different neurobiological mechanisms may be involved in the effects of paroxetine on women and female Wistar rats, it seems too early to conclude at the moment that hormonally (sub-) primed ovariectomized female Wistar rats are an inadequate model for SSRI-induced female sexual dysfunctions.

In the second part of our experiment, we have tested the putative of 5-HT_{1A} receptor desensitization. The 5-HT_{1A} receptor desensitization was also found in humans treated with SSRIs[28-30] and it is even proposed that receptor desensitization is required for therapeutic effects of SSRIs in depression.[31,32] In all vehicle treated female rats, (±)8-OH-DPAT clearly decreased proceptive behavior and lordosis score. This effect is in line with other studies that show the inhibiting effect of 5-HT_{1A} receptor agonists on female sexual behavior.[17,33-36] Furthermore, there is a clear right-shift in the dose response curve of females chronically treated with 10 and 20 mg/kg paroxetine, suggesting 5-HT_{1A} receptor desensitization. Previously we showed a similar phenomenon in SERT knockout rats.[12] Other studies also showed 5-HT_{1A} receptor desensitization after chronic SSRI treatment.[22,37-39]

(±)8-OH-DPAT is a 5-HT_{1A} receptor agonist with a slight co-affinity for the 5-HT_7 receptor. WAY-100635, on the other hand, is a silent selective 5-HT_{1A} receptor antago-

nist in that it prevents effects of 5-HT_{1A} receptor agonists, but has no effect in the absence of agonists.[12, 40, 41] In the sub-primed females, the effect of (±)8-OH-DPAT is only partially attenuated by WAY-100635 in the vehicle groups, while the effect was completely blocked in the paroxetine treated females (see figure 3 and 4). The finding clearly indicates 5-HT_{1A} receptor desensitization. Some studies have shown that repeated paroxetine and fluoxetine treatments do not alter 5-HT_{1A} receptor density in several brain regions.[39, 42-45] This suggests that changes in signal proteins downstream of the 5-HT_{1A} receptor are responsible for the functional desensitization. Thereby, a lower dose of WAY-100635 will be sufficient to fully attenuate the inhibiting effect of (±)8-OH-DPAT in chronic paroxetine treated females. However, the partial effect of WAY-100635 on control animals co-treated with (±)8-OH-DPAT may also suggest a role of the 5-HT_7 receptors in (±)8-OH-DPAT induced reduction in sexual behavior, while inhibitory effects of 5-HT_7 receptor activation on female sexual behavior have been reported before.[46]

At last, we would like to comment on the observation that there is no difference in effect of chronic paroxetine treatment in both sub-primed and fully-primed female rats; 5-HT_{1A} receptors are desensitized in both groups. Our sub-primed females show lower levels of proceptive behavior compared to fully-primed females and are, therefore, suggested as an animal model for female sexual dysfunction. Fully-primed rats, on the other hand, can be used as control females. From this study, we can conclude that initial level of sexual excitement does not influence the later effects of chronic paroxetine treatment on sexual functioning.

Conclusion

Overall, we conclude that chronic paroxetine treatment does not cause sexual side-effects in Wistar female rats. Furthermore, chronic treatment causes adaptive changes in the serotonin system such as desensitization of 5-HT_{1A} receptors, which may counteract the absence of sexual dysfunctions in chronic paroxetine treated rats.

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General discussion and summary

7

Eelke Snoeren

General discussion and summary

Female sexual dysfunction (FSD) is a disorder that affects between 33-48% of the population in the USA and in Europe.[1-3] In women, low arousal and low sexual desire are the most common problems.[4, 5]
The aim of this thesis was the development of animal models of female sexual dysfunction (FSD).
The thesis is divided into two parts. In the first part, two different models for FSD are described: the male-avoider model and the hormonally (estradiol) sub-primed model. In the second part, the potential sexual side effects of SERT blockade (either via chronic administration of a selective serotonin reuptake inhibitor (SSRI) or via gene knockout) are investigated. In this general discussion, we would like to discuss all models in a broader perspective and hypothesize about their contributions to future research directions.

The validity of animal models for FSD

The main goal of this research was the search for animal models of FSD. In the old days, lordosis was mainly used as measurement for sexual behavior. Lordosis is a hormone-dependent reflex, which is highly stereotyped and species specific. However, human sexual behavior is less hormone-dependent and stereotyped,[6] which makes it difficult to make direct generalizations from rats to humans. This was never considered as a problem, because the research would generate knowledge about how hormones act in the brain at the cellular and molecular level and which brain structures are involved in sexual behavior. But, nowadays, there is a growing need for effective treatment of human sexual disorders[3] and therefore, the research focuses on translational research elements of sexual behavior in order to have predictive models of human sexual pathology. Other parameters to study motivation in rats that relate more to sexual desire in human have been entering the research. Beach et al.[7] already pointed out that a meaningful model should not be based on formal similarities of behavior, but upon its causal mechanisms and practical use. One of the purposes of an animal model is to predict the effects of drugs in human. Therefore, it is necessary to validate an animal model using standard compounds that are known to be effective in humans. The animal model should be as sensitive to the agents as human are. It should also be selective so that drugs ineffective in human should also be ineffective in the animal model. Whereas this set of criteria is very useful in other animal models (of anxiety or depression), it is hard for animal models for FSD, because there are no drugs with proven clinical effects.
However, another method to judge the adequacy of animal models is by comparing the similarities of behaviors displayed in human and animals (face validity). It is not necessary to have similarity in the exact motor patterns, but it should refer to the purpose of the behavior. For instance, sexual desire or sexual motivation can be described as a successful approach to a potential mate.[8] This leads to the suggestion that approach behaviors, like darts and hops, are the better comparable behaviors. In addition, it is logical that the motivation to engage in sexual activities should gradually reduce in females during prolonged sexual interaction. Ågmo et al.[8] showed that the number of proceptive behaviors also slightly decreases during lengthened sexual behavior, which again suggests the usefulness of the number of darts and

hops as parameter of sexual behavior. The contact-return latencies also increased during prolonged sexual interaction, but this was accompanied by bigger variations, resulting in a less trustworthy parameter for studying sexual motivation.
A last criterion of animal models is construct validity, where the underlying mechanisms, causes or behavioral processes should be similar in human and animal models. It has been described before that the basic neural and behavioral mechanisms controlling sexual desire or motivation are similar in rodents and in humans,[9] which suggests that rodent models can be considered partly homologous to human sexual behavior.

Animal models for female sexual dysfunction

In our laboratory, we have developed models of male sexual dysfunctions that make use of the natural occurring differences in ejaculation frequency between individual Wistar rats. Three different endophenotypes are recognized: sluggish, normal and rapid.[10, 11] Sluggish rats have 0-1 ejaculation, normal rats 2-3 ejaculations, and rapid rats have 4-6 ejaculations during a 30-minute sex test. Based on the similarities between human and rat male sexual behavior, we proposed that sluggish rats could be preclinical models for delayed ejaculation, or low libido in men, whereas rapid ejaculators could model premature ejaculations.[11] In analogy with these male studies, we investigated the existence of such "endophenotypes" in female Wistar rats (**chapter 3**). We found that about 40% of the female Wistar rats are 'male-avoiders', because they spent less time with the male than the 'male-approachers'. Furthermore, they showed significantly less proceptive behaviors and this behavior was consistent over time. Both, low sexual desire and arousal disorders could be addressed here, but we suggest that the avoider-model reflects in particular the sexual desire disorder.
The tendency to avoid the male may correspond to the avoidance of sexual interactions by women suffering from low sexual desire. The tendency to avoid the male is based on the limited time the avoiders spent with the male in addition to increased contact-return latencies. Erskine et al.[12] suggested that the change in percentage of exits reflects the female's short-term response to the intensity of the copulatory stimulus, while contact-return latency is direct measure of the female's motivation to reinitiate mating. Therefore, it seems that the avoiders suffer from low sexual desire instead of low sexual arousal. But it should be taken into account that many women suffer from combinations of sexual dysfunctions, which makes it hard to distinguish between the different subtypes.
Unfortunately, there are some limitations of the avoiders-animal model. First of all, many rats have to be tested before a sufficient amount of avoiders are selected for further research, which makes it expensive and laborious. Second, our study did not exclude the possibility that the avoidance behavior of the females is not at all related to sexual behavior. For instance, increased levels of anxiety or a social deficit may lead to a similar avoidance behavior.

In **chapter 3**, we used time spent in male compartment as selection method. In **chapter 4**, we describe that non-estrus females also spent a lot of time with the male without having sex. Somehow, this sounds very contradictory. However, we hypothesize that time spent in the male compartment is a correct parameter as soon

as the females are hormonally primed. In **chapter 3,** intact (not ovariectomized) females were fully-primed with a high dose of estradiol. In these experiments, the male rat smelled the estrus female, which probably induced a very strong urge to copulate. Rejective behavior by male-avoiders might not be enough to stop the male from copulation. Therefore, the only way for the female to escape from sexual behavior is to run away to her own compartment. On the other hand, males will not try to copulate with a non-estrus female and thus there will be no reason for the female to escape from the male anymore. Overall, if this hypothesis is correct, it means that our method of selecting females by time spent with the male is plausible and a good indication of the willingness of the female to have sex.

The second animal model for FSD presented in this thesis (**chapter 4**) is the sub-primed model. In this model, ovariectomized females are primed with a small amount of estradiol. We showed that sub-primed females had significantly lower levels of sexual activity, both in proceptive and receptive behavior, compared to fully-primed (estradiol plus progesterone) females. The effects of hormones on female sexual behavior have been shown before.[13-15] But the sub-primed females can be perfectly used as a model to study the underlying mechanisms of FSD, while the lower sexual activity of the sub-primed female rats can model low sexual desire or arousal disorder. The biggest advantage of the sub-primed model above the avoiders-model is that it is very easy to make these 'dysfunctional females'.

Pharmacological studies in the animal models

In the avoiders model we tested (±)8-OH-DPAT (5-HT_{1A} receptor agonist), apomorphine (dopamine D_1/D_2 receptor agonist) and paroxetine (SSRI) (**chapter 3**). In the sub-primed model we tested testosterone and vardenafil alone and in combination (**chapter 4**).

The administration of (±)8-OH-DPAT in avoiders and approacher resulted in a decrease in number of darts and hops. This result is in agreement with other rat studies.[16-18] In addition, we showed that apomorphine caused an inhibition of sexual activity in both avoiders and approachers. This effect was more robust in avoiders, suggesting that these females are more sensitive to dopaminergic drugs than approachers. The inhibiting effect of apomorphine was in line with other studies,[19-21] except for some studies that showed an opposite effect.[22, 23] These differences in results with apomorphine may be due to differences in the doses tested: low doses might act via presynaptic receptors to inhibit dopamine release and thereby stimulate sexual behavior, while high doses act via postsynaptic receptors to inhibit sexual activity. The changes in dopaminergic systems in the avoiders might underlie their sexual dysfunctions. However, more research is needed to reveal the problems in dysfunctional females. The dopamine D_2 receptors is the best candidate, based on the hypothesis of Grierson et al.[24] that dopamine D_2 receptors are more involved in female sexual behavior than dopamine D_1 receptors.

In the second model, the sub-primed females, we have used testosterone and vardenafil as validating drugs. In women with low levels of sexual desire, this treatment seems to work.[25] Our results showed no effects of testosterone alone, but found prosexual effects of testosterone combined with vardenafil. So far, we were not able to detect the working mechanism or location of this combination treatment.

We were able to show in **chapter 4** that vardenafil does not act via the brain alone, because intracerebroventricular (i.c.v.) injections showed no prosexual effects in the sub-primed females. In males, i.c.v. injection of vardenafil has a pro-sexual effect.[26] Therefore, we have to conclude that the working location mechanism is probably different in male and female rats, although we can not exclude the possibility that we used an ineffective dose in our i.c.v. experiments.
Overall, the observed prosexual effects of the combination of testosterone and vardenafil validates the sub-primed model as a model of FSD. This model may be used in further research on female sexual dysfunction and the discovery of new treatments. The use of testosterone in our study also provides a new strategy for future research. Testosterone may create the right environment for certain drugs to stimulate behavior.

Effects of serotonin transporter blockade on female sexual behavior

In the second part of this thesis, the effects of serotonin transporter (SERT) blockade on female sexual behavior were investigated. From literature studies we know that women on SSRI treatment complain about sexual dysfunction.[27, 28] Whether this effect is really due to the drug treatment or to the depression illness itself is unclear. In male rats, it is clearly shown that several SSRIs do inhibit ejaculatory behavior.[29] In female rats, on the other hand, there are only some conflicting studies with fluoxetine.[30, 31] Therefore, we have further investigated the role of SERT in female sexual behavior.
First, we tested serotonin transporter (SERT) knockout rats (**chapter 5**) and second, chronic paroxetine (SSRI) treatment in normal rats (**chapter 6**). Both studies showed no effect on proceptive and receptive behavior after blockade (chronic paroxetine treatment) or absence (knockout rats) of the SERT. The lack of effect in both studies suggest that our results were trustworthy, but they are quite different from the other studies.[30, 31] Differences in drug (paroxetine vs. fluoxetine) or rat strain used may explain these differences in outcome. Wistar rats are known to be less sensitive to serotonergic drugs than other strains, like Fischer rats.[32] However, our study also suggests that more research should be done to ascertain that the sexual dysfunctions reported in women after chronic SSRI use are really due to the drug use and not just by their depression itself (discussed in **chapter 6**). A well designed double-blind placebo-controlled study in depressive women should be done, to clarify the effects of SSRI on female sexual functioning.
Another conclusion that can be drawn from our chronic paroxetine experiment is that female rats are less sensitive to paroxetine than male rats. Male rat studies with chronic paroxetine show robust effects on ejaculatory latencies.[29] Differences in responses to serotonergic drugs between males and females have been shown before.[33]

Adaptive changes following SERT blockade

An important finding in this thesis (**chapter 5** and **6**), was the desensitization of 5-HT_{1A} receptors in both SERT knockout rats and after chronic treatment with paroxetine. A recent study of Guptarak et al.[34] reported similar results after subchronic fluoxetine treatment in female rats. Also other studies confirmed 5-HT_{1A} receptors desensitization after chronic treatment with SSRIs[35, 36] or in SERT

knockout rats.[37] As described in **chapter 2**, several studies suggest that female sexual behavior depends on a balance between inhibitory (via 5-HT_{1A} receptors) and facilitory (via 5-$HT_{2A/2C}$ receptors) 5-HT receptors. It may be that this balance is still intact in the SERT knockouts and after chronic SSRI treatment.

Methodologies of female sexual behavior studies

In this thesis, the paced mating sex test was used to study female sexual behavior in rats. The most important factor in the sex test is the possibility of the female rat to pace her sexual behaviors. This was arranged by using a cage with two different compartments which were divided by a sheet with three holes. Because of the female's smaller size, only she was able to pass the holes and thereby pace her sexual behavior.

In female sexual behavior research it is important to use a paced mating set up, because non-paced mating is not rewarding.[38, 39] As soon as the female is able to pace her interactions, conditioned place preference is shown.[38] What the exact rewarding component in paced mating is that is rewarding is unclear. But it might be that control over the timing for the next stimuli is important.[40, 41]

The advantage of using the two-chamber paradigm, instead of a bilevel chamber[42] is that the female rat can relax in her own compartment and is not all the time chased by the male rat. Other parameters, like percentages of exits and contact-return latencies (CRL) can also be calculated. The only problem that comes with these extra parameters is that it is doubtful what they exactly mean. As mentioned before, Erskine et al.[12] suggested that the percentage of exits reflects the female's short-term response to the intensity of the copulatory stimulus, while contact-return latency is a direct measure of the female's motivation to reinitiate mating. However, there are some limitations to these measurements. First of all, the females should receive enough stimulation (mounts, intromissions and ejaculations) to calculate a real percentage of exits or contact-return latencies. Otherwise, the parameters will be based on only a few sexual stimuli and what will this tell us? Therefore, the second limitation is that it is impossible to study the percentages of exits and contact-return latencies when the rats are primed with inhibiting drugs that dramatically decrease the amounts of received stimuli. Overall, this parameter should be used as method to study sexual behavior in general, as a kind of additional information on the sexual behavior of female rats. It should not be used as the main measure for sexual excitement.

General thoughts

Finally, I would like to mention some general thoughts about the research performed the last decades on female sexual behavior. Many studies have been performed on sexual behavior, but it is clear that knowledge of female sexual behavior is still far lagging behind that of male sexual behavior. It is suggested that there are many differences between male and female sexual behavior that are even sometimes contradictory. As discussed in **chapter 2**, this difference may actually be not that big. The research in males mainly focuses on ejaculatory behavior, while in female research arousal is more the center of attention. Arousal and ejaculation are clearly mediated by different brain circuits. It could be that both in males and females arousal disorders have similar underlying mechanisms.

Conclusions on future perspectives

Overall, this thesis shows that the male-avoiders and the sub-primed model are good animal models for FSD. Whether or not the SERT knockout and chronic SSRI treatment models are suitable as model for SSRI-derived FSD, should be investigated in future. But with the help of all these models we discovered some interesting phenomena in female sexual behavior. First of all, we investigated the potential new treatment for FSD: a combination of testosterone and vardenafil. Second, we discovered some possible site of actions in mechanisms underlying FSD or the lack of FSD. Both the dopaminergic system (probably the D_2 receptors) and serotonergic system (5-HT_{1A} receptors) seems to be involved in sexual functioning. And therefore, future research should focus on these systems to unravel the problems underlying FSD. In addition, combination treatment with testosterone should not be forgotten in the development of new therapeutic drugs for FSD.

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-Samenvatting in het Nederlands
-List of abbreviations
-Dankwoord
-About the author
-List of publications

8

Eelke Snoeren

Samenvatting in het Nederlands

Vrouwelijke seksuele disfunctie (in het Engels afgekort tot FSD) wordt, door een groeiende hoeveelheid informatie erover, steeds meer gezien en erkend als een seksuele aandoening. Ongeveer 40% van alle vrouwen in de Verenigde Staten en Europa heeft last van deze aandoening in een mate waarin de kwaliteit van leven significant wordt aangetast.
FSD is onder te verdelen in vier verschillende categorieën: laag libido, lichamelijke opwindingsproblemen, problemen met orgasme en pijn tijdens de seksuele handelingen. Laag libido en opwindingsproblemen komen het meeste voor.
Vanwege ethische en praktische beperkingen van patiëntenonderzoek zijn diermodellen van groot belang bij het vergaren van wetenschappelijke kennis over FSD, met name bij psychofarmacologisch en neuroanatomisch onderzoek naar de onderliggende mechanismen van seksueel gedrag. Voor dit proefschrift is er gebruik gemaakt van 'Wistar' ratten, omdat deze seksueel gedrag vertonen dat zeer stabiel is en makkelijk kan worden geobserveerd. Bij een sekstest wordt een seksueel ontvankelijke vrouwtjesrat in een kooi bij een seksueel actief mannetje gezet. De kooi bestaat uit twee kamers die gescheiden zijn door een wand met drie openingen, waar alleen het vrouwtje doorheen kan. Zo kan zij ontsnappen aan het mannetje. Seksueel gedrag bij ratten wordt gekarakteriseerd door verschillende gedragingen die elkaar in korte tijd opvolgen. De gedragingen van het vrouwtje om het mannetje te verleiden heten darts en hops, wat respectievelijk gekenmerkt wordt door rennen waarbij de vrouwtjes plotselinge stoppen en hun achterlijf naar beneden doen om zich aan het mannetje te presenteren en door sprongen met vier poten van de grond. Lordoses, oftewel de kromming van de rug waarbij de kop omhoog wordt geheven, is gedrag dat gebruikt kan worden als maat voor de ontvankelijkheid voor seksuele stimuli. Mannelijk gedrag daarentegen, wordt gekenmerkt door mounts (bespringingen van het vrouwtje), intromissies (bespringing met penetratie) en ejaculaties. Na een serie van verschillende mounts, intromissies, darts, hops en lordoses volgt een ejaculatie. Na een korte rustperiode van ongeveer 5 minuten beginnen de opeenvolgingen van seksuele gedragingen opnieuw, wat in principe lang (uren) kan doorgaan maar in onze experimenten na 30 minuten wordt beëindigd.
Door alle gedragingen te observeren en te tellen, kunnen verschillende parameters bekeken worden. Zo wordt lordosequotiënt als maat gebruikt voor de frequentie dat een vrouwtjesrat lordoses laat zien en een lordose-score (van 0 tot 3) voor de mate van ontvankelijkheid. Ook kan de tijd die het vrouwtje bij het mannetje doorbrengt berekend worden en kan het aantal keren dat ze dart of hopt worden geteld. Al deze parameters samen geven de omvang van seksuele activiteit van het vrouwtje weer.
In dit proefschrift worden vier verschillende diermodellen besproken die bestudeerd en ontwikkeld zijn om verder onderzoek te kunnen doen naar FSD. Om de modellen te valideren en om meer inzicht te krijgen in de etiologie en pathogenese van de seksuele disfunctie, is ook psychofarmacologisch onderzoek gedaan.

Diermodellen voor FSD

Als eerste diermodel wordt het zogeheten "avoiders"-model besproken (hoofdstuk 3), waarin een onbehandelde populatie van 120 vrouwtjes ratten gescreend

werd op hun seksuele gedrag. Ongeveer 40% van zo'n groep zijn mannen-ontwijkers (ook wel "avoiders" genoemd), omdat ze significant minder tijd doorbrengen bij de mannen dan normale ratten ("approachers"). Dit gedrag, dat stabiel blijft gedurende alle testen, gaat gepaard met minder seksuele gedragingen (darts en hops) en langere pauzes tussen ontvangen stimuli (mounts en intromissies). Dit alles doet vermoeden dat het seksuele gedrag van de avoiders meer overeenkomt met een laag libido, dan met het onvermogen om seks te kunnen hebben.
Psychofarmacologisch onderzoek liet zien dat avoiders anders reageren op toegediende stoffen dan approachers. Beide groepen reageren hetzelfde op (±)8-OH-DPAT (serotonine 1A receptoragonist) en paroxetine (SSRI), maar de avoiders reageren beduidend extremer op apomorfine (een dopamine receptoragonist) dan approachers. Dit kan wijzen op een ander dopaminesysteem van disfunctionele ratten, waardoor zij gevoeliger zijn voor dopaminerge stoffen dan gezonde ratten.

Omdat het "avoiders"-diermodel ook nadelen heeft, zoals de hoeveelheid tijd en geld die het kost om de disfunctionele ratten te selecteren uit 120 vrouwtjesratten, is het tweede model ontwikkeld: het zogeheten "sub-primed"-model (hoofdstuk 4).
In dit model worden de eierstokken uit de vrouwtjesratten verwijderd (OVX-vrouwtjes), waardoor de productie van hormonen (oestrogeen en progesteron) stil komt te liggen. Door handmatige toediening van hormonen wordt de seksuele activiteit gemanipuleerd. Een lage dosering oestrogenen (bij sub-primed vrouwtjes) zorgt voor lage seksuele activiteit, terwijl toediening van oestrogenen en progesteron (bij fully-primed vrouwtjes) normaal seksueel gedrag oplevert. De sub-primed vrouwtjes dienen als model voor FSD, wat vervolgens gevalideerd werd met de toediening van een combinatie van testosteron (subcutaan) met vardenafil (oraal) (een pde-5 remmer met hetzelfde werkingsmechanisme als Viagra). De resultaten laten een proseksueel effect op seksueel gedrag in sub-primed vrouwtjes zien, wat mogelijk een aanknopingspunt kan zijn voor de ontwikkeling van een nieuwe medicijnen voor FSD. Testosteron en vardenafil hebben geen effect bij aparte toediening. De (voor)behandeling met testosteron creëert kennelijk een zodanige toestand in het lichaam (vermoedelijk in het brein), waardoor extra seksuele prikkels (veroorzaakt door vardenafil) beter opgemerkt worden wat leidt tot een proseksueel effect.
Hoe dit precies werkt is nog niet duidelijk. In hoofdstuk 4 wordt besproken hoe de injectie van vardenafil in de hersenen, gecombineerd met een subcutane injectie van testosteron, niet hetzelfde, gewenste effect heeft als bij orale toediening. Dit suggereert dat het proseksuele effect van vardenafil niet gereguleerd wordt in de hersenen, maar elders. Waar precies, is echter nog onduidelijk

Effect van SERT blokkade op seksueel gedrag

Vrouwen die een chronische behandeling met SSRI's (antidepressiva) ondergaan, klagen vaak over seksuele disfuncties (FSD) als bijwerking.Twee diermodellen voor deze medicatiegebonden FSD worden in het tweede deel van dit proefschrift besproken.

In hoofdstuk 5 worden genetisch gemodificeerde ratten gebruikt (de zgn. SERT knockout ratten), die de serotonine transporter (SERT) missen. De SERT is het transportmolecuul van serotonine (SERT) op serotonerge neuronen dat door SSRI's wordt geblokkeerd, waardoor serotonineniveaus in de hersenen stijgen en een antidepressieve werking in mensen bewerkstelligen. Dit effect wordt gesimuleerd in zowel ratten die chronisch behandeld worden met SSRI's, als in SERT knockout ratten die de SERT missen. De seksuele activiteit van SERT knockout ratten (zowel homozygote als heterozygote dieren) is echter normaal vergeleken met controlegroepen, waaruit opgemaakt kan worden dat de SERT onder normale condities niet belangrijk is bij het reguleren van vrouwelijk seksueel gedrag. Hoewel seksueel gedrag normaal is, blijken serotonine 1A (5-HT_{1A}) receptoren wel ongevoelig te zijn geworden omdat onder normale omstandigheden een 5-HT_{1A} receptoragonist ((±)8-OH-DPAT) een remmend effect op seksueel gedrag heeft (hoofdstuk 2 en 3), maar in SERT knockout ratten heeft deze stof geen effect. In de hersenen bestaat mogelijk een balans tussen activiteit van verschillende serotonerge receptoren op seksueel gedrag. Zo remmen 5-HT_{1A} receptoren seks, maar stimuleren bijvoorbeeld 5-$HT_{2A/2C}$ receptoren juist seks. Afhankelijk van de verhouding tussen activiteit van die receptoren ontstaat de mate van seksuele activiteit. Kennelijk worden de disfunctionele 5-HT_{1A} receptoren in de SERT knockout ratten gecompenseerd via een dergelijk mechanisme, waardoor er geen seksuele disfuncties optreden.
Hoofdstuk 6 toont een vergelijkbaar experiment als bij de SERT knockout ratten, maar dan met wildtype vrouwtjes die gedurende 56 dagen paroxetine (SSRI) toegediend kregen. Ook deze ratten vertonen, net als SERT knockout vrouwtjes, geen seksuele stoornissen. Zoals bij de SERT-knockout ratten, is wederom de 5-HT_{1A} receptor ongevoelig geworden. Deze verandering wordt, zoals eerder geschetst, kennelijk gecompenseerd door andere sytemen (zoals de 5-$HT_{2A/2C}$ receptor).

Toekomstig onderzoek

Uit de resultaten en conclusies in dit proefschrift kunnen een aantal aanbevelingen voor toekomstig onderzoek worden gemaakt.
Ten eerste zal onderzocht moeten worden of het ontwijkende gedrag in de avoiders slechts te wijten is aan seksuele disfunctie, of dat het mannetje ontweken wordt vanwege angst, sociale stoornis, depressie of iets dergelijks. Het diermodel kan in de toekomst beter gebruikt worden als er meer inzicht is in dit fenomeen.
Daarnaast lijkt onderzoek aan het het dopamine- en serotoninesysteem belangrijk voor het achterhalen van de onderliggende problemen van vrouwelijke seksuele disfuncties. Uit mijn onderzoek is duidelijk geworden dat deze systemen belangrijk zijn bij seksueel gedrag. Zo zit er mogelijk een storing in het dopaminesysteem, aangezien de ratten met een seksuele disfunctie gevoeliger zijn voor dopaminerge stoffen dan seksueel normale ratten. Het gebrek aan seksuele stoornissen na 5-HT_{1A} receptor desensitizatie daarentegen, wijst op de complexe rol van het serotoninesysteem in seksueel gedrag.
Met meer onderzoek naar de onderliggende hersenmechanismen van seksueel gedrag en eventuele disfunctionele componenten daarvan, zou in de toekomst een aanzet gegeven kunnen worden tot de ontwikkeling van een medicijn voor FSD.

De laatste, belangrijkste aanbeveling van dit proefschrift is dan ook de mogelijkheid van combinatiebehandeling van testosteron met vardenafil. Dit is mede aangetoond in een onderzoek met vrouwen. Naast dat deze combinatie op zichzelf een nieuw medicijn kan worden voor FSD, biedt dit ook perspectief voor toekomstige middelen. Testosteron kan de mediator zijn voor meer stoffen die uiteindelijk samen proseksuele effecten hebben. Het voordeel van deze combinatiebehandeling is de mogelijkheid tot acute inname (in plaats van chronisch), zodat de bekende bijwerkingen van testosteron achterwege blijven.

Abbreviations

Sexual behavior

CRL	contact-return latency
FSD	female sexual dysfunction
HSDD	hyposexual desire disorder
IELT	intravaginal ejaculation latency time
LQ	lordosis quotient
LS	lordosis score
MSD	male sexual dysfunction
PE	premature ejaculations
PEI	post ejaculatory interval

Others

5-HT	serotonin
DA	dopamine
EB	estradiol
ENU	N-ethyl-N-nitrosurea
Fos-IR	Fos-immunoreactivity
NA or NE	noradrenaline
NO	nitric oxide
P	progesterone
SERT	serotonin transporter
SERT+/+	wildtype serotonin transporter knockout rat
SERT+/-	heterozygous serotonin transporter knockout rat
SERT-/-	homozygous serotonin transporter knockout rat
TP	testosterone propionate
OVX	ovariectomized

Psychopharmacology

5,7-DHT	5,7-dihydroxytryptamine
5-MEO-DPAC	5-methoxy-3-(di-n-propylamino) chroman
5-MeODMT	5-methoxy-N,N-dimethyltryptamine
5-OH-DPAC	5-hydroxy-3-(di-n-propylamino)chroman
(±)8-OH-DPAT	(±)-8-hydroxy-2-(di-n-propylamino)tetralin
FG5893	diphenylbutylpiperazinepyridyl
LY-293,284	(4R)-6-acetyl-4-(di-n-propylamino)-1,3,4,5-tetrahydrobenz[c,d] indole
LY-228,729	(-)-4-(dipropylamino)-1,3,4,5-tetrahydrobenz-[c,d]indole-6-carboxamide
NAD-299	(R)-3-N,N-dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzo pyran-5-carboxamide hydrogen (2R,3R)-tartrate monohydrate
PDE5-inhibitor	phosphodiesterase type 5 inhibitor
p-MPPI	4-(2'-methoxy-phenyl)-1-[2'-(n-2"-pyridinyl)-p-iodobenzamido]-ethyl-piperazine

SSRI	selective serotonin reuptake inhibitor
(S)-UH-301	(S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin
WAY-100635	N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexane carboxamide 3HCL

Neuroanatomy

BNST	bed nucleus of the stria terminalis
BNSTpm	posteromedial part of BNST
CEA	central nucleus of amygdala
CTF	central tegmental field
DRN	dorsal raphé nucleus
IML	thoracolumbar intermediolateral cell column
LHA	anterior lateral hypothalamus
LSt neurons	lumbar spinothalamic neurons
MCG	midbrain central gray
MeA	medial amygdala
MeApd	posterodorsal part of MeA
mPFC	medial prefrontal cortex
MPN	medial preoptic nucleus
MPOA	medial preoptic area
MRN	median raphé nucleus
NAc	nucleus accumbens
nPGi	nucleus paragigantocellularis
PA	posterior nucleus of the amygdala
PD	posterodorsal preoptic nucleus
PAG	periaquaductal gray
PMV	ventral premammillary nucleus
PVN	paraventricular nucleus of the hypothalamus
RN	raphé nuclei
SPFp	parvocellular subparafascicular nucleus of posterior thalamus
SPN	sacral parasympathetic nucleus
VMN	ventromedial nucleus of the hypothalamus
VMNvl	ventrolateral part of VMN
VMNcv	caudoventral part of VMN

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Na stiekem toch wel wat bloed, zweet en tranen is mijn proefschrift dan eindelijk echt af! Natuurlijk had ik dit nooit voor elkaar gekregen zonder de steun van veel mensen. Het is daarom erg fijn dat een proefschrift ook altijd een dankwoord bevat, waardoor ik van deze gelegenheid gebruik kan maken om een aantal mensen in het bijzonder te bedanken.

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Berend: Je was mijn promotor, dus moest je me wel helpen. Maar dit deed je als een zeer fijne en prettige promotor. Je was altijd erg betrokken bij mijn onderzoek en zette, ondanks je drukke schema, direct alles aan de kant voor, zoals je zelf altijd al zei, "eindelijk weer wetenschap". Dank je wel dat de deur altijd open stond.

Marcel: Bedankt dat je mijn promotor wilde zijn. We hadden dan misschien niet heel vaak contact, maar toch ben ik blij dat je bij het proces betrokken bent geweest.

Johnny: Mijn partner in crime... Bij jou ben ik begonnen met mijn stage waar dit prachtige promotie-onderzoek uit voortkwam. Als begeleider was je al leuk, maar als collega nog leuker. Ik heb deze vier jaar veel aan je gehad als collega en vriend. Je trok me uit de diepste dalen met nieuwe theorieën over werk en floot me terug als ik weer eens te overdreven reageerde op dingen. Relativeren is jouw sterkste kant. Ik had me geen leuker en gezelliger kamergenootje kunnen wensen.

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Yuliya: Roommate... Het was erg gezellig in onze kamer. We zijn maar twee jaar collega's geweest, maar dit was genoeg voor leuke herinneringen aan bijvoorbeeld de gezellige Vooghel-avonden. Deze tekst is in het Nederlands, want de snelheid waarmee jij deze nieuwe taal hebt geleerd, is een voorbeeld voor mijn Noors.

Liesbeth: Jouw proefschrift is ook af! De afgelopen maanden waren een gekkenhuis, maar des te fijner was het om dit samen te doen. Samen lachen, samen huilen. Het waren vijf geweldige jaren om nooit te vergeten!

Jolanda: Dank je wel voor die geweldige discussieavonden in de Vooghel tot in de vroege uurtjes. Je was ook mijn kamergenoot op congressen. Heerlijk om eens iemand te hebben die net zo vlot naar bed gaat en opstaat als ik. Je bent een leuke vriendin en collega! En nogmaals bedankt voor dat ene verjaardagscadeau.

Tessa: Zwemmaatje...In het zwembad was het soms een strijd om wie de snelste was , maar op het werk was hier niets van terug te zien. Je bent een super collega/vriendin. Vergeet je vakanties niet!

Monica: Een gekkere collega kan ik me niet wensen. Ik heb genoten van alle maffe momenten die ik met je beleefd heb! Zowel op het werk als in de Vooghel. Maar nodig je voortaan geen gekke, eenzame vrouwen meer uit?

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Erik: Ik stond vaak bij je op de stoep met mijn historische momentjes. Dat zul je soms wel vervelend hebben gevonden. Maar toch stond je me iedere keer weer te woord. En dat met een grote portie humor. Lekker hè, die salades…

Gerdien: Bedankt dat je me iedere keer weer zo geduldig hielp als ik weer eens iets niet kon vinden. Je wist dan ook altijd precies waar alles lag. Het lab zonder jou zou maar een zooitje zijn.

Ruud: Ook jij bedankt voor alle goede tips die je me in de jaren hebt meegegeven. Je was een ideale coach met al jouw kennis en ervaring.

Marjolein: Met jou was het altijd lachen. Net toen het lab een beetje saai en burgerlijk begon te worden, kwam jij met je geweldige verhalen. Succes met de kippetjes!

Floor: Vanaf het eerste moment konden we het goed met elkaar vinden. Ook jij succes met je onderzoek.

Mechiel: Kletsen, dat kun je! Ik mocht mij er dan soms openlijk aan ergeren, maar jij weet, net zo goed als ik, dat we het prima met elkaar konden vinden. Twee tegenpolen, maar toch ook hetzelfde. Wat het was, weet ik niet, maar een magie was er wel.

Lucianne: Ook van jouw kennis heb ik regelmatig gebruik gemaakt. Het was prettig om regelmatig even snel bij jouw kamer naar binnen te kunnen glippen.

Christiaan: Ik kon soms aardig tegen je uitvallen, maar stiekem mag ik je wel!

Marga: Zonder jou was er niets terecht gekomen van mijn promotie. Jij regelde immers alles voor me. *Tusen takk* voor alles!

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Beste paranimfen,

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Lieve broertjes en zusjes,

Gineke: Je had altijd grote interesse in mijn onderzoek. Door jouw goede vragen werd ik altijd weer gedwongen terug te gaan naar de basis van het onderzoek.

Froukje: Ook jij bent nu aan het promoveren. Je wilt niet weten hoe leuk ik dit vind. Ik heb genoten van het zeuren over onderzoek en de daarbij behorende problemen. Misschien klinkt het gek, maar juist door deze kleinigheden kreeg ik meer vertrouwen in mijn werk.

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Jorma & **Israel**: Ik had me geen betere schoonbroertjes kunnen wensen. Thank you!

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About the Author

Eelke Snoeren was born on July 24th 1983 in Oirschot, the Netherlands, and lived in Bergen op Zoom during her childhood. In 2001 she passed her final exams at scholengemeenschap 't Rijks' and proceeded to study Biomedical Sciences at the Utrecht University (UU) in Utrecht. After graduating for her bachelor degree in 2004, she started her master Experimental and clinical neuroscience at the UU. In order to receive a master degree, she investigated the involvement of the dopamine system in addiction behavior under supervision of professor Louk Vanderschuren (Rudolf Magnus Institute in Utrecht) and the existence of potential endophenotypes for sexual behavior in female rats under supervision of dr. Johnny Chan and professor Berend Olivier (dept. psychopharmacology at UU). Under supervision of dr. Lucianne Groenink (dept. psychopharmacology at UU), she wrote her master thesis about interactions between corticotrophin-releasing factor and dopamine and anxiety behavior. She graduated in October 2006.
Since 2007, Eelke worked as a PhD-student at the department of psychopharmacology of the UU. Her project was about the search for animal models of female sexual dysfunction and the pharmacological drug effects in rats. Dr. Ronald Oosting, Prof. Berend Olivier and Prof. Marcel Waldinger helped her out by providing her with supervision.
Most of the results obtained in this project were described and discussed in the present thesis.

List of publications

Articles (First Author)

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