Sex, Drugs & Dual Control

A personalized sexual medicine drug development program based on the dual control model of sexual response

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ISBN 978-90-5335-894-8

Printed in the Netherlands by Ridderprint B.V.

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Sex, Drugs & Dual Control: een ontwikkelingsprogramma voor gepersonaliseerde geneesmiddelen tegen seksuele problemen, gebaseerd op het *dual control model* van de seksuele respons

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 1 oktober 2014 des middags te 4.15 uur

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De onderzoeken in dit proefschrift zijn gefinancierd door Emotional Brain B.V.

Dit proefschrift is mede-gefinancierd door Emotional Brain B.V.

Table of contents

CHAPTER 1

Introduction:

Female sexual dysfunction, pharmacotherapeutic options and understanding the role of Dual Control

Introduction

Low sexual desire, with or without sexual arousal problems, is the most common sex-related complaint reported by women [1–3]. As a result, many women suffer from sexual dissatisfaction, which often negatively interferes with their psychological wellbeing [4]. Unfortunately, the therapeutic arsenal for these complaints is limited. This thesis describes a research program which was directed at finding pharmacotherapeutic interventions for desire and arousal problems in women, which to date is still an unmet medical need. To find such interventions, a personalized sexual medicine approach was taken, seeking for altered function in different underlying mechanisms within one and the same indication, and designing and testing different pharmacotherapies that target the specific dysfunctional system. This approach led to the hypothesis that desire and arousal problems in women can originate from at least two distinct mechanisms. On one hand, it may be caused and sustained by a decreased sensitivity of the brain for sexual stimuli. It was found that these women benefit from the on-demand combined administration of sublingual testosterone and a phosphodiesterase type 5 (PDE5) inhibitor. On the other hand, desire and arousal problems may be caused and sustained by dysfunctional over-activity of sexual inhibitory mechanisms. These women were found to benefit from the on-demand combined administration of sublingual testosterone and a serotonin 1A receptor agonist.

The goal of the present introductory chapter is to describe the background in which this research program took, and is still taking, place. The patient population and the prevalence of female sexual dysfunctions are described, as well as the most notable potential pharmacotherapeutic solutions which have been investigated by other institutions and a rationale why these drugs will have a modest effect, at best. Subsequently, the scientific model which stands at the base of our personalized sexual medicine approach, the dual control model of sexual response, is introduced. I will discuss how this model guides us in the subdivision of sexual dysfunctions, and what that implies for potential pharmacotherapies in this indication. This leads to the two hypotheses on how sexual desire and arousal problems may originate in women and how specifically targeted pharmacotherapies can help tackle these problems. I will also introduce aspects of measurement and measurement validity that needs to be taken into account when investigating sexual function and dysfunction. The section will end with an overview of this thesis.

Classification and prevalence of sexual dysfunctions in women

In scientific literature, sexual dysfunctions are mostly classified and described in accordance with the Diagnostic and Statistical Manual of Mental Disorders (DSM), which is a widely used diagnostic handbook for clinicians and researchers, issued and published by the American Psychiatric Association. In May of 2013, its 5th edition was

published (DSM 5) and it introduced several relevant changes in the classification of sexual dysfunctions. The studies described in this thesis all predate DSM 5 and used the classification system of DSM's 4th edition (text revision; DSM IV-TR). Because of this, the DSM IV-TR classification is described, but the relevant changes in DSM 5 and its implications are also noted.

In DSM IV-TR, female sexual dysfunction (FSD) is divided into four major categories: sexual desire disorders, sexual arousal disorders, orgasmic disorders and sexual pain disorders. Sexual desire disorders are further divided into Hypoactive Sexual Desire Disorder (HSDD), which is a deficiency or absence of sexual fantasies and desire for sexual activity, and Sexual Aversion Disorder (SAD), which is an aversion to and active avoidance of genital sexual contact with a sexual partner. Sexual arousal disorders only contains Female Sexual Arousal Disorder (FSAD), which is a persistent or recurrent inability to attain or maintain until completion of the sexual activity, an adequate lubrication-swelling response or sexual excitement. Orgasmic disorders contain Female Orgasmic Disorder (FOD), which is a persistent or recurrent delay in, or absence of, orgasm following a normal sexual excitement. Finally, sexual pain disorders are divided into dyspareunia, which is genital pain associated with sexual intercourse, and vaginismus, which is the recurrent or persistent involuntary contraction of the perineal muscles surrounding the outer third of the vagina when vaginal penetration with penis, finger, tampon, or speculum is attempted. All these disorders have the further criteria that they must cause personal distress or interpersonal difficulties, and that they cannot be accounted for by other medical or psychiatric conditions or by medication or illicit drug use.

This thesis describes a program that is directed at the development of safe and effective pharmacotherapies in HSDD, the most common of the FSDs, but it is not restricted to HSDD alone. A common empirical finding is that sexual desire and arousal highly overlap, as do the dysfunctional states HSDD and FSAD [5]. The overlap between desire and arousal is much greater than that between other sexual domains. This overlap and the increasing evidence suggesting that the separation between HSDD and FSAD is artificial was a main reason to merge HSDD and FSAD into the single diagnosis Female Sexual Interest/Arousal Disorder (FSIAD)[1] in DSM 5. Thus, the HSDD patient population we are targeting is often also diagnosed with FSAD, and therefore the data we have gathered is generalizable to FSIAD providing these patients have absent/reduced interest in sexual activity and absent/reduced sexual erotic thoughts or fantasies. The transition from HSDD and FSAD to FSIAD will not have a substantial impact on the drug development program. This is discussed further in chapters 5 and 8.

Prevalence rates of FSD are very diverse. Percentages ranging from 15.6% to 53.8% have been reported for the prevalence of any FSD in general (see table 1). This

[1] Additionally, SAD has been removed because this diagnosis lacked empirical support. Dyspareunia and vaginismus have been merged into a single diagnosis, genito-pelvic pain/penetration disorder because a separation between these two was artificial and unreliable. Additionally, DSM5 diagnoses now also require a minimal symptom duration of 6 months, and some symptoms must be present 75-100% of the time. The disorder cannot be the consequence of severe relationship distress (e.g., partner violence) or other significant stressors.

extraordinary range is at least partly explainable by differences in demographics (age ranges and country), by prevalence type (point prevalence or period prevalence) and by FSD definitions used (e.g. duration of symptoms).

Table 1 Prevalence of female sexual dysfunction

Study	Population	N	Age	Prevalence type
Laumann et al. [1]	US	1749	18-59	Period (12 months)
Fugl-Meyer & Fugl-Meyer [2]	Sweden	1335	18-74	Period (12 months)
Bancroft et al. [6]	US	987	20-65	Period (4wks)
Mercer et al. [7]	UK	11161*	16-44	Period (12 months)
Leiblum et al. [8]	US	952	20-70	Point
Shifrin et al. [3]	US	31581	18-102	Point
West et al. [9]	US	2207	30-70	Period (30 days)

Table 1 Prevalence of female sexual dysfunction (continued)

Study	HSDD (%)	FSAD (%)	FOD (%)	PAIN (%)	FSD (%)
Laumann et al. [1]	31.6	20.6†	25.7	15.6	43
Fugl-Meyer & Fugl-Meyer [2]	33	12†	22	7	48
Bancroft et al. [6]	7.2	12.2/31.2‡	9.3	3.3	24.4
Mercer et al. [7]	10.2 / 40.6§	-	3.7/14.4§	3.4/11.8§	15.6/53.8§
Leiblum et al. [8]	14/26/9/14¶	-	-	-	-
Shifrin et al. [3]	37.7	25.3	21.1	-	43.1**
West et al. [9]	26.7/7.7/±40/±12***	-	-	-	-

* Total number of men and women that were included in the study

† Lubrication problems

‡ Arousal/lubrication

§ First number is percentage of subjects experiencing problems >6 months, second number is percentage of subjects experiencing problems >1 month.

¶ HSDD in premenopausal women/surgically postmenopausal women of 20-49 yrs/naturally postmenopausal women/surgically postmenopausal women of 50-70 yrs.

** 22.4% indicated as experiencing personal distress because of their FSD

*** Premenopausal with low desire / premenopausal with HSDD / postmenopausal with low desire / postmenopausal with HSDD

FOD = female orgasmic disorder; FSAD = female sexual arousal disorder; FSD = female sexual dysfunction; HSDD = hypoactive sexual desire disorder; UK = United Kingdom; US = United States.

It is not the goal of this chapter to define exactly the scale of the problem, but it is safe to say, that a very large group of women suffer, at one point or other, from sexual problems. As can be seen in table 1, the percentages for HSDD range from 7.7% to 40.6 %, and for FSAD from 12% to 31.2%. These numbers show that, even for the most conservative estimations, HSDD, with or without FSAD, (and thus also FSIAD) is a common problem. Unfortunately, treatment of HSDD with psychological interventions like cognitive behavioral therapy, sensate focus training or sex therapy show only modest treatment effects and limited maintenance of improvement over longer periods of time (see Basson et al. [10] for review).

'One size fits all' pharmacological treatment options for FSD

There has been a substantial amount of research performed on several different pharmacotherapies for this indication, but none have yet received marketing approval from the US and EU regulatory agencies (US Food & Drug Administration (FDA) and European Medicines Agency (EMA), respectively). One of the candidates is continuous

testosterone treatment because of its well-established role in sexual behavior (even though there is still debate about the exact mechanisms involved; see chapter 5 for a discussion on this topic). Continuous testosterone administration can increase sexual desire [11-15], which is why there are at least 4 million off-label prescriptions for continuous testosterone therapy written out in the US alone, for women with sexual problems [16]. The testosterone preparations that have been tested in women for HSDD have however failed to show an adequate safety/efficacy ratio. Another candidate is the daily use of flibanserin, a 5-HT_{1A} receptor agonist and 5-HT_{2A} receptor antagonist which was originally tested as an anti-depressant. It has been hypothesized that flibaserin increases sexual desire in HSDD by increasing dopaminergic and noradrenergic transmission, and decreasing serotonergic transmission in the prefrontal cortex [17]. However, in both New Drug Applications to the FDA, the agency deemed the safety/efficacy ratio of flibanserin inadequate, to date still withholding approval.

A last candidate that is in an advanced stage of clinical research is bremelanotide. This melanocortin receptor agonist, originally developed as a tanning agent, has an on-demand subcutaneous drug delivery system. It's mechanism of effect seems to be largely dependent upon its ability to increase dopaminergic transmission in the medial preoptic area, a hypothalamic center which innervates the ventral tegmental area and the nucleus accumbens, which are both implicated in reward related behavior [18]. Initially, intranasal administration was tested, but this administration method was discontinued because of concerns about its side effects on blood pressure. Bremelanotide is planned to enter Phase 3 clinical testing in the second half of 2014.

The efficacy of testosterone gels and patches, flibanserin and bremelanotide all seem modest. The reason for this may lie in the heterogeneous etiology of the targeted indication, HSDD. Pharmacotherapies that target the main symptom of HSDD, decreased desire, by chronically increasing testosterone levels, will likely be inefficacious in a substantial subgroup because desire is not only testosterone dependent. Pharmacotherapies that aim to 'normalize' central dopaminergic, noradrenergic and serotonergic transmission, will likely be inefficacious in a substantial subgroup because one or more of these systems may not be dysfunctional in these women; altering their activity could then have no or even reversed effects. Thus, clinical trials investigating the efficacy of these 'one size fits all' drugs will show modest effects at best because they are inefficacious in a substantial portion of the targeted indication. A drug development program is more likely to succeed if the possibility of different etiologies is explored and drugs are designed specifically to target these causes.

Personalized sexual medicine based on the Dual Control model of sexual response

We advocate a personalized medicine approach, developing different treatments for the same disorder under the assumption that different causal mechanisms underlie that single disorder. The dual control model of sexual response [19,20] offers a scientific

basis for the investigation and specification of different underlying neurobiological mechanisms and thus for pharmacotherapeutic targets. According to the dual control model, sexual behavior is dependent upon two separate but interacting systems: a sexual excitation system and a sexual inhibition system. The opposing sexual excitation and inhibition systems determine behavior depending on presence of external stimuli, internal state and outcome expectation (of reward and of negative consequences). Sexual stimuli (e.g. erotic video clips) trigger the sexual excitation system, but do not necessarily induce a sexual response. This is dependent upon the relative strength of the erotic stimulus, the internal state (e.g. a satiated or non-satiated state) and of other internal or external stimuli signaling (dis)advantageousness of a sexual response. If a sexual stimulus is potent enough to activate the sexual excitation system, and the consequences of sexual responding are not, consciously or subconsciously, perceived as negative, the sexual inhibition system will not be activated and the stimuli may induce a sexual response. If the same potent sexual stimulus is presented in a setting in which sexual responding is disadvantageous, stimuli signaling negative consequences (e.g. thoughts of negative consequences of sexual responding) will activate the sexual inhibition system and thus counter the sexual stimulus' activation of the sexual excitation system, ultimately causing a decimated or even no sexual response to that sexual stimulus.

Healthy sexual functioning is only possible if these two systems are in balance. If an individual has low propensity for sexual excitation, or a high propensity for sexual inhibition, a potent sexual stimulus will have a smaller chance to induce a sexual response under advantageous conditions for sexual responding, than it will in an individual with normal propensity for sexual excitation and inhibition. The dual control model therefore postulates that individuals who have low propensity for sexual excitation or a high propensity for sexual inhibition are more likely to experience problems of impaired sexual response or reduced sexual interest. HSDD/FSIAD may therefore be caused by dysfunction of either of these 2 distinct mechanisms, which means that at least 2 subgroups within HSDD/FSIAD may exist, depending on which system is out of balance. Therefore, when designing pharmacotherapies for these 2 subgroups, one must recognize that for one group, sexual excitation must be increased, whilst for the other group, sexual inhibition must be decreased.

In chapters 2, 3, 5 and 8 the underlying mechanisms of dysfunction in sexual excitation and sexual inhibition are elucidated, and it is described how this may cause HSDD/FSIAD. This understanding leads to the inception (and testing of; see chapters 2, 3, 6 and 7) of two on-demand pharmacotherapeutic drug combinations for two distinct subsets of patients with HSDD/FSIAD: on-demand combined administration of sublingual testosterone and a PDE5 inhibitor for women with HSDD/FSIAD and decreased sensitivity of the brain for sexual stimuli, and, on-demand combined administration of sublingual testosterone and a $5HT_{1A}$ receptor agonist for women with HSDD/FSIAD and dysfunctional over-activity of sexual inhibitory mechanisms.

At the basis of these two on-demand therapies lies testosterone. Testosterone plays an important role in sexual behavior and sublingual testosterone administration increases the sexual motivation with a delay in effect of approximately 4 hours [21]. The basic assumption for the two on-demand drug therapies is that sublingual testosterone administration induces a window of effect of approximately 3 hours during which the PDE5 inhibitor or the $5HT_{1A}$ can take effect. Hence, both on-demand combination drug therapies are administered in such a manner that the effects of both compounds of each drug combination coincide. Sublingual testosterone administration gives a rapid uptake of testosterone into the systemic circulation (T_{max} = 15 minutes) and a rapid elimination rate; after two to three hours the peak in testosterone has dissipated. It has a delay in effect in the increase of sexual motivation from approximately three up and to approximately six hours after it has been administered, giving a therapeutic window of 3 hours [21]. The PDE 5 inhibitors sildenafil and vardenafil, and the $5\text{-}HT_{1A}$ receptor agonist also have a therapeutic window of approximately 3 hours, but they have a quicker onset of action (approximately 1 hour after administration). To create maximal overlap in the pharmacodynamic effects of the two active constituents in both drug treatments, they have to be released in the systemic circulation at different time points. First, sublingual testosterone is administered, and two to two and a half hours later, the PDE5 inhibitor or the $5\text{-}HT_{1A}$ receptor agonist, depending on the drug combination, are administered orally. This method of administration timing ensures that both on-demand drug therapies have a therapeutic window of approximately 3 hours, starting 3 hours after the sublingual testosterone administration.

Validity of the laboratory assessment of sexual functioning

If sexual inhibition greatly influences sexual responses on a conscious and subconscious level, one might wonder how this could covertly affect a laboratory measurement and thus the validity of results obtained in the laboratory. Various procedures have been used to influence sexual excitation and/or inhibition in the laboratory. A common method to induce sexual excitation in the laboratory is by exposure to erotic film clips. Inducing inhibition of the sexual response is less straightforward, but has been induced in the laboratory through different subtle experimental manipulations during exposure to erotic stimuli, like undemanding cognitive distraction [22,23], seeing one's reflection in the mirror [24], inducing the feeling of being watched [25], and monitoring one's sexual arousal [26]. These manipulations can inhibit genital and subjective sexual arousal. This illustrates that relatively nonintrusive psychological manipulations can shift the delicate balance between excitatory and inhibitory factors influencing the sexual response. This implies that systematic factors present in the laboratory setting—such as presence of an experimenter in the other room, or the unfamiliar artificial situation—might influence the sexual response in an unknown manner, and thus bias the results. If people differ in their propensity for sexual inhibition, this could differentially affect the sexual response of these (different groups of) people in the laboratory. On the other hand, the inhibitory stimulus could be so strong that it clouds

any group differences. In chapter 4 this issue is addressed. An ambulatory laboratory was developed, so that the physiological sexual response could be measured at home and a comparison could be made to the physiological sexual response in the institutional lab in women with and without HSDD. It was hypothesized that, at home, potential (covert) lab-induced inhibitions would not be present. The home measurement would show stronger sexual responses in women without HSDD and this is then taken as evidence of increased ecological validity of the results of an ambulatory measurement setting.

General Outline

Chapter 2 describes the first study investigating the combined administration of sublingual testosterone 0.5 mg and the PDE5 inhibitor vardenafil 10 mg. This randomized, double-blind, placebo-controlled, cross-over study tested the efficacy of this combination, as compared to placebo and the monotherapies, in increasing VPA in response to sexual stimuli, in women with HSDD. It also investigated the influence of the centrally acting testosterone on preconscious attentional bias for erotic cues. This leads to the finding that HSDD patients who were sexually abused during their childhood react differently to testosterone administration, and to the combined therapy, and ultimately to the hypothesis that HSDD may be caused by two very distinct mechanisms.

Chapter 3 describes a replication study of the study described in chapter 2. This study also investigates the combined administration of sublingual testosterone 0.5 mg and the PDE5 inhibitor vardenafil 10 mg. This randomized, double-blind, placebo-controlled, cross-over study tested the efficacy of this combination, as compared to placebo and the monotherapies, in increasing VPA and subjective sexual arousal in response to sexual stimuli, in women with HSDD, without childhood sexual abuse. It also investigated preconscious attentional bias for erotic cues. The findings confirm and refine the hypothesis that HSDD may be caused by two very distinct mechanisms.

The studies described in chapters 2 and 3 were performed in the research institution's psychophysiological laboratory. Sexual responding however, is strongly susceptible to interfering, non-sexual stimuli, like being in a laboratory with female technicians measuring every response. Therefore, an ambulatory psychophysiological laboratory was developed. This portable lab could be taken home and operated by the patient. Chapter 4 describes a controlled study that investigates differences in subjective and physiological sexual responding of HSDD patients and healthy controls in the institutional laboratory setting and the ambulatory home laboratory setting. The findings provide evidence that measurements of sexual function in the institutional laboratory are less valid than measurements at home.

Chapter 5 is a theoretical substantiation of the hypothesis that different causal mechanisms are responsible for the emergence of HSDD. It describes the role of androgens and serotonin in sexual functioning, and provides an elaborate rationale for

the use of on-demand combined administration of sublingual testosterone and a PDE5 inhibitor in women with HSDD and insensitivity of the brain for sexual stimuli, and for the use of on-demand combined administration of sublingual testosterone and a serotonin 1A receptor agonist in women with HSDD and maladaptive activation of sexual inhibitory mechanisms.

Chapters 6 describes a randomized, double-blind, placebo-controlled, cross-over study on the efficacy and safety of on-demand combined administration of sublingual testosterone and a PDE5 inhibitor in women with HSDD and insensitivity of the brain for sexual stimuli. This study investigates the effects of this therapy on sexual responses as measured by the ambulatory laboratory at home, and of its effects on the sexual satisfaction of sexual encounters over a period of 3 weeks. The data show that this medication combination is an efficacious and safe potential therapy for this indication.

Chapters 7 describes a randomized, double-blind, placebo-controlled, cross-over study on the efficacy and safety of on-demand combined administration of sublingual testosterone and a 5-HT_{1A} receptor agonist in women with HSDD and dysfunctional activity of sexual inhibitory mechanisms. This study investigates the effects of this therapy on sexual responses as measured by the ambulatory laboratory at home, and of its effects on the sexual satisfaction of sexual encounters over a period of 3 weeks. The data show that this medication combination is an efficacious and safe potential therapy for this indication.

Chapter 8 describes an MRI study in women with HSDD and healthy controls that was set up to explore potential neuroanatomical correlates of HSDD. The results of this study show that HSDD coincides with anatomical differences in the central nervous system, in both gray and white matter. The areas which correlate with sexual desire and arousal problems play an important part in the brain's sensitivity for sexual cues and inhibitory mechanisms, and thus give additional evidence for the validity of the hypothesized HSDD subdivision.

Finally, in chapter 9 this thesis' hypotheses are discussed and possible future directions are given.

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CHAPTER 2

Childhood sexual abuse, selective attention for sexual cues and the effects of testosterone with or without vardenafil on physiological sexual arousal in women with sexual dysfunction: a pilot study

Journal of Sexual Medicine 2009;6:429-439

Abstract

Introduction. Female sexual dysfunction (FSD) may be associated with reduced central sensitivity for sexual cues. A single dose of testosterone might induce an increase in sensitivity for sexual stimuli, which in turn allows a PDE5 inhibitor to be effective in boosting the physiological sexual response. Negative sexual experience—like childhood sexual abuse (CSA)—might be an important intervening factor in these drugs-induced alterations.

Aim. To investigate if the combination of testosterone and vardenafil causes an increase in sensitivity for sexual cues and an increase in physiological sexual responding in women suffering from hypoactive sexual desire disorder (HSDD).

Methods. Thirteen women with HSDD underwent four different drug treatments: (i) placebo; (ii) vardenafil; (iii) testosterone; and (iv) combination of testosterone and vardenafil. During each treatment, they performed an emotional Stroop task and watched neutral and erotic film clips.

Main Outcome Measures. A masked version of the emotional Stroop task, and the vaginal pulse amplitude (VPA).

Results. We found different effects in women who had reported CSA (N = 5) compared with those who had not (N = 8). In women without CSA, testosterone induced an increase in their originally low levels of preconscious attention for sexual cues, while women with CSA showed a decrease in their originally high levels of attention. In these groups, we also found different effects of the combination of testosterone and vardenafil on the VPA: women without CSA revealed a statistically significant increase in their VPA during treatment with the combination of testosterone and vardenafil as compared with placebo. Women with CSA, however, showed no alterations in their physiological sexual responding during this combined drug treatment.

Conclusion. In women without CSA, testosterone appears to activate central sexual mechanisms resulting in higher VPA under the combination of testosterone and vardenafil. This effect did not occur in women with CSA.

Introduction

The present study was designed to investigate the effects of sublingual testosterone, the phosphodiesterase type 5 (PDE5) inhibitor vardenafil and the combination of these drugs on sexual function of women diagnosed as having hypoactive sexual desire disorder (HSDD), with or without sexual arousal problems.

It has been shown that one dose of sublingually administered testosterone (0.5 mg) in sexually functional women had a stimulating effect on physiological vaginal arousal and subjective indices of sexual functioning 4 hours after the peak in plasma testosterone levels [1,2]. We assume that these effects on physiological arousal occur as the result of an increase in activation of central sexual stimulation. Central sexual stimulation is a necessary condition for a PDE5 inhibitor to be effective; vardenafil is such a PDE5 inhibitor. Vardenafil can improve erectile function in men with erectile dysfunction, on average, close to normal function [3,4]. The working mechanisms of vardenafil and similar drugs are as follows. In the penis, during sexual stimulation, nitric oxide (NO) will be released from nerves and endothelium, which induces production of cyclic guanosine monophosphate (cGMP). cGMP is a key mechanism in relaxing smooth muscle necessary for the induction of an erection. This nucleotide is hydrolyzed by the phosphodiesterases (PDE) in the corpora cavernosa, for which phosphodiesterase5 is the most abundant PDE. Therefore, during sexual stimulation, the action of NO/cGMP on erectile function will be enhanced by PDE5 inhibitors [5]. The genitalia of both sexes have common embryological origins. The clitoris consists of an erectile tissue complex, which embeds the anterior vaginal wall. Clitoral tumescence and the anterior wall of the vagina are highly involved in female sexual arousal and response. Consequently, sexual stimulation will activate the NO/cGMP pathway also in women, which in turn is necessary for a PDE5 inhibitor to be effective. It has recently been shown that sildenafil—also a PDE5 inhibitor—improves sexual performance in sexually functional women [6].

Women suffering from HSDD, however, by definition suffer from low sexual desire and low central sexual stimulation, which is why PDE5 inhibitors will seldom have an effect on genital arousal [7–9], and why these women will not benefit from the use of PDE5 inhibitors for improvement of their sexual functioning. Treatment with centrally acting drugs that stimulate central sex mechanisms—like sublingual testosterone—might be a necessary condition for vardenafil to be effective.

In the present study, we are particularly interested in the influence of these drugs on the association or dissociation between indices of central sexual information processing and physiological sexual responding (as a preparatory response for sexual behavior). We assume that alterations in preconscious attentional bias for sexual cues reflect a change in the sensitivity of the brain for the processing of sexual information.

In the present placebo-controlled study, we investigated the effects of vardenafil, testosterone, and the combination of testosterone and vardenafil on preconscious attentional processes and physiological sexual responding. The emotional Stroop task measures attentional bias for emotional cues [10,11]. In this task, subjects are instructed to name the color in which emotional and neutral stimuli are printed as quickly as possible while ignoring the meaning of the word. The motivational state of the subject and the emotional content of the stimuli determine the performance on this task in terms of slowing down or speeding up in color naming. Attentional bias for emotional cues is demonstrated when color-naming latencies for emotional stimuli are greater or smaller than color-naming latencies for neutral stimuli. It has been assumed that attention is automatically allocated to the emotional value of the stimuli. Threatening cues, however, could also evoke withdrawal of attentional resources from emotional cues, indicating that the threat is so serious that it can elicit an avoidance response. A masked version of this task turned out to be a more reliable measurement of (preconscious) attentional bias for emotional cues [10,11]. Thus, deceleration in color naming of masks, preceded by sexual words, indicates increased resource allocation to the processing of sexual stimuli, while acceleration indicates withdrawal of attentional resources from erotic cues.

There are many factors that can influence sexual function, such as the use of selective serotonin reuptake inhibitors, and factors influencing the hormonal state (like phase of the menstrual cycle, use of oral contraceptive, obesity, smoking, and reproductive age). Using the above mentioned delay paradigm, however, it has been demonstrated that sublingual testosterone induces significant alterations in cognitive and affective functioning in heterogeneous groups of female subjects [1,2,12–20]. These effects can be established irrespective of the presence of factors mentioned above that influence testosterone (and the free fraction of testosterone) and sexual function. Consequently, we did not exclude women on the above-mentioned factors that influence sexual functioning.

We carried out a double-blind experiment in which each subject underwent four different drug treatments in randomly assigned order: placebo, the PDE5 inhibitor vardenafil, testosterone, and a combination of testosterone and vardenafil. In the presentation of the results, we will concentrate on the shift in preconscious attentional processing of the sexual information and on physiological sexual responding between two time moments on each drug treatment day (on each treatment day there are pre and post drug intake measurements). Because of the 4-hour delay in effect of sublingual testosterone, the first session was carried out just before the intake of the drugs, and the second session was 4 hours after the intake of testosterone.

We hypothesized that testosterone (with or without vardenafil) would induce an increase in preconscious attentional bias for erotic cues after a 4-hour delay. Moreover, based on this increase in sensitivity for sexual cues, we expected that the combination of

testosterone and vardenafil would induce an increase in genital blood flow (i.e., the vaginal pulse amplitude [VPA]) during exposure to visual erotic stimulation. However, in the initial exploration of the Stroop data, two subgroups with different response profiles in their preconscious attentional bias scores emerged. Further exploration proved that the histories of the subjects in these subgroups differed: subjects in one group had experienced sexual abuse during their childhood, while subjects in the other group had not. Depending on sexual history and activation of implicit or explicit memory processes, sexual stimuli can activate different sexual regulation mechanisms (e.g., sexual inhibitory or excitatory mechanisms), and can elicit different sexual responses (e.g., sexual withdrawal or approach behavior) [21,22]. Of course, circumstances are also important for activation of the different response possibilities. Because of CSA, sexual stimuli may become conditioned as negative and threatening. We assume that testosterone (alone or combined with a PDE5 inhibitor) will increase the sensitivity of the brain for sexual cues [23]. Consequently, this treatment might have different effects in women who experience sexual cues as threatening than in women for whom sexual cues are neutral (e.g., as a result of relatively insensitive system for sexual cues) or attractive. Because of these considerations, we have used these two subgroups in our analysis.

Methods

Subjects

Thirteen women diagnosed as suffering from HSDD, with or without arousal problems, participated in this study. Every woman signed a written informed consent and received reimbursement for her participation. This study was approved by a medical ethics committee, Stichting Therapeutische Evaluatie Geneesmiddelen, Medisch Ethische Toetsingscommissie (STEGMETC) in Almere, The Netherlands.

Design and Procedures

In a double-blind, placebo-controlled cross-over design, each subject underwent four different drug treatments in randomly assigned order at separate experimental days: (i) *placebo*: placebo for testosterone (cyclodextrin solution without testosterone) *and* placebo for vardenafil (powder-filled gelatin capsule without vardenafil); (ii) *vardenafil*: vardenafil (10 mg) (hidden in a powder-filled gelatin capsule) and placebo for testosterone (cyclodextrin solution without testosterone); (iii) *testosterone*: testosterone [0.5 mg] sublingually with cyclodextrin as carrier and placebo (for vardenafil); or (iv) *combination of testosterone and vardenafil*: sublingual testosterone (0.5 mg) with cyclodextrin as carrier and vardenafil (10 mg) (hidden in a powder-filled gelatin capsule). During each day, subjects underwent two experimental sessions (one before and one after the drug intake). See last paragraph of this section for details.

Women were recruited through printed announcements in a local hospital, advertisements in regional newspapers, and through the Internet. Potential participants were invited for a screening visit. During this screening visit subjects filled out the Female Sexual Function Inventory (FSFI) [24] and were interviewed by an experienced female psychiatrist and a gynecologist to diagnose for FSD, according to the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV-TR) criteria [25], and to determine eligibility for study participation. In addition, subjects were asked about childhood sexual abuse (CSA).

To exclude major medical and psychiatric illnesses, the general medical and gynecological history was taken. Contraindications to participate were pregnancy, breastfeeding, major surgery on the vagina or vulva, and hormonal contraception containing anti-androgen hormones. Weight, height, blood pressure (supine and standing), heart rate, respiration rate, and body temperature were measured. A supine, 12-lead electrocardiogram was recorded and examined by a physician (and when necessary, a cardiologist). A gynecological examination and urine pregnancy test were performed to exclude pregnancy, vaginal infections, and undetected major gynecological illnesses. Cultures were taken to exclude chlamydia or gonococcus infections. Standard blood chemistry and hematology tests were performed.

Women who had been experiencing HSDD with or without low sexual arousal, for at least the last 6 months before the screening visit were included in the study. When they had any significant medical illness or other contraindications, subjects were not enrolled into the study. A trained female experimenter familiarized the subject with study requirements and procedures. This included the use of the vaginal photoplethysmograph (a tampon-shaped device) to measure changes in VPA during different exposure conditions. Subjects viewed a 5-minute neutral film fragment followed by a 5-minute erotic film fragment. After this, they practiced a shortened masked version of the emotional Stroop task. No subjects dropped out after the familiarization trial. Participants were instructed not to use any alcohol or psychoactive drugs on the evening before and during the experimentation day and not to make appointments for the experimental trials during their period of menstruation.

Each experimental day started with a physical examination (measurements of vital signs, blood pressure, heart rate, temperature and respiration rate). The four experimental days were each separated by at least 3 days and at most 7 days. During each experimental day, subjects underwent two experimental sessions. The first session was carried out immediately before the intake of the sublingual testosterone (0.5 mg) (or placebo for testosterone). During this *first session*, subjects were seated in a sound attenuated, dimly lit experimental room, and the subjects carried out the first emotional Stroop task (15 minutes). Subsequently, the subjects received the vaginal probe and were left alone in the room to insert the probe [26]. During all trial sessions, the VPA was continuously measured (see paragraph measures). The subjects were instructed to

sit as motionless as possible while viewing the film fragments. After a 10-minute adaptation period, subjects were exposed to a 5-minute neutral film clip followed by a 5-minute erotic film clip. After these baseline measurements, the subjects applied the solution of testosterone (or placebo) sublingually using a syringe. They were instructed to rinse the solution sublingually and to swallow the solution upon the warning signal of the female experimenter. The subjects removed the vaginal probe and were taken to a waiting room for a pause. Two hours after the intake of testosterone, subjects ingested the vardenafil (or placebo for vardenafil) capsule. During the continuation of their pause, they could consume their low fat lunch. Four hours after intake of testosterone, women underwent the same psychophysiological trial (*session 2*) as before: the 10-minute adaptation period and the 5-minute neutral and erotic film clips. This trial was followed by a second execution of the masked emotional Stroop task. Each experimental day ended with a short physical examination and a report of adverse events. Throughout the experiment, different neutral and erotic films were used. The neutral film fragments were selected from a non-erotic popular movie. The different erotic film excerpts were selected to depict heterosexual vaginal intercourse, which were expected to evoke equal levels of sexual arousal.

Measures

VPA was measured using vaginal photoplethysmography [26]. VPA reflects phasic changes in the blood volume of the artery corresponding with each heartbeat; higher levels indicate higher levels of vaginal blood flow in the artery. The dependent variable used is the amplitude of the pulse wave. Before the mean VPA was calculated, the raw signal (sample rate was 20 Hz) was digitally bandpass filtered with a Butterworth filter (−3 dB cutoff frequency range 0.7–1.5 Hz; 40 dB down/octave). Movement artifacts were detected by visual inspection and removed manually. These artifacts were defined as a sudden increase in the VPA as compared with a 15-second period before this sudden increase. A sudden increase was defined as about 3 standard deviations above the mean of the 15-second period. Hereafter, the amplitude was measured as the distance between the top and bottom of a pulse wave. The peak-to-trough amplitude was calculated for each pulse and averaged over 5 minutes. This procedure resulted in one data point for each baseline (BA) (VPA : BA) and one data point for each erotic clip (EC) (VPA : EC) per trial. Because the vascular density of the vaginal wall differs between subjects, as well as within subjects, absolute values cannot be used. Therefore, we calculated the relative increase (percentage) in vasocongestion during the erotic condition as compared with the neutral condition:

$$VPA\ Relative\ change = (VPA : EC - VPA : BA)/VPA : BA) \times 100$$

To measure preconscious attention bias for sexual cues, a masked version of the emotional Stroop task was used [10]. In this task, words were presented (for 26.6 ms) in different colors (i.e., red, green, blue, and yellow) on a 75 Hz computer screen (Liteon

Technology Corp., Taipei, Taiwan) and then masked by randomly cut and reassembled letters in the same color. This procedure prevents conscious processing of the words. Subjects were instructed to name the color of the masks as quickly as possible. A microphone connected to a voice-level detector was placed in front of the subject. Initiation of vocal response was registered by the computer's clock and terminated the target (mask) presentation (with a no-response maximum of 3,000 ms). For the present experiment, we constructed an erotic version of this task. Thirty-two unambiguous neutral words from one category (furniture: examples are “chair” and “table”) and thirty-two unambiguous erotic words (examples are “penis,” “coitus,” and “vagina”) were presented in a blocked manner (eight words per block). The preconscious attentional bias scores for erotic cues were calculated by subtracting the reaction times to neutral words from the reaction to erotic words. The same words were used for each test; however, the sequence of words and colors differed all eight times this task was used. An extra set of stimuli consisting of meaningless letter strings was used for practice trials.

Here we will shortly highlight the experimental within-subjects design: All subjects underwent the four drug conditions at separate experimental days. At each experimental day, we carried out two sessions (the first session immediately before, and the second session a few hours after the intake of the drugs). During each session, subjects carried out an emotional Stroop task, and they were exposed to a neutral and an erotic film excerpts, during which VPA was measured.

Statistical Analyses

We performed four planned separate repeated measures analyses of variance (ANOVA) for the dependent variables “preconscious attentional bias” and “VPA.”

Our first hypothesis, concerning the testosterone-induced attentional bias for erotic cues as measured by an emotional Stroop task, was tested as follows. First, differences in preconscious attention were analyzed with a repeated measures ANOVA: a 2 (drug: no testosterone [i.e., placebo and vardenafil] vs. testosterone [i.e., testosterone and testosterone and vardenafil]) × 2 (session: sessions 1 [before drug intake] vs. session 2: [after drug intake]), in which drug and session were the within-subject factors. Second, in exploratory analyses, we investigated the possible influence of CSA: We carried out a second analysis in which two groups (nonsexual abuse vs. sexual abuse) were added to the analyses. In this analysis, group was the between-subject factor. The dependent variable—in both analyses—was the mean of the preconscious attentional bias scores for sexual cues.

Our second hypothesis, concerning the combined treatment of central mechanisms and peripheral mechanisms by means of combination of testosterone and vardenafil, influencing genital blood flow was tested as follows. First, three separate ANOVA repeated measures were performed on the relative change in VPA during both film

excerpts. Each of these comparisons comprised two within-subjects factors: drug with two levels (i.e., placebo vs. vardenafil, placebo vs. testosterone, and the crucial comparison, placebo vs. combination of testosterone and vardenafil), and session with two levels (before vs. after drug intake, i.e., first session vs. second session). Second, in order to investigate the influence of CSA on VPA, group (nonsexual abuse vs. sexual abuse) was added to the analyses as the between-subject factor.

We controlled for a possible influence of the menopausal status by carrying out the statistical procedures described in the above paragraph (testing the second hypothesis), with menopausal status (premenopausal vs. postmenopausal) as between-subject factor.

There were four missing values in the used database (less than 1% of the data). The following data were missing: in one subject, the reaction times on the neutral and erotic words of the Stroop task during the testosterone condition (after the drug intake), in one subject, one VPA measurement during the testosterone condition (after the drug intake), and in one subject, one value of a subscale of the FSFI. We used the method of Myers and Well [27] to impute these missing values.

Results

Sample Description

Thirteen heterosexual women participated in this study (mean age 40.6 years, SD 10.9; premenopausal: N = 8, postmenopausal: N = 5) with HSDD with (N = 5) or without arousal problems (N = 8) according to the DSM-IV-TR criteria [25] for at least 6 months prior to study entry. All fertile women used contraceptives. All women except one had steady heterosexual relationships of more than 1 year. There were no major medical illnesses. Sexual distress—an important criterion for the definition of FSD—was assessed during the clinical interview and also during the course of the experiment. Five women had been sexually abused in their childhood. From the women who had experienced CSA, two were raped and three reported being inappropriately touched repeatedly, all by family members. All women, CSA included, reported suffering from low sexual desire and negative emotions as a consequence of their sexual complaints. Moreover, besides the clinical interview, the FSFI also provided an indication of their sexual functioning. Three sexually abused women reported that they had not been active over the last 4 weeks before the start of the experiment. In the group of non-abused women, there was only one woman who had not been sexually active. These results indicate more sexual activity in the non-abused women than in the abused women.

A comparison between both groups of demographic variables and two subscales (Desire and Arousal, first two general questions of this subscale) of the FSFI is presented in Table 1.

Table 1 Comparison of demographic variables and indices of sexual functioning of the subjects with and without childhood sexual abuse (CSA)

Demographic variables	All subjects (N = 13)	Subjects without CSA (N = 8)	Subjects with CSA (N = 5)
Mean age (SD)	42.6 (9.9)	42.0 (11.2)	43.6 (8.5)
Race			
Caucasian	13	8	5
Marital status			
Married	7	3	4
Cohabitation	4	3	1
LAT	1	1	0
Unknown	1	1	0
Mean amount of children (SD)	2.3 (0.8)	1.8 (0.8)	2.8 (0.4)
Mean weight* (SD)	67.9 (12.3)	68.3 (14.4)	67.1 (9.6)
Mean length† (SD)	169 (7.0)	168.3 (7.9)	170.5 (5.1)
Mean body mass index (SD)	23.6 (2.9)	23.9 (3.2)	23 (2.4)
Educational requirement of current profession			
High school	1	0	1
Community college	4	2	2
University of professional education	4	3	1
Not applicable‡	4	3	1
Menopausal status			
Premenopausal	8	5	3
Postmenopausal	5	3	2
Smokers	4	3	1
SSRI users	3	2	1
Mean FSFI Desire General (SD)	1.7 (0.74)	1.6 (0.71)	1.8 (0.84)
Mean FSFI Arousal General§ (SD)	2.3 (1.85)	2.4 (1.88)	2.4 (2.03)
Sexual arousal problems	5	3	2

* Mean weight in kilograms.
† Mean length in centimeters.
‡ In this study we noted participants' professions. Some professions could not be categorized according to educational requirement, for example the profession "housewife".
§ The general arousal subscore of the FSFI consists of four questions, two of which can only be answered if the respondent has had sexual intercourse within 4 weeks prior to filling out this questionnaire. Because five of our participants did not have sexual intercourse during this period, a valid general arousal subscore could not be calculated over all participants. We therefore used the two questions that are independent of sexual intercourse frequency, and used the same conversion factor (0.6) as is used for the FSFI desire subscore, which also consists of two questions.
SD = standard deviation; LAT = living apart together; SSRI = selective serotonin reuptake inhibitor; FSFI = female sexual functioning index.

Preconscious Attentional Bias

With respect to preconscious attentional bias (first hypothesis), the following results were found. In the comparison of no-testosterone vs. testosterone, we found no statistically significant main effects for drug or session. Our second analysis: the interaction between drug (no testosterone vs. testosterone) by session (pre vs. post-

drug admission) × group (non-CSA vs. CSA) revealed a statistically significant effect (F[1,11] = 6.40, P < 0.03, partial η^2 = 0.37; see Figure 1).

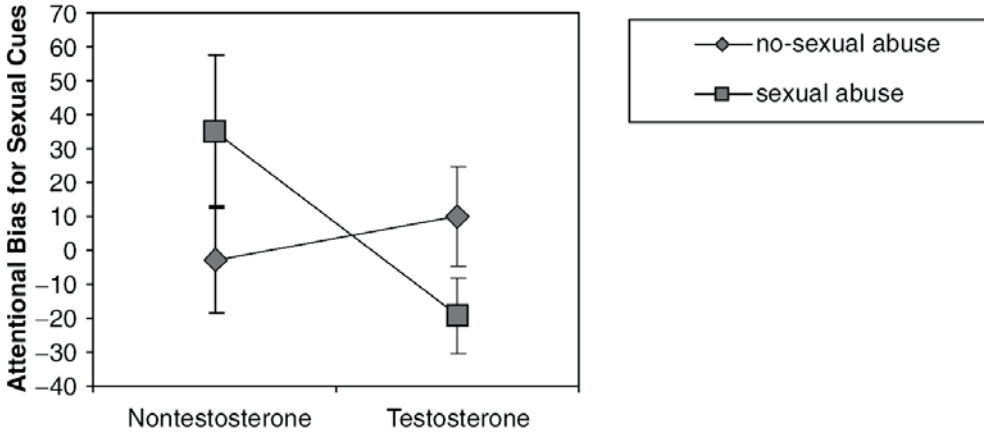

Figure 1 Preconscious attentional bias for sexual cues. The reaction times are presented in milliseconds. Mean preconscious attentional bias scores in women with (N = 5) or without (N = 8) Childhood Sexual Abuse (CSA) during non-testosterone and testosterone containing drug conditions.

Figure 1 shows that the originally low preconscious attentional bias for sexual cues of non-abused subjects, as compared with abused women, increased after admission of testosterone-containing medication. In contrast, in the abused subjects, this attentional bias relatively decreased after testosterone administration.

Physiological Sexual Arousal

For the comparisons of placebo vs. vardenafil and placebo vs. testosterone, there were no statistical significant main or interaction effects for the VPA measurement. This was also true when we used group as between-subjects factor.

For the crucial comparison of placebo vs. the combination of testosterone and vardenafil, there was a main effect only for session (F[1,12] = 8.11; P < 0.02). Furthermore, we found a statistically significant interaction effect for drug × session (F[1,12] = 5.62; P < 0.04, partial η^2 = 0.32) for the comparison between placebo vs. the combination of testosterone and vardenafil. In our second analysis, in which we tested the influence of sexual abuse on these relationship, we found a trend toward statistical significance for drug × session × group [F (1,11) = 3.63; P < 0.09, partial η^2 = 0.25]. These results indicate a possible influence of the experience of sexual abuse during childhood on the effects of the combination of testosterone and vardenafil on physiological sexual responding. Subsequently, we carried out a separate analysis in both groups. This analysis revealed a statistically significant effect for the interaction between drug × session in the non-abused women [F (1,7) = 7.49; P < 0.03, partial η^2 = 0.52],

while we found no such effect in the women who had experienced CSA. These results indicate a differentiation in effect of the combination of testosterone and vardenafil in women who had experienced sexual abuse during childhood and women who had not (see Figure 2).

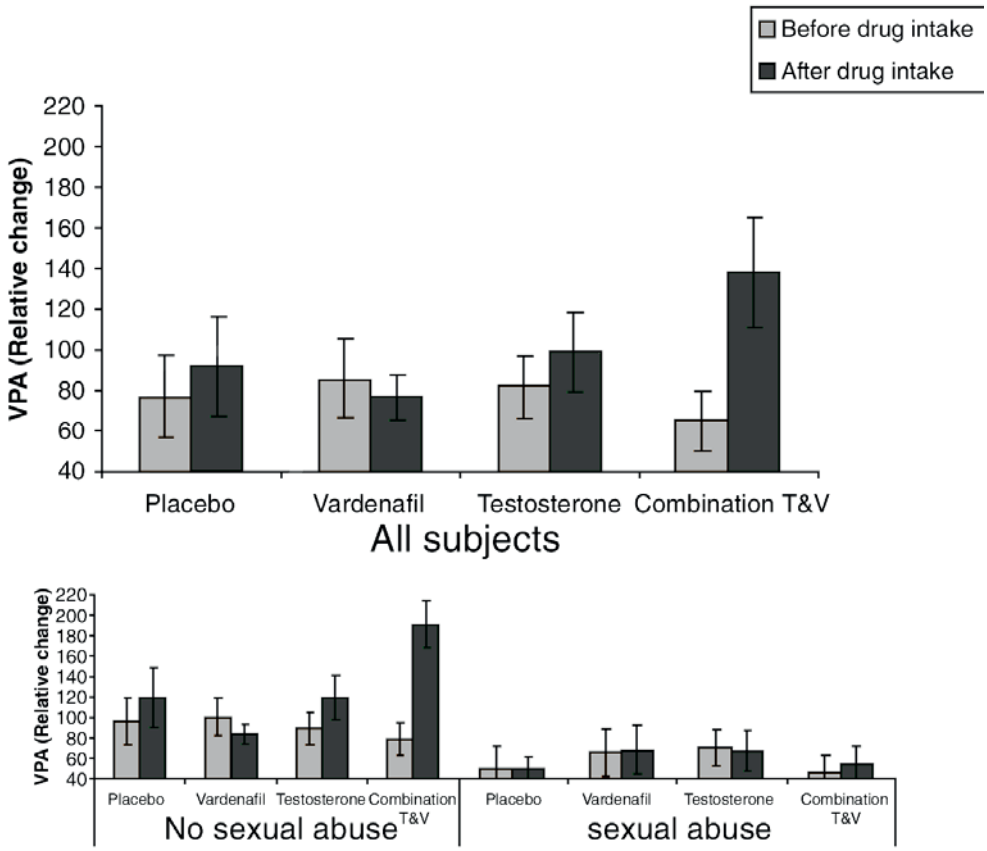

Figure 2 Mean vaginal pulse amplitude (VPA) pre vs. post-drug in four drug conditions in all women (upper), in women without childhood sexual abuse (left, below), and with childhood sexual abuse (right, below). T&V = testosterone and vardenafil.

Our analysis, in which we controlled for the influence of menopausal status on drug induced alterations in preconscious attentional bias scores and physiological sexual function, revealed no significant statistical effects.

Discussion and Conclusion

Analyzing the results of this pilot experiment on preconscious sexual information processing and physiological sexual responding, we established some remarkable findings. We had hypothesized that sublingual testosterone would influence the processing of sexual information, and that this treatment, combined with the PDE5

inhibitor vardenafil, would induce higher physiological sexual responding in women suffering from HSDD. This hypothesis was partly corroborated. In women suffering from HSDD, this combined treatment induced an overall statistically significant higher vaginal physiological arousal in response to an erotic film, as compared with the placebo, testosterone, or vardenafil alone. We noticed, however, a different response profile regarding the association between cognitive and physiological sexual functioning in women who reported CSA as compared with those who did not.

The non-abused and abused women had an opposite pattern regarding the testosterone-induced alteration in preconscious attention bias scores for sexual cues. Abused women in conditions containing testosterone showed a decrease in attention from their relatively high scores during placebo, while non-abused women revealed, during testosterone, an increase from their relatively low scores. From these results, we hypothesize the following model. In the abused women, the testosterone containing drug treatments induced a preconscious withdrawal of attention for erotic cues, while in the non-abused women, the testosterone administration might have induced increased allocation of attention to erotic cues. Moreover, these relative differences in information processing of sexual cues were accompanied by a corresponding different profile in physiological sexual responding. In the women who reported childhood sexual abuse, the withdrawal from sexual cues was associated with an absence of an effect on vaginal sexual responding. In contrast, in non-abused women, the relative increase in attention for sexual cues was accompanied by an increase in the preparatory physiological sexual response for sexual behavior when testosterone was combined with vardenafil.

The difference in results between both groups seem to reveal an important role for cognitive mechanisms in the central processing of erotic cues and the subsequent induction of sexual motivational processes. Attentional bias for emotional cues can be reliably measured with the emotional Stroop task [10]. In the present study, we used a masked version of this task. It has been demonstrated that the masked version—but not the unmasked version—prevents activation of prefrontal control mechanisms [10,11,28]. Since it has been suggested that sexual inhibition plays a pivotal role in sexual function [21,22], this masked version seems to be more suitable for our experimental purposes. The discordant pattern in sexual responding profiles in both groups might be evoked by a difference in activation of inhibitory and excitatory mechanism influencing sexual function [21,22,29]. We think that sexual abuse during childhood has induced conditioning of cognitive (or affective) mechanisms that can elicit sexual inhibition under particular circumstances. For example, the increased sensitivity for sexual cues under condition of testosterone might have evoked an avoidance response for sexual cues because these cues become too threatening. This avoidance response resulted in the absence or even inhibition of automatic physiological sexual responding in this group of subjects as compared with the non-abused women. In the non-abused women, testosterone also seems to have increased the sensitivity of the

brain for sexual cues, but here it induced an approach response, which combined with vardenafil, has increased the physiological sexual response.

A limitation of the present study is that it was a pilot experiment in which we did not use a control group. Tentatively, some comparisons can be made with sexually functional women. Our results demonstrate again that there is no effect of vardenafil alone in women suffering from FSD. In several experiments, it has been demonstrated that sublingual testosterone produces increases in physiological and subjective indices of sexual function in sexual functional women [1,2] and other aspects of cognitive and affective functioning [13–19] in heterogeneous groups of women. Although the present study also demonstrates that testosterone affects cognitive function (preconscious attention for sexual cues) in women suffering from FSD as well, no effect of testosterone was found on physiological sexual responding (in contrast to sexual functional women). Based on these results, we surmise that the combination of testosterone and vardenafil will produce comparable increases in attention for sexual cues and in VPA in sexually functional women. As a result, we hypothesize that after intervention of the combined use of testosterone and a PDE5 inhibitor, women suffering from HSDD (associated with low attention for sexual cues) cannot be differentiated from sexual functional women who underwent the same treatment.

Another limitation of the present study is the small sample size, which restricts the validity of these conclusions. The differential effects of testosterone, however, on preconscious attentional processes in the CSA and non-CSA women, and the difference in effects on physiological sexual responding when testosterone was combined with vardenafil, are at least consistent and remarkable. It might indicate a fundamental difference between these women in cognitive appraisal and subsequent autonomic processing, but more research is needed to explore the neurobiological and psychological background. Further experimentation is needed to explore the possibility for the combined use of testosterone and a PDE5 inhibitor as a potential fruitful “on demand” intervention for women suffering from HSDD without a history of CSA. In this context, it has to be mentioned that administering testosterone is presently only used (off-label) in the treatment of postmenopausal women, with unwanted adverse effects and unclear long-term safety data [30]. This treatment with continuous testosterone administration is, however, a completely different approach as the use of one dosage of testosterone in our approach. In our opinion, the results described here and their interpretation justifies further investigation of the proposed intervention for treatment of HSDD, irrespective of reproductive age.

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CHAPTER 3

The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction

Journal of Sexual Medicine 2009;6:777-790

Abstract

Introduction. Women with female sexual dysfunction have a reduced sensitivity to sexual stimuli. Activation of central mechanisms may open a window for phosphodiesterase type 5 inhibitors (PDE5) to be effective; as a consequence, the combination of testosterone and a PDE5 inhibitor will restore sexual function.

Aim. To demonstrate that the combination of testosterone and vardenafil will increase the sensitivity for sexual stimuli and will improve the desire and arousal components of the sexual response.

Methods. In a double-blind, randomly assigned, placebo-controlled, cross-over design, 28 women with desire and/or arousal disorder underwent four different drug treatments on four separate experimental days. A masked version of the emotional Stroop task with sexual and nonsexual words was used to measure sensitivity for sexual content. Neutral and erotic film fragments were used to determine genital–physiological and subjective reactions.

Main Outcome Measures. A masked version of the emotional Stroop task, vaginal pulse amplitude. For subjective measurement, responses were collected continuously with a lever and two self-report measures were used.

Results. In two subgroups, which were differentiated on the basis of their initial preconscious attentional bias for sexual cues, a different sexual response profile was found. In an initially low-attention group, preconscious attentional bias for sexual cues increased under the testosterone condition. In these women, the combination of testosterone and vardenafil caused an improvement in genital response and subjective indices of sexual functioning. In the group that had initially a high attention for sexual cues, preconscious attentional bias for sexual cues decreased under the condition of testosterone. In these women, the combination of testosterone and vardenafil had no effect on any of the indices of their sexual functioning.

Conclusion. In women suffering from low sexual desire—associated with low attention for sexual cues—the combination of testosterone and vardenafil may be a promising new treatment.

Introduction

Experiments testing the effects of phosphodiesterase type 5 (PDE5) inhibitors produced promising results in sexually functional women [1,2], but the majority of women suffering from female sexual dysfunction (FSD) showed an absence of response to this class of drugs [3,4]. Activation of central sexual mechanisms is necessary for the interpretation of stimuli as sexual, by which these stimuli can produce (behavioral) sexual responses (i.e., an increase in sexual desire and motivation; inducing sexual approach behavior). Activation of central "sexual" mechanisms is a necessary condition for activation of the nitric oxide (NO) pathway, which in turn is necessary for a PDE5-inhibitor to be effective. Reduced capacity to interpret stimuli as sexual will be accompanied by low sexual desire and/or arousal; indeed, reduced sexual desire is the most prevalent issue in women. Centrally working drugs increasing the sensitivity for sexual stimuli, which might influence sexual motivation, are required and may induce a condition sufficient for PDE5-inhibitors to be effective.

In earlier experiments, we demonstrated a 4-hour delay in the effect of testosterone (0.5 mg, sublingual) on physiological and subjective sexual responses in sexually functional women [5,6], which was replicated by Heard-Davison and colleagues [7]. In the present approach, we used this effect as a window for further experimentation with a PDE5-inhibitor. We assume that testosterone induces an increase in sensitivity, lowering the threshold for processing sexual stimuli and leading to alterations in attention for sexual cues. For this (sex) hormone induced increase in sensitivity for sexual stimuli, a steroid-responsive neural network has been postulated [8]. This network encompasses a highly interconnected group of sex hormone receptor-containing neurons in the brain. This network is not a closed circuit, but serves reproductive aims by functioning as an integrating and activating center between external sensory cues, hormonal processes, and reproductive behavior. This is partly accomplished by selective filtering of sensory input and amplification of signals that may facilitate sexual behavior.

It has been abundantly demonstrated that the emotional Stroop task can measure (alterations in) selective attention or attentional bias for emotional cues [9]. In the present study, we will use a masked version of the emotional Stroop task to determine the effects of pre-attentive processes on drug-induced alterations of sexual functioning. In this task, deceleration in color naming of masks preceded by erotic words (as compared with neutral words) indicates increased subconscious attentional resource allocation to the processing of sexual cues [9]. This masked version (see Methods section) turned out to be a more reliable index for our experimental purposes [9,10], i.e., to investigate the association between drug-induced alterations in preconscious selective attention for sexual cues and sexual function. In our argumentation, we follow LeDoux's proposal that sensory information, without becoming conscious, is quickly processed through the thalamic–amygdala pathway [11]. A second thalamic–prefrontal–

amygdala pathway allows conscious processing and has a regulatory function on emotional response. In addition, in an earlier experiment [12] we found striking differences in neuroendocrine activity induced by the processing of masked and unmasked angry faces. In particular, subjects showing preconscious attentional bias for angry faces demonstrated a post-task statistically significant increase in testosterone levels, which was absent after exposure to unmasked angry faces. Before this experiment, it has been predicted that processing of (features of) masked as compared with unmasked angry faces would elicit more "biologically prepared" responses through skipping the cortical route and merely travelling the thalamic–amygdala pathway [12,13]. Likewise, it has been shown that different neurobiologically determined personality traits are associated with differences in the processing of conscious and subconscious processing of emotional relevant words [10]. Thus, by measurement of subconscious attentional bias processes we surpass prefrontal control in sexual–emotion regulation. Consequently, this measurement method provides an adequate estimate of the sensitivity of the brain in the processing of sexual stimuli. Thus, because empirical evidence indicated that the masked version—but not the unmasked version—prevents activation of cortical (prefrontal) control mechanisms [12], this version seems to be more suitable for our experimental purposes.

In a recent pilot study, we demonstrated this association between testosterone-induced alterations in the sensitivity of the brain for sexual cues and physiological sexual responding in women with different sexual histories [14]. In this pilot study, we found opposite effects of testosterone on preconscious attentional bias for sexual cues and (when combined with vardenafil) physiological sexual responding in women with FSD who experienced childhood sexual abuse (CSA) compared with those who did not. Under placebo conditions, women without a history of CSA had a lower preconscious attentional bias for sexual cues than women who had suffered CSA. Testosterone induced an increase in this attentional bias in the non-CSA group and a decrease in the CSA group. The physiological sexual response showed an opposite pattern during the combination of testosterone and vardenafil: an increase in the non-CSA group and no alteration in the CSA group (see Discussion). We assumed that the difference in preconscious attentional bias for sexual cues represents a difference in central processing of sexual information, which in turn, affects drug-induced alterations in physiological sexual responding during the exposure of sexual stimuli.

Using the delay paradigm, it has been demonstrated that sublingual testosterone induces significant alterations in cognitive and affective functioning in heterogeneous groups of female subjects [5–7,14–22]. These effects can be established irrespective of the use of oral contraceptives, phase of the menstrual cycle, use of selective serotonin reuptake inhibitors, etc. Consequently, we assume that testosterone effects will occur irrespective of hormonal status associated with reproductive age, menstrual phase (with the exception of menstruation), and use of oral contraceptives.

The present placebo-controlled study was designed to investigate the effects of testosterone, vardenafil (a PDE5-inhibitor), and the combination of both drugs on alterations in preconscious attentional bias for sexual cues and physiological and subjective indices of sexual function in 28 women suffering from FSD. Because the results of our pilot study indicated that preconscious attentional bias affects drug-induced alterations in sexual function, we also investigated in the current study the influence of this variable on measured parameters.

Methods

Participants

Twenty-eight women with hypoactive sexual desire disorder (HSDD) (with or without female sexual arousal disorder [FSAD]) participated in this study; all signed a written informed consent and received reimbursement for expenses for their participation. Women were recruited through printed announcements in a local hospital, or advertisement in regional newspapers and on the Internet. Potential participants were invited for a screening visit. During this screening visit, women filled out the Sexual Function Questionnaire, and were interviewed by an experienced psychologist and a gynecologist to diagnose for FSD (i.e., HSDD and/or FSAD), according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition-Text Revision criteria [23], and to determine eligibility for study participation. Women who have been diagnosed as suffering from HSDD, with or without arousal problems, for at least 6 months prior to the screening visit, were included when they did not have any significant medical illness.

To exclude major medical and psychiatric illnesses, a general medical, gynecological, and psychiatric anamnesis was taken. Weight, height, blood pressure (supine and standing), heart rate, respiration rate, and body temperature were measured. A supine, 12-lead electrocardiogram was recorded and examined by a physician (and when necessary, a cardiologist). A gynecological examination and urine pregnancy test were performed, to exclude pregnancy, vaginal infections, major surgery on the vagina and/or vulva, undetected major gynecological illnesses, or unexplained gynecological complaints. Cultures were taken to exclude chlamydia or gonococcus infections. A venous blood sample was taken for hormonal analyses and standard hematology tests. Additionally women were asked about CSA and other negative sexual experiences.

Design and Procedure

We tested the effects of testosterone, vardenafil (a PDE5-inhibitor), and the combination of both drugs—as compared with a placebo—on alterations in attentional bias for sexual cues, as well as changes in physiological and subjective indices of sexual function during neutral and erotic visual stimulation. A Medical Ethics Committee, STEGMETC in Almere, approved this study. The study was carried out in agreement with ICH-GCP, and was controlled by a certified CRO (PSR Group, Hoofddorp, the Netherlands).

In a double-blind cross-over design, each subject underwent four different drug treatments in randomly assigned order on four separate experimental days: (i) Placebo: placebo for testosterone (cyclodextrin solution without testosterone) and placebo for vardenafil (powder-filled gelatin capsule without vardenafil); (ii) Vardenafil: vardenafil (10 mg [hidden in a powder-filled gelatin capsule]) and placebo for testosterone (cyclodextrin solution without testosterone); (iii) Testosterone: testosterone (0.5 mg) sublingually with cyclodextrin as carrier and placebo (for vardenafil); and (iv) Combination of testosterone and vardenafil: the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and vardenafil (10 mg [hidden in a powder-filled gelatin capsule]).

At a separate familiarization day, before these four experimental drug days, a trained female experimenter explained the study requirements and procedures to the participants. This included the practical use of the vaginal photoplethysmograph (see Measures) and a shortened version of the emotional Stroop task (see also paragraph Measures). None of the women dropped out after this familiarization trial. Women were instructed not to use psychoactive drugs 3 weeks before the first experimental day. Participants subsequently received several instructions regarding the experimental days: they were not allowed to use any alcohol either the evening before or during the experimental days, and they were asked only to eat a light fate free breakfast before they came to our laboratory. They were instructed not to make appointments for the experimental trials during their period of menstruation.

At the start of each experimental day, women received a short physical examination. The four experimental days themselves were each separated by at least 3 days and at most 7 days. Each experimental day consisted of two sessions. The first session was carried out immediately before, and the second session about 4 hours after the intake of sublingual testosterone (or placebo for testosterone). The vardenafil (or placebo capsule for vardenafil) was taken 2 hours after taking the sublingual testosterone or placebo. In every session, the women had three test episodes. The first episode started with a 10-minute return to baseline period, during which women watched a neutral film (i.e., a music film). In episode 1, women were exposed to a 3-minute neutral (music) film, followed by a 3-minute erotic film. In episode 2, the women were again exposed to 3-minute neutral and erotic film fragments, during both films there were distraction tasks superimposed, i.e., the masked version of the classic Stroop color-word task (see paragraph Measures). During episode 3, women were again exposed to 3-minute neutral and erotic film fragments and distraction tasks, which consisted of masked versions of an erotic Stroop task (see paragraph Measures), immediately followed by another erotic film with the instruction to focus on their vaginal sensations. During this last film, women used a lever to indicate their subjective vaginal arousal.

During all trial sessions, the vaginal pulse amplitude (VPA) was continuously measured (see paragraph Measures). The subject received the vaginal probe from the female

experimenter and the subject was left alone in the room to insert the probe [24]. The subject was instructed to sit as motionless as possible while viewing the film fragments. After the first session, the women removed the vaginal probe and were taken to a waiting room. There the women were given the solution of testosterone (or placebo), which they applied sublingually. They were instructed to swallow the solution after 1 minute upon a signal of the experimenter. After 2 hours, the women ingested the vardenafil (or placebo) capsule. During the next 2 hours, they could consume a fat-free lunch. After this pause, they continued with session 2 consisting of the same three episodes as in session 1. Each experimental day ended with a short physical examination and a report of adverse events. Venous blood samples were taken two times during each experimental day: the first one before the intake of the testosterone (or placebo), and the second one 20 minutes after the intake of the testosterone (or placebo).

Measures

Preattentional Bias for Sexual Cues

To measure speed or bias in preconscious attention to lexical sexual stimuli during episode 3, a masked version of the emotional Stroop task was used [9]. Each of the two emotional Stroop tasks carried out on each experimental day consisted of a fixation point which was shown for 750 ms, followed by a very short presentation of the target stimulus (the colored neutral or erotic word), which was immediately replaced by a pattern mask. In the erotic version of this task, color naming latencies were measured on masks, which were preceded by unambiguous erotic words (e.g., cunnilingus, erection, horny, fuck, orgasm, penis, sex, vagina) and neutral words (e.g., arrival, book, hammer, clock, spoon, metal, paper, telephone). Some translated words are in Dutch unambiguous, but might be ambiguous in translated form (for example, the word “fuck”). Words were presented (for 26.6 ms) in different colors (i.e., red, green, blue, and yellow) on a 75 Hz CRT computer screen (Liteon Technology Corp.) and then masked by randomly cut and reassembled letters in the same color. This procedure prevents conscious processing of the words [9].

A two-button computer mouse was placed in front of the subject. The women were instructed to click the right button as quickly as possible when the colors were red or green, or the left button when the colors were blue or yellow. The mouse click was registered by the computer's clock and terminated the target (mask) presentation (with a no-response maximum of 3,000 ms). Thirty-two neutral words and 32 erotic words were presented in blocks (eight words per block). The same words were used in each test; however, the sequence of words and colors differed all eight times this task was used. An extra set of stimuli consisting of letter strings was used for practice trials.

Vaginal Pulse Amplitude

Vaginal photoplethysmography was used to measure VPA [5,24]. VPA reflects phasic changes in the blood volume of the artery corresponding with each heartbeat; higher levels indicate higher levels of blood supply via the vaginal artery. The dependent variable used is the amplitude of the pulse wave. Before the mean VPA was calculated, the raw signal (sample rate was 20 Hz) was digitally bandpass-filtered with a Butterworth filter (−3 dB cutoff frequency range 0.7–1.5 Hz; 40 dB down/octave). Movement artefacts were detected by visual inspection and removed manually. Hereafter, the amplitude was measured as the distance between the top and bottom of a pulse wave. The peak-to-trough amplitude was calculated for each pulse and averaged over 3 minutes. This procedure resulted in one data-point for each baseline (VPA : BA) and one data-point for each erotic clip (VPA : EC) per trial. Because there are differences in vascular density of the vaginal wall between women (at different time points) as well as within women, absolute values cannot be used. Therefore, we calculated the relative change (percentage) in blood supply during the erotic condition as compared with the preceding neutral condition [5]:

$$VPA\ relative\ change\ = (VPA : EC - VPA : BA)/VPA : BA) \times 100$$

Subjective Sexual Function

Questionnaire

Self-report rating of sexual functioning was adapted from Morokoff and Heiman [25] and Heiman and Hatch [26]. Based on earlier research [5,6,27], subjective experiences related to “vaginal sensations” and “sexual desire” were collected at the beginning and after the last erotic film excerpt. Each of these constructs was assessed with 5 scales, consisting of five-point Likert scales (1 to 5, from “not at all” to “extremely”).

Lever

Via a lever, mounted on a table, women reported increase or decrease in vaginal arousal during the last erotic film excerpt by moving the lever up or down, respectively. They could visually monitor their indicated subjective arousal through a series of light emitting diodes [28].

Hormonal Measures

Serum total testosterone, serum estradiol, serum progesterone, serum prolactin, serum luteinizing hormone (LH), serum follicle-stimulating hormone (FSH), serum thyrotropin, and serum sex hormone binding globulin (SHBG) were measured through electrochemiluminescence radioimmunoassay with COBAS® kits of Roche Diagnostics (Mannheim, Germany), using a Modular E170 at OLVG Hospital (Amsterdam, The Netherlands). Free testosterone levels were calculated according to [29]. Serum albumin

was determined through photometric determination with the bromocresol green method, with COBAS® kits, using a Modular P at the community hospital of Almere ("Flevoziekenhuis", Almere, The Netherlands). In the results section, we will only present the results of total testosterone, SHBG, estrogens, and the calculated free fraction of testosterone.

Statistical Analyses

First, we investigated if we could differentiate the subject into two subgroups, on the basis of their attention for sexual cues, which could affect the testosterone-induced attentional bias for erotic cues. On the basis of their reaction times (RT) on erotic words compared with neutral ones during the placebo condition, two subgroups were formed. One group (N = 17) had lower RT to erotic words (Mean RT erotic words < RT neutral words) and thus—we assume—a lower preconscious attentional bias for sexual cues. We named this group Initially Low Sexual Attention. The other group (N = 11) had higher RT to erotic words (i.e., mean RT erotic words > RT neutral words), and thus we assume a higher preconscious attention for erotic cues. Possible differences between testosterone-induced alterations in preconscious attention between these groups were analyzed with a repeated measures MANOVA: a 2 (Drug: No-testosterone vs. testosterone × 2 (stimulus factor: neutral vs. erotic words) × 2 group (Initially Low Sexual Attention vs. Initially High Sexual Attention). Drug and stimulus factor were the within-subject factors, and group was the between-subject factor. The dependent variable was the mean of the preconscious attentional bias scores for sexual cues.

In our proof of concept experiment, the first Stroop task on each experimental day was carried out before the trials—thus women were not exposed to sexual stimuli—while in the present experiment the first Stroop task was carried out during the trial, thus when women were already exposed to the sexual stimuli and thus sexually primed. For the present experiment, we could not compare the Stroop results of the second session with the Stroop results of the first session, because these were in contrast to the first experiment contaminated with exposure to sexual stimuli. Thus, we used the results of the erotic emotional Stroop task of the second session.

To investigate the changes in physiological and subjective indices of sexual responding during the different drug conditions, we carried out three pairwise comparisons: each active drug was compared with placebo. Because we found no effects for the comparisons between placebo vs. vardenafil (P vs. V) and placebo vs. testosterone (P vs. T), we restricted ourselves to the comparison between placebo vs. combination of testosterone and vardenafil (P vs. C). Moreover, because in the last comparison (P vs. C) there was a clear multivariate effect for group (i.e., low attention vs. high attention), it was justified to analyze and describe both groups separately.

In each group (i.e., Initially Low Sexual Attention and Initially High Sexual Attention), we carried out again a doubly-MANOVA repeated measures: a 2 Drug (placebo vs.

combination of testosterone and vardenafil) × 2 session (first session vs. second session). The within-subjects factors were drug and session. The dependent variables were the relative change in VPA during the first film excerpt and the subjective reports "vaginal sensations", "sexual desire," and the "lever" results. To control for the possible influence of the endocrinological status associated with the pre- and postmenopausal phase of life, we extended our analyses with this differentiation of phase of life as between-subject factor.

Less than 2% of the data were missing, and for their estimation we used the expectation maximization method available on SPSS MVA (SPSS Inc., Chicago, IL, USA). For all analyses, sphericity was assumed.

In this article, we will not present the physiological results of the distraction tasks and attention manipulation task. We will prepare a separate manuscript to report the results on these tasks.

Results

Sample Description

Twenty-eight healthy women (mean age: 43.4 ± 9.5 years; premenopausal [LH < 20 and FSH < 20]: N = 20, postmenopausal: N = 8) experiencing FSD (i.e., low sexual desire N = 23, or a combination of low desire and arousal problems N = 5) for at least 6 months prior to study entry, participated in this study. Out of premenopausal women, five women used hormone-containing contraceptives. The other women, with the exception of two women, had other forms of contraceptives or their partners were sterilized. All women, with the exception of two women, had steady relationships of more than 1 year. In the group showing initially high levels of preconscious attentional bias for sexual stimuli (N = 11), there were seven women who experienced severe sexual abuse (four cases of rape, three cases of assault and/or inappropriate touching). In the other group showing initially low levels of preconscious attentional bias for sexual stimuli (N = 17), there were three women who experienced assault and/or inappropriate touching. Thus, in the group showing an initially higher preconscious attentional bias for erotic cues, there was a larger prevalence of experiences of sexual abuse (63%) compared with the low-attention group (17%).

Sexual Distress

Sexual distress is an important criterion for the definition of FSD [30]. First of all, we assessed sexual distress during the clinical interview. The clinician reviewed the sexual distress by asking about the burden and the consequences of the sexual dysfunction. All women reported moderate to severe suffering from their sexual complaints. Women were experiencing emotions like guilt, sadness, being ashamed, and frustrated. Consequences like acute or chronic stress, insecurity, and lack of understanding or even

ignorance by the partner were most commonly heard. All women wanted to improve their sexual functioning.

Attentional Bias: Two Groups

Our analyses of the RT of the erotic emotional Stroop revealed no significant effect for the interaction between drug (non-testosterone vs. testosterone-containing drugs) × stimulus variable (neutral vs. erotic words). We found, however, a clear group difference $F(1.26) = 17.44$; $P < 0.001$); partial $\eta^2 = 0.40$, regarding the testosterone-induced alterations in preconscious attention for sexual cues (Figure 1).

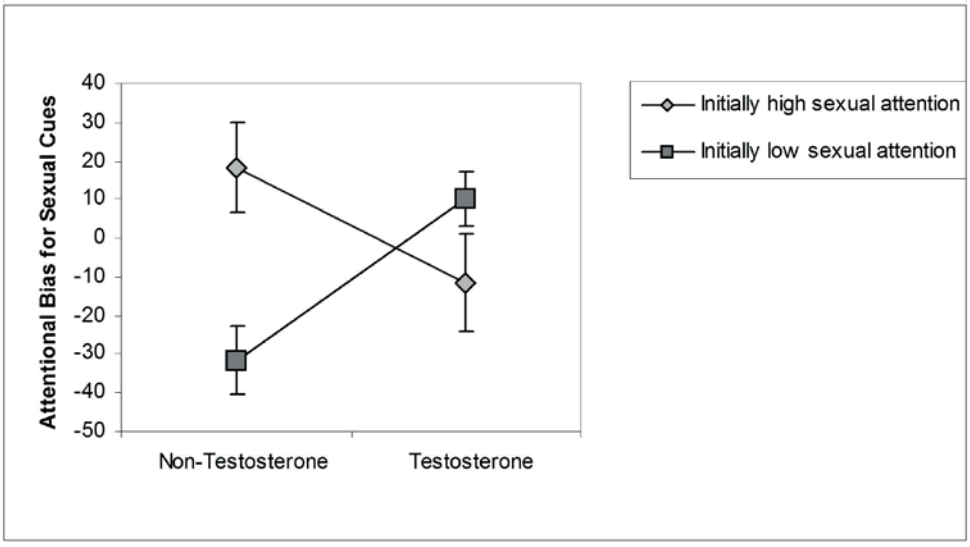

Figure 1 Preconscious attentional bias for sexual cues. This figure shows the mean difference (i.e., reaction times to erotic words minus reaction times to neutral words) scores under the drug conditions when no testosterone was used (i.e., placebo and vardenafil) compared with the drug conditions when testosterone was used (i.e., testosterone and the combination of testosterone and vardenafil).

These results indicate that in women with an initially low preconscious attention for sexual cues, testosterone induced an increase of preconscious attention allocation, while a reversed pattern occurred in women with an initially preconscious high attention for sexual cues. These different testosterone-induced alterations in attention allocation represented—in our interpretation— activation of different central mechanisms. As a consequence of this finding, we used this group division in our further analyses. For the sake of clarity, in the rest of this article, we will use the following designations for these groups: Initially Low Sexual Attention and Initially High Sexual Attention, respectively.

Hormonal Analyses

For the present discussion, we present only the results of the hormonal analyses of estrogens, SHBG, total testosterone, and the calculated Free Fraction Testosterone as measured during the screening phase. These results indicate a different hormonal profile for postmenopausal women as compared with premenopausal women. Results (nmol/L) will be presented as means and standard deviation. Postmenopausal as compared with premenopausal women had lower levels of estrogens (98 [134] vs. 447 [471]); $t = 2.04$; $P < 0.06$); lower levels of SHBG (48.3 [17.8] vs. 73.6 [26.8]), $t = 2.45$; $P < 0.025$; no difference in total testosterone (1.22 [0.50] vs. 1.17 [0.56]); not significant; and higher levels of the calculated Free Fraction Testosterone (0.0195 [0.012] vs. 0.0122 [0.005]), $t = 2.30$; $P < 0.035$. It is known that these decreasing levels of estrogens are accompanied by a decrease in levels of SHBG, which cause a subsequent increase in the free fraction of testosterone because there is less SHBG available for binding with testosterone [31].

Furthermore, the intake of testosterone (alone or in combination with vardenafil) was of course accompanied with a substantial increase in serum total testosterone levels 15 minutes after testosterone intake during these experimental days (not presented). We believe that the calculated free fraction of testosterone [29] is an unreliable measure directly following acute and sharp increases of serum testosterone levels (see Discussion and Conclusion). In our opinion, this method will underestimate serum free fraction testosterone. Equilibrium dialysis and liquid chromatography tandem mass spectroscopy (LCMS/MS) is the gold standard for assessing free testosterone accurately and is as such recommended [32]. The cost of this assessment has prohibited us from determining free testosterone accurately. Therefore, these data are not presented.

Vaginal, Subjective, and Behavioral Responding

The multivariate comparisons between placebo and vardenafil, and between placebo and testosterone, revealed no statistically significant effects, nor with group as between-subject factor. Our drug (placebo vs. combination of testosterone and vardenafil) × session (session 1: before drug intake vs. session 2: after drug intake) analyses reached no statistical significance ([$F = 4.23$] = 2.46; $P < 0.08$, $\eta^2 = 0.30$).

Our analyses revealed, however, a statistically significant multivariate interaction effect for the comparison drug × session × group (F[4.23] = 5.44; $P < 0.004$), partial $\eta^2 = 0.49$. (This effect was also significant within the FDR approach.) The univariate tests showed significant effects for the VPA measurement (F[1.26] = 4.31, $P < 0.05$), partial $\eta^2 = 0.14$ and for "genital sensations" (F[1.26] = 4.31, $P < 0.05$), partial $\eta^2 = 0.14$. No statistically significant effect, however, was found for "lever" (F[1.26] = 3.47, $P < 0.08$), partial $\eta^2 = 0.12$.

Based on these effects, we analyzed both groups separately and these results will be presented further.

Initially High Sexual Attention

Our analysis in the Initially High Sexual Attention group (N = 11) revealed no multivariate statistically significant effect, nor univariate effects for any of the dependent variables. These results indicate that the used drug treatments are not effective in this group of women with FSD, associated with preconscious initially high attention for sexual cues.

Initially Low Sexual Attention

In this group, our multivariate analysis demonstrated a significant drug × session effect (F[4.13] = 10.1; $P < 0.005$), partial $\eta^2 = 0.76$. The univariate tests showed statistically significant effects for "VPA" (F[1.16] = 7.47; $P < 0.02$), partial $\eta^2 = 0.32$; "Vaginal sensations" (F[1.16] = 4.44; $P < 0.05$), partial $\eta^2 = 0.22$ and "Sexual Desire" (F[1.16] = 5.1; $P < 0.04$), partial $\eta^2 = 0.24$. However, we found no statistically significant effect for "Lever" (F[1.16] = 3.90; $P < 0.07$), partial $\eta^2 = 0.20$. These results indicate that the combination of testosterone and vardenafil causes an increase in all measured physiological and subjective aspects of sexual functioning, with the exception of the "Lever" measurement, in women suffering from FSD associated with preconscious initially low attention for sexual cues. These results (together with the other drug conditions) are presented in Figure 2.

All postmenopausal women belonged to this low selective attention group. We controlled for a possible influence of endocrine age on the previously mentioned physiological and subjective indices of sexual function, but there were no statistically significant multivariate or univariate effects. These results indicate that the effects of the combination of testosterone and vardenafil occur irrespective of the differences in the hormonal state between pre- and postmenopausal women.

Discussion and Conclusion

In this study, we demonstrated that sublingual testosterone combined with vardenafil in a subgroup of patients suffering from HSDD and/or FSAD produced in a psychophysiological laboratory setting positive effects in their sexual function. This group was composed of women who preconsciously had a low attention for sexual cues. Testosterone induced an increase in their preconscious sexual attention, and—during exposure to erotic visual stimuli—the combination of testosterone and vardenafil enhanced their sexual motivation. These women demonstrated an increase in physiological sexual responding (i.e., VPA), sexual desire, as well as vaginal sensations as measured by subjective report. Thus, for this subgroup of women suffering from HSDD, our working hypotheses have been confirmed.

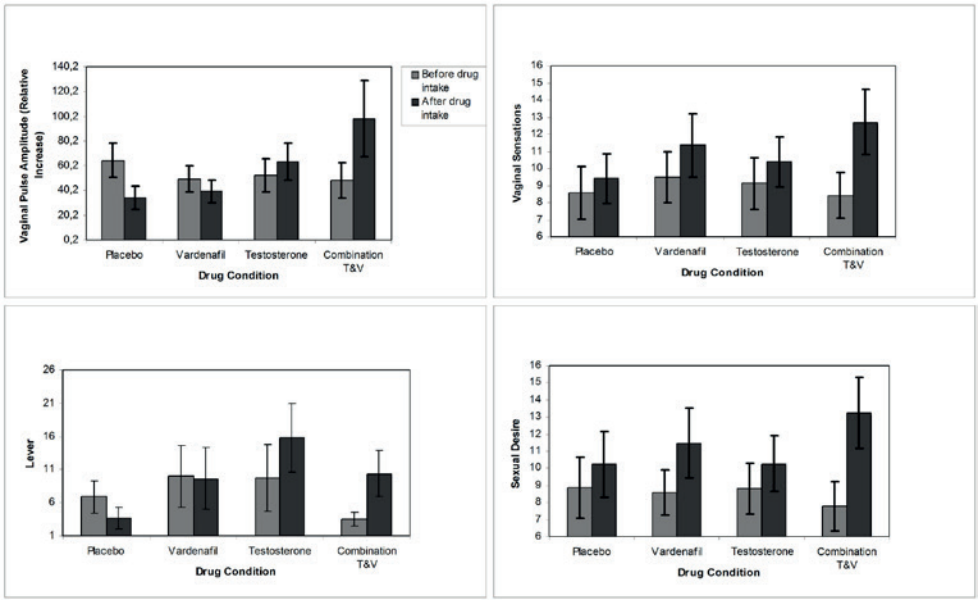

Figure 2 Results of the effects of placebo, vardenafil, testosterone and the combination of testosterone and vardenafil on several physiological and subjective indices of sexual functioning in women with initially low levels of preconscious attentional bias for sexual cues.

In the other group, which was composed of women who had initially high preconscious attention for sexual cues, testosterone induced a statistically significant lowering of (the originally high) preconscious attention for sexual cues. These women had no further benefit from the drug treatments.

Williams [33] recently described a model in which she emphasizes the central role of "stimulus significance" in organizing human information processing, and its effects on the balance of excitatory and inhibitory mechanisms. In her view, "significance is determined by the relevance of a stimulus to the core motivations of the human organism, and the emotional states associated with these motivations" (p. 6). With reference to Gordon [34], she highlights the temporal organization of brain function, from milliseconds to a whole lifespan. In experiments of her group, it was found that exposure to emotional stimuli under the threshold of conscious awareness, can cause alterations in brain functioning. The other way around, different responses after exposure to subliminal presentation of stimuli might be the result of a difference in the balance between excitatory and inhibitory mechanisms. Bancroft and Janssen [35,36] have conceptualized also the presence of dual control systems in regulating sexual functioning. They claim—influenced by trait properties and particular circumstances—that individual differences in sexual responding depend on a delicate interplay of such activating excitatory and inhibitory processes. In understanding the divergent patterns in preconscious attention for sexual cues and sexual response profiles, we will refer to a difference between both groups in the (dual) operation of such excitation and inhibition

mechanisms. To support our point of view, we will, firstly, describe some of the background of how testosterone can influence sexual functioning within 4 hours. Secondly, in addition to the introduction, we will elaborate on the Stroop task as a measurement method to determine a preconscious attention for sexual cues. Thirdly, we will explain that the meaning of subconscious attentional bias scores depends on textual and personal factors, and finally, how this is incorporated in our interpretation.

The Delay Effect of Testosterone

In the present study, we used alterations in the VPA elicited by sexual stimuli, as a physiological measure for sexual motivation. This genital responding is considered preparatory for copulatory behavior [27]. In a pervious study, we found in hypogonadotropic hypogonadal women that treatment with testosterone undecanoate, 40 mg orally per day during an 8-week period, enhanced this aspect of vaginal responsiveness [27]. Because women swallowed the capsules each morning, while the measurements were performed in the afternoon, we assumed that this effect on physiological sexual responding could be caused by a time-dependent effect of testosterone. To test this hypothesis, we investigated in eugonadal and sexually functional women whether administration of a single dosage of testosterone sublingually (0.5 mg) increases VPA in a time course manner. We demonstrated a 4-hour delay in effect of testosterone [5,6]. This effect has not only been established for sexual responding [5–7], but also for several other indices of cognitive and affective functioning [15–22]. These effects were established in highly heterogeneous groups of subjects. In the present study and in agreement with our assumption, the effects of the combined use of testosterone and vardenafil on physiological and subjective indices of sexual function occurred irrespective of the hormonal status associated with the reproductive age of our participants.

There are important differences between the usual chronic treatment with modest increased levels of testosterone (by means of patches, gels, pills, etc.) and our approach (i.e., sublingual administering of 0.5 mg testosterone). Chronic treatment with modest levels of testosterone is accompanied by a slight increase in absolute levels of testosterone, and in the long run (days/weeks), an increase in the free fraction of testosterone. This gradual increase of the free fraction of testosterone is the result of an alteration in a homeostatic state regarding testosterone (metabolism), and is responsible for its positive as well as negative effects. In contrast, in our approach we produce a considerable increase in absolute levels of testosterone (about 15–20 times higher than in the chronic treatment) within and during a short period, but we assume that this increase will not produce a proportional increase in the free fraction of testosterone. We postulate an SHBG (and to a lesser extent albumin) saturation threshold mechanism. The increase in influx of testosterone into the body will be first bound to SHBG and (to a lesser extent) albumin, before it can produce an increase in the free fraction. We have tentatively calculated that the increase of testosterone produced

by our method is large enough to pass this threshold and will produce consequently also a short peak of free testosterone. We believe that this short burst in the free fraction of testosterone is responsible for cognitive, affective, and behavioral effects a few hours later, presumably as the result of gene-expression at the androgen receptor level itself.

The Combined Use of Testosterone and Vardenafil

Sexual functions are the result of an interaction between central and peripheral processes. In several studies, it has been shown that selective PDE5 inhibitors improve erectile function in men with erectile dysfunction, on average to close to normal function [37]. In the penis, during sexual stimulation NO will be released from nerves and endothelium, which induces the production of cyclic guanosine monophosphate (cGMP). cGMP is a key mechanism in relaxing smooth muscle, necessary for the induction of an erection. This nucleotide is hydrolyzed by the phosphodiesterases, from which the main activity in the corpora cavernosa is as a result of PDE5. Therefore, during sexual stimulation, the action of NO/cGMP on erectile function will be enhanced by PDE5 inhibitors [38]. The genitalia of both sexes have common embryological origins. Recently, it has been shown that the clitoris consists of an erectile tissue complex, which embeds the anterior vaginal wall. Clitoral erection and the anterior wall of the vagina are highly involved in female sexual arousal and response. It has recently been shown that sildenafil—a PDE 5 inhibitor— improves sexual performance in sexually functional women [1].

Women diagnosed as having HSDD suffer, by definition, from low sexual interest, attentional deficits for erotic cues, avoidance of sexual stimulation, or other deficits in central sexual (motivational) systems. As has been stated extensively before, central sexual stimulation is a prerequisite for parasympathetic nervous system activation which in turn is necessary for NO induction and thus for action of a PDE5 inhibitor. In other words, in women, concurrent activation of central sexual motivational mechanisms is a necessary condition for a PDE5 inhibitor to be effective. In the present study, we combined use of testosterone meant to induce an increase in the sensitivity of the sexual motivation system and a PDE5 inhibitor to facilitate arousal of peripheral genitalia (induced by the activation of central sexual mechanisms). In this approach, there is no principal difference in the preference for available PDE5 inhibitors (for example, vardenafil, sildenafil, or tadalafil), with the exception of the timing of intake of these drugs.

Subconscious Attention for Sexual Cues

Testosterone determines that our cognitive system becomes more sensitive to sexual stimulation [5,10,21]. Attention is drawn towards sexual stimuli and motivational interest in sex is aroused. These processes occur involuntary and automatically, probably with little influence of learning or experience. Recently, Spiering and Everaerd [39] have emphasized the role of the sexual unconscious, which is “inaccessible for

phenomenal awareness and independent of voluntary control" (p. 168). They convincingly show that sexual features are subject to subconscious attentive processing and analyses, which can—under the right conditions—activate physiological sexual responding. Here we would like to emphasize that these subconscious attentive processes might also elicit inhibitory mechanisms. Moreover, trait and state conditions influence such subconscious attentive processes on excitatory and inhibitory mechanisms. For, instance, inherited biological factors (such as, sensitivity of the androgen receptor system or the activity of the mesoaccumbens dopaminergic system) or a particular subjective sexual experience might be involved in a specific outcome.

Selective attention, however, might be related to threatening as well as rewarding properties of the stimulus. Thus, an increase in selective attention for sexual cues might be the result of a positive or negative motivational stance towards these sexual cues or stimuli. The situation becomes more complicated when—the other way around—threatening cues could also trigger acceleration in color naming, which indicates withdrawal of subconscious attentional resources from the emotional cues. Thus, when subjects are faster in naming the color of masks preceded by threat stimuli compared with neutral ones, it is assumed that they avoid the processing of threat cues or automatically orient attention away from the threat [40]. Thus, an emotional stimulus might capture attention because of threatening properties, but when the threatening value becomes too high, it might produce an opposite reaction of avoiding that stimulus.

Individual Differences in Preconscious Attentional Bias for Sexual Cues

It has been convincingly demonstrated that exposure to the unmasked and masked versions of the emotional Stroop task—in which threatening cues were used—can elicit the activation of opposite conscious and subconscious attentive processes, respectively. Moreover, in these experiments, it has been shown that the direction of the allocation of subconscious attentional resources depends on trait properties of the individual. In these traits, neuro–endocrine regulatory mechanisms— and especially testosterone—are highly involved [12,41–43].

Subconscious Attention for Sexual Cues and the Consequences for the Present Interpretation

In the present experiment, as in our pilot experiment, we found a group difference in subconscious attentional processes regarding sexual cues during the placebo condition, and alterations herein during the testosterone containing drug conditions. We assume that the subconscious attentional bias for sexual cues during the placebo condition in this initially low attentional bias group, represents a factual low level of attention for sexual stimuli. In extension, we do not interpret the founded attentional bias in this group as an avoidance reaction because the stimuli were preconscious attentively analyzed as too threatening. Low levels of attention for sexual stimuli might be associated with low sexual desire [44]. In our interpretation, the testosterone-induced

increase in subconscious attentional bias represents an increase in (positive) attentional engagement for sexual cues. Thus, this group might not be interested in sex, simply because their emotional sexual system is not very sensitive for sexual cues; administering testosterone increases this sensitivity and receptivity. This interpretation is in agreement with the predicted results on physiological and subjective sexual responding under condition of the combined treatment with testosterone and vardenafil. Testosterone caused an increase in sensitivity of the brain for sexual stimulation, and our experimental induced sexual stimulation was sufficient to induce an effect of a PDE5 inhibitor on vaginal arousal. Awareness of this physiological vaginal responding might also induce an increase in sexual desire, possibly mediated by implicit and explicit memory mechanisms.

The results of the other group—i.e., women showing an initially high attentional bias for sexual cues—are different from those of the first group (and reveal the same pattern as the women in our pilot experiment who experienced sexual abuse during their childhood). We believe that the increased subconscious attentional bias for sexual cues in this group means that—in contrast to the first group—the sexual cues are subconsciously processed and analyzed as threatening. Exposure to sexual stimuli may then serve as a cue for the retrieval of implicit and/or explicit sexual memories. These sexual memories are associated with recollections of sexual encounters (for example, sexual abuse), attitudes towards sex, sexual fantasies, learned (automatized) sexual scripts, and classically conditioned sensations. Differences in sexual responding may be caused by variations in the content of sexual memories. The emotional valence of a sexual stimulus depends on matches with sexual memories and will determine the attentional resource allocation [45]. In the initially high sexual attention group, there were more women who reported sexual abuse than in the initially low sexual attention group. For the present reasoning, we assume that the women with initially high attention are not characterized by a relatively insensitive system for sexual cues, but negative associations for sex (for example, sexual abuse or other negative associations with sex), which have influenced their information processing system for sex. We have extensively argued that testosterone induces an increase in sensitive affective/cognitive system for sexual stimulation, which must also be true when these stimuli are threatening. Consequently, the increased sensitivity for sexual cues induced by the testosterone containing drugs, might have resulted in a brain state in which these sexual cues have become more threatening. We interpret the decrease in subconscious attentional bias for sexual cues during testosterone containing drug treatments as the result of activating an inhibition mechanism (responsible for avoiding the processing of the sexual threat cues). This conclusion of the induction of an inhibitory mechanism is strengthened by the absence of any effect of testosterone containing drug conditions (with the exception of attention) on physiological and psychological indices of sexual functioning. Furthermore, in the high attention group, a higher percentage of the women reported negative sexual experiences as compared with the initially low attention group (63% vs. 17%, respectively). Negative sexual experiences (in general) might have

induced conditioning of a sexual inhibition mechanism, which might—in turn—cause sexual arousal problems [46–48]. This interpretation is in agreement with a somewhat higher incidence of co-morbid arousal problems in the high attention group, as compared with the low attention group (36% vs. 12%). This statement, however, needs to be released from its hypothetical status by future experimental research. Furthermore, future research is also needed to investigate the influence of testosterone (treatment) on the assumed activation of conditioned inhibition mechanisms in women with negative sexual experiences as compared with the formerly described women who had experienced CSA [49].

In conclusion, the here presented results justify further investigation into the combined use of sublingual testosterone and vardenafil as a treatment of HSDD, when the sexual problems can be attributed to a relatively insensitive system for sexual stimulation. The combination of these drugs had, however, no effect in women who showed initially a high level of subconscious attentional bias for sexual cues (and a decrease during testosterone treatment). These women may have developed a higher sensitivity for the induction (of conditioning) of sexual inhibitory mechanisms. Recently, we started a study to investigate this hypothesis in depth.

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CHAPTER 4

Induction of sexual arousal in women under conditions of institutional and ambulatory laboratory circumstances: a comparative study

Journal of Sexual Medicine 2010;7:1160-1176

Abstract

Introduction. Measuring under naturally occurring circumstances increases ecological validity. We developed an ambulatory psychophysiological laboratory that allows experiments to be performed at home.

Aim. To compare institutional laboratory task measures with ambulatory laboratory task measures.

Methods. Physiological sexual arousal was measured in eight women with hypoactive sexual desire disorder (HSDD) and eight healthy controls while exposed to neutral and erotic film clips both in the institute's laboratory and at home. Before and after film clip presentations, subjects performed an emotional Stroop task and completed two questionnaires.

Main Outcome Measures. Vaginal pulse amplitude (VPA), clitoral blood volume (CBV), subjective report of sexual arousal, preconscious attentional bias for erotic stimuli, subjective reports about feeling at ease, tense, anxious or inhibited.

Results. In healthy controls, genital measures of sexual arousal were significantly increased at home compared with the institutional laboratory, whereas no differences were observed between the institutional laboratory and the at home measurements in women with HSDD. The responses at home were significantly higher in healthy controls compared with women with HSDD. Subjective experience of genital responding increased at home for both groups of women. Concordance between subjective experience and genital sexual arousal was more pronounced in the institutional laboratory setting. Preconscious attentional bias was stronger in the institutional laboratory for both groups of women. Healthy controls felt more at ease and less inhibited at home while subjects with HSDD did not.

Conclusions. The use of an ambulatory laboratory is a valuable tool allowing psychophysiological (sex) research under more natural circumstances (e.g., a participant's home). In this study, the increase in ecological validity resulted in a qualitative differentiation between the healthy controls and the women with HSDD in the home setting, which is not apparent in the artificial setting of the institutional laboratory.

Introduction

In sex research, it might be desirable to measure physiological and subjective sexual responses in the domestic environment of the participants. We introduce an ambulatory psychophysiological laboratory by which experiments can be performed under more naturally occurring circumstances (e.g., at home without the presence of an experimenter). This ambulatory laboratory can be fully controlled by a participant. Furthermore, new technological developments in remote data collection made it possible to securely send parallel data streams to a central database in real time. Conclusions of an empirical investigation using this methodology can be generalized with more confidence to the naturally occurring situations in which the phenomenon under investigation occurs (ecological validity) [1] because the measurement takes place in the natural environment [2].

Induction of sexual arousal and execution of sexual behavior results from a delicate balance between excitatory and inhibitory mechanisms [3,4]. Sexual cues, including erotic thoughts or erotic movies may activate sexual excitatory mechanisms. Other factors, like stress, sexual performance related concerns or sexual satiety might evoke inhibitory mechanisms. Contextual factors like environmental setting, physical health, and mood state also influence the induction of sexual inhibition or excitation in women [5]. Individual differences in the sensitivity for activation in such excitatory and inhibitory mechanisms induced by particular stimuli or cues are also likely to exist.

Various procedures have been used to influence sexual excitation and/or inhibition in the laboratory. A relatively straightforward and common method to induce sexual excitation in the laboratory is by exposing subjects to erotic film clips. Attenuation of the physiological and subjective sexual response is less straightforward, but has been induced in the laboratory through different subtle experimental manipulations. For example, during viewing of erotic film clips, the installment of undemanding cognitive distraction [6,7], seeing one's reflection in the mirror [8], inducing the feeling of being watched [9], and monitoring one's sexual arousal (at least in men) [10] can attenuate or inhibit genital and subjective sexual arousal. These findings illustrate that relatively nonintrusive psychological manipulations can shift the delicate balance between excitatory and inhibitory factors influencing the sexual response. This implies that systematic factors present in the institutional sex laboratory setting—such as presence of an experimenter in the other room, or the unfamiliar artificial situation—might influence the sexual response in an unknown manner, and thus bias the results.

Vaginal photoplethysmography has been widely used to measure the genital response to erotic stimuli (e.g., exposure to erotic film excerpts). This measurement method has been validated as specific to erotic stimuli [11]. Recently, we introduced the clitoral photoplethysmograph for the measurement of clitoral blood volume (CBV) [12]. This measure appeared more sensitive to inhibitory influences on the sexual response.

Moreover, it may also be a more valid measure of sexual arousal because it measures engorgement of the clitoral and surrounding tissues (see Gerritsen et al. [12] for a discussion on this matter).

Questionnaires are often used in the laboratory to assess subjective arousal. Experiments in men mostly show high concordance between their physiological and subjective arousal, and as a result these subjective measures are more valid in men [13,14]. In women this relationship is less clear, frequently showing discordance between measures of genital and subjective arousal [14–17]. This may be (partly) attributable to the measurement method. Studies using, e.g., thermography as a measure of genital arousal and a lever to indicate subjective arousal, report significant correlations between the two [18,19]. Significant correlations are also reported between laser Doppler imaging and post-stimulus subjective report [20]. It may, however, also be (partly) attributable to measurement setting.

To measure aspects of cognitive–affective information processing of sexual cues, the emotional Stroop task [21,22] may be used. In this task, subjects are instructed to name the color in which emotional and neutral stimuli are printed as quickly as possible while ignoring the meaning of the word. The motivational state of the subject and the emotional content of the stimuli determine the performance on this task in terms of slowing down or speeding up in color naming [23]. Attentional bias for emotional cues is demonstrated when color-naming latencies for emotional stimuli are greater or smaller than color-naming latencies for neutral stimuli. A masked version of this task turned out to be a more reliable measurement of (preconscious) attentional bias for emotional cues [21,22]. It has been suggested that automatic cognitive processes can lead to activation of a genital response when a sexual meaning is present, and that can lead to low levels of sexual arousal and/or the presence of negative affect when other sexually neutral or negative meanings are present [24]. Preconscious attentional bias for erotic cues may reflect such an automatic process and thus possibly influence (the relationship between) physiology and subjective experiences.

General sexual functioning can be measured by structured interviews and validated trait questionnaires such as the Female Sexual Functioning Questionnaire (SFQ) [25]. The SFQ assesses sexual functioning in the domestic setting and can be used to diagnose different types of female sexual dysfunction (FSD). Studies comparing women with different FSD diagnoses and healthy controls did not find consistent differences in genital or subjective arousal in the laboratory [17,26,27] (although Brotto et al. [26] did find differences between subgroups of patients suffering from female sexual arousal disorder). This lack in group differences in the laboratory is in stark contrast to the reported differences in the real-life experience of these women. This may be a result of a variety of factors, one of which can be that laboratory specific circumstances are different from domestic circumstances. The aforementioned systematic factors inherent to the laboratory may influence the balance between excitatory and inhibitory

mechanisms and may conceal group differences. To our knowledge, these discrepancies in observations in the institutional laboratory settings compared with the real-life experience of these groups have not been investigated before.

In the present study, we investigated the influence of the measurement setting (the institutional laboratory vs. the domestic measurement setting) on indices of physiological and subjective sexual responding. We investigated sexual responses in women suffering from hypoactive sexual desire disorder (HSDD) and in a control group of sexually functional women (healthy controls). We believe that the domestic setting might have fewer cues that initiate or sustain inhibitory mechanisms, although we are uncertain about the effects of the sexual history of the women with HSDD at home. We therefore hypothesized that both groups of women would (i) show more attention for subliminally presented erotic stimuli (as measured by a masked version of the Emotional Stroop Task), (ii) show more genital sexual arousal (as measured by vaginal pulse amplitude [VPA] and CBV), and (iii) experience stronger sexual arousal (as measured by the Sexual Arousal Response Self Assessment Questionnaire [SARSAQ]) at home. In addition, we hypothesized that all these effects would be more pronounced in healthy controls, because women with HSDD might have a less sensitive and/or less responsive system for sexual excitation or be more susceptible to activation of inhibitory mechanisms [3,4].

Methods

Participants

Twenty premenopausal heterosexual women were recruited in order to include a total of eight women with HSDD and eight healthy controls for participation in this study. They were selected from our database of women who had participated in previous studies. All participants underwent a new intake procedure. Participants were diagnosed for HSDD according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) criteria. All participants signed a written informed consent and received €120 reimbursement for their participation. This study was approved by the local ethics committee (Stichting Therapeutische Evaluatie Geneesmiddelen Medisch Ethische Toetsingscommissie, Almere, The Netherlands), carried out in agreement with the International Conference Harmonization-Good Clinical Practice and monitored by a contract research organization (PSR Group, Hoofdorp, The Netherlands).

Apparatus and Stimuli

VPA and CBV

VPA was measured using a vaginal photoplethysmograph, a tampon-shaped device containing an infrared light-emitting diode (LED) and a photosensitive light detector

(photodiode). The cabling is protected with silicon tubing. An additional clitoral photoplethysmograph is attached to the silicon tube (see no. 2 in Figure 1—section "Ambulatory laboratory") to measure CBV. The shape of the clitoral probe follows the anatomical curves of the area surrounding the urethral opening up to the clitoris, between the labia minora and just above the introitus. The distance between the clitoral probe and the vaginal photodiode is 5 cm and the vaginal probe is rotated 30° clockwise, as seen from behind the clitoral probe. The LED and photodiode for the clitoral probe are located inside the clitoral probe. In order to target the left clitoral bulb, the LED and photodiode are set at an angle of 45°, pointing to the right, as seen from behind the clitoral probe. The clitoral photoplethysmograph used in this study is an improved version of the one described in Gerritsen et al. [12]. The main difference between both clitoral photoplethysmographs is that the present one emits and receives the light through a LED and photodiode that are located inside the clitoral probe instead of in an external connection box with optical cables, which transport light to and from the probe. This improvement makes the probe easier to handle and more sturdy.

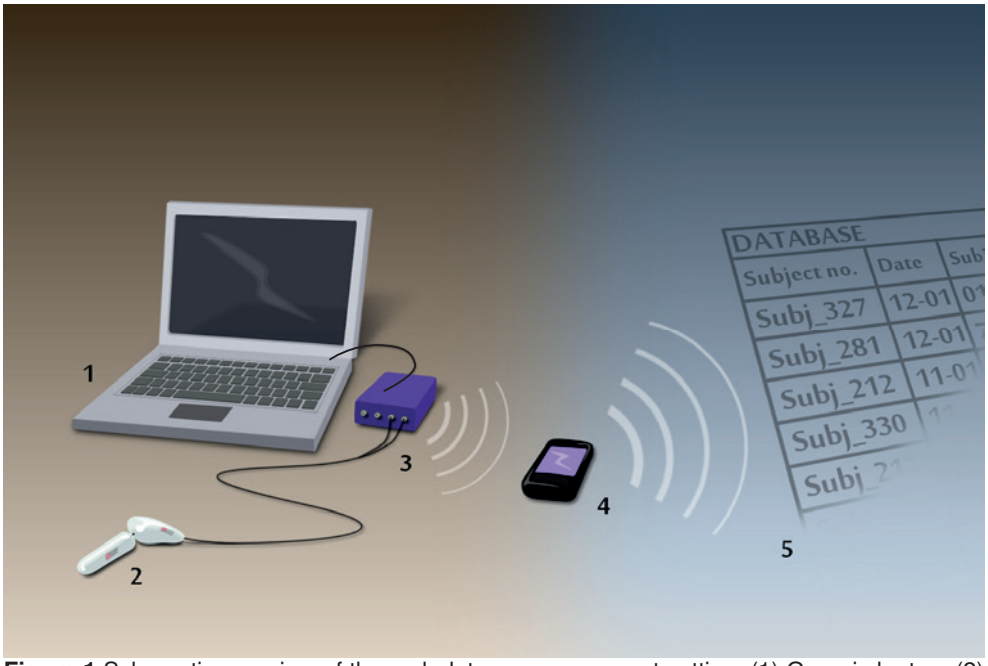

Figure 1 Schematic overview of the ambulatory measurement setting. (1) Generic laptop, (2) genital probe, (3) wireless sensor system, (4) handheld computer, and (5) secure central database. See text "Ambulatory laboratory" for detailed description.

Stimuli

For this study, neutral, erotic foreplay, and hardcore film clips were used. Neutral clips were 6-minute and 2-minute clips from Dutch action films. The 6-minute neutral film clips were used to establish a baseline VPA. Erotic foreplay clips were 2-minute clips

(two versions) showing kissing, caressing, and cunnilingus, but no fellatio. Erotic hardcore clips were 2-minute clips (two versions) showing cunnilingus and coitus, including visible penetration. The erotic film footage was selected and edited by female researchers. The hardcore footage was edited in order to meet the following criteria: the first 20–30 seconds consist of foreplay scenes with kissing, caressing, and cunnilingus but not fellatio; only heterosexual couples were shown, and visible vaginal intercourse was shown within 30 seconds following onset of the clip. Stroking of the penis and fellatio were not included, as these behaviors have been shown to be rated as less arousing by women [28]. Edited clips were judged by the other female investigators and female research assistants. All digitally sampled film clips were presented using Presentation software (Neurobehavioral Systems, Albany, CA, USA).

Emotional Stroop Task

To measure preconscious attentional bias for sexual cues, a masked version of the emotional Stroop task was used [21–23]. In this task, words were presented for 26.6 ms in four different colors (red, green, blue, and yellow) on a 75 Hz computer screen (Liteon Technology Corp., Taipei, Taiwan), or on a Dell Latitude D531 laptop (Dell Inc, Round Rock, TX, USA) for the domestic measurement setting, set at a 75 Hz refresh rate. Words were backwardly masked by randomly cut and reassembled letters in the same color. Backward masking prevents conscious processing of the words. Participants were instructed to name the color of the masks as quickly as possible. A microphone connected to a voice-level detector was placed in front of the participant. Initiation of vocal response was registered by the computer's clock and terminated the target (mask) presentation (with a no-response maximum of 3,000 ms). Accuracy of color naming was not scored. Thirty-two unambiguous neutral words from one category (furniture; examples are “chair” and “table”) and 32 unambiguous erotic words (examples are “penis,” “coitus,” and “vagina”) were presented in a blocked manner (eight words per block). The same words were used for each test; however, the sequence of words and their colors differed all eight times this task was used. These different versions were randomized over the participants. An extra set of stimuli consisting of meaningless letter strings was used for practice trials directly before each Stroop task.

SARSAQ

The SARSAQ is a 10-item self-report questionnaire using a 7-point Likert scale (ranging from “not at all” to “extremely”), adapted from Morokoff and Heiman [17] and Heiman and Hatch [29]. It measures current subjective feelings of sexual arousal and sexual desire. Five items concern subjective feelings of genital responding, and five items concern subjective feelings of sexual desire. In the institutional laboratory, participants completed the questionnaire using a touch screen monitor. In the ambulatory laboratory however, number keys were used to complete the questionnaire.

SFQ

The SFQ is a validated self-report questionnaire containing 34 items and assessing eight domains of sexual function: desire, arousal–sensation, arousal–lubrication, subjective arousal, enjoyment, orgasm, pain, and partner relationship [25]. The SFQ was administered once during the screening visit. A pen and paper version was used.

Subjective Experience Questionnaire (SEQ)

The SEQ is a questionnaire with four items which was construed to evaluate the participants' experienced comfort during the two experimental sessions. Participants were asked directly after each experimental session to rate on a 5-point Likert scale (i) how much at ease they were during the measurement; (ii) how anxious they were during the measurement; (iii) how inhibited they felt during the measurement; and (iv) how tense they were during the measurement. Higher scores meant that participants where more anxious, inhibited and tense, and felt less at ease. A pen and paper version was used in both sessions.

Institutional Laboratory

The experimental session in the institutional laboratory took place in a closed, dimly lit, and sound attenuated experimental room containing the signal amplifier and a computer screen on which the film clips were presented. Participants were seated in a comfortable chair and provided with a blanket to cover their lap, in order to prevent external light from interfering with the measurement. An intercom was present to allow for two-way communication (on demand) in case of possible problems or additional instructions.

Ambulatory Laboratory

The ambulatory laboratory is based on the MobiHealth Mobile remote monitoring system (MobiHealth B.V., Enschede, The Netherlands) [30]. Study specific functionality of this system enables VPA and CBV measurements, stimulus presentation, and execution of emotional Stroop tasks to be performed at an arbitrary time and location (e.g., house of a participant). This laboratory is operated autonomously by the participant. The ambulatory laboratory transmits all measured data to a secure central database server, at which the researcher can obtain the data for further analysis.

Figure 1 depicts a schematic overview of the whole ambulatory measurements setting. The ambulatory laboratory consists of four components: (i) a generic laptop for stimulus presentation and Stroop task execution; (ii) a genital probe for VPA and CBV measurement; (iii) a wireless sensor system; and (iv) a handheld computer (MobiHealth Mobile Base Unit; MBU). The handheld computer is a HTC P3600 (HTC, Taoyuan, Taiwan). Both the genital probe and generic laptop are attached to the wireless sensor

system ("Mobi system"; TMSi, Enschede, The Netherlands). The sensor system is controlled by the MBU. The MBU runs the software that controls the whole measurement process, including authorized and secure communication to the sensor system and the secure central database (v). After a measurement session is finished, the MBU automatically terminates the connection. During a session, all data is sent instantaneously (i.e., real time) to the central database server to prevent local data storage, and thus the possibility of unwanted and untraceable data manipulation.

Procedure

Participants were interviewed by a trained psychologist to diagnose for HSDD according to the DSM-IV-TR criteria. Additionally, participants filled out the SFQ. To exclude major medical and psychiatric illnesses, a general medical and gynecological anamnesis was taken. Weight and height were measured. Blood pressure (supine), heart rate, and body temperature were measured when the anamnesis indicated further examination. A gynecological examination and urine pregnancy test were performed to exclude pregnancy, vaginal infections, major surgery on the vagina and/or vulva, undetected major gynecological illnesses, or unexplained gynecological complaints. Cultures were taken to exclude Chlamydia or Gonococcus infections. Additionally, participants were asked about childhood sexual abuse and other negative sexual experiences. Twenty participants were screened. Two screened participants where excluded: one suffered from dyspareunia and the other did not meet the DSM-IV-TR criteria for HSDD. A third screened participant stopped prior to the measurements because of personal circumstances.

When participants met all inclusion criteria, they were randomized into one of two possible test sequences: first measurement at home, second measurement in institutional laboratory; or first measurement in institutional laboratory, second measurement at home. Participants of both subgroups were evenly distributed over these two sequences. No experimental days were planned when participants were menstruating.

At the beginning of the measurement in the institutional laboratory, participants were screened for drug and alcohol use. Participants were explained how to insert the genital probe and were subsequently left alone in the experimental chamber to insert the probe. The measurement session began when the participants indicated via intercom that they were ready. Participants first completed a SARSAQ, after which they completed the first emotional Stroop task. Then a 6-minute neutral film clip was shown in order to establish a VPA and CBV baseline. Participants where then instructed (on the computer screen) to fantasize about an erotic encounter. This could be from memory or imaginary. A short music fragment alerted participants when the fantasy condition ended. The volume of the music fragment started inaudible and steadily increased in volume over 2 seconds to an audible but low level. This was done so that participants who had their eyes closed

during the fantasy session would know that the session was at an end but would not be startled. Following the fantasy session, participants completed the SARSAQ again. Then, a 2-minute neutral film clip was shown, followed by a 2-minute erotic foreplay film clip. A third SARSAQ was completed. Then, a 2-minute neutral film clip was shown, followed by a 2-minute erotic hardcore film clip, and a fourth SARSAQ was completed. The erotic film clips were presented in a fixed order (fantasy, foreplay, and hardcore) in order to maximize the sexual response. The 2-minute neutral film clips in between were meant to separate the exposure to the three sexual stimuli without the intention to induce return to baseline. During all film clips and the fantasy condition, VPA and CBV were measured. The experimental session ended with a second emotional Stroop task and SEQ. A measurement session took approximately 30 minutes.

For the experimental session at home, participants took the ambulatory laboratory home. Participants were trained in the setup and use of the ambulatory laboratory beforehand. Only when participants had shown that they could connect and run a mobile laboratory by themselves were they considered sufficiently trained. This training process took 20 minutes on average. Duration of the training was mostly dependant on the subjects' previous experience with computers; experienced computer users were faster to learn the procedure. Also, an instruction manual with photographs and an instruction video was included in which the setup and use of the mobile laboratory was explained. Participants were instructed to replicate the institutional laboratory setting at home as much as possible. They were free to choose the room, as long as it was quiet and they would not be disturbed during the measurement. They were explicitly instructed to sit upright at a table or desk. The experimental session at home was the same as in the institutional laboratory. A cleansing kit for the genital probe (including instructions) was also taken home by the participants. All probes were cleansed a second time when they returned to the institution.

At the end of the study, a psychologist interviewed the participants concerning their experiences during the home measurement. They where asked if all went well and how they experienced the procedure at home in comparison to the institutional laboratory.

Data Reduction

The VPA reflects phasic changes in vaginal engorgement corresponding with each heartbeat. VPA was defined as the peak-to-trough amplitude of the pulse wave and was calculated by acquiring the means of all peaks and troughs and subtracting those. Data from the photoplethysmograph were sampled at 256 Hz and filtered offline (high-pass 1 Hz, 48 dB/oct and low-pass 1.5 Hz, 48 dB/oct), in order to isolate the alternating current (AC) component from the direct current (DC) coupled amplifier, reduce respiration artifacts and high-frequency oscillations. Large movement artifacts (more than 100% increase for a small number of isolated periodic cycles) were manually removed following visual inspection of the data. There were 36 movement artifacts

observed in the VPA data collected in the institutional laboratory, and 47 in the VPA data collected via the ambulatory laboratory. One participant accounted for eight of the movement artifacts in the ambulatory laboratory while only having one artifact in the institutional lab. The data were divided into 30-second epochs for each 2-minute film clip, thus yielding four discrete values reflecting VPA during different stages of the film clip. Finally, in order to eliminate interpersonal differences and obtain meaningful data, VPA scores during the foreplay and hardcore clips were related to activity during the first 6-minute neutral clip, using the following formula:

$$VPA_{rel} = ((VPA_x - VPA_{neu})/VPA_{neu})$$

with VPA_{rel} being the relative change in VPA related to the first neutral clip, VPA_x being any 30-second epoch value during the erotic clips, and VPA_{neu} being the average VPA score during the first neutral clip.

CBV was assessed by analyzing the DC component of the signal from the clitoral photoplethysmograph. The AC signal-to-noise ratio proved to be relatively small and the signal less sensitive to increasing sexual arousal, when compared with the DC signal. (Since we used a DC-coupled amplifier in the experimental setup, both AC and DC components were available for analysis.) The smaller signal to noise ratio is probably caused by differences in vaginal wall and clitoral/labial tissue. The capillaries in the vaginal wall in which the VPA is measured lie more at the surface of the tissue. Moreover, signal-to-noise ratio of the AC component in the clitoral complex may decrease more if the cavernous tissue fills with blood. CBV data were sampled at 256 Hz and filtered offline (low-pass 0.03 Hz, 24 dB/oct). Again, data were divided into 30-second epochs. The data were baseline corrected for each participant by subtracting the minimum value during the session from the actual values for each epoch. The CBV signal proved to be resistant to participant movement, especially when compared with VPA, therefore no additional artifact rejection was needed. The CBV values were then used to produce relative scores in a way similar to the VPA procedure, i.e., based on the first 6-minute neutral clip.

$$CBV_{corr\_x} = (CBV_x - CBV_{min})$$

$$CBV_{rel} = ((CBV_{corr\_x} - CBV_{corr\_neu})/ CBV_{corr\_neu})$$

with CBV_{corr_x} (“corrected CBV”) being the baseline corrected score, CBV_x being any 30-second epoch value during the erotic clips prior to correction, and CBV_{min} the minimum value of all the epochs following the return to baseline clip. CBV_{rel} is the relative increase in corrected CBV related to the first neutral clip, as expressed by CBV_{corr_neu}, the average corrected CBV score during the first neutral clip.

The Stroop reaction times for color naming were visually inspected for outliers. There were 115 outliers over all participants in the Stroop data in the institutional laboratory,

and 72 in the ambulatory laboratory. After these outliers were excluded, participants' mean reaction times for erotic and neutral words on each trial were calculated. Mean reaction times of erotic and neutral words on each trial were used in the analysis.

The SARSAQ was administered four times during one measurement session. For each administration the five-item scores for subjective feelings of genital responding (SARSAQ-GR) were added together and the five-item scores concern subjective feelings of sexual desire (SARSAQ-SD) were added together. This yielded two SARSAQ scoring domains, both having a range of 5–35. For each SARSAQ scoring domain, the relative increase was calculated of which the first SARSAQ was the baseline:

$$SARSAQ_{rel} = ((SARSAQ_x - SARSAQ_{base})/ SARSAQ_{base})$$

with $SARSAQ_{rel}$ being the relative change in SARSAQ score related to the first SARSAQ score, $SARSAQ_x$ being the second (which is completed directly after the erotic fantasy), third (after foreplay) or fourth (after hardcore) SARSAQ score, and $SARSAQ_{base}$ being the first SARSAQ.

Genital and subjective measures are, thus, analyzed with transformed variables, which denote the relative change from baseline of each specific measure giving three levels: baseline vs. erotic fantasy, baseline vs. foreplay film clip, and baseline vs. hardcore film clip. In the three levels of the subjective and genital measures, the means of the second level (nearly) always lie between the means of the first and last level (see Table 2), because stimuli of increasing potency (fantasy, foreplay, and hardcore) were presented in a fixed order. All analyses of genital and subjective arousal as described below therefore only describe the first and last of these three levels. For the VPA and CBV, the first and fourth (last) 30-second epochs were compared in order to investigate the maximum effect of the erotic fantasy condition and hardcore film clips on sexual arousal.

Statistical Analysis

All dependent variables were checked for normality. Outliers were discarded from analysis if they were significant ($z > 3.1$) [31].

Group homogeneity with respect to demographic variables was tested with independent samples *t*-tests with diagnosis as grouping variable (HSDD vs. healthy controls) on all continuous demographic variables and on the domains of the SFQ separately (see Table 1).

VPA and CBV data were analyzed separately, but in the same manner: dependent variables were analyzed in a 2 measurement setting (ambulatory lab vs. institutional lab) × 2 stimulus type (erotic fantasy vs. hardcore film clips) × 2 time (epoch 1 vs. epoch 4) × 2 group (HSDD vs. healthy controls) repeated measures anova, with measurement

setting, stimulus type, and time as within subject factors, and group as between subject factor.

The eight dependent variables of the Stroop where analyzed in a 2 measurement setting (ambulatory lab vs. institutional lab) × 2 stimulus type (erotic vs. neutral words) × 2 time (session 1 vs. session 2) × 2 group (HSDD vs. healthy controls) repeated measures anova, with measurement setting, stimulus type, and time as within subject factors, and group as between subject factor.

The dependent variables for SARSAQ-GR and SARSAQ-SD where analyzed separately in a 2 measurement setting (ambulatory lab vs. institutional lab) × 2 stimulus type (erotic fantasy vs. hardcore film clips) × 2 group (HSDD vs. healthy controls) repeated measures anova, with measurement setting and stimulus type as within subject factors, and group as between subject factor.

Interaction effects between measurement setting and group in the VPA, CBV, Stroop, SARSAQ-GR, or SARSAQ-SD were further examined by repeating the analysis for each group separately.

The SEQ dependent variables deviated from normality so these were tested with the Wilcoxon signed ranks test for nonparametric data. To test the hypothesis that measurement setting influences subjective experience, the four item scores in the two measurement settings (ambulatory lab vs. institutional lab) were compared with the Wilcoxon signed ranks test for nonparametric data twice; once for the HSDD group and once for the healthy controls. Exact probabilities were calculated because the number of possible signed ranks was smaller than 10 [32].

Pearson's correlation coefficient was calculated to investigate the relation between genital (VPA and CBV) and subjective measures (SARSAQ-GR and SARSAQ-SD) of sexual arousal. In these calculations, CBV and VPA data of the fourth 30-second epoch were used because these are likely to correspond most with the subjective measures that were reported following most closely to these epochs. A significance level of $\alpha = 0.050$ was used for all tests.

Results

Sample Description

Sixteen women completed this study (one participant was excluded on the first visit because she tested positive for cannabis and benzodiazepines), eight of which (mean age 34.6, sexual desire [SD] 7.2) were diagnosed with HSDD. Of these subjects with HSDD, one subject was also diagnosed with female sexual arousal disorder, one with female orgasmic disorder and one with both female sexual arousal disorder and female orgasmic disorder. The eight other women (mean age 36.3, SD 8.7) served as control

group. There were no major medical psychiatric illnesses. The group means of demographic variables, age, number of parity, and body mass index of both groups did not differ (see Table 1).

Table 1 Comparison of demographic variables and indices of sexual functioning between subgroups

Demographic variables	All participants (N = 16)	Participants with HSDD (N = 8)	Healthy controls (N = 8)	*P* values
Age (years)	35.4 (8.0)	36.3 (8.7)	34.6 (7.2)	ns
Number of parity	0.9 (1.1)	1.3 (1.2)	0.6 (0.9)	ns
Body mass index (kg/m^2)	26.2 (4.4)	24.1 (3.5)	28.2 (4.3)	ns
Contraceptives				
OAC	4	2	2	na
IUD (hormonal)	2	0	2	na
Injectable contraceptive	1	0	1	na
Vaginal ring	1	0	1	na
Condom	2	2	0	na
None	6	4	2	na
Ethnic origin				
Caucasian	14	8	6	na
Black	2	0	2	na
Amount of smokers	5	2	3	na
SFQ				
Desire	16.3 (4.8)	13.0 (1.8)	20.2 (4.1)	$P < 0.010$
Arousal (sensation)	11.1 (4.5)	9.0 (3.9)	13.5 (4.2)	ns
Arousal (lubrication)	7.0 (2.1)	6.6 (2.2)	7.5 (2.1)	ns
Total	105.8 (21.0)	92.7 (16.0)	121.2 (15.0)	$P < 0.010$

Note: Cell values represent group means and group standard deviations (in parentheses), except cell values for "Contraceptives," "Ethnic origin," and "Amount of smokers," which represent subject counts. The last column shows the *P* values of an independent sample *t*-test comparing the means of the two subgroups (women with HSDD and healthy controls) of the demographic variable of that row. The subgroup means of weight, "SFQ Desire" and "SFQ Total" differ significantly. HSDD = hypoactive sexual desire disorder; OAC = oral anti conceptive; IUD = intra uterine device; SFQ = Female Sexual Functioning Questionnaire; ns = not significant; na = not applicable. HSDD = hypoactive sexual desire disorder; OAC = oral anti conceptive; IUD = intra uterine device; SFQ = Female Sexual Functioning Questionnaire; ns = not significant; na = not applicable.

SFQ domain scores of three participants (two healthy controls and one HSDD) could not be computed. If someone has not had sexual activity in the last 4 weeks, most items are scored as missing, and therefore, domain scores cannot be computed. The SFQ domains desire, arousal (sensation), arousal (lubrication), orgasm, pain, enjoyment, partner, and the SFQ total score were compared. On average, women with HSDD scored lower on the domain "desire" (M = 13.0, SD = 1.8) than the healthy controls (M = 20.2, SD = 4.1). This difference was significant $t(11) = 4.17$, $P < 0.010$, $d = -2.32$. Women with HSDD also scored lower on the domain "partner" (M = 7.1, SD = 1.2) than the women without FSD (M = 10.0, SD = 0.0). This difference was significant $t(11) = 5.72$, $P < 0.001$, $d = -0.57$. Finally, women with HSDD scored lower on the SFQ total score (M = 92.7, SD = 16.0) than the women without FSD (M = 121.2, SD = 15.0). This difference was also significant $t(11) = 3.29$, $P < 0.010$, $d = -0.96$. The participants did not differ significantly on any of the other SFQ domain scores.

VPA

There was a main effect for "stimulus type" $F(1,14) = 8.051$, $P < 0.050$, partial $\eta^2 = 0.37$. All women had a stronger VPA increase to hardcore film clips (M = 0.86, SE = 0.25) in comparison to erotic fantasy (M = 0.24, SE = 0.08). Also, there was a main effect of "time," $F(1,14) = 13.912$, $P < 0.010$, partial $\eta^2 = 0.50$. Relative increase from neutral baseline was stronger in the fourth epoch (M = 0.73, SE = 0.18) in comparison with the first epoch (M = 0.37, SE = 0.12). There was an interaction effect among "stimulus type," "time," and "group" $F(1,14) = 6.808$, $P < 0.050$, partial $\eta^2 = 0.33$. Women with HSDD had a smaller increase in response to the hardcore stimuli than the healthy controls, and their response to the erotic fantasy condition did not differ from healthy controls. Finally, a trend was visible in the interaction between "stimulus type," "time," and "measurement setting" $F(1,14) = 3.169$, $P < 0.100$, partial $\eta^2 = 0.19$. All women showed a stronger VPA response to hardcore stimuli in the home measurement setting as compared with the institutional laboratory setting. Their response to the erotic fantasy condition did not differ between measurement settings (see Figure 2).

CBV

There was a main effect for "stimulus type," $F(1,14) = 21.176$, $P < 0.001$, partial $\eta^2 = 0.60$. All women had a stronger CBV increase to hardcore film clips (M = 1.60, SE = 0.24) in comparison with erotic fantasy (M = 0.68, SE = 0.13). There was also a main effect of "time," $F(1,14) = 19.198$, $P < 0.001$, partial $\eta^2 = 0.58$. Relative increase from neutral baseline was stronger in the fourth epoch (M = 1.41, SE = 0.21) in comparison with the first epoch (M = 0.87, SE = 0.13). There was an interaction effect among "measurement setting," "time," and "group", $F(1,14) = 6.795$, $P < 0.050$, partial $\eta^2 = 0.33$. Women with HSDD had much smaller increases in CBV in response to the erotic stimuli at home than healthy controls. The responses of both groups to all erotic conditions in the institutional laboratory were largely the same. There was an interaction effect between "stimulus type" and "time," $F(1,14) = 4.887$, $P < 0.050$, partial $\eta^2 = 0.26$. CBV increases to hardcore film clips, especially the fourth 30-second epoch were stronger than to erotic fantasy. There was an interaction effect among "measurement setting," "stimulus type," and "time," $F(1,14) = 9.487$, $P < 0.010$, partial $\eta^2 = 0.40$. Women's responses increased to erotic fantasy epochs in the home setting, but the response to the fourth 30-second hardcore epoch at home was much stronger. Finally, there was an interaction effect among "measurement setting," "stimulus type," "group," and "time," $F(1,14) = 5.621$, $P < 0.050$, partial $\eta^2 = 0.29$. In the institutional laboratory, both groups did not differ. However, in the home setting, healthy controls strongly increased their CBV response to all erotic stimuli, but much more pronounced for the hardcore stimuli, while the women with HSDD had an attenuated CBV response to all erotic stimuli (see Figure 2).

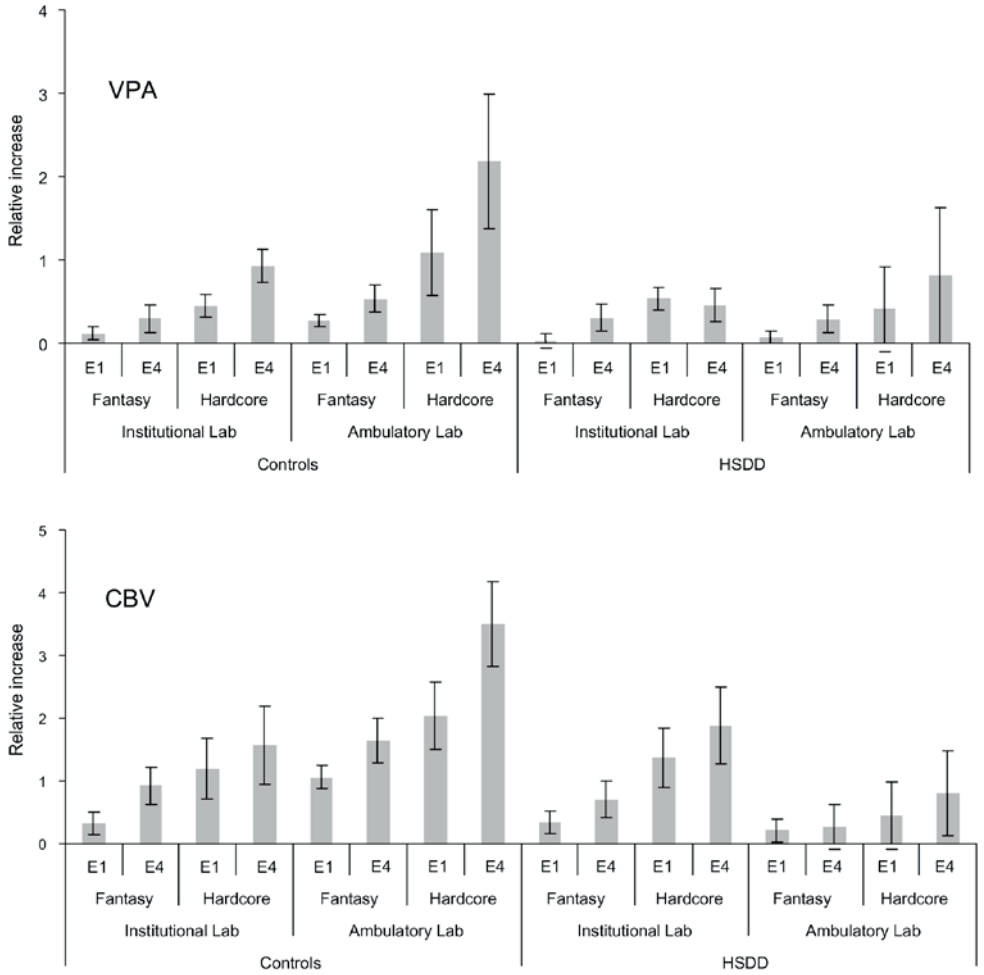

Figure 2 Upper panel: Mean (and standard error) relative increases in VPA to erotic stimuli. Epochs 1 and 4 (E1 and E4; 1st and 4th 30-second epochs) in the fantasy and hardcore conditions are shown for both laboratory settings in both groups of women (healthy controls vs. HSDD). Lower panel: Mean (and standard error) relative increases in CBV to erotic stimuli. Epochs 1 and 4 (E1 and E4; 1st and 4th 30-second epochs) in the fantasy and hardcore conditions are shown for both laboratory settings in both groups of women (healthy controls vs. HSDD). VPA = vaginal pulse amplitude; CVB = clitoral blood volume; HSDD = hypoactive sexual desire disorder.

To investigate the interaction effects of "measurement setting" and "group" further, the same analysis was run for each group separately. A "measurement setting" and "time" interaction was observed for CBV that was significant for the healthy controls, $F(1,7) = 5.903$, $P < 0.050$, partial $\eta^2 = 0.46$, but not for the HSDD group. The healthy controls showed a stronger increase over time at home, as compared with the institutional setting. In the healthy control group, there was also a "measurement setting," "stimulus type," and "time" interaction for the CBV, $F(1,7) = 15.997$, $P < 0.010$,

partial $\eta^2 = 0.70$, but not for the HSDD group. The healthy controls showed a stronger response over time for the hardcore stimuli at home, as compared with the institutional setting.

Stroop

There was a main effect for "time" $F(1,14) = 6.954$, $P < 0.050$, partial $\eta^2 = 0.33$. Women slowed down 34 ms in color naming at the end of the experiment (M = 552.9 ms, SE = 20.9 ms) compared with the start of the experiment (M = 528.7 ms, SE = 17.9 ms). There was an interaction effect between "measurement setting" and "stimulus type" $F(1,14) = 5.930$, $P < 0.050$, partial $\eta^2 = 0.30$. Women slowed down in color naming of neutral words in the home laboratory setting (M = 549.3, SE = 20.6) compared with the institutional laboratory (M = 526.4, SE = 20.4) in contrast to the mean reaction times of erotic words that were largely the same (M = 546.4, SE = 20.4 and M = 541.3, SE = 22.5, respectively) (Figure 3).

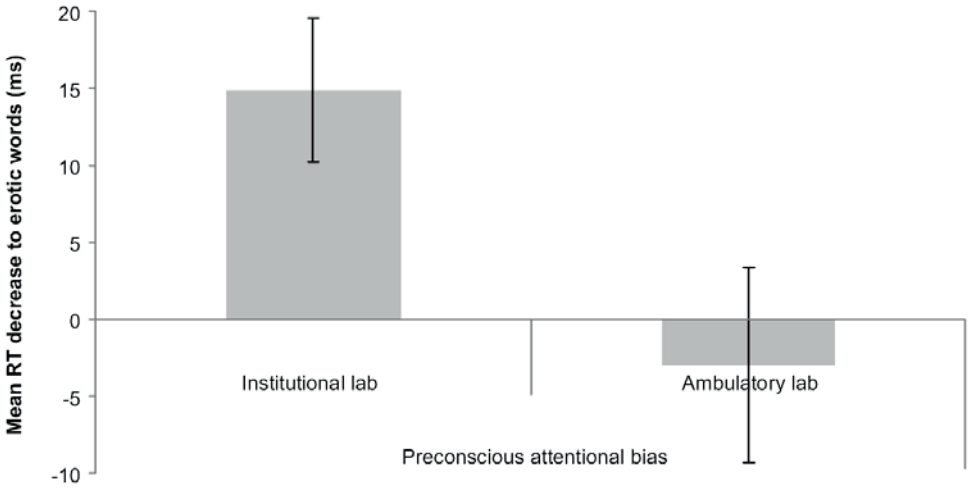

Figure 3 Whole sample means and standard errors of preconscious attentional bias scores in color naming for erotic words in both laboratory settings. RT = reaction time.

SARSAQ

Investigation of the SARSAQ data showed that one participant had four significant univariate outliers ($P < 0.001$). For this reason, this participant was omitted from the analyses of the SARSAQ data. Three SARSAQ dependent variables (two for the SARSAQ-GR and one for the SARSAQ-SD) showed different variances for the two groups. A more stringent α level ($\alpha = 0.025$) was used to control for Type I error [33]. For SARSAQ-GR there was a main effect for "stimulus type," $F(1,13) = 12.073$, $P < 0.010$, partial $\eta^2 = 0.48$. Participants reported more subjective experience of genital responding following the hardcore film clips (M = 1.05, SE = 0.23) than following the erotic fantasy (M = 0.46,

SE = 0.10). Also, there was an interaction effect between "stimulus type" and "measurement setting," $F(1,13) = 7.261$, $P < 0.025$, partial $\eta^2 = 0.36$. The relative increase in subjective experience of genital responding following the erotic fantasy conditions was the same in both measurement settings. Relative increases were stronger following the hardcore film clips and of the two settings, the strongest increase was at home (see Figure 4). For SARSAQ-SD, there was also a main effect for "stimulus type," $F(1,13) = 9.424$, $P < 0.010$, partial $\eta^2 = 0.42$. Participants reported more subjective experience of sexual desire following the hardcore film clips (M = 1.06, SE = 0.45) than following the erotic fantasy (M = 0.46, SE = 0.13). A trend was observed in the interaction between "stimulus type" and "measurement setting," $F(1,13) = 3.050$, $P = 0.100$, partial $\eta^2 = 0.19$. The relative increase in subjective experience of sexual desire following the erotic fantasy conditions was the same in both measurement settings. Relative increases were stronger following the hardcore film clips, and of the two settings was strongest at home.

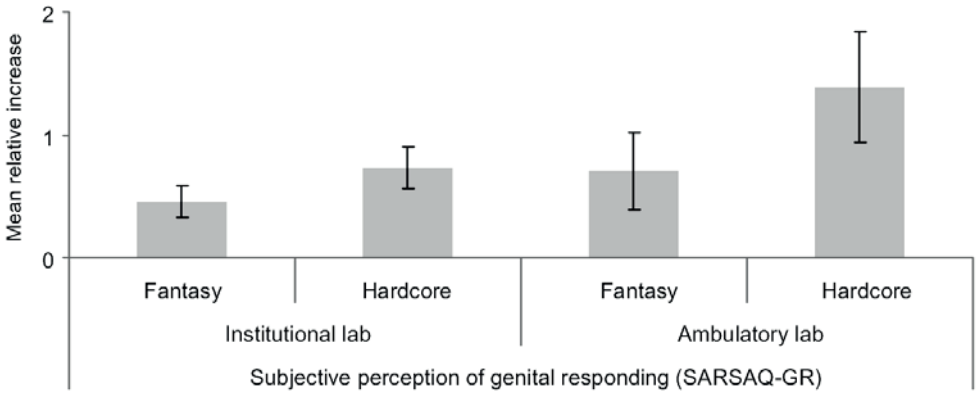

Figure 4 Whole sample mean relative increases and standard errors in subjective feelings of genital arousal during the fantasy condition and the hardcore condition in both laboratory settings.

SEQ

The healthy controls reported less inhibition at home (Median [Mdn] = 1) than in the institutional laboratory (Mdn = 2), N = 5, T = 15.0, $P < 0.05$ (one-sided), $r = -0.79$. The women without FSD also reported being more at ease at home (Mdn = 1) than in the institutional laboratory (Mdn = 2), N = 6, T = 21.0, $P < 0.05$ (two-sided), $r = -0.80$. The item used to measure how "at ease" people were, is asked in an inverse manner when asked in Dutch. Thus, the effect size is negative, and the median in the home setting is lower than the median in the institutional laboratory setting. Women with HSDD showed no differences in the SEQ items between measurement settings.

Table 2 Means and SE of the relative increases from baseline of VPA, CBV, SARSAQ-GR, and SARSAQ-SD

				Controls			HSDD		
Measure	Laboratory setting	Condition	RI epoch	N	Mean	SE	N	Mean	SE
VPA	Institutional	Fantasy	1	8	0.12	0.10	8	0.03	0.06
			4	8	0.30	0.15	8	0.31	0.17
		Foreplay	1	8	0.23	0.17	8	0.20	0.09
			4	8	0.78	0.19	8	0.50	0.16
		Hardcore	1	8	0.45	0.15	8	0.54	0.13
			4	8	0.93	0.23	8	0.45	0.18
	Ambulatory	Fantasy	1	8	0.27	0.08	8	0.07	0.07
			4	8	0.53	0.19	8	0.29	0.14
		Foreplay	1	8	0.68	0.44	8	0.23	0.43
			4	8	1.77	0.82	8	0.60	0.55
		Hardcore	1	8	1.08	0.52	8	0.41	0.51
			4	8	2.18	0.95	8	0.81	0.64
CBV	Institutional	Fantasy	1	8	0.33	0.14	8	0.34	0.21
			4	8	0.92	0.34	8	0.70	0.24
		Foreplay	1	8	0.88	0.21	7*	0.44	0.19
			4	8	1.31	0.33	7	1.12	0.36
		Hardcore	1	8	1.20	0.31	8	1.37	0.60
			4	8	1.58	0.46	8	1.88	0.74
	Ambulatory	Fantasy	1	8	1.06	0.22	8	0.21	0.15
			4	8	1.65	0.38	8	0.27	0.33
		Foreplay	1	8	1.27	0.34	8	0.49	0.30
			4	8	2.55	0.63	8	1.05	0.52
		Hardcore	1	8	2.04	0.64	8	0.45	0.41
			4	8	3.50	0.84	8	0.80	0.47
SARSAQ-GR	Institutional	RI Fantasy		8	0.39	0.19	7*	0.53	0.13
		RI Foreplay		8	0.73	0.21	7	0.92	0.28
		RI Hardcore		8	0.91	0.26	7	0.73	0.26
	Ambulatory	RI Fantasy		8	0.76	0.33	7	0.15	0.07
		RI Foreplay		8	1.07	0.41	7	0.94	0.28
		RI Hardcore		8	1.40	0.55	7	1.18	0.25
SARSAQ-SD	Institutional	RI Fantasy		8	0.32	0.20	7	0.60	0.18
		RI Foreplay		8	0.65	0.15	7	0.93	0.27
		RI Hardcore		8	0.72	0.22	7	0.74	0.26
	Ambulatory	RI Fantasy		8	1.04	0.55	7	0.37	0.22
		RI Foreplay		8	1.50	0.69	7	0.75	0.27
		RI Hardcore		8	1.80	0.80	7	0.98	0.30

* Significant outliers excluded (Z > 3.1). SE = standard error; VPA = vaginal pulse amplitude; CVB = clitoral blood volume; SARSAQ-GR = Sexual Arousal Response Self Assessment Questionnaire-genital responding; SARSAQ-SD = SARSAQ-sexual desire; HSDD = hypoactive sexual desire disorder; RI = relative increase.

Concordance Genital and Subjective Measures

Correlation analysis of the genital and subjective measures of sexual arousal (see Table 3) revealed a positive relationship between VPA and SARSAQ-SD for the healthy controls in the fantasy condition in the institutional laboratory, $r(6) = 0.84$, $P < 0.010$. The HSDD participants showed a positive correlation between VPA and SARSAQ-GR in the hardcore condition in the institutional laboratory, $r(5) = 0.88$, $P < 0.010$. Overall, VPA correlated with SARSAQ-GR, $r(13) = 0.59$, $P < 0.050$, and SARSAQ-SD, $r(13) = 0.73$, $P < 0.010$, in the fantasy condition in the institutional laboratory. Also, VPA correlated with SARSAQ-GR in the hardcore condition in the institutional laboratory, $r(13) = 0.61$, $P < 0.050$. At home, no significant correlations were observed.

Table 3 Pearson's correlations (*r*) between genital (VPA and CBV) and subjective (SARSAQ-GR and SARSAQ-SD) measures of sexual arousal for the control group, the HSDD group, and overall

		Institutional laboratory					
		Control (N=8)		HSDD (N=7)		Overall (N=15)	
		VPA	CBV	VPA	CBV	VPA	CBV
fantasy	SARSAQ-GR	0.67	0.08	0.57	0.42	0.59*	0.16
	SARSAQ-SD	0.84**	0.49	0.75	0.02	0.73**	0.28
foreplay	SARSAQ-GR	-0.11	0.32	0.56	0.57†	0.14	0.42‡
	SARSAQ-SD	0.22	0.66	0.25	0.53†	0.11	0.51‡
hardcore	SARSAQ-GR	0.54	0.18	0.88**	0.31	0.61*	0.23
	SARSAQ-SD	0.62	0.41	0.60	-0.28	0.49	-0.02
		Ambulatory laboratory					
		Control		HSDD		Overall	
		VPA	CBV	VPA	CBV	VPA	CBV
fantasy	SARSAQ-GR	0.36	0.38	0.23	-0.04	0.43	0.46
	SARSAQ-SD	0.32	0.47	0.21	0.17	0.37	0.47
foreplay	SARSAQ-GR	-0.15	0.40	0.74	0.23	0.12	0.34
	SARSAQ-SD	-0.12	0.26	0.35	0.01	0.05	0.27
hardcore	SARSAQ-GR	-0.13	-0.07	0.52	-0.09	0.03	-0.01
	SARSAQ-SD	-0.04	-0.07	0.01	0.11	0.04	0.11

*$P < 0.05$; **$P < 0.01$.
†N = 6, due to a significant outlier in the CBV (see also Table 2); ‡N = 14.
Note: The scores of seven HSDD participants were included in the calculation of the correlations because one participant's SARSAQ scores were mostly significant outliers (see results section).
VPA = vaginal pulse amplitude; CVB = clitoral blood volume; SARSAQ-GR = Sexual Arousal Response Self Assessment Questionnaire-genital responding; SARSAQ-SD = SARSAQ-sexual desire; HSDD = hypoactive sexual desire disorder.

Discussion

The results of this study support our hypothesis that in healthy controls, clitoral and subjective laboratory measures of sexual arousal show stronger increases to erotic stimuli in the home environment than in the environment of the institutional laboratory. This effect was apparent in response to hardcore stimuli, but not to erotic fantasy. Contrary to what we expected, VPA did not increase more strongly at home in the

healthy controls. In the institutional laboratory, women with HSDD and healthy controls did not differ in their genital and subjective response to erotic stimuli. However, the marked increase herein at home as observed in the healthy controls was absent in the women with HSDD. The institutional laboratory setting seemed to initiate or sustain certain inhibitory mechanisms in both groups of women. In healthy controls, but not in women with HSDD, these inhibition-inducing/enhancing factors were less pronounced at home, resulting in increased subjective and genital arousal. To our knowledge, this is the first study that investigates ecological validity of sexual psychophysiological measures by comparing those assessed in the institutional laboratory to those assessed at home with an ambulatory laboratory.

At home, as compared with the institutional laboratory setting, the healthy controls showed more pronounced increases in both genital arousal and the subjective perception of genital arousal in response to erotic stimuli (especially hardcore erotica) compared with neutral stimuli. As expected, they reported "feeling less inhibited" during the home session, as well as "feeling more at ease," as compared with the institutional laboratory setting. These findings are in agreement with the dual control model of sexual functioning [3,4], which states that decreased inhibition leads to increased sexual arousal. We also observed a decrease in preconscious attentional bias for erotic words at home.

The stronger increase in genital arousal at home in comparison with the institutional setting was especially apparent in the CBV response; the stronger VPA increase pointed in the same direction but did not reach statistical significance. In a previous study [12] we showed that participants' CBV decreased dramatically following an inhibitory stimulus—a sudden warning over the intercom—contrary to their VPA. The strength of the participants' CBV decline was related to the strength of the sympathetic response to the inhibitory stimulus. This was not true for VPA. It would thus appear that CBV is the more sensitive measure for sexual inhibition than VPA. Stronger influence of inhibitory stimuli on CBV may be caused by a stronger influence of sympathetic activity on smooth muscle contraction in the clitoral tissue complex as compared with the arterial plexus of the vaginal wall (see Gerritsen et al. [12] for a discussion). The decrease in the reported feelings of inhibition and the stronger increase of CBV compared with the slight increase of VPA in the home measurement setting, supports the idea that there are less inhibitory influences at home as compared with the institutional laboratory.

The second major finding of this study was that the genital arousal to erotic stimuli of women with HSDD and of healthy controls only differed from each other in the ambulatory laboratory setting. Many studies have compared participants with different FSD diagnoses to healthy controls in an institutional laboratory, but consistent differences in genital arousal between these groups have not been found [17,26,27]. In the present study, both women with and without HSDD did not show different responses in the institutional laboratory either, however, a clear difference was found at

home. Unlike the healthy controls, women with HSDD did not show more genital responding at home as compared with the institutional laboratory setting. This may be (partially) caused by a less sensitive/responsive sexual excitation system in women with HSDD. The comparable levels in preconscious attentional bias for sexual cues between both groups of women in both settings challenges this, but it has never been established that preconscious attentional bias for erotic cues is directly linked to sensitivity/responsivity of the sexual excitation system. Also, the women with HSDD did not report feeling significantly less inhibited or feeling significantly more at ease at home either. Cues that activate or sustain inhibitory mechanisms still may have been present for these women, at least more so than in controls. It is possible that these women were influenced by other inhibitory stimuli in the home setting, e.g., context dependent cues [34,35] that could induce negative memories of past—e.g., bedroom experiences. Such inhibitory stimuli may have a different influence on subjective experience, seeing that these women reported increased subjective perception of genital arousal while genital arousal itself did not increase. It seems less likely that distraction accounted for these findings because preconscious attentional bias was comparable with controls in both settings. Further research is necessary to elucidate these findings.

Concordance between genital and subjective measures of sexual arousal was observed in some conditions in the institutional laboratory but not in the home setting. We expected that creating a more appropriate context for genital and subjective sexual responding by measuring in the home setting would on one hand increase genital and subjective sexual responding, and on the other hand increase concordance between these measures. However, for most participants in the home setting, a strong increase in the subjective measures was accompanied by relatively smaller increases in physiological measures or vice versa. It is possible that this finding reflects less interdependence between genital and subjective sexual arousal in women, as compared with men (see Suschinsky et al. [36] and Chivers [37] for a discussion on this matter). Inspection of the scatter diagrams showed that the significant correlations in the institutional laboratory were partly the result of floor effects in the subjective and genital measures of sexual arousal, thus inflating correlations. These floor effects may in part account for the unexpected observed differences in concordance magnitude between the two measurement settings. According to Basson's model of female sexual response [38], sexual stimuli must have an appropriate context for sexual arousal to occur. An appropriate setting (e.g., home) forms a part of this appropriate context, as demonstrated by the present findings of increased genital and subjective arousal. It has been proposed that women's subjective arousal—in contrast to men's—is more dependent on the meaning that a sexual stimulus generates [39]. Increasing the appropriateness of the context by altering the meaning of sexual stimuli traditionally presented in a psychophysiological lab (e.g., erotic film clips) could result in more concordance. The present experimental procedure may be complemented by instructions from a trained psychologist/sexologist, focusing on how to fantasize or how to experience bodily responses during viewing of an erotic film clip. It must be kept in

mind that within-subject correlations could not be computed because a continuous measure of subjective arousal (e.g., a potentiometer during film clip viewing) was not used. Between-subject correlation is a less sensitive measure of concordance. Also, the low sample size renders these observations less reliable. Further research in the home setting is necessary to investigate these findings.

Attentional bias in the form of sexual content induced delay [40] seems to be dependent on magnitude of arousal induced by the sexual stimulus and independent of stimulus valence; both positive and negative stimuli can induce a delay, and the length of the delay is dependent upon the strength of the arousal [41]. In the current study, both groups of women showed preconscious attentional bias for erotic stimuli in the institutional laboratory, but not at home. Reaction times to neutral stimuli were larger at home compared with the institutional laboratory, while the reaction times to erotic stimuli did not differ between measurement settings. The surroundings of an experimental chamber in an institutional laboratory are purposely made stimulus-deprived to avoid distraction from the experimental stimuli. The home environment does not match the institutional laboratory environment in this respect. Possibly, the home environment is too distracting to measure subtle differences in Stroop reaction times. If this prolongation of reaction times to neutral stimuli at home is caused by the more distracting surroundings, a ceiling effect herein would explain why this was not observed for the erotic stimuli. Additional research is needed to test these hypotheses.

This study has several limitations. All of our participants were naive to the home laboratory setting, but not to the institutional laboratory setting. This may have influenced the results, but it cannot explain the differential results of the two subgroups. Other confounding factors are likely to have been present in the home measurement setting; e.g., environmental noise or the presence of children or a spouse in the house. These factors are unlikely to be systematic, reducing their influence on the (direction of our) results. Nevertheless, such unsystematic bias would be less influential with more participants. We did not control for phases of the menstrual cycle other than the menstruation phase, when our participants were not tested. Sexual experience has been shown to vary over the phases of the menstrual cycle (see Bullivant et al. [42] for an overview), which could have influenced the findings presented here. Finally, more direct and objective investigation of inhibitory mechanisms is recommended using biological markers of psychological stress (e.g., saliva cortisol or electrodermal activity) because simply asking people whether they feel “at ease” at home may be susceptible to social desirable answering [43].

This study was also a methodological exercise to look into the feasibility of using an ambulatory laboratory and real time plural data transmission as a tool for the study of variables that are influenced by their ecological surroundings; e.g., translational research. To ensure that the measurement at home is optimal, subjects had to be trained in the institution. The duration of the training was mostly dependent on the computer

skills of the subject, but a lack of computer skills did not give rise to serious problems. None of the subjects reported having any problems or difficulties in running the ambulatory lab. Movement or other measurement artifacts were comparable as in the institutional laboratory. There were more movement artifacts in the VPA at home, but this difference was attributable to two subjects. The number of outliers in the Stroop task was smaller at home. Data transmission was without problem. Both the method of data collection, as well as the results of this study warrants further investigation of this methodology. As a next step, we will investigate the influence of different pharmacological treatments for FSD on the psychological and physiological sexual response at home.

Conclusion

The ambulatory laboratory is a valuable tool that allows researchers to perform psychophysiological (sex) investigations under naturally occurring conditions (e.g., a participant's home). In the present study, the increase in ecological validity resulted in a qualitative differentiation between the healthy controls and women with HSDD in the home setting, which is not apparent in the artificial setting of the institutional laboratory.

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CHAPTER 5

Toward Personalized Sexual Medicine (Part 1): Integrating the "DualControl Model" into differential drug treatments for HSDD and FSAD

Journal of Sexual Medicine 2013;10:791-809

Abstract

In three related manuscripts we describe our drug development program for the treatment of Hypoactive Sexual Desire Disorder (HSDD). In this first theoretical article we will defend the hypothesis that different causal mechanisms are responsible for the emergence of HSDD: low sexual desire in women (with HSDD) could be due to either a relative insensitive brain system for sexual cues *or* to enhanced activity of sexual inhibitory mechanisms. This distinction in etiological background was taken into account when designing and developing new pharmacotherapies for this disorder.

Irrespective of circulating plasma levels of testosterone, administration of sublingual 0.5 mg testosterone increases the sensitivity of the brain to sexual cues. The effects of an increase in sexual sensitivity of the brain depend on the motivational state of an individual. It might activate sexual excitatory mechanisms in low sensitive women, while it could evoke (or strengthen) sexual inhibitory mechanisms in women prone to sexual inhibition. Sexual stimulation in the brain is necessary for phosphodiesterase type 5 inhibitor (PDE5i)-mediated increase in genital sexual response. Accordingly, a single dose of T+PDE5i might enhance sexual responsiveness, especially in women with low sensitivity to sexual cues. In other women sexual stimulation might elicit a prefrontal cortex (PFC)-mediated phasic increase in sexual inhibition, in which activity of 5-hydroxytryptamine (5-HT, serotonin) is involved. We hypothesize that a single dose of 5-hydroxytryptamine$_{1A}$ receptor agonist (5-HT$_{1A}$ra) will reduce the sexual-stimulation-induced PFC-mediated sexual inhibition during a short period after administration. Consequently, treatment with T+5-HT$_{1A}$ra will be more effective, in particular in women exhibiting sexual inhibition.

Based on the results of our efficacy studies described in parts 2 and 3 of the series, we conclude that tailoring on-demand therapeutics to different underlying etiologies might be a useful approach to treat common symptoms in subgroups of women with HSDD.

Introduction

Human sexual behavior has been extensively studied in biology and psychology, but so far there is very limited success in the development of drugs for treatment of sexual dysfunction in women. Low sexual desire, with or without sexual arousal problems, is the most common sex-related complaint reported by women [1–3]. As a result, many women suffer from sexual dissatisfaction, which often negatively interferes with psychological well-being [4]. This has been classified as a clinical condition, referred to as Hypoactive Sexual Desire Disorder (HSDD) [5].

To date, no medication for this condition has been approved by the U.S. Food and Drug Administration (U.S. FDA). In the present article we will describe our hypothesis that different causal mechanisms are responsible for the lack of sexual desire in women, which was taken into account when designing and developing two new medicines for HSDD. In particular, in some women with HSDD, sexual dysfunction results from a relative insensitivity for sexual cues, whereas in others, sexual complaints result from dysfunctional automatic activation of sexual inhibitory mechanisms during exposure to sexual stimulation. One drug has been developed for a subgroup of patients in which sexual dysfunction occurs as the result of a relatively insensitive system for sexual cues. The other drug has been developed for women in which the sexual complaints result from (dysfunctional) activation of sexual inhibitory mechanisms.

The efficacy of both drugs is based on a delay in effect of sublingual testosterone. That does not mean that low sexual desire can be attributed to an absolute deficiency of testosterone. There are other intervening biological and psychological variables influencing the sensitivity of the brain for sexual stimuli and mediating the effects of treatment with testosterone. In this first theoretical part we will try to clarify these assertions and our assumptions. The second and third parts of this trilogy are empirical studies, in which we will describe the results on the efficacy of both proposed treatments in two subgroups of women with HSDD, in which the complaints have a different etiological origin.

Administration of one single dose of 0.5 mg sublingual testosterone increases, with a delay in effect of about 4 hours, the sensitivity of the brain to cues associated with behavior driven by social interaction, including sexual behavior [6–8]. This effect on the brain's sensitivity to sexual cues affects mechanisms involved in sexual motivation, and has divergent effects in the proposed subgroups of women. A single-dose sublingual testosterone might increase central sexual stimulation, a necessary condition for the efficacy of a phosphodiesterase type-5 (PDE5) inhibitor on physiological sexual responding. Sublingual testosterone combined with a PDE5 inhibitor (dosed in such a manner that the time of the peak plasma concentration of the PDE5 inhibitor coincides with the 4-hour delay in behavioral effects of the testosterone) will enhance sexual responsiveness, especially in women with low sensitivity for sex [9,10]. On the other

hand, in women already prone to sexual inhibition, an increase in central sexual stimulation might elicit a phasic prefrontal cortex (PFC)-mediated serotonin-dependent increased response in sexual inhibition. A single dose of a 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor agonist will reduce the sexual stimulation-induced PFC-mediated sexual inhibition during a short period after intake. Consequently, treatment with sublingual testosterone and 5-HT$_{1A}$ receptor agonist (dosed in such a manner that the pharmacological effects of the 5-HT$_{1A}$ receptor agonist coincide with the behavioral window induced by the testosterone administration) will be more effective, in particular in women prone to sexual inhibition.

Multifactorial Determination of Human Sexual Behavior

A range of biological and psychological regulatory mechanisms control human social and sexual functions. However, the degree of activation of these mechanisms (i.e., their relative strength) varies within and between subjects. One possible reason for the limited success in developing drug treatments for HSDD could be the lack of consideration of multiple interacting biological *and* psychological mechanisms in the existence of individual differences in sexual (dys)function. In humans, a range of complex social, cognitive, and affective processes mediate the transition from a preparatory sexual motivational state to actual sexual behavior. These conscious and subconscious processes are partly the result of present and previous beneficial and/or adverse experiences and significantly influence the extent to which an individual is willing to engage in sexual activities and the extent to which those activities are enjoyable. Therefore, the delicate and relative balance between activation of central mechanisms governing sexual excitation and sexual inhibition controls sexual activities in humans, including fantasizing, masturbation, and sexual intercourse.

Human sexual behavior occurs in the context of social relationships. These social relationships themselves are influenced by (or can influence) biological (e.g., testosterone) and psychological variables. The relationship between endogenous or exogenous induced testosterone levels and social behavior is subject of extensive research. Here we will start to discuss some of the established relationships between this steroid and social behavior and highlight the reciprocal nature of this relationship and the implications this has on sexual behavior. In other words, we will discuss the influence of testosterone on social behavior in the context of the interrelationship between social and sexual behavior.

On the Physiological Control of Social and Sexual Behavior

In many mammalian species, female sex steroids are essential for the expression of female sexual behavior. As a result, the capability for copulation in these animals is limited to the period of ovulation [11,12]. In higher primates, like humans, sexual intercourse is not limited to the peri-ovulatory period, and it has been suggested that

testosterone plays an important role in sociosexual behavior of women [13]. This is supported by the fact that the depletion of testosterone in women following ovariectomy and adrenalectomy is accompanied by a complete loss of libido [14], while substitution of testosterone restores sexual desire and fantasies after surgical menopause [15]. Because testosterone plays a central role in the steroid-responsive neural network of human (males and) females, testosterone will influence the sensitivity of the brain for social and sexual cues in both sexes.

Although the testosterone production in adult men is about 10 to 15 times higher than in women, it is believed that women are more sensitive to this steroid [16]. Despite the large differences in circulating testosterone levels between the sexes, it has been shown that testosterone has similar effects on behavior in men and women [17]. Thus, conclusions about the influence of testosterone on behavior in men are generalizable to women. Testosterone has been associated with behavior driven by social interaction, in particular competitive and sexual behavior. Competitive behaviors are linked to the personality trait "dominance." This trait refers to the motivated behavior through which high status within a social hierarchy will be attained and maintained, and is partly determined by the production of endogenous testosterone [17,18].

For a long time it has been assumed that testosterone also encourages competition between males to attract attention of sexually attractive females. Dominance is often used by males in competition with others to get reproductive advantage [19,20]. They do so by using direct (e.g., self-promotion) and indirect (e.g., derogation of the competitors) dominance strategies. In animal studies it is often shown that testosterone is involved in various behaviors related to competition to get access to the opposite sex, including increased sexual motivation, territorial demarcation, mate guarding, increased aggression, and display behavior [21–23]. In human males it has recently been shown that precompetitive testosterone levels were positively associated with dominance behavior during a competition experiment, in which men had to compete with other men to attract attention of an attractive female confederate. High testosterone levels were associated with higher assertiveness and the degree of control these men took during an interview setting with these women, and the extent to which women had "felt a click" with these men. These effects of testosterone on the behavior of dominant men were especially pronounced in those who also reported a strong need for social dominance. Moreover, men with more testosterone and a high need for social dominance were found to be able to suppress the competitor's ability to also attract potential partners. In contrast, among the men who had indicated that they had little or no need for social dominance, there was neither a correlation between their testosterone levels and their dominant behaviors, nor in their perception of the behaviors of their competitors [21]. These findings emphasize an important difference between humans and animals. In humans—in contrast to animals—explicit and conscious motives influence the effects of testosterone on behavior.

Thus testosterone promotes approach behavior focused on dominance and status especially to get access to sexual rewards. Further evidence for this comes from a functional magnetic resonance imaging (fMRI) study, in which it was demonstrated that in anticipation of a reward, treatment with testosterone enhanced the activity of neural pathways involved in reward seeking [24]. However, not only does testosterone influence social behavior, but a reciprocal relationship also exists between social behavior and testosterone levels. In rhesus monkeys it has been demonstrated that an increase in social status induces an increase in testosterone [25,26], whereas a loss of social status is accompanied by a decrease in testosterone [25,26], which was also found in men [27,28]. Moreover, increases and decreases in testosterone levels in response to mating cues also influence men's mating behavior in a reciprocal fashion. Testosterone levels in men rise when they interact with female confederates in the lab, and the degree of increase of these testosterone levels correlates with the confederates' experience of how much they felt the men tried to impress them [29]. Furthermore, testosterone levels of men also increase during exposure to sexually explicit movies as compared with neutral ones [30]. In women it has also been demonstrated that salivary testosterone levels rise in anticipation of sexual intercourse compared with anticipation of nonsexual activities. These levels stayed higher up until 15 minutes after the intercourse [31]. Also, Hamilton and Meston [32] studied women in long-distance relationship and found that testosterone increases the day before women were reunited with their partners after a separation. These results seem to support the hypothesis that testosterone increases in anticipation of sexual activity. A recent study of Goldey and Van Anders [33] showed that sexual thoughts in women can also influence testosterone levels. These studies employed salivary testosterone measures, and it should be noted that salivary testosterone measurements as compared with serum testosterone levels may underestimate testosterone–behavior correlations in women [34].

Concluding, testosterone not only influences social behavior, but socially challenging stimuli and social behavior can also induce increases and decreases of testosterone levels, which can be interpreted as a functional adaptation of the action of this hormone to changing circumstances. Although many factors and circumstances elicit fluctuations in testosterone levels, the basal level of this steroid appears to be relatively constant over longer periods [35]. As a result, many researchers consider these basal levels as a trait-level factor. The increases and decreases elicited in these testosterone levels may be considered as transient states, which are also trait-dependent in size, and which are superimposed on the baseline trait levels.

Sex Steroids in Animals and Humans: Sensitivity for Sexual Cues

The idea of the existence of individual differences in sensitivity of the brain for sexual cues is an important concept for our hypothesis that different causal mechanisms are responsible for HSDD, and that sublingual testosterone can increase this sensitivity with different effects in the differentiated subgroups [10].

In animal experiments it has been shown that sex hormones affect sexual behavior, through alterations in functions and activity of the brain [36]. In the development of animal models, scientists have focused on the relationship between neurophysiological brain mechanisms and several indices of sexual behavior. Based on these experiments, a steroid-responsive neural network has been postulated, a highly interconnected group of sex hormone receptor-containing neurons in the brain [36]. According to the authors this network is not a closed circuit, but serves reproductive aims by functioning as an integrating and activating center between external sensory cues, hormonal processes, and reproductive behavior. This is partly accomplished by selective filtering of sensory input and amplification of signals that may facilitate sexual behavior. In human sex research ethical and practical barriers exist for observing sexual behavior directly, and of assessing the underlying neurophysiological brain processes in a similar fashion as in animal research. Although models of human and animal sexuality are to a large extent incomparable, they might bear in common that the influence of sex hormones operate through a steroid-responsive network which can vary in sensitivity.

Two Paradigms in Testosterone Treatment and Sex Research

There are two main research paradigms used for investigating the effects of treatments with testosterone on sexual behavior: (i) chronic increases of testosterone levels through continuous administration; and (ii) short transient increases of testosterone through single-dose sublingual administration. We will give a short description of the differences between both approaches.

Chronic Testosterone Treatment and Sexual Behavior in Women

Chronic testosterone preparations affecting sexual functioning of women have been prescribed by physicians for surgically and naturally postmenopausal women for decades. Clinical trials documenting positive effects of androgens on sexual desire and sexual responsiveness indicated a significant positive effect of testosterone on women's sexual function [15,37–41].

It is well known that during the natural menopause, testosterone levels decrease very gradually. However, this is more likely a result of declining ovarian and adrenal function due to aging than a function of natural menopause. In contrast, women who undergo bilateral oophorectomy (surgical menopause) experience a more rapid dramatic decline in testosterone production with levels that are 50% lower than they are for natural menopause. Since aging and menopause have been linked to low libido or HSDD, the decline in androgen levels in women with a natural menopause or following oophorectomy is supportive for the hypothesis that decreased testosterone is related to reduced sexual desire and/or satisfaction.

In the 1980s, the first experiments were conducted to support this hypothesis. Sherwin et al. examined the effect of intramuscular testosterone on sexual function in 53

surgically postmenopausal women. The subjects were randomized to testosterone alone, estrogen alone, testosterone (T) combined with estrogen (E) or no treatment. Participants in the T + E group showed significant higher rates of sexual desire, arousal, and sexual fantasy compared with E alone or the untreated group [15]. Since then, others reported increased desire and other parameters of sexual well-being in postmenopausal women receiving oral methyltestosterone in combination with esterified estrogens [42], and subcutaneous testosterone implants [43].

Considering the significant effect of testosterone therapy on sexual functioning in postmenopausal women, further studies explored the most efficacious route of administration for testosterone. Transdermal administration has the advantage of avoiding first-pass hepatic metabolism, of delivering consistent and reliable doses of testosterone, and it is less invasive than intramuscular administration. Shifren et al. conducted the first randomized, double-blind, placebo-controlled trial of a transdermal testosterone patch in 75 women who had undergone surgical menopause. The participants were randomized to receive a 150 μg/day testosterone patch, 300 μg/day testosterone patch or placebo for 12 weeks. Only the highest dose group showed significant higher scores for frequency of sexual activity, sexual fantasies, masturbation, and orgasm j[39]. Since then, several studies reported beneficial effects of the transdermal testosterone patch in surgically menopausal women [38,40] and in naturally menopausal women [44]. Also, others studies have demonstrated that administration of transdermal testosterone gel in surgical and natural menopausal women increases sexual desire and frequency of sex [45,46].

In premenopausal women only a few studies have been performed on chronic testosterone treatment for HSDD as compared with the large database of studies for postmenopausal women. Davis et al. conducted a study in 261 premenopausal women with decreased sexual satisfaction who received one of three different daily doses of transdermal testosterone spray (50 μg testosterone per μL: one 56-μL spray, one 90-μL spray, and two 90-μL sprays) or placebo for 16 weeks. Only the treatment group on the single 90-μL dose showed a significant increase in satisfactory sexual events as compared with placebo [47].

The above described studies show promising results for testosterone as treatment for women with HSDD. However, chronic testosterone treatment is accompanied with adverse events due to a long-lasting higher androgen status. Transdermal testosterone preparations result in physiologic serum testosterone levels and the most common side effects reported are increased hair growth and acne [47,48]. When supraphysiological testosterone levels are reached, cardiovascular adverse events have been described [49]. Low-dose testosterone is safe in postmenopausal women with regard to endometrial effects and breast cancer risk [50–53]. However, safety studies in premenopausal women are sparse, and long-term safety studies regarding exogenous testosterone preparations are necessary. At this moment there is no complete long-term

safety profile for a testosterone-based product in premenopausal women and therefore the U.S. FDA has not yet approved any for treatment in women with HSDD.

Sublingual Single Dose Administration of Testosterone

An important aspect of sexual motivation is physiological sexual responding. An increase in vaginal vasocongestion (as measured by vaginal pulse amplitude [VPA]) elicited by sexual stimuli is considered to be preparatory for copulatory behavior [54]. Increases in VPA are consistently observed during exposure to erotic stimuli. In eight hypogonadotropic hypogonadal females we found that substitution with testosterone undecanoate 40 mg orally per day during an 8-week period enhanced the VPA during exposure to erotic visual stimuli [54]. This effect was not found in another group of eight women with panhypopituitarism (also hypogonadotropic hypogonadal) (unpublished data). In both studies subjects received testosterone each morning, but patients in the first experiment were tested during the afternoon and patients in the second experiment during the morning. The different outcomes on physiological responding between these experiments may be caused by the difference in patient population, but also by a delay in effect of testosterone on physiological sexual responding. In a third experiment, we examined if administration of a single dosage of sublingual testosterone (0.5 mg), as compared with a placebo, increases the VPA during exposure to visual erotic stimuli as compared with neutral stimuli [6]. On the 2 treatment days, eight sexually functional women viewed a neutral and erotic film clip 25 minutes before drug intake, and at five time points after drug intake (at $t = 0$, 90, 180, 270, and 360 minutes). The intake of testosterone caused a sharp increase in plasma levels of testosterone of short duration. About 3.5 hours after this testosterone peak, we found a striking increase in VPA and subjective sexual experiences (self-report ratings of sexual lust and genital sensations) when the subjects were exposed to the visual sexual stimuli. These findings indeed demonstrated a time lag in the effect of sublingually administered testosterone on genital arousal in sexually functional women. A subsequent study was designed to investigate the influence of repeated measurements on the physiological and subjective sexual responses. The same experimental design was used as in the former study, but with only two measurement points: at $t = -25$ minutes and $t = 270$ minutes. The same results were obtained for the physiological sexual response in 10 sexually functional women. However, the subjective indices of sexual arousal showed no significant increase. Repeated exposure to sexual stimuli seemed to be an important condition for the subjective arousal to also increase under sublingual testosterone [7]. This delay in effect of testosterone on sexual arousal was replicated in 10 sexually functional postmenopausal women [8], and for other cognitive and affective functions [8,55–64].

It is not fully understood which exact mechanism is responsible for this delay in behavioral effect, it could be that testosterone exerts its effect via an increase in free levels of T, androgenic metabolites (e.g., 5α-dihydrotestosterone [DHT], androsterone glucoronide), via genomic or non-genomic mechanisms or a combination of these factors

[65]. There is some debate as to the importance of testosterone aromatization to estradiol in determining testosterone effects in human sexual behavior [66–68]. However, the delay in effect of about 4 hours of sublingual testosterone on behavior cannot be explained by the process of aromatization of testosterone to estradiol. In animal experiments it is well documented that the time course of aromatization of testosterone to estradiol starts about 16 hours after the increase in testosterone and reaches its maximum level after about 48 hours. In these experiments it has been demonstrated that activation of copulatory behavior follows a similar time course, and occurs with a delay of 24–48 hours after the induced increase of testosterone [69,70]. So, it is not plausible that aromatization to estradiol accounts for the at least 20 replications of the 4-hour delay in effect of sublingual testosterone. Direct evidence that testosterone's effect on sexual motivation is not due to aromatization to estradiol comes from Davis et al. [71]. In this study, it was evaluated if the effects of transdermal testosterone in postmenopausal women is the direct effect of testosterone itself or indirectly influenced by the aromatization of testosterone by aromatase. Sixty postmenopausal women using transdermal estrogen therapy with low sexual satisfaction were treated with transdermal testosterone gel and randomly assigned to receive treatment with the aromatase inhibitor letrozole, or placebo. It was demonstrated that treatment with testosterone, which induced an increase in the total and free testosterone levels, was associated with improved sexual satisfaction, mood, and well-being in these women. These increases were not due to aromatization to estradiol because testosterone administration with simultaneous treatment with an aromatase inhibitor did not have any effect on these outcomes. Further studies are necessary to investigate what then causes this delay in effect of testosterone.

During chronic administration of testosterone the behavioral effects occur often only after several weeks of treatment. This time-effect interval is in stark contrast to the time lag of about 3 to 4 hours induced by the sublingual dosage form. This difference in time on effects may be partially caused by the differences in the pharmacokinetic profile of both treatment forms.

Chronic vs. Sublingual Administration of Testosterone

Only few pharmacokinetic studies have been performed with sublingual testosterone administration, and mostly in hypogonadal men [72,73]. Besides the different influence of chronic and single-dose administration of testosterone on sexual behavior, pharmacokinetic differences exist between these administration methods. According to Salehian et al. [73], investigating the pharmacokinetic profiles of testosterone enanthate (intramuscular 200 mg) and two doses of sublingual testosterone (2.5 and 5.0 mg) in hypogonadal men, free and total levels of testosterone increased much faster in the sublingual group compared with the group receiving the testosterone enanthate. Only two pharmacokinetic studies of sublingual testosterone administration in women have been described [6,74]. These two studies demonstrated that after administration of

0.5 mg sublingual testosterone, peak concentrations of serum testosterone were reached at 15 minutes post dose, and that baseline levels were reached within 150 minutes. The time to reach maximum concentrations is consistent with that found in men [73].

It is widely accepted that the unbound fraction of testosterone is the most bioactive and therefore responsible for the effects on behavior. If free fraction testosterone levels increase much later in the chronic administration method it is expected that pharmacodynamic effect, e.g., on sexual behavior, will also occur in such a time-dependent manner. This is consistent with several studies describing the effects of a testosterone patch on sexual behavior. Women who started with the testosterone patch treatment reported improvements in sexual functioning over placebo after 4–8 weeks [75]. This is in sharp contrast with the series of sublingual testosterone (0.5 mg) experiments, demonstrating a delay in effect of about 4 hours after the peak in circulating testosterone [6–9,55–64].

Free Fraction and Sex Hormone Binding Globulin (SHBG) Saturation Threshold Mechanism

As mentioned earlier, chronic administration of testosterone causes a slow increase of total and free testosterone levels, and therefore a slow increase of the free fraction, in contrast to the rapid increase of these parameters after sublingual administration. The free levels and free fraction of testosterone is partly dependent upon circulating SHBG levels. In the study of Salehian et al. [73], it was shown that free testosterone levels in the testosterone enanthate condition only increased when SHBG levels were suppressed after administration by day 7. Apparently, chronic treatment of testosterone induces a rise in free fraction levels, after the installment of a new equilibrium between testosterone and SHBG. Moreover, there is some evidence that a rise in free fraction levels following sublingual testosterone administration depends on circulating SHBG levels [74]. In the latter study it was demonstrated that the free fraction levels increased in a dose-dependent fashion (after 0.25 mg, 0.50 mg, and 0.75 mg of sublingual testosterone), but only in subjects with relatively low SHBG levels. In the group of women with high SHBG levels, no increase of free fraction levels was observed after sublingual testosterone administration. This observation is partly consistent with the SHBG saturation threshold hypothesis postulated by van der Made et al. [10]. According to this hypothesis, the free fraction of testosterone only increases after saturation of SHBG. However, van der Made et al. state that when testosterone levels are high enough to pass this saturation threshold, this will result in a short supraphysiological peak of free testosterone, and subsequently induce behavioral effects after approximately 4 hours. This is not consistent with the results described by van Rooij et al. Besides the fact that free fraction levels only increased in women with low SHBG, which is in line with the saturation part of the hypothesis, increases in the free testosterone levels were also observed in the women with high SHBG. However, maximum concentrations of free

testosterone in the women with low SHBG were significantly higher compared with the women with high SHBG. Apparently, rapid influx of testosterone does induce a peak in free and total testosterone irrespective of SHBG level. See also Figure 1.

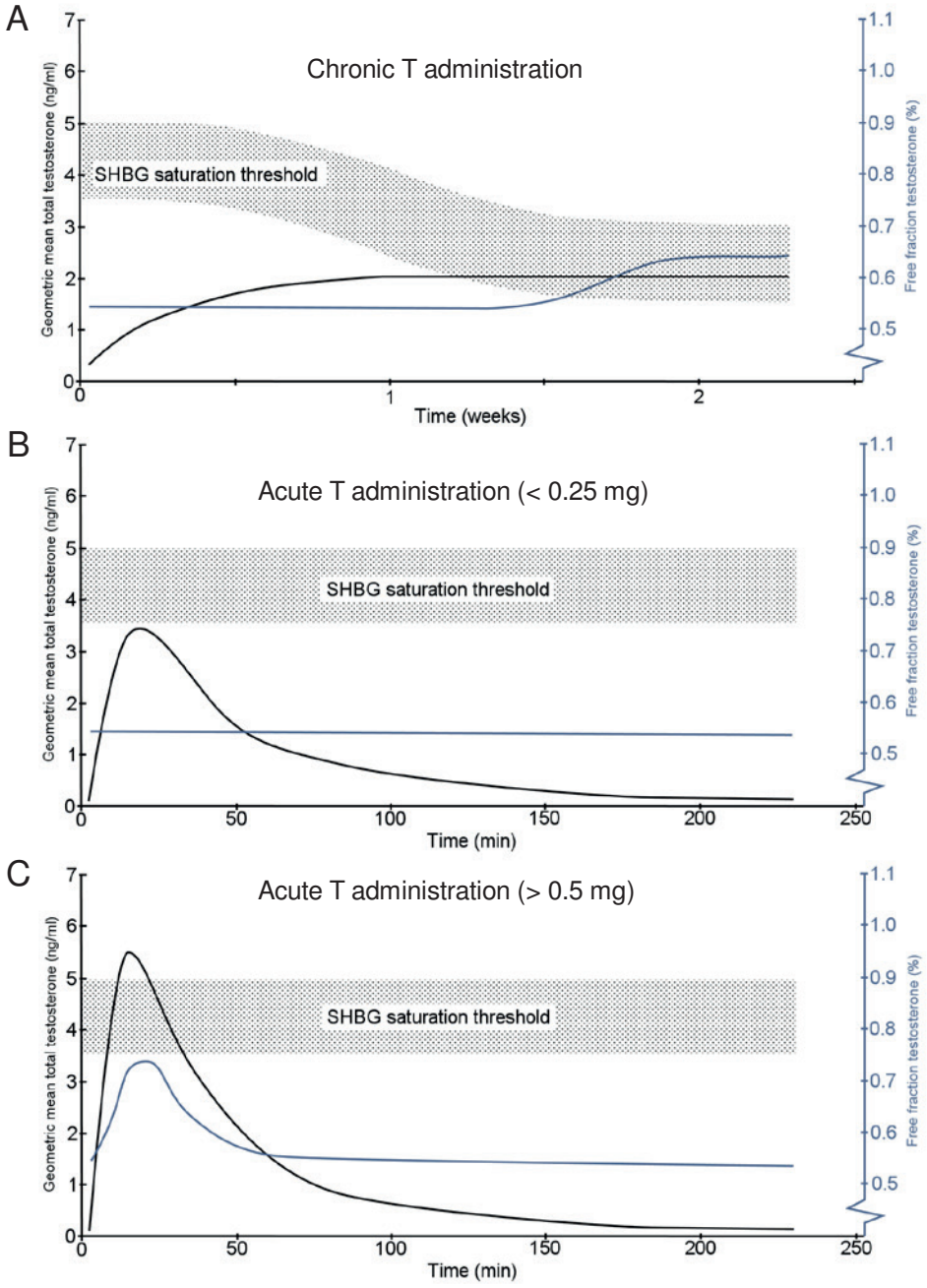

Figure 1 Free fraction testosterone and the Sex Hormone Binding Globulin (SHBG) saturation hypothesis. (A) A slow increase in testosterone levels following chronic administration. This decreases SHBG after several days—weeks, and thereby also the SHBG saturation threshold. Once this threshold is lowered, the free fraction increases and behavioral effects become manifest. (B) A fast transient increase in testosterone levels following acute sublingual administration of doses too low to reach SHBG saturation, thus not influencing the free fraction. (C) A fast transient increase in testosterone levels following acute sublingual administration of doses that are high enough to reach SHBG saturation, thereby also transiently increasing the free fraction. SHBG saturation threshold is depicted in bandwidth to indicate that this threshold is depends on a person's endogenous SHBG level. Testosterone levels are shown as baseline corrected levels. (A) A hypothetical figure adapted from several studies reporting pharmacokinetic and efficacy measures of chronic testosterone administration (see text). (B) and (C) Adapted from van Rooij et al. [74].

It is unclear at this point, whether the increase of free testosterone levels or the free fraction is responsible for the induced behavioral effects of the sublingual testosterone dosage form. Future studies specifically designed for this purpose are needed to clarify the influence of free testosterone and free fraction levels on behavior.

Automatic Responses and Cognitive Factors in Sexual Functioning of Women

Models of human sexuality are mainly based on subjective reports, sometimes combined with manifestations of physiological sexual arousal. An increase in vaginal vasocongestion induced by sexual stimuli results from activation of neural pathways involved in reward-seeking and associated areas. Visual exposure to sexual intercourse between members of the species of the onlooker is a potent releasing stimulus for such a preparatory motivational response. Both men and women have a marked capacity to respond to erotic films with a genital response, irrespective of whether they found such exposure pleasant or not [76,77]. Discordance between genital responses and subjective sexual functioning, as monitored in the laboratory, has been frequently reported [54,78]. As a result, an increase in vaginal responding has been considered as an automatic preparatory physiological response for copulatory behavior [54].

As stated above, the capability of sexual cues to evoke an increase or decrease in the sexual motivational brain activity depends on individual biological and psychological characteristics and social circumstances, such as the sensitivity of the steroid receptor system (e.g., polymorphism of the androgen receptor [79]), prenatal testosterone exposure influencing organization of the sexual brain (measured by the second to fourth digit ratio [80]), levels of sex steroids, and environmental factors, e.g., the proximity of an attractive mate. Moreover, the transition of preparatory motivational brain activity to the execution of copulatory behavior is, at least in humans, mediated by higher cortical processes (which can feedback on the motivational systems involved in reward-seeking). Aside from the induction of a preparatory physiological response during conscious perception of erotic stimuli, it has been shown that subliminally presented

erotic stimuli can activate the limbic reward circuitry involved in sexual motivation [81]. Thus, even erotic cues which do not enter conscious awareness induce alterations in (sexual) reward-related motivational brain areas. A sexual cue belongs to the domain of stimuli—e.g., an odor, a thought, a memory, a word, a sound, a picture, flirtatious body language, or someone's physical appearance, etc.—which can elicit sexual responses in the brain or body. Individual differences in sexual-cue sensitivity might be reflected in differences in responses induced by exposure to subliminal sexual cues. One method to investigate this is by the measurement of preconscious attentional bias for sexual cues, using an emotional Stroop task. The emotional Stroop task measures attentional bias for emotional cues [9,10]. In this task, subjects are instructed to name the color in which emotional and neutral stimuli are printed as quickly as possible, while ignoring the meaning of the word. The motivational state of the subject and the emotional content of the stimuli determine the performance on this task, in terms of slowing down or speeding up in color naming. Attentional bias for emotional cues is demonstrated when color-naming latencies for emotional stimuli are greater or smaller than color-naming latencies for neutral stimuli. It has been assumed that attention is automatically allocated to the emotional value of the stimuli. In the context of sexual function, with another cognitive task it has been demonstrated that low levels of attention for sexual stimuli is associated with low sexual desire [82].

A masked version of this task turned out to be a more reliable measurement of (preconscious) attentional bias for emotional cues [9,10]. Thus, deceleration in color-naming of masks, preceded by sexual words and as compared with neutral words, indicates increased resource allocation to the processing of sexual stimuli, while acceleration indicates relatively decreased resource allocation to the processing of sexual stimuli as compared with neutral ones. In the masked version of this task measuring preconscious attentional bias for sexual cues words with erotic content (“orgasm”) and words without erotic content (“chair”) are presented for 26 milliseconds on a computer screen in different print colors. Directly after presentation, the word is masked (i.e., covered) by a scrambled letters in the same print color. This method of presentation ensures that the target word does not enter the subject's conscious awareness. The subject is instructed to name the print color of the word mask as fast as possible. Thus, deceleration in color-naming of masks which were preceded by erotic words (as compared with neutral words) indicates increased preconscious attentional resource allocation to the processing of sexual cues. In other words, subconscious attentive processing capacity is automatically allocated to the emotional value of the cues [83]. Differences in this preconscious attentional bias between subjects then indicate differences in sexual cue sensitivity, and thus differences in the strength with which these subjects respond sexually to sexual stimuli as the examples mentioned above. The sexual response can be anything ranging from facilitation or activation of brain motivational systems to full blown sexual arousal and desire, depending on the sensitivity of the brain for sex and on stimulus intensity.

Sexual behavior in humans depends on a range of interacting biological and psychological regulatory mechanisms, which are influenced by situational cues. The absolute and relative degree of activation of these mechanisms varies within and between subjects. Psychological mechanisms (including conscious and subconscious processes) are partly established by present and previous beneficial and/or adverse experiences, and significantly influence the extent to which an individual is willing to engage in sexual activities and the extent to which those activities are enjoyable. Therefore, the delicate and relative balance between activation of neurobiological and psychological mechanisms governing sexual excitation and sexual inhibition, controls sexual activities in humans, including fantasizing, masturbation, and sexual intercourse.

Sublingual Testosterone and Treatment of Female Sexual Dysfunction

Single administration of sublingual testosterone (0.5 mg) can induce an increase in physiological and subjective indices of sexual responding in sexually functional women [6,7]. This might indicate this dosage form can be part of a potential pharmacotherapy for HSDD.

In a randomized, placebo-controlled, double-blind, cross-over study, we investigated the efficacy of sublingual testosterone (0.5 mg), the PDE5 inhibitor vardenafil and the combination of these drugs on preconscious attentional bias for sexual cues and physiological sexual function in women diagnosed as having HSDD [9]. We assume that the delay in effect of testosterone on physiological sexual arousal in sexually functional women occurred as the result of an increase in activation of central sexual motivational mechanisms. Central sexual stimulation is necessary for a PDE5 inhibitor to induce an increase in the amount of blood in erectile tissue of the genitals. This occurs in the following way: In genital erectile tissue of both men and women, sexual stimulation will induce the release of nitric oxide (NO) from nerves and endothelium. NO induces an increase in production of cyclic guanosine monophosphate (cGMP). cGMP is a key mechanism in relaxing smooth muscle necessary for the induction of enlargement of the erectile tissue. cGMP is hydrolyzed by the phosphodiesterases in the corpora cavernosa, in which PDE5 is the most abundant PDE. Therefore, during sexual stimulation, the action of NO/cGMP on erectile function will be enhanced by PDE5 inhibitors [84]. Thus, without adequate central stimulation, i.e., activation of central sexual motivational mechanisms, a PDE5 inhibitor cannot be effective, which is likely why the trials investigating PDE5 inhibitor efficacy in FSD failed.

Indeed, the PDE5 inhibitor alone had no effect on physiological sexual functioning of our patients, but neither did testosterone alone. In contrast, the combined use of sublingual testosterone and the PDE5 inhibitor produced an increase in physiological sexual responding about 4 hours after the intake of the testosterone. Apparently, in addition to testosterone-mediated facilitation of central sexual stimulation, peripheral facilitation of the physiological sexual response was needed. Interestingly, we found a striking

difference in effect between women who had and women who had not reported the experience of childhood sexual abuse (CSA). In women without CSA, testosterone treatment induced an increase in their originally low levels of preconscious attentional bias for sexual cues, while women with CSA showed a decrease in their originally high levels of attention. The effects of the combination of testosterone and the PDE5 inhibitor on the physiological sexual response also differed between these groups. Women without CSA revealed an increase in their physiological response, while the women with CSA showed no alterations in this response.

These results were reproduced in a second randomized, double-blind, cross-over, placebo-controlled study [10]. This study was designed to investigate the effects of testosterone, a PDE5 inhibitor, and the combination of both drugs on alterations in preconscious attentional bias for sexual cues in 28 women suffering from HSDD. Moreover, we investigated the influence of these drugs on physiological and subjective indices of sexual function during neutral and erotic visual stimulation. In this study women who reported experience of CSA were excluded. Based on their initial preconscious attentional bias for sexual cues scores, we construed two groups: women with a high and women with a low sensitive brain system for sexual cues. Testosterone treatment produced in the low sensitive group an increase in their attentional bias for sexual cues, while in the high sensitive group a reversed pattern was found. We examined in both groups the effects of the different treatments on physiological and subjective sexual responding. Again, neither testosterone (0.5 mg) nor the PDE5 inhibitor (vardenafil, 10 mg) alone had an effect on measures of physiological and subjective arousal. The combined administration of testosterone and vardenafil in the low sensitive group produced 4 hours post dose a significant increase in physiological and subjective sexual functioning, while in the latter group no drug induced alterations in these measures were observed. This study had no subjects with a history of CSA, but the latter group did have a higher prevalence of negative sexual experiences (63% vs. 17%), which implies that this group's past negative sexual experiences may have contributed to the development of HSDD as a result of activation of central inhibitory mechanisms. Vardenafil was used as the PDE5 inhibitor in these studies, but other PDE5 inhibitors such as sildenafil can also be used (see Poels et al. this issue) as the mechanism of action is the same.

Testosterone and Sensitivity of the Brain for Socially—Including Sexually—Relevant Cues

As described above, female sexual behavior in many mammalian species depends on female sex steroids. In human females, testosterone appears to be highly involved in sexual motivation and behavior. Consequently, testosterone plays a central role in the steroid-responsive neural network involved in the regulation of social and sexual behavior from both men and women, and might exert its influence on these behaviors by selective filtering and amplification of cues important for behavioral responses. In other

words, testosterone influences the sensitivity of the brain for cues (status, challenge, sex) relevant for an individual's interest. The above described relationships between preconscious attentional biases, testosterone treatment, and sexual responsiveness can be conceptualized in terms of testosterone-induced alterations in sensitivity of the brain for sexual cues [9,10]. The low preconscious attention scores before treatment with testosterone reflected a relatively insensitive brain system for sexual cues in women without CSA. Sublingual testosterone increased this sensitivity about 4 hours after dosing, reflected in an increase in subconscious attention for sexual cues. It might be assumed that an increase in the brain's sensitivity for sexual cues is accompanied by an increase in central sexual stimulation, making it possible for a PDE5 inhibitor to produce an increase in physiological sexual responding. On the other side, the absence of an effect on physiological sexual response in the women with negative sexual experiences (including a history of CSA) can also be interpreted as the result of an increase in sensitivity of the brain for sexual cues. Because of the negative association with sex in these women, this increased sensitivity resulted in an automatic activation of a sexual inhibitory mechanism [9,10]. Thus, testosterone increases sensitivity of the brain for socially relevant cues, which might produce different behavioral outcomes depending on other involved characteristics of an individual.

Testosterone and Sexual Inhibition

Janssen and Bancroft [85] conceptualized that individual differences in sexual responding depend on a delicate interplay of the earlier mentioned excitatory and inhibitory processes. It is widely accepted that the PFC is involved in the inhibitory control of human behavior [86], including sexual behavior [87,88]. A sexual event which is consciously or subconsciously negatively valenced or which induces inappropriate responses in, for that response, inappropriate situation, can be expected to induce a phasic increase in PFC-mediated sexual inhibition. Amplifying negative valence or response sensitivity in an inappropriate setting would be expected to increase inhibition even further. Indeed, in two studies (unpublished data) we found support for this hypothesis, in that testosterone can actually induce an inhibitory sexual response.

In the first experiment we examined whether treatment with testosterone would cause an increase in neural activity in brain areas associated with sexual behavior. In a randomized, placebo-controlled, double-blind, cross-over design, an fMRI-VPA study was conducted in 12 sexually functional eugonadal female volunteers. Four hours after the intake of the placebo or 0.5 mg of testosterone sublingually, the blood-oxygen-level-dependent (BOLD) MRI response was measured while subjects viewed neutral and erotic film excerpts. Immediately after the fMRI session, VPA in response to another comparable set of neutral and erotic film excerpts was measured. As expected, we found comparable BOLD responses to erotic stimuli in the placebo condition, as in other imaging studies [89,90]: the amygdala and temporal pole, hippocampus, hypothalamus, brain stem, orbitofrontal cortex, anterior cingulate cortex, and decreased BOLD

response of dorsal prefrontal areas (Figure 2, top row). We expected this pattern to become more pronounced following sublingual testosterone administration, followed by a concomitant increase in VPA relative to placebo.

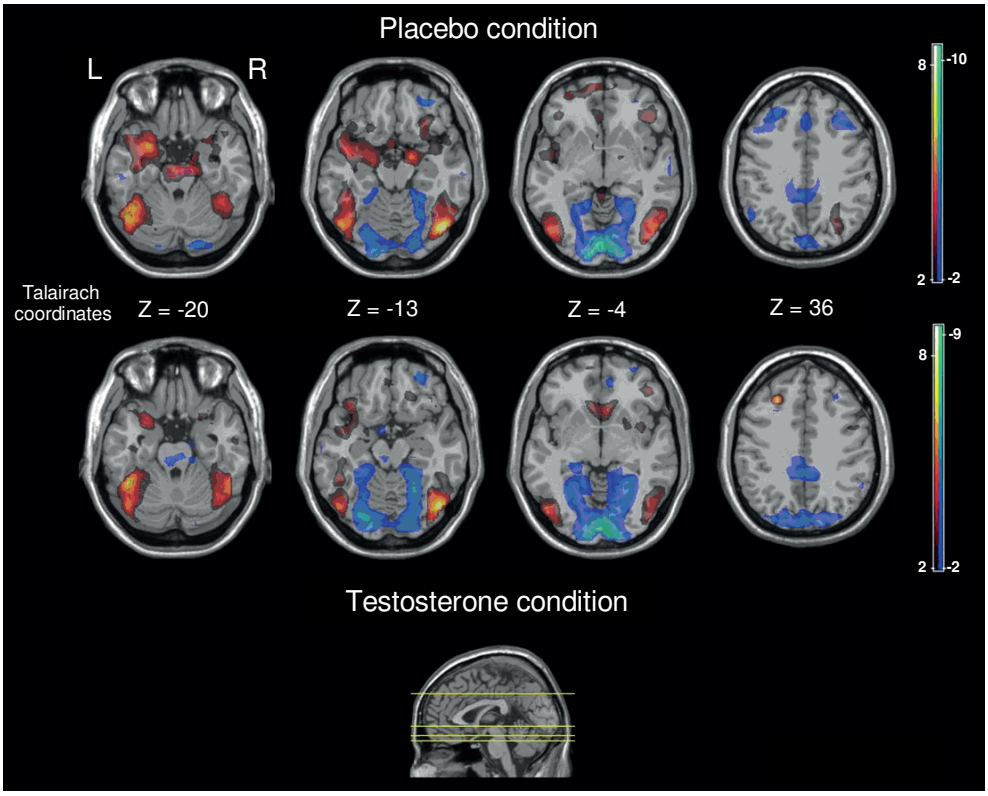

Figure 2 Blood oxygen level dependent responses to erotic stimuli under placebo and testosterone. Transverse images of the brain. Z-coordinates in Talairach space. (Source: unpublished results)

In the testosterone condition, however, women showed decreased BOLD responses in all brain structures implicated in the normal sexual response (Figure 2, bottom row). Additionally, there was an increased BOLD response in the superior part of the middle frontal gyrus, a portion of the dorsolateral PFC (DLPFC), which mediates inhibition of task-irrelevant responses [91–95] and willful inhibition of the sexual response [87]. Also, there was increased BOLD response of the septal nuclei, which have been postulated to play a constrictive role on subcortical structures, reducing emotional and arousal extremes, thus preventing emotional overshoot [96]. Moreover, and contrary to our former findings [6,7], the VPA showed a relatively blunted response in the testosterone condition as compared with placebo (see Figure 3), as compared with our former studies on the delay effect of testosterone.

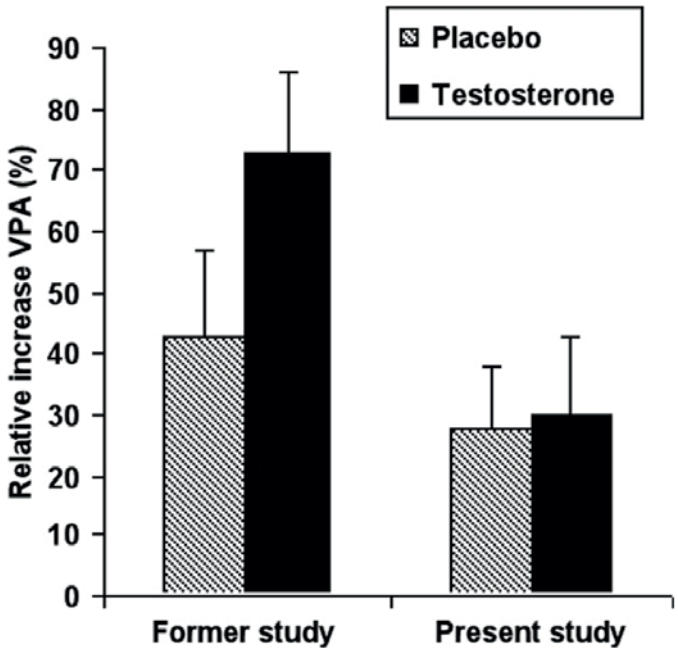

Figure 3 Relative changes in vaginal pulse amplitude (VPA). Comparison between a former [6] and the present experiment for the average relative increases in VPA induced by an erotic film fragment viewed about 4 hours after placebo and testosterone (0.5 mg) intake. A univariate analysis revealed a significant interaction effect [$F(1, 17) = 6.49$; $P < 0.025$] for Drug (Placebo vs. Testosterone) × Study (Former vs. Present), indicating a relatively blunted VPA response in the testosterone condition for the fMRI-VPA experiment as compared with the former VPA-alone experiment. (Source: unpublished results)

We hypothesized that exposure of our subjects to the experimental conditions—i.e., a noisy dark narrow cylinder with male technicians and scientists all around—seemed to have demanded induction of an inhibitory mechanism in order to dampen the inappropriate effects of the testosterone-induced increase in sensitivity of the sexual response system.

The amount of attentional resources directed at sexual stimuli greatly determines the strength of the physiological and subjective sexual response. Focusing on sexual stimuli facilitates the sexual response, while withdrawing attention attenuates it. In subjects with sexual problems, however, the opposite relationship has been observed [97]. Apparently, focusing attention on sexual stimuli can induce inhibition under certain circumstances. Varying attentional resource allocation in the sexually inappropriate MRI measurement setting should then be able to amplify and/or reduce (testosterone-induced) situation-dependent inhibitory BOLD responses.

In a second pilot experiment [98], the hypothesis of testosterone-induced sexual inhibition was further investigated while varying the level of attention for sexual stimuli,

in a group of women with HSDD (N = 14). In this randomized, double-blind, placebo-controlled, cross-over fMRI study, the influence of varying levels of attention (Stroop task superimposed on an erotic film clip, erotic film clip without additional instruction, an erotic film clip and the instruction to monitor bodily sexual response preceded by a neutral film clip, and an erotic film and the instruction to monitor bodily sexual response preceded by an erotic film clip with Stroop task superimposed) for sexual stimuli on BOLD MRI response to erotic vs. neutral film clips were investigated in three treatment conditions: placebo, testosterone (0.5 mg), and the combined administration of testosterone (0.5 mg) and sildenafil (50 mg).

In the placebo condition where subjects viewed erotic film clips without additional instruction, we observed activation patterns comparable to former imaging studies on sexual arousal [87,89,90], but with three noteworthy differences: subjects did not show increased amygdala (stimulus salience perception/attribution) and right frontal insula (interoception) BOLD response, but they did show increased BOLD response in the left DLPFC (Figure 4) indicating that subjects inhibited their sexual (amygdala and insula) response.

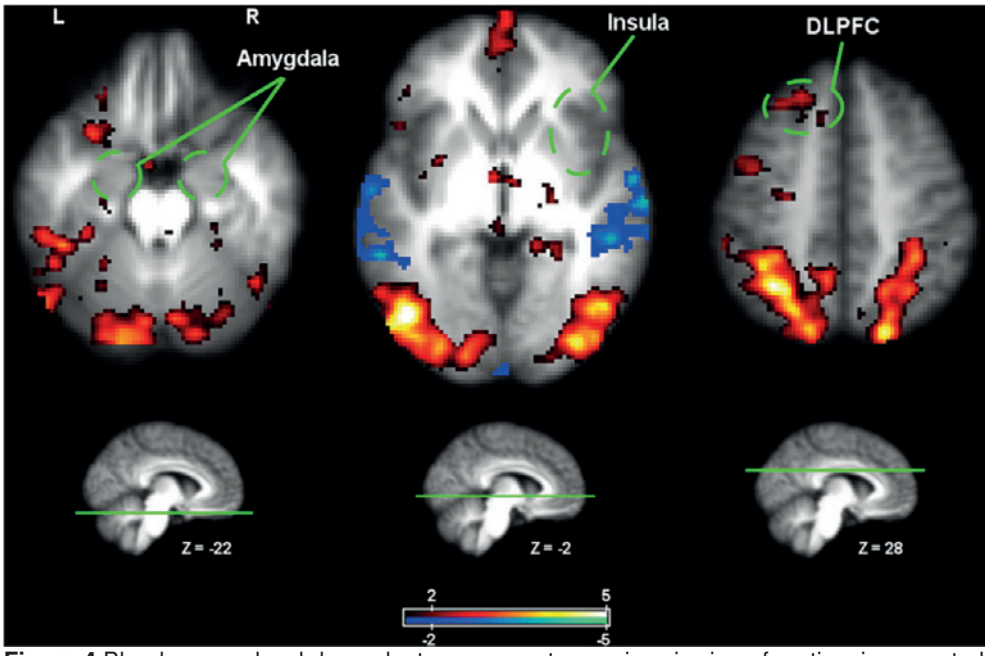

Figure 4 Blood oxygen level dependent responses to passive viewing of erotic minus neutral film clips under placebo. Transverse images of the brain. Z-coordinates in Montreal Neurological Institute space. Source: unpublished results. Presented at the Congress of the International Society for the Study of Women's Sexual Health, 2007 [98]. DLPFC = dorsolateral prefrontal cortex

In the condition when subjects watched an erotic film clip while monitoring their bodily sexual response preceded by a Stroop-superimposed erotic film clip as compared with the condition where a neutral film clip preceded the erotic film clip left DLPFC BOLD response decreased and right insula BOLD response increased under placebo and even more so under testosterone (Figure 5). However, the combination of testosterone plus sildenafil reversed this pattern; adding the sildenafil apparently increased the sexual response to inappropriate levels, thereby necessitating inhibition.

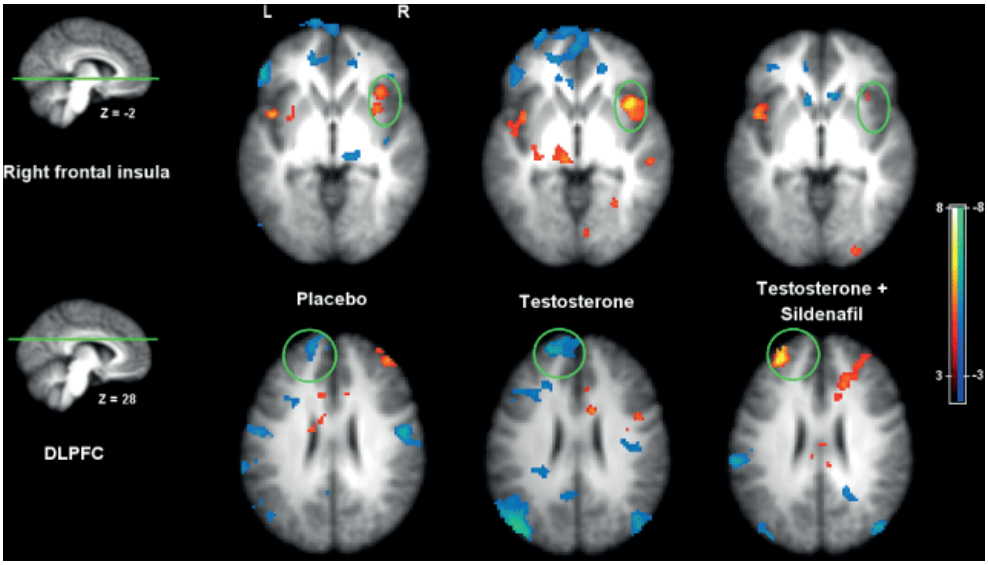

Figure 5 Blood oxygen level dependent responses to attentional engagement under conditions of increased sexual stimulation. BOLD responses to attentional engagement under conditions of increased sexual stimulation, under placebo, testosterone, and the combination of testosterone and sildenafil. Transverse images of the brain. Z-coordinates in Montreal Neurological Institute space. Source: unpublished results. Presented at the Congress of the International Society for the Study of Women's Sexual Health, 2007 [98]. DLPFC = dorsolateral prefrontal cortex

In many situations, it is inappropriate to act on a sexual cue, for example, because of social conventions (i.e., there is a threat of consequences that acting on such a cue in the wrong circumstance implies). Under such circumstances, sexual cues can still elicit preparatory sexual responses, but these then have to be inhibited [99]. The need for, or strength of this inhibitory response is dependent on the elicited sexual response. Thus, if a sexual cue is relatively weak (due to the nature of the cue, or to the neurobiological disposition of the individual), no inhibition may be needed. However, if the processing of a relatively weak sexual cue is enhanced through testosterone administration, inhibition may be needed. Therefore, the net effect of testosterone administration could be an inhibitory response.

In conclusion, depending on circumstances, testosterone can produce effects that deviate from the expectations concerning the functional (i.e., facilitation) role of testosterone in the regulation of sexual behavior.

The Influence of Testosterone and Serotonin on Sexual Inhibition

Given that sublingual testosterone increases the sensitivity of the brain to sexual cues, women prone to sexual inhibition are expected to exhibit stronger inhibitory activity in the PFC during exposure to a sexual stimulus following testosterone administration. An important mediator of inhibitory mechanisms in the brain is the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) [100], that also exerts abundant inhibitory effects via the PFC [101]. After acute treatment with a 5-HT_{1A} receptor agonist, the agonist binds to somatodendritic autoreceptors of the raphe nuclei in the midbrain. The hyperpolarizing effect of activated 5-HT_{1A} auto receptors decreases serotonergic firing activity [102] and inhibition of serotonin release from the presynaptic terminal [103], and subsequently, reduced extracellular serotonin levels in the PFC [104]. There are differences in effects on serotonergic activity between acute and prolonged treatment with a 5-HT_{1A} receptor agonist, resulting in opposed effects on behavior mediated by 5-HT_{1A}. For example, acute buspirone causes an increase in impulsivity, which effect is reversed following chronic treatment [104]. Accordingly, acute treatment with a 5-HT_{1A} receptor agonist might decrease sexual stimuli induced phasic serotonergic inhibitory control in the PFC, which in turn might prevent or reduce the inhibitory response to sexual cues in women with HSDD as the result of activation of sexual inhibitory mechanisms. Consequently, when exposed to sexual stimuli (whether internally or externally induced), women prone to sexual inhibition might show an increased sexual response when treated with sublingual testosterone and a 5-HT_{1A} receptor agonist.

Two studies above [9,10] describe the use of the emotional Stroop task to differentiate between high and low sensitive women. The high sensitive women did not show an increase, or showed decreases in physiological and subjective sexual responding following the combined administration of testosterone and vardenafil. These women also showed a higher incidence of negative sexual experiences, which led to the hypothesis that these women suffered from HSDD as a result of (over)activation of sexual inhibitory systems. Women who have negative sexual experiences are about twice as likely to develop a sexual dysfunction, but it is not a given [105]. A potential risk factor for developing a sexual dysfunction after negative sexual experiences is, according to our hypothesis, a person's sensitivity to sexual cues, which will affect positive and negative sexual experiences. Irrespective of the absolute levels of testosterone, different levels of sensitivity for sexual cues might occur, for example, as the result of a difference in the sensitivity of the receptor system for androgens (i.e., different length of CAG repeats [79] and/or different levels in androgenic intracellular activity [106]). Other systems might interfere with functioning of the androgen systems in the brain; for example, differences in polymorphisms of the serotonin transporter gene, and the

serotonin1A receptor gene have been linked to a higher likelihood of developing affective disorders [107,108] due to the altered processing of emotional stimuli (i.e., more reactive to negative stimuli) [107]. High sensitive women are more sensitive for positive and negative sexual experiences. It is possible that the high sensitive women can develop HSDD (partly) because of altered serotonergic transmission, making them more sensitive to negative sexual experiences leading to subsequent overactivation of sexual inhibitory mechanisms in response to sexual arousal. This is in contrast to the low sensitive women, who have a higher chance to develop HSDD because their insensitivity causes a lack of adequate activation of sexual excitatory mechanisms (see Figure 6). In the near future we will report on the results of our research program into these relationships between the above mentioned brain variables/mechanisms and the vulnerability to develop HSDD.

Conclusions

Summarizing, we formulated the hypothesis that different causal mechanism are responsible for HSDD. Based on this hypothesis, we designed and developed two new medicines for HSDD. Sublingual testosterone combined with a PDE5 inhibitor has been developed for women who suffer from low sexual motivation and low sexual desire (HSDD), as the result of a relatively insensitive system for sexual cues. This combination should increase the sensitivity for internal and external sexual cues, activates central sexual motivation mechanisms and subsequently the physiological sexual response. The combination of sublingual testosterone and a 5-HT_{1A} receptor agonist was designed to treat HSDD induced by dysfunctional sexual inhibition mechanisms. This combination should also increase sexual motivation, but inhibits overactive sexual inhibition mechanisms in the prefrontal areas.

To investigate whether the testosterone combined with the PDE5 inhibitor was also efficacious at home in women who suffer from HSDD as the result of an insensitive system for sex, and to test the hypothesis that testosterone plus a 5-HT_{1A} receptor agonist are efficacious in women with HSDD who are prone to sexual inhibition, we conducted a randomized, double-blind, placebo-controlled cross-over study in 56 women with HSDD with or without Female Sexual Arousal Disorder. In an ambulatory experiment at home (see also [109]), preconscious attentional bias for sexual cues, physiological and subjective indices of sexual functioning were measured three times during the first week of each treatment. In a subsequent bedroom experiment during a 3-week period under condition of each treatment, we evaluated sexual satisfaction following each sexual event. The results of the efficacy of sublingual testosterone combined with a PDE5 inhibitor in women with HSDD as the result of an insensitive system for sexual cues are described in part 2 (chapter 6) of this series. In part 3 (chapter 7) we will describe the results of treatment with sublingual testosterone combined with a $5HT_{1A}$ receptor agonist in women with HSDD as the result of activation of sexual inhibitory mechanisms.

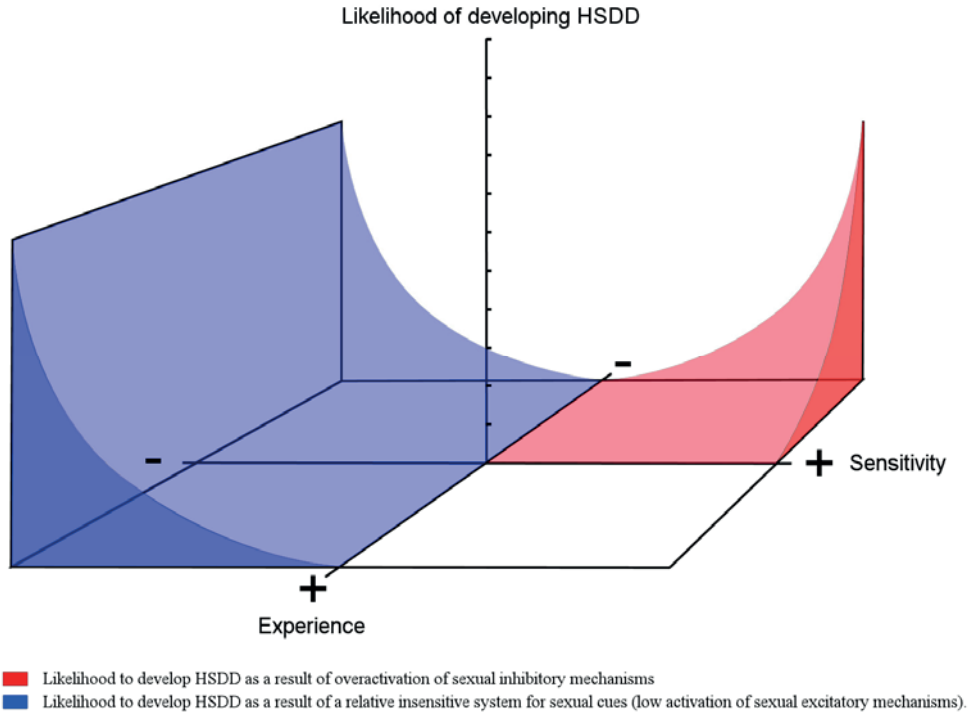

Figure 6 Representation of our hypothetical sexual (low–high) sensitivity-inhibition model. According to the model, HSDD is correlated with low sensitivity to sexual cues (blue area), or by overactivation of sexual inhibitory mechanisms (red area). Subjects with high sensitivity for sexual cues will be more sensitive for positive sexual stimuli and experiences, which can lead to a hedonic sex life. However, high sensitivity subjects are also more vulnerable to negative sexual experiences, and as a result more susceptible for learning a negative association with sex. Exposure to stimuli that make an appeal to the sexual motivational system, can then automatically elicit an inhibitory response (e.g., a phasic increase in serotonin activity in the dorsolateral prefrontal cortex [PFC]) to diminish conscious or subconscious negative affective state induced by the undesired sexual motivational state. The strength of the inhibitory response might be a function of the sensitivity of the sexual brain system, and the duration and severity of negative sexual experiences. Low sensitivity for sexual cues might be the result of a low sensitive androgenic receptor system in the brain, and/or a low level of intracellular androgenic activity, and/or tonic high serotonergic activity in particular areas in the PFC (which may function as a filter for emotional positive and negative stimuli). Subjects with a low sensitive system will have decreased levels of activation of sexual excitatory mechanisms, resulting in low sexual desire. This state of low sensitivity can be interpreted as a biological trait, and is unlikely to be caused by sexual experiences. Different combinations of various levels of sensitivity and inhibition are possible, but high inhibition is more likely to occur in sexually high sensitive subjects (resulting in HSDD). Subjects with HSDD and a low sensitive sexual brain are less likely to suffer from high inhibition because they already have low propensity to respond to sexual stimuli; they have less need to inhibit their sexual response.

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CHAPTER 6

Toward Personalized Sexual Medicine (Part 2): Testosterone combined with a PDE5 inhibitor increases sexual satisfaction in women with HSDD and FSAD, and a low sensitive system for sexual cues

Journal of Sexual Medicine 2013;10:810-823

Abstract

Introduction. Low sexual desire in women may result from a relative insensitivity of the brain for sexual cues. Administration of sublingual 0.5 mg testosterone (T) increases the sensitivity of the brain to sexual cues. Sexual stimulation in the brain is necessary for phosphodiesterase type 5 inhibitor (PDE5i)-mediated increase in genital sexual response. Accordingly, a single dose of T+PDE5i might enhance sexual responsiveness, especially in women with low sensitivity for sexual cues.

Aim. To assess the hypothesis that treatment with on-demand use of T+PDE5i improves sexual functioning, particularly in women who suffer from Hypoactive Sexual Desire Disorder (HSDD) as the result of a relative insensitivity for sexual cues.

Methods. In a randomized, double-blind, placebo-controlled, cross-over design, 56 women with HSDD underwent three medication treatment regimes (placebo, T+PDE5i, and T with a serotonin $_{1A}$ receptor agonist; see also parts 1 and 3), which lasted 4 weeks each. In a participant-controlled ambulatory psychophysiological experiment at home (the first week of each drug treatment), physiological and subjective indices of sexual functioning were measured. In a bedroom experiment (the subsequent 3 weeks), sexual functioning was evaluated following each sexual event after the self-administration of study medication. Subjective evaluation of sexual functioning was also measured by weekly and monthly reports.

Main Outcome Measures. Subjective: sexual satisfaction, experienced genital arousal, sexual desire. Physiological: vaginal pulse amplitude. Cognitive: preconscious attentional bias.

Results. T+PDE5i, as compared with placebo, significantly improved physiological and subjective measures of sexual functioning during ambulatory psychophysiological lab conditions at home and during the sexual events, in women with low sensitivity for sexual cues.

Conclusions. The present study demonstrated that on-demand T+PDE5i is a potentially promising treatment for women with HSDD, particularly in women with low sensitivity for sexual cues.

Introduction

Low sexual desire is the most common sexual complaint in women [1]. It has been classified as the clinical condition Hypoactive Sexual Desire Disorder (HSDD), which is characterized by chronic or recurrent loss or decrease in interest in sexual activity, causing sexual dissatisfaction and severely affecting a woman's quality of life [2]. Bancroft and Janssen have described the involvement of dual control systems in the regulation of sexual functioning. They argue that individual differences in sexual responding depend on a delicate interplay of such activating excitatory and inhibitory processes [3,4]. We have hypothesized that in some women with HSDD low sexual desire could be due to a relatively insensitive system for sexual cues, resulting in impaired activity of excitatory mechanisms involved in sexual motivation [5]. In these women, administration of sublingual 0.5 mg testosterone might increase the brain's sensitivity for sexual cues, which might activate excitatory mechanisms in the brain involved in sexual motivation and desire [5–8]. Sufficient sexual stimulation of the brain is necessary for a phosphodiesterase type 5 inhibitor (PDE5i)-mediated increase in the genital sexual response [9,10]. Because central mechanisms (sexual motivation) play a large role in sexual stimulation, PDE5i's will generally have little to no effect when activation of central sexual mechanisms is reduced or absent. In previous studies it was shown that PDE5i administration increases physiological [6] and subjective [5] sexual responding under psychophysiological lab conditions in women with HSDD and low sensitivity for sexual cues, only when the PDE5i administration was preceded by administration of a single dose of sublingual testosterone. It was also shown that testosterone by itself did not increase sexual responding [5,6]. In the present study we measured sexual functioning under the more ecologically valid condition of an ambulatory psychophysiological lab setting at home and sexual satisfaction following sexual events. We hypothesize that treatment with T+PDE5i improves physiological and subjective indices of sexual functioning, particularly in women who suffer from HSDD as the result of an insensitive system for sex.

Methods

Participants

The 56 heterosexual women who participated in this study signed a written informed consent and received reimbursement for their participation. The local medical ethics committee (Stichting Therapeutische Evaluatie Geneesmiddelen Medisch Ethische Toetsingscommissie, Almere, the Netherlands) approved this study, which was carried out in agreement with International Conference on Harmonization-Good Clinical Practice (ICH-GCP), and monitored by a certified Contract Research Organization (CRO) (PSR Group, Hoofddorp, the Netherlands). Women were eligible if they were healthy, between 21 and 70 years, and had a diagnosis of HSDD or Female Sexual Arousal Disorder (FSAD) according to the Diagnostic and Statistical Manual of Mental Disorders-

Fourth Edition (Text Revision) (DSM-IV-TR) criteria [2]. Subjects were diagnosed by an experienced psychologist. If the subject was diagnosed with any psychiatric disorder other than HSDD or FSAD they were excluded; also any treatment for female sexual dysfunction within 7 days before or during the study was excluded. Furthermore women were excluded if they were using oral contraceptives containing anti-androgens or more than 50 μg estrogen, Cytochrome P450 3A4 (CYP3A4) inhibitors, CYP3A4 inducers, nitrates, monoamine oxidase inhibitors, calcium channel blockers, antidepressants, opiates, and medicinal herbs like St. John's wort. Cardiovascular exclusions included a history of myocardial infarction, stroke or life-threatening arrhythmia within the prior 6 months, uncontrolled hypertension, atrial fibrillation/flutter or any other significant abnormality observed on electrocardiogram (ECG). Gynecological exclusions included pelvic inflammatory disease, vaginal infection, previous prolapse and incontinence surgery affecting the vaginal wall, abnormal uterine bleeding patterns, perimenopausal hormonal status, pregnancy and breastfeeding in the past 6 months. Lastly women were excluded with clinically relevant endocrine disease, neurological disease, severe or acute liver disease, history of severe hepatic impairment, body mass index above 35, and vision impairment. Women were recruited and enrolled from referrals, newspaper advertisements, the Internet, and our own database. To determine eligibility, participants were screened 4 weeks prior to study entry. In addition to an assessment of medical history with detailed sexual, gynecological, and psychological history, all subjects received a physical examination including a 12-lead ECG, a vaginal culture test to exclude infection, standard biochemistry and hematological laboratory tests, and pelvic examination. Biochemical parameters including hematology, liver function tests, electrolytes, creatinine, uric acid, glucose and lipids were collected at baseline, following each treatment period, and at the final follow-up visit. Serum concentrations of total testosterone, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, and prolactin were measured at baseline. All subjects also had their thyroid status assessed; thyroid stimulating hormone and free thyroxine. A urine pregnancy test was administered to all women of childbearing potential. Investigators monitored and followed up any reports of adverse events. On a pretreatment visit, a trained female experimenter explained the study requirements and procedures to the participants. A practice trial was conducted, including insertion of the vaginal photoplethysmograph and a practice session of the emotional Stroop task.

Study Design

In a randomized, double-blind, placebo-controlled, cross-over design, each participant underwent three different medication treatments: (i) **placebo**: placebo for testosterone (cyclodextrin solution without testosterone) and placebo for the PDE5i (PDE5i = sildenafil) and 5-HT_{1A} receptor agonist (5-HT_{1A}ra = buspirone) (powder-filled gelatin capsule without sildenafil/buspirone); (ii) **T+PDE5i**: the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and sildenafil (50 mg,

hidden in a powder-filled gelatin capsule); (iii) **T+5-HT$_{1A}$ra**: the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and buspirone (10 mg, hidden in a powder-filled gelatin capsule). A single medication regime lasted 4 weeks, and the order of the three medication regimes was randomized. All treatment orders were possible. Between each medication regime there was a washout period of 1 week. Participants received 14 units of medication (throughout the manuscript, 1 medication or 1 unit of medication means the combination of the sublingually administered solution *and* the oral capsule) during each medication regime; instructions were given to have at least 48 hours between two medication intakes (see Figure 1). Participants were instructed to take the cyclodextrin solution (testosterone or placebo) sublingually 4 hours prior to each measurement session or sexual event and rinse it under the tongue for 1 minute. They were instructed to ingest the capsule (sildenafil, buspirone, or placebo) 2.5 hours later. The sequence of the drugs and time frame were such that the pharmacological effects of the PDE5i and 5-HT$_{1A}$ra coincide with the window of T-induced behavioral effects, approximately 3–6 hours after T administration. Following the completion of a drug regime, the research physician took in any leftover medication of the previous regime and handed out the next regime's medication box.

Study Procedure

Ambulatory Psychophysiological At-Home Experiment

Participants visited the study site 12 times during a period of 26 weeks for screening and safety control visits. In the first week of each 4-week medication regime, participants underwent three experimental psychophysiological measurement sessions (under condition of study medication) at home using participant-controlled (i.e., no experimenters were present) ambulatory laboratory [11]. In premenopausal women not using oral contraceptives, experimental days were within the first 10 days after their menstruation. Users of oral contraceptives were not measured during their pill-free period. During each experimental session, subjects engaged in 2 minutes each of: (i) self-induced sexual fantasy; (ii) viewing an erotic film clip depicting sexual foreplay; and (iii) viewing an erotic film depicting explicit heterosexual intercourse. The sexual fantasy condition was preceded by a 6-minute baseline establishment period during which subjects viewed a neutral film clip. The foreplay and heterosexual intercourse films were preceded by 2 minutes return to baseline periods (see Stimuli, Apparatus, and Measures below, for a detailed description of the neutral and erotic stimulus material). At the start of the experimental session, and after each exposure to sexual stimuli (the fantasy session and film clips), we measured “experiences of genital arousal” and “sexual desire” by means of the Sexual Arousal Response Self-Assessment Questionnaire (SARSAQ; see Stimuli, Apparatus, and Measures below, and [5]). During all neutral and sexual stimulus sessions, the vaginal pulse amplitude (VPA) was continuously measured (see Stimuli, Apparatus, and Measures below). Before the baseline establishment period and finally as

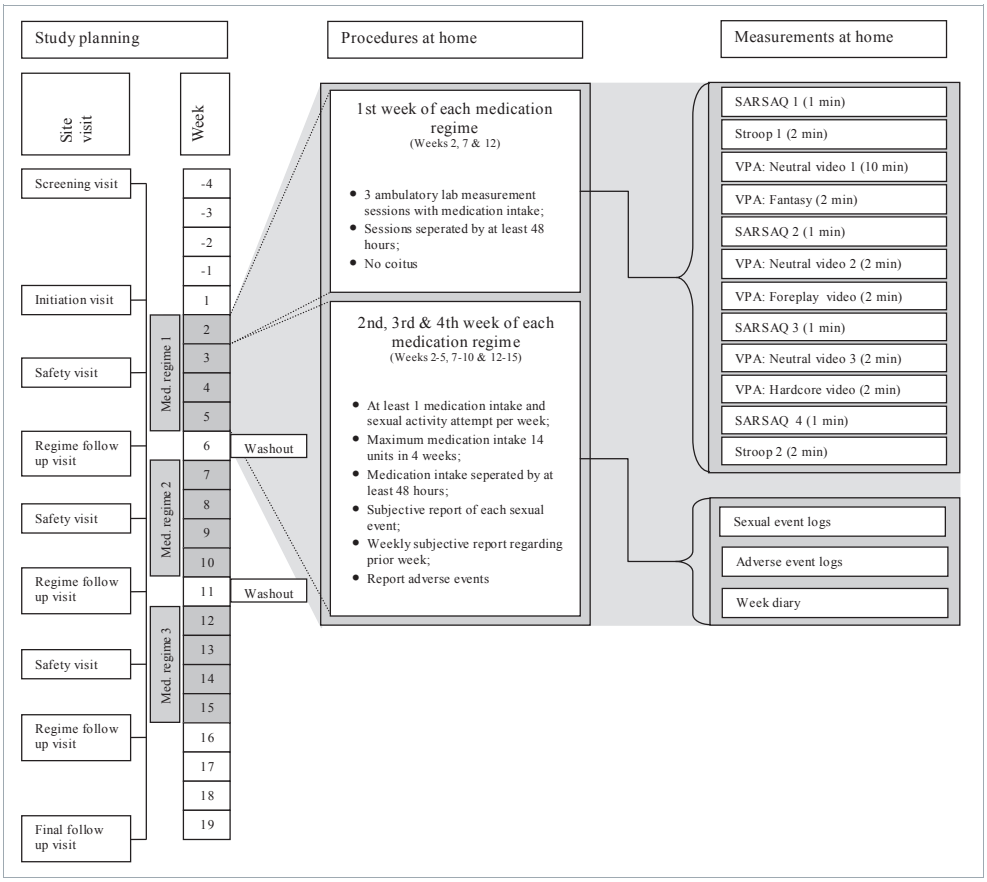

Figure 1 Overview of study design and procedures. Subjects were randomized before the initiation visit and received seven units of medication to take home for the subsequent 2 weeks. At each safety visit, which was planned halfway of each regime, subjects received the other seven units of medication for the last 2 weeks of that regime. Adverse events (AEs) and vital signs were recorded at each visit. Blood safety was also determined at all follow-up visits. During the first week of each regime, subjects received an ambulatory laboratory for three psychophysiological measurements at home. During the subsequent 3 weeks subjects were free to take the medication when they wanted. They were instructed to attempt sexual activity at least once a week with medication during this period. Medication intake was on-demand but separated by at least 48 hours. Subjects reported their subjective experience within 24 hours following each sexual activity via a secure Web-based questionnaire. At the end of each week, a Web-based week diary was completed. AEs experienced at home could also be reported in this manner. AEs which were reported through the Web-based questionnaire were discussed with the subject the following visit. SARSAQ = Sexual Arousal Response Self-Assessment Questionnaire; VPA = vaginal pulse amplitude

the last element of the experimental session, we measured biases in preconscious attention for sexual cues by means of an emotional Stroop task (see Figure 1).

Bedroom Experiment

For the remaining 3 weeks, participants were instructed to use at least one medication per week, with an attempt at a sexual event (cuddling, coitus, or masturbation). For the remainder, they were free to take their medication whenever they wanted but not during two consecutive days. When they had experienced a sexual event they were asked to fill out an event diary within 24 hours and a diary at the end of each week.

Stimuli, Apparatus, and Measures

Audiovisual Stimuli

For this study, neutral, erotic foreplay, and explicit sex film clips were used. Neutral clips were 6-minute and 2-minute clips from Dutch action films. The 6-minute neutral film clips were used to establish a baseline VPA. Erotic foreplay clips were 2-minute clips showing kissing, caressing, and cunnilingus, but no fellatio. Explicit sex clips were 2-minute clips showing cunnilingus and coitus, including visible penetration. The erotic film footage was selected and edited by female research associates and then rated by other female research associates to ensure that all clips were within set specifications. All digitally sampled film clips were presented using Presentation software (Neurobehavioral Systems, Albany, CA, USA). For a more detailed description see Bloemers et al. [11].

Ambulatory Laboratory

The ambulatory laboratory is based on the MobiHealth Mobile remote monitoring system (MobiHealth B.V., Enschede, The Netherlands) [12]. Study-specific functionality of this system enables VPA measurements, stimulus presentation, and execution of emotional Stroop tasks to be performed at an arbitrary time and location (e.g., house of a participant). This laboratory is operated autonomously by the participant. The ambulatory laboratory transmits all measured data to a secure central database server, at which the researcher can obtain the data for further analysis. This method allows for a more ecologically valid psychophysiological measurement of sexual responding. See for a detailed description the study by Bloemers et al. [11].

Main Outcome Measures: Ambulatory Laboratory

VPA

The VPA reflects phasic changes in vaginal engorgement corresponding with each heartbeat. VPA was measured using a vaginal photoplethysmograph, a tampon-shaped device containing an infrared light-emitting diode and a photosensitive light detector (photodiode). VPA was defined as the peak-to-trough amplitude of the pulse wave and was calculated by acquiring the means of all peaks and troughs and subtracting those.

Data from the photoplethysmograph were sampled at 256 Hz and filtered offline (high-pass 1 Hz, 48 dB/oct and low-pass 1.5 Hz, 48 dB/oct), in order to isolate the AC component from the DC coupled amplifier, reduce respiration artifacts and high-frequency oscillations. Large movement artifacts (more than 100% increase for a small number of isolated periodic cycles) were manually removed following visual inspection of the data. The data were divided into 30-second epochs for each 2-minute film clip, thus yielding four discrete values and for the 6-minute film clip 12 values reflecting VPA during different stages of the film clip. Finally, in order to eliminate interpersonal differences and obtain meaningful data, mean VPA scores over the four epochs of the fantasy condition, foreplay clips, and explicit clips were related to activity during the last epoch of the first 6-minute neutral clip, using the following formula:

$$VPA_{rel} = ((VPA_x - VPA_{neu})/VPA_{neu})$$

with VPA_{rel} being the relative change in VPA related to the first neutral clip, VPA_x being the mean of the four 30-second epochs of either the fantasy condition, foreplay clips, or explicit clips, and VPA_{neu} being the last 30-second epoch of the first 6-minute neutral clip.

Emotional Stroop Task

To measure preconscious attentional bias for sexual cues, a masked version of the emotional Stroop task was used [13]. In this task, words were presented for 26 milliseconds in four different colors (red, green, blue, and yellow) on a Dell Latitude D531 laptop (Dell Inc., Round Rock, TX, USA) set at a 75 Hz refresh rate. Words were backwardly masked by randomly cut and reassembled letters in the same color. Backward masking prevents conscious processing of the words. Participants were instructed to name the color of the masks as quickly as possible. A microphone connected to a voice-level detector was placed in front of the participant. Initiation of vocal response was registered by the computer's clock and terminated the target (mask) presentation (with a no-response maximum of 3,000 milliseconds). Accuracy of color naming was not scored. Thirty-two unambiguous neutral words from one category (furniture; examples are “chair” and “table”) and 32 unambiguous erotic words (examples are “penis,” “coitus,” and “vagina”) were presented in a blocked manner (eight words per block). The same words were used for each test; however, the sequence of words and their colors differed all eight times this task was used. These different versions were randomized over the participants. An extra set of stimuli consisting of meaningless letter strings was used for practice trials directly before each Stroop task. The Stroop reaction times for color naming were visually inspected for outliers. After these outliers were excluded, participants' mean reaction times for erotic and neutral words on each trial were calculated. The differences between the mean reaction times of erotic and neutral words on each trial were used in the analysis.

Sensitivity of the Brain to Sexual Cues

Based on their mean reaction times of erotic and neutral words on the emotional Stroop task taken over the 3 experimental days, we divided patients in two groups: one group with low (negative score; reaction time to neutral words > erotic words) and one group with high (positive score; reaction time to neutral words < erotic words) sensitivity for sexual cues.

SARSAQ

The SARSAQ is a 10-item self-report questionnaire using a seven-point Likert scale (ranging from "not at all" to "extremely"), adapted from Morokoff and Heiman [14] and Heiman and Hatch [15]. It measures current subjective feelings of sexual arousal and sexual desire. Five items concern subjective feelings of genital responding, and five items concern subjective feelings of sexual desire. The SARSAQ was administered via the ambulatory laboratory laptop whereby the number keys (from 1 to 7) were used to complete the questionnaire.

Main Outcome Measures: Bedroom Experiment

Event Diary

In this secure web-based diary, subjects were asked 10 questions (one open-ended, four multiple-choice, five 5-point Likert scale items) concerning the type, duration, pleasantness, and intensity of the sexual event. Participants were instructed to fill out the event diary within 24 hours following each sexual event (e.g., cuddling, coitus, masturbation).

Week Diary

Women were also instructed to fill out the secure Web-based week diary once a week during each medication regime. Subjects were asked their experiences during the past week regarding: (i) sexual desire (six-point Likert scale: 1 = nothing to 6 = much); (ii) vaginal arousal (six-point Likert scale: 1 = nothing to 6 = much); (iii) sexual improvement/deterioration (four-point Likert scale; 1 = nothing to 4 = much); and (iv) if they could attribute the improvement/deterioration to the medicines (yes/no).

Subjective Evaluation of Improvement (SEI)

The SEI questionnaire was used to determine, after each medication regime, if the subject felt there had been an overall improvement in sexual desire, sexual arousal or both, and if they attribute this improvement to the medication. Subjects answered yes or no to both questions. The SEI was administered as a pen and paper test.

Subjective Evaluation of Gain (SEG)

The SEG questionnaire was used to determine, after each medication regime, if the subject felt that she had any meaningful benefit from the study medication, and if she would use it if it were available by prescription. Subjects answered yes or no to both questions. The SEG was administered as a pen and paper test.

Measures: On Site

Hormonal Measures

Serum total testosterone, serum estradiol, serum progesterone, serum prolactin, serum LH, serum FSH, and serum SHBG were measured through electrochemiluminescence radioimmunoassay with COBAS kits of Roche Diagnostics (Mannheim, Germany), using a Modular E170 at OLVG Hospital (Amsterdam, the Netherlands). The measuring range for total testosterone was 0.087–52.0 nmol/L. The coefficient of variation was 8%.

Data Transformation and Reduction

Ambulatory Experiment

For each drug treatment condition, we used the mean levels of the 3 experimental days of the experience of genital arousal, sexual desire, preconscious attentional bias for sexual cues and VPA, respectively, for further analyses.

Bedroom Experiment

Based on high Cronbach's alphas of the six items measuring duration, pleasantness, and intensity of the sexual events (measured by the event diary) during the different drug conditions (placebo: $\alpha = 0.95$; T+PDE5i: $\alpha = 0.95$) we calculated the mean of these items, as a measure of "sexual satisfaction." Each question of the week diary, SEG and SEI questionnaire were analyzed separately. To analyze sexual satisfaction and the week diary items, we calculated the average of each dependent variable over the 3 weeks of drug treatment.

Statistical Analysis

Baseline characteristics and demographics of both subgroups were compared using the Student's *t*-test, Mann–Whitney *U*-test or chi-square test when appropriate.

For each of the dependent variables, separate repeated measures analyses of variance (anovas) were carried out. The within-subject factor had two levels (placebo and T+PDE5i). The between-subject factor also had two levels (low and high sensitivity for sexual cues). For all analyses menopausal status (premenopausal vs. postmenopausal)

and hormonal contraception (hormonal contraception vs. non-hormonal contraception) were analyzed in a repeated measures ANOVA for each dependent variable.

We have chosen to report an ANOVA for each of these dependent variables separately, instead of analyzing the variables together in a multivariate analysis (MANOVA). The reasons for this choice are twofold: first, our interest is into effects on each of the dependent variables separately as they are quite different in nature (e.g., we have a physiological measure such as VPA as well as self-report measures such as diaries under different experimental conditions); second, when missing data are scattered over all dependent variables (e.g., sometimes women did not have sexual activities, at other times a VPA measurement failed), participants are eliminated completely from MANOVA leading to unnecessary loss of power.

An assumption of ANOVA is that the means of the variables are normally distributed. Tabachnick and Fidell indicate that the F-test is robust against violations of normality of variables if there are at least 20 degrees of freedom for error in a univariate anova, provided that there are no outliers [16]. In most of our analyses the number of degrees of freedom is 55 (in some of the analyses the number of degrees of freedom is a bit lower because sometimes women did not have sexual activities). The dependent variables have no outliers, except for the six dependent variables of the VPA, who have a few, so, except for the six dependent variables of the VPA the assumptions of anova are met. For VPA we created log-transformed variables and this removed almost all of the outliers. To investigate the robustness of the results for VPA we carried out an analysis on the original variables as well as on the log-transformed variables. The log-transformed variables yielded an almost identical pattern of significant results as the original analysis. We report the results of the original analysis because the original variables are more easily interpretable.

The alpha level was set at 0.05 for all tests carried out. All tests were two-tailed. We made no adjustment for multiple testing. In this article many tests have been carried out and the application of, e.g., the popular Bonferroni correction would lead to conservative results and extreme loss in power. Although we do not control for chance capitalization, nearly all analyses show significant results in the same direction, showing that these findings are not by chance.

There were no data exclusions. Less than 5% of the data were missing. When data from an experimental day during the ambulatory experiment were missing, the data were estimated by taking the averaged data over the other 2 experimental days of the same drug condition.

Results

This article only reports about the T+PDE5i condition compared with placebo in relation to sensitivity for sexual cues. In part 3 the results of the third drug condition (T+5-

HT_{1A}ra) will be described along with the subdivision in low and high inhibition (see chapter 7).

Participants

Fifty-six otherwise healthy patients (Mean age 39.8 [±10.36]; premenopausal: N = 43, postmenopausal: N = 13) diagnosed as having HSDD or FSAD for at least 6 months prior to study entry completed the study (for a trial profile see Figure 2).

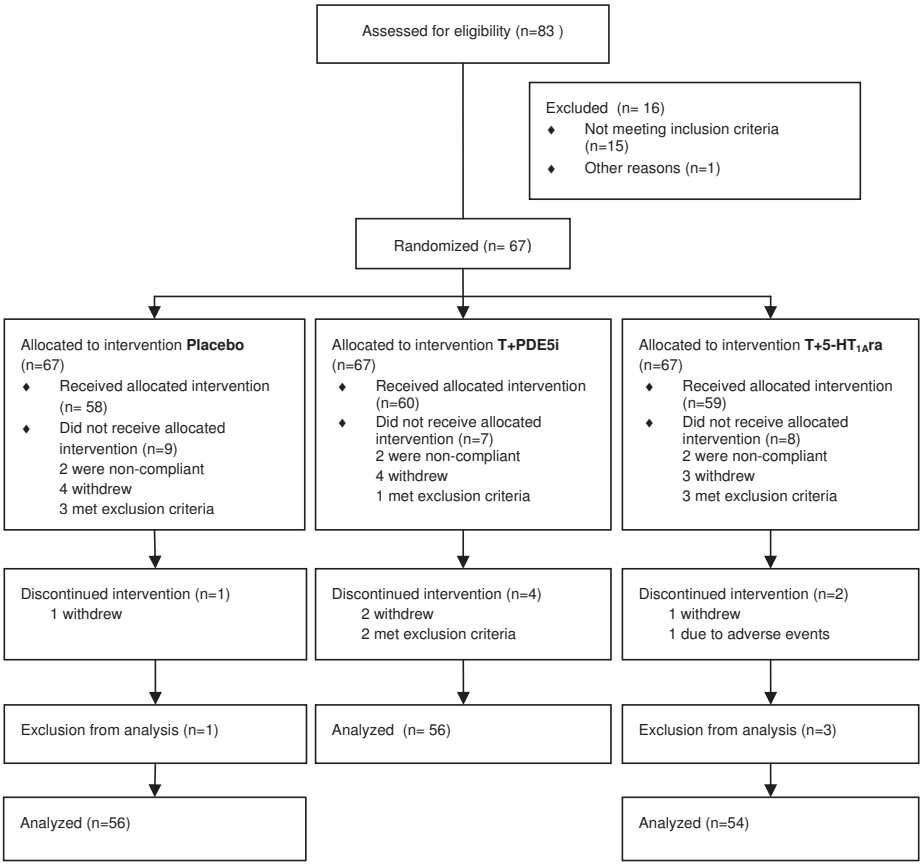

Figure 2 Clinical trial profile and disposition of women. Three women were not analyzed in the T+5-HT_{1A}ra condition. One participant did not receive T+PDE5i, therefore could not be determined as either a high or low inhibitor for analysis in the T+5-HT_{1A}ra condition. The remaining two subjects' data were unreliable; one subject owned a company which went bankrupt during the course of the study and one subject had severe maxillary sinusitis which in her opinion influenced her sexual functioning during one of the treatment regimes (the T+5-HT_{1A}ra regime, as determined after unblinding).

Women diagnosed with primary FSAD (N = 11), reported experiencing low sexual desire. Women rarely perceive desire and arousal as two separate entities of the sexual response cycle, which was also true for the women in our study. This is also one of the reasons for the proposed revision in DSM-V of the combining of the diagnoses HSDD and FSAD into "Sexual Interest/Arousal Disorder." Because of this, these women were included in the study, which had no effect on the results. During the clinical interview all women reported moderate to severe distress from their sexual complaints. Thirty-one women used hormone-containing contraceptives, 15 used other forms of contraceptives, and 10 used no contraceptives. Forty-nine women were Caucasian, three were Black, two were Asian, one was mixed Black/Caucasian, and one was mixed Asian/Caucasian. All baseline hormonal values were in the normal female reproductive and/or postmenopausal range. None of the dependent variables were influenced by menopausal status and hormonal contraception (data not shown). Demographics and baseline characteristics of the subgroups are presented in Table 1.

Effects of T+PDE5i Relative to Placebo: Preconscious Attentional Bias for Sexual Cues

The results on the emotional Stroop task revealed a clear group difference regarding the T+PDE5i induced alterations in preconscious attention for sexual cues. In women with a low sensitive system for sexual cues, T+PDE5i induced an increase of preconscious attention allocation [$F(1, 54) = 13.27$, $P < 0.001$] (Figure 3). This reversed pattern is consistent with the results reported by van der Made et al. [5,6].

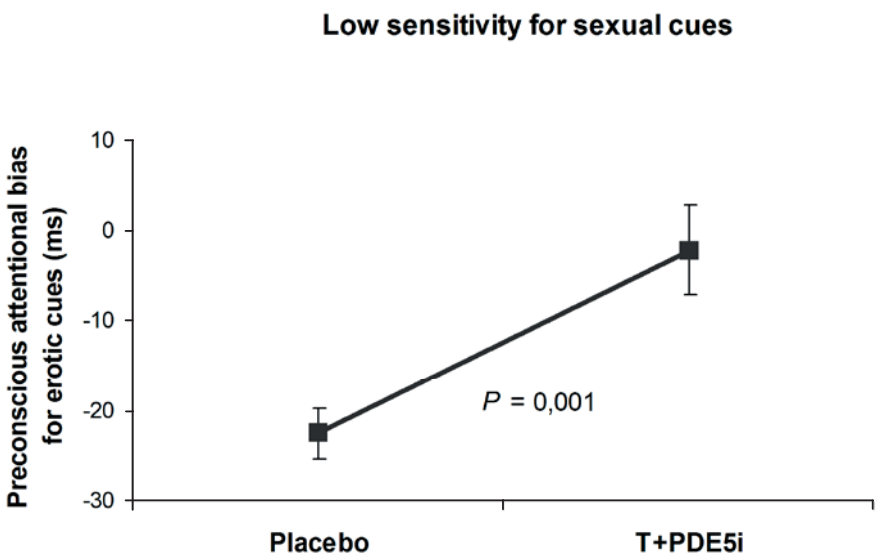

Figure 3 Preconscious attentional bias for sexual cues. Treatment with T+PDE5i relative to placebo produced an increase in the preconscious attentional bias (the differences between the mean reaction times of erotic and neutral words) for sexual cues in women with a relative insensitivity for sexual cues.

Table 1 Baseline characteristics and demographics

	Low sensitive women	High sensitive women	*P*
N	29	27	
Age, years	39.3 ± 10.4	40.3 ± 10.4	n.s
Body mass index, kg/m^2	25.1 ± 4.0	24.0 ± 3.5	n.s
Race, no (%)			n.s.*
Caucasian	25 (86.2)	24 (88.9)	
Black	2 (6.9)	1 (3.7)	
Asian	0 (0)	2 (7.4)	
Other	2 (6.9)	0 (0)	
Parity, no (%)			n.s.*
Para 0	9 (31)	7 (25.9)	
Para 1	5 (17.2)	3 (11.1)	
Para ≥ 2	15 (51.8)	17 (63)	
Menopausal status, no (%)			n.s.*
Premenopausal	22 (75.9)	21 (77.8)	
Postmenopausal	7 (24.1)	6 (22.2)	
—Surgical menopause	1 (3.4)	0 (0)	
Contraception, no (%)			n.s.*
Hormonal	18 (62.1)	13 (48.2)	
—Combination Pill	8 (44.4)	7 (53.8)	
—Progestagen (IUD, implanon)	8 (44.4)	5 (38.5)	
—Vaginal ring (progestin and estrogen)	2 (11.1)	1 (7.7)	
Nonhormonal	6 (20.7)	9 (33.3)	
—Condoms	1 (16.7)	1 (11.1)	
—Sterilization	3 (50.0)	3 (33.3)	
—Sterilization partner	2 (33.3)	5 (55.5)	
None	5 (17.2)	5 (18.5)	
FSD diagnosis, no (%)			n.s.*
Primary HSDD	24 (82.8)	21 (77.8)	
Primary FSAD	5 (17.2)	6 (22.2)	
Negative sexual experiences (inappropriate touching, [attempted] rape, other), no (%)			n.s.*
Yes	11 (37.9)	12 (42.9)	
No	18 (62.1)	15 (53.6)	
Duration of current relationship, years	12.5 (38)	18.0 (29)	n.s.†

Age and Body Mass Index are represented as mean ± standard deviation. Duration of current relationship did not fit the normal distribution and therefore is represented as median (range).
*Chi-square test
†Mann–Whitney *U*-test
IUD = intra uterine device; FSD = female sexual dysfunction; HSDD = Hypoactive Sexual Desire Disorder; FSAD = Female Sexual Arousal Disorder; n.s. = not significant

Effects of T+PDE5i Relative to Placebo: Ambulatory Experiment

During the first part of the study T+PDE5i as compared with placebo produced statistically significant overall increases in the subjective indices of sexual function (experienced genital arousal and sexual desire) in women with a relative insensitivity for sexual cues. There were no treatment-order effects observed.

Low Sensitive Women

The results of the VPA were only significant in the fantasy condition showing higher levels of the VPA in the T+PDE5i treatment (M = 0.39, SE = 0.11) compared with placebo (M = 0.14, SE = 0.05), [F(1,28) = 7.68, $P = 0.010$] in women with low sensitivity for sexual cues (Figure 4).

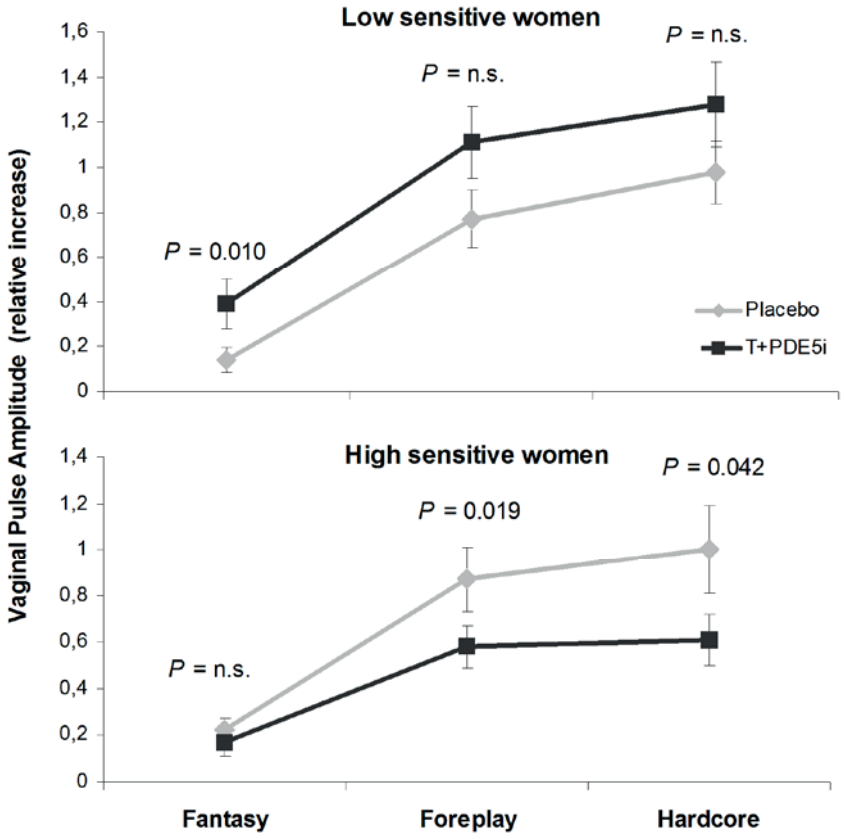

Figure 4 Ambulatory experiment: The effects of T+PDE5i relative to placebo on vaginal pulse amplitude. There was a significant interaction effect between the drug (placebo vs. T+PDE5i) and the group (low vs. high sensitivity for sexual cues), [F(1, 54) = 8.50, $P = 0.005$].

In the subgroup of women with low sensitivity for sexual cues (N = 29) the experience of genital arousal was significantly higher during the T+PDE5i treatment in the fantasy (M = 14.41, SE = 1.21), foreplay (M = 21.00, SE = 1.36) and explicit sex condition (M = 22.34, SE = 1.49) compared to placebo (fantasy; M = 9.59, SE = 1.02, [F(1,28) = 21.95, $P < 0.001$], foreplay; M = 14.34, SE = 1.23, [F(1,28) = 31.92, $P \leq 0.001$], explicit sex; M = 16.31, SE = 1.44, [F(1,28) = 21.11, $P < 0.001$], Figure 5).

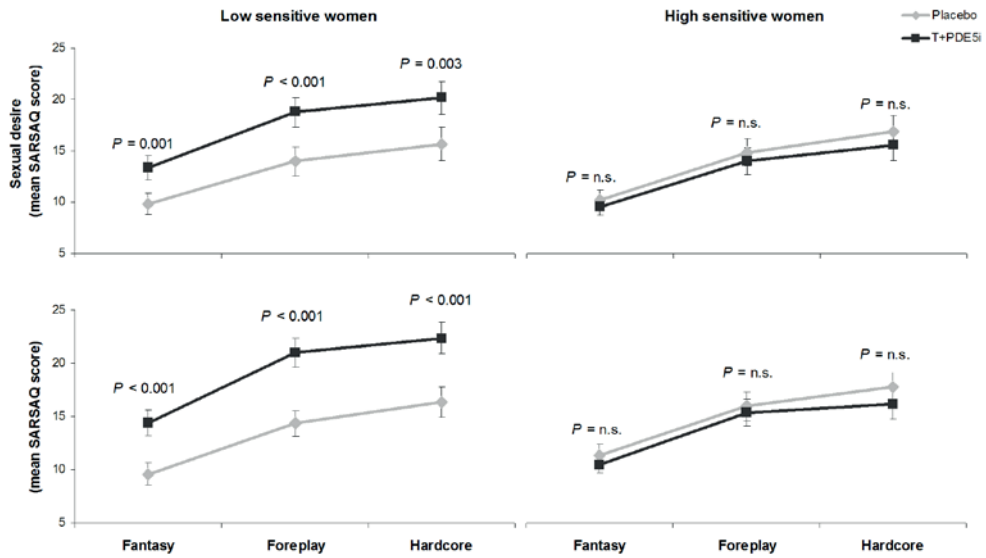

Figure 5 Ambulatory experiment: The effects of T+PDE5i relative to placebo on the experience of genital arousal and sexual desire. T+PDE5i, relative to placebo and under all stimulus conditions, produced higher levels of the experience of genital arousal and sexual desire in women with a relative insensitivity for sexual cues. There was a significant interaction effect between the drug (placebo vs. T+PDE5i) and the group (low vs. high sensitivity for sexual cues) for both measures (experience of genital arousal: [F(1, 54) = 21.24, $P < 0.001$], subjective report of sexual desire: [F(1, 54) = 13.48, $P < 0.001$]). SARSAQ = Sexual Arousal Response Self-Assessment Questionnaire

The experience of sexual desire was significantly higher during the T+PDE5i treatment in the fantasy (M = 13.34, SE = 1.18), foreplay (M = 18.76, SE = 1.47) and explicit sex condition (M = 20.14, SE = 1.62) compared with placebo (fantasy; M = 9.97, SE = 1.01, [F(1,28) = 13.39, $P = 0.001$], foreplay; M = 13.97, SE = 1.44, [F(1,28) = 16.65, $P < 0.001$], explicit sex; M = 15.69, SE = 1.58, [F(1,28) = 10.86, $P = 0.003$], Figure 5).

High Sensitive Women

During the T+PDE5i treatment within the high sensitive subgroup (N = 27), the VPA showed lower levels in the foreplay (M = 0.58, SE = 0.09) and explicit sex condition (M = 0.61, SE = 0.11) compared with placebo (foreplay; M = 0.87, SE = 0.14,

[F(1,26) = 6.28, $P = 0.019$], explicit sex; M = 1.0, SE = 0.19, [F(1,26) = 4.60, $P = 0.042$], Figure 4).

Effects of T+PDE5i Relative to Placebo: Bedroom Experiment

During the bedroom experiment treatment with T+PDE5i relative to placebo produced overall statistically significant increases in sexual functioning. There were no treatment-order effects observed.

Low Sensitive Women

Sexual Satisfaction During Sexual Events. Treatment with T+PDE5i produced a statistically significant [F(1,23) = 6.34, $P = 0.019$] increase in sexual satisfaction (M = 3.36, SE = 0.16) as compared with placebo (M = 2.96, SE = 0.20), Figure 6.

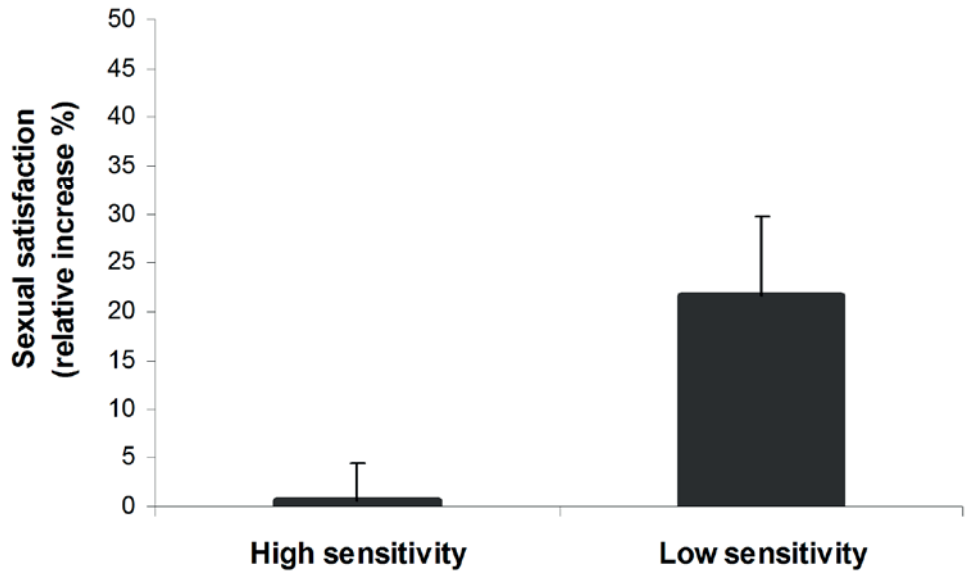

Figure 6 Bedroom experiment: The relative increase in sexual satisfaction following T+PDE5i treatment. In women with a relative insensitivity for sexual cues treatment with T+PDE5i increased sexual satisfaction with 22%, relative to placebo, while in the high sensitive group there was no such relative increase

Weekly Diary. Our analysis of the weekly reports revealed somewhat higher levels (statistically not significant) during treatment with T+PDE5i as compared with placebo for sexual desire (M = 2.72, SE = 0.16 vs. M = 2.54, SE = 0.16), genital arousal (M = 2.80, SE = 0.14 vs. M = 2.59, SE = 0.15), and sexual improvement (M = 1.40, SE = 0.16 vs. M = 1.19, SE = 0.09).

Monthly Diary. The results of the SEI questionnaire showed that during the T+PDE5i treatment participants perceived more improvement in sexual arousal/desire (M = 2.07, SE = 0.18) compared with placebo (M = 1.62, SE = 0.14), [F(1,28) = 7.04, *P* = 0.013]. Moreover, this improvement was more frequently attributed to the medication during the T+PDE5i treatment (M = 1.52, SE = 0.09) compared with placebo (M = 1.21, SE = 0.08), [F(1,28) = 7.66, *P* = 0.010], Figure 7.

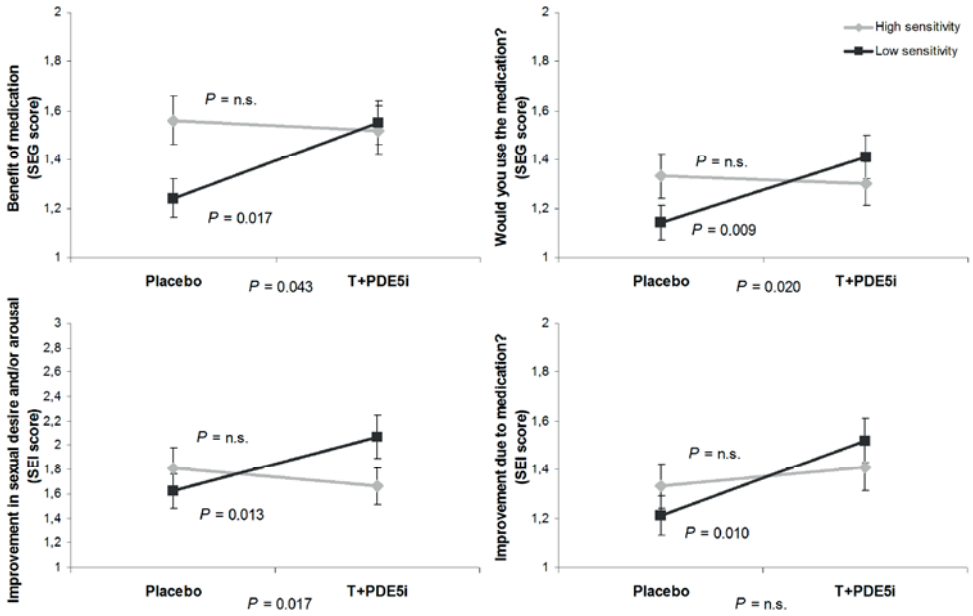

Figure 7 Bedroom experiment: The effects of T+PDE5i relative to placebo on the subjective indices of the Subjective Evaluation of Gain (SEG) and Subjective Evaluation of Improvement (SEI) questionnaire. There was a significant interaction effect between the drug (placebo vs. T+PDE5i) and the group (low vs. high sensitivity for sexual cues) for all dependent measures, with exception of "improvement due to medication." Benefit from medication: [F(1, 54) = 4.30, *P* = 0.043], would you use the medication: [F(1, 54) = 5.80, *P* = 0.020], and improvement in sexual desire and/or arousal: [F(1, 54) = 6.03, *P* = 0.017].

The results of the SEG questionnaire showed that during the T+PDE5i treatment, low sensitive participants experienced more benefit from the study medication (M = 1.55, SE = 0.09) than from the placebo (M = 1.24, SE = 0.08), [F(1,28) = 6.41, *P* = 0.017]. Low sensitive women also indicated that when the drugs are available via prescription they would use T+PDE5i (M = 1.41, SE = 0.09) more than placebo, (M = 1.14, SE = 0.07), [F(1,28) = 7.93, *P* = 0.009], Figure 7.

High Sensitive Women

The results of the high sensitive subgroup showed no statistically significant drug effects in the bedroom experiment.

Treatment with T+PDE5i was well tolerated. Only "mild" to "moderate" adverse were reported. All adverse events were caused by the PDE5i component (see Table 2). During the study, no serious adverse events were reported.

Table 2 Treatment related adverse events

	T+PDE5i (%)*	Placebo (%)†	Total (%)‡
Flushing	23.0	3.7	9.6
Headache	15.9	2.4	7.4
Lightheadedness	0.9	0.6	3.9
Dizziness	1.1	0.2	4.2

* Percentage T+PDE5i medication = AE T+PDE5i/552 units
† Percentage placebo medication = AE placebo/542 units
‡ Percentage total medication = AE total/1,636 units
T = testosterone; PDE5i = phosphodiesterase type 5 inhibitor; AE = adverse events

Discussion

We suggested different causal mechanisms for the lack of sexual desire in women with HSDD, which was taken into account when designing and developing new medicines for HSDD. In the present article, we have focused on the results of treatment with sublingual testosterone combined with a PDE5i, developed for women with HSDD and a relatively insensitive brain system for sexual cues.

In earlier experiments, we have shown that sublingual testosterone activates central sexual mechanisms thereby increasing salience of sexual stimuli, which affects central sexual stimulation and can thereby increase sexual motivation [7,8]. Central sexual stimulation is a prerequisite for PDE5i to be effective. In the present study, we again showed that sublingual testosterone influences central sexual processing, reflected by the increase in preconscious attentional bias in low sensitive women. Treatment with T+PDE5i affects mechanisms involved in processing of sexual information, and improves physiological and subjective sexual responding under institutional psychophysiological lab conditions, in particular demonstrated for women with HSDD and low sensitivity for sex [5,6]. The present results again show that T+PDE5i influences central sexual information processing, as reflected in an increase in preconscious attentional bias for sex in low sensitive women and that this treatment caused increased physiological sexual responding in the fantasy condition, and more pronounced, significant effects in subjective measures of sexual functioning under all stimulus conditions in an ambulatory psychophysiological lab in the domestic setting. These results demonstrate that the findings of van der Made et al. determined under institutional lab conditions also hold in a psychophysiological measurement setting at home [5,6]. In extension, and more importantly, treatment with T+PDE5i, as compared with placebo, caused a statistically significant higher level of sexual satisfaction during sexual events in women with a relatively low sensitive system for sex. In the active drug condition, these women also reported improvement on other subjective measures of sexual functioning; they indicated that they experienced positive effects, that they attributed these effects to the

medication, and that they would use it if available via prescription. These findings are of importance because they indicate that T+PDE5i's positive effect was also meaningful to the subjects, and not just a statistically significant increase which was not perceived as such by the subjects.

An important difference between our on-demand approach and chronic testosterone treatments is that the effects in our treatment form occur nearly directly (about 3.5 hours post-dose) compared with chronic formulations, which take weeks to take effect. As described in chapter 5, the unbound fraction of testosterone is the most bioactive testosterone and therefore responsible for the effects on behavior. If free fraction testosterone increases much later under chronic treatment, it is expected that the pharmacodynamic effect, i.e., increases in sexual motivation and desire, will occur in the same time-dependent manner [17]. This is consistent with several studies describing the effects of a testosterone patch on sexual behavior as described in chapter 5. As stated in chapter 5, one decisive factor in the time-dependent difference in behavioral effects between sublingual or chronic treatment with testosterone is the influence of an SHBG saturation threshold. This saturation threshold mechanism will ensure that the use of sublingual testosterone is accompanied by an increase in free levels of testosterone within 15 minutes after administration. These differences between the acute, on-demand dosage forms containing testosterone and the chronic dosage forms are of such importance because the amount of exposure to testosterone is much less in the former. Less exposure means fewer side effects, and in the case of testosterone, this would mean that the chance of adverse events like increased hair growth and acne are much less likely to occur. Indeed, testosterone-related side effects were not observed during this study, and are neither to be expected to occur during long-term treatment. The medication was well tolerated, with only mild to moderate, transient adverse events which were caused by the PDE5i component. The majority of studies investigating chronic testosterone administration in the treatment of women's sexual behavior have been done in naturally or surgically menopausal women [18–20]. The present study demonstrates that T+PDE5i is well tolerated and effective in premenopausal as well as in postmenopausal women. The results revealed no interaction with menopausal status or hormonal contraception.

The medication regimes in the present study were relatively short (3 weeks for the bedroom experiment). However, seeing that drug efficacy in a phase III testosterone gel trial was not statistically significant higher than placebo during 6 months of therapy [21], the current results are promising. More extensive research is needed to establish the effects of T+PDE5i over a longer period of time, but we expect the large placebo effects, which were also encountered in the current study, to decrease over time. Since the present study already shows significant superior efficacy as compared with placebo, this can even be expected to increase when tested during a longer period of time.

In the present study, the ratio between low and high sensitive subjects was approximately 50/50 (N = 29 vs. N = 27). This 50/50 division between low and high sensitivity is not inherent to the calculation which we used to define sensitivity; if a subject is faster in color naming of masked erotic words than of nonerotic words, she is low sensitive. If she is slower in color naming of masked erotic words, she is high sensitive. In our previous studies this division was approximately 40/60 [5,6]. Together, these studies measured 41 women with HSDD. With the present data, we have determined the sensitivity to sexual cues in 97 women. Taken together, our study population revealed a 45/55 division in low and high sensitive subjects. More research in a higher number of women is necessary to determine if this ratio between low and high sensitive subjects with HSDD is generalizable.

The low and high sensitive women only differed from each other on the Stroop task, and on the measures of sexual arousal under condition of T+PDE5i, as compared with placebo. They did not differ in severity of their sexual complaints or any other trait measure. As sexual cue sensitivity is testosterone dependent (see chapter 5), combinations of biological markers reflecting (and influencing) the activity of the androgen and serotonin systems (see chapter 5) may well be able to differentiate between high and low sensitive women. Without such markers, or without the emotional Stroop task, assessing cue sensitivity in the broader HSDD population will be difficult.

The use of an on-demand therapy for HSDD seems counterintuitive. Why would women with low desire bother to take an on-demand sex drug if she is not “in the mood” for sex? One of the main DSM-IV-TR criteria for HSDD is that the desire problems cause marked distress or interpersonal difficulties. Women seeking help for HSDD do so because of their distress, i.e., they want to want to have sex. These women are thus prepared to take a medicine on-demand, in the hope that several hours later, they will be more receptive to their spouses' advances or more willing to initiate sex.

Based on these results we tentatively conclude that the combination of sublingual testosterone and a PDE5i is a safe and promising potentially effective pharmacotherapy for women with HSDD as the result of an insensitive system for sexual cues. Future studies will have to confirm this.

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CHAPTER 7

Toward Personalized Sexual Medicine (Part 3): Testosterone combined with a serotonin1A receptor agonist increases sexual satisfaction in women with HSDD and FSAD, and dysfunctional activation of sexual inhibitory mechanisms

Journal of Sexual Medicine 2013;10:824-837

Abstract

Introduction. Among other causes, low sexual desire in women may result from dysfunctional activation of sexual inhibition mechanisms during exposure to sex. Administration of sublingual 0.5 mg testosterone (T) increases the sensitivity of the brain to sexual cues, which might amplify sexual inhibitory mechanisms further in women already prone to sexual inhibition. Sexual stimulation might elicit a prefrontal cortex (PFC)-mediated phasic increase in sexual inhibition, in which activity of 5-hydroxytryptamine (5-HT, serotonin) is involved. A single dose of 5-HT_{1A} receptor agonist (5-HT_{1A}ra) might reduce the sexual stimulation induced PFC-mediated sexual inhibition during a short period after administration. Consequently, treatment with a single dose of T+5-HT_{1A}ra might enhance sexual responsiveness, particularly in women exhibiting sexual inhibition.

Aim. To investigate if treatment with a single dosage of T+5-HT_{1A}ra will produce improvement in sexual functioning in women with Hypoactive Sexual Desire Disorder (HSDD) as the result of dysfunctional high sexual inhibition.

Methods. Fifty-four women were divided on the basis of their excitatory or inhibitory responses during T+phosphodiesterase type 5 inhibitor (PDE5i) in low (N = 26) and high inhibitors (N = 28). Physiological and subjective indices of sexual functioning were measured in a participant-controlled ambulatory psychophysiological experiment at home (the first week of each drug treatment). In a bedroom experiment (the subsequent 3 weeks), sexual functioning was evaluated by event, week, and monthly diaries.

Main Outcome Measures. Subjective: sexual satisfaction, experienced genital arousal, sexual desire. Physiological: vaginal pulse amplitude.

Results. Women with high inhibition show a marked improvement in sexual function in response to treatment with T+5-HT_{1A}ra relative to placebo and relative to T+PDE5i.

Conclusions. The present study demonstrated that on-demand T+5-HT_{1A}ra is a potentially promising treatment for women with HSDD, particularly for those women who are prone to sexual inhibition.

Introduction

Sexual dissatisfaction is a complaint with a high prevalence which negatively interferes with psychological and social well-being. In women, the most common complaint is low sexual desire, which is classified as Hypoactive Sexual Desire Disorder (HSDD) [1]. As stated in previous chapters, HSDD might result from a relative insensitivity for sexual cues or might be caused by dysfunctional activation of sexual inhibitory mechanisms during sexual stimulation. In chapter 6, it is demonstrated that combined treatment with testosterone and a phosphodiesterase type 5 inhibitor (T+PDE5i) improves sexual functioning in women with HSDD, and who have a relatively insensitive system for sexual cues. As expected, some women deteriorated by treatment with T+PDE5i. This subgroup of women might be prone to sexual inhibition during sexual stimulation (see part 1 of this series, chapter 5). In the present study, our focus is on treatment of these women who did not respond or who had a decreased response to on T+PDE5i, for which we assume, as the result of increased activation of central inhibitory mechanisms.

It is widely accepted that the prefrontal cortex (PFC) is involved in the inhibitory control of human behavior [2], including sexual behavior [3,4]. (see also chapter 5). An important mediator of inhibitory mechanisms is the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) [5], which also exerts abundant inhibitory effects via the PFC [6]. We assume that a sexual event, which is consciously or subconsciously negatively valenced—which is dependent on an individual's trait properties, their experiences, and the particular circumstance in which an event occurs—can induce a phasic increase in serotonergic activity in areas of the PFC involved in sexual inhibition. Acute treatment with a serotonin$_{1A}$ receptor agonist (5-HT$_{1A}$ra) decreases serotonergic activity for a short time after administration. Accordingly, acute treatment with a 5-HT$_{1A}$ra might decrease sexual-stimuli-induced phasic serotonergic inhibitory control in the PFC, which in turn might prevent or reduce the inhibitory response to sexual cues in women with HSDD and prone to sexual inhibition. We hypothesize that for this subgroup of women with HSDD, and enhanced activation of sexual inhibitory mechanisms elicited by sexual stimulation, treatment with T+5-HT$_{1A}$ra will improve their physiological and subjective sexual responses during ambulatory lab conditions, and their sexual satisfaction during sexual events.

Methods

The 56 heterosexual women who participated in this study signed a written informed consent and received reimbursement for their participation. The local medical ethics committee (Stichting Therapeutische Evaluatie Geneesmiddelen Medisch Ethische Toetsingscommissie, Almere, the Netherlands) approved this study, which was carried out in agreement with International Conference on Harmonization-Good Clinical Practice (ICH-GCP), and monitored by a certified Contract Research Organization (CRO) (PSR Group, Hoofddorp, the Netherlands).

Women were eligible if they were healthy, between 21 and 70 years, and had a diagnosis of HSDD or Female Sexual Arousal Disorder (FSAD) according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (Text Revision) DSM-IV-TR criteria [1]. Subjects were diagnosed by an experienced psychologist. If the subject was diagnosed with any psychiatric disorder other than HSDD or FSAD they were excluded, also any treatment for female sexual dysfunction within 7 days before or during the study was excluded. Furthermore women were excluded if they were using oral contraceptives containing antiandrogens or more than 50 μg estrogen, Cytochrome P450 3A4 (CYP3A4) inhibitors, CYP3A4 inducers, nitrates, monoamine oxidase inhibitors, calcium channel blockers, antidepressants, opiates, and medicinal herbs like St. John's wort. Cardiovascular exclusions included a history of myocardial infarction, stroke or life-threatening arrhythmia within the prior 6 months, uncontrolled hypertension, atrial fibrillation/flutter or any other significant abnormality observed on electrocardiogram (ECG). Gynecological exclusions included pelvic inflammatory disease, vaginal infection, previous prolapse and incontinence surgery affecting the vaginal wall, abnormal uterine bleeding patterns, perimenopausal hormonal status, pregnancy and breastfeeding in the past 6 months. Lastly women were excluded with clinically relevant endocrine disease, neurological disease, severe or acute liver disease, history of severe hepatic impairment, body mass index above 35, and vision impairment.

Women were recruited and enrolled from referrals, newspaper advertisements, the Internet, and our own database. To determine eligibility, participants were screened 4 weeks prior to study entry. In addition to an assessment of medical history with detailed sexual, gynecological, and psychological history, all subjects received a physical examination including a 12-lead ECG, a vaginal culture test to exclude infection, standard biochemistry and hematological laboratory tests, and pelvic examination. Biochemical parameters including hematology, liver function tests, electrolytes, creatinine, uric acid, glucose and lipids were collected at baseline, following each treatment period, and at the final follow-up visit. Serum concentrations of total testosterone, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, and prolactin were measured at baseline. All subjects also had their thyroid status assessed; thyroid stimulating hormone and free thyroxine.

A urine pregnancy test was administered to all women of childbearing potential. Investigators monitored and followed up any reports of adverse events. On a pretreatment visit, a trained female experimenter explained the study requirements and procedures to the participants. A practice trial was conducted, including insertion of the vaginal photoplethysmograph and a practice session of the emotional Stroop task.

Study Design

In a randomized, double-blind, placebo-controlled, cross-over design, each participant underwent three different medication treatments: (i) **placebo**: placebo for testosterone (cyclodextrin solution without testosterone) and placebo for the PDE5i (PDE5i = sildenafil) and 5-HT_{1A}ra (5-HT_{1A}ra = buspirone) (powder-filled gelatin capsule without sildenafil/buspirone); (ii) **T+PDE5i:** the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and sildenafil (50 mg, hidden in a powder-filled gelatin capsule); (iii) **T+5-HT_{1A}ra**; the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and buspirone (10 mg, hidden in a powder-filled gelatin capsule). A single medication regime lasted 4 weeks and the order of the three medication regimes was randomized. Between each medication regime there was a washout period of 1 week. Participants received 14 units of medication (throughout the manuscript, 1 medication or 1 unit of medication means the combination of the sublingually administered solution *and* the oral capsule) during each medication regime; instructions were given to have at least 48 hours between two medication intakes. Participants were instructed to take the cyclodextrin solution (testosterone or placebo) sublingually 4 hours prior to each measurement session or sexual event and rinse it under the tongue for 1 minute. They were instructed to ingest the capsule (sildenafil, buspirone, or placebo) 2.5 hours later. The sequence of the drugs and time frame were such that the pharmacological effects of the PDE5i and 5-HT_{1A}ra coincide with the window of T-induced behavioral effects, approximately 3–6 hours after T administration. Following the completion of a drug regime, the research physician took in any leftover medication of the previous regime and handed out the next regime's medication box.

Study Procedure

Ambulatory Psychophysiological At-Home Experiment

Participants visited the study site 12 times during a period of 26 weeks for screening and safety control visits. In the first week of each 4-week medication regime, participants underwent three experimental psychophysiological measurement sessions (under condition of study medication) at home using participant-controlled (i.e., no experimenters were present) ambulatory laboratory [7]. See Figure 1 in chapter 6, for an overview of the study design and procedures.

In premenopausal women not using oral contraceptives, experimental days were within the first 10 days after their menstruation. Users of oral contraceptives were not measured during their pill-free period.

During each experimental session, subjects engaged in 2 minutes each of: (i) self-induced sexual fantasy; (ii) viewing an erotic film clip depicting sexual foreplay; and (iii) viewing an erotic film depicting explicit heterosexual intercourse. The sexual fantasy

condition was preceded by a 6-minute baseline establishment period during which subjects viewed a neutral film clip. The foreplay and heterosexual intercourse films were preceded by 2 minutes return to baseline periods (see Stimuli, Apparatus, and Measures below, for a detailed description of the neutral and erotic stimulus material). At the start of the experimental session, and after each exposure to sexual stimuli (the fantasy session and film clips), we measured "experiences of genital arousal" and "sexual desire" by means of the Sexual Arousal Response Self-Assessment Questionnaire (SARSAQ; see Stimuli, Apparatus, and Measures below, and [8]). During all neutral and sexual stimulus sessions, the vaginal pulse amplitude (VPA) was continuously measured (see Stimuli, Apparatus, and Measures below). Before the baseline establishment period and finally as the last element of the experimental session, we measured biases in preconscious attention for sexual cues by means of an emotional Stroop task.

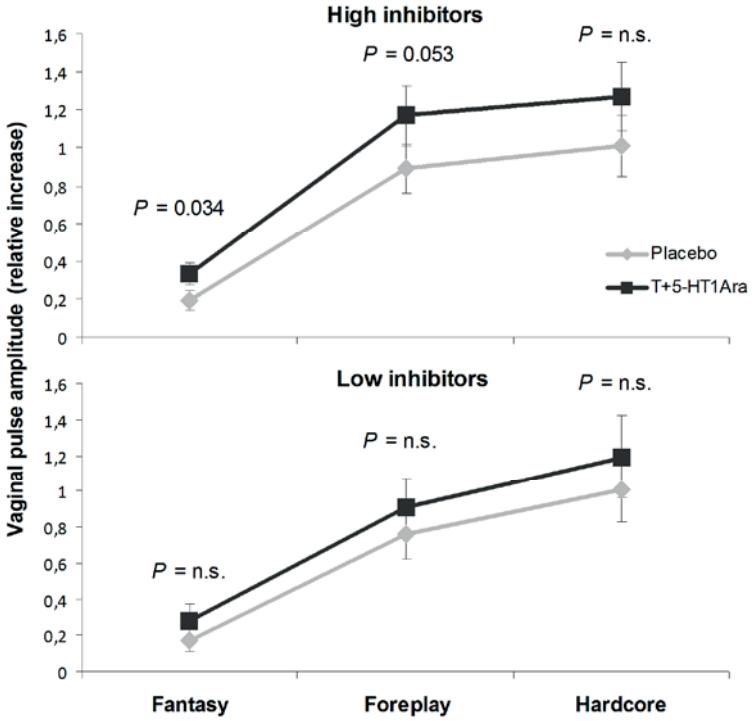

Figure 1 Ambulatory experiment: The effects of T+5-HT_{1A}ra relative to placebo on vaginal pulse amplitude. There was no significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors), [$F(1, 52) = 0.151$, P = n.s].

Bedroom Experiment

For the remaining 3 weeks, participants were instructed to use at least one medication per week, with an attempt at a sexual event (cuddling, coitus, or masturbation). For the remainder, they were free to take their medication whenever they wanted but not

during 2 consecutive days. When they had experienced a sexual event they were asked to fill out an event diary within 24 hours and a diary at the end of each week.

Stimuli, Apparatus, and Measures

Audiovisual Stimuli

For this study, neutral, erotic foreplay, and explicit sex film clips were used. Neutral clips were 6-minute and 2-minute clips from Dutch action films. The 6-minute neutral film clips were used to establish a baseline VPA. Erotic foreplay clips were 2-minute clips showing kissing, caressing, and cunnilingus, but no fellatio. Explicit sex clips were 2-minute clips showing cunnilingus and coitus, including visible penetration. The erotic film footage was selected and edited by female research associates and then rated by other female research associates to ensure that all clips were within set specifications. All digitally sampled film clips were presented using Presentation software (Neurobehavioral Systems, Albany, CA, USA). For a more detailed description see Bloemers et al. [7].

Ambulatory Laboratory

The ambulatory laboratory is based on the MobiHealth Mobile remote monitoring system (MobiHealth B.V., Enschede, The Netherlands) [9]. Study-specific functionality of this system enables VPA measurements, stimulus presentation, and execution of emotional Stroop tasks to be performed at an arbitrary time and location (e.g., house of a participant). This laboratory is operated autonomously by the participant. The ambulatory laboratory transmits all measured data to a secure central database server, at which the researcher can obtain the data for further analysis. This method allows for a more ecologically valid psychophysiological measurement of sexual responding. See for a detailed description the study by Bloemers et al. [7].

Main Outcome Measures: Ambulatory Laboratory

VPA

The VPA reflects phasic changes in vaginal engorgement corresponding with each heartbeat. VPA was measured using a vaginal photoplethysmograph, a tampon-shaped device containing an infrared light-emitting diode and a photosensitive light detector (photodiode). For a detailed description of the VPA, see chapter 6.

SARSAQ

The SARSAQ is a 10-item self-report questionnaire using a seven-point Likert scale (ranging from “not at all” to “extremely”), adapted from Morokoff and Heiman [10] and Heiman and Hatch [11]. It measures current subjective feelings of genital arousal and sexual desire. Five items concern subjective feelings of genital responding, and five

items concern subjective feelings of sexual desire. The SARSAQ was administered via the ambulatory laboratory laptop whereby the number keys (from 1 to 7) were used to complete the questionnaire.

Main Outcome Measures: Bedroom Experiment

Event Diary

In this secure Web-based diary, subjects were asked 10 questions (one open-ended, four multiple-choice, five 5-point Likert scale items) concerning the type, duration, pleasantness, and intensity of the sexual event. Participants were instructed to fill out the event diary within 24 hours following each sexual event (e.g., cuddling, coitus, masturbation).

Week Diary

Women were also instructed to fill out the secure Web-based week diary once a week during each medication regime. Subjects were asked their experiences during the past week regarding: (i) sexual desire (six-point Likert scale: 1 = nothing to 6 = much); (ii) vaginal arousal (six-point Likert scale: 1 = nothing to 6 = much); (iii) sexual improvement/deterioration (four-point Likert scale: 1 = nothing to 4 = much); and (iv) if they could attribute the improvement/deterioration to the medicines (yes/no).

Subjective Evaluation of Improvement (SEI)

The SEI questionnaire was used to determine, after each medication regime, if the subject felt there had been an overall improvement in sexual desire, sexual arousal or both, and if they attribute this improvement to the medication. Subjects answered yes or no to both questions. The SEI was administered as a pen and paper test.

Subjective Evaluation of Gain (SEG)

The SEG questionnaire was used to determine, after each medication regime, if the subject felt that she had any meaningful benefit from the study medication, and if she would use it if it were available by prescription. Subjects answered yes or no to both questions. The SEG was administered as a pen and paper test.

Measures: On Site

Hormonal Measures

Serum total testosterone, serum estradiol, serum progesterone, serum prolactin, serum LH, serum FSH, and serum SHBG were measured through electrochemiluminescence radioimmunoassay with COBAS kits of Roche Diagnostics (Mannheim, Germany), using a Modular E170 at OLVG Hospital (Roche Diagnostics, Amsterdam, the Netherlands). The

measuring range for testosterone was 0.087–52.0 nmol/L. The coefficient of variation was 8%.

Data Transformation and Reduction

Ambulatory Experiment

For each drug treatment condition, we used the mean levels of the 3 experimental days of the experience of genital arousal, sexual desire, preconscious attentional bias for sexual cues and VPA, respectively, for further analyses.

Bedroom Experiment

Based on high Cronbach's alphas of the six items measuring duration, pleasantness, and intensity of the sexual events (measured by the event diary) during the different drug conditions (placebo: $\alpha = 0.95$; T+PDE5i: $\alpha = 0.95$; T+5-HT_{1A}ra: $\alpha = 0.93$) we calculated the mean of these items, as a measure of "sexual satisfaction." Each question of the week diary, SEG and SEI questionnaire were analyzed separately. To analyze sexual satisfaction and the week diary items, we calculated the average of each dependent variable over the 3 weeks of drug treatment.

Different Drug Treatment Responders

The T+PDE5i negative responders were defined as follows. We calculated the reversed scores of items measuring sexual satisfaction and other positive experiences of sexual functioning during the T+PDE5i treatment period (for items of both the event diary and week diary). We used "sexual desire" and "improvement in sexual function" items from the week diaries and "pleasure,""horny," and "genital arousal" of the event diaries. The raw item scores were standardized and summated, representing the variable "Sexual Inhibition." Cronbach's alpha over the five items was sufficiently high ($\alpha = 0.90$) to summate. Subjects with negative summated z-scores were termed "low inhibitors" and subjects with positive summated z-scores were termed "high inhibitors." We used this division of high and low inhibitors as between-subject factor in analyzing the effects of T+5-HT_{1A}ra compared with placebo and to T+PDE5i, respectively, on sexual experiences during the bedroom experiment.

Statistical Analysis

Demographic data were analyzed to investigate possible group differences (low vs. high inhibitors) with an independent *t*-test if the data were normally distributed. For non-normally distributed data, the Mann–Whitney test was used. Categorical data were compared between the groups with a chi-square test.

For each of the dependent variables, separate 2 × 2 repeated measures analyses of variance (repeated measures anovas) were carried out. The within-subject factor had two levels (placebo and T+5-HT_{1A}ra or T+PDE5i and T+5-HT_{1A}ra). The between-subject factor also had two levels (low inhibition vs. high sexual inhibition). For all analyses menopausal status (premenopausal vs. postmenopausal) and hormonal contraception (hormonal contraception vs. nonhormonal contraception) were analyzed in a repeated measures anova for each dependent variable.

We have chosen to report an anova for each of the dependent variables separately, instead of analyzing these variables together in a multivariate analysis (manova). The reasons for this choice are twofold: first, interest goes out into effects on each of the dependent variables separately as they are quite different in nature (e.g., we have a physiological measure such as VPA as well as self-report measures such as diaries under different experimental conditions); second, when missing data are scattered over all dependent variables (e.g., sometimes women did not have sexual activities, other times a VPA measurement failed), participants are eliminated completely from manova leading to unnecessary loss of power.

An assumption of ANOVA is that the means of the variables are normally distributed. Tabachnick and Fidell indicate that the F-test is robust against violations of normality of variables if there are at least 20 degrees of freedom for error in a univariate ANOVA, provided that there are no outliers [12]. In most of our analyses the number of degrees of freedom is 52 (in some of the analyses the number of degrees of freedom is a bit lower because sometimes women did not have sexual activities). The dependent variables have no outliers, except for the nine dependent variables of the VPA (three drug conditions: placebo, T+PDE5i, and T+5-HT_{1A}ra and three stimuli conditions), who have a few, so, except for the nine dependent variables of the VPA the assumptions of ANOVA are met. For VPA we created log-transformed variables and this removed almost all of the outliers. To investigate the robustness of the results for VPA we carried out an analysis on the original variables as well as on the log-transformed variables. The log-transformed variables yielded an almost identical pattern of significant results as the original analysis. We report the results of the original analysis because the original variables are more easily interpretable.

An alpha level of 0.05 was set for all analyses. We made no adjustment for multiple testing. In parts 2 and 3 of this series, many tests have been carried out and the application of, e.g., the popular Bonferroni correction would lead to an extreme loss in power. Although we do not control for chance capitalization, nearly all analyses show significant results in the same direction, showing that these findings are not by chance.

Less than 5% of the data were missing. When a data for an experimental day during the ambulatory experiment were missing, the data were estimated by taking the averaged data over the other 2 experimental days of the same drug condition.

Results

As described in chapter 6, the analysis in the T+PDE5i condition was done with 56 women, while for the T+5-HT_{1A}ra condition the data of 54 women have been used. There were two data exclusions, both exclusions were caused by factors that retrospectively biased the study results during the T+5-HT_{1A}ra drug regime; one subject lost her own business due to financial problems and another woman had severe maxillary sinusitis, which in her opinion influenced her sexual functioning during the T+5-HT_{1A}ra regime. All baseline hormonal values were in the normal female reproductive and/or postmenopausal range. None of the dependent variables were influenced by menopausal status and hormonal contraception (data not shown). For a detailed description of the study population, see Table 1.

Ambulatory Experiment

When the participants were divided on the basis of their response on T+PDE5i in low (N = 26) and high inhibitors (N = 28), the results showed that treatment with T+5-HT_{1A}ra relative to placebo (or to T+PDE5i) produced statistically significant increases in most dependent variables of the ambulatory experiment in high inhibitors compared with low inhibitors. The interaction effects between drug and group in the ambulatory experiment are described in the caption for each figure.

Effects of T+5-HT1Ara Relative to Placebo

Low Inhibition Group. Our analysis in the low inhibition group (N = 26) revealed no statistically significant effects of the dependent variables in the ambulatory experiment.

High Inhibition Group. The results of the high inhibition group (N = 28) of the VPA during the fantasy condition revealed a statistically significant effect. In the T+5-HT_{1A}ra condition VPA was statistically significant higher (M = 0.33, SE = 0.06) compared with the placebo condition (M = 0.19, SE = 0.05) [F(1,27) = 4.97, $P = 0.034$]. In the foreplay condition this effect was marginally significant (M = 1.17, SE = 0.16) vs. (M = 0.89, SE = 0.13), respectively, [F(1,27) = 4.10, $P = 0.053$]. There were no significant results in the explicit sex condition (see Figure 1).

The experience of genital arousal was higher after T+5-HT_{1A}ra administration as compared with placebo in the fantasy condition (M = 11.71, SE = 1.01 vs. M = 9.64, SE = 0.94) [F(1,27) = 8.87, $P = 0.006$], the foreplay condition (M = 16.14, SE = 1.26 vs. M = 13.29, SE = 1.08) [F(1,27) = 8.35, $P = 0.008$], and the explicit sex condition (M = 17.86, SE = 1.42 vs. M = 15.18, SE = 1.31) [F(1,27) = 7.03, $P = 0.013$], and see Figure 2.

Table 1 Baseline and clinical characteristics of the participants

	Low inhibitors	High inhibitors	*P*
N	26	28	
Age, years	39.1 ± 11.3	40.07 ± 9.8	n.s
Body mass index, kg/m^2	25.5 (15.5)	22.2 (15.5)	n.s*
Race, no (%)			
Caucasian	20 (76.9)	27 (96.4)	n.s.†
Black	3 (11.5)	0 (0)	
Asian	2 (7.7)	0 (0)	
Other	1 (3.8)	1 (3.6)	
Parity, no (%)			0.038†
Para 0	12 (46.2)	4 (14.3)	
Para 1	3 (11.5)	5 (17.9)	
Para ≥ 2	11 (42.3)	19 (67.8)	
Menopausal status, no (%)			n.s.†
Premenopausal	18 (69.2)	23 (82.1)	
Postmenopausal	8 (30.8)	5 (17.9)	
—Surgical menopause	0 (0)	1 (20)	
Contraception, no (%)			n.s.†
Hormonal	16 (61.5)	15 (53.6)	
—Combined oral contraceptive pill	6 (37.5)	8 (53.3)	
—Progestagen (IUD, implanon)	7 (43.8)	7 (46.7)	
—Vaginal ring (progestin and estrogen)	3 (18.8)	0 (0)	
Nonhormonal	5 (19.2)	9 (32.1)	
—Condoms	1 (20.0)	1 (11.1)	
—Sterilization	3 (60.0)	3 (33.3)	
—Sterilization partner	1 (20.0)	5 (55.6)	
None	5 (19.2)	4 (14.3)	
FSD diagnosis, no (%)			n.s.†
Primary HSDD	21 (80.8)	23 (82.1)	
Primary FSAD	5 (19.2)	5 (17.9)	
Duration of current relationship, years	11.56 ± 8.4	16.9 ± 9.2	0.033
Negative sexual experiences (inappropriate touching, [attempted] rape, other), no (%)			0.025†
Yes	7 (26.9)	16 (57.1)	
No	19 (73.1)	12 (42.9)	

Age and relationship duration are in are means ± standard deviation. For the body mass index (BMI) median (range) is described. The BMI is the weight in kilograms divided by the square of the height in meters.
* Mann–Whitney test
† Chi-square test
IUD = intra uterine device; FSD = female sexual dysfunction; HSDD = Hypoactive Sexual Desire Disorder; FSAD = Female Sexual Arousal Disorder; n.s. = not significant

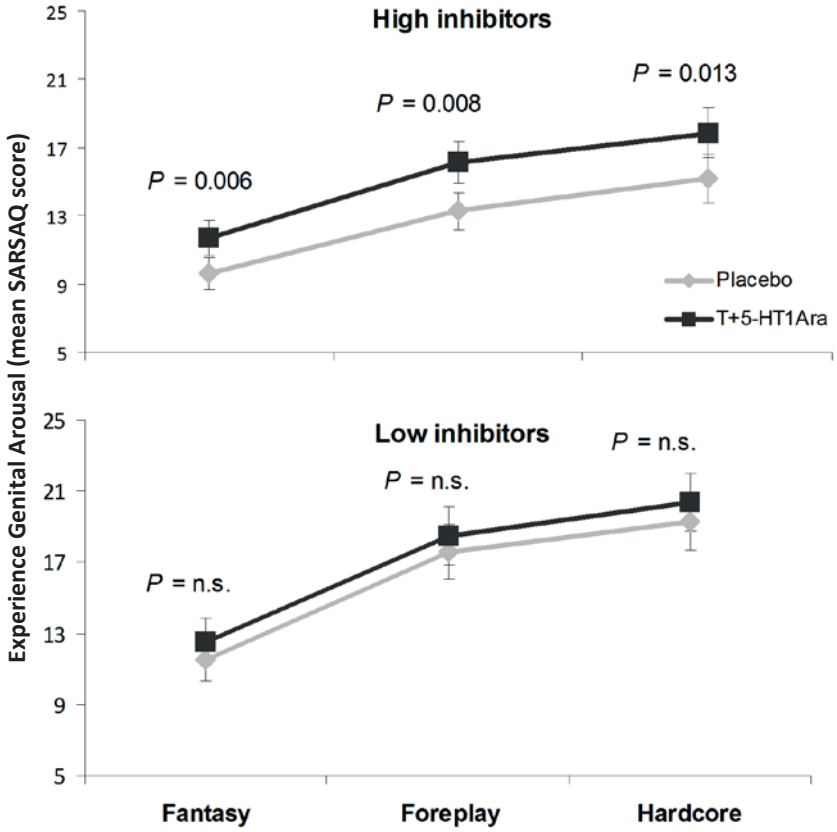

Figure 2 Ambulatory experiment: The effects of T+5-HT_{1A}ra relative to placebo on the experience of genital arousal. T+5-HT_{1A}ra, relative to placebo and under all stimulus conditions, produced higher levels of the experience of genital arousal in women in the high inhibition group. There was no significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors) for the experience of genital arousal, [F(1, 52) = 1.30, *P* = n.s]. SARSAQ = Sexual Arousal Response Self-Assessment Questionnaire

Subjective reports of sexual desire were significantly higher during T+5-HT_{1A}ra treatment in the fantasy condition (M = 10.96, SE = 1.03) compared with placebo (M = 8.96, SE = 0.80) [F(1,27) = 6.90, *P* = 0.014]. There were no significant results in the foreplay and explicit sex condition.

Effects of T+5-HT1Ara Relative to T+PDE5i

The results demonstrate that treatment with T+5-HT_{1A}ra (as compared with T+PDE5i) produced no statistically significant differences between levels of physiological (VPA) and subjective indices of sexual functioning (experience of genital arousal and subjective reports of sexual desire) in low and high inhibitors, with the exception of subjective reports of sexual desire in the explicit sex condition which were higher after T+5-HT_{1A}ra

administration (M = 15.61, SE = 1.46) compared with the T+PDE5i condition (M = 13.86, SE = 1.25) [F(1,27) = 4.38, *P* = 0.046].

Bedroom Experiment

The results show that treatment with T+5-HT_{1A}ra relative to placebo produced statistically significant increases in all measures of sexual functioning in high inhibitors compared with low inhibitors. The interaction effects between drug and group in the bedroom experiment are described beneath each figure.

Effects of T+5-HT1Ara Relative to Placebo

Low Inhibition Group. Our analysis in the low inhibition group (N = 26) revealed no statistically significant effects of the dependent variables in the bedroom experiment.

High Inhibition Group

Sexual Satisfaction During Sexual Events. In the T+5-HT_{1A}ra condition "sexual satisfaction" was statistically significant higher (M = 2.98, SE = 0.13) compared with the placebo condition (M = 2.51, SE = 0.14) [F(1,25) = 9.51, *P* = 0.005]. See Figure 3.

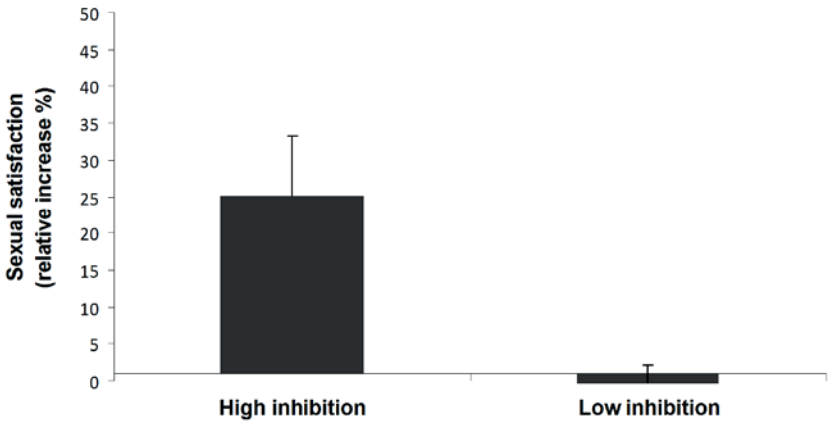

Figure 3 Bedroom experiment: The relative increase in sexual satisfaction following T+5-HT_{1A}ra treatment. In women with high inhibition, treatment with T+5-HT_{1A}ra increased sexual satisfaction with 25%, relative to placebo, while in the low inhibition group there was no such relative increase.

Weekly Diary. According to the weekly diaries, participants in the high inhibitor group reported more sexual desire in the T+5-HT_{1A}ra condition (M = 2.45, SE = 0.11) compared with placebo (M = 2.19, SE = 0.11) [F(1,27) = 6.49, *P* = 0.017]. This effect was also seen for genital arousal (M = 2.60, SE = 0.09 vs. M = 2.29, SE = 0.11) [F(1,27) = 10.08, *P* = 0.004]. In the T+5-HT_{1A}ra condition, more participants reported an improvement of

sexual functioning (M = 1.31, SE = 0.09) compared with placebo (M = 1.09, SE = 0.06) [F(1,27) = 4.35, *P* = 0.047]. This improvement was more frequently attributed to T+5-HT_{1A}ra medication (M = 1.48, SE = 0.09) compared with placebo (M = 1.22, SE = 0.07) [F(1,27) = 4.80, *P* = 0.037]. See Figure 4.

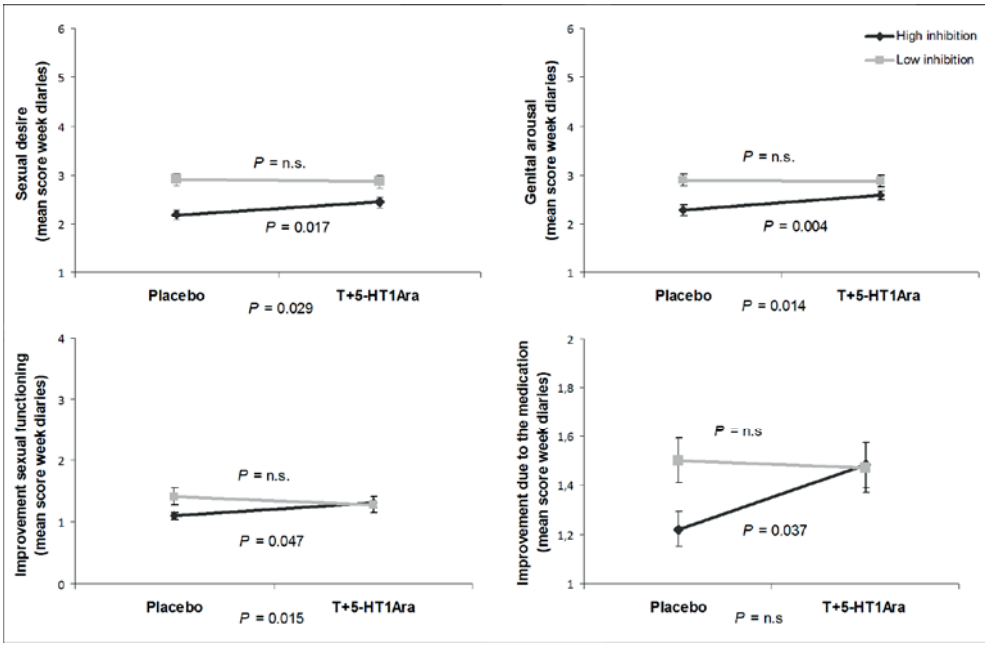

Figure 4 Bedroom experiment: The effects of T+5-HT_{1A}ra relative to placebo on the subjective indices of the week diaries. There was a significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors) for all dependent measures with exception of "improvement due to medication." Sexual desire: [F(1,52) = 5.05, *P* = 0.029]; genital arousal: [F(1,52) = 6.41, *P* = 0.014]; and improvement of sexual functioning: [F(1, 52) = 6.32, *P* = 0.015].

Monthly Diary. The results of the SEI questionnaire revealed that in the condition high inhibitors reported more sexual improvement in desire and/or arousal during treatment with T+5-HT_{1A}ra (M = 1.93, SE = 0.17) compared with placebo (M = 1.25, SE = 0.10) [F(1,27) = 11.56, *P* = 0.002]. They attributed this improvement to the medication more frequently in the T+5-HT_{1A}ra condition (M = 1.57, SE = 0.10) compared with placebo (M = 1.11, SE = 0.06) [F(1,27) = 18.18, *P* < 0.001].

The results of the SEG questionnaire showed that high inhibitors experienced more benefit from the T+5-HT_{1A}ra medication (M = 1.61, SE = 0.09) as compared with placebo (M = 1.25, SE = 0.83; F(1,27) = 9.25, *P* = 0.005). Regarding the question if they would use the medication if available via prescription, high inhibitors would use T+5-HT_{1A}ra (M = 1.43, SE = 0.10) more compared with placebo, (M = 1.04, SE = 0.36) [F(1,27) = 17.47, *P* < 0.001]. See Figure 5.

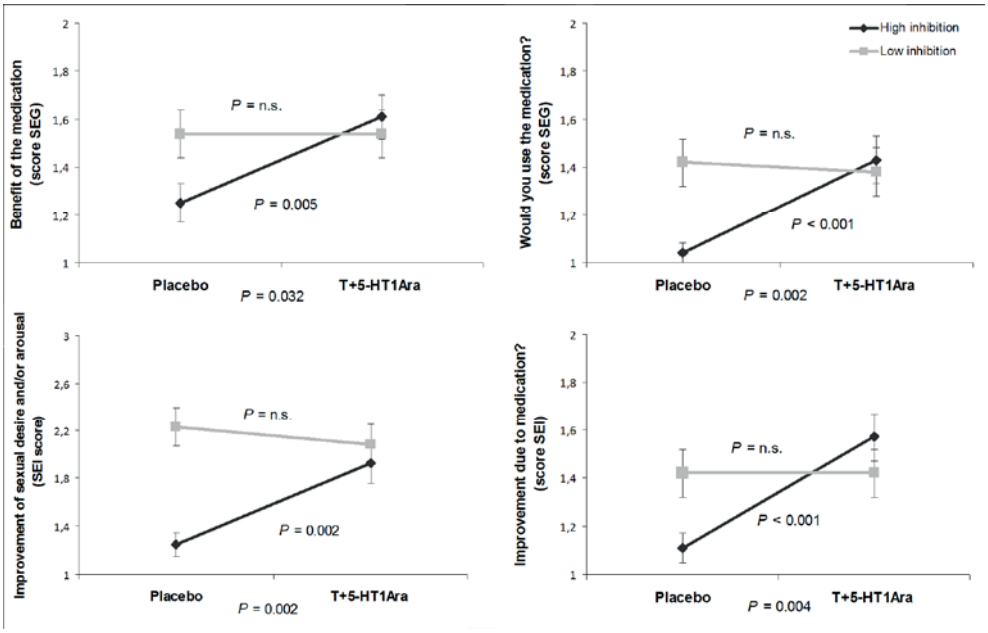

Figure 5 Bedroom experiment: The effects of T+5-HT_{1A}ra relative to placebo on the subjective indices of the Subjective Evaluation of Gain (SEG) and Subjective Evaluation of Improvement (SEI) questionnaire. There was a significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors) for all dependent measures. Benefit from medication: [F(1, 52) = 4.85, P = 0.032], would you use the medication: [F(1, 52) = 11.20, P = 0.002], improvement in sexual desire and/or arousal: [F(1, 52) = 10.68, P = 0.002], and improvement due to the medication: [F(1, 52) = 8.91, P = 0.004].

Effects of T+5-HT1Ara Relative to T+PDE5i

The results demonstrate that participants in the low inhibitor group reported more beneficial effects of T+PDE5i treatment, while the high inhibitors have more effect of T+5-HT_{1A}ra administration.

Low Inhibition Group

Sexual Satisfaction During Sexual Events. Our analysis in the low inhibition group (N = 26) revealed that treatment, "sexual satisfaction" was statistically significant higher during T+PDE5i (M = 3.82, SE = 0.11) compared with the T+5-HT_{1A}ra condition (M = 3.37, SE = 0.18) [F(1,22) = 8.84, P = 0.007]. See Figure 6.

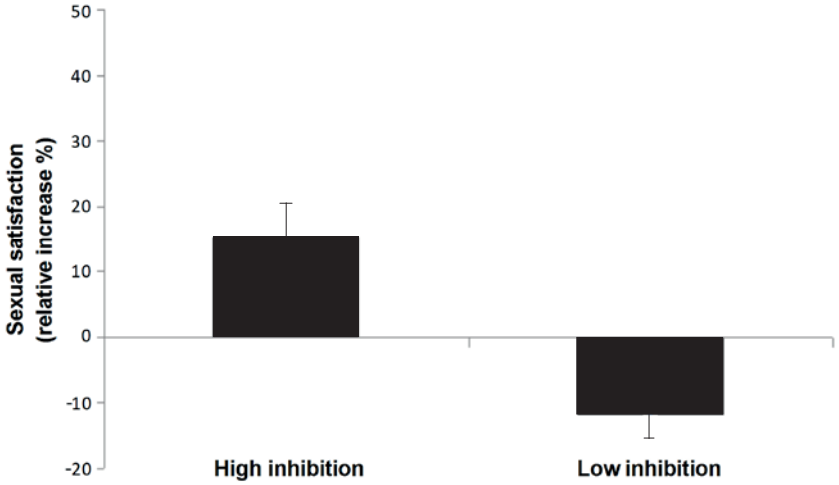

Figure 6 Bedroom experiment: The relative increase in sexual satisfaction following T+5-HT_{1A}ra treatment compared with T+PDE5i treatment (sexual satisfaction T+5-HT_{1A}ra minus satisfaction T+PDE5i). Treatment with T+5-HT_{1A}ra, relative to T+PDE5i increased sexual satisfaction significantly with 15% in women with high inhibition (while women in the low inhibitor group had more effect of T+PDE5i administration).

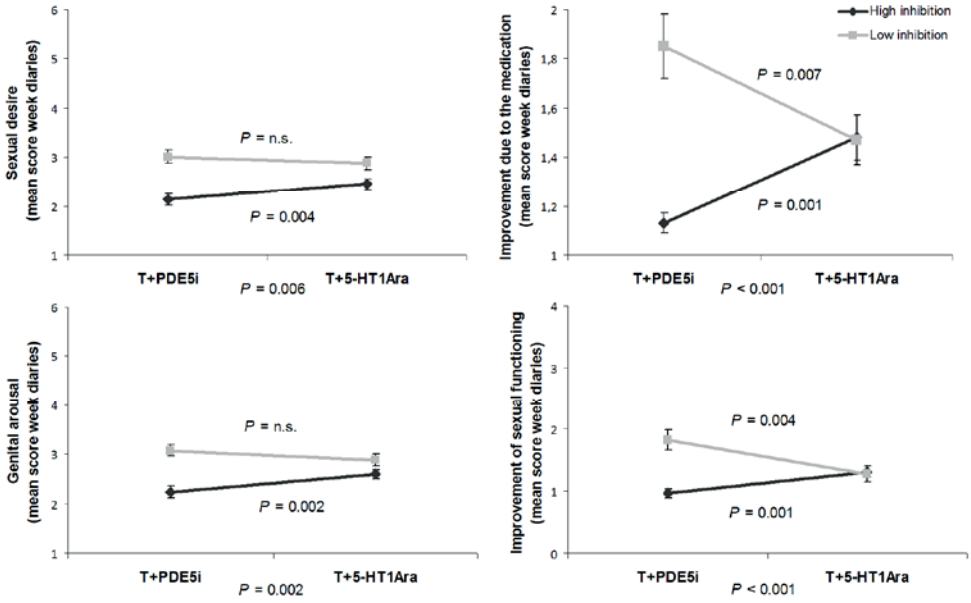

Figure 7 Bedroom experiment: The effects of T+5-HT_{1A}ra relative to T+PDE5i on the subjective indices of the week diaries. There was a significant interaction effect between the drug (T+5-HT_{1A}ra vs. T+PDE5i) and the group (low vs. high inhibitors) for all dependent measures. Sexual desire: [$F(1, 52) = 8.27$, $P = 0.006$], genital arousal: [$F(1, 52) = 11.12$, $P = 0.002$], improvement of sexual functioning: [$F(1, 52) = 21.72$, $P < 0.001$], and improvement due to the medication: [$F(1, 52) = 21.01$, $P < 0.001$].

Weekly Diary. According to the week diaries, no statistically significant differences were observed between the T+PDE5i and T+5-HT_{1A}ra condition for sexual desire or genital arousal. In the T+PDE5i condition, more low inhibition participants reported an improvement of sexual functioning (M = 1.82, SE = 0.16) compared with T+5-HT_{1A}ra (M = 1.28, SE = 0.13) [F(1,25) = 9.94, P = 0.004]. This improvement was more frequently attributed to T+PDE5i medication (M = 1.85, SE = 0.13) compared with T+5-HT_{1A}ra (M = 1.47, SE = 0.10) [F(1,25) = 8.58, P = 0.007]. See Figure 7.

Monthly Diary. The results of the SEI questionnaire revealed that in the T+5-HT_{1A}ra condition, low inhibitors reported no more sexual improvement in desire and/or arousal compared with T+PDE5i.

The results of the SEG questionnaire showed that low inhibitors experienced more benefit from the T+PDE5i medication (M = 1.81, SE = 0.08) as compared with T+5-HT_{1A}ra (M = 1.54, SE = 0.10) [F(1,25) = 5.17, P = 0.032]. Regarding the question if they would use the medication if available via prescription, low inhibitors would use T+PDE5i (M = 1.65, SE = 0.10) more compared with T+5-HT_{1A}ra, (M = 1.38, SE = 0.10) [F(1,25) = 6.62, P = 0.016]. See Figure 8.

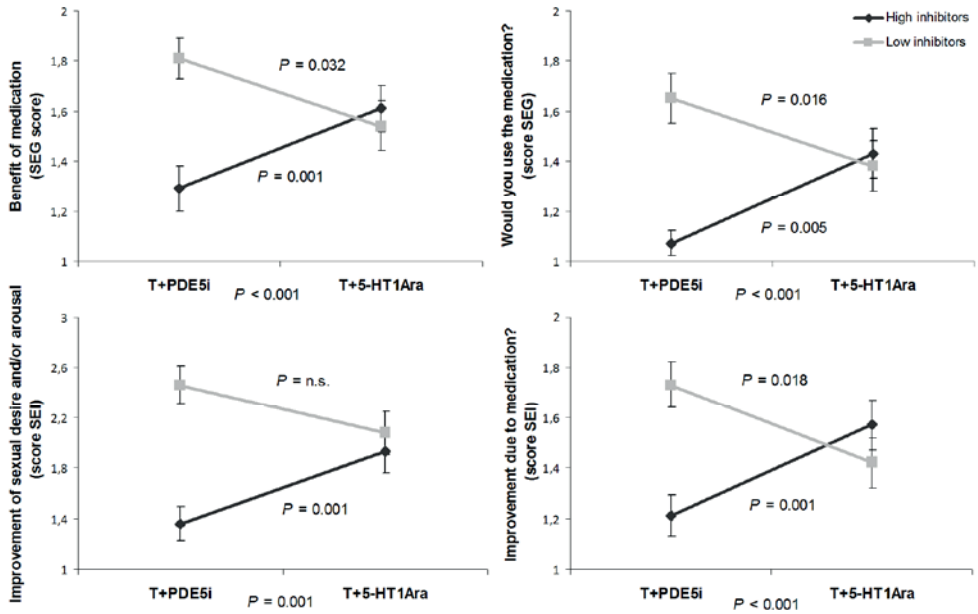

Figure 8 Bedroom experiment: The effects of T+5-HT_{1A}ra relative to T+PDE5i on the subjective indices of the Subjective Evaluation of Gain (SEG) and Subjective Evaluation of Improvement (SEI) questionnaire. There was a significant interaction effect between the drug (T+5-HT_{1A}ra vs. T+PDE5i) and the group (low vs. high inhibitors) for all dependent measures. Benefit from medication: [F(1, 52) = 16.07, P < 0.001], would you use the medication: [F(1, 52) = 15.68, P < 0.001], improvement in sexual desire and/or arousal: [F(1, 52) = 11.24, P = 0.001], and improvement due to the medication: [F(1, 52) = 19.41, P < 0.001].

High Inhibition Group

Sexual Satisfaction During Sexual Events. "Sexual satisfaction" was statistically significant higher during T+5-HT_{1A}ra treatment (M = 2.98, SE = 0.14) compared with the T+PDE5i condition (M = 2.64, SE = 0.13) [F(1,23) = 8.88, *P* = 0.007]. See Figure 6.

Weekly Diary. According to the week diaries, participants in the high inhibitor group reported more sexual desire in the T+5-HT_{1A}ra condition (M = 2.45, SE = 0.11) compared with T+PDE5i (M = 2.13, SE = 0.12) [F(1,27) = 10.08, *P* = 0.004]. This effect was also seen for genital arousal (M = 2.60, SE = 0.09 vs. M = 2.23, SE = 0.12) [F(1,27) = 11.50, *P* = 0.002]. In the T+5-HT_{1A}ra condition, more participants reported an improvement of sexual functioning (M = 1.31, SE = 0.09) compared with T+PDE5i (M = 0.96, SE = 0.07) [F(1,27) = 13.92, *P* = 0.001]. This improvement was more frequently attributed to T+5-HT_{1A}ra medication (M = 1.48, SE = 0.09) compared with T+PDE5i (M = 1.13, SE = 0.04) [F(1,27) = 13.50, *P* = 0.001]. See Figure 7.

Monthly Diary. The results of the SEI questionnaire revealed that in the condition high inhibitors reported more sexual improvement in desire and/or arousal compared during treatment with T+5-HT_{1A}ra (M = 1.93, SE = 0.17) compared with T+PDE5i (M = 1.36, SE = 0.13) [F(1,27) = 13.10, *P* = 0.001]. They attributed this improvement to the medication more frequently in the T+5-HT_{1A}ra treatment condition (M = 1.57, SE = 0.10) compared with T+PDE5i (M = 1.21, SE = 0.08) [F(1,27) = 15.00, *P* = 0.001].

The results of the SEG questionnaire showed that high inhibitors experienced more benefit from the T+5-HT_{1A}ra medication (M = 1.61, SE = 0.09) as compared with T+PDE5i (M = 1.29, SE = 0.87) [F(1,27) = 12.89, *P* = 0.001]. Regarding the question if they would use the medication if available via prescription, high inhibitors would use T+5-HT_{1A}ra (M = 1.43, SE = 0.10) more compared with T+PDE5i, (M = 1.07, SE = 0.05) [F(1,27) = 9.25, *P* = 0.005]. See Figure 8.

Both T+PDE5i and T+5-HT_{1A}ra were tolerated well. Only "mild" to "moderate adverse" were reported. All adverse events were caused by the PDE5i or 5-HT_{1A}ra components. See Table 2. During the study, no serious adverse events were reported.

Table 2 Treatment-related adverse events

	T+PDE5i (%)*	T+5-HT_{1A}ra (%)†	Placebo (%)‡	Total (%)§
Flushing	23.0	1.9	3.7	9.6
Headache	15.9	3.7	2.4	7.4
Lightheadedness	0.9	10.3	0.6	3.9
Dizziness	1.1	11.3	0.2	4.2

* Percentage T+PDE5i medication = AE T+PDE5i/552 units
† Percentage T+5-HT_{1A}ra medication = AE 5-HT_{1A}ra/542 units
‡ Percentage placebo medication = AE placebo/542 units
§ Percentage total medication = AE total/1,636 units
T = testosterone; PDE5i = phosphodiesterase type 5 inhibitor; 5-HT_{1A}ra = 5-HT_{1A} receptor agonist; AE = adverse events

Discussion

We suggested (chapter 5) the existence of different causal mechanisms for the lack of sexual desire in women with HSDD, which were taken into account when designing and developing new medicines for HSDD. In chapter 6 it is demonstrated that women with a relative insensitivity of the brain for sexual cues benefit from T+PDE5i. In this part, women with high inhibition show a marked improvement in sexual function in response to treatment with T+5-HT_{1A}ra relative to placebo and relative to T+PDE5i. These results demonstrate that, in accordance with our hypothesis, the efficacy of the treatments seems to depend on the etiological origin of the complaints.

The majority of studies investigating chronic testosterone administration or other medicines in the treatment of women's sexual behavior have been done in naturally or surgically menopausal women [13–15]. Based on the results of chapter 6 and the present study, T+PDE5i and T+5-HT_{1A}ra are well tolerated and effective in premenopausal as well as in postmenopausal women. The results revealed no interaction with menopausal status or hormonal contraception.

As described in chapter 6, the medication regimes in the present study were relatively short (3 weeks for the bedroom experiment). However, given that in most drug efficacy studies have a much longer treatment period, the current results are promising. More extensive research is needed to establish the effects of T+PDE5i and T+5-HT_{1A}ra over a longer period of time, but we expect the large placebo effects to decrease, while the drug effects are expected to increase over time. Thus, since the present study already shows significant effect of both treatment forms as compared with placebo in the subgroups which these treatments were designed, this may even be expected to increase when tested during a longer period of time.

In the present study, the ratio between low and high inhibitors was approximately 50/50 (N = 26 vs. N = 28). This near 50/50 division between low and high inhibitors is at least in part attributable to the calculation which we used to define inhibition, and thus, we are unable to determine at this time if this ratio reflects the ratio in the general HSDD population. In chapter 6, the ratio between low and high *sensitive* subjects was also approximately 50/50 (N = 29 vs. N = 27). This 50/50 division between low and high sensitivity was *not* inherent to the calculation which we used to define sensitivity and also roughly corroborated our previous findings of a division in sensitivity in HSDD subjects of approximately 40/60 (low/high sensitivity) [8]. So in the case of sensitivity, there is good reason to believe that approximately 40 to 50% of the HSDD population is low sensitive, although this will have to be confirmed in a larger population. In the present series, we have treated the analysis population as belonging to one of two ends of either continuum (a sensitivity or inhibition continuum). Future studies should take into account the interaction between sensitivity and inhibition, both in terms of prevalence as in terms of this interaction's influence on sexual behavior.

Our former studies showed a relationship between negative sexual experiences and high sensitivity for sexual cues [8,16]. The results of these studies led to the hypothesis that these women suffered from HSDD as a result of (over)activation of sexual inhibitory systems. A potential risk factor for developing a sexual dysfunction after negative sexual experiences is, according to our hypothesis, a person's sensitivity to sexual cues, which will affect positive and negative sexual experiences. High sensitive women are more sensitive for positive and negative sexual experiences. It is possible that the high sensitive women can develop HSDD (partly) because of altered serotonergic transmission, making them more sensitive to negative sexual experiences leading to subsequent over-activation of sexual inhibitory mechanisms in response to sexual arousal. In the present study we observed that women with high inhibition reported negative sexual experiences significantly more often than women with low inhibition, but we observed no such difference between the high and low sensitive women (chapter 6). It has to be noted that negative sexual experiences by themselves do not induce HSDD or high inhibition.

Number of parity was also related to group assignment, where women who were high inhibitors tended to have more children. As stated in chapter 5, women with over-activation of sexual inhibitory mechanisms are more sensitive to emotional stimuli, so any type of negative or emotionally laden experience will impact these people more profoundly. Raising children can be very demanding and can increase the pressures a woman or a couple endures in their lives and is known to affect the sexual relationship between couples [17]. On the other hand, women in the high inhibition group also had a longer relationship duration compared with the women in the low inhibitor group, this could also influence the parity and might influence induction of inhibitory mechanisms. However, further research on this topic is needed.

As stated in chapter 6, one could think that on-demand therapy for HSDD is counterintuitive. We think that because these women with HSDD experiencing distress or interpersonal difficulties with their sexual complaints, which is one of the main criteria of the DSM-IV-TR, they want to want to have sex because of the fact they seek help. We contend that different causal mechanisms could give rise to common symptoms in HSDD (see also chapter 5). While biological factors play an important role in human social interactions, psychosocial factors should also be taken into account when attempting to understand and explain these phenomena. In the present study (see also chapter 6), a common biological factor (testosterone) had opposing effects, depending on the subject's psychological state and/or psychosocial circumstances. In low sensitive women, sublingual testosterone boosted sexual motivation, while in others it caused sexual inhibition. In this latter group of women, HSDD is most likely caused by dysfunctional activation of sexual inhibitory systems. For a potential pharmacotherapy for women with HSDD and prone to sexual inhibition, the mode of action should thus be directed at alleviating this dysfunctional inhibition. Indeed, as the present study shows,

in these subjects, T+5-HT_{1A}ra could be a potentially effective pharmacotherapy. Future studies will have to confirm this.

Considerable effort has been put into developing drugs for HSDD, but with little success to date. A main assumption in these endeavors is to find one generally applicable drug for the improvement of all women with HSDD, irrespective of the different causal mechanisms involved in the sexual suffering. Halford et al. [18] recently described a similar situation with regard to drug development programs for obesity. One of the main reasons for the limited success in the development of antiobesity drugs is that too little emphasis is placed on behavioral analyses of eating behavior, in particular the heterogeneity in the motives for eating. While biological factors play an important role in human behavior, psychosocial factors should also be taken into account when attempting to understand and explain these phenomena (see also Eisenegger et al. [19]) and thus also when developing drugs for psychopathology. Along the same lines, we described in chapter 5 that different causal mechanisms could give rise to common symptoms in HSDD. In order to obtain a comprehensive understanding of human sexual (dys)function, research should be extended to conceptual analyses and empirical control of the reciprocal influences of biological and psychological mechanisms. Ideally, sexual dysfunction in human subjects should be described in terms of a constellation of interacting mechanisms, both biological and psychological, which at the same time should provide an adequate indication for treatment. Based on such an analyses, we have demonstrated that our approach of tailoring on-demand therapeutics to different underlying etiologies can be used to treat common symptoms in subgroups of women with HSDD.

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CHAPTER 8

Reduced gray matter volume and increased white matter fractional anisotropy in women with HSDD

Journal of Sexual Medicine 2014;11:753–767

Abstract

Introduction. Models of hypoactive sexual desire disorder (HSDD) imply altered central processing of sexual stimuli. Imaging studies have identified areas which show altered processing as compared with controls, but to date, structural neuroanatomical differences have not been described.

Aim. The aim of this study is to investigate differences in brain structure between women with HSDD and women with no history of sexual dysfunction, and to determine sexual behavioral correlates of identified structural deviations.

Methods. Sexual functioning and gray matter (GM) and white matter (WM) were assessed in 29 women with HSDD and 16 healthy control subjects of comparable age and socioeconomic status with no history of sexual dysfunction.

Main Outcome Measures. WM properties were measured using diffusion-weighted imaging and analyzed using fractional anisotropy (FA). GM volume was measured using three-dimensional T1-weighted recordings and analyzed using voxel-based morphometry. Sexual functioning was measured using the Sexual Function Questionnaire.

Results. Women with HSDD, as compared with controls, had reduced GM volume in the right insula, bilateral anterior temporal cortices, left occipitotemporal cortex, anterior cingulate gyrus, and right dorsolateral prefrontal cortex. Also, increased WM FA was observed within, amongst others, the bilateral amygdalae. Sexual interest and arousal correlated mostly with GM volume in these regions, whereas orgasm function correlated mostly with WM FA.

Conclusion. HSDD coincides with anatomical differences in the central nervous system, in both GM and WM. The findings suggest that decreased salience attribution to sexual stimuli, decreased perception of bodily responses and sexual emotional stimulus perception, and concomitant altered attentional mechanisms associated with sexual response induction.

Introduction

The execution of human sexual behavior depends on a delicate balance within and between biological mechanisms [1], for example, (neuro)endocrinological, (neuro)physiological and vascular, and (socio)psychological mechanisms (e.g., cognitive, affective, sociocultural, and interpersonal) [2]. Imbalance in one or more of these systems can disturb sexual behavior; thus, many medical, psychiatric, and psychological conditions can cause sexual dysfunction as can medication and illicit drug use. There is a large group of women who suffer from sexual problems which are not accounted for by medical or psychiatric conditions or by drug (ab)use. Before the introduction of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders *(*DSM 5*)* [3], these sexual problems were classified into four major categories of women's sexual dysfunction in DSM 4th Edition—Text Revision *(*DSM 4-TR*)* [4]: hypoactive sexual desire disorder (HSDD), female sexual arousal disorder (FSAD), female orgasmic disorder, and sexual pain disorders. In DSM 5, HSDD and FSAD have been merged into female sexual interest/arousal disorder (FSIAD). The etiology of sexual disorders, irrespective of the DSM 4 or *5* classification, is comprised of a large array of biological and psychological factors. Investigations into (common subsets of) biological and/or psychological markers for sexual dysfunctions (as defined by DSM 4-TR; research into FSIAD is still lacking) has yielded inconsistent results [5]. Nonetheless, each disorder's core symptoms are the same for each patient suffering from that disorder, suggesting common ground at some level.

To date, no investigations have been made into possible structural neuroanatomical deviations in HSDD. A noninvasive method of investigating possible neuroanatomical differences is the determination of gray matter (GM) volume and white matter (WM) fractional anisotropy (FA). GM contains neural cell bodies, and WM consists mostly of myelinated axon tracts. GM properties can be determined by voxel-based morphometry (VBM) of magnetic resonance images, which is sensitive to GM volume and density. WM properties can be determined by FA of diffusion-weighted images (DWI), which is mainly determined by axonal fiber diameter and density and fiber tract coherence. By investigating differences between women with and women without HSDD in GM and WM, we can infer how these GM and WM deviations impact information-processing within and between different brain areas and how this influences sexual behavior.

Sexual behavior is mediated by specific neurobiological networks. In these networks, incoming sensory information is integrated with the internal state influencing the behavioral state of the individual, which ultimately determines sexual behavior (for review, see [6]). Functional neuroimaging studies investigating women's and men's sexual behavior often show erotic stimulus-dependent (de)activation of the amygdala, insula, anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), and/or the nucleus accumbens [7–15]. These areas form a network which is involved in evaluation of the emotional significance of stimuli and the production of an

affective state elicited by these stimuli [16]. For psychopathologies in general, a deviation in the function or structure in one or more of these areas can be interpreted as a neurobiological correlate of this disorder [17]. Following this same line of reasoning, differences between people with and without HSDD in the structure in one or more of these areas could be indicative of a neuroanatomical correlate for this disorder. To date, there is no direct evidence that one or more of these areas are impaired in women with HSDD, but an fMRI study by Arnow and colleagues [14] provides evidence that for one or more (associated) areas, this may be the case. In their study, women with HSDD had significantly lower activation in bilateral entorhinal cortices and significantly higher activation in the right medial frontal gyrus, the right inferior frontal gyrus, and bilateral putamen, in response to erotic film fragments. The authors concluded that HSDD subjects may differ from non-HSDD subjects in the encoding of erotic stimuli and/or retrieval of past erotic experiences (entorhinal cortex), and that attentional focus to sexual responses may be increased in HSDD (medial and inferior frontal gyri). Investigating neuroanatomical differences between subjects with and without HSDD in GM and WM may substantiate these findings. It may also elaborate on them as certain structural differences may not become manifest in fMRI because of the artificial setting and simplified stimulus-response paradigm.

Sexual desire, arousal, orgasm, and (lack of) pain all contribute to a successful sexual event and are at least partially interdependent in their occurrence and strength. The co-occurrence of these behavioral states is not necessary for a satisfying sexual event (e.g., one can have satisfying sex without reaching orgasm), but dysfunction in one of these domains can affect functioning of another, which often leads to secondary diagnoses. Indeed, a common empirical finding is that sexual desire and arousal highly overlap, as do the dysfunctional states as described by the diagnoses HSDD and FSAD [18]. The overlap between these two is much greater than that between other sexual domains. This overlap was a main reason to merge HSDD and FSAD into the single diagnosis FSIAD (in DSM 5). If there are differing degrees of interdependence between sexual desire, arousal, orgasm, and pain, these may be mirrored in differing degrees of interdependence of underlying neurobiological systems, which should be taken into account when studying neuroanatomical correlates of sexual (dys)function.

In the present study, GM volume and WM FA were measured to investigate structural brain differences between women with HSDD and healthy controls. The study was largely exploratory, but it was expected that subjects with HSDD, as compared with women who did not have sexual problems, would show decreased GM volume and/or decreased WM FA in the amygdala, insula, the nucleus accumbens, and areas of the ACC, mPFC, and OFC which have all been implicated in sexual function. In order to further investigate possible differences in neuroanatomical correlates of functional domains in women with HSDD, the sexual function questionnaire (SFQ) was used to assess different aspects of sexual function. To ensure that interdependency of functional domains would

not bias the results, the SFQ desire, SFQ arousal, and SFQ enjoyment domains were collapsed into a single sexual interest and arousal (SIA) domain.

Methods

Forty-five premenopausal heterosexual women between 21 and 45 years of age completed the study: 29 women with HSDD (with or without FSAD) and 16 female controls of comparable age and socioeconomic status but with no history of sexual dysfunction. Subjects received reimbursement for their participation. This study was approved by a Medical Ethics Committee (Stichting Therapeutische Evaluatie Geneesmiddelen Medisch Ethische Toetsingscommissie, Almere, The Netherlands).

Participants were recruited from the community through advertisements in the local newspaper or selected from our research institute's volunteer database, containing women with and without sexual problems. All participants provided written informed consent to participate in this study at the start of the screening visit. During the screening visit, participants were interviewed by a trained psychologist or physician to diagnose for HSDD according to *DSM 4-TR*. Blood chemistry and hematology, electrocardiogram, and vital signs were assessed. A physician performed a physical and gynecological exam, and interviewed subjects to exclude pregnancy (via urine pregnancy test) or the intention to become pregnant during the study, lactating subjects or subjects who gave birth in the previous 6 months, pelvic inflammatory disease or an untreated vaginal infection, previous prolapse and incontinence surgery affecting the vaginal wall, other unexplained gynecological complaints, such as abnormal uterine bleeding patterns, (history of) endocrinological disorders, (history of) neurological disorders, (history of) psychiatric disorders, history of childhood sexual abuse, or substance abuse (including assessment via drug dipsticks and alcohol breath test). Eligible participants filled out the SFQ [19,20].

Qualified subjects were enrolled in the study protocol and participated in three experimental visits which were separated by 1-week intervals. During the visits, participants were placed supine in a 3T Philips Intera MRI scanner (Academic Medical Center, Amsterdam, The Netherlands). Soft cushions were placed around the head to help participants not to move during the recordings. A mirror was placed above the heads of the participants which enabled them to look out of the scanner (front end). A single scanning session took approximately 50 minutes. MRI and DWI were recorded at each experimental day.

A contract research organization (PSR Group, Hoofddorp, The Netherlands) monitored study conduct to assure protocol adherence and data collection in compliance with International Conference on Harmonization—Good Clinical Practice guidelines. PSR Group also conducted clinical database management and audited all study documentation.

Main Outcome Measures

SFQ

The SFQ is a validated 34-item self-report questionnaire that assesses sexual functioning over the past 4 weeks. Seven domains of sexual functioning were measured: (i) desire; (ii) arousal–sensation; (iii) arousal–lubrication; (iv) enjoyment; (v) orgasm; (vi) pain; and (vii) partner relationship. The SFQ domain scores were calculated according to Quirk et al. [20]. Several items have the response option "I did not take part in sexual activity," referring to the preceding 4 weeks. If a subject did not have sexual activity during this period, these items have to be scored as "missing." These missing-by-design items are the same for each subject who did not take part in sexual activity. In these cases, specific domain scores cannot be calculated, and the group mean (HSDD vs. control) was imputed per missing domain score [21].

HSDD and FSAD have been combined into a single DSM 5 diagnosis, FSIAD [3], because there is little evidence that desire and arousal are strictly separate sexual responses. We therefore compiled SFQ desire, arousal-sensation, arousal-lubrication, and enjoyment domains into a SIA domain score to optimally match the functional domain with its possible underlying biological mechanisms. The SIA domain score was calculated using SFQ domain *z*-scores as follows:

$$SIA\ domain\ score = (z\text{-}desire + z\text{-}enjoyment + ((z\text{-}arousal_S + z\text{-}arousal_L)/2))/3$$

where $arousal_S$ and $arousal_L$ are the SFQ domain scores for arousal-sensation and arousal-lubrication, respectively. The mean of the two arousal domains was used as one score in order to have comparable weight for desire, arousal, and enjoyment domain scores in the SIA domain score. *z*-Scores were used because SFQ domain scores have different ranges. The SIA domain score together with the orgasm and pain SFQ domains covers the three major *DSM 5* diagnoses for women's sexual dysfunction: FSIAD, female orgasmic disorder, and Genito-pelvic pain/penetration disorder. SFQ domain internal consistency reliability was determined for all domains including the SIA domain using Cronbach's alpha.

The means of the population characteristics of both groups were compared using one-way anova for the continuous variables and a chi-squared test for the categorical variables. If categorical variables contained fewer than five observations in a group, the Fisher's exact test was used. An alpha of 0.05 was adopted to test for significance in group differences.

GM Volume and WM FA

For each subject, the following images were acquired: three MRI (3D T1, Turbo Field Echo, TE 4.6 ms, TR 9.6 ms, FA 8°, 182 sagittal slices of 1.2 mm, FOV 2,502 mm, reconstruction matrix 256^2) and three DWI (TR 7,720 ms, TE 94 ms, flip angle 90°, FOV 224 × 224 mm, matrix size 128 × 128, 40 slices, b = 600, 94 ms).

The T1 recordings were analyzed with FMRIB Software Library (FSL; FMRIB, The Oxford Centre for Functional Magnetic Resonance Imaging of the Brain)-VBM, a VBM style analysis [22] carried out with FSL 4.1 [23]. VBM is sensitive to differences in the GM volume and density. GM-segmented brains (using all 16 control subjects and 16 randomly selected subjects from the HSDD group) were used to generate a subject-specific template. Next, we nonlinearly transformed all nonlinearly GM-segmented brains to this template and used the inverse of the Jacobian to modulate the intensity values. In this way, a higher value comes to mean that the patch of the brain from which a voxel originated was larger than in the standard template and vice versa for lower values. The exact procedure used is described in Van Gaal et al. [24], with the exception that the procedure was executed separately on the first and the average of the second and the third T1 recordings independently. FA was calculated using FSL 4.1 and tract-based spatial statistics [25]. FA is mainly determined by the properties of WM fibers and fiber tract coherence. The exact procedure used is described in Rouw & Scholte [26] with the exception that the procedure was executed separately on the first and the average of the second and the third DWI recordings independently. This procedure yielded two independently recorded datasets for both the VBM and the FA analysis. The first dataset was used to determine which regions of interest (ROIs) in the brain differed between the HSDD group (n = 29) and the control subjects (n = 16) while controlling for brain size and age as nuisance covariates. The VBM template was generated using 16 randomly selected HSDD subjects. False positives were controlled for by selecting only those regions which contained more than 1,600 mm^3 [27] with an alpha of 0.05. Group differences were evaluated using a permutation test in which group membership is shuffled with a subsequent evaluation of the difference between the dummy groups [28]. If this difference is smaller than the observed differences between the real groups, it is considered as support for a true difference. The P value is determined by dividing the number of times the dummy groups differed more than the real groups by the total number of permutations.

The second dataset (consisting of two recordings per individual) was subsequently used to determine to what degree these ROIs could be predicted by the SFQ domains that were calculated. Stepwise multiple regression analyses were used to predict GM volume and WM FA for each ROI with all three SFQ domain scores (SIA, orgasm, and pain) as predictors per model. Default settings of spss 17.0 (SPSS Inc., Chicago, IL, USA) for stepwise multiple regression were used (decision criteria: probability of F to enter ≤0.05, probability of F to remove ≥0.10). A corrected alpha of 0.007 was adopted to test

for significance in group differences. This alpha level coincides with the false discovery rate multiple comparisons correction using the false discovery rate which keeps the proportion of type 1 errors constant [29]. Pearson's correlation coefficients were calculated to further investigate relationships between SFQ domains, symptom duration, and the GM volume and WM FA. Correlations with SFQ domains were calculated over the whole group. Correlations with symptom duration were calculated only with HSDD subjects, as including controls would inflate correlations because of extreme clustering (all controls score "0" for symptom duration). A corrected alpha of 0.012 using false discovery rate was adopted to test for significance of correlations. Symptom duration was not a predefined question during the study, so it was ascertained if possible after the final visit by rechecking the diagnostic interview source documentation. Symptom duration was missing for five subjects. SPSS 17.0 was used for all statistical analyses.

Results

Participants

A total of 57 subjects were screened. Twelve subjects screened failed: four subjects reported one or more instances of childhood sexual abuse, three subjects had increased blood pressure, two subjects had sexual problems but did not meet DSM 4-TR criteria for HSDD, one subject had a history of manic-depression, one subject had hyperthyroidism, and one subject used contraindicated medication (selective serotonin reuptake inhibitor). Of the 46 subjects enrolled, one subject discontinued after the first experimental visit. A total of 29 subjects diagnosed with HSDD and 16 control subjects completed the study. Of the 29 subjects with HSDD, two were also diagnosed with primary FSAD, three with secondary FSAD, and 18 others reported sexual arousal problems to a lesser degree. Four subjects with HSDD could not reach orgasm (anymore), and five other subjects with HSDD reported having difficulties in reaching orgasm but were still able to achieve it. Three HSDD subjects had comorbidity: one subject had back problems, one subject had mild seasonal affective disorder, and one subject had vulvar complaints because of labor rupture. One control subject had lower back problems. The HSDD and control groups did not differ significantly on demographics or gynecological history. Group means for the SFQ domains (except "pain"; see Table 1) were significantly lower in the HSDD group than in the control group.

SFQ

SFQ domains showed moderate to high internal consistency reliability: SFQ desire, $\alpha = 0.92$; SFQ arousal-sensation, $\alpha = 0.90$; SFQ arousal-lubrication, $\alpha = 0.83$; SFQ orgasm, $\alpha = 0.65$; SFQ pain, $\alpha = 0.89$; SFQ enjoyment, $\alpha = 0.94$; and SFQ partner relationship, $\alpha = 0.77$. For the SIA domain score, Cronbach's alpha of the SIA domain was determined with the four SFQ domain *z*-scores used in the calculation of the SIA domain ($\alpha = 0.91$)

but also with all SFQ items of the four domains which comprise the SIA domain ($\alpha = 0.96$). SFQ desire, SFQ arousal-sensation, SFQ arousal-lubrication, and SFQ enjoyment correlated strongly (ranging from $r = 0.61$ to $r = 0.84$), underlining the substantial overlap between these functions and advising against multiple regression analysis using these domains separately as predictors because the high correlations will give unreliable results because of multicollinearity.

Table 1 Population characteristics of the HSDD and control groups

Population Characteristics	HSDD	Controls	*p* Value[‡]
Demographic Variables			
Subjects	29	16	
Yrs of age, mean ± SD	35.6 ± 6.5	35.1 ± 7.6	0.80[§]
BMI, mean ± SD	24.4 ± 4.5	24.5 ± 3.2	0.93[§]
Gynecological History			
Age of menarche, mean ± SD	13.6 ± 1.5	13.3 ± 2.2	0.60[§]
No. of Parity, mean ± SD	1.14 ± 1.0	1.25 ± 1.3	0.75[§]
Gynecological surgery, No. (%)[*]	12 (41%)	9 (56%)	0.34[¶]
Oral contraceptives, No. (%)[*]	10 (34%)	3 (19%)	0.32[**]
Sexual Functioning Questionnaire			
Desire, mean ± SD	10.9 ± 3.5	21.1 ± 4.8	<0.001[§]
Arousal-sensation, mean ± SD	8.3 ± 2.4	12.9 ± 3.2	<0.001[§]
Arousal-lubrication, mean ± SD	4.8 ± 1.6	7.2 ± 1.3	<0.001[§]
Orgasm, mean ± SD	9.0 ± 2.0	11.1 ± 1.7	0.001[§]
Pain, mean ± SD	12.7 ± 2.9	14.0 ± 1.7	0.09[§]
Enjoyment, mean ± SD	13.9 ± 4.3	22.6 ± 2.8	<0.001[§]
Partner Relationship, mean ± SD	7.2 ± 2.3	9.7 ± 0.6	<0.001[§]
SIA, mean ± SD [†]	-0.53 ± 0.51	0.95 ± 0.56	<0.001[§]

[*] Percentage based on group N.
[†] Calculated variable based on z-scores of SFQ domains (see Methods section 'Analysis of behavioral data').
[‡] Independent samples t-test was used for continuous variables, Chi-square test for categorical variables and Fisher's exact test when categorical variables contained less than 5 observations in one group.
[§] Independent samples t-test.
[¶] Chi-square test.
[**] Fisher's exact test.
BMI = body mass index; HSDD = hypoactive sexual desire disorder; SIA = sexual interest/arousal.

GM Volume and Sexual (Dys)function

Analysis of GM volume revealed six regions where GM volume was smaller in patients with HSDD than in controls: the bilateral anterior cingulate gyrus (further analyzed as a single region), the right insula, bilateral anterior temporal cortices, the left lateral occipitotemporal cortex (OTC), and the right middle frontal gyrus (which is part of the dorsolateral prefrontal cortex [DLPFC]) (see Table 2 and Figure 1).

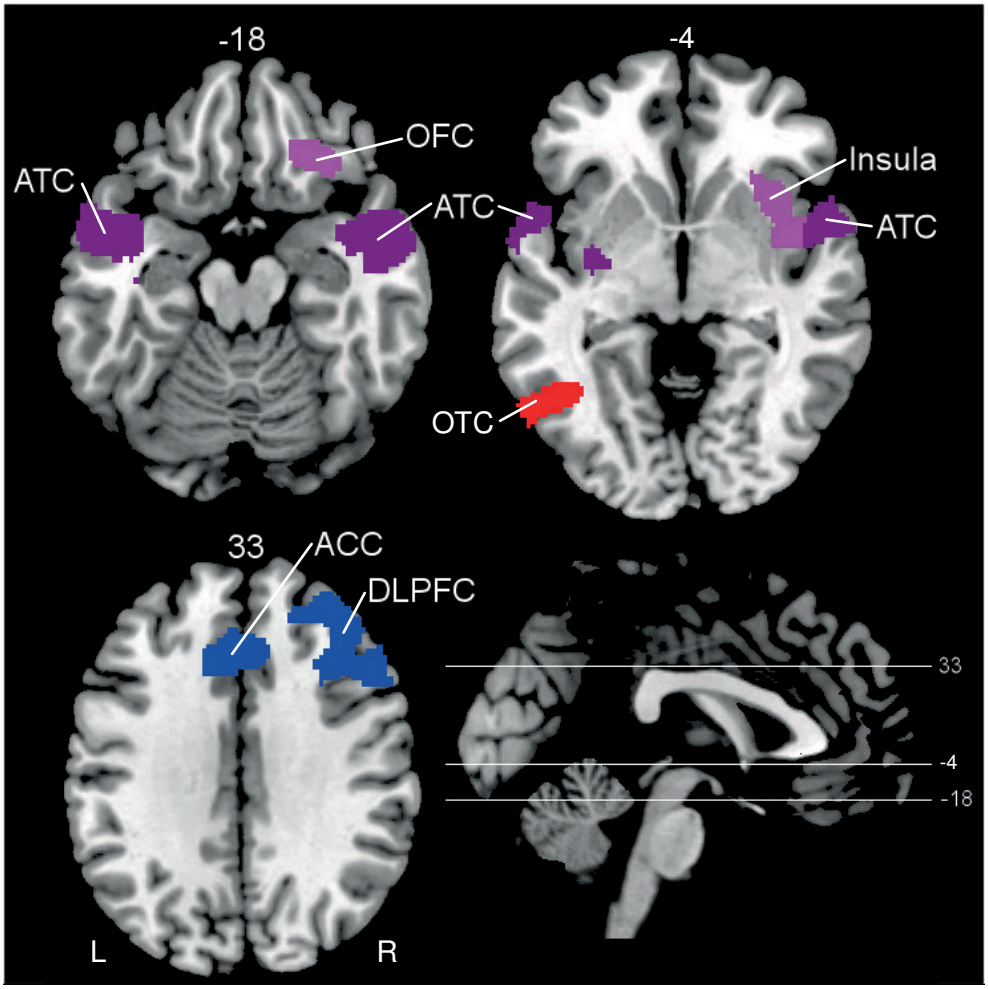

Figure 1 ROIs with increased GM volume in subjects with hypoactive sexual desire disorder compared with controls. Red, ROIs implicated in salience perception and attribution (see also Figure 2); purple, ROIs implicated in perception of bodily/visceral responses and subjective awareness of sexual emotional stimuli; blue, ROIs implicated in response selection and inhibition. Decreased OFC GM volume ($z = -18$) is a part of the insula ROI GM volume.
z-Coordinates in MNI space.
ACC = anterior cingulate cortex; ATC = anterior temporal cortex; DLPFC = dorsolateral prefrontal cortex (middle frontal gyrus); GM = grey matter; MNI = Montreal Neurological Institute; OFC = orbitofrontal cortex; OTC = occipitotemporal cortex; ROI = regions of interest

Table 2 Group differences of GM volume and WM fractional anisotropy between participants with HSDD and controls.

ROI GM	Group Difference	X	Y	Z	Number of Voxels	mm3	*p* Value
Anterior cingulate gyrus (bilateral)	control>HSDD	0.93	31.21	31.24	875	7000	0.02
Insula (right)	control>HSDD	33.44	18.22	-9.07	928	7424	0.01
Anterior temporal cortex (left)	control>HSDD	-47.06	-2.51	-21.21	2391	19128	0.007
Anterior temporal cortex (right)	control>HSDD	45.69	0.89	-24.18	2569	20552	0.005
Lateral OTC (left)	control>HSDD	-50.86	-60.80	-1.71	333	2664	0.003
Middle frontal gyrus (right)*	control>HSDD	36.32	32.88	32.31	1027	8216	0.01
ROI FA							
Amygdala (left)	HSDD>control	-27.17	-6.48	-11.54	319	319	<0.001
Amygdala (right)	HSDD>control	29.19	-5.96	-13.89	95	95	0.001
Cerebellum	HSDD>control	24.87	-50.84	-36.05	338	338	<0.001
Cerebellum 2	HSDD>control	-1.05	-54.01	-23.21	455	455	<0.001
Cortico-spinal tract (left)	HSDD>control	-23.71	-10.00	34.00	237	237	0.002
Forceps major (left)	HSDD>control	-21.17	-50.39	18.13	227	227	0.004
Forceps major (right)	HSDD>control	28.40	-67.69	2.25	305	305	0.001
OFC, gyrus rectus (left)	HSDD>control	-7.20	28.08	-15.99	244	244	<0.001
SLF (left)	HSDD>control	-42.28	-25.50	30.28	269	269	<0.001
SLF, temporal part (left)	HSDD>control	-46.87	-51.69	-2.58	258	258	<0.001
Brainstem	control>HSDD	-2.51	-28.37	-28.46	314	314	<0.001
Cortico-spinal tract (left)	control>HSDD	-25.14	-21.60	11.27	211	211	0.001

*The middle frontal gyrus is part of the dorsolateral prefrontal cortex

Coordinates in MNI space

FA = fractional anisotropy; GM = gray matter; HSDD = hypoactive sexual desire disorder; MNI = Montreal Neurological Institute; OFC = orbitofrontal cortex; OTC = occipitotemporal cortex (posterior middle temporal gyrus, Brodmann's area 37); ROI = region of interest; SLF = superior longitudinal fasciculus; WM = white matter

Table 3 Pearson's correlation coefficients for SFQ domain scores (n=45) and symptom duration (n=24) by GM volume and by WM FA.

ROI - GM	SFQ SIA	SFQ Orgasm	SFQ Pain	SFQ Partner	Symptom duration[a]
Anterior cingulate gyrus	0.42***	0.10	0.07	0.29	-0.40
Insula (right)	0.33*	0.01	-0.25	0.13	-0.07
Anterior temporal cortex (left)	0.53****	0.22	0.15	0.41***	-0.24
Anterior temporal cortex (right)	0.64****	0.25	0.13	0.42***	0.09
Lateral OTC (left)	0.45***	0.24	-0.12	0.13	-0.29
Middle frontal gyrus (right)†	0.39***	0.37**	0.12	0.21	0.07
ROI - FA					
Amygdala (left)	-0.30*	-0.36*	-0.16	0.02	0.01
Amygdala (right)	-0.13	-0.29	-0.12	0.16	0.01
Cerebellum	-0.34*	-0.42***	-0.27	-0.13	0.07
Cerebellum 2	-0.29*	-0.17	-0.32*	-0.25	-0.14
Cortico-spinal tract (left)	-0.24	-0.34*	-0.21	-0.16	0.11
Forceps major (left)	-0.25	-0.37**	-0.2	-0.15	0.30
Forceps major (right)	-0.27	-0.43***	-0.23	-0.10	0.06
OFC (left)	-0.48****	-0.37*	-0.28	-0.27	0.18
SLF (left)	-0.39***	-0.4***	-0.33*	-0.23	0.02
SLF, temporal part (left)	-0.55****	-0.63****	-0.41***	-0.33*	0.41*
Brainstem	0.28	0.05	0.23	0.11	0.29
Cortico-spinal tract (left)	0.28	0.38***	0.01	-0.06	0.05

*P<.05; **P<.012; ***P<.01; ****P<.001. Correction of the p-value using false discovery rate gave P<0.012
[a] n=24, symptom duration was assessed after the final visit using source documentation of the diagnostic interview. Data for 5 subjects was missing.
† The middle frontal gyrus is part of the dorsolateral prefrontal cortex.
FA = fractional anisotropy; GM = gray matter; HSDD = hypoactive sexual desire disorder; OFC = orbitofrontal cortex; OTC = occipitotemporal cortex; ROI = region of interest; SFQ = sexual function questionnaire; SIA = sexual interest/arousal; SLF = superior longitudinal fasciculus; WM = white matter.

We subsequently used multiple regression analyses to investigate the relationship between these GM (and WM; see below) areas and SFQ domains independently from the group subdivision of HSDD and controls. The SIA domain score was the best predictor for GM volume in all six regions (see Table 3) where higher SIA domain score (better sexual functioning) was associated with more GM volume.

Further analysis using Pearson's correlation coefficients showed that SFQ orgasm was also associated with GM volume of the middle frontal gyrus (higher orgasm scores associated with larger GM volume). Also, SFQ partner relationship scores correlated positively with GM volume of the bilateral anterior temporal cortices (see Table 4).

Table 4 Prediction models of GM volume and WM FA.

ROI GM	Model (SFQ domains)	R	R^2	Adjusted R^2	Δ R^2	β1	β2	*F Value*	*p* Value
Anterior cingulate gyrus	SIA	0.418	0.174	0.155		0.418		9,087	0.004*
Insula (right)	SIA	0.327	0.107	0.086		0.327		5,143	0.03
	SIA (1) & Pain (2)	0.481	0.232	0.195	0.125	0.429	-0.368	6,328	0.004*
Anterior temporal cortex (left)	SIA	0.530	0.281	0.265		0.530		16,833	<0.001*
Anterior temporal cortex (right)	SIA	0.644	0.415	0.401		0.644		30,469	<0.001*
Lateral OTC (left)	SIA	0.452	0.204	0.185		0.452		11,017	0.002*
Middle frontal gyrus (right)†	SIA	0.392	0.153	0.134		0.392		7,790	0.008
ROI FA									
Amygdala (left)	Orgasm	0.355	0.126	0.106		-0.355		6,208	0.02
Amygdala (right)	-	-	-	-		-		-	-
Cerebellum	Orgasm	0.420	0.176	0.157		-0.420		9,201	0.004*
Cerebellum 2	Pain	0.323	0.105	0.084		-0.323		5,021	0.03
Cortico-spinal tract (left)	Orgasm	0.337	0.114	0.093		-0.337		5,525	0.02
Forceps major (left	Orgasm	0.374	0.140	0.119		-0.374		6,971	0.01
Forceps major (right)	Orgasm	0.427	0.183	0.164		-0.427		9,613	0.003*
OFC (left)	SIA	0.480	0.230	0.212		-0.480		12,855	0.001*
SLF (left)	Orgasm	0.403	0.162	0.143		-0.403		8,323	0.006*
SLF, temporal part (left)	Orgasm	0.628	0.395	0.381		-0.628		28,046	<0.001*
	Orgasm (1) & Pain (2)	0.689	0.475	0.450	0.080	-0.567	-0.290	18,985	<0.001*
Brainstem	-	-	-	-		-		-	-
Cortico-spinal tract (left)	Orgasm	0.383	0.147	0.127		0.383		7,405	.009

* Significant prediction models, corrected alpha of .007 (see Methods section)

† The middle frontal gyrus is part of the dorsolateral prefrontal cortex

FA = fractional anisotropy; GM = gray matter; OFC = orbitofrontal cortex; OTC = occipitotemporal cortex; ROI = region of interest; SFQ = sexual function questionnaire; SIA = sexual interest/arousal; SLF = superior longitudinal fasciculus; WM = white matter

WM FA and Sexual (Dys)function

Analysis of the WM FA revealed 10 regions where WM FA was larger in participants with HSDD than in controls: the bilateral amygdalae (see Figure 2), two cerebellar regions, the left corticospinal tract, bilateral forceps major, the gyrus rectus of the OFC (see Figure 2), the left superior longitudinal fasciculus (SLF), and the temporal part of the left SLF. In two regions, we observed a reversed pattern, where WM FA was smaller in women with HSDD than in controls: the brainstem and the left corticospinal tract (see Table 2).

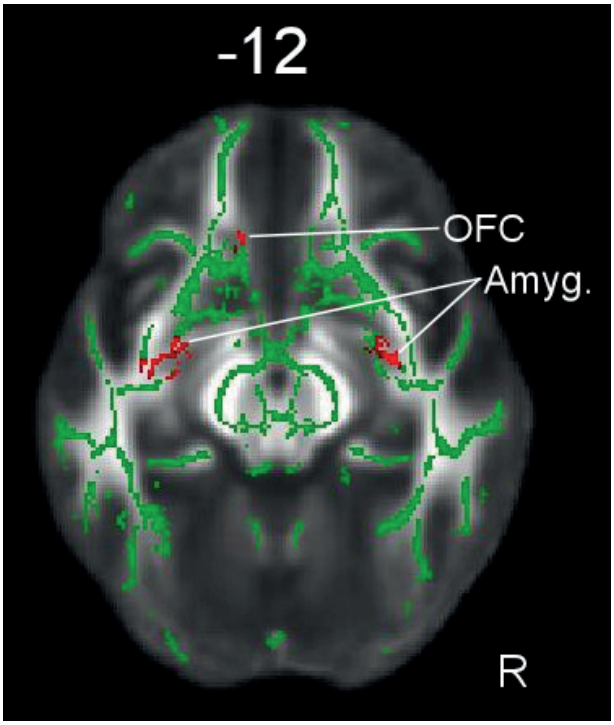

Figure 2 Increased myelination of the amygdalae (Amyg.) and orbitofrontal cortex (OFC) in subjects with hypoactive sexual desire disorder (HSDD) as compared with controls. Significant higher mean fractional anisotropy (FA) values for HSDD subjects (red) within the mean FA skeleton (green) projected onto Montreal Neurological Institute (MNI) brain.
z-Coordinates in MNI space

Multiple regression analyses showed that orgasm was the best predictor for WM FA for eight of the 12 regions (see Table 3), where lower orgasm domain scores (less orgasm) were associated with more WM. WM FA of one of the cerebellar regions was best predicted by pain, with lower pain domain scores (i.e., less pain) being associated with more WM FA. WM FA of the OFC was best predicted by SIA, where lower sexual function (i.e., lower SIA domain score) was associated with increased WM FA. For the temporal

part of the left SLF, a two-predictor model with orgasm and pain as predictors predicted WM FA significantly better than the one-predictor model (orgasm). Inspection of scatter plots of all GM and FA data with SFQ domains showed that the prediction models were not because of group clustering (see Figure 3 as example).

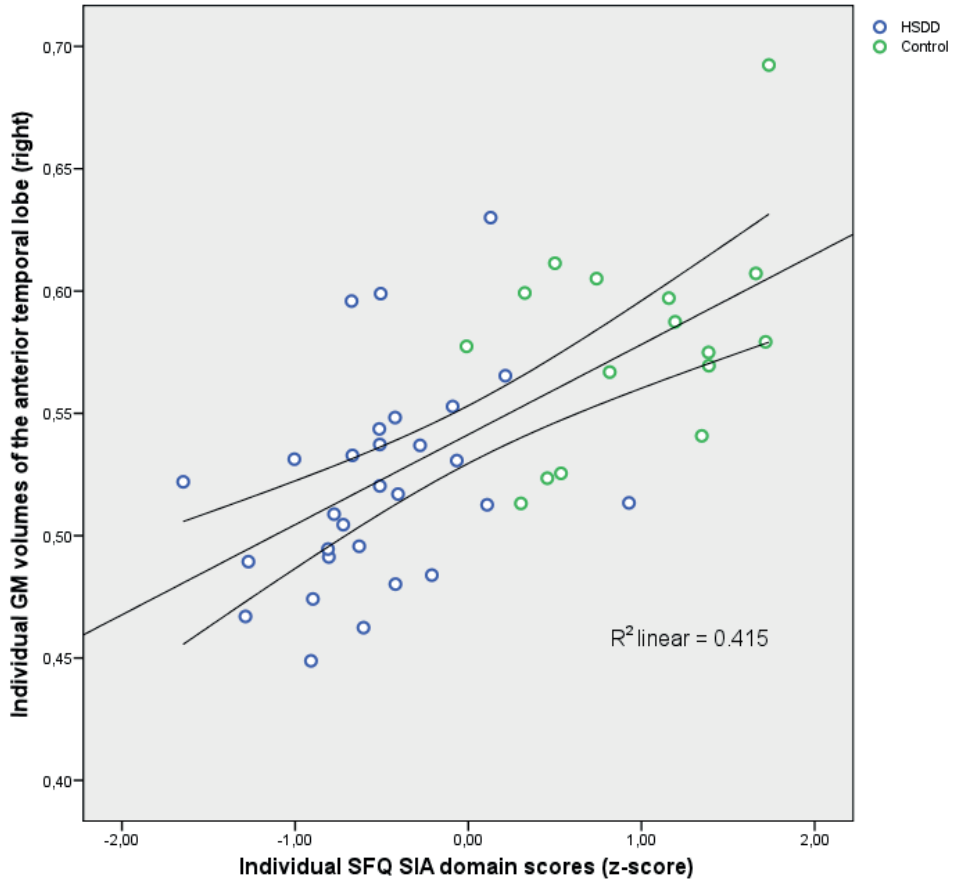

Figure 3 Scatter plot of gray matter (GM) data of the right anterior temporal pole with the sexual function questionnaire (SFQ) derived domain sexual interest/arousal (SIA). HSDD = hypoactive sexual desire disorder

Further analysis using Pearson's correlation coefficients showed that SFQ SIA was also negatively related to WM FA of the left amygdala, both cerebellar regions, and both SLF regions; thus, higher SIA scores (better sexual function) were associated with lower WM FA values (see Table 4). Furthermore, the SFQ pain score was associated with FA value of both SLF regions, where higher SFQ pain scores (i.e., less pain) were associated with lower WM FA value. SFQ partner relationship correlated negatively with WM FA value of the left SLF.

These results indicate that sexual functioning in women with and without HSDD is positively correlated with GM volume in different cerebral areas. In general, less GM volume is related to lower SIA, and more GM volume is related to higher levels of SIA. Our analyses revealed a reversed pattern for the association between orgasm function and WM; specifically, decreased orgasm function was associated with more WM FA.

Discussion

The present study identifies differences in GM volume and in WM FA between a group of women with HSDD and a control group. The brain regions showing group differences are areas associated with salience attribution, perception of bodily responses, emotional awareness, and attention. We will argue that women with HSDD may have hampered sexual stimulus salience attribution, lower perception of bodily sexual responses and sexual awareness, and concomitant altered function of attentional mechanisms. Furthermore, we show a relationship between different domains of sexual functioning and the *types* of structural differences: GM volume in all ROIs is best predicted by SIA, whereas WM FA is best predicted by orgasm function.

Stimulus Salience and Sexual Cue Sensitivity

The amygdalae signal the behavioral relevance of incoming stimuli (i.e., attributes stimulus salience) and have been shown to be more active under condition of erotic stimuli as compared with neutral stimuli in imaging studies in men [10,11,15,30] and women [14,30]. Women with HSDD may have altered processing of sexual cues resulting in decreased sensitivity for sexual cues in the brain [31,32], which is in line with information-processing models of sexual functioning [33,34]. This could be related to altered amygdala function (i.e., decreased salience attribution), and the increased WM FA in the bilateral amygdalae of the HSDD subjects in the present study (Figure 2) provides evidence for this hypothesis. Increased WM FA generally reflects facilitation of signal conduction, but it does not necessarily mean better functioning [35]. It can also be the result of microscopic deficits of axonal structures, decreases in axonal diameter, axonal packing density, and axonal branching, aside from increased myelination [36]. Moreover, it can reflect compensation of functional deficits, where tracts which “take over” functions of impaired tracts show increased myelination [37]. At this point, we cannot determine the cause of the increased WM FA of the HSDD subjects. It may be because of axonal deficits, but it may also be a functional compensatory, albeit insufficient, adjustment. Alternatively, increased WM FA may also reflect increased functionality of inhibitory fibers which exert inhibitory control within the amygdalae, thereby decreasing salience detection and consequently sexual cue sensitivity.

More evidence that sexual cue sensitivity is altered in HSDD lies in the observed decreased GM volume of the left OTC, a structure which is heavily involved in the processing of visual object information. Processing activity within the OTC increases

with increased stimulus salience (see Kastner and Ungerleider [38] for a review), for example, during the processing of highly salient erotic stimuli [9,10,12,14,30]. Decreases in GM volume can be the consequence of loss or atrophy of neurons or glia, an altered ratio of small to large cell types, a decreased density of neuronal processes [39], or increased myelination of intracortical axons [40]. Unfortunately, it cannot determined what exactly has caused the present GM differences, but it can safely be assumed that in our HSDD sample, decreased GM volume is associated with decreased sexual function. Taken together, the decreased GM volume observed in the present study suggests deficient processing of visual (salient/sexual) object information, which may thus contribute to decreased sexual cue sensitivity in HSDD.

Perception of the Physiological Sexual Response and Sexual Emotional Awareness

According to Damasio's "somatic marker" hypothesis [41], the perception of visceral stimuli (interoception) is key in the subjective experience of emotion. Interoception is largely mediated by the insula [42], where interoceptive stimuli are remapped with increasingly complex information, in a posterior to anterior gradient, accumulating in the anterior insula and forming subjective awareness of a global emotional state [43]. There is ample evidence that interoception of the sexual response is also of importance in subjective awareness of sexual arousal [44], and the insula is one of the most consistently activated areas in response to sexual stimuli in imaging studies [8,9,11–14,30]. Taken together, the finding that the GM volume of the right insula is decreased in the present HSDD sample could suggest suboptimal perception of bodily sexual response and of subjective sexual awareness.

The anterior temporal cortex (ATC) receives and integrates highly processed visual, auditory, and olfactory information, and thus mediates conceptual object knowledge [45]. The temporal pole, the most anterior part of the ATC, integrates interoceptive information from the insula with highly processed sensory information from multimodal association areas. Because of this coupling of visceral–emotional and sensory information, and because the temporal pole is often active in tasks with social narrative, it has been hypothesized to be important in socioemotional processing [46]. The ATC is activated in response to sexual stimuli [15], and the authors hypothesized that the temporal pole may be necessary for physiological arousal to enter full conscious awareness. In this light, the decreased GM volume in the ATC in the present group of women with HSDD may also reflect decreased subjective sexual awareness. The ATC GM volume, however, also correlates with the SFQ partner score in the present study indicating that women lower in GM volume of the ATC (subjects with HSDD) worry more about their partners' attitudes towards their sexual problems. This relationship fits with the notion that the ATC is involved in socioemotional processing, possibly via decreased subjective sexual awareness. Responsive desire (as opposed to spontaneous desire) is when sexual motivation begins after sexual behavior has started, and its absence is an additional diagnostic criterion for FSIAD in *DSM 5*. A prerequisite for responsive desire

to occur is subjective sexual awareness. If there is no awareness of a sexual state, desire cannot occur. It is feasible that if in a relationship a woman has no spontaneous desire *and* no responsive desire, the burden on their relationship of their unfulfilling sex life is heavier, which is why these women with HSDD worry more about their sexual problems.

Attentional Control and Inhibition

The ACC is well-known for its role in attentional control and performance monitoring [47] and is reactive to sexual stimuli [7–9,11–14,30]. It has been hypothesized that the anterior insula and the ACC are both core components of a salience network (together with the amygdalae and other structures) which detects salient (socio)emotional stimuli, facilitates the processing of related relevant internal and external stimuli, and engages appropriate systems to optimally guide behavior [48]. Both the insula and ACC show decreased GM volume in the HSDD group in the present study. This finding may imply that subjective awareness of a sexual state is dysfunctional in these women, and also concomitant engagement of relevant bodily/emotional responses through ACC attentional control. Thus, not only perception but also induction of relevant bodily/emotional responses may be biologically compromised in the present group of women with HSDD. This notion is supported by the increased FA value of fiber tracts in the left gyrus rectus, which forms a border between the OFC and the mPFC. The OFC receives information from different perceptual modalities, integrating it and synthesizing a representation of reward and hedonic value ("liking") of biologically significant stimuli [49], including sexual stimuli [7,8,13,30]. The mPFC is important in emotion regulation [50], modulating bodily responses to emotional stimuli [51]. The gyrus rectus borders on these two areas and is implied in both OFC and mPFC processing. It has been suggested that this area transfers information between these two key areas of emotional processing [52]. In the present context, this may mean that a hedonic (sexual) stimulus' ability to induce a visceral emotional response in women with HSDD is hampered.

Finally, decreased GM volume was observed in the right DLPFC, which mediates executive functioning including response inhibition [53–55]. The DLPFC plays a key role in emotional self-regulation [56,57] including self-regulation of sexual responses [15,58]. This could suggest that in the present HSDD group, the self-regulation of sexual behavior may be altered. If sexual responses are less frequent or weaker in HSDD (e.g., because of decreased sexual cue sensitivity), self-regulation of sexual behavior (e.g., inhibiting sexual responses in inappropriate situations) may be less developed because the need for or strength of such regulation is lower. Conversely, there is also evidence that a subset of subjects with HSDD may overinhibit their sexual responses via the DLPFC [59]. According to this subdivision, HSDD can be caused by low sensitivity to sexual cues or by overactivation of sexual inhibitory systems. The overinhibitors, however, possess normal sexual cue sensitivity which does not seem to be the case with the present subset [59]. Possibly, these inhibitors were underrepresented in the present

subset, or the decreased GM volume may not reflect decreased inhibitory function but rather increased efficiency of inhibitory function.

The present study could not determine whether the observed GM volume and WM FA differences cause or are caused by sexual dysfunction. Genetic, molecular, or cellular causes may directly impact GM volume and make these women more susceptible to the development of a sexual dysfunction. It may be more likely that such factors have an indirect influence on GM volume and WM FA. For example, testosterone administration can influence sexual cue sensitivity [31,32] and testosterone fluctuations impact sexual responsiveness [1], but testosterone's role in the etiology of HSDD is not clear [5]. Testosterone exerts its androgenic effect through its action at the androgen receptor. The strength of the androgenic effect that testosterone induces is dependent upon the cytosine-adenine-guanine (CAG) repeat length polymorphism of the androgen receptor gene (AR[CAG]n), where long AR[CAG]n is associated with weak receptor transactivation [60]. It is reasonable to assume that testosterone's influence on sexual cue sensitivity is dependent on AR[CAG]n, and it may be that deficiencies in this mechanism are important in the etiology of HSDD. Low sexual cue sensitivity through such an androgen AR[CAG]n interaction may then impact GM volume and WM FA in relevant areas. Such changes would most probably become manifest during puberty when there is a sharp increase in hormone production and when sexual stimuli become highly salient.

Deviations in GM volume and WM FA could also be induced later in life through (negative) sexual experience. If structural brain differences are induced by sexual experience, it would be expected that a correlation exists between structural differences and symptom duration, assuming a linear relationship. A marginally significant correlation was found only for the SLF (temporal), implying congenital causes. However, this is highly speculative, and it is likely that a nonlinear relationship exists because GM and WM matter changes can be induced by short-term (weeks) learning effects [61,62] but can also reflect long-term influences of congenital abnormalities [63,64]. Treatment studies or longitudinal studies measuring GM volume and WM FA over time in larger groups of women may differentiate between cause and consequence.

In summary, we identified neural correlates of HSDD in brain regions associated with salience attribution, body response perception, emotional awareness, and response selection. The data suggest that HSDD is associated with decreased salience attribution to sexual stimuli (amygdala, OTC), decreased sexual response perception and awareness (insula, ATC), and altered response selection/inhibition (ACC, DLPFC). We hypothesize that in women with HSDD in general, less salience is perceived and attributed to sexual stimuli because of/leading to altered processing in the amygdala and OTC, leading to lower sexual cue sensitivity. Because sexual stimuli are less salient, the subsequent automatic sexual (visceral) response is less potent. A less potent response is less likely to be perceived (insula) and less likely to enter awareness (insula and ATC), and

concomitantly, attention cannot be directed towards these stimuli, and appropriate sexual response systems will not be engaged (ACC) (see Figure 1). Because sexual responses are less frequent and less potent, inhibition of sexual responses may be less developed (DLPFC), at least in the present subset of women. Our hypothesis is in agreement with information-processing models of sexual behavior, which emphasize the interdependency of stimulus salience and attentional mechanisms for an optimal sexual response [34,65]. Our hypothesis is also in agreement with a generalized model for emotion perception and regulation by Phillips et al. [16] which posits that these areas identify emotional significance of stimuli and produce and regulate an affective state in response to emotionally significant stimuli. Importantly, Phillips et al. [17] also show that differential dysfunction within these neurobiological networks is related to different psychopathologies. The present neuroanatomical data suggest this may also be the case for HSDD, and, if so, can have important implications for selecting or designing new treatments.

Orgasm Function

A second finding of the present study is that GM volume and WM FA values are differentially predicted by SFQ domain scores in women with HSDD. Higher SIA was predominantly associated with larger GM volume, and better orgasm function was associated with lower FA of fiber tracts in the cerebellum, forceps major (right), the SLF (left), and the temporal part of the SLF (left). The correlation matrix (Table 3) shows that higher SIA is related to both higher GM volume and lower WM FA values. SIA's relationship to GM volume is stronger however. Orgasm does not significantly correlate with GM volume, except with that of the DLPFC (better orgasm function with increased GM volume). We therefore hypothesize that functioning of specific neuronal populations is most important for sexual interest and sexual arousal, but signal conduction between these (and other) areas—as reflected by negative correlations with FA values—is also relevant for SIA, which is not surprising for a complex behavior. For orgasmic function, the present results imply that altered communication between brain areas is more important than the processing within the specific neuronal populations themselves. Unfortunately, we cannot determine if the increased FA found in the present HSDD sample is the result of axonal deficits or compensation of functional deficits, or if it is the result of facilitated signal conductance of a related but antagonistic process. Also, the data are not based on women with female orgasmic disorder, but there was variation in orgasm capability, and nine women did report some level of orgasm problems. Nevertheless, hypotheses can be formulated regarding this intriguing relationship between brain connectivity and orgasm function.

Orgasm is preceded by a sexual arousal plateau phase, where sexual arousal is maintained at a high, steady level for a prolonged period [66,67]. Leading up to and during this plateau phase, different cortical and subcortical structures are continuously and increasingly processing sexual stimuli and producing the according responses in an

intricate interplay between and among structures. This continuous cyclic/reiterative communication between structures needs to be sustained for a prolonged period for orgasm to occur. This cyclic/reiterative communication may be impaired in women with orgasm problems, as reflected by the negative correlation between FA values—but not GM volume—of most ROIs and the orgasm domain scores. It is also possible that the increased FA values reflect facilitated signal conductance of an antagonistic process, for example, pain transmission or sexual inhibition. The SFQ pain score however did not show a comparable or stronger relationship with FA values than orgasm, making this possibility unlikely. Sexual inhibition was not measured, but it is intriguing that the DLPFC, known for inhibitory control of the sexual response [15], was the only GM area which correlated with the FA values and orgasm function. We hypothesize tentatively that orgasm problems in women with HSDD are mostly dependent on altered cyclic/reiterative communication between cortical and subcortical structures, as opposed to dysfunction within structures. To investigate the validity and generalizability of this hypothesis, a larger group of women with HSDD having more variation in their ability to orgasm should be compared with a group of women with female orgasmic disorder and adequate control subjects who do not have sexual problems.

Limitations

As stated previously, the present study cannot determine if the observed group differences are a cause or consequence of HSDD. We believe it is likely that the differences are the result of a direct relationship with HSDD because we have controlled for factors which are known to influence sexual functioning. Frequent comorbidities of HSDD such as depression and anxiety disorders, other psychiatric disorders, major medical disorders, endocrinological disorders, neurological disorders, unexplained gynecological complaints, and childhood sexual abuse were grounds for exclusion from the study. The presence of any of these exclusionary criteria was assessed during the screening visit by a physician who performed a physical and gynecological examination and interviewed the subjects using a structured interview tool. Blood chemistry and hematology, ECG, and vital signs were also assessed, but some potential disorders (e.g., affective or anxiety disorders) were not assessed through direct measurement using specific structured interviews or validated questionnaires. It is possible that subjects were included in the present study who should have been excluded. We believe that inclusion of subjects with these conditions is unlikely because of the elaborate screening procedure. If there was an unjust inclusion, it is unlikely that the observed results were caused by these subjects because these subjects would have been few and diverse. The relatively low number of subjects, especially in the control arm, is another limitation of the present study. Replication of this study should be done with a significantly larger number of subjects before reliable generalizations to the total HSDD population can be made. Also, it would be of great interest to investigate and compare subjects with different sexual diagnoses. A core assumption of the present study is that desire and arousal have strong neuroanatomical overlap; it would be of interest to investigate the

strength of this overlap. Comparing subjects with primary HSDD, FSAD and female orgasmic disorder, without further secondary sexual problems, in a comparable study design could shed light on if and how desire and arousal are neuroanatomically separated. Such a study would test our claims regarding orgasm function. Future replication studies may also benefit from a lower cluster size threshold, which was 1,600 mm^3 in the present analysis. This size makes it less likely that any subcortical group differences will be observed, for example in the nucleus accumbens or the hypothalamus, both of which are important in sexual stimulus processing and sexual behavior. Finally, the results presented here may seem outdated, as by the time of publication, the diagnoses HSDD and FSAD have been replaced by FSIAD. However, we believe that our present population would have largely met the *DSM 5* criteria for FSIAD, which is why the present results are likely generalizable to that disorder. Replication studies are necessary, where subjects who meet the FSIAD criteria should be investigated.

In conclusion, we identify neuroanatomical correlates of HSDD in both GM and WM, in a symptom-dependent manner. Even though cause or consequence could not be determined, these structural differences suggest that subjects with HSDD have decreased sexual cue sensitivity, decreased perception of sexual responses and sexual emotional awareness, and altered attentional mechanisms which are needed to induce an adequate sexual response. This understanding can have vast clinical implications in the future treatment of HSDD. It is highly likely that individual differences exist between women with HSDD in their predisposition for sexual cue sensitivity, perception of sexual responses, sexual emotional awareness, and attentional mechanisms/inhibition. If diagnoses of HSDD can be further refined, taking into account an individual's propensity for these various functions, thus giving several subtypes of HSDD, selective pharmacological and/or psychological therapies can be designed to tackle each underlying cause of these HSDD subtypes instead of a “one-size-fits-all” approach.

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CHAPTER 9

Discussion

The present thesis describes a series of studies that were performed as a part of a personalized sexual medicine drug development program. This program is based on the dual control model of sexual response. The main premise of this program is that Hypoactive Sexual Desire Disorder (HSDD) and Female Sexual Interest/Arousal Disorder (FSIAD)[2] is caused and sustained by dysfunction in one of two separate but interacting systems, the sexual excitation and the sexual inhibition system, as described by the dual control model of sexual response [1,2]. The dual control model postulates that individuals who have low propensity for sexual excitation or a high propensity for sexual inhibition are more likely to experience problems of impaired sexual response or reduced sexual interest. HSDD/FSIAD may therefore be caused by dysfunction in either one of these two distinct mechanisms, which means that at least two subgroups within HSDD/FSIAD may exist, depending on which system is out of balance. Knowledge of how these systems operate, and where they fail, should guide the development of potential drugs for HSDD/FSIAD. The drug development program described here investigated underlying neurobiological mechanisms for dysfunctional sexual excitation and dysfunctional sexual inhibition, which led to two subgroups: HSDD/FSIAD as the result of low sensitivity for sexual cues, or HSDD/FSIAD as the result of dysfunctional over-activation of sexual inhibitory mechanisms. This also led to the inception of two on-demand drug therapies: combined administration of testosterone (0.5 mg) and a phosphodiesterase 5 (PDE5) inhibitor (sildenafil, 50 mg) for the former group and combined administration of testosterone (0.5 mg) and a serotonin (5HT) 1A receptor agonist (buspirone, 10 mg) for the latter group.

Both on-demand combination drug therapies are administered in such a manner that the pharmacodynamic effects of both compounds coincide. Sublingual testosterone administration gives a rapid uptake of testosterone into the systemic circulation (time to maximum concentration, T_{max} = 15 minutes) and a rapid elimination rate; after two to three hours the peak in testosterone has dissipated. It has a delay in pharmacodynamic effect however, of approximately four hours. It increases the sexual motivation from approximately three up and to approximately six hours after it has been administered, giving a therapeutic window of 3 hours [3]. The PDE 5 inhibitors sildenafil and vardenafil, and the 5-HT_{1A} receptor agonist also have a therapeutic window of approximately 3 hours, but they have a quicker onset of action (approximately 1 hour after administration). To create maximal overlap in the pharmacodynamic effects of the two active constituents in both drug treatments, they have to be released in the systemic circulation at different time points. First, sublingual testosterone is administered, and two to two and a half hours later, the PDE5 inhibitor (figure 1) or the 5-HT_{1A} receptor agonist (figure 2), depending on the drug combination, are administered orally. This method of administration timing ensures that both on-demand drug therapies have a therapeutic window of approximately 3 hours, starting 3 hours after the sublingual testosterone administration. In this final chapter, the main findings and conclusions of

[2] HSDD and FSIAD refer to the same disorder. FSIAD has replaced HSDD in the 5th edition of DSM [4].

the studies investigating these two on-demand drug combinations will be summarized and discussed.

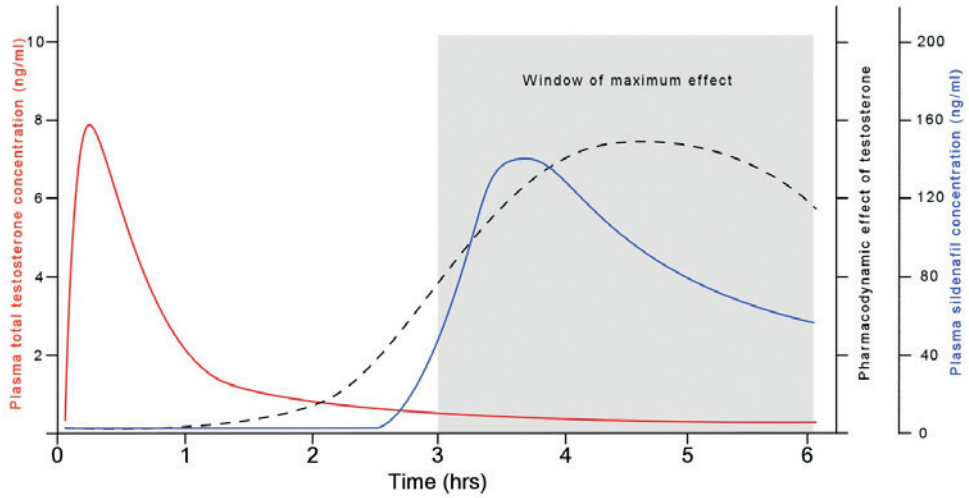

Figure 1 Time-course of the pharmacokinetic and pharmacodynamic effects of testosterone and the PDE5 inhibitor sildenafil. The gray box indicates the therapeutic window (from Poels et al. [12]).

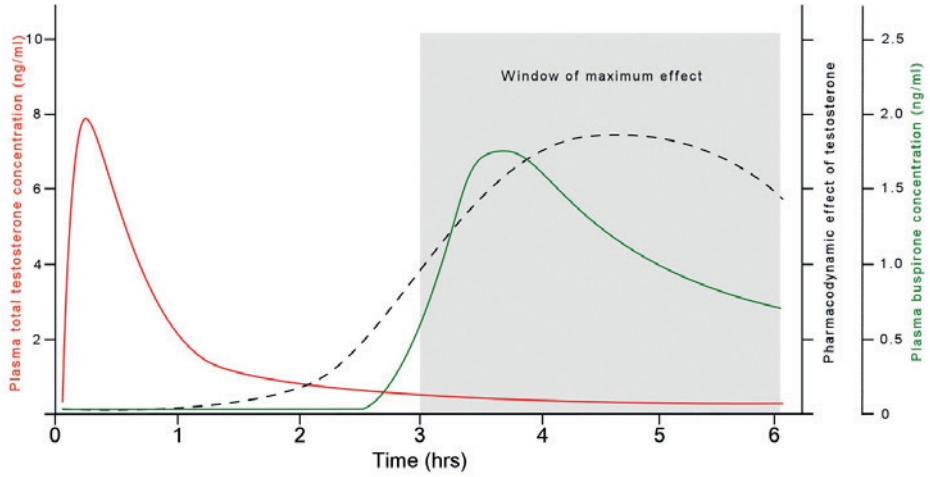

Figure 2 Time-course of the pharmacokinetic and pharmacodynamic effects of testosterone and the 5-HT_{1A} receptor agonist buspirone. The gray box indicates the therapeutic window (from Poels et al.[12]).

Influence of sensitivity for sexual cues on efficacy of combined treatment with testosterone and a PDE5 inhibitor

In chapter 2, the influence of a single dose of the combined administration of testosterone (0.5 mg) and the PDE5 inhibitor vardenafil (10 mg) on the physiological sexual response to erotic film clips of 13 women with HSDD was compared to placebo and the monotherapies separately. It was demonstrated that the combination of testosterone and vardenafil significantly increased the vaginal pulse amplitude (VPA) response during the viewing of erotic film clips, compared with placebo. The VPA response showed a time-dependent increase, with a maximum at 4.5 hours. Neither vardenafil nor testosterone alone led to a significant increase in VPA as compared to placebo. Effects differed between women who had reported being victim of childhood sexual abuse (CSA) (n = 5) and those who had not (n = 8). In women with no CSA, testosterone induced an increase in their *initially low* levels of preconscious attentional bias for sexual cues (as measured by an adapted version of the emotional Stroop task), while women with CSA showed a decrease in their *initially high* levels of attention. The effects of the combined administration of testosterone and vardenafil on the VPA also differed between these groups. Women without CSA revealed a statistically significant increase in their VPA during treatment with the combination of testosterone and vardenafil as compared with placebo. Women with CSA, however, showed no alterations in their physiological sexual responding during this combined drug treatment.

We hypothesized that the low preconscious attention scores, for women with no CSA, before testosterone treatment reflect a relatively insensitive brain system for sex. Testosterone increases the brain's sensitivity for sex [5] so the increase in preconscious attentional bias four hours after sublingual testosterone administration is reflective of increased sensitivity (making it possible for a PDE-5 inhibitor to take effect). Because testosterone increases sensitivity of the brain for sexual stimuli, the decrease in attentional bias following testosterone administration in the CSA group is probably caused indirectly (i.e. not directly by testosterone induced increase in sensitivity). CSA may have induced conditioning of cognitive (or affective) mechanisms that can elicit sexual inhibition under particular circumstances. For example, the increased sensitivity for sexual cues under condition of testosterone might have evoked an avoidance response for sexual cues because these cues become too threatening. This avoidance response resulted in the absence or even inhibition of automatic physiological sexual responding in this group of subjects as compared with the non-abused women. Thus, the testosterone-induced withdrawal of preconscious attention and the absence of an effect on physiological sexual response in the abused women were hypothesized to be the result of the activation of a sexual inhibitory mechanism.

A second randomized, double-blind, cross-over, placebo-controlled study (chapter 3) also investigated the effect of a single dose, combined administration of 0.5 mg testosterone and 10 mg vardenafil, again compared to placebo and to the monotherapies

separately. VPA and subjective measures of sexual arousal were measured during the viewing of erotic film clips, in 28 women with HSDD, this time excluding women with CSA. The subjects were differentiated based on their initial preconscious attentional bias for sexual cues. In the group that had initial (i.e., pre-testosterone dose) low attention for sexual cues (n = 17), preconscious attentional bias for sexual cues increased during testosterone treatment, and the combination of testosterone plus vardenafil caused an increase in VPA and subjective indices of sexual functioning relative to placebo. In the group that had initially high attention for sexual cues (n = 11), preconscious attentional bias for sexual cues decreased following testosterone treatment. Moreover, the combination of testosterone plus vardenafil had no effect on any of the indices of sexual functioning. These results replicate those of the previous study described in chapter 2. There was a difference between the non-responding groups of the two studies. The group of non-responders of the former study were victim of CSA whilst the non-responders of the present study were not, but they did report a higher prevalence of negative sexual experiences (e.g., rape, inappropriate touching) later in life, than did the responders of the present study (63% vs 17%). Both groups of non-responders had a history of negative sexual experiences that likely affected their current sexuality, presumably in a comparable manner. The high sexual abuse rates in both non-responder groups, together with the similarity between the two non-responder groups in psychophysiological response profiles to testosterone and the combination of testosterone and vardenafil in the studies reported in chapters 2 and 3, led to the hypothesis that both groups of women suffer from HSDD due to dysfunctional over-activity of sexual inhibitory mechanisms.

A neuroanatomical substrate for sexual inhibition

The validity for the existence of a subgroup of women with HSDD due to dysfunctional over-activity of sexual inhibitory mechanisms was further substantiated by two functional magnetic resonance imaging (fMRI) studies (described in chapter 5). In the first study it was shown that testosterone indeed induced inhibition under certain circumstances. In this randomized, double-blind, placebo-controlled, cross-over study, the delayed effect of a single dose of sublingual testosterone (0.5 mg) on alterations in functional (brain oxygenation level dependent) brain-activity and vaginal arousal were investigated in 12 sexually functional women. In the placebo condition, subjects showed activation of the expected (sub)cortical areas associated with sexual stimulation and a moderate increase in vaginal arousal. Contrary to our hypothesis, testosterone compared to placebo led to deactivation of these (sub)cortical brain regions, and to a relatively blunted vaginal response. In the testosterone condition we found significantly more activation of the superior part of the middle frontal gyrus (the dorsolateral prefrontal cortex (DLPFC)) and the septal nuclei, regions associated with inhibition of motivated behavior. Apparently, the situation in the testosterone condition necessitated inhibition of the sexual response. We concluded that testosterone increases the sensitivity of the sexual regulatory system, but because the experimental circumstances - i.e. being

in a noisy fMRI apparatus surrounded by male technicians and scientists - are inappropriate for testosterone induced amplification of sexual responding, inhibitory mechanisms (DLPFC and septal nuclei) were activated.

The second fMRI study showed that the combined administration of testosterone 0.5 mg and sildenafil 50 mg can induce a comparable inhibitory response in women with HSDD (n=14). In this randomized, double-blind, placebo-controlled, cross-over study, the effects of attentional manipulations on the blood oxygenation level-dependent (BOLD) response under placebo, testosterone, and the combined administration of testosterone and sildenafil was investigated. Focusing attention on the genital response under conditions of increased sexual stimulation decreased left DLPFC activity (response inhibition) and increased right insula activation (interoceptive awareness) with placebo treatment and more so with testosterone. The combination of testosterone plus sildenafil reversed this pattern, indicating inhibition of the bodily and/or central sexual response. Adding the sildenafil apparently increased the sexual response to inappropriate levels, thereby necessitating inhibition.

In many situations, it is inappropriate to act on a sexual cue, for example, because of social conventions (i.e., implying there is a threat of negative consequences on acting to such a cue in the wrong circumstance). Under such circumstances, sexual cues can still elicit preparatory sexual responses, but these then have to be inhibited [2]. The need for, or strength of this inhibitory response is dependent on the elicited sexual response. Thus, if a sexual cue is relatively weak (due to the nature of the cue, or to the neurobiological disposition of the individual), no inhibition may be needed. But, if the processing of a relatively weak sexual cue is enhanced through testosterone administration, inhibition may be needed. Therefore, the net effect of testosterone administration could be an inhibitory response. The evidence so far suggests that the DLPFC plays a very important part in this inhibition. In conclusion, depending on circumstances, testosterone can produce effects that deviate from the expectations concerning the functional (i.e., facilitatory) role of testosterone in the regulation of sexual behavior: it can induce inhibition, and this is largely mediated by the DLPFC.

Serotonin and its role in (dis)inhibition

Given that sublingual testosterone increases the sensitivity of the brain to sexual cues, women prone to sexual inhibition are expected to exhibit stronger inhibitory activity in the DLPFC during exposure to a sexual stimulus following testosterone administration. Serotonin exerts abundant inhibitory effects via the prefrontal cortex (PFC) [6]. The observed inhibition mediated by the DLPFC may therefore be decreased by transiently altering 5HT levels in the PFC. After acute treatment with a 5-HT_{1A} receptor agonist, the agonist binds to somatodendritic auto-receptors of the raphe nuclei in the midbrain. The hyperpolarizing effect of activated 5-HT_{1A} auto-receptors decreases serotonergic firing activity [7] and inhibition of serotonin release from the presynaptic terminal [8], and subsequently, reduced extracellular serotonin levels in the PFC [9]. Prolonged treatment

with a 5-HT_{1A} receptor agonist results in an opposite effect. For example, acute buspirone causes an increase in impulsivity, which effect is reversed following chronic treatment [9]. Thus, it was postulated that acute treatment with a 5-HT_{1A} receptor agonist might decrease sexual stimuli induced phasic serotonergic inhibitory control in the PFC, which in turn might prevent or reduce the inhibitory response to sexual cues in women with HSDD as the result of dysfunctional over-activation of sexual inhibitory mechanisms. Consequently, when exposed to sexual stimuli, women prone to sexual inhibition might show an increased sexual response when treated with sublingual testosterone and a 5-HT_{1A} receptor agonist.

The hypothesis that the combined on-demand administration of testosterone 0.5 mg and a 5-HT_{1A} agonist is effective in women with HSDD and dysfunctional over-activation of sexual inhibition was tested in a randomized, double-blind, placebo-controlled, cross-over study. This study tested both medication combinations in both subgroups, which enabled us to determine whether each drug combination is indeed effective only in the subgroup that it was designed for. The study tested the efficacy of the drug combinations at home over a longer period of time (chapters 6 and 7). 57 Subjects with HSDD underwent three, 4-week medication regimes (testosterone 0.5 mg + sildenafil 50 mg; testosterone 0.5 mg + buspirone 10 mg; placebo). They received 14 units medication per regime so that they could take it, on-demand, maximally every other day. The order of the treatments was randomly allocated. Subjects were randomized on the first visit after inclusion. During the first week of each treatment period, subjects measured their physiological and subjective responses to erotic film clips using the ambulatory psychophysiological laboratory (see chapter 4 which describes the increase in ecological validity that is gained by the use of this portable laboratory). During the remaining 3 weeks, subjects could use the medication on-demand before sexual activity. Both combinations (testosterone 0.5 mg + sildenafil 50 mg and testosterone 0.5 mg + buspirone 10 mg) significantly improved sexual functioning in both study parts (i.e., ambulatory psychophysiological laboratory experiment and bedroom experiment). The level of improvement attained depended on the underlying etiology: testosterone 0.5 mg + sildenafil 50 mg worked best in women with HSDD and low sensitivity to sexual cues, whereas testosterone 0.5 mg + buspirone 10 mg was most effective in women with HSDD and dysfunctional over-activation of sexual inhibitory mechanisms.

A neuroanatomical substrate for HSDD/FSIAD

Finally, in chapter 8, an MRI study is described in which we investigated differences in brain structure between 29 women with HSDD and 16 sexually healthy control subjects.

White matter (WM) properties were measured using diffusion weighted imaging and analyzed using fractional anisotropy (FA), gray matter (GM) volume was measured using 3D-T1 weighted recordings and analyzed using voxel-based morphometry, and sexual functioning was measured using the Sexual Function Questionnaire. The results showed that HSDD is associated with decreased salience attribution to sexual stimuli

(increased FA of the bilateral amygdalae, and decreased GM of the left occipitotemporal cortex (OTC)). It is also associated with decreased sexual response perception and awareness (decreased GM of the right insula and the bilateral anterior temporal cortices (ATC)) and altered response selection/inhibition (decreased GM of the anterior cingulate cortex (ACC) and the right DLPFC). We hypothesize that in women with HSDD in general, less salience is perceived and attributed to sexual stimuli due to or leading to altered processing in the amygdala and OTC, which results in lower sexual cue sensitivity. Because sexual stimuli are less salient, the subsequent automatic sexual (visceral) response is less potent. A less potent response is less likely to be perceived (insula) and less likely to enter awareness (insula and ATC) and concomitantly, attention cannot be directed towards these stimuli and appropriate sexual response systems will not be engaged (ACC). Because sexual responses are less frequent and less potent, inhibition of sexual responses may be less developed (DLPFC), at least in the present subset of women. At the time that this study was conducted, we were unfortunately unable to determine to which HSDD subgroup the patients belonged. We assume that the present subset contains mainly low sensitive HSDD patients. In a subset of high inhibitors, we would expect to observe increased gray matter volume in the DLPFC, reflecting increased propensity for inhibition of sexual responses.

This interdependency between stimulus salience and attentional mechanisms is essential for an optimal sexual response [10,11]. In terms of the dual control model for sexual response, these findings give a possible structural neuroanatomical substrate for dysfunction in sexual excitation and sexual inhibition. In HSDD/FSIAD, the sexual excitation system has propensity for lower activity because sexual stimuli are less salient, sexual response perception is decreased (i.e., internal sexual stimuli are also less salient), and response selection is altered. The sexual inhibition system is affected because, logically, neural mechanisms for response inhibition (DLPFC) are altered. A limitation of this study is that no division of subgroups (low sensitive vs high inhibition) was possible. A future study should include and *a priori* differentiate between low sensitive and high inhibition patients to establish whether these areas are differentially affected in these groups.

Concluding remarks

The studies described in this thesis give cognitive, psychophysiological, subjective, neuroanatomical and pharmacological evidence, that at least two subgroups in HSDD/FSIAD do indeed exist. This has major consequences for the field. To date, HSDD/FSIAD is assumed by most to be a homogenous disorder, and the pharmacotherapeutic treatments in development adhere to this assumption. If this disorder should indeed be divided into two or more subgroups, which the evidence in this thesis strongly suggests, pharmacotherapeutic treatments which treat HSDD/FSIAD as a homologous disorder will be (largely) ineffective in one of the two proposed

subgroups. Consequently, they will fail to show efficacy because of the substantial size of the non-responder groups in clinical trials.

Acknowledging that there are patients with HSDD/ FSIAD due to low sensitivity to sexual cues, or due to dysfunctional over-activity of sexual inhibitory systems, generates two therapeutic targets. Selective pharmacotherapeutic- and/or psychological therapies can be designed to tackle each underlying cause of these HSDD/FSIAD subtypes, instead of a "one size fits all" approach. This is the main premise of our personalized sexual medicine drug development program, which has led to two potential on-demand drug therapies. The first is the combined administration of testosterone (0.5 mg) and the PDE5 inhibitor sildenafil (50 mg) which increases the sensitivity for internal and external sexual cues, activates central sexual motivation mechanisms and subsequently the physiological sexual response and is intended for HSDD/FSIAD as the result of low sensitivity for sexual cues. The second is the combined administration of testosterone (0.5 mg) and the 5-HT_{1A} receptor agonist buspirone (10 mg) which increases sexual motivation, but inhibits overactive sexual inhibition mechanisms in the prefrontal areas and is intended for HSDD/FSIAD as the result of dysfunctional activation of sexual inhibitory mechanisms.

Future studies will have to confirm these drugs' long-term safety and efficacy in larger sample sizes. In these studies, an easy and reliable method of differentiating between the subgroups will have to be used. The studies described in this thesis used a modified version of the emotional Stoop task to determine if a subject had low sensitivity for sexual cues. It was also used as an indicator for high inhibition, as was subjects' response to the combined administration of testosterone and sildenafil (see chapter 7). These indirect methods for establishing HSDD/FSIAD subtype need to be refined, making demarcation more reliable. Moreover, it should be user-friendly so that if these combination therapies enter the market, a clinician can easily determine the HSDD/FSIAD subtype, and therefore which drug should be prescribed. One avenue our laboratory is exploring is the use of combined hormonal and genetic markers as a demarcation tool. Many factors can potentially affect the brain's sensitivity to sexual cues, and it's propensity for inhibition, for example androgens, estrogens, dopamine, serotonin, and noradrenaline, to name but a few. These neuromodulators must be considered in their interaction with their respective receptors, and the sensitivity thereof. For example, testosterone exerts its androgenic effect through its action at the androgen receptor. The strength of the androgenic effect that testosterone induces is dependent upon the cytosine-adenine-guanine (CAG) repeat length polymorphism of the androgen receptor gene (AR[CAG]n), where long AR[CAG]n is associated with weak receptor transactivation [13]. It is reasonable to assume that testosterone's influence on sexual cue sensitivity is dependent on AR[CAG]n, and it may be that deficiencies in this mechanism are important in the etiology of HSDD/FSIAD , specifically through low sexual cue sensitivity.

More research into the functional neuroanatomical correlates of HSDD/FSIAD is also warranted. As the studies in this thesis show, knowledge of functional neuroanatomical correlates of sexual problems helps to narrow the scope when searching for the root cause. It can also contribute to the diagnosis of HSDD/FSIAD, and its subtypes. A future study into the (functional) neuroanatomy of low sensitive and high inhibitory subjects may show that low sensitive subjects have altered amygdala and OTC processing, while high inhibitory subjects have altered DLPFC processing. Such a study may also benefit from a lower cluster size threshold (which was 1,600 mm^3 in the analysis of the study described in chapter 8). Subcortical group differences may then be observed, for example in the nucleus accumbens or the hypothalamus, both of which are important in sexual stimulus processing and sexual behavior. Such a study would further validate the here proposed HSDD/FSIAD subgroups. Moreover, this methodology could then be used to investigate long-term beneficial effects of targeted therapies.

Finally, the described personalized sexual medicine approach focusses on finding causes and therapies for HSDD/FSIAD, based on the dual control model of sexual response. The dual control model however, also describes male sexual (dys)function, in essentially the same terms as female sexual (dys)function. This raises the question if a comparable subtyping can be made in men, and if comparable medication combinations would be effective (albeit likely with higher testosterone doses). A program investigating this would be a valuable contribution to the field of male sexual dysfunction.

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Samenvatting

Verminderd seksueel verlangen is de meest voorkomende seks-gerelateerde klacht bij vrouwen [1-3]. Veel vrouwen ervaren hierdoor psychisch leed [4]. In die gevallen wordt er gesproken van een stoornis in het seksueel verlangen. Deze diagnose wordt gesteld aan de hand van het meest gebruikte diagnostisch handboek voor clinici, de *Diagnostic and Statistical Manual for Mental Disorders* (DSM). In dit handboek wordt deze stoornis aangeduid als *hypoactive sexual desire disorder* (HSDD). Een tweede seksuele stoornis welke in DSM wordt beschreven is *female sexual arousal disorder* (FSAD), oftewel een stoornis in de lichamelijke seksuele opwinding. Omdat HSDD en FSAD vaak samen gaan, en er slechts beperkt bewijs is dat het om separate stoornissen gaat [5] zijn in de meest recente, 5e uitgave van de DSM, HSDD en FSAD samengevoegd tot de diagnose *female sexual interest / arousal disorder* (FSIAD).

HSDD en FSAD komen vaak voor. De schattingen lopen zeer uiteen, van 7,7% tot 40,6 % voor HSDD en van 12,0% tot 31,2% voor FSAD [1-3,6-9]. Deze prevalentiecijfers lopen uiteen, mede doordat de studies die dit hebben onderzocht verschillende criteria hanteren (zie tabel 1 van hoofdstuk 1). Desalniettemin kan men stellen dat HSDD en FSAD, en dus ook FSIAD, zeer vaak voorkomen, zelfs volgens de meest conservatieve schattingen. Ondanks de omvang van dit probleem zijn de behandelmogelijkheden beperkt. De beschikbare psychologische interventies zijn over het algemeen beperkt tot matig effectief [10] en zijn er nog geen door *United States Food & Drug Administration* (FDA) en *European Medicines Agency* (EMA) goedgekeurde medicijnen op de markt.

Dit proefschrift beschrijft een serie studies welke zijn uitgevoerd als onderdeel van een gepersonaliseerd, medicijn ontwikkelingsprogramma voor middelen tegen stoornissen in het seksuele verlangen van vrouwen. Het programma is gebaseerd op de *'dual control model'* van de seksuele response. Dit model stelt dat stoornissen in, onder andere, het seksueel verlangen (HSDD dan wel FSIAD) worden veroorzaakt en in stand gehouden door het disfunctioneren van één van twee verschillende maar interacterende systemen, het seksueel activatie systeem en het seksueel inhibitie systeem. Individuen met een laag vermogen voor activatie van het seksueel activatie systeem of juist een sterk vermogen tot activatie van het seksueel inhibitie systeem zullen grotere kans hebben problemen te ondervinden in hun seksueel verlangen of hun seksuele respons. HSDD/ FSIAD kan dus worden veroorzaakt door disfunctioneren in een van deze systemen. Dit betekent dat HSDD/FSIAD kan bestaan uit minimaal twee verschillende subgroepen, afhankelijk van welk systeem in disbalans is. Als we weten welke breinmechanismen schuil gaan achter deze systemen, en wat er gebeurt wanneer zij haperen, dan kunnen we doelgericht medicijnen ontwikkelen om problemen in het seksueel verlangen tegen te gaan. In het medicijn ontwikkelingsprogramma dat hier wordt beschreven werd gezocht naar deze onderliggende breinmechanismen. Deze zoektocht leidde tot twee subgroepen binnen de HSDD / FSIAD patiëntenpopulatie. Eén subgroep heeft problemen in hun seksueel verlangen omdat zij een ongevoelig zijn voor seksuele prikkels (hun brein kan deze prikkels minder adequaat verwerken). Wanneer seksuele prikkels aanwezig zijn, zal hun brein er nauwelijks op reageren, waardoor seksuele activatie en motivatie niet op gang komen. De tweede subgroep heeft problemen in hun seksueel

verlangen omdat er bij hen sprake is van overactiviteit van hun seksueel remsysteem. Zodra seksuele prikkels aanwezig zijn, zal hun seksueel activatie systeem geactiveerd worden, maar dit leidt meteen tot sterke activiteit van hun seksueel remsysteem, waardoor de seksuele activatie en motivatie meteen direct afgeremd wordt. Deze bevindingen leidde tot de ontwikkeling van twee verschillende 'on-demand' (je neemt het in wanneer je zin wil krijgen om te vrijen) medicijncombinaties: de gecombineerde toediening van sublinguaal toegediende testosteron (0,5 mg) en de fosfodiesterase 5 (PDE5) remmer sildenafil (50 mg) voor de groep vrouwen met een ongevoelig systeem, en de gecombineerde toediening van sublinguaal toegediende testosteron (0,5 mg) en de serotonine 1A receptor agonist buspiron (10 mg) voor de groep vrouwen met een overactief remsysteem.

Voor elk van deze medicijn combinaties worden de twee actieve componenten (testosteron en een PDE5 remmer, dan wel testosteron en een serotonine 1A receptor agonist) op een zodanige wijze toegediend dat het farmacodynamisch effect van beide actieve componenten grotendeels overlapt. Sublinguaal toegediend testosteron wordt heel snel opgenomen in het lichaam (de maximale concentratie wordt na 15 minuten bereikt) en ook snel afgebroken. Na 2 tot 3 uur zijn de testosteron waarden in het bloed weer teruggekeerd naar de oorspronkelijke niveaus. Er is bij deze toedieningsvorm van testosteron echter een verlaat farmacodynamisch effect op de seksuele stimulusverwerking en seksuele motivatie welke 3 uur na toediening begint en dat ongeveer 3 uur voortduurt tot omennabij 6 uur na toediening [11]. De duur van het therapeutisch effect van sildenafil en buspiron is ook ongeveer 3 uur, maar deze start al +/- 1 uur na inname. De actieve componenten in beide medicijncombinaties moeten daarom op verschillende momenten in de bloed circulatie vrijkomen. Eerst wordt testosteron sublinguaal toegediend, waarna 2 tot 2½ uur later sildenafil (zie figuur 1 van hoofdstuk 9), of buspiron (zie figuur 2 van hoofdstuk 9) oraal worden toegediend. Deze toedieningsmethode zorgt er voor dat beide 'on-demand' medicijncombinaties een 3 uur durend therapeutisch effect hebben, welke in werking treedt ca. 3 uur na testosterontoediening.

Hoofdstuk 2 beschrijft een gerandomiseerd, dubbel blind, placebo gecontroleerd experiment waarin de invloed werd onderzocht van één enkele dosering van de gecombineerde toediening van testosteron (0,5 mg) en de PDE5 remmer vardenafil (10 mg) (in vergelijking met placebo, en in vergelijking met beide monotherapieën afzonderlijk) op de fysiologische seksuele respons in vrouwen met HSDD. De vaginale puls amplitude (VPA) tijdens visuele seksuele stimulatie was sterker in de conditie met de gecombineerde toediening van testosteron en vardenafil dan in de andere condities. Dit effect werd gedragen door een groep vrouwen (n=8) die *niet* seksueel misbruikt waren tijdens hun jeugd. In de groep vrouwen (n=5) die dit wel was overkomen (*childhood sexual abuse*, CSA), werden er geen verschillen in fysiologische responsen waargenomen tussen de vier drug condities. De patiënten uit de twee groepen verschilden ook van elkaar in de mate waarin zij onderbewuste aandacht hadden voor seksuele prikkels. De CSA groep had aanvankelijk hoge aandacht voor seksuele prikkels

maar dit daalde aanzienlijk na testosteron toediening. De niet-CSA groep had aanvankelijk lage aandacht, welke steeg na testosteron toediening. Deze observaties leidde tot de hypothese dat vrouwen met een aanvankelijk lage aandacht voor seksuele prikkels HSDD hebben omdat de relevante systemen in hun brein relatief ongevoelig zijn voor seksuele prikkels. Testosteron kan de gevoeligheid voor seksuele prikkels verhogen [12], dus de toename in aandacht na testosteron toediening is een weerspiegeling van toename in gevoeligheid voor seksuele prikkels in deze vrouwen. Deze toename in gevoeligheid zorgt er voor dat een PDE5 remmer effectief kan zijn. In de groep vrouwen met CSA, werd er een daling van aandacht waargenomen. We concludeerden dat dit het gevolg was van een vermijdingsreactie (de door testosteron versterkte en, sinds kinds af aan, al dan niet bewust, negatief geïnterpreteerde seksuele prikkels zorgen hiervoor), wat zorgt voor remming van hun seksuele respons. Dit verklaart het ontbreken van een fysiologische respons op de gecombineerde medicijntoediening van deze groep vrouwen in deze studie.

In een tweede gerandomiseerd, dubbel blind, placebo gecontroleerd experiment (hoofdstuk 3) werd wederom de invloed onderzocht van één enkele dosis van de gecombineerde toediening van testosteron (0,5 mg) en de PDE5 remmer vardenafil (10 mg) (in vergelijking met placebo, en in vergelijking met beide monotherapieën afzonderlijk) op de fysiologische en subjectieve seksuele respons in 28 vrouwen met HSDD, nu zonder vrouwen met CSA. De vrouwen werden ingedeeld in twee groepen, wederom gebaseerd op hun onderbewuste aandacht voor seksuele prikkels vóór testosterontoediening. De resultaten waren vergelijkbaar met die van de studie uit hoofdstuk 2. De vrouwen met een aanvankelijk lage aandacht (n=17) verhoogden hun aandacht na testosteron toediening, en hadden een sterke toename in hun fysiologische (VPA) en subjectieve (vragenlijst) respons tijdens visuele seksuele stimulatie in de conditie met gecombineerde toediening van testosteron en vardenafil, maar niet in de placebo conditie, noch in de monotherapie condities. De vrouwen met aanvankelijk hoge aandacht (n=11) toonden het tegenovergestelde effect. De twee *non-responder* groepen (vrouwen die niet positief reageerde op de gecombineerde toediening van testosteron en vardenafil) uit de studies uit hoofdstuk 2 en 3, verschilde enkel in CSA verleden. De ene groep had CSA ervaren, terwijl de anderen dat niet hadden ervaren. De groep non-responders zonder CSA rapporteerden echter wel vaker seksueel misbruik op latere leeftijd dan de *responders* in die studie (63% vs 17%). Beide non-responder groepen hebben dus een geschiedenis van seksueel misbruik gehad. Dit, tezamen met hun vergelijkbare psychologische en fysiologische responsen op testosteron en de gecombineerde toediening van testosteron en vardenafil als beschreven in hoofdstuk 2 en 3, leidde tot de hypothese dat deze vrouwen HSDD hebben door een overactief seksueel remsysteem.

De hypothese dat er een subgroep vrouwen bestaat welke HSDD heeft door een overactief seksueel remsysteem werd verder bevestigd door 2 *functional magnetic resonance imaging* (fMRI) studies (zie hoofdstuk 5). In de eerste van deze twee fMRI studies, kregen 12 seksueel gezonde vrouwen erotische filmfragmenten te zien onder

condities van placebo en sublinguaal testosteron (0,5 mg). Er werd verwacht dat onder conditie van testosteron, de hersengebieden welke belangrijk zijn voor seksuele opwinding sterker geactiveerd zouden zijn. Het tegendeel werd geobserveerd. Deze gebieden vertoonden juist minder sterke activatie in vergelijking tot de placebo conditie. Tevens waren er 2 gebieden, welke belangrijk zijn bij inhibitie van motivationeel gedrag, sterk geactiveerd: de dorsolaterale prefrontale cortex (DLPFC), en de septale kernen. De omstandigheden in de testosteron conditie zorgden blijkbaar voor remming van de seksuele respons. We concludeerden dat de testosteron toediening de sensitiviteit van het brein verhoogde, maar doordat de omstandigheden niet geschikt waren voor een seksuele respons/seksueel gedrag (de vrouwen lagen in een lawaaierig MRI apparaat met mannelijke techneuten en proefleiders die hen bekeken), werden inhibitie mechanismen (DLPFC, septale kernen) geactiveerd.

De tweede fMRI studie toonde aan dat gecombineerde toediening van testosteron en de PDE5 remmer sildenafil een soortgelijke inhibitierespons teweeg kan brengen in vrouwen met HSDD. In dit gerandomiseerd, dubbel blind, placebo gecontroleerd, cross-over experiment werd de invloed van mate van aandacht voor seksuele prikkels op breinactivatie onderzocht, onder condities van placebo, testosteron en de gecombineerde toediening van testosteron en sildenafil. Uit dit experiment bleek dat wanneer de aandacht werd gericht op de genitale respons, verminderde DLPFC activatie en verhoogde insula activatie optrad (toename in interoceptieve gewaarwording) onder placebo, en dit werd versterkt onder testosteron condities. Onder condities van de gezamenlijke toediening van testosteron en sildenafil werd dit patroon omgekeerd, en was dus sprake van inhibitie van de seksuele respons. We concludeerden dat de additieve sildenafil component de seksuele activatie in het brein verhoogde, wat blijkbaar (bewust of onbewust) ongepast bleek waardoor inhibitie noodzakelijk was.

Er zijn veel situaties waarin het ongepast is om seksueel te reageren op een seksuele prikkel, bijvoorbeeld vanwege sociale normen. Ondanks deze normen kan een seksuele prikkel alsnog een respons uitlokken, maar deze moet dan afgeremd worden [13]. De mate waarin moet worden afgeremd is afhankelijk van de sterkte van de seksuele respons welke geremd dient te worden. Als een seksuele stimulus zwak is (door de aard van de stimulus, of de predispositie van de patiënt voor verminderde sensitiviteit voor seksuele prikkels) zal een seksuele response relatief zwak zijn, waardoor remming mogelijk niet noodzakelijk is. Maar wanneer een stimulus wordt versterkt door testosteron toediening, dan zal de response sterker zijn, en dus ook de mate van activiteit noodzakelijk om deze respons te remmen. Hierdoor kan dus het eindeffect van testosterontoediening, remming zijn. Het lijkt erop dat de DLPFC een belangrijke rol speelt in dit remmechanisme.

Vrouwen met HSDD die overmatige activiteit van seksuele remmechanismen hebben, worden verondersteld overmatige inhibitie activiteit van de DLPFC te hebben (al dan niet na testosteron toediening), en zouden dus baat kunnen hebben bij een tijdelijke vermindering van deze remming. Serotonine (5-HT) is een neuromodulator welke een

belangrijke rol speelt in prefrontale cortex (PFC) gemedieerde inhibitie [14]. Wanneer 5-HT output in de PFC tijdelijk wordt verlaagd, zou er minder DLPFC-gemedieerde remming ontstaan. Buspiron is een serotonine 1A receptor agonist welke dit effect kan bewerkstelligen. We veronderstelden dat de gecombineerde toediening van testosteron en buspiron aan vrouwen met HSDD door overmatige activatie van het seksueel remsysteem, de remming van hun seksuele respons op seksuele prikkels zou verminderen waardoor zij vervolgens een relatief normale seksuele respons zouden kunnen hebben.

Beide 'on-demand' combinatiebehandelingen werden getest in twee groepen vrouwen met HSDD (HSDD als gevolg van een ongevoelig systeem, of als gevolg van overmatige remming). Deze gerandomiseerde, dubbel blind, placebo gecontroleerde, cross-over studie testte het effect van meermalig, 'on-demand' gebruik thuis van de gecombineerde toediening van testosteron en sildenafil, en de gecombineerde toediening van testosteron en buspiron (deze studie staat beschreven in hoofdstukken 6 en 7). 57 vrouwen met HSDD kregen de drie onderzoekmedicijnen (testosteron 0,5 mg + sildenafil 50 mg; testosteron 0,5 mg + buspirone 10 mg en placebo) elk gedurende een vier weken durend medicatieregime. Tijdens de eerste week uit elk regime werd de subjectieve en psychofysiologische respons van de patiënt gemeten tijdens het kijken naar erotisch beeldmateriaal, door middel van ambulante meting thuis met het draagbare, door patiënt aangestuurde psychofysiologisch laboratorium (zie hoofdstuk 4 voor een beschrijving van dit draagbare laboratorium, en een discussie over de toename in ecologische validiteit van de resultaten hierbij). De daarop volgende drie weken konden patiënten zelf bepalen wanneer en hoeveel van het 'on-demand' medicijn ze wilde gebruiken (met een maximum van één medicatiecombinatie per twee dagen). Beide actieve studiemedicaties verhoogden de subjectieve en psychofysiologische respons op erotische films, en verhoogden de seksuele satisfactie thuis, in die subgroep van patiënten waar het combinatiemiddel voor ontwikkeld was. Dus testosteron 0,5 mg + sildenafil 50 mg werkte in vrouwen met HSDD en een lage sensitiviteit voor seksuele prikkels, en testosteron 0,5 mg + buspirone 10 mg werkte in vrouwen met HSDD en overactiviteit van seksuele remmechanismen.

In hoofdstuk 8 wordt een MRI studie beschreven waarvan het doel was om potentiële neuro-anatomische verschillen in grijze (celkernen) en witte stof (axonverbindingen) te onderzoeken tussen vrouwen met (n=29) en zonder (n=16) HSDD. De resultaten van deze studie toonden dat in de breinen van vrouwen met HSDD seksuele stimuli minder opvallen dan in de breinen van vrouwen zonder HSDD. Dit bleek uit de grotere witte stof waarden van beide amandelkernen, en het lagere grijze massa volume in de linker occipitotemporale cortex van vrouwen met HSDD. Daarnaast bleek dat het waarnemen van de lichamelijke seksuele respons en de bewustwording hiervan ook minder goed verloopt in de breinen van de HSDD groep. Dit bleek uit het lagere grijze massa volume in respectievelijk de rechter insula en de bilaterale anterior temporale cortices. Als laatste bleek dat de vrouwen met HSDD minder grijze massa volume in de anterior cingulate cortex en de dorsolaterale prefrontale cortex (DLPFC) hadden t.o.v. de

controlegroep, wat mogelijk wijst op afwijkende (seksuele) respons selectie en inhibitie van seksuele responsen. We concludeerden dat vrouwen met HSDD seksuele prikkels als minder opvallend ervaren (amandelkernen en occipitotemporale cortex), wat mogelijk overeenkomt met de eerder beschreven verlaagde gevoeligheid voor seksuele prikkels. Omdat seksuele prikkels minder opvallen, zal seksuele respons hierop minder vaak voorkomen, dan wel minder sterk zijn. Een zwakkere seksuele response geeft een zwakkere lichamelijk respons. Een zwakkere lichamelijk response wordt minder goed waargenomen (insula), en zal minder makkelijk door dringen tot de bewuste gewaarwording (anterior temporale cortices). Doordat de seksuele prikkels, zowel de externe prikkels als de interne lichamelijk seksuele responsen, over het algemeen minder goed worden waargenomen, kan aandacht niet op deze stimuli worden gericht. Hierdoor worden seksuele respons systemen minder vaak actief aangewend (anterior cingulate cortex). Omdat seksuele responsen minder vaak voorkomen (dus ook minder vaak in ongepaste situaties), is de inhibitie van de seksuele response ook onderontwikkeld (DLPFC). Dit laatste is gebaseerd op de aanname dat de huidige HSDD steekproef met name bestaat uit vrouwen met een laag sensitief systeem. We verwachten dat in vrouwen met overmatige activiteit van seksuele remmechanismen, de DLPFC juist meer grijze massa volume heeft.

Deze bevindingen geven een mogelijk neuro-anatomisch substraat voor het *dual control model*. Het seksueel activatie systeem van vrouwen met HSDD/FSIAD heeft een lagere tendentie om adequaat geactiveerd te raken omdat het brein seksuele prikkels niet als 'opvallende' prikkels verwerkt en potentiele lichamelijke reacties op seksuele prikkels worden minder goed waargenomen. Hierdoor verloopt de respons selectie (bijvoorbeeld een seksueel respons script) minder adequaat. Het seksueel inhibitie systeem is ook aangetast wat blijkt uit het afwijkende grijze massa volume van een van de belangrijke structuren welke inhibitie medieert, de DLPFC.

Conclusie

Dit proefschrift geeft cognitieve, psychofysiologische, subjectieve, neuro-anatomische en farmacologische evidentie dat er op zijn minst twee HSDD/FSIAD subgroepen bestaan. Deze bevinding is van groot belang voor het vakgebied. Over het algemeen wordt HSDD/FSIAD als een homogene stoornis behandeld. De farmacologische behandelingen die elders in ontwikkeling zijn, lijken uit te gaan van deze premisse. Maar als HSDD/FSIAD inderdaad bestaat uit twee verschillende subgroepen, dan zullen potentiele medicamentueze behandelingen die uitgaan van een homogene stoornis waarschijnlijk ineffectief zijn in minimaal één van de twee subgroepen. Klinische trials die deze medicijnen onderzoeken zullen een grote kans hebben om te falen omdat er een grote groep non-responders geïncludeerd wordt.

Als we erkennen dat er twee (of meer) HSDD/FSIAD subgroepen zijn, namelijk één subgroep waarin de patiënten gekenmerkt worden door hun lage gevoeligheid voor seksuele prikkels, en één subgroep waarin de patiënten gekenmerkt worden door hun

overmatige activiteit van seksuele remmechanismen, dan geeft dit niet één maar twee therapeutische indicatiegebieden. Met deze kennis kunnen dan selectievere farmacotherapeutische of psychologische interventies worden ontwikkeld, in plaats van een '*one size fits all*' benadering. Dit was een van de fundamenten van Emotional Brain's medicijn ontwikkelingsprogramma, welke heeft geleid tot twee medicamenteuze 'on-demand' behandelingen voor HSDD/FSIAD. De eerste is de gecombineerde toediening van sublinguaal testosteron (0,5 mg) met sildenafil (50 mg) voor vrouwen met HSDD/FSIAD laag gevoelig voor seksuele prikkels, en als tweede, de gecombineerde toediening van sublinguaal testosteron (0,5 mg) met buspiron (10 mg) voor vrouwen met HSDD/FSIAD met een verhoogde activiteit van hun seksueel remsysteem. Toekomstig onderzoek, in grotere behandelingsgroepen, zal moeten uitwijzen wat de lange termijn effectiviteit en veiligheid is van deze middelen. Als deze middelen op de markt komen, zal er ook een verbeterde en meer betrouwbare methode om de subgroepen van elkaar te onderscheiden moeten worden ontwikkeld. Deze methode dient ook makkelijk en hanteerbaar te zijn voor de clinicus, die uiteindelijk moet bepalen welk van de twee medicijnen voorgeschreven dient te worden.

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Acknowledgements / Dankwoord

Er zijn veel mensen die direct of indirect betrokken zijn geweest bij het tot stand komen van dit proefschrift. Ik zal proberen hen recht te doen.

Adriaan Tuiten – zonder jouw inspirerende ideeën, lef, tomeloos enthousiasme, en onvoorstelbaar doorzettingsvermogen was er geen Emotional Brain, geen Lybrido, geen Lybridos en dus ook geen proefschrift. Ik heb ontzettend veel van je geleerd, door onze vele discussies (vaak tot in de kleine uurtjes), maar ook door het gewoon af te kijken. Een voor mij belangrijke selectie: het lef om van de betreden paden af te wijken, voorbij die beren op de weg te kijken, doorzetten en er boven op te blijven zitten, whisky te waarderen, en niet in de minste plaats, beter te analyseren en strakker te schrijven.

Berend Olivier – ondanks de afstand tussen Almere en Utrecht ben je altijd heel benaderbaar geweest. Je bent een zeer betrokken promotor, maar je liet me de afgelopen jaren ook mijn eigen gang gaan, wetend hoe belangrijk mijn andere werkzaamheden waren, en dat deze nou eenmaal vaak voorrang hadden. Toch weerhield jou dit niet om op een altijd-prettige wijze de druk erop te houden ("je moet wel promoveren voor ik met pensioen ga!"). Bijna gelukt.

Steven Scholte – het is inspirerend om met jou te discussiëren Steven. Je bruist van de ideeën (ik moet toegeven dat ik niet altijd die kermis van gedachten kon volgen) en energie en dat is zeer aanstekelijk. Ik had graag wat meer projecten met je gedaan de afgelopen jaren, maar wie weet wat de toekomst brengt.

Marcel Waldinger – jouw bijdrage aan ons onderzoeksveld is bewonderenswaardig. Jij zit in 'de mannenhoek' maar ik weet zeker dat de grote raakvlakken tussen de seksen op gebied van de regulatie van seksueel gedrag voor raakvlakken in ons onderzoek en werk zal zorgen, en daar verheug ik me op.

Walter Everaerd – ik bewonder jouw onbegrensde kennis van de psychologische en seksuologische literatuur. Er lijkt geen artikel te zijn dat je niet hebt gelezen. Dit heeft mij in het begin van mijn promotietraject vaak geholpen. Ik zal altijd met een glimlach terugdenken aan onze redigeersessies van mijn eerste stuk. Jij naast me zittend ("wat wil je nou zeggen? … schrijf dat dan op!") terwijl ik typte.

Hans Koppeschaar, Leo de Leede en Peter van der Heijden – ik heb de afgelopen jaren ontzettend veel van jullie geleerd over de endocrinologie, farmacie/farmacologie en statistiek, respectievelijk. Daarnaast zijn jullie alle drie ook gewoon hele fijne mensen om mee samen te werken! Ik draag jullie alle drie een warm hart toe.

Leonard DeRogatis, Irwin Goldstein and Don Rubin – three greats in their areas. I have always loved the discussions we've had and I've learned so much from you guys. You are an example to all of us and I sincerely hope that our collaboration will continue for years to come.

Members of the dissertation committee – thank you!

Alle mensen van Emotional Brain (maar ook ATIA en FlevoResearch) – Emotional Brain is al 12 ½ jaar mijn 2e thuis. Het is een plek waar ik altijd met veel plezier heb gewerkt en dat komt niet alleen door het prachtige onderzoek wat we doen. Het komt ook door alle lieve, hardwerkende mensen die er werken en hebben gewerkt (Els, Brenda, Donna & Hans werk ik al heel lang met veel plezier mee samen, maar er zijn er nog veel meer). Vooral team EB-FSD (present & past: Cliviante, Diana, Jeroen, Kim, Martine, Monique, Petra, Saskia, Marijke, Inge, Yvonne, Nikki, Fenna, Gert, Flip, Crista, Hester, Frederique, Janske, Wadi, Eva en Viktoria) ben ik dankbaar, het fijne groepje mensen dat alle studies naar seksueel disfunctioneren uitvoert of heeft uitgevoerd. Jullie zijn me allen dierbaar en zonder jullie hadden we nooit datgene bereikt wat we tot nu toe hebben bereikt. De dagelijkse lunch zorgde altijd voor de broodnodige ontspanning, en ik hoop dat de gesprekken over seks, zombies, en intelligente TV series als Goeie Tijden en Holland's next Topmodel nog vaak gevoerd zullen worden. Van deze lieve groep verdienen Kim van Rooij en Jeroen Gerritsen wat extra aandacht. Jeroen en Kim – dank jullie beiden voor de steun die jullie voor mij zijn geweest. Onze woensdagavond schrijfavonden hebben mij de laatste extra zet gegeven om dit proefschrift af te ronden, en het squashen was voor mij de perfecte manier om alle opgebouwde cortisol uit m'n lichaam weg te spoelen.

En nu een allerlei aan mensen met wie ik de afgelopen jaren via mijn werk bij EB mee in contact ben gekomen en die op hun vakgebied (en manier) mij iets hebben bijgebracht. Amy Criswell (thanks for all the photos of your 'kids'), Marilyn Agney (thanks for the puns & the spell check), Henrik Rasmussen ("absolutely phenomenal"), de slimme mensen van V.O. Patents & Trademarks (Mark, Frits & Tamara – jullie vakgebied blijft een mysterie voor mij), het hardwerkend team van PSR Group dat de kwaliteit van onze studies bewaakt (Roger, Christa, Hansje, Dinny – onze ICH-GCP goeroes die een discussie over de regelgeving altijd verwelkomen) en de kundige mensen van ACE Pharmaceuticals die de onderzoeksmedicatie hebben bereid (Bas, Cees – altijd een plezier om mee samen te werken).

De aandeelhouders van EB – dank voor jullie geloof in ons. Zonder jullie steun was dit allemaal onmogelijk geweest.

Alle vrijwilligsters die meededen aan onze studies – dank jullie wel! Ik hoop dat we in de toekomst nog meer kunnen bijdragen aan het begrip over seksuele problemen zodat de behandelingsmogelijkheden beter worden.

En natuurlijk mijn lieve familie, vrienden en the Clan. Deze mensen hebben mij gevormd, gesteund en heerlijk afgeleid de afgelopen jaren en ik hoop dat jullie dat nog vele jaren blijven doen. Pap – dank je. Ik ben zo dankbaar dat je nog het moment hebt mee mogen maken dat ik genoeg artikelen had om te kunnen promoveren. De tranen die we toen deelden zullen me altijd bijblijven. Mam – dank je. Pap en jij hebben mij alle kansen gegeven zodat ik kon gaan studeren 'in de grote stad'. Na wat vallen en opstaan is het

dan toch nog goed gekomen. Arna, Jos, Jet, Puk & Sam – onze feestavonden en vakanties met onze gezinnen zijn me zeer dierbaar. Proost! Theo en Femke –dank voor jullie aanhoudende interesse en steun, en voor de tig keren dat jullie hebben bijgesprongen als ik weer eens naar de VS moest. Bram en Ingrid – dank ook voor jullie aanhoudende interesse en steun. Niet alleen onze jongens zijn dol op jullie.

Daniël, Christan en Alexander – mijn echte meesterwerken. Ik ben best wel trots op dit boekje. Ik heb er namelijk heel hard voor gewerkt. Maar het verbleekt bij de trots die ik voel voor jullie. Laten we dit vieren met een Legodag en mannenavond!

En Karin – tja… last but not least? Last *and most* is veel accurater. Jij bent mijn rots geweest al deze jaren. Je hebt mijn keus om en een drukke baan te hebben en een promotie daarnaast te doen altijd gesteund. Je hebt nooit aan mij getwijfeld. Je hebt mij een goed geplaatste schop onder de kont gegeven waardoor ik uiteindelijk neuropsychologie ben gaan studeren en het zover heb geschopt. Je hebt mij 3 prachtige zoons geschonken. 'Dank je' is ineens een leeg begrip. Ik hou van je.

Curriculum Vitae

Jos Bloemers was born on the 22nd of March, 1974, in Cuijk and Sint Agatha, the Netherlands. He completed secondary school in 1994 at Elde College (at that time, Skinle College), Schijndel, the Netherlands. He attended Radboud University in Nijmegen (at that time, Katholic University Nijmegen) where he majored in Neuro- & Rehabilitation Psychology. He obtained his degree in 2001, and on January 1st, 2002, started work at Emotional Brain as a junior scientist. He first conducted research on the influence of dopamine on motivated behavior. In 2005, he became manager of the FSD R&D department at Emotional Brain. This department conducts research on the fundamental mechanisms of female sexual (dys)function, and performs clinical trials investigating potential pharmacotherapeutic interventions for HSDD. During this period, he started his work on his PhD thesis. In 2011, he became Director Scientific Operations at Emotional Brain, and he presently still holds this position. Aside from his scientific work, Jos has also co-authored international patents and has (co-)authored Regulatory submissions (pre-IND meeting, Investigational New Drug applications, End of Phase 2 meetings, Requests for Scientific Advice, Special Protocol Assessments) for, and held meetings with the U.S. Food and Drug Administration and the European Medicines Agency.

Jos Bloemers is geboren op 22 maart 1974, te Cuijk en Sint Agatha, Nederland. Hij behaalde zijn VWO diploma in 1994 op het Skinle College te Schijndel (het huidige Elde College). Na het behalen van zijn VWO diploma is hij psychologie gaan studeren aan de Katholieke Universiteit Nijmegen (de huidige Radboud Universiteit). Hij studeerde af in 2001 op de afstudeerrichting Neuro- en Revalidatiepsychologie. Op 1 januari 2001 startte hij als junior onderzoeker bij Emotional Brain. Hij begon met onderzoek naar de invloed van dopamine op motivationeel gedrag. In 2005 werd hij manager van de afdeling FSD R&D van Emotional Brain. Deze afdeling doet fundamenteel wetenschappelijk onderzoek naar seksuele (dis)functies bij vrouwen, en ontwikkelt en onderzoekt potentiele geneesmiddelen daarvoor. Gedurende die periode is hij gestart met zijn proefschrift. In 2011 is hij Director Scientific Operations geworden bij Emotional Brain. Deze positie bekleedt hij nog steeds. Naast zijn wetenschappelijke werkzaamheden is Jos coauteur van internationale patenten. Hij is ook (co)auteur van regulatoire documentatie (pre-IND meeting, Investigational New Drug applications, End of Phase 2 meetings, Requests for Scientific Advice, Special Protocol Assessments) voor en heeft besprekingen gehad met de Amerikaanse en Europese regelgevende autoriteiten (U.S. Food and Drug Administration en de European Medicines Agency).